Heavy metals in tattoo inks

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BfR-Symposium "First International Conference on Tattoo Safety"
Berlin, June 6–7, 2013

Metals as colorants - additives - contaminants

- White: Ti oxide, Zn oxide, Ba sulphate, Pb carbonate
- **Black**: Fe oxides, C
- **Blue**: Cu carbonate (azurite), Na-Al silicate (lapis lazuli), Ca-Cu silicate (Egyptina blue), Cu phthalocyanine, Co oxides, Cr oxides
- **Brown**: Fe oxide (ochra), Cd salts, ferric sulfate
- **Green**: Cr oxides, Cu phthalocyanine, Pb chromate (chrome yellow)
- **Red**, **orange**, **yellow**: Hg sulphide, Cd sulphide, Cd selenide, Fe oxides
- **Violet**: some Mn salts

- Al silicate, Mg-Al silicate, Ba sulphate: to preserve the products or to influence some physical properties (viscosity, tixotropy)

  Nano-sized metals (Ti dioxide, Fe oxides): to have a higher color strength, the desired transparency, low oxidation/photocatalytic activity

- Inks using non-metal colorants can include traces of toxic metals because they are naturally found in the environment and they can be present in raw materials and/or because they are residues deriving from the ink production process

Medical literature

- The FDA has received in one year (2003-2004) 150 reports of bad reactions to tattoo inks right after tattooing or even years later
- Cutaneous allergies due to the presence of Ni in inks
- Photosensitivity in yellow tattoos attributed to Cd sulfide in inks
- Granulomatous reactions with Hg, Cr, Co, Cd and Al as causative agents
- Pseudolymphomas occurring mainly in reddish tattooed areas (HgS) but also in green (Cr salts) and blue (Co salts) areas

But, the clinical picture is rather manifold, and reactions cannot be related to one single metal, one single physical property or one single release or interaction mechanism

Legislation on metals

- Today, no specific regulation exists concerning metals in tattoos
- The resolution ResAP (2008)1 stated a list of permitted metals, namely As, Ba, Cd, Co, Cr(VI), Hg, Ni, Pb, Sb, Se, Sn and Zn, with maximum permitted concentrations - but the resolution is only a proposal
- Tattoo inks have to meet the regulations of labeling and classification that apply to all chemical products, and so metals that are carcinogenic, mutagenic and toxic for reproduction (classified in the categories 1A, 1B or 2 of the Regulation 1272/2008) should not be used in inks
- So the situation is “half-solved” as, in principle, metals are permitted below some threshold levels, but at even very low concentration the metals can be toxic, and, most of all, tattoo remains on the body for life and long-term effects cannot be excluded

Effects of metals

	Reg. 1272/2008	IARC	Health effects
Ti	-	TiO_2 Group 2B	TiO_2 biologically inert; mild irritant; nano TiO_2 pulmonary effects
Al	$AlCl_3$: Skin corr 1B	-	Reproductive, developing nervous and cardiovascular systems
Cu	$CuSO_4$: Skin irrit 2	-	Irritant (nose, mouth, eyes)
Ba	-	-	Cardiovascular system
Zn	$ZnCl_2$: Skin corr 1B $ZnSO_4$: Eye dam 1	-	Retardation of growth
Cr	CrO_3: Carc 1; Muta 1B; Repr 2; Skin sens 1 Cr (VI) compounds (except $BaCrO_4$): Carc 1B; Skin sens 1	Cr(VI) compounds Group 1	Allergies, sensitization
Mn	-	-	Manganism and other neurodegenerations
Ni	Ni: Carc 2; Skin sens 1 Ni oxides: Carc 1A; Skin sens 1 Ni sulphides: Carc 1A; Skin sens 1 $NiSO_4$: Carc 2; Skin sens 1 $Ni(OH)_2$: Carc 2; Skin sens 1 $NiCO_3$: Carc 2; Skin sens 1	Ni(II) compounds Group 1 Metallic Ni Group 2B	Allergies, sensitization
Cd	Cd and CdO: Carc 1B; Muta 2; Repr 2 CdS: Carc 1B; Muta 2; Repr 2 $CdSO_4$: Carc, Muta 1B, Repr 1B	Cd and Cd compounds Group 1	Kidney, bone, reproductive system
Sb	-	SbO_3 Group 2B	Respiratory and gastrointestinal systems
Pb	$PbCrO_4$: Carc 2; Repr 1A $Pb(COOH)_2$: Repr 1A Pb sulfochromate yellow (CI pig. 34): Carc 2; Repr 1A Pb chromate molybdate sulfate red (CI pig. Red 104): Carc 2; Repr 1A	Inorganic Pb compounds Group 2A	Brain, kidney, reproductive system
Hg	Hg_2Cl_2: Skin irrit 2 $HgCl_2$: Skin corr 1B		Brain, kidney, skin sensitizer
Co	Co: Skin sens 1 CoO: Skin sens 1 CoS: Skin sens 1 $CoCl_2$: Carc 1B; Skin sens 1 $CoSO_4$: Carc 1B; Skin sens 1	Metallic Co, Co compounds, $CoSO_4$, other soluble salts of Co(II) Group 2B	Respiratory tract, skin sensitizer

