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18 May 2020

The Secretary

Joint ACCS-ACMS Scheduling Secretariat

E-mail: medicines.scheduling@health.gov.au

Dear Sir/Madam:

Submission to the Proposed Amendments to the Poisons Standard

for June 2020 meeting of the Joint ACMS-ACCS

Procter & Gamble (P&G) is the name behind some of the leading brands in the Australian household. Our products are diverse and cover home cleaning, cosmetics and personal care products such as Olay, SKII, Herbal Essences, Pantene, Head & Shoulders, Gillette, Braun, Oral B, Ambi Pur and Fairy amongst others. Since P&G was founded over 182 years ago, our primary concern remains to be the safety of the people who use our products, and the safety of the environment we live in. Our commitment to human safety and environmental sustainability is both our heritage and our future. Our safety standards for all our products and ingredients are the same throughout the world. Hence, P&G is aligned with the Australian government's objective of ensuring that consumer products sold in the market are safe to both consumers and the environment and do not jeopardise the health and trust of our consumers.

We refer to the notice published on 17 April 2020 inviting public submissions with respect to certain substances, addressing a matter raised in s.52E of the Therapeutic Goods Act 1989. P&G Australia Pty. Ltd. provides this submission in response to the consultation on the following chemicals for consideration at the June 2020 meeting of the ACMS-ACCS:

- Methylisothiazolinone and Methylchloroisothiazolinone
- Isothiazolinones

P&G Australia Pty. Ltd. is a member of the industry association Accord. We have been actively involved in industry discussions around these scheduling proposals and we express our strong support for the general principles reflected in the Accord submission.

Please see attached submission for details, including our feedback and counterproposal, for the committee's consideration. We look forward to further advice from the ACMS-ACCS committee. Should the Committee require any additional information, please do not hesitate to contact me at kam.ad@pg.com.

Procter & Gamble Australia Pty. Ltd.

In general, we do not support the proposed scheduling. We believe that the restriction on Isothiazolinone levels, as well as the mandated warning statements, are overly restrictive to the detriment of the cleaning and hygiene industry. Consistent with the general scheduling of skin sensitisers, we propose introducing an additional intermediate restriction level of 0.015% (for Methylisothiazolinone, Benzisothizolinone, and Methylbenzisothiazolinone) and 0.0015% (for Methylchloroisothiazolinone), below which there is no risk of induction of skin sensitisation to consumers as supported by safety data, and therefore exempt from the proposed sensitisation warning statements.

1. <u>The scheduling proposal mandating sensitisation warning statements on labels of products not intended for direct skin application are not warranted if the levels of Isothiazolinones are kept at or below safe levels.</u>

   The warning statement "CONTAINS ISOTHIAZOLINONES. REPEATED EXPOSURE MAY CAUSE SENSITISATION." on products NOT intended for direct skin exposure are not warranted for a number of reasons. Firstly, the level of isothiazolinones for these products are kept at a safe level below which there's no expected skin sensitisation even following repeated exposures. Secondly, restrictions placed for risk management should be chemical-specific and should hence consider the individual toxicity of the isothiazolinones which can differ significantly.

   a. <u>Different isothiazolinones have different maximum safe levels based on their potency.</u>

      Different isothiazolinones have different skin sensitisation potency, and therefore a general blanket restriction level of 0.05% with any level below requiring mandatory warning statements across all isothiazolinones is not appropriate from a scientific point of view. Isothiazolinones have very different skin sensitization potencies which are inherent based on their chemical structures, and therefore they have different sensitising induction thresholds. Induction thresholds can span over various orders of magnitude. The isothiazolinones methylisothiazoline (MIT), benzisothiazolinone (BIT), and methylbenzisothiazolinone (MBIT) are strong sensitisers with a non-expected sensitisation induction level (NESIL) of approximately 15 ug/cm2/day. On the other hand, Methylchloroisothiazolinone (MCIT[1]) is an extreme skin sensitiser with a NESIL of about 1 order of magnitude lower, around 1 ug/cm2/day.

      The induction of skin sensitization is a threshold effect. Based on an exposure-based quantitative risk assessment approach (QRA), taking into account the inherent hazard and potency of the Isothiazolinones (the NESIL), the specific exposure to which the consumer is exposed to per product type (the consumer exposure level, CEL), the application of Sensitisation Assessment Factors (SAF) to address uncertainty around the values, and the derivation of acceptable exposure levels (AEL), one can determine safe isothiazolinones levels across different product applications. Any AEL/CEL >1 (equivalent to a Margin of Safety or MOS > 1) indicates that there is no expected risk of sensitisation based on the consumer exposure via that product type. Such an approach is in line with general risk assessment principles, deriving a MOS.

[1] MCIT (CAS# 26172-55-4) is used as representative for MCIT/MIT (CAS# 55965-84-9). Hazard and potency data are all taken from MCIT/MIT mixture (CAS # 55965-84-9).

For the specific isothiazolinones MIT, BIT and MBIT, a QRA shows that exposures to levels of 0.015% (150ppm) Isothiazolinone and below across all cleaning product types (Table 1) are safe and are not expected to induce skin sensitization upon repeated use. On the other hand, for MCIT, the safe use level in a product is much lower due to the different inherent sensitisation potential of MCIT vs. MIT. Considering that MCIT is a more potent skin sensitizer, safe use levels are reduced to 0.0015% (15ppm) and below (Table 1). Below these indicated levels, data show there is no expected risk of sensitisation upon repeated exposure, and therefore do not justify the requirement for the proposed warning statements.

Table 1. Comparsion of AEL/CEL ratios across different cleaning & hygiene product types. (Exposure estimations are based on publicly available information and models.)[2]

	Isothiazolinones	**MIT, BIT, MBIT,**		**MCIT**	
	NESIL	**15 ug/cm2**		**1 ug/cm2**	
	SAF	**100**		**100**	
	AEL (= NESIL/SAF)	**0.15**		**0.01**	
	Isothiazolinone level in product	**150 ppm (0.015%)**		**15 ppm (0.0015%)**	
	Product category	**CEL[3]**	**AEL/CEL**	**CEL**	**AEL/CEL**
Cleaning products	Dish washing liquid – full sink	0.0045	33	0.00045	22
	Dish washing liquid -– direct application (sponge)	0.0705	2.1	0.007	1.4
	Liquid laundry detergent	0.015	10	0.0015	6.7
	Fabric softener	0.015	10	0.0015	6.7
	Hard surface cleaner	0.0195	7.7	0.00195	5.1
	Air freshener	0.03	5	0.003	3.3

It must be noted that among the different product categories with incidental skin contact, hand dish wash via direct application on the sponge represents the worst case in terms of exposure. The estimated value for this direct application scenario is conservative as it assumes the consumer would not rinse their hands after the washing, which is highly unlikely. The vast majority of consumers will rinse their hands if there is foam present, thus the real-life exposure would be much lower. Further, based on Australian Habits and Practices study

[2] Sources of information: Values and estimations based on publicly available exposure values; e.g., RIVM exposure factsheets, AISE exposure values, as below:

Rothe et al., 2017;

HERA, 2005. Human and Environmental Risk Assessment on ingredients of household cleaning products. www.heraproject.com.

A.I.S.E., 2017., A.I.S.E. / FEA Specific Consumer Exposure Determinants ("SCEDS") https://echa.europa.eu/documents/10162/22788232/aise_sceds_factsheets_v1-1_oct2017_en.pdf/b7f2c8df-d8fc-4243-a37c-620637fe40ff

RIVM, 2006. Cleaning Products Fact Sheet to assess the risks for the consumer, Report 320104003/2006.

IFRA Cat 10B, GUIDANCE FOR THE USE OF IFRA STANDARDS, December 12, 2019; https://www.rifm.org/downloads/RIFM-IFRA%20Guidance-for-the-use-of-IFRA-Standards.pdf

[3] CEL (Consumer exposure level) adjusted to 150ppm Isothiazolinones concentration. The CEL's reported in the references are based on 100ppm Isothiazolinones concentration.

conducted by P&G in 2013, the majority of respondents dilute the hand dish liquid in the sink or bowl (45%) rather than apply it directly on the sponge (21%).

Please refer to Appendix 1 (Safety Assessment of Isothiazolinones) for technical details, including the skin sensitisation induction thresholds of different isothiazolinones, and the methodologies used in the safety assessment.

b. Incidental skin products are the least contributor of IT exposure and likely sensitisation.

In a study to track the rate of MIT contact allergy diagnosed in clinics in Australia from Jan 2011 to Dec 2017, it was concluded that the likely sources of exposure to MIT are mostly cosmetic products, particularly leave-on products like skin moisturisers and wet wipes. (Flury *et. al*., 2018). It must be noted that the use of MIT and MCIT in leave-on products has been banned since 2017. Out of the total 127 cases investigated, only 2 were due to dishwashing liquids and 7 were due to occupational exposure such as use of paints (Table 2).

Table 2. Suspected sources of methylisothiazoline exposure in 2014 (Flury *et. al.,* 2018).

2014 (n = 84)[a]

Suspected source of exposure	No. of reports[a] (%)
Shampoos/conditioners	27 (32.2)
Moisturizers/body lotions/face creams/hand creams	23 (27.4)
Wet wipes	22 (26.2)
Hand/body washes	20 (23.8)
Unknown	11 (13.1)
Occupational (paints, biocides)	7 (8.3)
Face cleansers/scrubs	7 (8.3)
Deodorants	4 (4.8)
Hair gels	2 (2.4)
Dishwashing detergents	2 (2.4)
Sunscreens	1 (1.2)
Mouth washes	1 (1.2)
Total	127

[a] Some people were exposed to >1 source.

This finding is supported by a study that shows a great disparity in the consumer exposure of Isothiazolinones between household cleaning products and cosmetic products. The frequency and level of exposure from household cleaning products is generally lower to the cosmetic rinse-off products, and much lower in about an order of magnitude lower compared to the cosmetic leave-on products (Table 3). This is mainly due to indirect and incidental skin contact when consumers use these household cleaning products.

Table 3. Comparison of consumer exposure level (CEL) across different product categories.[4]

Product category / Usage scenario	Product type	CEL[5] (ug/cm2/day)
Shampoo + conditioner (1x/day)	Rinse-off cosmetic	0.068
Hand soap (5x/day)	Rinse-off cosmetic	0.035
Face/Skin cream (2x/day)	Leave-on cosmetic	0.63
Dishwashing liquid – full sink (3x/day)	Household cleaning	0.0045
Laundry detergent (1x/day)	Household cleaning	0.015
Fabric softener	Household cleaning	0.015
Hard surface cleaner	Household cleaning	0.0195

In Australia, we have undergone several regulatory changes for the use of MIT and MCIT in 2016 and 2017. The MIT limit of 0.01% was imposed for both leave-on and rinse-off cosmetic products in 2016, and then later in 2017, the use of MIT in leave-on cosmetics was banned. Since then, the threshold has been reduced further to the current 0.0015% for rinse-off cosmetic products. With restrictions put in place to reduce consumer's exposure to MCIT and MIT in cosmetic products, we expect incidence of skin sensitisation in Australia to have significantly reduced and coupled with the above data showing that incidental skin products are smaller contributors of exposure, we believe that warning statements are not warranted if we stay within the maximum safe levels calculated.

c. Restrictions including warning statements are intended to protect human health, and caution should be taken to avoid unintended consequences.

In addition to using safety data to guide the right application of warning statements depending on the level cut-offs, caution should also be exercised in mandating warning statements on product labels especially of everyday household, cleaning, hygiene and air care products. The proposed warning statements "CONTAINS ISOTHIAZOLINONES. REPEATED EXPOSURE MAY CAUSE SENSITISATION" may unnecessarily cause fear that the product will trigger sensitization (induction) and considered unsafe when that is not the intent of the risk management control.

d. Consistent risk management approaches should be applied across all consumer product categories.

The same general approach in risk management used for rinse-off cosmetic products should be consistently applied to products with indirect/incidental skin contact. The point of difference in risk management between direct and indirect exposure should relate to the acceptable threshold below which there is little risk of induction of sensitisation.

[4] Rothe *et al*., 2017;

[5] CEL (Consumer exposure level) adjusted to 150ppm Isothiazolinones concentration. The CEL's reported in the Rothe *et al* paper are based on 100ppm Isothiazolinones concentration.

MIT and MCIT for use in topical rinse off products are currently scheduled as follows:

Schedule 6:
Methylisothiazolinone except:
(a) in rinse-off cosmetic preparations or therapeutic goods intended for topical rinse-off application containing 0.0015 per cent or less of methylisothiazolinone;

Schedule 6:
Methylchloroisothiazolinone except:
(a) in rinse-off cosmetic preparations or therapeutic goods intended for topical rinse-off application containing 0.0015 per cent or less of methylchloroisothiazolinone and methylisothiazolinone in total;

The restriction for cosmetic rinse-off products is placed only on the level of MIT and MCIT, and importantly, no warning statements are mandated below that threshold level. This is to recognize that 0.0015% level is considered safe for the consumer from the point of view of induction of sensitisation. Considering that products with no direct skin application have even more limited skin exposure, we feel that mandating warning statements in addition to a restriction level is overly restrictive.

2. <u>The proposed concentration restrictions and mandatory statements for products that are not intended for direct application to the skin are not in line with international practice.</u>

The USA does not have any restrictions or mandatory statements on the use of isothiazolinones in home care products such as those used for laundry, dish and air care where there's indirect and incidental skin contact. In Europe, MIT and BIT have no maximum allowed limits in detergents but have CLP (local implementation of globally harmonised hazard classification system (GHS)) classification and labelling limits. CLP does not mandate a "MAY CAUSE SENSITISATION" statement at any level. The current proposed SUSMP restrictions and label requirements are also not in line with GHS that is in use for similar products in New Zealand.

