



PRESERVATIVES IN TATTOO AND PMU INKS IN THE FRAME OF REACH REGULATION: RESULTS OF AN ITALIAN STUDY

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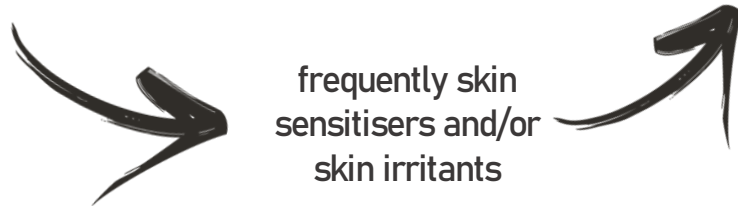
Preservatives in tattoo & PMU inks

Preservatives are often added to the mixture to avoid microbiological contamination of inks after opening favoured by the high content of water and organic substances.

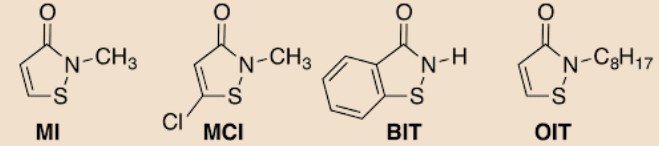


Composition:

- Pigments ($\leq 60\%$ by weight)
- Water (main solvent)
- Alcohols (eg ethanol, isopropylalcohol) ($\leq 30\%$)
- Additives (eg. surfactants, thixotropic agents, binding agents, fillers) ($\leq 30\%$)
- **Preservatives** ($\leq 1,5\%$)



Isothiazolinone derivatives



As pointed out by the results of market surveillance studies in Switzerland and Germany in the past years (2007, 2012), isothiazolinone derivatives may be found in tattoo inks

- MCI/MI mixture is a strong sensitiser among the EU population. MI in MCI/MI is a stronger sensitiser even more than MI alone. MI has been banned in leave-on cosmetics since 2017.
- **Large use of BIT.** BIT is a skin sensitiser in animal models with potency similar to MI.
- The presence of isothiazolinone derivatives in inks could cause a reaction in people who have been sensitised before the tattooing procedure or prime elicitation in not yet sensitised ones

Allergic reactions as adverse effects after tattoo/PMU application

- Instant or delayed allergic reactions and, to a lesser extent, acute dermatitis and inflammatory reactions represent common acute adverse effects;
- Possible chronic skin reactions lasting more than 3-4 months ;
- 2.9-8% of the tattooed people reported allergic reactions, but they are underestimated. Contact allergic reactions can not be precisely estimated (Annex to Background Document to RAC and SEAC Opinion).

Preservatives in tattoo & PMU inks: REACH Reg. and BPR

Preservatives with harmonised classification under CLP or banned by CPR (Annex II) or enlisted in Annex IV to CPR



Over 4.000 substances covered by REACH restriction (entry No. 75) after 4 January 2022

Concentration limits		
Skin corrosive 1, 1A, 1B, 1C or skin irritant 2 or serious eye damage 1 or eye irritant 2:	skin sensitiser 1, 1A, 1B:	enlisted in Annex II to CPR:
0.01% by weight (100 mg/kg)	0.001% by weight (10 mg/kg)	0.00005% by weight (0.5 mg/kg)

According to RAC & SEAC Final Opinion on proposed restriction, preservatives as part of the tattoo ink mixture are regulated under the EU Biocides Regulation (BPR) and fall under the authorisation regime of the BPR. *“the in-can preservative (PT-06) used in tattoo inks are de facto subject to the BPR rules [...] According to the Dossier Submitter, the proposed restriction would not change the obligations under the BPR but would limit the type of preservatives that can be authorised for the use”*

PT 6	Preservatives for products during storage	Used for the preservation of manufactured products, other than foodstuffs, feeding stuffs, cosmetics or medicinal products or medical devices by the control of microbial deterioration to ensure their shelf life. Used as preservatives for the storage or use of rodenticide, insecticide or other baits.
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No preservatives (PT-06) have been authorised for their use in tattoo/PMU inks under BPR!!!