Effects of metal-nanoparticles

- Nanoparticles have special biological effects that are conditioned by their small size and high surface area

- TiO_2 nanoparticles in mice can induce harm and instability in the genetic code of the cells whereas TiO_2 normally is assumed to be inert
  Trouiller B et al. Titanium dioxide nanoparticles induce DNA damage and genetic instability in vivo in mice. Cancer Res 2009;69, 8784

- Tests in rats with subcutaneous injection of nano-Ag (50-100 nm) have shown that nanoparticles reach the blood circulation and distribute themselves to the kidneys, liver and spleen, while larger particles are not transferred to the blood

Tang J et al. Distribution, translocation and accumulation of silver nanoparticles in rats. J Nanosci Nanotechnol 2009;8:4924

- ZnO nanoparticles are toxic to human lung cells in lab tests even at low concentrations

Weisheng et al.Toxicity of nano- and micro-sized ZnO particles in human lung epithelial cells, J Nanoparticle Res, 2009;11:25

Activities

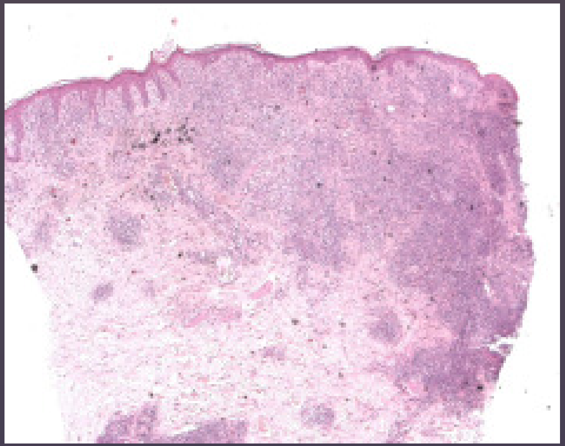
1. To study people with tattoo-induced pseudolymphomas and analyze the applied inks in order to find a relationship between clinical risk and metals

2. To quantify metals in a variety of commercial inks to test their conformity to ResAP(2008)1 limits and to evaluate human exposure

3. To classify the particle sizes in tattoo inks and characterize them in order to know if tattooing is a method of introducing nanoparticles into human body

1. The clinical study





- Patients with pseudolymphoma showed lesions in the red colored areas; lesions appeared 1-year or 2-years after the tattooing
- Patch tests with metals had negative results indicating that tattoo inks are in a particulate form and are captured under the stratum corneum
- The hystopathological findings showed a top-heavy dense infiltrate in the upper and medium dermis consisting of medium to small sized lymphocytes with a predominant T-phenotype (CD3+, CD8+)
- Scattered within the infiltrate isolated histiocytes (CD68+) were observed
- Red pigment particles were found both intra-cellularly (inside the histiocytes) and extra-cellularly

Analysis of biopsies taken from the red area

mg/kg					
Cd	Co	Cr	Hg	Ni	Pb
0.006-0.02	0.003-0.01	0.1-0.23	0.02-0.10	0.1-2.5	0.008-0.01

Analysis of the red inks taken from the tattoo shop

mg/kg					
Cd	Co	Cr	Hg	Ni	Pb
0.002-0.025	0.01-0.04	1.4-3.8	<0.01-0.17	0.10-0.49	0.05-0.39

- Mercury (HgS) which was, in the past, considered as the responsible for adverse reactions, did not play a role in the observed cases but other metals as Cr and Ni could be more implicated

2. Metal analysis of inks

- 56 tattoo inks were bought in Internet and 10 different colors were selected (orange, white, blue, yellow, gray, brown, black, red, green, violet)

- 4 different suppliers (3 US and 1 Germany)

- 20 of the 56 inks had no safety data sheets

- The pigments declared on the label were in accordance with those permitted by the ResAP(2008)1, but there was no information regarding metals content

- In 1 color series a general safety data sheet specified that the tattoo inks have been tested for selected heavy metals (<1 mg/kg)

SF-ICP-MS method (Element 2, ThermoFinnigan)

Microwave digestion		
ca. 0.25 g	4 ml HNO_3 suprapure 1 ml HF suprapure 1 ml H_2O_2 suprapure	5 min a 250 W 5 min a 400 W 10 min a 600 W

SF-ICP-MS parameters	
Nebulizer	Cross Flow
Spray chamber	Scott Double Pass
Neb gas flow	Optimized for <3% oxides
Sample flow	1 ml/min
RF power	1.2 KW
Dwell time	100 ms

