

SysDEA:
Systematic analysis of dermal exposure to hazardous chemical agents at the workplace

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Research
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R. Franken
S. Spaan
K. Kasiotis
A. Tsakirakis
I. Chartzala
D. Nikolopoulou
P. Anastasiadou
A. Snippe
E. Schoen
J. Baan
R. Engel
J. Turkenburg
K. Machera
R. Gerritsen-Ebben

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Authors:

Remy Franken, MSc (TNO)
Suzanne Spaan, PhD (TNO)
Konstantinos Kasiotis, PhD (BPI)
Angelos Tsakirakis, PhD (BPI)
Ilianna Chartzala, MSc (BPI)
Dimitra Nikolopoulou, PhD (BPI)
Pelagia Anastasiadou, MSc (BPI)
Almar Snippe, Ir (TNO)
Eric Schoen, Dr. (TNO)
Jan Baan, Ir (TNO)
Roel Engel, Ing (TNO)
Jaap Turkenburg (TNO)
Kyriaki Machera, PhD (BPI)
Rianda Gerritsen-Ebben, MSc (TNO)

TNO – Location Zeist
Utrechtseweg 48, NL-3704 HE Zeist

Benaki Phytopathological Institute (BPI)
8 Stefanou Delta Street, Kifissia, Athens, 14561

Cover figures:

Ilianna Chartzala, Pelagia Anastasiadou, Angelos Tsakirakis
Benaki Phytopathological Institute

Cover design:

Milena Marano
Federal Institute for Occupational Safety and Health

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Federal Institute for Occupational Safety and Health
Friedrich-Henkel-Weg 1 – 25, 44149 Dortmund, Germany
Postal address: Postbox 17 02 02, 44061 Dortmund, Germany
Telephone +49 231 9071-2071
Fax +49 231 9071-2070
Email *info-zentrum@baua.bund.de*
Web *www.baua.de*

Berlin: Nöldnerstraße 40 – 42, 10317 Berlin, Germany
Telephone +49 30 51548-0
Fax +49 30 51548-4170

Dresden: Fabricestraße 8, 01099 Dresden, Germany
Telephone +49 351 5639-50
Fax +49 351 5639-5210

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Contents

SysDEA: Systematic analysis of dermal exposure to hazardous chemical agents at the workplace

Abstract

The overall objective of the SysDEA study is to generate scientific knowledge for improvement and standardization of measurement methods for dermal exposure to chemicals at the workplace. To this end, five different tasks (transfer, spreading, spraying, handling immersed objects, and handling contaminated objects) were performed with three different product types: a dusty powder (solids) and high viscosity (HV) and low viscosity (LV) liquids. The investigated exposure situations (product-task combinations) were: dumping powder, pouring LV and HV (transfer), rolling LV and HV (spreading), surface spraying LV and HV (spraying), manually handling objects immersed in LV and HV (immersion/dipping) and handling objects contaminated with powder. The measurement methods investigated were: whole body dosimeter (coverall) versus patches for body exposure, gloves versus hand wash for hand exposure, and head bands versus head wipes for head exposure. In addition, a fluorescence method was used for all body parts. Each of these exposure situations was performed four times by four different test subjects each for all of the three different measurement methods (including body, hand and head exposure). In total 320 individual experiments were performed.

Statistical analysis of the measurement results led to the following results:
For body exposure, the patch method resulted in higher measured exposures than the use of overalls for exposure situations with liquids, except for rolling. No significant difference was found for powders.
For hand exposure, significantly higher exposure values were measured with the glove method for rolling and manually handling objects with liquids. For spraying and pouring, also higher values were measured with the glove method compared to the hand washing method, but these differences were not statistically significant. In the case of exposure situations with powders, the glove method resulted in significantly higher exposure values for handling contaminated objects, but not for dumping powders.
For head exposure, the wipe method resulted in higher values than the headband method, except for spraying and rolling, where no significant difference was observed.
Estimates of body exposure using the fluorescence method resulted in severely lower exposure values compared to the methods based on chemical analysis for both liquids and powders.

Key words:

dermal exposure, dermal measurement methods, exposure factors, workplace exposure

SysDEA: Systematische Untersuchung der dermalen Exposition gegenüber Gefahrstoffen am Arbeitsplatz

Kurzreferat

Das übergeordnete Ziel der SysDEA-Studie ist es, wissenschaftliche Erkenntnisse zur Verbesserung und Standardisierung von Messmethoden für dermale Exposition gegenüber Chemikalien am Arbeitsplatz zu gewinnen. Hierzu wurden fünf verschiedene Tätigkeiten (Transfer, Ausbringung, Sprühen, Handhabung eingetauchter Objekte, Handhabung kontaminierter Objekte) mit drei verschiedenen Produkttypen durchgeführt: einem staubigen Pulver, sowie hochviskosen (HV) und niedrigviskosen (LV) Flüssigkeiten. Die untersuchten Expositionssituationen (Produkt-Tätigkeits-Kombinationen) waren: Umfüllen von Pulver und Umgießen von LV und HV (Transfer), Rollen von LV und HV (Ausbringung), Sprühen von LV und HV (Sprühen), Handhabung von in LV und HV eingetauchten Objekten (Eintauchen) und Handhabung von mit Pulver kontaminierten Objekten. Die untersuchten Messmethoden waren: Ganzkörper-Dosimeter (Overall) versus Patches für Körperexposition, Handschuhe versus Handwäsche für Handexposition, sowie Kopfbänder versus Stirnabwischen für Kopfexposition. Darüber hinaus wurde für alle Körperstellen eine Fluoreszenzmethode angewendet. Jede dieser Expositions-situationen wurde insgesamt viermal von vier Probanden für alle drei verschiedenen Messmethoden (bei denen Körper-, Hand- und Kopf-Exposition einbezogen wurden) durchgeführt. Insgesamt wurden so 320 Einzelexperimenten durchgeführt.

Die statistische Auswertung der Messergebnisse lieferte folgende Ergebnisse:
Bei der Körperexposition führt die Patch-Methode zu höheren gemessenen Expositionen, als die Verwendung von Overalls für Expositionssituationen mit Flüssigkeiten, außer beim Rollen. Für Pulver wurde kein signifikanter Unterschied festgestellt.
Für die Handexposition wurden mit der Handschuhmethode beim Rollen und Handhaben von Gegenständen mit Flüssigkeiten deutlich höhere Expositionswerte gemessen. Für Sprühen und Gießen wurden mit der Handschuhmethode ebenfalls höhere Werte im Vergleich zur Handwaschmethode gemessen, aber diese Unterschiede waren statistisch nicht signifikant. Im Falle von Expositionssituationen mit Pulvern liefert die Handschuhmethode deutlich höhere Expositionswerte beim Umgang mit kontaminierten Objekten, nicht aber beim Umschütten.
Bei der Kopfexposition führte die Wischmethode zu höheren Werten als die Kopfbändermethode, mit Ausnahme von Sprühen und Rollen, wo kein signifikanter Unterschied festgestellt wurde.
Die Abschätzung der Körperexposition mit der Fluoreszenzmethode führte zu deutlich geringeren Expositionswerten, als die Methoden, die auf chemischen Analysen beruhen, sowohl für die Expositionen gegenüber Flüssigkeiten als auch gegenüber Pulver.

Schlagwörter:

dermale Exposition, dermale Messmethoden, Expositionsfaktoren, Arbeitsplatz-exposition

1 Introduction

1.1 Background information

Assessment of dermal exposure originates from the field of assessment of exposure to pesticides. However, the relevance of dermal exposure for industrial chemicals was also recognized, resulting in a boost of conceptual, methodological and field investigations, for instance the development of a conceptual model on dermal exposure (Schneider et al., 1999). Furthermore, a Working Group on Dermal Exposure Assessment (WG 6) within the CEN TC 137 was founded. With respect to the standardization of dermal exposure assessment methodology the CEN working group published two standardization documents: NPR-CEN/TR 15278 (exposure assessment strategy), and NPR-CEN/TS 15279, (measurement principles and methods). These were merged into the ISO report ISO/TR 14294:2011 (Workplace atmospheres - Measurement of dermal exposure - Principles and methods).
Over the last 10 to 15 years assessment of dermal exposure and development and validation of dermal exposure models for pesticides and industrial chemicals has been an issue for both, research and regulations. In general, the development of dermal exposure modelling is hampered by scarcity of sound data and limited possibilities for pooling data due to different methods used to generate the data. Exposure studies sometimes addressed a comparison of measurement methods, however, focused studies on the performance of measurement methods are scarce. Recently, Gorman Ng et al. (2014) published a relatively small scaled and controlled study were three dermal exposure sampling methods were compared. A comprehensive study focusing on the generation of scientific knowledge as a basis for an improvement and a standardization of measurement methods for dermal exposure to chemicals at the workplace, has not yet been performed.
In principle, three sampling principles are distinguished (see Table 1.1). Each sampling principle shows a diversity of methods, each with degrees of freedom for selection of substrate, solvent, tracer, body location, etc. Different measurement principles will result in inherent different measurement results, representing so called potential exposure mass, estimation of the actual exposure mass. Interception methods, and especially whole body method/outer cloths and patches, theoretically capture all contaminant en route to the body, so all potential exposure. The spatial and temporal resolution is depending on the number of subsamples taken from first, a surface and second, in time. Key elements are the collection efficiency and the ability to retain the contaminant over the sampling period. Actual exposure is mimicked by extraction of a second layer of clothing, e.g. long-sleeved T-shirt, long trousers, worn underneath (protective) clothing. Removal methods estimate the amount of contaminant that has reached the skin either directly or by penetration or permeation through the (protective) clothing and was not redistributed, absorbed by the skin, or removed otherwise (“actual exposure”). Usually these type of methods are used for those body parts that are usually not covered by normal (work) clothing, e.g. hands, wrist, fore head or the neck. The spatial and temporal resolution is depending on the number of subsamples taken from first, a body surface and second, in time, however, the barrier function of the skin can be disrupted by (frequent) sampling. Critical issues here are the removal efficiency in relation to the time of residence (interval between contamination and removal), where properties of the contaminant are important parameters, e.g. volatility, permeation/penetration and

adherence to skin layers. In situ methods do not need sampling, thus actual exposure can be determined without sampling losses. In addition, high spatial and temporal resolution can be obtained. However, like for other methods, the efforts related to the measurements will be a limiting factor. In case a visualization method is used, e.g. a fluorescent tracer in combination with UV irradiation quantification of exposure (i.e. the amount of tracer) will be achieved by quantification of the intensity of the emitted light by the tracer after radiation. Usually these type of methods are used for those body parts that are not covered by normal (work) clothing, e.g. hands, wrist, forehead, V of the neck. Critical issues are calibration and interaction of the contaminant or tracer with the skin and the limited dynamic range for quantification. In case of a tracer, the comparability of the tracer behavior in the exposure process and a contaminant can be an issue. A schematic overview of most commonly used methods with regard to their application for various body parts is presented in Table 1.2.