Only PT-06 preservatives (for «in-can» preservation) authorised under BPR to be used for preserving tattoo and PMU inks or avoid the growth of microorganisms in the product after opening

Preservatives: uncertainties about joint application of REACH Reg. and BPR

- PT-06 does not include substances for products to be applied on human skin or injected into derma

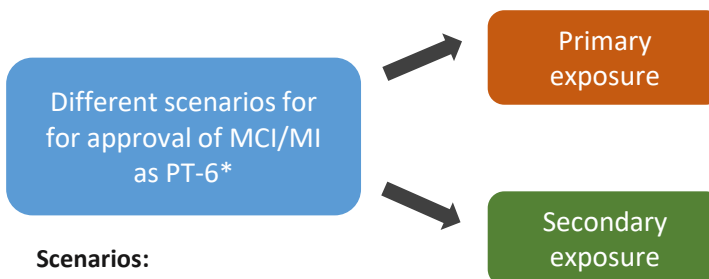


Table 1: Sub-categories in PT 6 according to MOTA v.6 (2013)

Sub-category	Description
6.1	Washing and cleaning fluids and human hygienic products
6.1.1	Washing and cleaning fluids (human hygienic products)
6.1.2	Washing and cleaning fluids (general) and other detergents
6.2	Paints and coatings
6.3	Additives used in paper, textile and leather production
6.3.1	Additives used in paper production
6.3.2	Additives used in textile production
6.3.3	Additives used for leather production
6.4	Metal working fluid
6.4.1	Lubricants
6.4.2	Machine oils
6.5	Fuel
6.6	Glues and adhesives

MOTA v.6 (2013), p. 46

- Risks assessment and toxical evaluation during product type revision (PT-06) under BPR do not take into account this kind of human exposure (injection into the dermis).



Scenarios:

- formulation of product to be preserved,
- paint and coatings,
- liquid detergents,
- fuel preservation,
- textile, leathers treatment, inks, polymer latex preservation,
- household and insustrial products

Primary exposure: during formulation of CMI/MI into product to be preserved and the use of these products by a professional/non professional

Secondary exposure: when consumers are exposed to products preserved with CMI/MI by an indirect path, i.e people eating into dishes cleaned with liquid detergents containing CMI/MI or people wearing clothes cleaned with detergents containing CMI/MI, etc...

A biocidal product can be used only for its authorised use... and exposure after tattoo/PMU application?

* Opinion on the application for approval of the active substance: Reaction mass of 5-chloro-2-methyl-2h-isothiazol-3-one and 2-methyl-2h-isothiazol-3-one (3:1)
 Product type: 6
 ECHA/BPC/46/2015

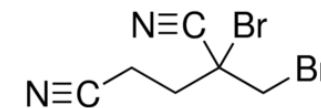
Preservatives: uncertainties about joint application of REACH Reg. and BPR


Why should it be safer to use PT-06 preservatives than preservatives for leave-on cosmetics? Both BPR and CPR do not take into account this human exposure (injection into dermis) but which one would be more reasonably applicable?

PT-06 preservatives (for «in-can» preservation) authorised under BPR to be used for preserving tattoo and PMU inks



Methyldibromo glutaronitrile



[-] Active substance	
Substance name:	2-bromo-2-(bromomethyl)pentanedinitrile (DBDCB) Product-type: 
EC number:	; 252-681-0
CAS number:	35691-65-7
IUPAC name:	2-bromo-2-(bromomethyl)pentanedinitrile
BAS number:	1226
Candidate for substitution:	No
Regulatory categorisation:	Review programme substance
Approval/Assessment status:	Approved

No harmonised classification but not approved in cosmetics

Methyldibromo glutaronitrile is not on the list of preservatives approved for use in cosmetic products since it has been shown “to cause elicitation of reactions by repeated open exposures with a rinse-off preparation at the maximum concentration allowed in rinse-off products (0.1%)” (SCCP Opinion, 2005)



REACH Reg. and new obligations

Up to now, chemical requirements were those of Generally Product Safety Directive (Directive (EC) No. 2001/95) in line with recommendations of ResAP(2008)1. Preservatives have to be used:

- not for compensating for poor microbiologic quality or inadequate hygiene
- at lowest effective concentration
- only after safety assessment.

With REACH restriction...

