BfR Commissions

Minutes | 29th October 2024

4th Meeting of the BfR Commission on Tattoo Inks

The BfR Commission on Tattoo Inks advises the German Federal Institute for Risk Assessment (BfR) as an honorary and independent expert body on issues of tattoo ink safety and risk assessment by giving counsel to the BfR on the development and adjustment of analytical and toxicological methods suitable for inks and pigments. Furthermore, the Commission ensures a continuous dialogue with the state surveillance agencies.

With its scientific expertise, the Commission advises the BfR and can assist the Institute as a network of experts in the event of a crisis. The Commission consists of 23 members appointed for a four-year term through an open tender and application procedure. They distinguish themselves through scientific expertise in their respective field. The members of the Commission are obliged to preserve confidentiality towards third parties and to fulfil their duties impartially. Any conflicts of interest regarding individual agenda items discussed in the meeting are subject to transparent queries and disclosure. The meeting minutes below reflect the scientific opinion of the BfR Commission. The Commission's recommendations are entirely advisory in nature. The Commission itself does not issue any decisions or expert opinions and is not authorised to issue instructions to the BfR (and vice versa) nor involved in its risk assessments.

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Item 1 Welcome and approval of agenda

The managing director opens the commission´s 4th meeting and welcomes the participants, acknowledging the subcommission meetings held the previous day. The managing director informs that the number of subcommission meetings will be reduced. Alternatively, workgroups can be established to address specific topics. He announced the new appointment period of the commission 2026-2029. The call for experts for the new appointment period will be published by the beginning of 2025. Experts are encouraged to apply. Attendees do not raise objections or propose additions to the agenda.

Item 2 Declaration on conflicts of interest

The participants declare to have no conflict of interest.

Item 3 Summary of previous subcommission meetings held on 28th of October

The chairperson, Prof. Dr. Marilena Carbone, gives a summary about the previous subcommission meetings.

Subcommission Analytics

An external guest speaker presents his work on the application of MALDI-ToF-MS – and especially data evaluation tools – for identification of tattoo pigments. One focus of this work is on sharpening the fingerprint when using MALDI-ToF-MS and creating more defined reference spectra for libraries. Spectra or fingerprint comparison can then be carried out using similarity matrices, for example cosine similarity. The presented approach – although not originally designed for pigments – was applied to tattoo pigments. The samples used for this test were provided by the BfR within a laboratory comparison study between several state surveillance agencies and consisted of several inks and the respective pigments, which were prepared using a clean-up procedure. In theory, the properties of tattoo pigments should provide good conditions for MALDI-ToF-MS analysis and data evaluation, as they exhibit in general strong chromophores and many functional groups. Identification of pigments in tattoo inks though proved to be

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more complex. While some pigments were easy to identify with the aforementioned evaluation approach, others only showed low matching similarities. Whether this is due to pigments only be present in low concentration or due to pigments being hard to detect by MALDI-ToF-MS still needs to be investigated.

In a second presentation, a commission member brings forward new information from ongoing research in his institute. Firstly, he reports on progress regarding the development of an HPLC method for pigment screening in inks and on the identification of forbidden pigments in investigated inks. Secondly, the application of colorimetry in sulfuric acid for pigment identification and quantification is presented. And lastly, it is reported, that the use of “old” restricted pigments is decreasing, so is the occurrence of the associated impurities. Likewise, the use of new pigments may introduce new impurities based on possible by-products from their synthesis. Here, BfR agrees that this is an important point to look at.

Lastly, the BfR summarises its recent activities with regard to pigment analysis. This includes a general update regarding the development of a tiered approach for tattoo pigment screening and analysis. Furthermore, two techniques, FTIR and HPLC-DAD, and progress in the respective method development and testing are discussed in more detail. For the FTIR screening method a laboratory comparison with several state surveillance agencies was organised by BfR. The results show, that the tattoo ink and pigment samples provided by the BfR could successfully be analysed by the participants and similar results were obtained on a qualitative level. However, the data evaluation in regard to the presence of specific pigments in the inks, proved difficult. No data evaluation protocols or transparent automated FTIR identification protocols or software are available at the moment. Therefore, this laboratory exchange will be continued focussing on extending the scope of samples and the data evaluation. Regarding the HPLC method, BfR reports on the successful improvement of the separation method and the continued validation. Furthermore, the BfR reports on a cooperation with a third party to investigate the dynamic dissolution of tattoo pigments in body fluid simulants. Here, the analysis of Pigment Blue 15:3 eluents by ICP-MS indicates that copper which was detected in the first fractions is likely to originate from ionic remnants from synthesis adsorbed at the pigment. Furthermore, over the duration of the one-week trial run, a moderate increase of copper release was detected, indication a slow dissolution over time.