Table 1.1 Sampling and measurement methods for dermal exposure assessment

Sampling principle	Method	Estimates
Interception Interception of agent mass transport by the use of collection media placed at the skin surface or covering (or replacing) work clothing during the sampling period	Media (substrates) - Whole body (coverall / clothing) - Gloves - Patches	Exposure mass (in g or g/cm²)
Removal Removal of the agent mass from the skin surface, the skin contaminant layer, at any given time	Manual wipe; dry or wetted Tape-stripping Hand wash Hand rinse	Exposure loading (in g/cm²)
In situ Direct assessment of the agent or a tracer at the skin surface, for example by image acquisition and processing systems at a given time. No actual sampling takes place	Detection of UV/fluorescence of agent or added tracer as a surrogate for the agent by video imaging; Attenuated Total Reflection ATR-FTIR, or using a light probe	Exposure loading (in g/cm²) and surface area contaminated (in cm²)

Table 1.2 Overview of sampling methods and application for different body parts

Principle	Method *	Body part **							
		hands	head	V-neck	torso	UA	FA	UL	LL
Interception	Gloves	+	-	-	-	-	(+)	-	-
	Coverall	-	-	-	+	+	+	+	+
	Underwear (incl. long pants, long sleeved T-shirt)	-	-	-	+	+	+	+	+
	Headband	-	+	-	-	-	-	-	-
	Patches	(wrist)	+	+	+	+	+	+	+
Removal	Wash	+	-	-	-	-			
	Rinse	+	-	-	-	-	(+)	-	-
	Wipe	+	+	+			+	-	-
	Tape strip	+	+	+	-?	+	+	-	-
In situ	Natural fluorescence (probe)	+	+	+	-	-	(+)	-	-
	FWA Tracer + Video imaging	+	+	+	***	***	+	+	+
	FWA Tracer + UV probe	+	+	+	-	-	(+)	-	-

* FWA=fluorescent whitening agent
** UA=upper arm, FA=forearm, UL=upper leg, LL=lower leg
*** Usually not applied / T-shirt

1.2 Goal and objectives of the study

The overall objective of the study is to generate scientific knowledge that forms the basis for improvement and standardization of measurement methods for dermal exposure to chemicals at the workplace.
By performing well-designed experiments to be able to perform tasks in a reproducible way and thereby reducing the variability in outcomes due to variation in behavior and environmental conditions, the data gathered can be used to investigate the following specific objectives:

- Description of the advantages and disadvantages of each of the measurement methods;
- Identification of the best method to be applied for specific tasks;
- Investigate the differences in measured dermal exposure outcomes between different measurement techniques;
- Investigate to which extend the results of the different measurement methods can be converted to one another by means of conversion factors;

1.3 Structure of the report

In the following chapters of this report the basic elements of the SysDEA project are depicted. Initially, in chapter 2 the study design is provided, which entails an overview of the experimental design, including the choice of exposure situations, products, and measurement methods included in the study, as well as the final work plan, protocols and standard operating procedures that were used during the course of this study.
Then in chapter 3, the preparation of the experimental work is presented, which constitutes the backbone of the SysDEA project. Hence, test substances and test formulations considered are presented, and details are provided with regard to the preparation of all formulations used in the experiments. Furthermore, a detailed report on the considered and selected matrices is provided in relation to the substance(s) and formulations and measurement methods. Also a description of the test location and UV set-up used for the fluorescence part is provided to facilitate the understanding of the overall experimental progress. In addition, the recruitment of the volunteers is described, followed by the development of some of the standard operating procedures and the development of the final protocols for each of the exposure situations.
In chapter 4, the validation of the analytical method is reported, including criteria and metrics, in which also the field spiking is incorporated. Consequently, in chapter 5 the description of the development of the fluorescent tool is provided, followed by a description of the execution of the experimental work in chapter 6. The statistical comparison and evaluation of the dermal exposure data as collected during the study is described in chapter 7, and finally the report ends with a discussion in chapter 8 and a conclusion in chapter 9.

2 Study design

2.1 General study design

In table 2.1 an overview of the general experimental design is presented. This design includes five different task groups and three different products/substances. The main focus of this design is an efficient comparison of the measurement methods, thus optimizing the number of experiments needed and the analytical effort needed.
This design leads to a total of 320 individual experiments, in which body, hand and head exposure are included.

Table 2.1 Overview general experimental design

Task group	Product	Specific task	Dermal exposure measurement methods *				# volunteers	# repeats	#experiments
			Body	Hands **	Head **	Fluo res-cen ce			
A. Transfer	A1. Dusty solid	Dumping	Cotton coverall	Gloves	Headband	Yes	4	4	16
			Cotton patches	Wash	Wipe	Yes	4	4	16
	A2. Liquid - low viscosity	Pouring	Tyvek coverall	Gloves	Headband	Yes	4	4	16
			Tyvek patches	Wash	Wipe	Yes	4	4	16
	A3. Liquid - high viscosity	Pouring	Tyvek coverall	Gloves	Headband	Yes	4	4	16
			Tyvek patches	Wash	Wipe	Yes	4	4	16
B. Sprea-ding	B1. Liquid - low viscosity	Rolling	Tyvek coverall	Gloves	Headband	Yes	4	4	16
			Tyvek patches	Wash	Wipe	Yes	4	4	16
	B2. Liquid - high viscosity	Rolling	Tyvek coverall	Gloves	Headband	Yes	4	4	16
			Tyvek patches	Wash	Wipe	Yes	4	4	16
C. Spraying	C1. Liquid - low viscosity	Surface spraying	Tyvek coverall	Gloves	Headband	Yes	4	4	16
			Tyvek patches	Wash	Wipe	Yes	4	4	16
	C2. Liquid - high viscosity	Surface spraying	Tyvek coverall	Gloves	Headband	Yes	4	4	16
			Tyvek patches	Wash	Wipe	Yes	4	4	16
D. Handling of immersed objects	D1. Liquid - low viscosity	Manually handling immersed objects	Tyvek coverall	Gloves	Headband	Yes	4	4	16
			Tyvek patches	Wash	Wipe	Yes	4	4	16
	D2. Liquid - high viscosity	Manually handling immersed objects	Tyvek coverall	Gloves	Headband	Yes	4	4	16
			Tyvek patches	Wash	Wipe	Yes	4	4	16

Task group	Product	Specific task	Dermal exposure measurement methods *				# volunteers	# repeats	#experiments
			Body	Hands **	Head **	Fluo res-cen ce			
E. Handling of contami-nated objects	E. Dusty solid	Handling contami-nated objects	Cotton coverall	Gloves	Headband	Yes	4	4	16
			Cotton patches	Wash	Wipe	Yes	4	4	16

* Throughout this report, the combination of measurement methods for body, hands and head as applied during the experiments will be indicated as the **WBD method** (coverall / headband / gloves) and **patch method** (patches / head wipe / hand wash).
** During all experiments cotton gloves and cotton headbands were used for monitoring.

2.2 Exposure situations

In table 2.2 the measured exposure situations are presented with the relevant test substances and the parameters considered in the experimental setting. In total ten different exposure situations have been measured. It has been demonstrated that both within- and between-person variances of dermal exposures are substantial. From that perspective it is considered important to have a certain number of persons performing the tasks and a certain number of repeats per person with regard to statistical significance. Therefore, it is proposed to include little variation in and over parameters (determinants of dermal exposure) within the exposure situations. For each of these exposure situations, a fixed set of parameters leading to (assumed) maximal dermal exposure under practical experimental conditions that are considered representative for a relatively large group of tasks performed on a regular basis are chosen.

Table 2.2 Measured exposure situations with relevant test substances and parameters considered in the experimental setting

Task group	Product / substance	Specific task	Key parameter(s)	Experimental set-up
A: Transfer	A1: Dusty solid	Dumping	Amount used, dropping height	Amount used (1 kg, six repetitive dumpings). From 20 L capacity bin at table (50 cm height) into another 20 L capacity bin at other table (50 cm height) Distance between tables is 100 cm and dumping height is approximately 15-25 cm above rim (opening)

Task group	Product / substance	Specific task	Key parameter(s)	Experimental set-up
	A2: Liquid - low viscosity	Pouring	Amount used, container size	Amount used (i.e. 10 L). Manual pouring from small neck container via 1 L jug in vessel on another work bench. Average work bench height (100 cm) and distance between work benches is 300 cm
	A3: Liquid - high viscosity	Pouring	Amount used, container size	Amount used (i.e. 10 L). Manual pouring from small neck container via 1 L jug in vessel on another work bench. Average work bench height (100 cm) and distance between work benches is 300 cm
B: Spreading	B1: Liquid - low viscosity	Rolling	Use rate, surface area treated, direction application	Rolling flat surface (both sides of door; 3.5 m^2) up- and downward directions, fixed use rate
	B2: Liquid - high viscosity	Rolling	Use rate, surface area treated, direction application	Rolling flat surface (both sides of door; 3.5 m^2) up- and downward directions, fixed use rate
C: Spraying	C1: Liquid – low viscosity	Surface spraying	Spray technique, spray direction(s), surface, surface area	Spray pressure, smooth surface - both sides of door; 3.5 m^2; up- and downward directions
	C2: Liquid – high viscosity	Surface spraying	Spray technique, spray direction(s), surface, surface area	Spray pressure, smooth surface - both sides of door; 3.5 m^2; up- and downward directions
D: Handling of immersed objects	D1: Liquid - low viscosity	Manually handling of immersed objects	Number of objects handled; object size	Smooth immersed object surface; average number of pieces handled (i.e. 15x); high surface contamination level; medium sized objects
	D2: Liquid – high viscosity	Manually handling of immersed objects	Number of objects handled; object size	Smooth immersed object surface; average number of pieces handled (e.g. 15x); high surface contamination level; medium sized objects
E: Handling of contami-nated objects	E: Dusty solid	Handling contami-nated objects	Number of objects handled; object size	Smooth immersed object surface; average number of pieces handled (e.g. 12x); high surface contamination level; medium sized objects

2.3 Study plan, protocols and standard operating procedures

Before the experiments were carried out, an extensive work plan was prepared, in which the details of the study design are described (TNO report R11110 - Systematic analysis of dermal exposure to hazardous chemical agents at the workplace (SysDEA) - Work plan, 2015).
For each exposure situation a protocol was prepared, in which in detail the experimental set-up is described. In the preparation and pilot phase, each protocol was tested and adapted accordingly if necessary. The final protocols for each of the exposure situations are presented in Annex 1.
For each procedure that was followed during the experiments, like dressing / undressing of the volunteers or skin wiping, a Standard Operating Procedure (SOP) was prepared (see Annex 1), in order to make sure that these procedures were carried out in a standardized and reproducible manner.