- inks shall not contain substances at concentration > REACH concentration limits
- complete list of ingredients will be mandatory
- inner packaging shall be labelled in accordance with CLP Regulation

For skin sensitisers CLP Regulation sets concentration limits for elicitation



CLP Reg.: concentration limits for elicitation

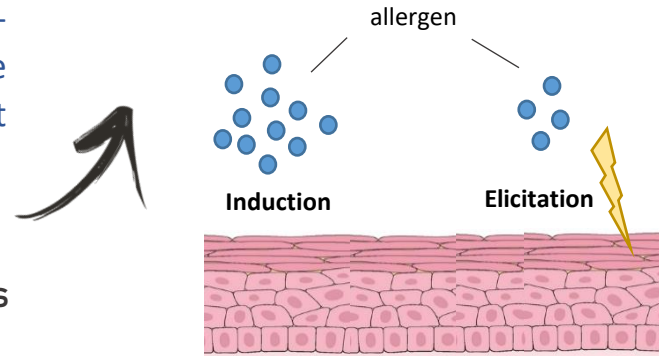
For the purpose of section 3.4 CLP,
Skin sensitisation includes two phases.

1. **INDUCTION** An allergenic substance primes the immune system.



2. **ELICITATION** The second phase takes place after re-exposure to the allergen and is associated with the manifestation of allergy (i.e. the allergic contact dermatitis).

Induction and elicitation of skin sensitisation in humans are generally regarded to be threshold phenomena

A lower level of exposure is generally considered to be required for elicitation than for induction to occur.



A concentration for a skin sensitiser < concentration limit for elicitation will protect:

-  already sensitised individuals from manifestation of allergic contact dermatitis (elicitation)
-  subjects from induction.

Substance	Generic or specific concentration limits under CLP (mg/kg)	Concentration limits for elicitation under CLP (mg/kg)
BIT	500,0	50,0
MCI/MI	15,0	1,5
MI	15,0	1,5
OIT*	15,0	1,5

The concentration limit for elicitation corresponds to 1/10 of the specific/generic concentration limits

Special labelling requirements of section 2.8 of Annex II to CLP will have to apply to protect already sensitised individuals and the hazard statement on the label “Contains [name of sensitising substance]. May produce an allergic reaction” (EUH208) shall be mandatory

* specific limit according to Delegated Regulation (EU) no.2020/1182 scheduled to apply after 1st March 2022

Italian research projects on tattoo & PMU inks

3 Research projects funded by Ministry of Health were carried out in Italy in the period 2017-2021

Main goals were:

- Risk evaluation for human health related to substances present in tattoo/PMU inks and evaluation of analytical methods available for detection of some substances with hazardous properties
- Development and validation of analytical methods for detection of substances with hazardous properties in tattoo/PMU inks



Analytical methods to support the enforcement of upcoming restriction on substances in inks:

- Building up an international working group to address technical or scientific issues concerning analytical activities

Main substances of interest:

Preservatives

Phthalates

Azocolourants

Primary aromatic amines

4 involved Institutions:



Investigated preservatives

Preservative	CAS No.	Relevant CLP classification /CPR conditions	REACH conc. limit % by weight	PT06 under BPR
Benzisothiazolinone (BIT)	2634-33-5	Skin sens 1; Skin Irrit. 2; Eye Dam. 1	0,001	Initial application for approval in progress
Methylisothiazolinone (MI)	2682-20-4	Skin sens 1A; Skin Corr. 1B; Eye Dam. 1	0,001	Initial application for approval in progress
Chloromethylisothiazolinone /Methylisothiazolinone (MCI/MI)	55965-84-9	Skin Sens. 1A; Eye Dam. 1; Skin Corr. 1C	0,001	approved
Octylisothiazolinone (OIT)	26530-20-1	Skin sens. 1A*; Skin Corr. 1; Eye Dam. 1	0,001	Initial application for approval in progress
Chloromethylisothiazolinone (MCI)	26172-55-4	//		Initial application for approval in progress
o-phenylphenol (o-PP)	90-43-7	Skin irrit. 2 Eye irrit. 2	0,01	approved
Phenoxyethanol (PE)	122-99-6	Eye Irrit. 2**	0,01	Cancelled application (UK)
Isobutylparaben (iButP)	4247-02-3	Annex II CPR	0,00005	//
Isopropylparaben (iPrP)	4191-73-5	Annex II CPR	0,00005	//
Pentylparaben (pentP)	6521-29-5	Annex II CPR	0,00005	//
Benzylparaben (BnzP)	94-18-8	Annex II CPR	0,00005	//
Methylparaben (MetP)	99-76-3	Authorized /Annex V to CPR	//	//
Etylparaben (EtP)	120-47-8	Authorized /Annex V to CPR	//	//
Propylparaben (PrP)	94-13-3	Authorized /Annex V to CPR	//	//
Butylparaben (ButP)	94-26-8	Authorized /Annex V to CPR	//	//