Noting that the government has adopted the "Accepting Trusted International Standards" policy which promotes acceptance of products from other comparable economies, we believe that the proposed scheduling of Isothiazolinones through the Poisons Standard should be reconsidered to be more internationally aligned.

3. <u>This unique Australian requirement will impose higher regulatory burden, unnecessarily impose barrier to trade, and reduce availability of products to the Australian consumers.</u>

Australia, being a small market in relation to other first world economies, normally shares labels with other countries. It is reasonable to assume that other markets are unlikely to commit to sharing a label that unduly impose such harsh warning statements regarding inducing sensitisation. Mandating an Australian unique warning statement will require an Australian-specific label which will be difficult to justify from a commercialisation point of view. Such labelling changes will also translate to higher product costs that will inadvertently be passed on to the general public. This will also impact on business decisions for future innovations that can be brought into Australia.

4. The immediate business impact of this proposed scheduling, in particular the label requirement, is immense as it will affect the majority of our home care products currently sold in Australia. In addition, there are also significant cost implications in terms of product retrieval, over-stickering, and scrapping in order to comply with the proposed scheduling in the standard implementation time.

   If this scheduling proposal proceeds as is, the majority of our laundry, dish cleaning and air deodorising products will be impacted. The value of impacted products is in the magnitude of millions. Customisation or over-labelling of existing stocks within our control alone is estimated to be up to AUD$500 000. Costs will be even higher if we are compelled to retrieve products from our customers. There are additional costs that are difficult to quantify associated with resources required to retrieve from market and product destruction.

5. Any scheduling decision should allow at least 24 months transition time for compliant products to be available to consumers in the marketplace. Furthermore, we propose no action needs to be taken for products already in-market, recognizing there is no immediate harm to consumers that will merit a major business disruption.

   Once the final decision is released to the public on 25th November 2020, the effective date of compliance will be with the release of the 1st February 2021 instrument. This is an extremely unrealistic timeline for industry and the supply chain logistics must be considered. Adequate time is needed to instigate artwork updates, commence production and ship products to the market from overseas manufacturing sites. Consideration should also be given to stocks already produced, that are still safe for consumers and labelled compliant with the current SUSMP instrument. Adequate transition time is also needed to allow exhaustion of products in the market to avoid product recall which will create unnecessary fear, business disruption and economic costs.

Our counterproposal, for the committee's consideration:

Please see below our counterproposal to ACCS-ACMS's proposed scheduling on Methylisothiazolinone, Methylchloroisothiazolinone and Isothiazolinones in general. Our proposed changes are highlighted and underlined.

Proposed ACCS-ACMS Scheduling	**Counterproposal**
Schedule 6: Methylisothiazolinone except: a. in rinse-off cosmetic preparations or therapeutic goods intended for topical rinse-off application containing 0.0015 per cent or less of methylisothiazolinone; or b. in other preparations that are not intended for direct application to the skin containing 0.05 per cent or less of isothiazolinones in total when labelled with the statements: Contains isothiazolinones. Repeated exposure may cause sensitisation	Schedule 6: Methylisothiazolinone except: a. in rinse-off cosmetic preparations or therapeutic goods intended for topical rinse-off application containing 0.0015 per cent or less of methylisothiazolinone; or b. in other preparations that are not intended for direct application to the skin containing 0.05 per cent or less of isothiazolinones, and if containing more than 0.015% of methylisothiazolinone, benzisothiazolinone, methylbenzisothiazolinone in total, when labelled with the statements: Contains

	isothiazolinones. Repeated exposure may cause sensitisation.
Schedule 6: Methylchloroisothiazolinone except: a. in rinse-off cosmetic preparations or therapeutic goods intended for topical rinse-off application containing 0.0015 per cent or less of methylchloroisothiazolinone and methylisothiazolinone in total; or b. in other preparations that are not intended for direct application to the skin containing 0.05 per cent or less of isothiazolinones in total when labelled with the statements: Contains isothiazolinones. Repeated exposure may cause sensitisation	Schedule 6: Methylisothiazolinone except: a. in rinse-off cosmetic preparations or therapeutic goods intended for topical rinse-off application containing 0.0015 per cent or less of methylisothiazolinone; or b. in other preparations that are not intended for direct application to the skin containing 0.05 per cent or less of isothiazolinones, and if containing more than 0.0015% of methylchloroisothiazolinone and other isothiazolinones in total, when labelled with the statements: Contains isothiazolinones. Repeated exposure may cause sensitisation.
Schedule 6: ISOTHIAZOLINONES not elsewhere specified in these Schedules, except: in preparations that are not intended for direct application to the skin containing 0.05 per cent or less of isothiazolinones in total and if containing more than 0.015 per cent, when and labelled with the statements: CONTAINS ISOTHIAZOLINONES. REPEATED EXPOSURE MAY CAUSE SENSITISATION.	Schedule 6: ISOTHIAZOLINONES not elsewhere specified in these Schedules, except: a. in preparations that are not intended for direct application to the skin containing 0.05 per cent or less of isothiazolinones, and if containing more than 0.015% of methylisothiazolinone, benzisothiazolinone, methylbenzisothiazolinone in total, when labelled with the statements: Contains isothiazolinones. Repeated exposure may cause sensitisation. b. in preparations that are not intended for direct application to the skin containing 0.05 per cent or less of isothiazolinones, and if containing more than 0.0015% of methylchloroisothiazolinone and other isothiazolinones in total, when labelled with the statements: Contains isothiazolinones. Repeated exposure may cause sensitisation.

References

Flury U, Palmer A, Nixon R. (2018) The methylisothiazolinone contact allergy epidemic in Australia. chromium and cobalt in consumer products: revisiting safe levels in the new millennium. *Contact Dermatitis.* **(79):** 189-191.

Rothe H, Ryan C, Page L, Vinali J, Goebel C, Scheffler H, Toner F, Roper C, Kern P. (2017) Application of in vitro skin penetration measurements to confirm and refine the quantitative skin sensitisation risk assessment of methylisothiazolinone. *Regulatory Toxicology and Pharmacology* **(91)**: 197-207.

Appendices:

Appendix 1: Technical paper on the safety of Isothiazolinones.

Appendix 2: Flury *et. al.,* (2018).

Appendix 3: Rothe *et. al.,* (2017).

Appendix 1

Technical paper on Isothiazolinones Safety

Isothiazolinone Safety

The critical safety endpoint for isothiazolinone (IT) preservatives is skin sensitization (i.e. Allergic Contact Dermatitis ACD). Consumer products can be safely formulated taking into account the use pattern of the products, and concentration of the ITs in product and the skin sensitization potencies of the different IT preservatives. This document will provide some information on the mechanism of ACD, the hazard and potency of various isothiazolinone preservatives and the principles of the quantitative risk assessment for skin sensitization.

1. Allergic Contact Dermatitis (ACD): Sensitization induction and elicitation

ACD is the clinical manifestation of a cell-mediated immune response known as 'skin sensitization' or 'skin allergy'. The development of ACD following dermal exposure to an allergen is a two-stage process: induction and elicitation. During the *induction phase*, the immune system is primed and sensitized for an allergic response, and during the *elicitation phase* subsequent contacts with the allergen trigger the adverse skin reactions (the ACD).

Dose response relationships can be observed for both the induction and elicitation phases of skin sensitization (Kimber *et al.*, 1999). They are considered to be threshold phenomena and, as such, a level of chemical exposure below which sensitization will not be induced or below which an allergic response will not be elicited in a sensitized individual can be determined. In general, the amount of chemical required to induce sensitization is usually greater than the amount of the same chemical needed to elicit a response in a sensitized subject.

Induction of skin sensitization (allergy) can be thought of as causing a naïve (non-allergic) person to become allergic to an ingredient. The induction of skin sensitization (allergy) does not produce any noticeable skin reactions.

The induction of Skin Sensitization is a threshold effect. Skin sensitizers can be grouped according to their inherent skin sensitization potency/ potential. Not all sensitizing chemicals induce skin sensitization at the same threshold level. The sensitizing (inducing) threshold ranges across several orders of magnitude. (Basketter et al. 2014) (Loveless et al. 2010)

To protect against induction of allergic reactions (i.e. sensitization), a quantitative risk assessment (QRA) is done to demonstrate that the consumer exposure level is below the threshold level at which induction would be expected to occur. If induction can be avoided, then the risk for elicitation is negligible.

Elicitation of an *allergic reaction* can be thought of as triggering an allergic skin response *in a person who already has an existing allergy to the ingredient*. Manifestation of skin sensitization in the clinic is based on elicitation reactions after patch testing.

2. Quantitative Risk Assessment (QRA) methodology

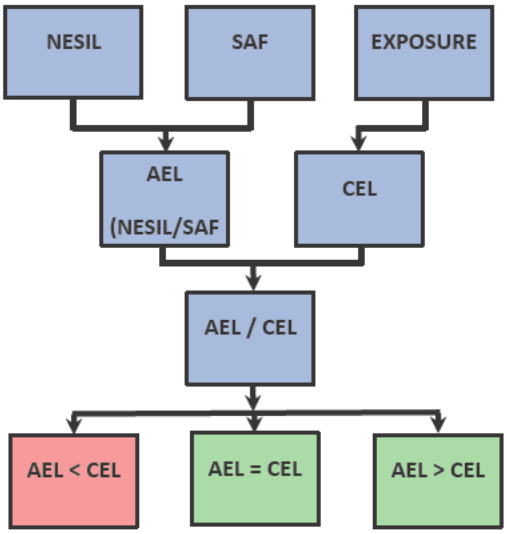
Consumer products are formulated with the minimum amount of preservative possible to ensure the products remains safe (1) from microbial contamination during its use lifetime and (2) for consumer not to be sensitized when using the products. The latter is done via a Quantitative Risk Assessment (QRA) which is a methodology applicable for all allergens (not only to IT).

The QRA methodology was initially developed and applied in collaboration with the fragrance industry (Api et al., 2008) and allows a prediction of a maximum (or upper) safe level for each allergen in a specific consumer product, which is not predicted to induce skin sensitisation (the acceptable exposure level AEL). It is derived from the hazard characterisation data of the allergen (No expected sensitisation induction level or NESIL) and adjusted using appropriate sensitisation assessment factors (SAFs) to address uncertainties around those data. It has since been applied to other established human contact allergens besides fragrance materials (Novick et al., 2013; Basketter et al., 2008; Jowsey, 2007; Basketter, 2010a; Basketter et al., 2003a); including preservatives, where the outcome of these QRA analyses predicted that exposures resulting from the use of some product types could induce skin sensitisation in line with corresponding clinical observations.

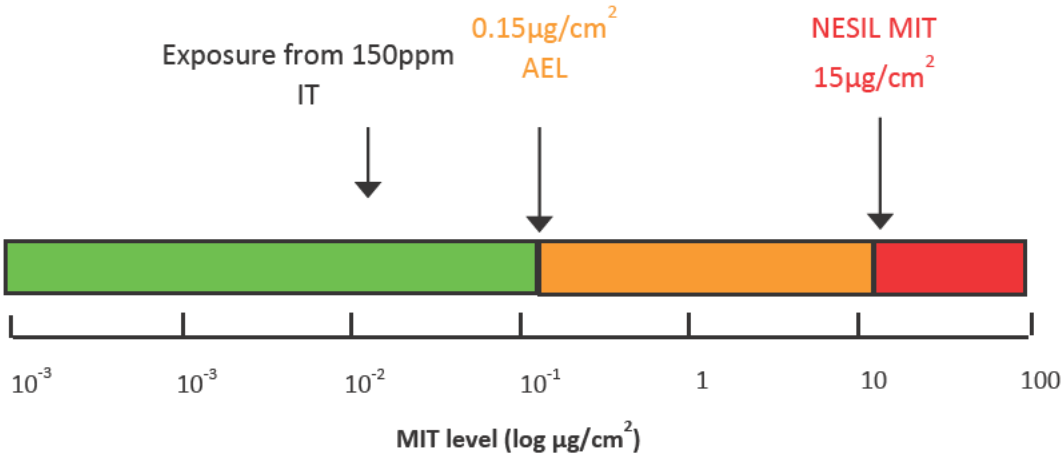
The QRA methodology currently represents the best tool available to ensure that the levels of sensitising ingredients in consumer products are safe with respect to induction of skin sensitisation.

The QRA approach for allergens in consumer products follows the same four fundamental steps as identified for general toxicology risk assessment: a) Hazard identification, b) Dose-response assessment or hazard quantification c) Exposure assessment and d) Risk characterisation.
The induction of skin sensitisation is a threshold-based event, the metric for risk assessment for this toxicological endpoint is accepted to be dose of the allergen per unit area of skin (or $\mu g/cm^2$) (Friedman, 2007; Kimber et al., 2008).

The key steps of the QRA process are determination of known safe benchmarks (the NESIL), application of sensitisation assessment factors (SAF) to address uncertainties, and calculation of consumer exposure (CEL) through normal product use. With these parameters, a reference dose (the acceptable exposure level AEL) can be calculated and compared with the CEL. When the AEL exceeds the CEL, it is predicted that induction of skin sensitisation is unlikely to occur (see flowchart below).



The figure below illustrates QRA: When the consumer exposure (CEL) is in the "green" area, then the risk for sensitization is extremely low, as the CEL is < AEL. If the CEL is in the "red"zone, then it is > NESIL, there is a high risk of induction. The "orange" area of exposure is the uncertain exposure area, which should be avoided as well, as a risk for induction cannot be excluded.