2.4 Project scientific committee and expert workshops

For the duration of the project a project scientific committee was installed, with experts from the field of dermal exposure assessment and dermal measurement methods. The following key experts joined this project scientific committee:

- Dr. Karen Galea (IOM)
- Prof. Dr. Ing. Udo Eickmann (BGW)
- Ir. Jan Urbanus (Shell Health)
- Prof. Dr. rer. nat. Thomas Göen (IPASUM)

During the course of the project two expert consultation workshops were organised in order to discuss the methodology and the results of individual project activities with the project scientific committee. The aim of the first consultation workshop was to discuss the work plan and the results of the validation/calibration of the methods and the preparation of the experiments. In the second workshop the results of the experiments and the (dis)advantages of each measurement method, the best applicable methods for specific tasks, and the differences in measured dermal exposure outcomes were discussed and it was investigated whether the results of the different measurement methods can be converted to one another by means of conversion factors. In addition to these workshops, the experts in the project scientific committee were consulted during the course of the project if considered necessary, which was initiated by BAuA and/or TNO.

3 Preparation of experimental work (pilot)

3.1 Introduction

In order to make sure that the actual experiments were conducted in the best way, both from a practical and an efficiency point of view, all exposure situations have been tested during the pilot phase. Furthermore, some other practical issues have been tested, like the most suitable low and high viscosity liquid and the dustiness of the test products, the most suitable monitoring clothing and the optimal way for head wiping. In the paragraphs below the results of the preparations as performed during the pilot phase are described.

3.2 Test substance

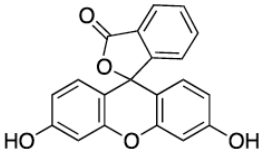
In order to be able to combine interception and/or removal measurement techniques with an in situ measurement technique, a fluorescent tracer substance was chosen as the substance of choice for all the experiments. Choosing one substance also enables a more direct comparison with the different types of products to be included (a dusty solid and a high and low viscosity liquid). Furthermore, using one substance for all experiments results in the application of one optimal chemical analytical method that needs to be validated for one substance. However, there are several fluorescent tracer substances available that could be used for this purpose. Therefore, an evaluation was made of the tracer substances that could be used for this purpose.
In case of application of the in situ sampling principle, no active sampling takes place. Exposure is more or less “visualized” by using a fluorescent tracer in combination with UV irradiation. Exposure is quantified by measuring the intensity of the emitted light by the tracer during radiation. The selection of a tracer which acts similar under the different circumstances of the experiments is crucial.
For the selection of an appropriate fluorescent tracer the following substances were evaluated (including some exploratory tests):

- Fluorescein
- Quinine
- Uvitex OB
- Tinopal SWN
- Tinopal CBS

More information about the materials, the possible harmfulness for the user by working with this tracer and the appropriateness of the substances are given below.

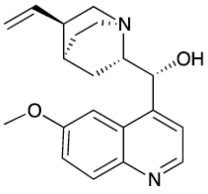
Fluorescein

Fluorescein (CAS 2321-07-5) is a synthetic organic compound available as a dark orange/red powder slightly soluble in water and alcohol. For dermal exposure, no human health risks were found when searching in ToxNet. A strong difference was observed with regard to the fluorescence capacity between fluorescein in the wet and dry form. For the dry form, hardly any fluorescence was observed, making this tracer not suitable for all experiments.



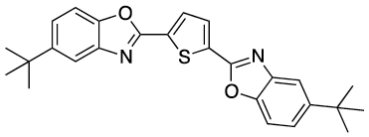
Quinine

Quinine (CAS 130-95-0) is a white crystalline alkaloid having antipyretic (fever-reducing), antimalarial, analgesic (painkilling), and anti-inflammatory properties and a bitter taste. In addition, it can be used as a fluorescent tracer. For dermal exposure, no human health risks were found when searching in ToxNet. A strong difference was observed with regard to the fluorescence capacity between quinine in the wet and dry form. For the dry form, hardly no fluorescence was observed, making this tracer not suitable for all experiments.



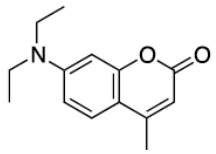
Uvitex OB

UVITEX (CAS 7128-64-5, 2,5-thiophenediylbis(5-tert-butyl-1,3-benzoxazole)) is used as fluorescent whitening agent for fibers, molded articles, films and sheets. For dermal exposure to Uvitex no human health risks were found when searching in ToxNet. However, a strong organic solvent is required to create a solution with Uvitex, which would introduce possible health risks for the volunteers when performing the experiments with liquid products. Therefore, this tracer is not preferred.



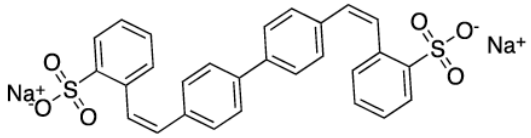
Tinopal SWN

Tinopal SWN (CAS 91-44-1, 7-diethylamino-4-methylcoumarin) is a fluorescent whitening agent for incorporation in laundry detergents, giving white effects on polyamide, polyester, secondary acetate and triacetate fibers, wool, silk and polyvinyl chloride fibers. For dermal exposure, no human health risks were found when searching in ToxNet (note: For coumarin (CAS 91-64-5) we did find health adverse effects). Good and comparable test results were found for dry and wet forms of this tracer. Tinopal SWN has a density of 1.177 g/cm³ at 20 °C.



Tinopal CBS

Tinopal CBS (CAS 27344-41-8, 4,4'-Distyryl biphenyl derivative (DSBP)) is a fluorescent whitening agent for incorporation in detergents, giving white effects on cellulosic fibers over the whole temperature range, especially from cold to medium working temperatures. For dermal exposure, no human health risks were found when searching in ToxNet. Good and comparable test results were found for dry and wet forms of this tracer.



In the past, TNO has used both UVITEX and Tinopal (both CBS and SWN) as fluorescent tracer substances. In these studies, these substances were used for visualization and/or quantification of the substance as well as the component for a chemical analysis of the measured concentration (Brouwer et al. 1999). From these studies, we know that these substances are stable, and that the substances do not break down after elimination. Furthermore, we have had good results with regard to removal/extraction of the substances from the used matrices in the past, as well as good results with regard to the chemical analysis of these substances.

Based on our evaluation (screening and test results combined), either Tinopal CBS or SWN were selected as our fluorescent tracer of choice. Tinopal is also an US FDA approved fluorescent whitening agent, and thus meets the F2349 tender requirement that the selected substance should not be harmful for the user (in this case both the volunteers as well as the technical personnel involved for executing the experiments).

Further testing with Tinopal SWN and/or Tinopal CBS as substance for the experiments has been carried out in order to investigate the possible interaction with the monitoring matrices, possibilities for combination of the substance in the test products (dusty solid, low viscosity liquid, high viscosity liquid), the efficiency with regard to removal from the skin, cleaning of surfaces and extraction of matrices and the chemical analysis. The results of these tests are presented in Table 3.1.

Table 3.1 Results tests with Tinopal SWN and Tinopal CBS

Test	Tinopal CBS	Tinopal SWN	Remarks
Solubility (solutions of 8 gram/l)			
solubility in demineriliized water	Good	Bad	forms an emulsion
solubility in ethanol	Bad	Good	forms an emulsion
solubility in ethanol/water (1:1)	Good	Good	
solubility in water/glycerol 70/30.	Good	Bad	forms an emulsion
solubility in water/glycerol 30/70	Good	Bad	forms an emulsion (quite viscous)
solubility in water/glycerol 70/30 + 10% 2-propanol	Good	Bad	forms an emulsion
solubility in water/glycerol 30/70 + 10% 2-propanol	Good	Bad	forms an emulsion
solubility in cellulose, 100 mg/25 mL.	Good	Bad	forms an emulsion (very viscous)
Illumination under black light			
Fluorescence in solution	Good	Good	
Fluorescence dry powder	Good	Good	
Removal from skin after washing with ethanol/demi (1:1)			
Exposure to solution in demi	Bad	Good	3x times washing; bad washing efficiency (visual inspection)
Exposure to solution in water/glycerol 30/70	Bad	Good	3x times washing; bad washing efficiency (visual inspection)
Exposure to solution in cellulose 100 mg/25 mL	Bad	Good	3x times washing; bad washing efficiency (visual inspection)
Level of contamination of skin after washing			
Directly after washing	High	Low	
One day later	High	Low	
Two days later	Medium	Low	
Dustiness			
Dustiness powder	Not dusty (coarse powder / crystals)	A little dusty (fine powder)	

Based on these results Tinopal SWN was chosen as the tracer substance to be used during all experiments.

3.3 Test formulations

3.3.1 Determining the most suitable liquid formulations

In the preparatory phase various tests have been performed with Tinopal SWN, in order to find the appropriate dilutions for the liquid formulations and to have a high viscous and a low viscous liquid (depending on ratio water : glycerol). Since Tinopal SWN forms an emulsion in water (settling of the larger particles), several tests have been performed during which various options were tried: varying with the mixing procedure (shaker, ultrasonic bath, etc.), first dissolving Tinopal SWN (in methanol, ethanol, acetone) before adding to glycerol / water formulation, heating the liquid when mixing and reducing the concentration of Tinopal SWN in the formulation (from 8 to 4 to 2 g/L). This resulted in some improvement, but the liquid formulations were not satisfactory enough to be used in the experimental phase.
Also, manual grinding the Tinopal SWN before adding it to liquid gave some improvement but not substantial enough to proceed. Therefore, different carriers or emulsifiers were added to the solution like carboxymethyl cellulose (CMC), Triton-X and Tween 20. The use of Triton-X (non-ionic surfactant-emulsifier) gave the best results, and therefore the final formulations for the preparation of 2 L of formulation consist of the following:

- Low viscous solution: 4 g Tinopal SWN, 200 mL acetone, 40 mL Triton-X, 400 mL glycerol, 1360 mL H_2O;
- High viscous solution: 4 g Tinopal SWN, 200 mL acetone, 40 mL Triton-X, 800 mL glycerol, 960 mL H_2O.