Among the investigated preservatives, only 2 are approved as PT-06 under BPR and most of them can be potentially found in inks

Chloromethylisothiazolinone (MCI) is used at a concentration ratio 3:1 with methylisothiazolinone (MI)

*Classification according to Delegated Regulation (EU) no.2020/1182 scheduled to apply after 1st March 2022

**Classification of Eye Irrit. 2 will be replaced with Eye Dam. 1 as established by Delgated regulation (EU) No. 2021/849 scheduled to apply after 17th December

Analytical methods: development and validation (1/2)

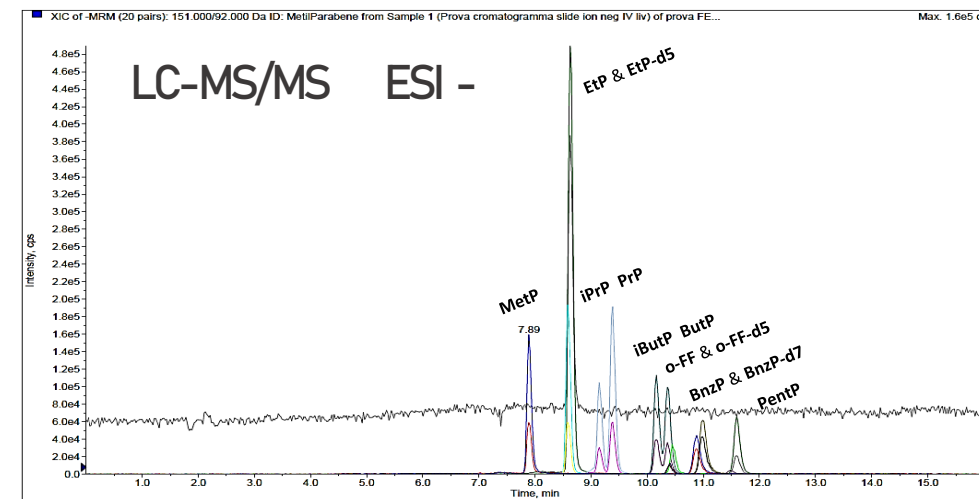
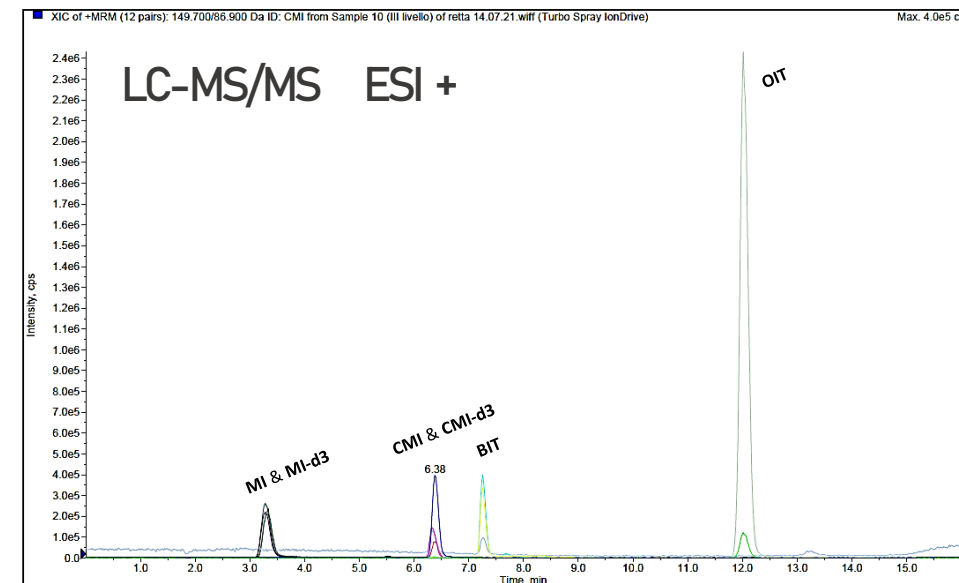
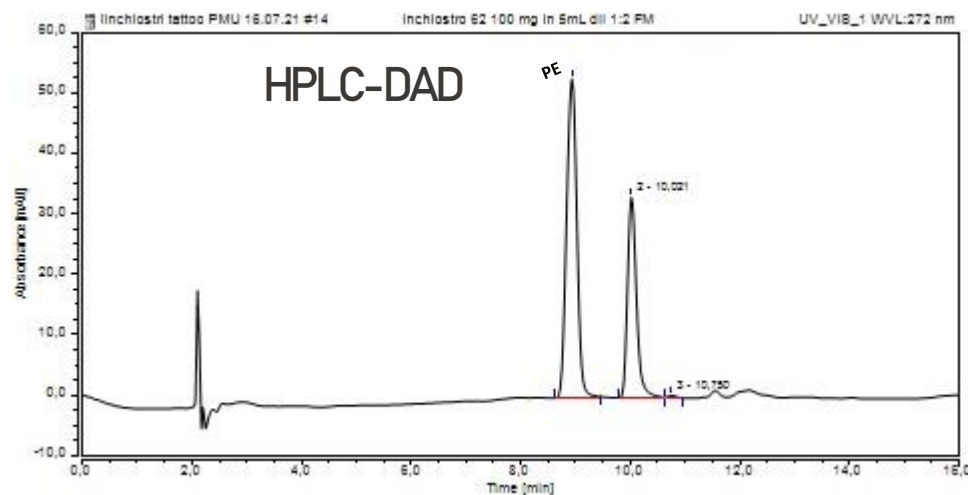
Two analytical methods were developed and validated for quantification of investigated preservatives.