Subcommission Toxicology

A BfR employee presents the application of the OECD Test Guideline (TG) 487 in genotoxicity testing, focusing on its relevance for tattoo pigment safety assessment. The

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speaker outlines a novel approach for adapting the micronucleus assay to assess the genotoxic potential of pigments considering them as nanomaterials. The speaker describes in detail a comparison of conventional vs. nano-adapted methods. Such specific modifications are emphasised as these are needed to address unique properties of nanomaterials in biological systems and to ensure the reliability of the test for the identification of genotoxic risks. A specific focus is given to photogenotoxicity testing of tattoo pigments, accounting for potential reactions under UV and visible light exposure. The modified OECD TG 487 protocol for photogenotoxicity is described, incorporating light exposure setups to simulate realistic conditions.

The presentation is followed by a discussion. Following challenges are identified: Members raise questions and discuss the scalability of adapted assays and their validation for regulatory acceptance.

There is consensus on the need to integrate advanced testing strategies, especially for nano-enabled tattoo pigments and their photogenotoxicity.

Subcommission Technology & Hygiene

A guest speaker presents the US FDA guidance document on tattoo inks: Insanitary Conditions in the Preparation, Packing, and Holding of Tattoo Inks and the Risk of Microbial Contamination - Guidance for Industry. Here, the microbiological safety of tattoo inks is introduced more generally giving insights into US survey data. This is followed by recommendations about hygienic conditions and best practices for tattoo ink preparation. In addition, the US system, in which customer report on adverse effects are reported directly to the FDA, and possible follow-up measures are discussed. Finally, it is mentioned that the FDA recommendations are not legally binding and that there is no information on the practicability of the proposed sterilization criteria.

Item 4 Guest speaker – Challenges in tattoo ink manufacturing

A manufacturer of tattoo inks reports on discontinuing its production of tattoo inks and provides background information on this topic.

The implementation of 3 internal standards to their ink manufacturing was initially set: 1) The inks must be legal and even meet criteria beyond to guarantee the safety of their use. 2) Use of a reduced number of ingredients to minimise chemical risk. Technical solutions in production were implemented, instead of using defoaming agents. Sterilised

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mono-dose inks were produced to avoid preservatives. The formulation was reduced to 8 pigments, water, glycerine and propylene glycol and two dispersion agents – all together resulting in 13 substances needed for the formulation of all colours. 3) Every ingredient shall be toxicologically evaluated using existing data considering also the safety guidelines of the BfR.

No new toxicological data could be created to meet the safety requirements as the costs for the conduction of such tests were too high. Moreover, 4000 substances are estimated to be affected by the entry 75 of the REACH Regulation. Targeting all of them is expensive, even by using external analytic service providers. Therefore, the following group of substances were monitored in the raw materials: primary aromatic amines, nitrosamines, heavy metals, polycyclic aromatic hydrocarbons, and few more substances. Dispersion agents had to be changed as they contain isopropanol, which is prohibited by the REACH regulation. Raw materials contained impurities such as diethylene glycol, phenol, 1,4-dioxane, acetone, xylene and dichlorobenzene. Adjustment of the inks to the recent requirements according to the REACH regulation, would take years and was therefore economically inefficient.

A further major problem mentioned was the absence of pure lots of pigments. Formaldehyde and acetaldehyde were present in raw materials such as glycerin but also formed upon gamma-sterilisation. The concentration limit of 0.5 ppm set under the REACH regulation is way stricter compared to other areas. Not even pharmaceutical grade materials would be sufficient to meet the requirements for the regulation. No purification process of raw materials could be found economically sufficient.

It is suggested by a commission member to write an open letter to the EU commission to address the above-mentioned points. Another ink producer mentions that heavy metal cannot be cleaned up easily and they also think about leaving the market. A joint supply for tattoo pigments is suggested. In the past, Ink producers tried to get support by pigment producers but have not been successful.

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Item 5 Guest speaker – Tattoos as a risk factor for cancer

A publication on lymphoma cases in tattooed people is presented. The paper by Nielsen et al. was published in the beginning of 2024 and was debated intensively by experts.[1] The study was intended to answer the question whether tattoos pose a risk factor for the development of malignant lymphoma. Nielsen et al. found that the number of lymphoma cases was increased by 21 % in tattooed people, indicating a higher risk in developing a lymphoma compared to non-tattooed subjects. All other investigations in the study have been exploratory. An introduction to the Swedish national registers that have a long history of keeping data for the total population is given. In the study, exposure was assessed by a questionnaire in terms of tattoo size, tattoo colour, time between tattooing and disease onset. An exposure-response relationship could not be verified. Infections are not seen as confounders but may play a role as mediators. Hence the study does not answer the question whether the ink components or other factors such as infections or poor hygiene during wound healing lead to the lymphoma development. In cancer development there are the tumour initiators that start the cancer process and develop over a long time until clinical manifestations are shown up, and there are the tumour promoters that may accelerate an already ongoing process. Hence, the short exposure times until diagnosis may be explained by previous tumour initiation, which was promoted by tattooing. This hypothesis is supported by the fact that short exposures observed for the Hodgkin's lymphoma, having larger genetic components, rather than other types of cancer appearing at later ages and are more influenced by environmental factors. Other life style factors, like other body modifications, BMI and nutrition are important factors that could not be considered in the study.