Both test and final viscous Tinopal SWN solutions were assessed for their viscosity by respective measurements conducted using a rotating viscometer (see Figure 3.1 and Table 3.2). The surface tension of the final formulations was also determined (see Table 3.2).

Figure 3.1 Measuring viscosity of Tinopal SWN solution using a rotating viscometer

Table 3.2 Surface tension and viscosity measurements for liquid formulations

Formulation	Viscosity (cP) *	Surface tension, γ (mN/m) **
Low viscosity	16.8±1.2 (n=3)	32.508±0.055 (n=13)
High viscosity	313.4±10.5 (n=3)	32.068±0.044 (n=12)

* Rotating viscometer, Brookfield Engineering.
** Surface tension apparatus, Sigma 70, KSV Instruments LTD (Helsinki, Finland), measurements at 16 °C.

3.3.2 Performance of dustiness tests to determine the most suitable solid formulation

3.3.2.1 Rotating drum procedure

There are several ways to measure the dustiness of a powder. In this project the rotating drum procedure was used, since the process of this system (generating dust by repeated lift and drop agitation of a powder sample) is more or less comparable to the way dust is generated during the dumping exposure situation (repeated dumping of 1 kg of pure Tinopal SWN) which is part of the experimental phase, whereas the continuous drop procedure (generating dust from continuous feed and drop of 'undisturbed' powder to simulate continuous dust generation processes) is less comparable with this exposure situation, and also is not comparable to the handling contaminated objects exposure situation (repeated handing of plates contaminated with dust).
Thus, dustiness tests were performed using a rotating drum. A rotating drum is an instrument used to determine the dustiness of dry products. The instrument consists of a stainless steel drum with two openings on each side. During a dustiness test the drum rotates for 65 seconds with a speed of 4 rounds per minute. The drum is hooked to a pump that creates an airflow in the drum with 38 liter/minute. During the rotation, eight fins in the drum cause a dust cloud within the drum. The air is sucked in through an inlet-opening provided with a glass fiber filter (Whatman GF/A, 150 mm, art. 1820-150) which filters the incoming air. At the exit-opening a case is mounted which hold different filters allowing the measuring of different particle sizes. The filters/foams are weighed prior to the dustiness test and at the end of the dustiness test to determine the amount of test substance on the filter. By weighing the tube with the substance before the test and after the test the dustiness of the test sample can be calculated.
By means of a dustiness test with a rotating drum the dustiness of the product can be defined for different dust fractions, namely the inhalable fraction, the thoracic fraction, the respirable fraction. For the current study, the dustiness was determined without further differentiation into different size fractions, since this was considered sufficient information for the purpose of the study (determining whether the test substance / test product of choice is a dusty powder).
The dustiness is calculated by dividing the increased filter weight in mg (difference between pre- and post-weighing of the filter) by the amount of the test substance in kg (difference between the weighing of the tubes prior to and after the dustiness test), as is seen in the equation below:
$W_{TOT} = \Delta M_f / M_s$

where W_{TOT} is the total dustiness, ΔM_f is the increased weight on the filter and M_s the total amount of test substance. Based on the result, the tested product can then be classified into one of the dustiness categories as defined for the rotating drum method (see NEN-EN 15051-2:2013).

3.3.2.2 Results of dustiness tests

During the course of the project four series of dustiness tests were performed, of which the results are presented in Table 3.3. Most of these series were performed to determine whether it would be possible to mix Tinopal SWN with another powder (in this case talc), resulting in a more or less homogeneous dusty powder.
During the first series samples from two types of talc and from two types of Tinopal SWN were tested. Furthermore, different treatments of Tinopal SWN were tested. The two types talc showed very different results, while the results of the two types of Tinopal SWN were fairly comparable. Based on these results it was concluded that pure Tinopal SWN seemed to be more dusty then talc, and thus could be used for the exposure situations with powders without further treatment. Treatment of the Tinopal SWN with a grinder seemed to have a negative result on the dustiness of the powder (seems to stick more together, and sticks to the wall of the grinder). Treatment of Tinopal SWN with a ball mill after grinding seemed to make the powder more dusty again, but did not fully reverse the effect of the grinder.
Because the dustiness of the type 2 talc and the type 2 Tinopal SWN (which refers to the Tinopal SWN that was purchased for this study) seemed to be fairly comparable, it was assumed that these two powders could be mixed to form a more or less homogeneous dusty powder.

Table 3.3 Results of the dustiness tests performed

Series	Description	Number of replicates	Dustiness *	Dustiness category
1	Pure talc (type 1)	2	3773	high
1	Pure talc (type 2)	2	9003	high
1	Pure Tinopal SWN (type 1)	1	16663	high
1	Pure Tinopal SWN (type 1) - grinder	1	1381	moderate
1	Pure Tinopal SWN (type 1) - grinder + ball mill	1	1962	moderate
1	Pure Tinopal SWN (type 2) **	2	10976	high
2	Pure talc (type 1)	2	3381	high
2	Pure talc (type 2)	2	9177	high
2	Pure talc (type 3)	2	1927	moderate
2	Pure talc (type 3)	2	1589	moderate
2	Pure talc (type 3)	2	1450	moderate
2	Pure Tinopal SWN (type 2)	2	12414	high
3	Pure talc (type 2)	2	10677	high
3	Pure Tinopal SWN (type 2)	2	12837	high
3	Mixture 50% Tinopal SWN (type 2) and 50% talc (type 2)	2	4313	high
3	Mixture 20% Tinopal SWN (type 2) and 80% talc (type 3)	2	2036	moderate
3	Mixture 10% Tinopal SWN (type 2) and 90% talc (type 3)	2	2676	moderate
4	Pure talc (type 1)	3	2967	moderate
4 ***	Pure Tinopal SWN (type 2), before dumping, run A	1	9410	high
4 ***	Pure Tinopal SWN (type 2), after 6 dumpings of 1 kg, run A	1	8693	high
4 ***	Pure Tinopal SWN (type 2), before dumping, run B	1	8808	high
4 ***	Pure Tinopal SWN (type 2), after 6 dumpings of 1 kg, run B	1	8294	high

* In most cases a dustiness test for a certain sample was duplicated or even triplicated. The result presented in this column represents the average of the number of replicates mentioned in the column next to it.
** Tinopal SWN from the same batch as is used in this study.
** These samples relate to the experiments described in paragraph 3.11.1.1.

During the second series of dustiness tests again different types of talc and one type of Tinopal SWN were tested, which were partially repetitions of the samples also tested during the first series. These results confirmed that the type 2 talc and type 2 Tinopal SWN seemed to be most suitable to be combined into a mixture.
As initially the aim was to use a mixture of Tinopal SWN and talc during the experiments, as pure Tinopal SWN is very costly, during the third series different mixtures of Tinopal SWN and talc were tested. The selected types of Tinopal SWN and talc were considered individually very dusty products, and mixtures with different

proportions (1:1, 4:1 and 9:1) were tested to be able to classify the dustiness of a mixture for the purpose of this study. As can be observed from Table 3.3, the different mixtures tested were less dusty than the individual products. It is assumed that an agglomeration effect occurs when mixing the Tinopal SWN with the talc, which results in a considerable reduction of the dustiness of the mixture. For the 1:1 mixture, the dustiness remained high. The other two mixtures showed only moderate dustiness.
Based on the results of the dustiness tests it is proposed to use the 1:1 mixture of Tinopal and talc for the experiments with dusty solids. However, apart from to the dustiness tests, it was also checked whether the composition of the mixture remained the same during use of the mixture (e.g. after becoming airborne) (see also paragraph 3.11.1.1), which did not show the desired results. Furthermore, in practice it was considered hard to maintain a homogeneous mixture to be used during the experiments. Based on this, and the results of the other pilot tests as described in paragraph 3.11.1.1, it was decided to use pure Tinopal SWN for the exposure situations with dusty solids.
During the fourth series a control sample of the type 1 talc was tested, for which the results were more or less in line with the results from previous dustiness tests (although a little lower). In addition, the difference in dustiness of samples of type 2 Tinopal SWN before dumping and after six consecutive dumpings was tested, which turned out to be relatively small. Although a reduction in dustiness was observed after repetitive dumpings, the dustiness of the Tinopal SWN was still considerable after the dumpings. Therefore, it was concluded that the proposed protocol for the dumping exposure situation could be applied. It was however noted that the dustiness of the type 2 Tinopal SWN before dumping in this series was lower than the dustiness as measured during previous series. It was assumed that this was probably a result of the level of moistness of the powder.

3.3.3 Preparation of the liquid formulations

Tinopal SWN is sieved at 38 µm for 20 minutes and then used without further treatment for the preparation of the liquid formulations. The calculated appropriate amount is weighed in a small beaker (e.g. 20 mL), and transferred to a 5 L beaker by decanting with the aid of a spatula. Then, 200 mL of acetone (pro analysis) is placed into a volumetric cylinder. Consequently, residues of non-transferred Tinopal SWN are dissolved in a low volume of acetone (from 200 mL) inside the small beaker, and then all Tinopal SWN is dissolved in acetone under continuous stirring (in the large beaker). After dissolving, Triton-X, glycerol and water are added sequentially (under continuous stirring) to form the final liquid product. However, Tinopal SWN can also be used in its non-sieved form since we did not find evidence for different behavior in the prepared liquid.

3.3.4 Preparation of the solid formulations

Pure Tinopal SWN was used for exposure situations with a dusty solid. For the dumping exposure situation pure Tinopal SWN, without any further treatment, was used. For the handling of contaminated objects exposure situation pure Tinopal SWN was sieved at 38 µm prior to loading it onto plates.