Sample preparation procedure:

1. An aliquot of ink sample extracted in a mixture of solvents
2. Precipitation of pigments
3. Filtration
4. Dilution and injection.

Analytical techniques:

HPLC-DAD (for PE) and LC-MS/MS (switching ESI+/ESI-).

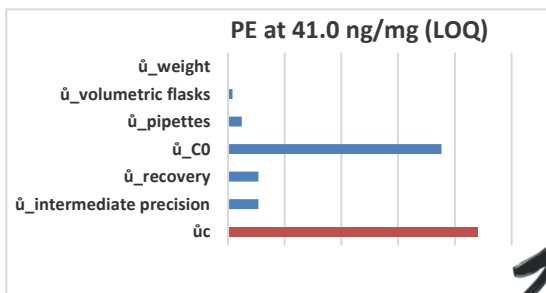


Analytical methods: development and validation (2/2)

Validation Study

Main performance characteristics were calculated in accordance with EN ISO IEC 17025 standard.

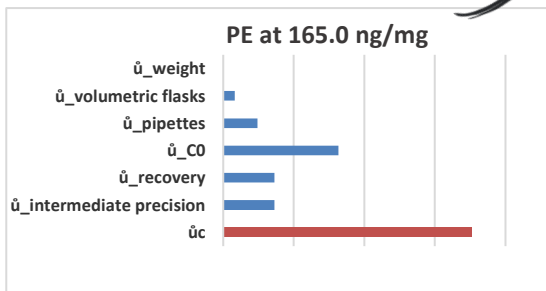
Measurement uncertainty was calculated on the basis of bottom-up approach according to UNI CEI 70098-3:2016 (GUM) and Eurachem/CITAC guide.



Main sources of uncertainty of the overall procedure

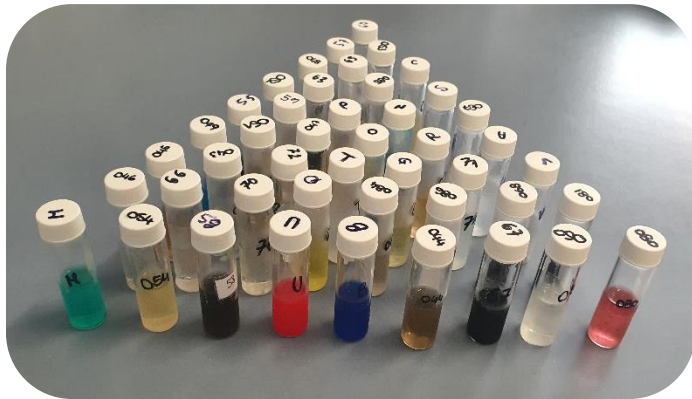
Relative combined uncertainty was mainly affected by