The expert refers to another recent study that found similar results by investigating twins.[2] Also studies on malignant melanoma and cutaneous squamous-cell carcinoma from the Nielsen group are about to be published soon.

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[1] Nielsen, C., M. Jerkeman, and A.S. Jöud, Tattoos as a risk factor for malignant lymphoma: a population-based case-control study. EClinicalMedicine, 2024. **72**: p. 102649.

[2] Clemmensen, S.B., et al., Tattoo ink exposure is associated with lymphoma and skin cancers – a Danish study of twins. medRxiv, 2024: p. 2024.07.05.24309993.

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Item 6 Guest speaker – PSLT properties of organic pigments – applicable animal and non-animal methods

An external guest starts her presentation by outlining the definition of inks, dyes and pigments and stressing important differences such as the low solubility of pigments in most media. Organic pigments consist of planar, hydrophobic molecules that are densely packed within the particle or crystal structure. Strong intermolecular forces result in high densities and binding energies. Toxicological studies indicate low overall toxicity based on the limited solubility and bioavailability which is reflected in the non-harmonised term Poorly Soluble Low Toxicity (PSLT). The speaker emphasises that these properties must be assessed on a case-by-case basis, as exceptions exist. PSLT is a strongly debated topic focusing on the inhalation route with thresholds and definitions still to be negotiated. A tiered approach of the testing procedure for PSLT substances is presented. The producer used a read-across strategy to minimise the extent of animal testing, which was approved by the ECHA. The dynamic dissolution assay, which uses biofluids to mimic the dissolution rate in vivo, is described. Pigment Yellow 14 as a realistic example for the tattooing scenario is presented and exemplary TEM pictures of crystals are shown, revealing no changes in particle structure or size before and after the dynamic dissolution test. These results are contrasted with partially soluble substances such as barium sulfate, where clear dissolution could be detected. The presenter then shifts to the particle toxicity of pigments that can be measured by several abiotic assays, one of which is the highly sensitive Ferric Reduction Ability of Serum (FRAS) assay. In 90 % of tested substances, no oxidative properties (referred to as "interferences") were observed. Electron Paramagnetic Resonance (EPR) has been used as a complementary method to support these results. The alveolar macrophage assay is mentioned as a third abiotic in vitro assay. The limits and obstacles of working with insoluble pigments are explained, including the infeasibility of liquid chromatography and artifact disturbances caused by pigment precipitation on glassware and cells. Moreover, the insolubility of the pigments not only affected the feasibility of analytical HPLC but also constituted significant challenges for preparative LC applications.

In addition, pigments may be contaminated with impurities, sometimes even encapsuled within the particle. Copper chloride, detected as a leaking substance, was mentioned as an artifact example in the case of phthalocyanines. Due to their low solubility, pigments require careful handling, and some methods such as QSAR might be ill-suited to analyse potential hazards.

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Item 7 Guest speaker – What's in my ink: Raman analysis of tattoo inks on the US and European markets

A guest speaker presents a study on a market analysis of tattoo inks in the USA. In this study, analytical techniques for the analysis of the carrier matrix (e. g., NMR, HPLC-DAD, GC-MS) as well as the pigments (e. g., Raman, XRF) were applied. Inks of 6 different colours from different brands available on the US market were purchased leading to all together 54 different inks. In summary, in both investigated analyte groups non-compliances were found (i.e., organic additives in the carrier and the pigments). Numerous inks contained additives in the carrier matrix, that were not declared. This included substances such as propylene glycol or polyethylene glycol, preservatives which are not approved for tattoo inks, or antibiotics. Furthermore, several inks also contained substances that could not be identified. Approximately half of the investigated samples contained pigments that were not declared and 11 inks were even labelled completely inaccurate. In summary, most inks exhibit a non-correct declaration, containing undeclared substances – either pigments or additives. In addition, so-called “REACH-compliant” inks which were acquired after January 1st 2024 were analysed using Raman to investigate if non-approved pigments are used. Several of these inks still contained Pigment Green 7 despite its ban. In this context the speaker also shortly discusses the use of Raman for pigment identification. Raman is a highly suitable method for the qualitative identification of tattoo pigments. Moreover, Raman allows the identification of pigments in a mixture. However, the accuracy of the method for quantification is not known. Yet challenges arise when distinguishing between different crystalline structures of one pigment (i. g. Pigment Blue 15:1, 15:3 and 15:6). Here, using analytical standards and sensitive instruments did not allow a precise clustering of the variants. Using a combination of techniques seems to be the most appropriate approach.

Item 8 Any other business

Possible dates for the next general meeting of the BfR Commission on Tattoo Inks are discussed and it is decided that 5th meeting of the BfR Tattoo Commission for Tattoo Inks should be scheduled for May 2025. BfR will sent out a poll to all members to determine the exact dates.

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Item 9 Planning of the next meeting

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