Isotopes	Interferences	Resolution
^{27}Al	$^{11}B^{16}O$, $^{54}Fe^{++}$, $^{13}C^{14}N$	MR (m/Δm 4000)
^{59}Co	$^{40}Ar^{18}OH$, $^{42}Ca^{16}OH$, $^{43}Ca^{16}O$, $^{42}Ca^{17}O$, $^{36}Ar^{23}Na$, $^{24}Mg^{35}Cl$, $^{40}Ar^{19}F$	MR
^{52}Cr	$^{35}C^{17}O$, $^{35}C^{16}OH$, $^{36}Ar^{16}O$, $^{38}Ar^{14}N$, $^{36}Ar^{15}NH$, $^{40}Ar^{12}C$	MR
^{63}Cu	$^{40}Ar^{23}Na$, $^{47}Ti^{16}O$, $^{27}Al^{36}Ar$	MR
^{55}Mn	$^{37}Cl^{18}O$, $^{40}Ar^{15}N$, $^{40}Ar^{14}NH$, $^{38}Ar^{17}O$, $^{36}Ar^{16}OH$, $^{39}K^{16}O$	MR
^{60}Ni	$^{44}Ca^{16}O$, $^{23}Na^{37}Cl$, $^{120}Sn^{++}$	MR
^{47}Ti	$^{31}P^{16}O$, $^{40}Ar^{7}Li$	MR
^{64}Zn	$^{48}Ti^{16}O$, $^{40}Ar^{24}Mg$, $^{48}Ca^{16}O$, $^{36}Ar^{28}Si$	MR

Concentration (mg/Kg) in inks by suppliers (A-D)

	A	B	C	D
	Median-Max	Median-Max	Median-Max	Median-Max
Ti	119,435-154,704	33,763-159,436	6,597-180,893	15.5-82,082
Al	1766-2987	1540-5893	428-3762	40-254
Cu	37-1606	6.80-4540	53-31,310	75-14,759
Ba	20-161	1.10-9.74	9.20-1226	11-1030
Zn	1.22-48.4	1.10-2.27	1.10-6.49	2.60-11.3
Cr	1.20-147	1.10-3.34	1.80-4.72	1.20-2.01
Mn	0.33-98.8	1.03-1.53	0.53-1.80	0.37-1.70
Ni	0.25-9.60	0.14-1.07	0.43-2.32	0.45-5.05
Cd	0.32-2.99	0.12-0.52	0.05-1.15	0.05-0.14
Sb	0.14-4.11	0.05-0.58	0.10-0.18	0.16-2.26
Pb	0.09-14.8	0.09-0.42	0.08-0.83	0.38-1.45
Hg	0.02-0.15	0.02-0.11	0.05-0.18	0.14-0.25
Co	0.02-6.40	0.02-0.057	0.02-0.12	0.03-0.11

Tattoo ink compositions varied as a function of the manufacturer, with a general trend:

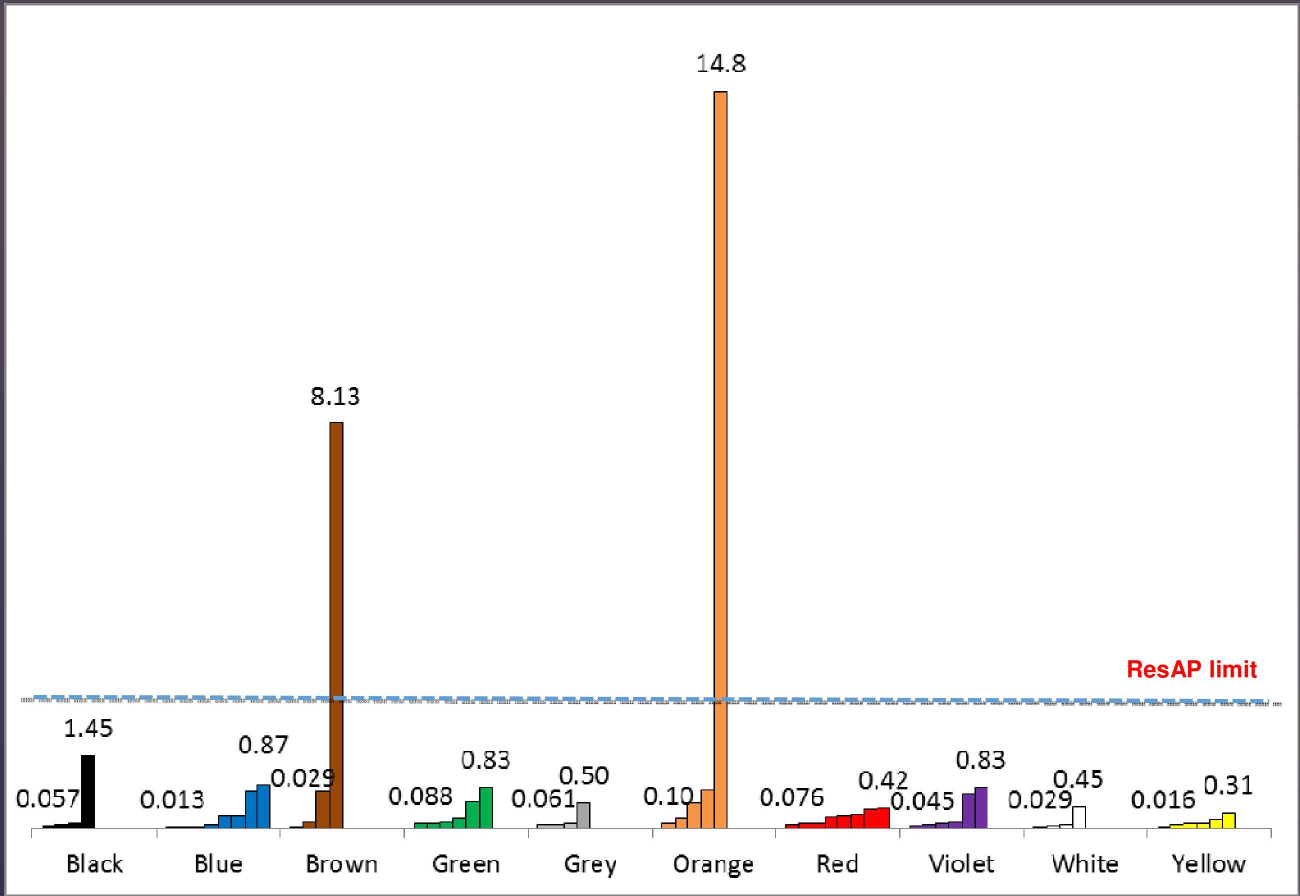
- Ti (Max, 18.1%), Al (Max, 0.58%), Cu (Max, 3.1%) and Ba (Max, 0.12%) were the highest and Hg the lowest (<LoD in 11 inks)
- Zn, Cr, Mn, Ni, Cd ranged 0.1-2.6 mg/Kg (medians)
- Sb, Pb, Co ≤ 0.1 mg/Kg (medians)
- Past used inorganic ingredients (*i.e.*, HgS, CdS, Mn salts, Co and Cr oxides) have been now mainly replaced by organic ones