- Intermediate precision
- Recovery
- Calibration curve uncertainty



Name	Calibration curve ranges (mg/kg)	LOD/LOQ (mg/kg)	Intermediate precision (CV%) (6 replicates per 3 days)	Recovery (%) (6 replicates per 3 days)	Percentage Measurement Uncertainty, $k = 2$ (%)
MI	0.36 – 5.81	0.12/0.36	1.64 – 6.90	90.0 – 91.9	≤ 14.1
MCI	0.40 – 6.32	0.13/0.40	2.36 – 4.85	91.0 – 92.4	≤ 15.2
BIT	0.34 – 5.48	0.11/0.34	4.97 – 6.96	96.6 – 101.2	≤ 16.4
OIT	0.71 – 14.12	0.24/0.71	4.56 – 5.37	101.7 – 105.7	≤ 15.3
PE	41.0 – 1475.0	13.66/41.0	1.41 – 4.54	92.3 – 99.8	≤ 17.7
o-PP	16.0 – 255.0	5.33/16.0	5.16 – 6.68	97.3 – 101.1	≤ 22.6
MetP	0.091 – 1.46	0.030/0.091	2.87 – 6.56	98.3 – 103.9	≤ 16.3
EtP	0.32 – 5.11	0.11/0.32	2.01 – 2.79	98.8 – 103.1	≤ 13.1
iPrP	0.079 – 1.26	0.026/0.079	2.75 – 5.13	99.8 – 102.7	≤ 13.4
PrP	0.080 – 1.28	0.027/0.080	2.20 – 4.83	99.8 – 103.1	≤ 12.9
iButP	0.033 – 0.54	0.011/0.033	2.98 – 4.13	100.0 – 103.1	≤ 11.5
ButP	0.035 – 0.56	0.012/0.035	2.72 – 7.06	98.7 – 100.2	≤ 16.3
PentP	0.024 – 0.38	0.0080/0.024	1.98 – 7.27	96.3 – 101.5	≤ 17.1
BnzP	0.027 – 0.43	0.0088/0.027	1.99 – 2.39	97.2 – 103.1	≤ 12.2

Analysis on samples available on the market



Analyses on n=75 inks available on the Italian market selected among the most popular brands

n= 52 tattoo inks; n= 23 PMU inks

different brands (n = 13) and colours

Purchased in the period 2020-2021 at different professional vendors

- Only 2 inks were labelled as containing the investigated preservatives (PE)
- Produced in the USA (mainly), Italy, Germany, Taiwan

Analyses are ongoing on a larger number of inks

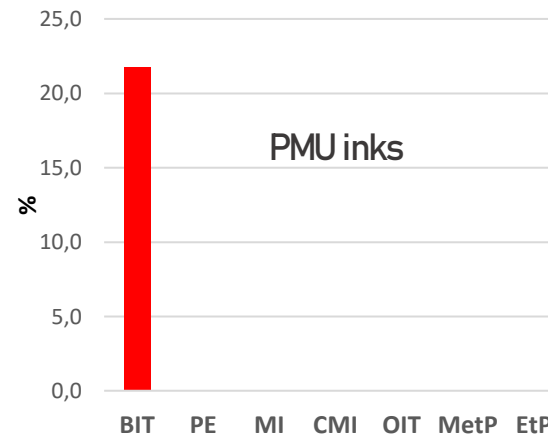
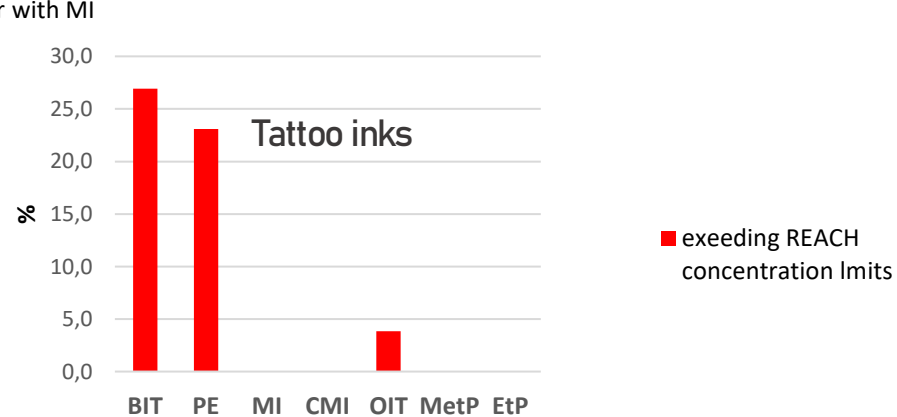
Results (1/3)