3.4 Sampling matrices

3.4.1 Choice of sampling matrices

For the conduct of the experiments it was decided to use Tyvek and cotton materials. The Tyvek coveralls and Tyvek patches were used for exposure situations in which liquid formulations were applied, while cotton coveralls and a Tyvek coverall in combination with cotton patches were used for exposure situations with a dusty solid.
In order to make a choice for the exact material for the experiments, different materials from different suppliers were tested. The initial Tyvek and cotton materials selected and tested for the chemical analysis showed good results. However, these were not suitable for the fluorescence method. First, the Tyvek coverall contained some coding in certain parts of it that were only visible under UV light. Since that coding was randomly placed on arms and legs of the Tyvek coveralls, this was undesirable. Second, the cotton material was white and contained whiteners, which interfered with the photographs under UV light.
For the Tyvek coverall, another supplier was found. For these coveralls the tests showed good results and thus, these Tyvek coveralls were finally selected to be used during the experimental phase. The supplier of Tyvek coveralls was “Leading products S.A.” [Emmanouil Pappa 11 str., PC 122 42, Aegaleo, Greece]. More precisely, this matrix that was chosen was a one-piece protective clothing Category 3 “Tyvek” type coverall with a hood attached, model “SprayGuard” in white color, manufactured by “Indutex, SA”.
For the cotton material, dark blue cotton fabric was found as an alternative, which also performed well for the chemical analysis. For the cotton material both two-piece and one-piece garments were available. In order to make the comparison between the different exposure situations as comparable as possible, it was decided to use the one-piece garments (hood attached) for the cotton coverall and they were ordered to be tailor-made by the supplier (“Safework ltd”, Tenedou 7 str., PC 17778, Tavros, Greece). This type of coverall was 100 % cotton. Since also some two-piece garments were initially purchased, these were used during some of the pilot experiments.
For the gloves, pairs of 100 % cotton gloves (no stretch), light green color, free of whiteners, were used. The supplier was “Tsismetzoglou Co.” (Mesogeion Ave. 105, PC 11526, Athens, Greece).
The headbands were made of the same cotton fabric that was used for the cotton coveralls. The fabric was provided by the same supplier as for the cotton coveralls and the headbands were prepared by BPI scientists (see paragraph 4.3.2 below on the preparation of matrices).
The wipes chosen for wiping the forehead, “wet hankies” (MEGA DISPOSABLES SA, Dekelias 148, 13678, Aharnes, Greece), showed the best results for removal of Tinopal SWN from the skin, were commercially available and contained ± 20 g ethanol / 100 g liquid.

3.4.2 Preparation of sampling matrices

The Tyvek coveralls were one-piece white coveralls. They included zippers at the front (from the waist up to the neck) as well as a narrow layer of adhesive strip folding over and along the zipper to cover it. It also had a piece of adhesive tape on the neck to ensure that no part of the neck remains uncovered. These two adhesive tapes

were removed by the field scientists during dressing of the volunteer, since they showed fluorescence under UV light. The Tyvek coverall included a hood which was used to protect the head of the volunteer. The Tyvek coveralls had elastics at the end of the sleeves as well as at the end of the legs, which could not be removed; however, these elastics showed no fluorescence. Tyvek coveralls were checked under UV light for background fluorescence and were proved suitable.
Cotton coveralls were one-piece dark blue coveralls and were tailor-made according to the design of the Tyvek coverall, but without elastics at hands and legs to avoid potential fluorescence. In order to tighten the sleeves onto the volunteers' hand (amongst others to avoid dust mitigating underneath the sleeve) a couple of safety pins were used.
The (no stretch) cotton gloves did not contain whiteners. However, a preliminary optical check at the UV was made to ensure that no faulty items were included in the purchased batch. No other special preparation was required.
The headbands, made of the same cotton fabric as the cotton coveralls, were cut by BPI in a clean laboratory area away of the experimental area. The headbands were 6 cm wide and 70 cm long to make sure that they fitted on every head (it was considered necessary to use the same size of headband for all volunteers). The headband was placed in the right position on top of the hood (to cover the forehead area) and was steadily attached with a safety pin (no stretch cotton, almost 1 cm was cut off at the end).
The wipes did not require any special preparation.
The Tyvek and cotton patches were made of the same material as the Tyvek and cotton coverall, respectively. In order to be attached at a Tyvek coverall – leaving an exposure area of 10 cm x 10 cm clear - various approaches were examined. An option proposed was to make plastic envelopes (for instance using showcases and join them at the sides with adhesive tape). Although this option would allow to reuse the envelopes after cleaning them, it was not favored as the field scientists would have to stick the patch in, or remove the tape at one or more sides to attach the patch to the volunteer which would not be practical. Finally, it was decided to cut patches of 12 cm x 12 cm, and place these in a square "nest" covering the outer 1 cm of the patches to provide a back-supporting layer. For this supporting layer, various paper types as well as aluminum foil were tested, but were rejected for several reasons, e.g. background fluorescence, bad performance in supporting the fabric weight, difficulties in folding (normal aluminum foil may not be firm enough, it was necessary to fold it double first), sticking/firmly attaching to the coverall, etc. In the end, whitish thin non-fluorescent semi-transparent square paper was chosen as material for the back-supporting layer. Examples of patches were prepared this way and were put to the coverall of the volunteer for demonstration purposes with regard to the way of preparing them, sticking/fixing them on the coverall, and defining the location that they should be put. The parameters that were checked during the preparation of the patches in order to confirm fit-for-purpose were:
a) Use double sided tape (or other) to attach the patches to the volunteers on Tyvek coveralls;
b) Check if the patches would stay on during a longer period of time, and during movement;
c) Check if the patches could be easily removed (preferably without applying too much force, since this may result in loss of test substance);
d) Check ease of removal of the patches with a set of tweezers from the supporting background layer (nest), in order to put them in the extraction pot.

For the confirmation of the above, a dry run with cotton clothing and Tyvek patches being attached to it was carried out. It was concluded that a) the patches stuck well to the surface, b) the placement of the patches always requires some attention to be put more or less at the same place each time, and c) the patches should be at the front of the body (not at the sides). For all exposure situations, the patches were attached to a Tyvek coverall. The patches were prepared in a clean area, with clean scissors and wearing gloves, to prevent them from getting contaminated beforehand. It was considered practical to prepare large quantities, and store them in plastic bags.

3.4.3 Determination of surface area of parts of coverall

In this study, potential dermal exposure is being measured on either a coverall or on patches placed on a coverall. To be able to convert the amount of Tinopal SWN measured on a certain part of this coverall into an amount per cm² and/or to be able to extrapolate an amount measured on a patch that was placed on a certain body part to the amount for that body part as a whole to be compared with the amount measured on the corresponding part of the coverall, the actual surface areas of the different parts of the Tyvek and cotton coveralls that were used in this study were determined.
The surface areas of the different parts of the Tyvek and cotton coveralls was estimated by dividing (cutting) an unused and clean coverall of each type into the different body part samples in the same way as for the coveralls that were worn during the experiments (for the procedure, see Annex 1), and then weigh each of these body part samples one-by-one on a balance in the laboratory. The division of the parts was made the same way as they were cut as in the real trials. The same approach was also followed for the cotton gloves. Non-fabric parts, such as metal buttons in case of the gloves, were removed before weighing.
Furthermore, the specific weight of both, the Tyvek and cotton coverall fabric was determined by weighing a piece of 30 x 30 cm² (= 900 cm²) to calculate the ratio of mass (based on the weight on the balance) to area. For the glove material a piece of glove fabric of 4 x 4 cm² (= 16 cm²) was used.
The surface area of the different body parts is then estimated by dividing the weight of the respective sample of the coverall (in g) by the specific weight of the fabric (in g/cm²). The determined surface areas are presented in paragraph 7.3.

3.5 Test location

All experiments were carried out at BPI premises. The preparatory actions regarding the set-up of the experimental site included the placement of containers at the BPI premises. Two identical containers (2.6 m × 7.1 m) equipped with a shower, air conditioning and a window were purchased and established at BPI premises. The containers were placed adjacent to each other (see Figure 3.2). One was used for the conduction of the experiments (container 1), while the other (container 2) was appropriately equipped inside with UV lamps, photographic camera and computer device and was used as UV-room as well as for the field spiking. Inside container 1, a ventilation system was installed with a measured ventilation rate of 1282 m^3/hour (KIMO LN101 instrument). In the area opposite to the containers a separated closed

area was constructed that was used as the dressing-undressing room (see Figure 3.3).

Figure 3.2 Containers at BPI premises

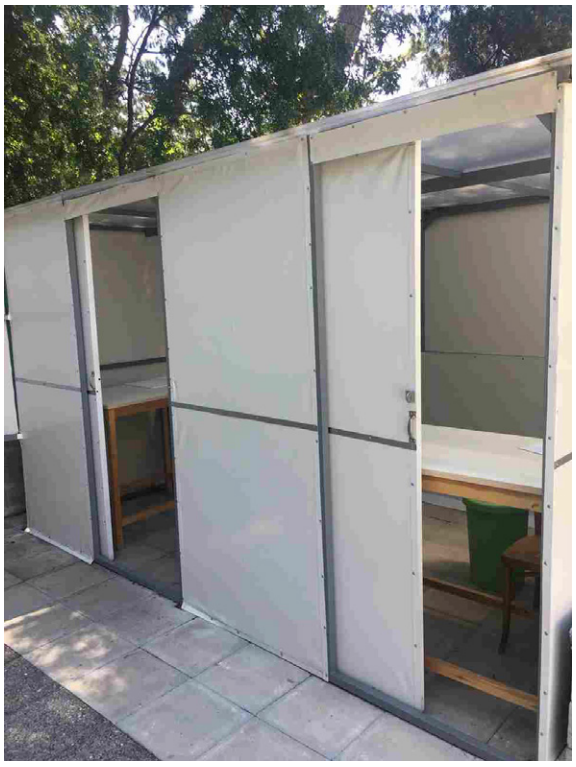


Figure 3.3 Dressing-undressing area at BPI premises

3.6 UV set-up

3.6.1 UV set-up at TNO

The fluorescence method used consists of two parts: a set-up under UV light used to photograph the exposure to the fluorescent tracer of the volunteers (described here), and the software used to analyze the recorded images and convert measured light intensity into exposure in mass (described in chapter 5).
At the premisis of TNO a test set-up of the UV-room was created to develop a set-up which provides a most optimal and diffuse lighting of the volunteer. This set-up consisted of aluminium pipes which could be freely moved, on which the double TL-armatures could be placed. A projection screen was placed in the middle against the wall, where the volunteers stands (directly in front of the screen). The pipe construction was placed in such a way that TL-armatures were facing the volunteers from different angles to provide a diffuse lighting of the volunteers (semicircle).
White squared pieces of paper were placed in a cross on the black background screen to test whether the set-up provided diffuse lighting. Software was used to analyze the photographs, which selected the square with the highest lighting and calculated the relative difference in lighting of the other squares.
To test the angular dependency of the set-up, a round bucket with a piece of paper wrapped around the bucket was placed on the stepping stones where volunteers would stand on. Angles of 25, 45, 60 and 90 degrees were written on the paper in both directions from the middle, and the photos were analyzed using software, which showed the light intensity for the entire range of the paper.
Photographs were taken using a Canon EOS 700D with a Canon EF-S 18-55 mm lens. The camera was positioned on a tripod at the height of the calculated estimated middle of a volunteer. Camera settings, such as the shutter time and auto-bracketing, were varied to find the optimal camera settings (in which no over-exposure occurred on the images).
Different set-ups were built and evaluated to find the set-up with the most diffuse lighting. Within these set-ups the angles of the TL-armatures, reflective screens on floor and ceiling, and the number of TL lamps active in each TL-armature were varied. For each set-up a photo of the cross and the bucket was taken and analyzed on the most optimal settings.