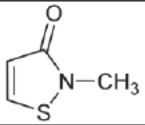
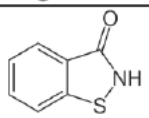


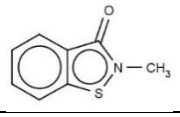
3. Isothiazolinone Skin Sensitization Induction threshold

The induction of Skin Sensitization is a threshold effect and not all sensitizing chemicals induce skin sensitization at the same threshold level. The sensitizing (inducing) threshold (NESIL; Non-Expected Sensitization Induction Level) ranges across several orders of magnitude. (Basketter et al. 2014) (Loveless et al. 2010). The NESIL is a benchmark, reflecting the human sensitisation threshold, that is typically derived from animal and human data or other data through the application of a Weight of Evidence (WoE) approach using all the relevant data. The NESIL is expressed as a dose per unit area (e.g. µg/cm^2/day) value.

Considering the proposed regulatory change to restrict total IT level, it is important to consider that different IT have different NESILs. For each of the IT substances, an individual NESIL can be determined which forms the basis for a risk assessment and can be used to determine safe limits in products. For the group of IT preservatives the NESIL values which have already been determined vary about 1 order of magnitude. In general, the IT preservatives could be split into 2 groups. (This a simplification which could help setting general safe limits in products)

Group A: IT preservatives with a strong sensitization potential and a NESIL of around 15 ug/cm2. These cover: MIT, BIT and MBIT.

Isothiazolinone	Chemical structure
Methylisothiazolinone (MIT, MI) CAS# 2682-20-4	
Benzisothiazolinone (BIT) CAS# 2634-33-5	

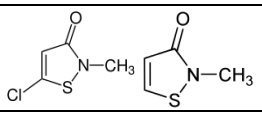
Methyllbenzisothiazolinone (MBIT) CAS# 2527-66-4	

MIT: The WoE NESIL for MIT is derived from five Human Repeat Insult Patch Tests (HRIPT) (Burnett et al., 2010) and 4 Local Lymph Node Assays (LLNA) (SCCNFP, 2004; Basketter et al., 2003b; Gerberick et al., 2005). In the LLNA studies MIT had EC_3 values (threshold values) between 0.4% (100 µg/cm^2) and 11% (2750 µg/cm^2) depending on the vehicle used. These values already indicate that MIT is a strong sensitiser (Kimber et al., 2003). In the HRIPTs, no positive responses were observed up to an exposure level of 15 µg/cm^2 MIT in water. Sensitisation was induced at exposures of 20 and 25 µg/cm^2. Taken all data together, MIT can be considered to be a strong sensitiser (Gerberick et al., 2005) with a NESIL of 15 µg/cm^2.

BIT: For the determination of the WoE NESIL for BIT the data from three Human Repeat Insult Patch Tests (HRIPT) (Basketter et al 1999), two LLNA (Basketter et al 1999, Ashby et al 1995) and guinea pig maximisation and Buehler tests were considered (Zissu 2002, Yamano 2001, SCCNFP 2004). The weight of evidence NESIL for BIT is around 10 to 50 µg/cm^2 . In line with MIT, assessment of Human Repeat Insult Path Test, (HRIPT) data were more conservative. Local Lymph Node Assay (LLNA) and guinea pig data, which would point to a higher NESIL were considered as supporting information.

MBIT: The weight of evidence (WoE) No Expected Sensitization Induction Level (NESIL) for N-methyl-1,2-benzisothiazol-3(2H)-one (MBIT) [2527-66-4] is 100 µg/cm^2 based on an assessment of Local Lymph Node Assay and Buehler data. For the determination of a WoE NESIL for MBIT the data from 2 local lymph node assays (LLNA) and one Buehler study were considered (CLH Report) .

<u>Group B:</u> IT preservatives with a strong to extreme sensitization potential and a NESIL around 1 ug/cm2. (the 3:1 mixture of CMIT/MIT)

Isothiazolinone	**Chemical structure**
Mixture of CMIT/MIT (Kathon CG) CAS# 55965-84-9	

MCI/MIT: MCI/ MIT is a defined mix in a 3:1 ratio of MCI and MI. (*To note: The preservative is available in this mix under the CAS# 55965-84-9. The MCI alone has a different CAS# 26172-55-4. So far this was not yet commercially available.)*

For the determination of a WoE NESIL for MCI/MIT the data from 10 Human Repeat Insult Patch Tests (HRIPT) and 6 LLNAs (Warbrick et al. 1999) and were evaluated. For MCI/MIT the lowest reported LLNA EC3 value, 1.25 µg/cm^2, is very close to the NOEL identified in HRIPTs conducted by Weaver et al (1986) and Maibach (1985), 0.83 and 2.5 µg/cm^2, respectively. Taking all data into consideration a WoE NESIL of 1 µg/cm^2 can be defined which classifies MCI/MIT as an extreme sensitizer, and a more potent sensitizer compared to the non-chlorinated IT structures.

4. Household care IT products exposure

The table below provides an overview of consumer exposure for a range of household care products assuming they contain an IT preservative at 150ppm. The consumer exposure relating to the higher end of the typical use level of the product, in dose per unit area to IT (in µg/cm^2/d), is shown. Consumer exposure is calculated using publicly available information (AISE, HERA, RIVM ref.) The highest exposure within the household care category is coming from using the hand dish washing product in a direct sponge application. The value for this direct application scenario is conservative as it assumes the consumer would not rinse their hands after the washing, which is not realistic considering there will be foam on the sponge and/or hands after the washing.

PRODUCT CATEGORY	150 ppm IT exposure (µg/cm^2)
Hand dish washing products	
Hand dish washing: full sink application	0.0045
Hand dish washing: direct application*	0.071
Laundry Detergents	
Laundry detergent handwashing scenario	0.015
Fabric enhancer	
Fabric enhancer / softener handwashing scenario	0.015
All-Purpose cleaners	
Hard surface cleaner (diluted)	0.020
Air Care products	
Febreze air effects	0.03

* conservative scenario, see text.

5. No sensitization risk expected based on the quantitative risk assessment (QRA) Group A IT preservatives

As mentioned previously, the WoE NESIL for Group a IT is 15 µg/cm^2.
In line with general toxicology, for skin sensitisation risk assessments it is equally necessary to extrapolate from the experimental (defined and controlled exposure conditions) to real life consumer exposure (variable exposure controlled by the consumer). This is achieved by the application of SAFs which take account of three parameters: a) inter-individual variability (the same as in general toxicology), b) vehicle/product matrix effects and c) use considerations (specific for skin sensitisation). (Basketter 2016) They are pragmatically set as 1, 3.16 (half log of 10) or 10. These values chosen are consistent with the approach used by EPA for general risk assessment (Dourson, 1996). In a conservative approach, we have chosen a total SAF of 100 for all product categories. The total SAF of 100 addresses uncertainty relating to human variability, product / matrix effects and in use considerations.

The total SAFs are applied to the NESIL to derive the AEL (NESIL/total SAF = AEL). Therefore, an AEL is a conservative estimate of a dermal exposure (in ug/cm^2 skin), corrected for associated uncertainty

around the value, that would not be expected to result in the induction of sensitisation in the general population, including more responsive subpopulations.
Applying a Safety Assessment Factor (SAF) of 100 to the NESIL for MI (15 µg/cm^2) will result in an AEL of 0.15 µg/cm^2 which is then compared with the CEL. If the AEL is > CEL, the risk for the induction of skin sensitization is extremely low/ negligible.

The below table indicates that a total IT level (Group A) of 150 ppm is supportable and the risk of sensitization is extremely low/negligible.

PRODUCT CATEGORY	150 ppm MI (ug/cm^2)	AEL/CEL (150 ppm)
Hand dish washing products		
Hand dish washing: full sink application	0.0045	33.3
Hand dish washing: direct application	0.0705	2.1
Laundry Detergents		
Laundry detergent handwashing scenario	0.015	10
Fabric enhancer		
Fabric enhancer / softener handwashing scenario	0.015	10
All-Purpose cleaners		
Hard surface cleaner (diluted)	0.0195	7.7
Air Care products		
Febreeze Air effect	0.03	5

To note:

Using an IT preservative like MCIT/MIT with a lower NESIL (1 ug/cm2) at 150ppm would result in a consumer exposure higher than the AEL (closer to the NESIL) and a risk for the induction of skin sensitization could not be avoided. Using MCIT/MIT only at 15 ppm concentration would lead to a AEL/CEL ratio > 1 for all the above products.

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Appendix 2

Flury U, Palmer A, Nixon R. (2018). The methylisothiazolinone contact allergy epidemic in Australia. chromium and cobalt in consumer products: revisiting safe levels in the new millennium. Contact Dermatitis. (79): 189-191.

The methylisothiazolinone contact allergy epidemic in Australia

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Methylisothiazolinone (MI) is a preservative that was released in early 2000 for occupational uses, particularly in paints, adhesives, and cleaning agents. It was widely used in cosmetics and household products from 2005,[1] although it had been known to cause sensitization in humans and guinea-pigs since the mid-1980s.[2,3] The first case of occupational contact dermatitis caused by MI was published in 2004,[4] and MI allergic contact dermatitis (ACD) caused by cosmetic products was first reported in 2010.[5] European regulations, introduced in February 2017, completely prohibited the use of MI in leave-on cosmetics.[6] From the end of April 2018, the acceptable MI concentration in rinse-off cosmetics on the European market was lowered to 0.0015%.[7] In Australia, the MI limit of 0.01% applied to both leave-on and rinse-off cosmetics; however, the use of MI in leave-on cosmetics was banned from October 2017.[8] The aim of this study was to track the rate of MI contact allergy diagnosed at our institution and investigate the likely sources of exposure to MI at the height of the epidemic.

METHODS

This retrospective study included all patients who underwent patch testing with MI from January 1, 2011 to December 31, 2017 in our Occupational Dermatology and Contact Dermatitis Clinics, Melbourne, Australia. These are tertiary referral clinics for the investigation of patients with suspected contact dermatitis.

Patients were patch tested with allergens from Chemotechnique Diagnostics (Vellinge, Sweden) or Allergeaze (SmartPractice, Tucson, Arizona) by the use of either Finn Chambers or Allergeaze test chambers (SmartPractice). MI was tested 0.2% aq. The patches were removed on day (D) 2, and read on D2 and D4. Later readings were not routinely performed unless patients noted additional reactions. Readings were performed according to ICDRG guidelines.

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For the subgroup of patients who reacted to MI in 2014, details of exposure to MI were ascertained from their medical records.

RESULTS

The number of patients tested and the positive results are summarized in Table 1. Across 7 years from 2011 to 2017 inclusive, 2787 patients were tested with MI, and 14.5% (404 patients) had positive test results. Seventy-seven per cent had a relevant reaction with documented exposure to MI in a product used and/or a positive patch test reaction to their product. The percentage of positive reactions to MI in those attending the clinic rose from 4.1% in 2011 to 20.3% in 2015. In 2016, the percentage decreased to 18.8%, and in 2017 it decreased to 11.4%.

Table 2 summarizes the suspected sources of exposure in 2014, the year in which the most reactions occurred (n = 84). The most important sources of MI were shampoos/conditioners (27 cases, 32.2% of the total cases for that year), moisturizers/body lotions/face creams/hand creams (23 cases, 27.4%), wet wipes (22 cases, 26.2%), and hand/body washes (20 cases, 23.8%). In 11 cases (13.1%), the source of MI exposure was either not identified by the attending dermatologist or not recorded in the patient file. It was not usually possible to identify the source of sensitization, but only the current exposure.

TABLE 1 Numbers of patients patch tested with methylisothiazolinone (MI) and relevant reactions

Year	No. tested	Total reactions to MI (%)	Relevant reactions to MI (%)	Old/ unknown reactions
2011	419	17 (4.1)	14 (82.4)	3
2012	452	56 (12.4)	41 (73.2)	15
2013	372	58 (15.6)	49 (84.5)	9
2014	428	84 (19.6)	70 (83.3)	14
2015	389	79 (20.3)	60 (76.0)	19
2016	361	68 (18.8)	45 (66.2)	23
2017	366	42 (11.4)	31 (73.8)	11
Totals	2787	404 (14.5)	310 (76.7)	94

TABLE 2 Suspected sources of methylisothiazolinone exposure in 2014 (n = 84)[a]

Suspected source of exposure	No. of reports[a] (%)
Shampoos/conditioners	27 (32.2)
Moisturizers/body lotions/face creams/hand creams	23 (27.4)
Wet wipes	22 (26.2)
Hand/body washes	20 (23.8)
Unknown	11 (13.1)
Occupational (paints, biocides)	7 (8.3)
Face cleansers/scrubs	7 (8.3)
Deodorants	4 (4.8)
Hair gels	2 (2.4)
Dishwashing detergents	2 (2.4)
Sunscreens	1 (1.2)
Mouth washes	1 (1.2)
Total	127

[a] Some people were exposed to >1 source.

DISCUSSION

Our prevalences of contact allergy to MI are higher than reported elsewhere, with a peak of 20.3% of those patch tested in 2015. In contrast, the prevalence of MI allergy in the United States was 10.9% in 2013 to 2014.[9] In Leeds, United Kingdom, MI allergy increased from ~1% in 2009 to ~10% in 2014, and decreased to 4% in 2015.[10] Data from 11 centres in Europe in 2015 showed proportions of 5% to 13%.[11]

This study has shown that the frequency of sensitization to MI has decreased since 2015, but is still high, at 11.4% in 2017. The reason for the decrease may be related to the increasing removal of MI from products, especially from wet wipes. It is not known whether there has indeed been more exposure to MI in Australia, or whether the high prevalences of MI allergy that we report have been influenced by lower rates of patch testing.[12]

Finally, from approximately 2015, patients attending for patch testing were instructed to avoid MI before attending, and this may have resulted in some patients not proceeding to patch testing because their dermatitis improved. There has also been increased awareness of MI allergy, with greater understanding among dermatologists and local publicity. This would mean that our high prevalences of MI allergy may actually be an underestimate.