	TATTOO (n= 52)				PMU (n=23)			
Name	Concentration ranges (mg/kg)	Average concentration \pm SD (mg/kg)	No. % \geq LOQ	REACH NC %	Concentration ranges (mg/kg)	Average concentration \pm SD (mg/kg)	No. % \geq LOQ	REACH NC %
BIT	0.60 – 139.63	43.26 \pm 40.33	40.4 (21/52)	26.9	0.57 – 392.12	84.68 \pm 136.9	43.5 (10/23)	21.7
PE	100.68 - 6475.12	1542.30 \pm 2064.69	23.1 (12/52)	23.1	--	--	--	--
MI	0.38 – 0.81	0.60 \pm 0.16	15.4 (8/52)	0	--	--	--	--
MCI	0.13 – 1.46	1.04 \pm 0.39	15.4 (8/52)	0*	--	--	--	--
OIT	5.16 – 18.57	10.07 \pm 6.23	7.7 (4/52)	3.8	--	--	--	--
MetP	--	1552.52	1.9 (1/52)	0	--	--	--	--
EtP	--	967.0	1.9 (1/52)	0	--	--	--	--

Main remarks

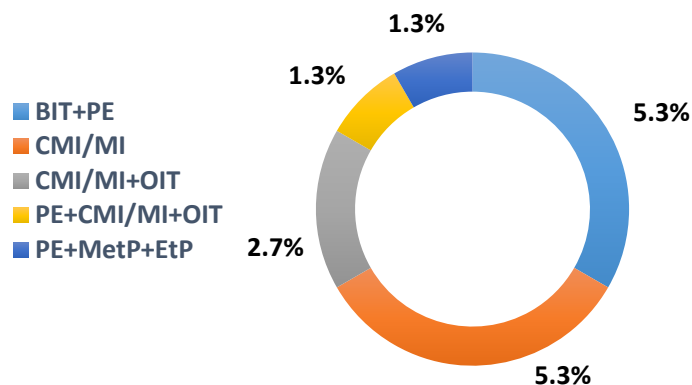
- BIT was the most frequently used preservatives followed by PE;
- Parabens other than MetP and EtP and o-PP were never detected
- In one tattoo sample, PE was found at a concentration of 6475.12 mg/kg, 65-fold the permitted limit of 100 mg/kg for Eye Irrit. 2;
- 26.9% and 21.7% of tattoo and PMU samples would contain BIT exceeding the concentration limit for skin sensitisers (10 mg/kg).

* together with MI



Results (2/3)

The most frequently detected preservative combinations



The most frequent associations were CMI/MI (5.3 %) and BIT+PE (5.3%);

The MCI/MI ratio was not always the expected ratio of 3:1. Similar results in studies on detergents and cosmetics.

Possible explanations:

1. MCI might react with other formulation ingredients
2. Inks preserved with MCI/MI might contain ingredients preserved with MI alone, and vice versa, thus changing the ratio

Main remarks

- Sample 24. Mixture containing OIT at concentration > REACH concentration limit. MCI/MI and OIT exceeding the concentration limit for elicitation.
- Sample 28. MCI/MI and OIT exceeding the concentration limits for elicitation. OIT at concentration > REACH concentration limit
- Sample 36. One sample labelled as containing PE actually did not contain PE but other preservatives (MCI/MI) at concentrations exceeding the concentration limit for elicitation.

Sample	BIT (mg/kg)	PE (mg/kg)	MI (mg/kg)	MCI (mg/kg)	OIT (mg/kg)	MetP (mg/kg)	EtP (mg/kg)
24	< LOD	< LOD	0,81 ± 0,11	1,01 ± 0,15	18,57 ± 1,75	< LOD	< LOD
28	< LOD	< LOD	0,78 ± 0,11	1,13 ± 0,17	10,86 ± 1,19	< LOD	< LOD
36	< LOD	< LOD	0,38 ± 0,05	1,11 ± 0,17	< LOD	< LOD	< LOD