Non conformity *vs.* ResAP(2008)1

	Limit (mg/Kg)	Samples %
Ba	50	27% (15/56)
Cd	0.2	32% (18/56)
Co	25	none
Cr	0.2 (CrVI)	not applicable
Cu	25 (Cu soluble)	not applicable
Hg	0.2	1.8% (1/56)
Ni	"as low as technically achievable"	0.4 mg/Kg - 9.6 mg/Kg
Pb	2.0	3.6% (2/56)
Sb	2.0	3.6% (2/56)
Zn	50	none

- It was not possible to distinguish between Cr(III) and Cr(VI) and between extractable Cu and total Cu, so a complete comparison with ResAP(2008)1 could not be made
- If the sentence "as low as technically achievable" means the "LoD" level, all the samples were >LoD so they could be regarded as not conform

Pb content by similar colors and used colorants



- Pb can be present at concentrations many times lower than the ResAP limit (so, it is feasible for manufacturers to eliminate these impurities from tattoos)
- As the EFSA's CONTAM Panel concluded that there is no evidence for a Pb threshold for the neurotoxicity and nephrotoxicity in adults, the Pb content in inks should be reduced to 'technically unavoidable amounts'

Dermal sensitization threshold

- Dose–response studies showed that 90% of sensitized patients fail to react to Co, Cr and Ni below 1 mg/kg even on irritated skin
  Basketter DA, et al. Nickel, chromium and cobalt in consumer products: revisiting safe levels in the new millennium. Contact Dermatitis 2003;49:1

- So it is generally stated that the level of each of these metals should not exceed 1 mg/kg in products that come in contact with the skin

	Samples > 1 mg/kg
Co	1.8% (1/56)
Cr	62% (35/56)
Ni	16% (9/56)

- A content of allergenic metals was demonstrated in all inks and same samples were above the limit considered at risk for sensitized people

- Moreover, none of the tattoo inks had a label stating that they contained Ni and Cr, as requested by the ResAP

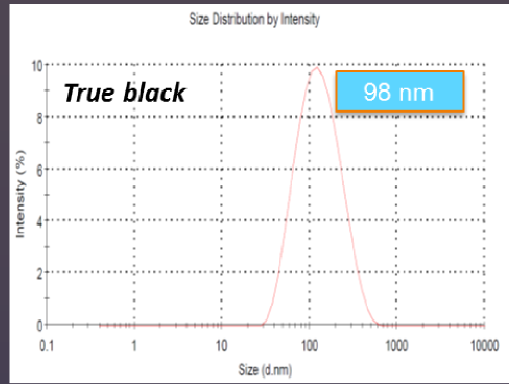
3. Nanomaterials

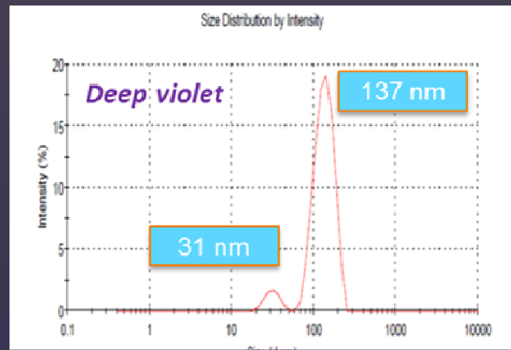
DLS method (Zetasizer Nano ZS, Malvern)