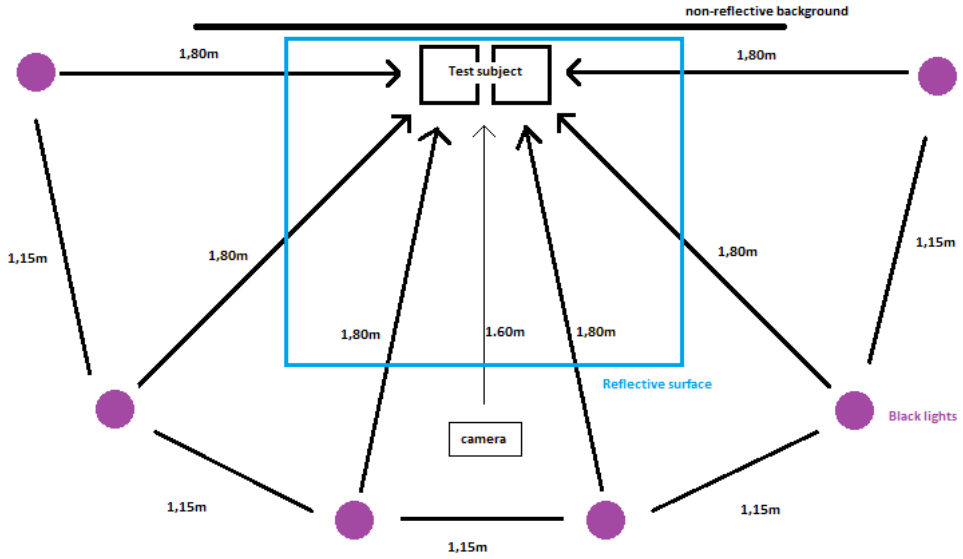


Figure 3.4 Final set-up of the lights and armatures

Ultimately, the TL-armatures were placed in a semicircle with each armature facing the volunteer, as shown in
Figure 3.4. Each armature was placed 1.80 m from where the volunteer would stand and 1.15 m apart from the next armature. A reflective surface was only used on the floor, since a reflective surface on the ceiling decreased the diffusion of the lighting. The camera was placed 1.60 m from where the volunteer would stand on a tripod which was set at 95 cm height.
The camera was set on ISO 3200, F/8, and three consecutive photos were taken with a shutter time of 1/80, 1/20 and 1/5, respectively. The reason for the three different shutter times was to ensure at least one photo would have sufficient exposure when for example photos with the higher shutter time would possibly be over-exposed.
Figure 3.5 shows the diffusion of the light on the white paper patch cross attached to the background screen. Here, the light is almost evenly distributed in the middle and on the lower patches, while the light intensity drops for the higher patches. If necessary, a correction factor can be applied for the higher body parts to account for these differences in lighting.

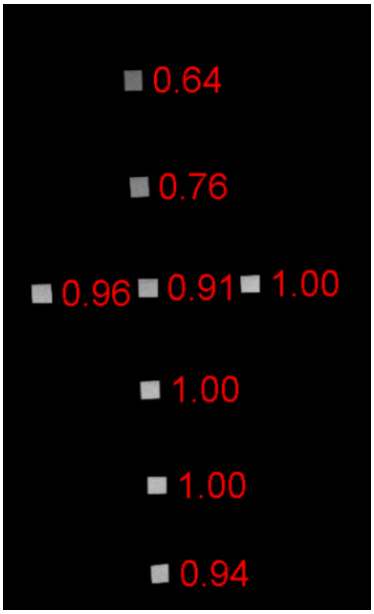


Figure 3.5 Diffusion of the light on the cross attached to the background screen

The angular dependency as defined for the TNO set-up is shown in Figure 3.6. The light intensity decreases over the bucked gradually to a relative intensity of 0.7 (70 % light intensity compared to the middle). For all the set-up possibilities both, cross and bucket results were the most diffuse in lighting.

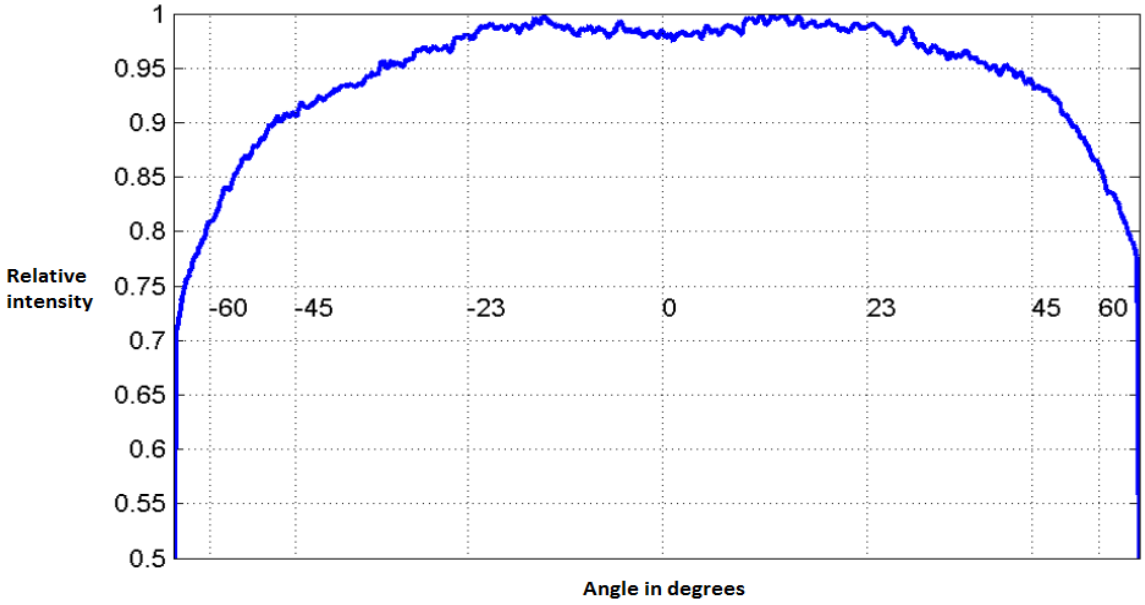


Figure 3.6 Angular dependency of UV light set-up at TNO

3.6.2 UV set-up at BPI

The characteristics of the set-up at TNO were sent to BPI, where the set-up was exactly reconstructed , to be used to photograph the volunteers under UV light before and after each experiment. The only difference was that at BPI a different camera was used, namely a Nikon D90. Figures 3.7 and 3.8 show the set-up inside the UV-room at the BPI premises.

Figure 3.7 and 3.8 Set-up inside the UV-room at BPI premises

3.7 Temperature and relative humidity

BPI used a digital sensor (Trevi, electronic meteo station, ME3160) for measuring temperature and relative humidity (see Figure 3.9). The device was used throughout the project and it performed well. Spraying mist or dust generated during the experiments did not create any problems with the sensor.

Figure 3.9 Device for measuring temperature and relative humidity

3.8 Recruitment of volunteers

A consent form was compiled considering the aims of SysDEA project also based on previous participation of the research team in several field trials (in the frame of research projects). In this document, the institutes that conducted this study were explicitly stated, and a full explanation was given to the candidates before their possible participation. The aim of the project, the exposure situations and the chemical(s) used were made transparent during the communication with candidates. In particular, the implementation of a fluorescent tracer substance commercially used as an optical brightener for detergents and cleaners (which is commercially available in Greece as well) was clarified both, verbally and in the document.
The candidates were informed that they could take time to reflect on whether they want to participate or not, and that they can ask questions at the initial stages or later.
Volunteers were recruited based on several criteria. Pivotal criterion was the previous participation in field trials and/or the proximity of their profession and everyday tasks to the ones scheduled in the SysDEA project. However, the latter was not a prerequisite for their final acceptance and enrollment. In general, easiness and willingness in performing tasks, “lack of fear” after all information was provided (on the nature of the tasks and chemicals) were priority criteria when selecting the volunteers. Furthermore, material safety data sheets (MSDSs) of all chemicals selected in SysDEA were provided in the native language.
A physician and the safety supervisor officer of BPI were available during the recruitment and at the onset of pilots and experimental phase (of each exposure situation) to guarantee its safe commencement and to be available for questions regarding health and safety issues that could emerge from the described uses of Tinopal SWN substance during the performance of the experiments.
The occupational physician of BPI has provided his consent (medical ethical approval, see Annex 2) for all chemical substances used within SysDEA after inspecting the corresponding MSDSs. Prior to the conduction of the pilot phase and the actual experiments all volunteers were asked for their physical condition, and only after their consent the experiments commenced. Lastly, the occupational physician was also informed by BPI personnel for the dermal sampling methods that were also approved by his side.
Similarly, the safety officer of BPI studied the MSDSs of all chemicals regarded and used within the project and provided his consent for their use within the project.

3.9 Safety of volunteers and staff

Apart from the matrices used as dosimeters, additional personal protective equipment (PPE) was purchased to protect the volunteers and the staff during the experiments. PPE made available were UV light protection (glasses, Honeywell, North T2400 tactile), safety goggles (see Figure 3.10, 3M SF200 series), rubber boots, filter masks (pro2000 PF10) for respiratory protection (Scott Safety, UK, see Figure 3.11), short-sleeved black nitrile protective gloves (EN 374) and cream for skin protection (Vaseline, pure petroleum jelly, Unilever UK).
Both, the safety officer and the occupational physician of BPI were present during the evaluation of potential PPE and approved with this selection.