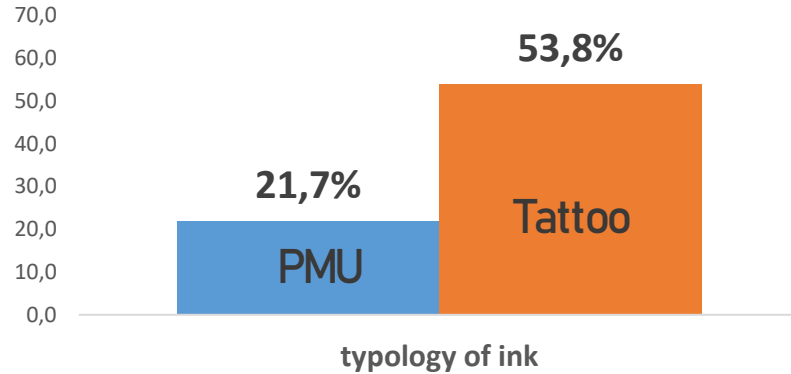
> the conc. limit for elicitation*

> REACH concentration limit for Skin Sens. 1, 1A, 1B

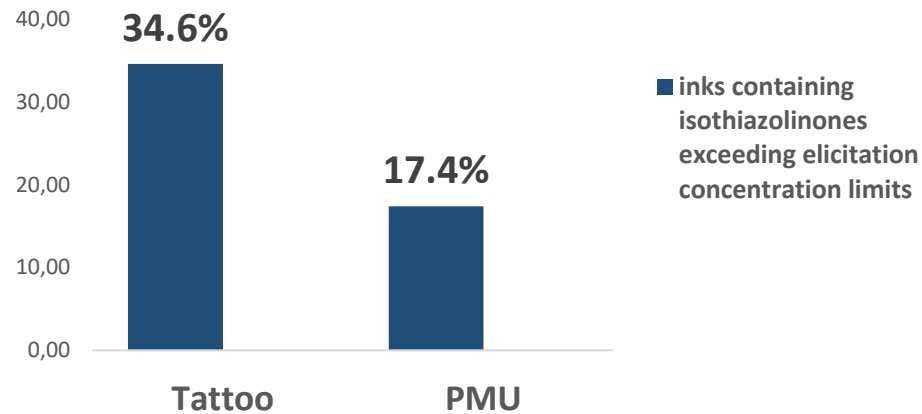
*Decision rule applied to conformity assessment with a single tolerance without guard band

Results (3/3)

Tattoo and PMU inks exceeding REACH concentration limits for skin sensitising/irritant and eye irritant preservatives



Tattoo and PMU inks requiring special labelling (EUH208)



Main remarks

- The number of non-compliant tattoo ink samples would be greater (53.8%) than non-compliant PMU ink samples (21.7%) if we consider the REACH concentration limits for skin sensitisers and eye irritant substances

- 34.6% of tattoo inks (18 out of 52) and 17.4% of PMU inks (4 out of 23) would contain BIT, CMI/MI or OIT at concentrations greater than CLP concentration limits for elicitation



Already sensitised individuals should avoid these inks

Conclusions (1/2)

- There are still open questions about REACH Reg. and BPR applications on preservatives in inks
- Over 75 analysed inks, about 44% would not be compliant with the concentration limits set by REACH for skin sensitiser/eye irritant preservatives.
- Particular attention should be drawn to the utilisation of BIT and PE, which exceeded the REACH concentration limits in the majority of samples;
- Manufacturers have to consider new strategies to reduce levels of skin sensitising/eye irritant preservatives in their inks. At concentration limits set by REACH, preservatives could not have effective preserving action. Manufacturers have to consider new alternatives.



in the «Annex to Background document to RAC and SEAC opinion» it has been suggested to refer to preservatives enlisted in Annex V to CPR, which do not have hazardous properties and with a historical use in tattoo inks (a dozen).

Industry can apply for their authorisation under BPR for the use in tattoo/PMU inks, as alternative to preservatives with harmonised classification.

Other proposed alternatives:

Reduce water content? (JRC)

Maintaining sterility (and reducing the need for using preservatives) by using sterile inks supplied in a container which maintains the sterility of the product until application (i.e., single use container)?(ANNEX XV RESTRICTION REPORT)



Conclusions (2/2)

- Preservatives carry over. In some cases, low levels of preservatives found in inks and the absence of indication on labels could be due to the fact that preservatives are not intentionally added to formulations, but they may arise from raw materials. Preservatives arising from raw materials could affect the compliance of the final products.
- 29.3 % of inks might cause a response in already sensitised individuals (elicitation).
- Manufacturers have to provide labelling of their inks under CLP accordingly (special labelling to protect already sensitised individuals, i.e. phrase EUH208 on the label will be mandatory).





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