- DLS measures the hydrodynamic diameter (nm) that determines how fast a particle moves in a suspension through Brownian motion
- The intensity of the scattered light is inversely proportional to the six power of the radius of the nanoparticles
- One drop of the ink was added to 30 ml of deionized H_2O
- Particle dispersions were analyzed unfiltered
- Measurements at 25 ºC and the scattering angle registered was 173°

DLS particle size of 9 inks

Commercial name	Z-Avg (nm)	Pigment chemical class
Brite orange	147	Azocompound
Ice blue	421	Inorganic, phthalocianine
Dark chocolate	418	Inorganic, phthalocianine, azocompunds
True Black	98	Inorganic
Black outliner	150	Inorganic
Monthly Red	211	Azocompound
Grasshopper green	277	Inorganic, phthalocianine, azocompound
Mean green	337	Inorganic, phthalocianine, azocompound
Deep violet	31; 137	Inorganic, azocompound





- Samples existed as a distribution of sizes ranging 98-421 nm
- Inks consisting of only one pigment had the smallest mean diameters (98-211 nm)
- Inks containing a mixture of pigments (inorganic and organic) revealed the highest mean diameters (277-421 nm) probably because of the masking of coarse particles from TiO_2 that is a strong scattered of light and is added to the majority of pigments

Mean diameters of the 50% of particles

Commercial name	D(50) nm
Brite orange	56.2
Ice blue	255
Dark chocolate	305
True Black	67
Black outliner	102
Monthly Red	143
Grasshopper green	144
Mean green	230
Deep violet	24.2

- A few pigments (orange, true black, black outlier, violet) contained the 50% of particles with diameters <100 nm
- EC (18 October 2011) defined “nanomaterial” as a “material containing the 50% or more particles in the number size distribution between 1 and 100 nm”, so 4 of the analyzed inks could be defined as “nanomaterials”
- Increasing the degree of “blackness” (true black vs. black outliner) the number of particles with size <100 nm increased

FFF principle

- A laminar flow of the eluent carries the sample down a narrow channel, then a cross-flow field is applied to the laminar flow, and size separation is obtained by the balance between the cross-flow force and the different diffusivities determined by particle sizes. Smaller particles diffuse higher, into a faster portion of the laminar-flow profile, and elute before larger particles

- The retention time (min) of particles is inversely proportional to the hydrodynamic radius (nm)

FFF-MALS information

- The scattered light is detected at different angles at the same time, and the angle of scattering is inversely dependent by the particle size

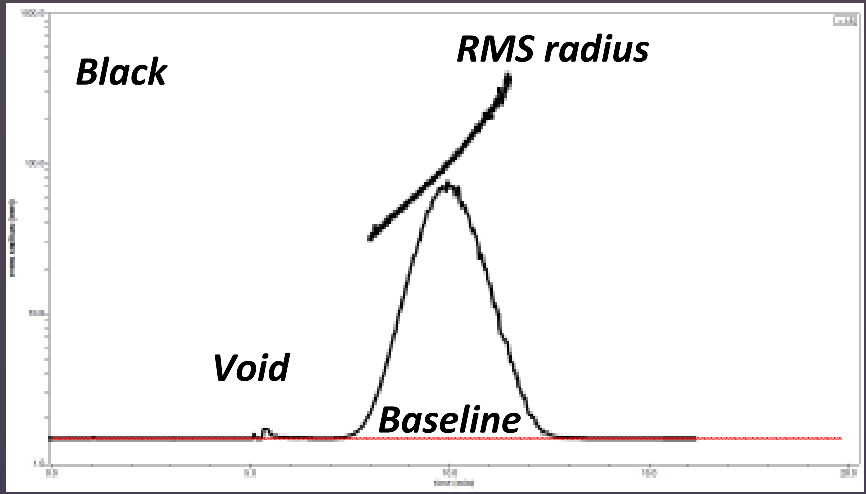
- The size measurement is known as the root mean square (RMS) radius (nm) that is the root mean square of all the radii making up the molecule multiplied by the mass at that radius