Although we can only comment on likely sources of exposure of MI, and do not know for certain how patients became sensitized, it is interesting that the most important sources of exposure to MI in our study were rinse-off products (shampoos/conditioners).

As shown in Table 2, wet wipes were also frequent sources of MI. In 2013, Boyapati from our group reported contact dermatitis involving the hands in parents and carers of babies, and hypothesized that it was the result of the use of a particular brand of baby wipes that was widely used in Australia.[13] It is unknown how many babies may have been sensitized through the use of wet wipes, as none were tested.

The use of MI in cosmetic products accounts for the majority of cases of contact allergy to MI, with it, surprisingly, being present in a mouthwash, but we have also reported 7 cases of definite occupational exposure to MI in paints and biocides. MI was also found in a number of work hand cleaners (number not specified) and shampoos used by hairdressers (number not specified). MI is volatile, and may cause airborne ACD, asthmatic symptoms and even systemic allergic dermatitis in newly painted rooms.[14] In contrast to the more regulated market for MI in cosmetic products, the use of MI in industrial products, including paints and detergents, is not restricted, but in the EU, as a result of REACH decisions in February 2018, products with more than 0.0015% (15 ppm) MI must be labelled as "may cause allergic sensitization".

Every cloud has a silver lining, and 1 upside of the MI epidemic has been a resurgence of interest in patch testing in Australia, aided by our centre proposing the first Australian baseline series, which includes MI,[15] and establishing a Contact Allergen Bank, which has facilitated patch testing, especially for remote and rural dermatologists.[16]

CONCLUSION

Australia appears to have experienced the highest prevalences of MI allergy reported in the literature. Our data show that the frequency of sensitization to MI in Australia is now decreasing. Clinicians need to be aware of the possibility of ACD caused by MI, especially from shampoos/conditioners, lotions and creams, wet wipes, and skin cleansers, and also from occupational sources.

Conflict of interest

The authors declare no potential conflict of interests.

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Appendix 3

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Application of *in vitro* skin penetration measurements to confirm and refine the quantitative skin sensitization risk assessment of methylisothiazolinone

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ABSTRACT

Use of quantitative risk assessment (QRA) for assessing the skin sensitization potential of chemicals present in consumer products requires an understanding of hazard and product exposure. In the absence of data, consumer exposure is based on relevant habits and practices and assumes 100% skin uptake of the applied dose. To confirm and refine the exposure, a novel design for *in vitro* skin exposure measurements was conducted with the preservative, methylisothiazolinone (MI), in beauty care (BC) and household care (HHC) products using realistic consumer exposure conditions. A difference between measured exposure levels (MELs) for MI in leave-on versus rinse-off BC products, and lower MELs for MI in HHC rinse-off compared to BC products was demonstrated. For repeated product applications, the measured exposure was lower than estimations based on summation of applied amounts. Compared to rinse-off products, leave-on applications resulted in higher MELs, correlating with the higher incidences of allergic contact dermatitis associated with those product types. Lower MELs for MI in rinse-off products indicate a lower likelihood to induce skin sensitization, also after multiple daily applications. These *in vitro* skin exposure measurements indicate conservatism of default exposure estimates applied in skin sensitization QRA and might be helpful in future risk assessments.

1. Introduction

A good understanding of hazard as well as consumer exposure to a chemical present in a beauty care (BC) or household care product (HHC) is required to evaluate the potential risk of inducing skin sen sitization in consumers. Some ingredients in consumer products that are known to have skin sensitization potential can be safely formulated as long as skin exposure to them is sufficiently lower than their defined threshold for inducing contact allergy (Felter et al., 2003; Api et al., 2008). Determining accurate consumer exposure levels can be very challenging as standard exposure values are based mainly on the total dose applied externally without consideration of the exact amount re levant for skin sensitization. For aggregate exposures, simple summa tion of values based on repetitively externally applied doses are an ticipated to be unrealistic as this tends to overestimate exposure. Standard *in vitro* absorption studies routinely examine a single chemical or product exposure at a time, determining usually the systemic ex posure (e.g. the amount present in the receptor fluid). This standard study design does not meet the requirements for measuring the skin exposure under consumer relevant usage conditions for HHC or BC products. In reality, consumers commonly experience complex repeated exposure scenarios to multiple product types/matrices with cycles of product removal. When measuring consumer exposure under product use conditions, a single application of the products is done for most situations, while some exposure scenarios require the repetitive appli cation of products, such as multiple usage of e.g. a hand soap during a single day, or beauty salon professionals' exposure to ingredients (e.g. preservatives) in shampoos, conditioners, and body care products used during their work day. Standard *in vitro* dermal penetration studies using human or pig skin are designed to reflect operational exposure scenarios (OECD, 2004a and b; SCCS, 2010) and typically measure the exposure to a single applied amount at a fixed dose of a chemical over 24 h, which is entirely unrelated to the amount used in a product for mulation or the actual exposure time during product use. However, the study design can be adapted to best simulate different exposure sce narios, including simulation of rinse off or leave on products, as well as

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repeated application of products over periods up to 96 h. Exposures determined in those modified skin exposure studies can also inform the quantitative risk assessment for skin sensitization (QRA) and can be used as replacement for consumer exposure calculations based on Ha bits & Practice (H & P) data.

The QRA approach for skin sensitization is based on the determi nation of the amount of chemical per unit area of exposed skin that is not expected to result in the induction of skin sensitization (e.g. the 'No Expected Sensitization Induction Level' or NESIL) followed by the ap plication of sensitization assessment factors (SAFs) (Gerberick et al., 2001; Api et al., 2008) to derive the acceptable exposure level (AEL) for consumers. The NESIL is derived using a weight of evidence approach, and is often based on data from a local lymph node assay (LLNA) or a well conducted confirmatory Human Repeat Insult Patch Test (HRIPT). The SAFs are used to extrapolate from the experimental conditions of the NESIL (defined and controlled exposure) to real life consumer ex posure (variable exposure controlled by the consumer) and to account for variations between subjects, matrices (other components causing skin irritation or enhanced skin penetration), and product use patterns affecting exposure considerations e.g. frequency, occlusion and skin condition or body sites (Felter et al., 2002; Api et al., 2008; Basketter and Safford, 2016). The QRA approach for contact allergens in con sumer products compares the calculated consumer exposure level (CEL, expressed as dose per unit area (μg/cm^2)) to the ingredient of interest in the product, with an AEL. When the AEL/CEL ratio is greater than 1, the exposure to the ingredient is unlikely to result in the induction of skin sensitization. More recently, the use of actual measured exposures to compounds applied to the skin under relevant exposure conditions, namely the "Measured (consumer) Exposure Level" (MEL) (Goebel et al., 2010) has been used in the QRA, replacing or refining the CEL. The MEL is the total amount of chemical recovered in the epidermis, dermis and receptor fluid (RF) plus amounts found in the stratum corneum (SC) when applied under consumer relevant conditions, at the end of the exposure (i.e. after rinsing, or drying). While this gives a realistic picture of the actual skin exposure, it is still a conservative approach since, normally, the SC is not considered to contribute to biological effects involved in skin sensitization (SCCS, 2010). This concept uses *in vitro* skin exposure measurements as a refinement for the applied dose in the context of skin sensitization QRA, and has been used to evaluate the skin sensitizing potential of certain hair dye in gredients (Goebel et al., 2012, 2014). This QRA approach involves a direct comparison of the NESIL (or the AEL) with the MEL. When ap plying a MEL instead of a CEL to inform the QRA, one needs to ensure that the NESIL and, subsequently the AEL, may also require adequate adjustment from the applied dose. Correct exposure information is critical and provides reassurance of the QRA and with that supports the risk assessment decisions.

Preservatives are key ingredients in consumer, professional and industrial products, serving to not only prevent microbial contamina tion of the product itself but also to protect the consumer from adverse effects including infection resulting from use of contaminated products. However, because of their anti microbial activity, preservatives are naturally reactive substances and are often responsible for inducing contact allergy (Yim et al., 2014; Beene et al., 2017). Balancing their use concentration with their efficacy is important since too high con centrations may lead to the induction of skin sensitization (Basketter et al., 2008); while concentrations that are too low will render it in effective as a preservative. Methylisothiazolinone (MI), a preservative, is a strong human and animal skin allergen (Roberts, 2013; Castanedo Tardana and Zug, 2013) which is also classified as a skin sensitizer (1A, H317 according to GHS/CLP, CLP regulation, 2008) and has been identified as the causative agent in numerous cases of allergic contact dermatitis (Lundov et al., 2013; Castanedo Tardana and Zug, 2013). Given the widespread use of MI in a large number of consumer products it is difficult to identify the products which have contributed the most to the outbreak of the MI skin allergy epidemic in Europe and beyond. Until recently the use of MI in cosmetics in Europe was restricted to an upper use limit of 100 ppm (0.01%) (SCCNFP, 2004) for rinse off and leave on products *via* the Annex V of the cosmetic directive. It was subsequently banned from leave on products and its use concentration in rinse off cosmetic products was reduced up to a maximum of 15 ppm (SCCS, 2013). The concentration of MI in non cosmetic products such as paints or household care products (all rinse off products, or not in tended to be in contact with skin) has been reported to range from 0.7 to 180.9 ppm, with most products containing MI at 100 ppm or lower (Schwensen et al., 2015; Garcia Hildago et al., 2017). Classification and labelling restrictions for non cosmetic products are currently finalised to ensure safe usage of MI in those products.

When a QRA was conducted for a number of cosmetic products with 100 ppm of MI, it was concluded that for the majority of leave on products, including wet baby wipes, there was a risk for inducing skin sensitization in consumers (i.e., AEL/CEL < 1), and MI should clearly not be used for these product categories at 100 ppm (CIR, 2014) or lower levels which result in unfavourable AEL/CEL ratios. For rinse off products such as shampoos, hair conditioners and bath soaps, the QRA provides a favourable AEL/CEL ratio > 1, which supports the use of 100 ppm MI in those product types. In addition, applying the QRA methodology to HHC products with MI concentrations of 100 ppm or lower, produces an AEL/CEL ratio > 1, which indicates a low risk for the induction of sensitization due to the use of those products (e.g., laundry products (Kwon et al., 2009)). Exposure to HHC products is generally lower than to cosmetic rinse off products as skin exposure is not intended and rinsing of exposed skin areas is done thoroughly (Corea et al., 2006). While there is usually an adequate margin of safety for MI in HHC rinse off products, there are limited data available to confirm the exposure assumptions and the overall risk assessments. Hence, for this investigation MI was chosen to demonstrate how mea surement of the skin exposure (determined as MEL) can be used to confirm and possibly refine the QRA by providing a more accurate determination of the in use skin exposures. When comparing the actual measured value (MEL) with the calculated CEL, if the MEL values are in line or lower than CELs, then the safety assessment for MI present in different cosmetic and household care products gains additional sup port.

2. Materials and methods

2.1. Materials

[^{14}C] Methyl isothiazolinone ([4,5 ^{14}C] RH 573); [^{14}C] MI; with specific activity of 5.57 mCi/mmol (legs 2 4)), was purchased from Rohm and Haas Research Laboratories, Spring House, PA, USA. [^{14}C] MI with specific activity of 58 mCi/mmol (legs 1, 5 10) was purchased from Quotient Bioresearch, Cardiff, UK Water. Various product for mulations were spiked with [^{14}C] MI at a final concentration of 100 ppm (0.1 g/l). The product formulations used were either com mercially available Procter & Gamble product variants without MI (face cream, liquid hand soap, liquid laundry detergent) or products made specifically for this study based on the formulas of commercial Procter & Gamble products (shampoo and conditioner, hand dish washing liquid). All products are representative formulations for their respective product category using standard cosmetic or household care ingredients. (It is recognized that, for the product types examined in this study, formulation differences may impact the skin penetration of MI.)

The [^{14}C] MI spiked formulations were then used neat or further diluted depending on the habits & practices (H & P) data (Supplementary Table 1 Coty H & P study, HERA, 2005; SCCS, 2015) for each of the use scenarios (Table 1). All preparations for scenarios 1 6 (shampoo, conditioner, hand soap and face cream), as well as for sce narios 9 and 10 (water dilution for HRIPT), were made with 0.1 g/l MI and were used neat; For scenario 7, liquid laundry detergent rinse off,

Table 1
Application amounts of different formulations. All formulations contained 100 ppm (0.01% w/v) MI.