Safety goggles, for which was made sure that they did not cover the forehead, were worn by the volunteers during the experiments. The UV protective glasses were worn by both, volunteers and technicians when they were in the UV-room. The UV lamps were turned on as short as possible, to reduce the risk of being exposed to UV-A radiation. When the photographs were taken (before and after the experiments), technical staff and field scientists were standing behind the UV armatures, and were thus not directly in the light direction. The switch for the UV lights was positioned at the door and handled by the field scientist. Protective nitrile gloves were worn underneath the cotton monitoring gloves. Filter masks for respiratory protection suitable for repeated use were used throughout the experimental phase of all the exposure situations. Especially in case of high exposure situations, such as dumping, handling contaminated objects and spraying, masks with a detachable filter were used. The volunteers were provided a pair of working boots, that were used during the experiments, and were stored in the vicinity of the containers to make sure that the surroundings were not contaminated. Furthermore, shoe covers were used when entering the UV-room to prevent contamination. A cream to moisturize volunteers' skin after washing was also available. The technical staff that entered the UV-room on a daily basis was provided with an appropriate sun protection lotion with SPF for UV-A.

Figure 3.10 and 3.11 Goggles (left) and filter mask (right) used during the experiments

3.10 Standard operation procedures (SOPs)

For all the procedures that needed to be carried out during the experimental phase of the project Standard Operation Procedures (SOPs) were developed, in order to make sure that these procedures were carried out in the same way during the experiments. The tests that were performed during the pilot phase in order to develop and/or optimize a certain SOP are described in more detail. All SOPs can be found in Annex 1.

3.10.1 Preliminary tests head wipe procedure

For the performance of the head wipe, first the use of the same cotton as used for the headband and the cotton clothing was considered. However, this fabric showed a low recovery efficiency of Tinopal SWN applied to the skin when used for wiping (by rubbing the cotton fabric against the skin). Furthermore, wiping with this fabric was considered to be a potential source of discomfort for the volunteers. Moreover, the cotton fabric had to be impregnated manually with water or ethanol before use to increase the wipe efficiency, and in practice, this impregnation part appeared hard to standardize. Secondly, medical gauzes were considered, but these also did not prove efficient for this purpose. Thirdly, hygienic wet wipes (commercially available baby hankies containing a water-ethanol solution) were tested and proved very suitable for removing Tinopal SWN formulations from the skin by means of wiping. More details about the tests that were performed with both liquid and solid formulations can be found in paragraphs 3.10.1.1 and 3.10.1.2.
During testing of the wiping procedure, it was ensured that comparable surface areas were used for each wipe. Fixating a template or marking a certain surface are on the volunteer's forehead - to specify the forehead area of interest in terms of a dosimeter - was not considered feasible due to the following reasons: a) drawing the wiping surface area on the forehead using a flexible template could result in ink coming off when wiping, which in turn could influence the extraction and analytical procedure, and b) the surface of the forehead was too small to use tape to mark a layout, as the aim was to have more or less the same surface area considered for wiping as the respective part of the headband that covers the forehead. After these tests, it was decided to predefine the part of the forehead to be included in the wiping procedure in the following way: from the hairline to the eyebrows, and from the outer side of the left eye to the outer side of the right eye. Based on this description each volunteer's surface area of this part of the forehead of was measured, which was more or less the same for each volunteer (no significant differences were observed).
Although it was considered to perform the wiping procedure in the UV-room to be able to check the wiping procedure, this was not considered within the context of the study as this would not result in the application of a standardized measurement method (to be compared with another standardized measurement method). Therefore, during the pilot phase also a standardized wiping procedure was developed, to make sure that each wipe sample was collected in the same way, resulting in a good removal and an even distribution of the pressure applied during wiping. It appeared to be most practical to fold the wipe after each "wiping pass" in such a way that the contaminated part always remained inside, while the remaining clean part of the wipe after each folding was enough to be held firmly when wiping the forehead. One wiping of the selected surface of the forehead more, and thus the use of only one wipe, did not result in a sufficient sampling efficiency. However, a total of three wiping passes, using a new wipe for each pass, thus three wipes in total, was considered sufficient for a complete removal of the substance from the skin. To prevent transfer, the field scientist who performs the wiping procedure should wear gloves when wiping, and as a last step also wipe the gloves to make sure that all Tinopal SWN is part of the sample. Thus four wipes (three for the forehead and one for the gloves) per experiment were needed, which were combined into one sample, and extracted and analyzed as one sample. Due to the content of the wet hankies, it was also decided to use ethanol/water for extraction instead of methanol/water. During the dressing procedure, special care was given to make sure

that the hood of the Tyvek coverall did not cover the forehead during the experiments, because this would affect the requirement of a constant wiping surface area.
Finally, the wet hankies were included as a new matrix in the matrix validation and stability tests.

3.10.1.1 Wiping tests with liquid formulations

The first test with a solution containing Tinopal SWN, for which Tinopal SWN was dissolved in an ethanol/water solution, was performed on the skin of a BPI technician who volunteered for this purpose. The aim was to compare the wiping of the skin with two cotton wipes of 3 cm x 10 cm with one cotton wipe of 6 cm x 10 cm, to see if it was possible to evenly distribute the pressure when wiping. The process involved the following sequence of events: folding the cotton wipe, applying 500 µL of ethanol/water 1/2 v/v on the cotton wipe, and then wipe the spiked surface area from one side to the other, without rubbing, three times with the same cotton wipe. This procedure was proved not effective as 50 % or more of the spiked Tinopal SWN remained on the skin. Additional tests with modification in the wash solution proportion (ethanol / water 1/1 v/v), as well as the size of the cotton wipe, process of moisturizing the cotton wipes, or duration of wiping were performed without any improvement of the results. It was decided not to spike skin with this form of solution anymore, as it was assumed that the solvent in the solution influenced the adherence of the Tinopal SWN to the skin and this solution did not resemble the formulations that were to be used during the experimental phase.
This test was repeated on the skin of a TNO scientist who volunteered for this purpose, with the modification of applying Tinopal SWN as glycerol/water 80/20 v/v formulation, using ethanol/water to moisturize the cotton wipes, and rubbing the skin firmly with each wipe. This procedure was not very effective, as the high viscous liquid applied in this did result in another stripe on the skin, which was not evenly divided as it was the case for the Tinopal SWN in ethanol/water, but resulted in more spots on the skin.
This test was repeated with the modification of wiping skin spiked with Tinopal SWN as glycerol/water 80/20 v/v formulation using wet hankies (commercially available, 20 % alcohol content). During this test three wet hankies were used for wiping, applying three strokes per wipe and folding the wipe after each stroke (‘wiping pass’). It was observed (using UV light) that when these wet hankies were used, the Tinopal SWN was efficiently removed from the skin, and most of the Tinopal SWN was already removed after wiping with the first wet hanky. Each wipe was stored separately, as well as the glass plate that was used for application of the spiked amount of formulation, and was analyzed. Analysis confirmed the qualitative observation when using UV light.
The test was repeated on the skin of a BPI scientist with the modification of applying Tinopal SWN as glycerol/water 70/30 v/v formulation. However, the Tinopal SWN content in the volume spiked on the skin was difficult to be quantified as this formulation was a dispersion rather than an emulsion and larger particles of Tinopal SWN seemed to precipitate in the formulation rather fast. It was observed again that most of the Tinopal SWN was removed from the skin, with the first wipe (qualitative observation using UV light). Each wet hanky was stored separately, as well as the glass plate that was used for application of the spiked amount of formulation, and

was sent for analysis. Analysis confirmed the qualitative observation when using UV light.

3.10.1.2 Wiping tests with powder formulation

During the first test, pure Tinopal SWN powder was spiked directly on the skin of a TNO scientist using a fingertip. Ethanol/water was used to moisturize the cotton wipes. The wiping procedure involved rubbing firmly on the skin with each wipe (ca 30 seconds). However, the results indicated that this procedure was not effective. The test was repeated with the modification of using demi-water as a moisturizing liquid, but this procedure was again not considered to be effective.
The test was performed again, but now, first some glycerol was applied on the forearm that was to be spiked, which was left to dry for a while. A significant amount of powder was applied with a fingertip on the skin with a layer of glycerol. The cotton wipes (first 6 x 6 cm, later bigger) were moisturized with larger quantities of ethanol/water 1/1 v/v. Several cotton wipes were used to rub the treated skin, but these cotton wipes did not seem to remove any of the Tinopal SWN from the skin. It was concluded that these cotton wipes were not suitable for the purpose of the study. Possible alternatives considered were medical gauzes and wet hankies.
The next test was performed on the skin of a TNO scientist, for which an unknown amount of grinded Tinopal SWN powder was applied with a fingertip. During this test three wet hankies were used for wiping, applying three strokes per wipe and folding the wipe after each stroke ('wiping pass'). Using UV light, it was observed that Tinopal SWN was efficiently removed from skin, and thus it was concluded that these wet hankies were suitable to be used. However, it was also observed that during wiping parts of the Tinopal SWN transferred to the hand of the person performing the wiping. Therefore, it was decided that the person performing the wiping procedure should wear gloves and the fingertips of this person should also be wiped as a final step of the wiping procedure (4^{th} wipe).
The last test was a repetition of the previous one (use of grinded Tinopal SWN, applied on the skin with a fingertip). During this test four "wet hankies" were used, applying more or less 3 strokes per wipe and folding the wipe after each stroke ('wiping pass').Now a different person performed the wiping procedure. It was observed again (using UV light) that the Tinopal SWN was removed from the skin, and most of the powder seemed to be removed with the first wipe. Wipes were sent for analysis as separate samples. Similarly, chemical analysis was in line with optical observations using UV light.

3.10.2 Preliminary tests hand wash procedure

For the hand wash procedure as applied during the study three aliquots with 500 mL of ethanol/water 1/1 v/v (the hand wash solution) were used. The washing of hands was carried out inside a plastic bag that was filled with the washing solution and held steady in a comfortable position by a field scientist for the volunteer to dip, rub and thoroughly wash his hands for 30 seconds per washing (a sequence of three washing was performed). All three hand wash samples derived per trial (extracts) were combined and analyzed as one sample.
In order to investigate the most efficient hand wash procedure, and especially how many washes needed to be performed to reach an optimal recovery, some tests

were performed, which also served as input for the recovery study (see paragraph 4.4.5).
Based on the results of the tests performed (see below), it was observed that a sequence of three washes was necessary to make sure all Tinopal SWN was removed from the hands. Furthermore, the wash procedure should have to make sure that the whole of the hands were included in the wash, and the technician should assist in this, by squeezing the bag a little to raise the level of the wash solution. Also the volunteers needed to be instructed in how to wash their hands. Preferably two technicians are present during this procedure, for the timing of the washing, putting the wash solution in the bag, etc. The bags with wash solution should be prepared before the sampling is performed. In addition, it was decided to combine the three wash solutions into one sample, to be extracted and analyzed as one sample.