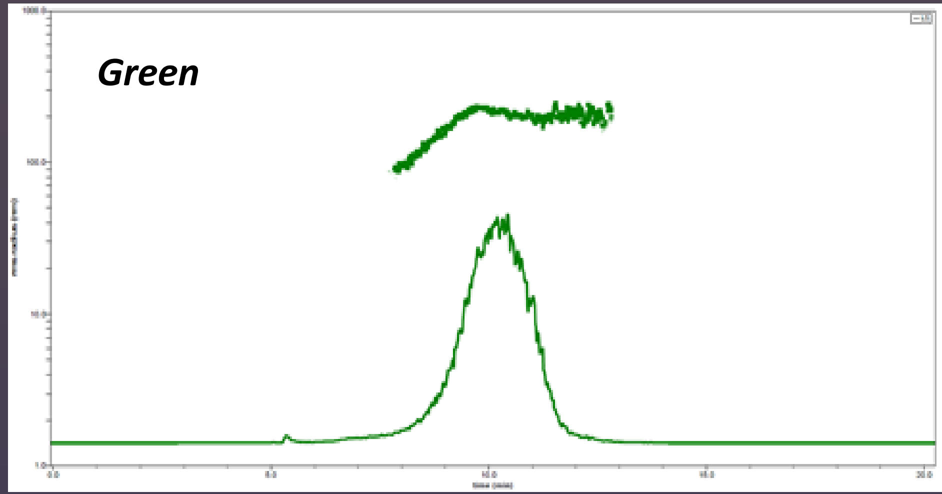
FFF-MALS method (AF4 Wyatt, DAWN Heleos)

- Samples were diluted 1:2000 in Triton X-100 and sonicated for 15 min in order to disrupt as much as possible large particles aggregates
- H_2O as the ideal mobile phase for compatibility with inks and ICP-MS coupling
- The separations were programmed to start after 4 min of focus time followed by 15 minutes of elution using a linear gradient from 1.0 ml/min to 0.1 ml/min

Parameter	
Channel length	26.5 cm
Membrane	10 KDa, Regenerated cellulose
Spacer width	250 µm
Sample injection	2 µl
Mobile phase	Deionized H_2O
Detector flow	1.0 ml/min
Focus flow	1.0 ml/min
Focus time	4 min
Laminar flow	1 ml/min
Cross flow	15 min: from 1.0 ml/min to 0.1 ml/min; 5 min: 0.1 ml/min
Detector	18-angle light scattering

FFF-MALS (90°) for black and green inks



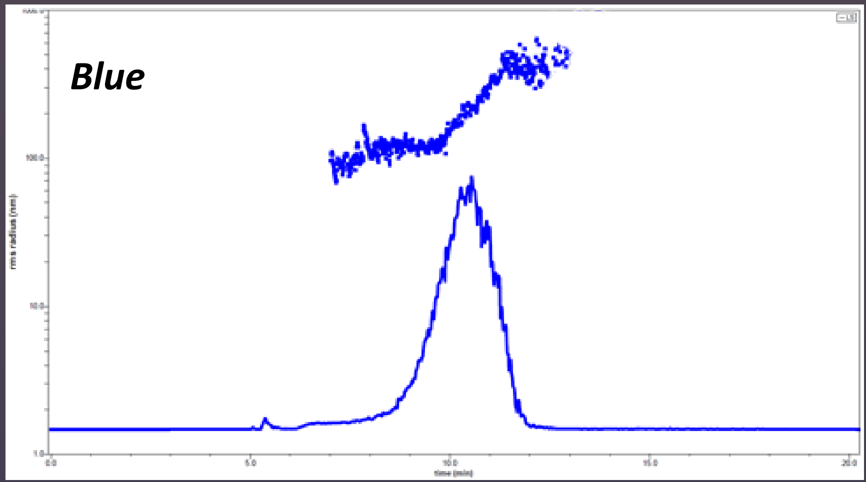


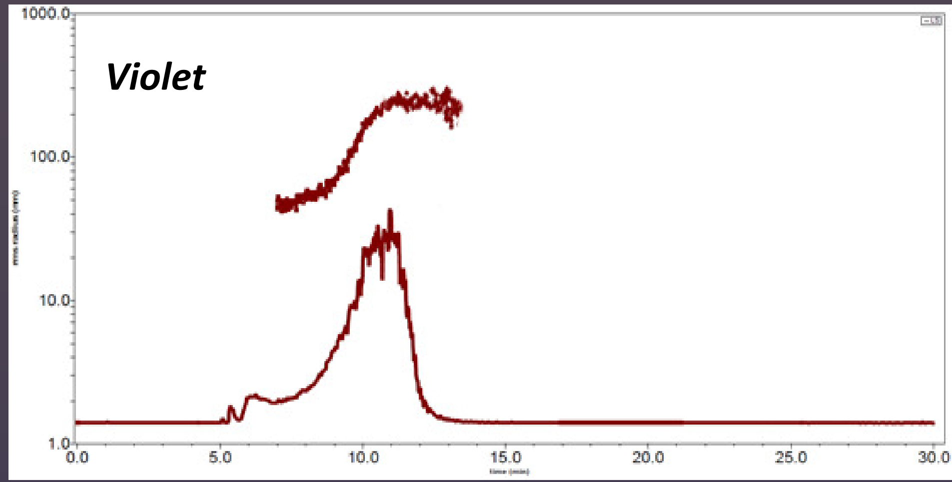
- the baseline signal demonstrated that there were no artifacts or micelles, and Triton X-100 was ideal as dispersant medium
- the void peak had a very low intensity so the entire injected sample was focused correctly and for a sufficient amount of time
- no sample remained in the injection line (no significant interaction sample-membrane)