Scenario	Formulation	User Scenario	Occluded?	Amount product/cm^2/ application	µg MI/cm^2/application	No. of applications per day	Total µg MI/ cm^2 applied per day
1	Shampoo (S) & conditioner (C)	Consumer	Non-occluded	S = 18 mg; C = 27 mg	S = 1.8; C = 2.7	1	4.5
2	Shampoo & conditioner	Professional hairdresser	Non-occluded	S = 18 mg + C = 25-30 (C = 27 mg (x3), 30 mg (x2) & 25 mg (x1))	S = 1.8 + C = 2.5–3.0 (C = 2.7 (x3), 3.0 (x2) & 2.5 (x1))	6	27.4
3	Shampoo & conditioner	Professional hairdresser	Non-occluded	S = 9.3 mg + C = 11 mg	S = 0.93 + C = 1.1	6	12.18
4	Shampoo & conditioner	Professional hairdresser	Non-occluded	S = 9 mg + C = 9-10 mg (C = 9 mg (x11) & 10 mg (x2))	S = 0.9 + C = 0.9–1.0 (C = 0.9 (x11) & 1.0 (x2))	13	23.6
5	Hand soap	Consumer	Non-occluded	4.8 mg	0.48	5	2.4
6	Face/skin cream	Consumer	Semi-occluded	2.1 mg	0.21	2	0.42
7	Laundry detergent – hand wash	Consumer	Non-occluded	0.96 ml of a 1%v/v solution	0.96	1	0.96
8	Hand dish washing liquid	Consumer	Non-occluded	0.96 ml of a 0.15%v/v solution	0.144	3	0.432
9	Water	Single Patch test	Occluded	0.15 g	15	1	15
10	Water	Repeated Patch test	Occluded	0.15 g	15	1 application on day 1 and 1 on day3	15 per day, 30 in total

the 0.1 g/l MI detergent preparation was diluted in ultrapure water to 1% (v/v); and for the liquid dish washing soap (scenario 8), the soap containing 0.1 g/l MI was diluted in ultrapure water to 0.15% (v/v) before application on the diffusion cells. The final amount of MI in the diffusion cell was based on the amount of the neat or diluted product formulation applied in the cell.

In accordance with OECD 428 and SCCS guidelines (OECD, 2004a and b; SCCS 2010, full thickness human skin from 14 donors aged 24 59 years old (13 from Plastic Surgery Unit, St John's Hospital, West Lothian NHS Trust, Livingston, UK, and 1 from Nottingham City Tissue Bank) was used. The skin was dermatomed to a thickness of 200 400 µm using a Zimmer® electric dermatome (Zimmer, Swindon, UK). An electrical resistance barrier integrity test was performed (Fasano and Hinderliter, 2004; White et al., 2011 and White et al., 2013) and any skin sample exhibiting an electrical resistance lower than 4 kΩ was excluded from subsequent absorption measurements.

2.2. Use of skin absorption assays to determine skin exposure

Static diffusion cells (PermeGear Inc) were used and the scenarios were conducted to be compliant with OECD guideline 428 (OECD, 2004a and b). Briefly, human skin samples were placed in a diffusion cell with the stratum corneum uppermost and the skin temperature maintained at *ca* 32 ± 1 °C. The exposed surface area of the skin was 3.14 cm^2. The receptor fluid was phosphate buffered saline (sodium azide (0.01%, w/v), streptomycin (0.1 mg/ml) and penicillin (100 units/ml) added as needed). MI has a water solubility of ≥1000 g/L (SCCNFP, 2004); therefore, the receptor fluid was not considered to be rate limiting for solubility of MI under any of the test scenarios. Ten different exposure scenarios (Table 1) were investigated distributed over two studies to control complexity of the dosing and handling of the samples. For all legs 12 samples were used from 4 to 6 donors (legs 2 4: 6 donors, legs 6 and 7: 4 donors and all other legs 5 donors). The amounts applied (Table 1) on the skin in the *in vitro* experiments are intended to match single and multiple exposures to products by con sumers in home and/or professional uses. The amounts were based on conservative H & P data: For the hair care scenarios, product amounts and use frequency were based on a study done with hair care profes sionals under maximized usage conditions (Supplementary Table 1). For all other scenarios, amounts were either based on SCCS Notes of Guidance (NoG) (SCCS, 2015) or on in house H & P information. The amount applied for the face cream scenario (Scenario 5) is higher than the amount listed in the SCCS NoG but is in line with a skin/hand cream exposure scenario as described in Api et al. (2008). In order to evenly cover the skin surface in the diffusion cell about 2 mg/cm^2 were needed which resulted in the higher level applied. However, this does not im pact the study results as the applied amount (as listed in Table 1, and as CEL in Table 4) is directly compared with the measured exposure (MEL) under those conditions. This leg will hence reflect a leave on skin cream irrespective of the body site. The applied amounts are in line with the calculated consumer exposure values (CEL) as used for QRA (Table 4, CEL). To note, for hair products, the amounts applied do not consider the retention factor of 0.01 per the SCCS NoG (SCCS, 2015), as skin is rinsed during the experiments, hence the applied amounts are about 100 fold higher than the CEL which was calculated using the retention factor. For the HHC products and hand soap scenarios the products were applied and then rinsed off during the study. The CELs in Table 4 include the retention factor in the calculation to account for the rinsing steps, which result in a difference was about 100 fold.

2.3. Dosing regimens for hair care products (subsequent application of shampoo and conditioner) scenarios 1 4

Four exposure scenarios were tested: Scenario 1 represented a ty pical consumer use of a shampoo followed by conditioner, whereas scenarios 2 4 represented exposures experienced by professional hair dressers (occupational) from repeated use of shampoos and condi tioners during a working day. Scenario 1 (consumer home use) re presented a single cycle of the 95th percentile of the amount of product used. For the professional use by hairdressers, three different scenarios (scenarios 2 4) were used based on an unpublished hairdressers use study (unpublished in house data, ENIGMA, 2007a and b; 2008, see Supplementary Table 1): Scenario 2 represented the 95th percentile of the amount of product used and mean frequency of application from country with highest application rate and amount/day; Scenario 3 re presented the mean amount of product used and the mean frequency of application from the county with highest application rate and amount/ day; and Scenario 4 mimicked the 95th percentile of the amount of product used and the 95th percentile of the frequency of applications from the country with highest application rate and amount/day. A summary of the amounts applied per day for each application scenario is shown in Table 1. In the professional test legs (scenarios 2 4) higher conditioner amounts are taking into account the large amounts of a “leave on” conditioning products, which is applied to the consumer's

hair, and subsequently rinsed off by the professional.

All 4 scenarios followed the same routine but involved different doses and cycles and total exposure times. The undiluted shampoo formulation (Conservative H & P scenario as shampoo is usually applied diluted with water) was applied to the skin surface (un occluded throughout) for 4 min and then removed by rinsing ten times with 1 ml aliquots of water and drying the skin surface with tissue swabs. Five minutes after washing, the Conditioner was applied undiluted for 2 or 3 min (details see below) with the exposure terminated by rinsing with water and drying as described above. At 24 h post initial dose, each skin sample was washed and dried a final time. The cells were dismantled, the skin removed and the samples prepared and analysed as described below. Details of the scenarios are summarised below and in Table 1.

Scenario 1: Shampoo (18 mg/cm^2) and conditioner (27 mg/cm^2) for a single application. One cycle was completed to mimic home use by a consumer.

Scenario 2: Shampoo (18 mg/cm^2) and conditioner (~27 mg/cm^2 for cycles 1 3 and 30 mg/cm^2 for cycles 4 and 5, and 25 mg/cm^2 for cycle 6, mimicking use amounts of different types of conditioner, like "rinse off" conditioner, "treatment" conditioner or "leave on" condi tioner, which are rinsed off by the professional after application to the consumers' head). Exposure was terminated by washing after 2 (cycles 1 3, and 6) or 3 min (cycles 4 and 5, representing the treatment con ditions) post application. In total 6 cycles of shampoo and conditioner application were completed. After each cycle a "break" of 30 min was mimicking time of further services by the professional, with exception of cycle 3, where a 60 min "break" was mimicking a lunch break as well.

Scenario 3: Shampoo (9.3 mg/cm^2) and conditioner (~11 mg/cm^2) for 6 cycles. Exposure was terminated by washing after 2 (cycles 1 3, and 6) or 3 min (cycles 4 and 5, representing the treatment conditions) post application. A total of 6 applications per day of shampoo and conditioner were completed, with a "break" of 30 min (mimicking time of further services by the professional) after each cycle with exception of cycle 3, where a 60 min "break" was mimicking a lunch break as well.

Scenario 4: Shampoo (9 mg/cm^2) and conditioner (~9 mg/cm^2 for 11 cycles and 10 mg/cm^2 in 2 cycles). A total of 13 cycles of shampoo and conditioner application were completed, with a break of 10 min after each cycle. To mimic a typical working day of a hairdresser, all products were applied within 8 h considering typical product exposure times, washing steps, etc. as described above, plus breaks of a typical hairdresser's day (A 30 min "coffee break" was included after cycles 3 and 11 and a 60 min "lunch break" after cycle 8.)

2.4. Dosing regimens for skin care products

These scenarios represented a typical usage of each product in terms of the concentration of MI (100 ppm as allowed until recently for cosmetics), the amount of product used, and the daily frequency of usage. A summary of the amounts applied per day for each application scenario is shown in Table 1 and detailed below.

Scenario 5: Hand washing soap (4.8 mg/cm^2) used 5 times per day. This scenario mimics the soap repeated open application test (ROAT) published by Yazar et al. (2015) and the total exposure is in line with the SCCS NoG (SCCS, 2015), except that the daily amount was applied over 5 times, while the NoG applies 10 times. Applications of 5 times a day are also in line with common H & P data (Sanderson et al., 2006). After exposure for 1 min, the skin was rinsed with ultrapure water, dried, and the hand soap was applied to the skin again at 1 h, 4 h, 7 h and 8 h post initial dose. After each application (1 min) the rinsing procedure was repeated.

Scenario 6: Face/skin cream (2.1 mg/cm^2) was applied twice per day to freshly washed skin to cover the entire skin surface area in the diffusion cell. The amount applied represents a worst case scenario application, as it is higher than the amount listed in the SCCS NoG for face cream (SCCS, 2015). Diffusion cells were covered immediately after dosing with semi occlusive carbon filter traps since MI was found to be volatile over a 24 h period under these experimental conditions. After exposure for 10 h, the skin was washed with four 1 ml aliquots of aqueous 1% sodium dodecyl sulfate (mimicking face washing with soap during the day), aspirating the solution after each wash, then rinsed with four 1 ml aliquots of ultrapure water, and dried. The same face cream preparation was applied to the skin again immediately after washing (at 10 h post dose), and the occlusive carbon filter traps were replaced. At 24 h after the first application the washing procedure was repeated.

2.5. Dosing regimen for a hand wash laundry detergent

Scenario 7: Typical hand wash usage of a laundry detergent: im mersing hands in a 1% solution of a liquid laundry detergent containing 100 ppm MI, used once per day (HERA, 2005). To simulate this, 3 ml of a 1% solution of a liquid laundry detergent (in water, 0.96 ml/cm^2) was applied to the skin. After exposure for 20 min, the solution was re moved and the skin was rinsed with ultrapure water and dried. In the *in vitro* experimental design, the total amount of MI applied on the skin was 0.1 µg/cm^2.

2.6. Dosing regimen for a hand dish washing liquid

Scenario 8: Typical hand dish washing liquid diluted in a sink, used three times per day with the hands wiped off (not rinsed, simulating the most conservative hand dish washing H & P) after use. Three ml of a 0.15% solution of a hand dish washing liquid (in water, 0.96 ml/cm^2) was applied to the skin. After exposure for 20 min, the dose was re moved and the skin was dried. The same preparation was applied to the skin again at 4 h and 8 h post dose. After each application (20 min) the product was removed and the skin surface was dried again.

2.7. Dosing regimen mimicking human repeat insult patch test conditions

The NESIL for MI which was used in the QRA is based on an oc clusive HRIPT with an applied exposure of 15 µg/cm^2 (Burnett et al., 2010; Rohm and Haas, 2003). During this HRIPT, the MI solution (at 15 µg/cm^2) was applied 9 times over a 3 week period. Scenarios 9 and 10 are designed to mimic the HRIPT conditions as close as possible to determine the NESIL using the measured amount. For technical reasons, inherent to such an *in vitro* skin penetration study design, dosing could only be done for a maximum of 3 days due to skin stability. Therefore, scenario 9 was conducted with a single application and scenario 10 was conducted with an application on day 1 and a second one on day 3.

Scenario 9: Single dose, 0.15 ml/cm^2 of an aqueous solution of MI (100 ppm) was applied to the skin, and the diffusion cells were oc cluded with Parafilm® to simulate occlusive HRIPT conditions. Exposure was terminated at 24 h post dose by rinsing then drying the skin.

Scenario 10: Two patch test exposures, simulating HRIPT condi tions on days 1 and 3. 0.15 ml/cm^2 of an aqueous solution of MI (100 ppm) was applied to the skin, and the cells were occluded with Parafilm®. At 24 h post dose, the Parafilm® was removed and the skin was dried with a tissue swab. The skin was then left un occluded. At 48 h post dose, the same solution (100 ppm in water) was re applied to the skin, and the cells were occluded with Parafilm®. After a further 24 h exposure (72 h post initial dose), exposure was terminated by rinsing then drying the skin.

2.8. Post application sample collection and analysis

Pre dosing and 0.5, 1, 2, 4, 8 and 24 h post initial dosing, aliquots (200 µl or 300 µl) of receptor fluid was collected from each diffusion cell for all test scenarios except scenario 10 (which was also collected at 36, 48, 48.5, 49, 50, 52, 56, 60, 72, 84 and 96 h post initial dose) as

samples were applied twice over 3 days.