	Range time (min)	Range RMS radius (nm)	Total peak Avg RMS radius (nm)
True Black	7-12	30-277	73
Grasshopper green	7-12	71-210	148

Both inks indicated a mono-modal distribution of a single population of nanoparticles continuously distributed from smaller to larger particles eluting from 7 to 12 min with average RMS radius of 73 nm for black and 148 nm for green

FFF-MALS (90°) for blue and violet inks



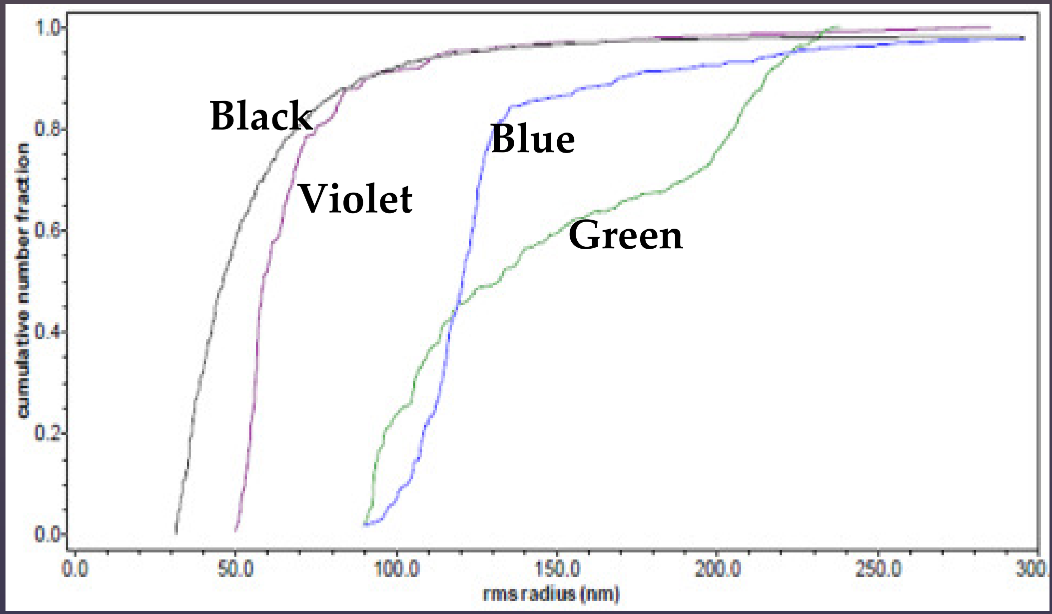


	Range time (min)	Range RMS radius (nm)	Total peak Avg RMS radius (nm)
Ice blue	8-12	100-387	158
Deep violet	8-12	59-256	92

In the blue, the fractogram showed an almost symmetric band with a maximum at 10 min, fronted with some samples eluting at 6 min but the scattering intensity was very low to calculate any RMS radius. The main peak eluted from 8 to 12 min and had an average RMS radius of 158 nm

In the violet, two population of particles were visible, the first eluting after the void peak at 6 min, followed by peak eluting from 8 to 12 min with an average RMS radius of 92 nm. This distribution seemed to be bi-modal but separation needs improvements

Cumulative number fraction



	Particles count		Particles count
True black	93% <100 nm	Ice blue	6% <100 nm
Deep violet	91% <100 nm	Grasshopper green	21% <100 nm

According to the EC definition for nanomaterials, black and violet inks could be defined “nanomaterials”, as already showed by the DLS results