After each collection, fresh receptor fluid was added to each diffu sion cell to return them to the calibrated level. All receptor fluid sam ples were mixed with scintillation fluid (10 ml for Scenarios 2 4) or with methanol: scintillation fluid (1:5, v/v; 12 ml)) for all remaining test scenarios. At 24 h post initial dose (except for Scenario 10 which was 96 h post initial dose), each skin sample was washed. For Scenario 6 (face/skin cream) the Parafilm® was removed, and the cells for all scenarios dismantled and the skin removed. For Scenario 6, the carbon filters and traps were also retained for analysis. The skin was dried with tissue paper swabs. Residual [^{14}C] MI in the donor chamber was ex tracted using methanol. Skin wash, swabs and Parafilm® were added to the respective scintillation fluid or methanol: scintillation fluid mixes, as described above. The skin under the cell flange (unexposed skin) was cut away from the exposed skin. The SC was removed with 20 succes sive tape strips (Scotch Tape 3M). The exposed skin was then further separated into dermis and epidermis by heat separation (65 °C) or by scraping the epidermis from the dermis using a scalpel (for scenario 6 only, as the test item was volatile in this small dose volume of for mulation applied to skin (0.96 ml/cm^2)). The unexposed and exposed skin samples were solubilised with Solvable® tissue solubiliser (Perkin Elmer, Beaconsfield, UK). The radioactivity in all samples was mea sured by scintillation counting with automatic quench correction by external standard and counted for 5 min together with representative blanks (using a Packard 2100 TR liquid scintillation analyser (Perkin Elmer, Beaconsfield, UK)). Representative blank sample values were subtracted from sample count rates to give net dpm per sample. The detection limit (1 dpm above background) was 19.3 pg/cm^2 of [^{14}C] MI for receptor fluid samples in scenarios 1, 5 7, 8 10 and 2.9 pg/cm^2 of [^{14}C] MI for scenarios 2 4, calculated based on specific activity and amount of [^{14}C] MI spiked into the individual formula tions. Mass balance was calculated relative to the actual total cumula tive administered dose of [^{14}C] MI and was within 100 ± 10% of the applied dose for all skin samples used in all application scenarios.

2.9. Calculations and statistical analysis

2.9.1. In vitro skin penetration studies

The systemically bioavailable amount is the amount of a chemical (here MI) in the epidermis, dermis and RF following exposure to the skin.

The MEL, as described here however, accounts for the fact that a potential allergen that penetrates or sticks to the SC or reaches the epidermis and/or dermis is available to cause induction or elicitation. Amounts of the chemical (here MI) detected in the SC need to be added to the systemically bioavailable amounts as those amounts could sub sequently penetrate into deeper skin layers. The MEL is therefore the sum of the amount of ingredient measured in SC, epidermis, dermis and RF and is reported as ng/cm^2:

MEL = Σ (ng/cm^2 in SC + ng/cm^2 in epidermis + ng/cm^2 in dermis + ng/cm^2 in RF)

The MEL was then converted to units of μg/cm^2 per day for use in the QRA calculations.

2.9.2. Calculation of the CEL

The calculated consumer exposure level CEL (MI skin surface ex posure in μg/cm^2) as listed in Table 4 under CEL estimates were mainly derived according to calculations based on SCCS NoG or in house ha bits & practice data (shown for each scenario in Supplementary Table 2), with the exception of the higher level for face/skin cream CEL which is in line with the hand cream exposure values (Api et al., 2008). The CELs in Table 4 match the applied amount in the study, with the considerations of the retention factors as described above.

2.9.3. Skin surface exposure, general formula for beauty care products (scenarios 1 6)

CEL(μg/cm^2) = AP*CP/100*F* *1/CBSA*RS/100*1000*1000

CP = Concentration (%) of ingredient in Product; AP = Amount of Product applied per use (g); F = Frequency of product use per day; CBSA = Covered Body Surface Area (cm^2); RS = amount of product Remaining on Skin (%) (e.g., retention factor);

For all of these scenarios, CP is 0.01 (100 ppm MI in product for mulation).

2.9.4. Skin surface exposure for hand dish and laundry applications (HERA, 2005)

CEL (μg/cm^2) = F1 x C′ x F2 x F3 x F4 x 1000

C' = C x T_{der} (product load in mg/cm^2), C = mg/cm^3 (the product use concentration), T^{der} = 0.01 cm (thickness of the aqueous layer in contact with skin), F1 = weight fraction of MI in product, F2 = weight fraction transferred from medium to skin, F3 = weight fraction re maining on skin, F4 = frequency used/day.

The acceptable exposure level (AEL) for MI was derived from the NESIL of 15 μg/cm^2 and a total SAF of 100. The NESIL is based on a weight of evidence considering data from five HRIPTs and four local lymph node assays (CIR, 2014). considering the more conservative values in the HRIPT as the primary basis for the NESIL. The total SAF for each product type investigated here, was based on uncertainty factors described by Felter et al. (2002) to account for inter individual variability (a factor of 10), product use patterns (a factor of 3) and matrices (a factor of 3). (To note: For practical purposes in SAF as signments the number 3 is used, while it actually is 3.16, the half log of 10.).

The scientific basis for the SAFs used in skin sensitization QRA have been recently reviewed (Basketter and Safford, 2016).

Statistically significant differences were determined by two sample equal variance student's t test.

3. Results

3.1. Beauty care products exposure scenarios: single versus multiple applications of a shampoo/conditioner

The distribution of MI in various skin layers and the receptor fluid after exposure to different dosing scenarios of shampoo and conditioner as well as the MEL are shown in Fig. 1A. In the scenarios with multiple applications, the mean and highest numbers of cycles and product amounts per application were tested, based on data from an internal study with hairdressers in 3 EU countries (Germany, UK, Italy; Supplementary Table 1). Nearly all (95 100%) of the applied MI was removed by the washing procedure. No accumulation of MI in the SC was observed with an increasing number of application cycles or doses, ranging between 20 and 28 ng/cm^2 across all four application sce narios. Very little MI entered the RF, such that 0.03 0.13% of the ap plied dose (1.47 ng/cm^2 in scenario 1 and 18.17 ng/cm^2 in scenario 3) was recovered in this compartment (Table 2). The kinetics of the pe netration of MI into the RF is shown in Fig. 1B. For repeated application scenarios, the kinetics of penetration of MI into the RF was not statis tically different regardless of the number of application cycles or doses of shampoo and conditioner (Fig. 1B). There was an increase in the amount of MI in the skin and RF when the number of cycles was in creased from a single cycle to multiple cycles, such that the MEL in creased from 44.72 ng/cm^2 after a single cycle to 133.43 ng/cm^2 after 6 cycles of 18 mg/cm^2 shampoo followed by 27 mg/cm^2 conditioner (a 4.6 fold increase between 1 and 6 cycles). By contrast, the amount of MI recovered in the skin and RF did not increase further by increasing the

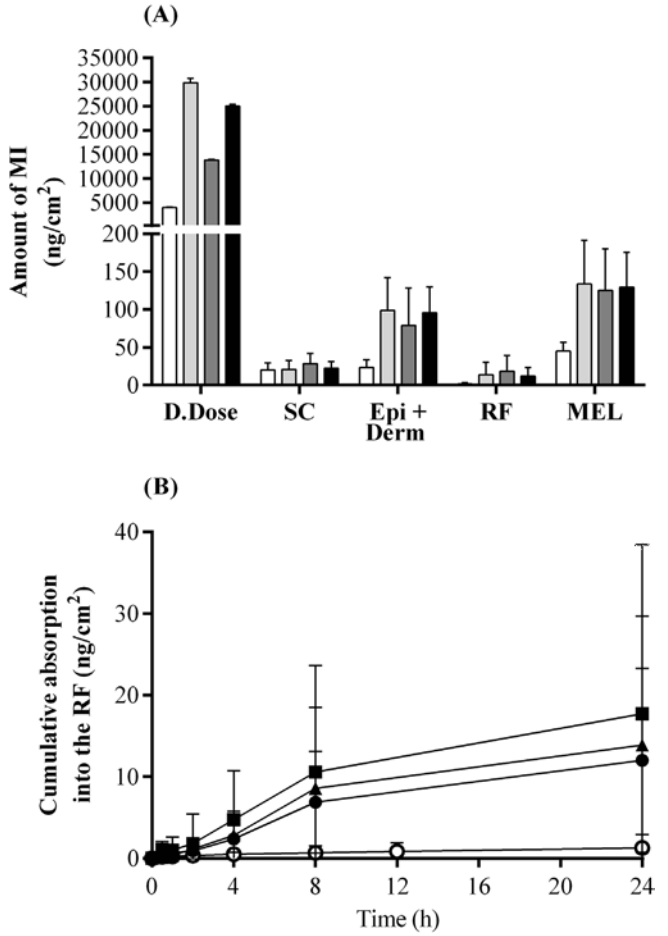

Fig. 1. (A) Distribution of MI across skin compartments (dislogeable dose (D.Dose), stratum corneum (SC), epidermis + dermis (Epi + Derm), receptor fluid (RF)) and MEL after different application scenarios of shampoo and conditioner to human *ex vivo* skin. White bars = Scenario 1; light grey bars = Scenario 2; dark grey bars = Scenario 3; and black bars = Scenario 4. Values are expressed as a mean ng/cm2 plus the SD, n = 12 skin discs from 5 donors. (B) The cumulative amount of MI in the RF over time (ng/cm2, mean ± SD) using an application Scenario 1 (○); Scenario 2 (▲); Scenario 3 (■); and Scenario 4 (●).

number of cycles from 6 to 13 (comparing Scenarios 3 and 4, in which the MEL was 125.36 and 129.68 ng/cm^2 after 6 and 13 cycles of similar application doses, respectively) (Table 2). This suggests a maximum MEL of 133 ng/cm^2 for MI in shampoo and conditioner used under the worst case exposure scenario for hairdressers (Table 2).

3.2. Comparison of rinse off and leave on beauty care products

Scenarios representing beauty care product exposure conditions for a consumer under standard H & Ps were compared: shampoo/conditioner (rinse off, single application scenario 1), hand soap (rinse off, scenario 5) and face/skin cream (leave on, scenario 6) (Fig. 2). In contrast to the two rinse off scenarios, in which most of the applied dose (98 100%) containing the MI was recovered in the skin wash (i.e. the dislodgeable dose), the amount of MI in the skin wash after 2 applications of leave on skin/face cream was much lower (19% of the applied dose, (Table 2)). Consequently, the amount of MI recovered in the epidermis/dermis and RF after 2 cycles of application of the face/skin cream was significantly higher than that recovered after the rinse off product scenarios. Interestingly, there was no reservoir effect of MI in the SC since the amount present in this layer after application of the leave on face/skin cream (25.85 ng/cm^2) was similar to the maximum amount present after application of 1 cycle (scenario 1) of shampoo and conditioner (20.4 ng/cm^2) (Table 2). Even after 6 cycles of shampoo and conditioner (scenario 3) the amount in the SC is not different (18.4 ng/cm^2). The MEL for MI in face/skin cream was 326.89 ng/cm^2 (Table 2) and was higher than that for hand soap and shampoo/conditioner applied for one cycle (15.54 and 44.1 ng/cm^2, respectively) or even after six cycles (125.36 ng/cm^2).

3.3. Household care product exposure scenarios

Two HHC product exposure scenarios were investigated, use of a liquid laundry detergent in a hand washing scenario (Scenario 7) and use of a hand dish washing liquid in a full sink washing scenario (Scenario 8). As with the other rinse off scenarios investigated, almost all the applied dose of MI was recovered in the skin wash (i.e. dislodgeable dose) at the end of the experiment (965 and 450 ng/cm^2 for the laundry detergent and for the hand dish wash liquid, respectively (Table 3). Less than 0.22% of the applied dose (as in Table 1) was present in the SC, skin or RF, resulting in a MEL of 2.1 ng/cm^2 for MI in the liquid laundry detergent scenario and 1.32 ng/cm^2 (0.29%) for MI in the hand dish wash scenario (Table 3).

3.4. Human repeat insult patch test conditions

There were two scenarios tested that were designed to investigate the exposure to MI under HRIPT conditions. In these scenarios, the solvent was water and the single MI dose, representing the NESIL (15 μg/cm^2, externally applied), was applied for 24 h once (scenario 9) or twice (scenario 10). The aim of this experiment was to determine the actual amount of MI that was available in the skin and systemic circulation when applied once or repetitively in water. In case values are measured to be < 15 μg/cm^2, the NESIL would need to be adjusted accordingly. When the consumer exposure determination is based on the bioavailable dose of MI, and not on the applied dose, and an MEL is determined, it should be compared with the bioavailable dose (and not the applied dose), which was derived under the conditions that determined the NESIL, in this case the HRIPT.

For both HRIPT patch test dosing scenarios, the majority of the applied dose was recovered in the skin wash (i.e. dislodgeable dose) (77% and 74% of the applied dose after 1 and 2 applications, respectively) (Table 3). Interestingly, the amounts of MI in the epidermis and dermis indicate saturation of the skin, because amounts recovered after one application (1277 ng/cm^2) were comparable and not statistically different from two applications (815 ng/cm^2). After a single application, 368 ng/cm^2 was recovered in the SC and 3156 ng/cm^2 was systemically available within 24 h. After 2 applications, 199 ng/cm^2 was recovered in the SC and 6994 ng/cm^2 was systemically available within 96 h. Consequently, the MEL is 3.5 μg/cm^2/24 h exposure for both scenarios. This MEL, as determined under these HRIPT conditions, can be seen as equivalent to a measured NESIL or mNESIL. Therefore, for any QRA, one should either use the NESIL of 15 μg/cm^2 based on the applied dose in the HRIPT and compare that with the CEL (AEL) or use the mNESIL of 3.5 μg/cm^2 in combination with a measured exposure (MEL) instead of the CEL which is based on an applied dose.

3.5. Predicted versus measured exposure (CEL versus MEL) and QRA calculations

From this point forward, for our refined QRA using the MEL, the mNESIL of 3.5 μg/cm^2 was used. As such, the AEL should also be adjusted to a modified AEL (mAEL) by applying the appropriate SAFs that reflect the fact that the NESIL and the consumer exposure (MEL) are now determined by measurements under relevant exposure conditions. In this case, the SAFs were slightly different from the previously used SAF of 100. The SAF of 10 for inter individual variation and the product matrix SAF of 3 remained. The use considerations SAF was assigned a value of 1 because mNESIL and the MELs were determined under

Table 2
Dermal distribution of MI 24 h post dose after single and multiple applications of rinse-off (shampoo and conditioner, hand soap) and leave-on (face cream). Values are expressed as ng/cm^2 and as % applied dose.