Coupling FFF with ICP-MS

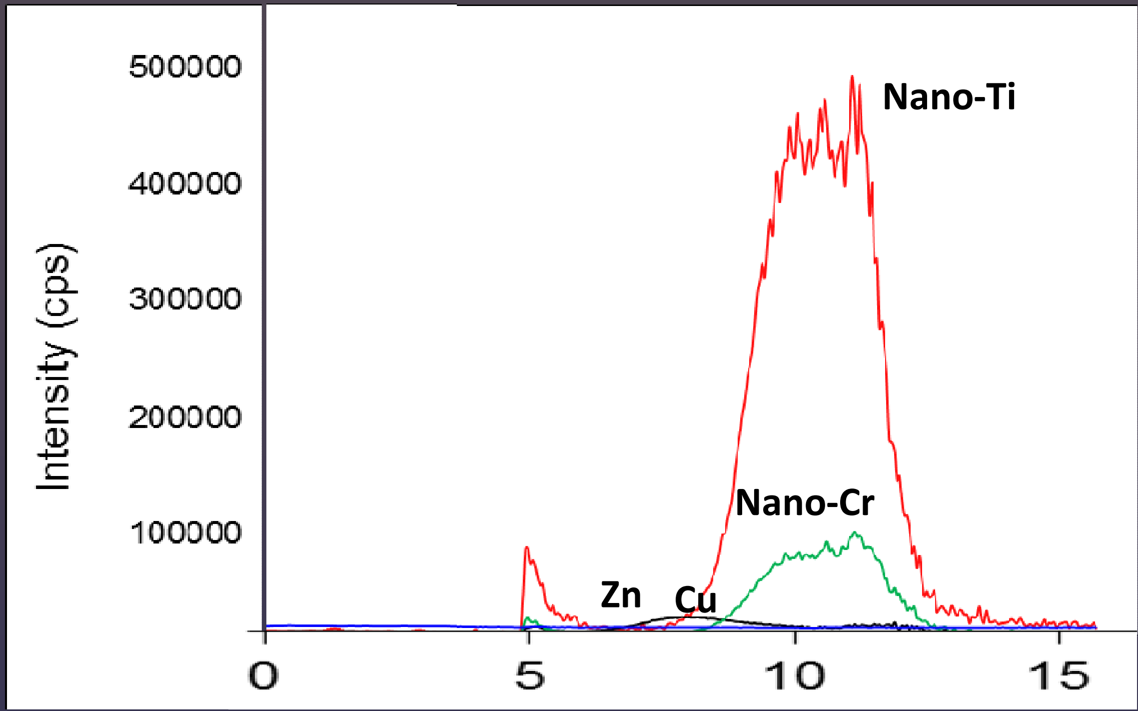
Metal mass in each nanoparticle is proportional to ions detected in the corresponding ICP-MS counts, while the dissolved metal produces a continuous low background signal

The NexIon used a collision cell with He to remove the solvent- and Ar-based polyatomic interferences and a detector dwell time very low in order to capture the time-resolved nanoparticles pulses that last only a few millisec

NexIon ICP-MS method (NexIon 300 Perkin Elmer)

Parameters	
Nebulizer	Cross Flow
Spray chamber	Scott Double Pass
Neb gas flow	Optimized for <3% oxides
Sample flow	1 ml/min
RF power	1.2 KW
Dwell time	1 ms
Analytes	^{63}Cu, ^{64}Zn, ^{52}Cr, ^{47}Ti
Collision cell	He

FFF-ICP-MS of the green ink



	SF-ICP-MS	FFF-ICP-MS
Ti	197,000 mg/kg	insoluble nanoparticles of Ti
Cr	2.5 mg/kg	insoluble nanoparticles of Cr
Cu	1600 mg/kg	dissolved Cu
Zn	1987 mg/kg	dissolved Zn

1. Final considerations - analytical methods

The development of a method for the separation of Cr(III) and Cr (VI) in inks is urgent

DLS and FFF-MALS seems to give similar results, both indicating the presence of particles continuously distributed from few nanometers to hundreds of nanometers

Coupling FFF-ICP-MS appears to be a useful for simultaneous determination of particle size and metal composition of inks even at trace concentration levels

Fractionation and ICP-MS analysis needs be improved and validated, but some important practical aspects have been yet solved: a good dissolution of samples in Triton-X, no run to run carry-over, no interactions sample-membrane, H_2O mobile phase compatible with ICP-MS

2. Final considerations – safety of metals

The cases of pseudolymphomas do not unambiguously point at one specific metal but a certain content of sensitizing metals (Cr, Ni) are in the biopsies and applied inks

In inks, some metals can be regarded as impurities but also trace levels of these metals may be troublesome when present in live tissues for a life, therefore there is a difference between what is the “ResAP technical limit” and what is “safe”

Some metals found in the inks are carcinogenic with no threshold (CrVI, Cd compounds, Ni compounds), others are skin irritants (Co, Cr, Ni) and others (Pb) are neurotoxic even at very low concentration

Moreover, it can be assumed that metals are released into the areas surrounding the tattoo, into the lymph stream and possibly even into the blood stream and thus constantly distributed in the body

Thus, the ResAP limits suggested for impurities do not necessarily provide a high level of protection for humans and, from a precautionary approach, all the metals should be reduced to “technically unavoidable amounts”

3. Final considerations – safety of metal nanoparticles

The finding of metal nanoparticles in tattoo inks is particularly important in contributing to the uncertainty in terms of which organs in the body (in addition to the skin and the lymph nodes that drain the tattooed area) can be exposed to metal nanoparticles

This is because particles with a particle size < 100 nm can distribute themselves differently in the body than soluble substances and larger particles

Further studies on a more representative number of inks and the fate of metal nanoparticles in the body are strictly recommended

Working group

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Acknowledgements

Alice Brun (Alfatest, Malvern) for DLS measurements
Barbara Roda (byFlow) for FFF-MALS measurements
Riccardo Magarini (Perkin Elmer) for the FFF-ICP-MS measurements

Pubblications

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THANKS FOR THE ATTENTION

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