Compartment	Exposure scenario 1 1 application: shampoo/conditioner (4.5 µg/cm^2 MI)		Exposure scenario 2 6 applications shampoo/conditioner (27.4 µg/cm^2 MI)		Exposure scenario 3 6 applications shampoo/conditioner (12.18 µg/cm^2 MI)		Exposure scenario 4 13 applications shampoo/conditioner (23.6 µg/cm^2 MI)		Exposure scenario 5 5 applications hand soap (2.4 µg/cm^2 MI)		Exposure scenario 6 2 applications face/skin cream (0.42 µg/cm^2 MI)	
	ng/cm^2 (% applied dose)		ng/cm^2 (% applied dose)		ng/cm^2 (% applied dose)		ng/cm^2 (% applied dose)		ng/cm^2 (% applied dose)		ng/cm^2 (% applied dose)	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Dislodgeable dose	3989.46 (94.87)	77.71 (1.85)	29894 (102.92)	833 (2.87)	13840 (100.40)	196 (1.42)	25046 (99.34)	316 (1.25)	2415.52 (98.82)	16.68 (0.68)	84.52 (18.71)	48.61 (10.76)
Stratum corneum (SC)	20.02 (0.48)	9.26 (0.22)	20.68 (0.07)	12 (0.04)	28.4 (0.21)	13.51 (0.10)	22.07 (0.09)	9.12 (0.04)	5.6 (0.23)	3.14 (0.13)	25.85 (5.72)	12.89 (2.85)
Epidermis + Dermis (E + D)	23.22 (0.56)	10.56 (0.22)	98.71 (0.39)	43.33 (0.16)	78.78 (0.57)	49.74 (0.36)	95.53 (0.38)	34.24 (0.14)	9.56 (0.39)	4.44 (0.16)	205.54 (45.49)	55.45 (13.56)
Receptor Fluid (RF)	1.47 (0.03)	1.84 (0.04)	14.05 (0.05)	15.99 (0.06)	18.17 (0.13)	21.08 (0.15)	12.08 (0.05)	11.27 (0.04)	0.37 (0.02)	0.73 (0.03)	95.50 (21.14)	55.62 (12.32)
Mass balance (% Applied dose)	95.94	1.84	103.39	2.92	101.31	1.42	99.87	1.3	99.45	0.75	91.44	5.59
Systemically available fraction (E + D + RF)	24.69 (0.59)	10.11 (0.24)	112.76 (0.39)	49.98 (0.17)	96.95 (0.70)	57.63 (1.42)	107.61 (0.43)	40.97 (0.16)	9.94 (0.41)	4.35 (0.18)	301.04 (66.64)	37.62 (8.33)
MEL (SC + E + D + RF)	44.72 (1.07)	14.17 (0.32)	133.43 (0.46)	47.72 (0.17)	125.36 (0.91)	55.69 (1.42)	129.68 (0.44)	37.18 (0.15)	15.54 (0.64)	5.49 (0.22)	326.89 (72.36)	79.59 (22.21)

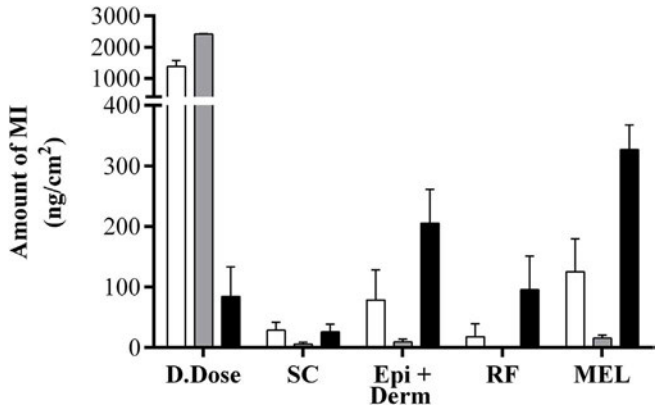

Fig. 2. Distribution of MI across skin compartments (dislogeable dose (D.Dose), stratum corneum (SC), epidermis + dermis (Epi + Derm), receptor fluid (RF)) and MEL after rinse-off (shampoo and conditioner and hand wash soap) or leave-on (face/skin cream) scenarios to human *ex vivo* skin. White bars = Scenario 3 (shampoo and conditioner); light grey bars = Scenario 5 (hand wash soap); and black bars = Scenario 6 (face/skin cream). Values are expressed as a mean ng/cm^2 plus the SD, n = 12 skin discs from 5 donors.

controlled experimental conditions without differences of skin sites or integrity (Api et al., 2008). Taking all considerations into account the overall SAF for each of the product types investigated is 30, which resulted in a mAEL of 3.5 µg/cm^2/30 = 0.12 µg/cm^2. All MEL values as listed in Table 2 in ng/cm^2 were converted to MELs in µg/cm^2 per day for use in the QRA (Table 4).

The MEL was compared to the calculated consumer exposure level estimates (CELs). For all exposure scenarios with shampoo and conditioner (single and repeated applications, Scenarios 1 4), the MEL (Table 4) was over 100 fold lower than the applied amount (Table 1, last column) as the undiluted products were applied and only rinsed off during the study based on H & P data. Furthermore, for scenario 1 and 3, the MEL is comparable to the CEL (which includes a retention factor in the calculation) and for scenarios 2 and 4, the MELs are 2.1 fold and 1.7 fold lower than the CELs. The MEL for Scenario 3, representing the mean dose and frequency of use for hairdressers, was similar to the CEL, confirming the estimated levels used for the MI QRA for shampoo and conditioner scenarios. Consequently, the ratio of the AEL/CEL and mAEL/MEL were comparable for this use condition (1.5 and 0.96, respectively). The AEL/CEL ratio for Scenarios 2 and 4 (representing scenarios with maximal dose or frequency, respectively) were less than 1, suggesting these exposure scenarios may induce skin sensitization in some individuals. However, the MELs for these scenarios, which were approximately 2 fold lower than the CEL, resulted in mAEL/MEL ratios that were similar to Scenario 3 and very close to an AEL/CEL of 1. Thus, these adjusted values based on measured exposure under maximized professional use conditions indicate that there is no difference in the likelihood of skin sensitization for hairdressers using shampoo and conditioner containing 100 ppm MI for all described scenarios with varying product usages.

For liquid laundry detergent and hand dishwashing scenarios, the MEL (Table 4) was 459 and 326 fold lower than the applied amounts (Table 1), respectively. The MELs incorporate rinsing of the applied doses in line with H & P, and hence result in approximately 5 fold lower amounts compared to the CELs for both scenarios. Therefore, the exposure assessment for liquid laundry detergent and hand dish washing liquid were overestimated using the standard calculations, resulting in AEL/CEL ratios which, while much higher than 1, were 3.5 to 4.8 fold lower than the mAEL/MEL ratios. Despite this difference in the ratios, both assessments support the use of MI at 100 ppm.

As with the laundry detergent and hand dish liquid scenarios, the rinse off scenario with hand soap demonstrated that despite 5 applications in 24 h, the overall MEL (Table 4) was 154 fold lower than the amount applied (Table 1). In this scenario, the MEL for hand soap was similar to the calculated CEL, resulting in ratios of AEL/CEL and mAEL/MEL that were in the same order of magnitude.

While the MEL for the face/skin cream leave on scenario was lower than the applied dose (or the CEL), the mAEL/MEL ratio for this application scenario was still below 1 (0.37 for mAEL/MEL and 0.28 for AEL/CEL). This suggests that under these conditions, MI at 100 ppm in a face/skin cream applied twice per day could induce skin sensitization.

4. Discussion

There are multiple studies that have investigated the skin sensitization potential of MI (referenced in SCCS, 2013; Schwensen et al., 2015; Schnuch et al., 2011; Lundov et al., 2011; Uter et al., 2012; Geier et al., 2015; Aerts et al., 2015; Latheef and Wilkinson, 2015), all

Table 3
Dermal distribution of MI 24 h post dose after rinse-off hand wash laundry detergent, hand dish washing liquid and under HRIPT conditions. The total duration of the exposure was 24 h for Scenario 7–9 and 96 h for Scenario 10.

Compartment/parameter	Exposure scenario 7 1 application Hand Wash Laundry Detergent (0.96 μg/cm² MI)		Exposure scenario 8 3 applications Hand Dish Washing Liquid (0.432 μg/cm² MI)		Exposure scenario 9 1 application HRIPT MI in water (15 μg/cm² MI)		Exposure scenario 10 2 applications over 3 days: HRIPT MI in water (30 μg/cm² MI total)	
	ng./cm² (% applied dose)		ng/cm² (% applied dose)		ng/cm² (% applied dose)		ng/cm² (% totally applied dose)	
	mean	SD	mean	SD	mean	SD	mean	SD
Dislodgeable dose	965.36 (100.56)	1.93 (0.20)	450.12 (100.03)	1.50 (0.33)	12205.83 (77.29)	1995.53 (12.64)	22391.59 (73.52)	3625.95 (11.91)
Stratum corneum (SC)	1.52 (0.16)	0.83 (0.09)	0.95 (0.21)	0.17 (0.04)	368.12 (2.33)	358.12 (2.27)	199.30 (0.65)	50.52 (0.17)
Epidermis + Dermis (E + D)	0.50 (0.06)	0.51 (0.03)	0.34 0.08)	0.16 (0.03)	1276.94 (8.09)	516.19 (2.65)	815.28 (2.68)	174.60 (0.55)
Receptor Fluid (RF)	0.09 (0.01)	0.06 (0.01)	0.03 (0.01)	0.02 (0.00)	1879.40 (11.9)	1775.34 (11.24)	6178.95 (20.29)	3264.11 (10.72)
Mass balance (% Applied dose)	100.78	0.21	100.33	0.34	100.03	1.24	97.41	1.01
Systemically available fraction (E + D + RF)	0.58 (0.06)	0.57 (0.06)	0.37 (0.08)	0.17 (0.04)	3156.34 (19.99)	2012.94 (12.75))	6994.23 (22.96)	3393.30 (11.14)
MEL (SC + E + D + RF)	2.10 (0.22)	0.976 (0.52)	1.32 (0.29)	0.24 (0.23)	3524.46 (22.32)	1883.22 (11.58)	7193.53 (23.61)	3269.17 (10.76)

Table 4
Comparison of the assessment of the potential for MI to cause skin sensitization when used in rinse-off and leave-on products using standard calculations and measured exposure values. The AEL (0.15 μg/cm²) was calculated using 15 μg/cm² as NESIL and 100 as the SAF. The mAEL was 0.12 μg/cm² and was calculated using the mNESIL of 3.5 μg/cm² and 30 as the SAF.

Scenario	In-use condition		Assessment based on standard calculations/applied dose		Assessment based on measured exposures	
		User scenario	CEL (μg/cm²/day)	AEL/CEL	MEL (μg/cm²/day)	mAEL/MEL
1	Shampoo and conditioner 18 mg + 27 mg ×1 cycle	Consumer	0.045	3.33	0.045	2.67
2	Shampoo and conditioner 18 mg + 25-30 mg ×6 cycles	Professional hairdresser	0.28	0.54	0.133	0.90
3	Shampoo and conditioner 9.3 mg + 11 mg ×6 cycles	Professional hairdresser	0.10	1.50	0.125	0.96
4	Shampoo and conditioner 9 mg + 9-10 mg ×13 cycles	Professional hairdresser	0.22	0.68	0.130	0.92
5	Hand soap, ×5 cycles	Consumer	0.0233	6.44	0.016	7.5
6	Face/skin cream, ×2 cycles	Consumer	0.4200	0.28	0.327	0.37
7	Hand Wash Laundry Detergent ×1 cycle	Consumer	0.0100	15.00	0.002	60
8	Hand Dish Washing Liquid, ×3 cycles	Consumer	0.0045	33.33	0.001	120

indicating a high risk for skin sensitization when consumer exposure to MI is above threshold levels and reporting a high incidence of MI sensitized individuals. Therefore, the thorough application of a QRA to all consumer products for sensitizing ingredients is of utmost im portance. Kimber et al. (2017) stated “Accordingly, use of skin sensi tization QRA is encouraged, not least because the essential feedback from dermatology clinics can be used as a tool to refine QRA in situa tions where this risk assessment tool has not been properly used”.

Investigations using the previous maximum amount of MI permitted by EU regulations (Cosmetic Directive Annex V, entry 57, European Commission No 1223/2009), 100 ppm, in cosmetic rinse off products (e.g. shampoo and conditioner) illustrate how to integrate a detailed understanding of consumer and occupational H & P into an experi mental model to measure skin exposure and to inform the QRA. The skin exposure experiments were carefully designed to mimic typical and maximized consumer and occupational use conditions and to determine how these affect the measured exposure (*i.e.*, the MEL) to 100 ppm MI used in different cosmetics and HHC products. A mass balance of 100 ± 10% for all test legs of the exposure study, including those with repeated product application and rinsing steps, demonstrated the ro bustness of the *in vitro* test system used (OECD 428 2004a and b, SCCS, 2010).

These *in vitro* skin exposure studies investigating the MEL of MI present in shampoo and conditioner indicate that determining the total daily exposure using standard additive calculation methods may not be accurate and tend to overestimate realistic exposure levels. During the studies, the shampoo and conditioner was applied undiluted to the skin and only rinsed after a few minutes, which led to a higher concentration of product on the skin as compared to a normal use practice where product is immediately diluted with water. Therefore, measured ex posure levels as shown here are anticipated to be a good reflection, if not conservative, of the actual exposure. Although there was an in crease in the measured exposure between a single and 6 cycles of shampoo and conditioner use, there was no further increase in the MEL when more than 6 cycles or a higher dose of both were applied. Moreover, the MEL for repeated application scenarios was not propor tional to the dose or number of cycles; thus, the assumption that ap plication of larger amounts of product or a higher frequency of use leads to an additive dermal exposure is not supported by these ex periments, probably as a result of the efficient removal of MI after each dose application. Fig. 3 shows the impact of incorporating the measured exposure to MI in a shampoo and conditioner after single and multiple cycles in one day (scenario 1 4) and the patch test procedure used to derive the mNESIL for use in the QRA. When the associated exposures

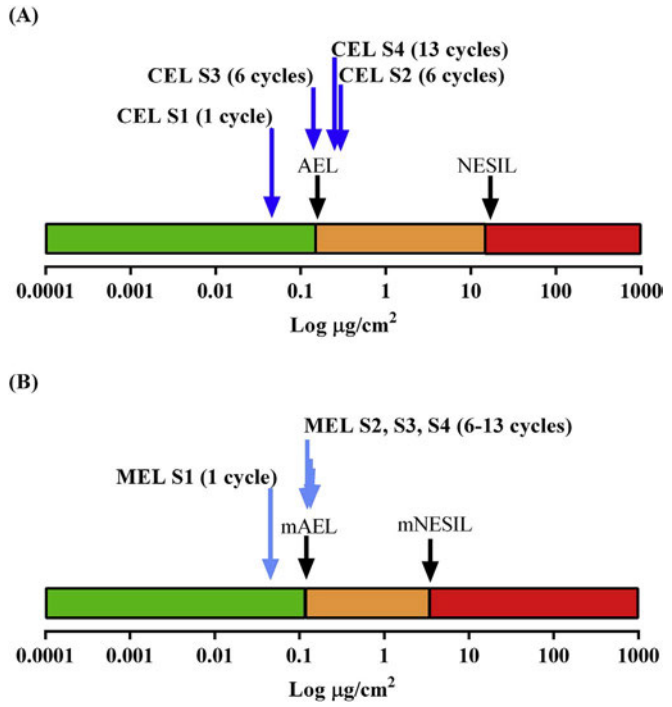

Fig. 3. Quantitative comparison of exposure doses for skin sensitization induction and shampoo and conditioner product single and multiple usage per day. Logarithmic comparison of the sensitization induction potency indicated as NESIL, AEL and the CEL (A) or the mNESIL, mAEL and MEL based on measured exposures. Red bar: exposure considered to be associated with induction of skin sensitization, green bar: exposure not considered being associated with the induction of skin sensitization, orange bar: uncertainty of the values, represented by a SAF of 100 for (A) and 30 for (B). Different application scenarios are shown from a single use (Scenario 1 (S1)) to multiple use (Scenarios 2, 3 and 4 (S2, S3 and S4)). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

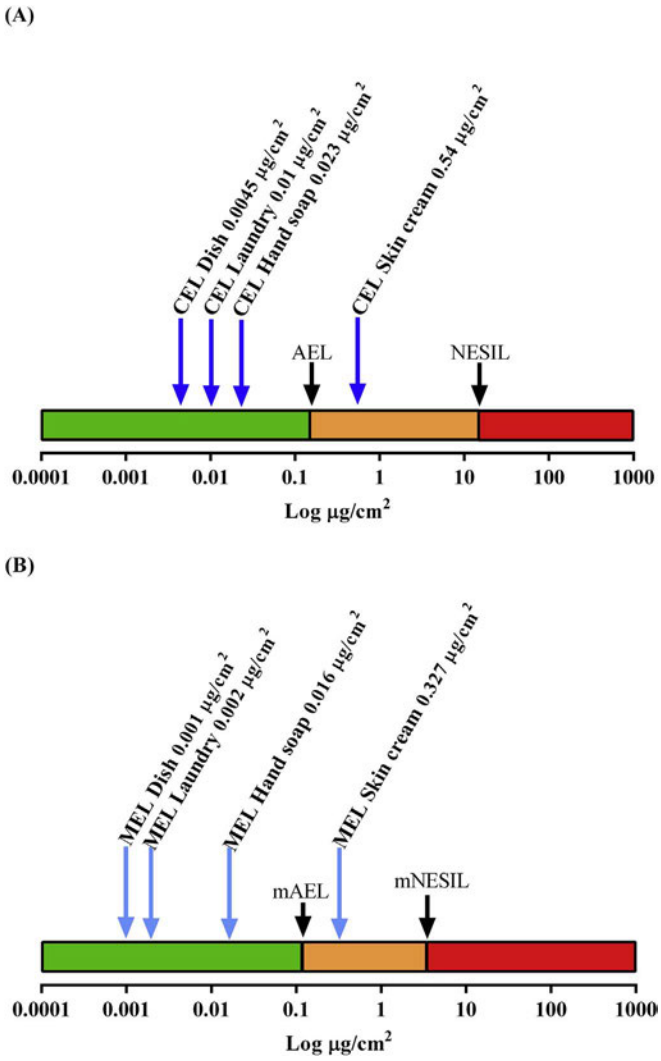

Fig. 4. Quantitative comparison of exposure doses for skin sensitization induction and laundry detergent product hand wash usage, hand dish washing liquid product usage, liquid hand soap product usage and face/skin cream (leave-on) product usage. Logarithmic comparison of the sensitization induction potency indicated as NESIL, AEL and the CEL (A) or the mNESIL, mAEL and MEL based on measured exposures. Red bar: exposure considered to be associated with induction of skin sensitization, green bar: exposure not considered being associated with the induction of skin sensitization, orange bar: uncertainty of the values, represented by a SAF of 100 for (A) and 30 for (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

are calculated (CEL) based on the applied amount, the exposure to MI in shampoo was below the NESIL of 15 µg/cm^2 and the AEL of 0.15 µg/cm^2 (Fig. 3A, CEL Scenario 1 (S1 1 cycle)). This suggests that induction of skin sensitization would not occur during usage at a concentration of 100 ppm. When the measured exposure (MEL) was used in this assessment, mAEL and MEL are equivalent (Fig. 3B MEL S1 (1 cycle)), confirming the exposure and supporting the QRA indicating that the product is not likely to induce skin sensitization. Taking this further, these experiments also showed that complex exposure scenarios experienced by hairdressers as part of their daily occupational exposure to MI can be recapitulated in an *in vitro* experimental set up. This refinement of the exposure assessment for increased application frequencies or higher doses of shampoo and conditioner containing 100 ppm MI better reflects the actual exposure and it does not indicate an increased skin sensitization induction risk under the applied rinse off applications (Fig. 3B, MEL S2, S3 and S4).

As with shampoo and conditioner usage scenarios, the MEL for MI in HHC products is extremely low compared to the measured exposure under patch test conditions (1 2 µg/cm^2 vs. 2248 3359 µg/cm^2, respectively). The impact of measuring the exposure to MI in hand wash laundry and hand dish washing under use conditions is illustrated in Table 4 and Fig. 4, respectively. The ratio of the mAEL/MEL is approximately 3 fold larger than the AEL/CEL. For hand soap, the AEL/CEL ratio is comparable to the mAEL/MEL ratios (Table 4; Fig. 4). All three assessments indicate that under the evaluated use conditions these products containing MI at 100 ppm result in low skin exposures, which are probably unlikely to induce skin sensitization.

The scenario of repeated hand soap applications also mimicked the daily application conditions used in a ROAT conducted by Yazar et al. (2015) using 19 MI allergic subjects and 19 controls without MI allergy. Ten of the 19 MI allergic patients used a hand soap containing 100 ppm MI and 9 used a hand soap containing 50 ppm MI. The results from that ROAT identified levels of MI exposure that are tolerated by MI allergic patients when exposed repeatedly. Yazar et al. applied a dose of 0.48 µg/cm^2 per application 5 times per day (total daily dose of 2.4 µg/cm^2), which is in line with the amount applied in the study on one day. All 10 subjects (100%) sensitized to MI suffered from allergic contact dermatitis (ACD) reactions that were elicited by a hand soap containing 100 ppm MI. Seven out of 9 MI allergic subjects (78%) suffered from ACD elicited by the soap containing 50 ppm MI. No positive reactions occurred in the 19 control subjects who used the soap containing 100 ppm MI over the 21 days of the test. The results of this study indicated that MI in rinse off soap at concentrations of either 100 ppm or 50 ppm was capable of eliciting reactions in MI allergic individuals. The MELs determined in the hand soap scenario 5 can also provide some insights on levels to which MI sensitive people clearly elicited an allergic response after repeated exposures. The MEL for the

use of a hand soap with 100 ppm of MIT is 0.016 μg/cm^2/day. While the MEL for a product with 50 ppm was not measured, it can be assumed to be lower. Hence, the elicitation threshold can be estimated to be lower than 0.016 μg/cm^2/day but no exact value can be derived.

The MELs determined in this study for the BC scenarios are higher than 0.016 μg/cm^2/day while the two HHC scenarios resulted in MELs < 0.016 μg/cm^2/day. It cannot be excluded that also single or repetitive shampoo/conditioner use, or a face/skin cream usage would elicit skin reactions in MI sensitive consumers. Whether pre sensitized consumers could safely use a dish or a hand wash product, which have the lowest MELs cannot be determined with certainty, but the consumers might be able to tolerate them better than e.g. hand soap products.

Measured exposure of MI from our studies support the hypothesis that use of a soap containing 100 ppm MI would fail to induce skin sensitization in non allergic individuals since the MEL is lower than the mAEL (Table 4; Fig. 4). However, under the ROAT study conditions the lack of responses in the non MI allergic control subjects does not allow a conclusion about the risk of induction from the use of MI containing hand soaps and cannot confirm or reject the hypothesis.

Lundov et al. (2011) also tested MI in a ROAT, simulating a skin cream exposure (Api et al., 2008) applying it at 0.21, 0.11 and 0.01 μg/cm^2 twice per day for 3 weeks to 11 MI allergic patients. Seven out of 11 subjects reacted to the highest dose of MI (0.21 μg/cm^2) and the same 7 also reacted to the middle dose (0.11 μg/cm^2). Two reacted to the lowest dose (0.01 μg/cm^2). The daily doses applied are 0.42, 0.22 and 0.02 μg/cm^2/day respectively which is in line with the daily amount applied for the skin cream during the study (Table 4, last column). The measured values in our study for MI from skin cream application showed a good correlation between the applied doses and the MELs, and we can hypothesise that MEL levels in this ROAT would also be in the same order of magnitude as the applied amounts. As some panelists still reacted to the lowest dose (~0.02 μg/cm^2/day) this study also confirms that the elicitation threshold is somewhere below the 0.016 μg/cm^2/day, indicating that a leave on skin cream, even with lower amounts of MI could probably not be tolerated by pre sensitized consumers.

The novel experimental design applied provides measured exposure levels, which are in line with consumer exposure estimates for QRA determined by the standard exposure estimation procedures. The measured data demonstrated good reliability of existing exposure calculation models and can be used to confirm and refine a QRA. The exposure models remain conservative due to missing algorithms considering amounts remaining on or in skin after short exposure times and or skin saturation effects. Skin measurements as conducted here, can circumvent the current limitations of modelling or estimating total exposure from the sum of applied doses or individual product experiments.

The study methodology used here is not specific for MI in the simulated exposure scenarios. It was used previously to examine exposure to hair dyes under hair dye use conditions. We have used the skin penetration study protocol and modified it to simulate various consumer exposure scenarios, but the study design is not limited to those presented here. The critical step is the translation of a consumer habit into an *in vitro* study design tying to simulate as close as possible the consumer exposure conditions *in vivo*. One could also envision designing a study for simple aggregate exposure measurements. While the choice of the chemical to be evaluated is mainly limited by the detection limit for the material in the study, a similar study could be designed for e.g. other preservative materials.

In conclusion, the exposure measurements clearly show a large difference in MI levels relevant for skin sensitization depending on consumer use conditions and the product matrix used. The amounts measured showed that under certain conditions the induction of skin sensitization cannot be excluded and that levels of 100 ppm MI in a leave on product (as shown in scenario 6) cannot be supported. The measurements also show that for rinse off products, also considering repeated exposures, the levels are lower than the MI induction threshold indicating a low likelihood for the induction of skin sensitization in consumers. Household care products show a significantly lower exposure compared to cosmetic rinse off products and based on our results are not expected to be a relevant contributing factor in the induction of skin sensitization. In general the generation of MEL values is recommended as a refinement step of default product exposure values to support the QRA in cases where more realistic exposure information is needed.

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2017.10.024.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.yrtph.2017.10.024.

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Abbreviations:

AEL: Acceptable Exposure Level
AP: Amount of Product applied per use (g)
BC: Beauty Care
CBSA: Covered Body Surface Area (cm2)
CEL: calculated Consumer Exposure Level
CF: Conversion Factor (mg/g)
CP: Concentration (%) of ingredient in Product
D: Dilution factor (%)
F: Frequency of product use per day
H & P: Habits & Practice (H & P)
HHC: HouseHold Care
HRIPT: Human Repeat Insult Patch Test
LLNA: Local Lymph Node Assay
MEL: Measured Exposure Levels
MI: Methylisothiazolinone
NESIL: No Expected Sensitization Induction Level
QRA: Quantitative Risk Assessment
RF: Receptor Fluid
ROAT: Repeated Open Application Test
RS: amount of product Remaining on Skin (%) (e.g., retention factor)
SAF: Sensitization-Assessment-Factors
SC: Stratum Corneum
T^{der}: Thickness of the aqueous layer in contact with skin