3.10.2.1 Washing tests with liquid formulations

The application amount of 2 x 100 µL of liquid formulation was applied as 1 x 100 µL was not considered enough. It should be noted that when these tests were conducted, the high and low viscosity liquids were not of the same composition as the final ones used in the exposure trials, and especially the low viscosity liquid did not have the desired homogeneity yet. Applying the high viscosity liquid with an ordinary automated laboratory pipet was not practical due to the viscosity of the liquid and particles in the liquid. Therefore, first a Hamilton syringe was tested as an alternative, but did not show any improvement. Secondly, cutting of the lower end of a pipet tip was tested, as was considered more practical, although it should be noted that this affected the accuracy of determining the volume applied on the skin. The spiked volume was applied to the hands, and then spread a little by the volunteer. After spreading, the hands were allowed to dry for 3 min before three washes were applied in 500 mL of hand wash solution each time, to recover the active substance from the skin. It was decided to perform the washings in plastic bags, as this would allow the field scientist to make the wash solution available for the volunteer without having to use a large amount of wash solution by holding the bag in his/her hands. Furthermore, as plastic bag was considered easy to transfer the wash solution towards the pots for collection of the sample.
The first test was performed with spikes of a low viscosity liquid on the hands of another volunteer from the BPI technical team. Checks were carried out before and after spiking by taking photographs under UV light. The procedure used was as described above. The quantity of low viscosity liquid applied to the skin could be determined with the limitations as mentioned above. However, in comparison to the high viscosity solution (see below), applying a spike with the low viscosity solution was harder, since this solution/emulsion was not optimal yet. The solution still contained particles, with the larger particles settling quite fast, and, if there was a larger particle in the spike, this might easily influence the results. Again, it was observed that almost no Tinopal was present in the second washing solution. It was also observed that at the edges of the hand of the volunteer not all Tinopal was removed after washing three times. Therefore, it was considered necessary that the field scientist provided some kind of assistance during the washing procedure, by squeezing the bag a little to make sure that all of the hands were covered with the washing solution. Furthermore, it was considered necessary to provide an instruction to the volunteers how to wash their hands, which should include the edges of their

hands when washing. The samples in the extraction bags were directly poured back into the pots and sent for analysis, which verified the visual observation.
Secondly, a test with spikes of a high viscosity liquid was performed on the hands of a volunteer from the BPI technical team. Checks were carried out before and after spiking by taking photographs under UV light. The quantity of high viscosity liquid applied to the skin could be determined with the limitations as mentioned above. It was observed (using UV light) that almost no Tinopal was present in the second washing solution. The samples in the extraction bags were poured back into the pots and sent for analysis, which verified the visual observation.

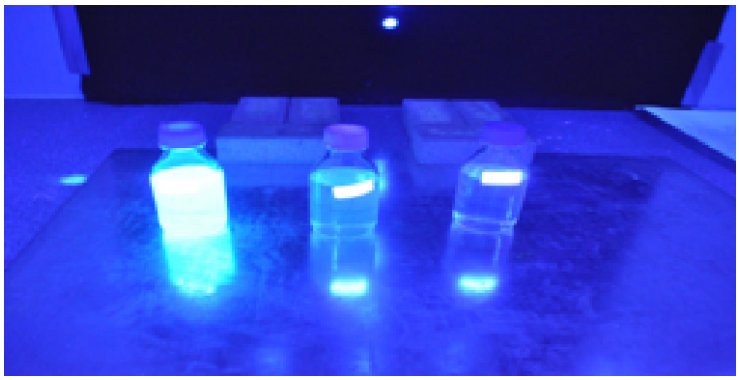


Figure 3.12 Hand wash solutions after a sequence of three consecutive washes (from left to right) under UV light

Based on these results, it was concluded that the washing procedure seemed to work well, as both visual inspection under UV light and chemical analysis of the three separate wash samples showed that most of the Tinopal SWN was found in the first wash solution (see Figure 3.12). Based on this observation it was decided to spike the skin with an ethanol/water 1/2 v/v Tinopal SWN solution instead of with the liquid formulation, to eliminate viscosity issues since Tinopal SWN dissolves very well in the ethanol/water solution, and to spike the material matrices with a methanol solution as it was also done in validation tests.

3.10.2.2 Washing tests with powder formulation

The washing procedure was also tested with pure Tinopal SWN spiked on the skin (powder). This test also provided valuable information for the wiping procedure. The test was performed on the hands of a field scientist who volunteered. Checks were carried out before and after spiking by taking photographs under UV light. The pure Tinopal SWN powder was loaded and applied on the skin via the fingertips of another scientist. The disadvantage of this approach was that the amount of powder applied could not be quantified. It was observed that Tinopal SWN was still present in the second washing solution, based on which it was decided to perform three washes instead of two after each experiment, to make sure that all Tinopal SWN present on the skin would be removed and be available in the extract.

3.10.3 Optimization of the cleaning procedure

During the pilot phase it became apparent that a good cleaning procedure was necessary to prevent any (cross-)contamination during the performance of the actual

experiments. After several tests the cleaning process was standardized and portrayed in the respective SOP. In the same context, a suitable cleaning procedure was considered necessary for both the field part (with regard to checking the test location) and the collection of samples (with regard to checking the volunteers and possible contamination that might interfere with the analytical results).
For the field part, it was considered necessary to carry out a cleaning procedure a) after each experiment, b) before the first experiment of a daily or weekly sequence, and c) after any observed contamination incident, to ensure that all locations, equipment and surfaces involved in the experiments were free from contamination with Tinopal SWN. As Tinopal SWN cannot be removed by water and soap, solvents such as ethanol and acetone were used in the cleaning procedure. It was also decided to use disposable plastic thick films and cardboard to prevent spillage on the floor and/or the walls of the container where the experiments took place. After the conduction of an experiment these were carefully folded, to make sure that any contamination was kept inwards, and disposed as waste.
The cleaning procedure was carried out only by a dedicated-to-purpose technician, who did not enter the UV-room and did not participate in specific “contamination sensitive experimental procedures”, such as dressing/undressing, carrying of samples, video recording, and operating the UV-room.
After performing the cleaning procedure, the container was optically checked using a portable UV light to detect possible contaminated areas. If these were identified, they were cleaned locally with either a vacuum cleaner (solid residues) or ethanol/water solution (liquid residues). The respective cleaning SOP that was compiled in the frame of the SYSDEA project was followed throughout the whole course of the project (see Annex 1).
After performing the experiment, taking the “after” photographs in the UV-room and the process of undressing (including collection of samples), all volunteers were immediately provided with wet hankies to clean “bear skin surfaces” of the body parts that were more vulnerable of being exposed to Tinopal SWN during the experiments (for instance the rest head). Afterwards, the volunteers entered the UV-room to check their exposure status, and to ensure that they were free of Tinopal SWN, or that there were at least no substantial quantities of Tinopal SWN (visual when using UV light) left on their skin. Consequently, they took a shower, which was installed in the UV-room and experiments container for this purpose. Furthermore, the volunteers were provided with a cream to moisturize their skin after the cleaning procedure.

3.11 Development of protocols for exposure situations

3.11.1 Dumping powder (A1)

3.11.1.1 Optimization of the protocol

The protocol for the exposure situation “dumping powder” (A1) was developed keeping in mind the agreed initial work plan (see Annex 1) and the amounts of Tinopal SWN that would be required for the experiments.
Since Tinopal SWN is discarded as waste after the experiments, carrying out a realistic exposure situation needed to be balanced with detectable exposure levels on one hand and not creating too much waste on the other hand. Based on the initial work plan, the first approach was to use three batches of 5 kg (pure Tinopal SWN

powder, or mixed with talc) that would be dumped in a specific container, leading to an amount of 15 kg per experiment. Amongst others based on the results of dustiness tests (see paragraph 3.3.2.2) it became clear that Tinopal SWN could not be mixed with another powder to generate a homogenous dusty mixture. Therefore, it was decided to use pure Tinopal SWN for the exposure situations with dusty solids. Considering the overall number of experiments (32), a total amount of approximately 500 kg of pure Tinopal SWN or its mixtures with other substances (such as talc) would be required. The use of such an amount, and thus production of such an amount of an organic pollutant, was regarded environmentally unacceptable and could not be justified. Furthermore, high costs would be involved in acquiring such an amount. During the pilot tests problems of discomfort (irritation of eyes and nose) were experienced, even though full protection was worn (including respiratory protection). Therefore, several tests and experiments were performed to establish a proper protocol for this exposure situation, taking into account the issues mentioned above.

Since it was critical that during the performance of this exposure situation detectable exposure levels would be generated, together with the reproduction of a realistic industry application, the performance of repetitive dumpings with a certain amount came up as an adequate alternative to the use of large quantities of Tinopal SWN.

It was decided to perform repetitive dumping tests on the basis of assessing the dustiness of Tinopal SWN. Tinopal SWN is not a free floating solid *(*as the one used by Brouwer et al.,1999) and not uniform in terms of particle size in the form it was purchased. Therefore, it was anticipated that after a dumping task, variance in its dustiness may occur. In this context, and also to determine the critical airborne concentration resulting in dermal exposure of the volunteers, airborne samples were collected during pilot dumping experiments (first on laboratory scale and under laboratory conditions). The sampling was performed using a Büchner type funnel and a pump, aiming to collect dust (total dust faction). The dusty powders used for these pilot dumping tests were a mixture of Tinopal SWN: Talc (25:75) and pure Tinopal SWN (non-grinded) (see Figure 3.13). The planned number of 32 consecutive dumpings was based on the respective number of actual experiments. Parameters monitored during these experiments were a) appropriate execution of the experiment following the initial dumping protocol, b) proper function (constant pressure, etc.) of the pump attached to the Büchner type funnel, c) collection of the airborne total dust fraction on the filter, d) gravimetric measurement of the filter. After these initial tests, chemical analysis of the amount of Tinopal SWN deposited on the filters was also performed. The number of collecting filters (Whatman paper) varied from three to nine per experiment in order to cover the whole process (from first dumping till the end) and be representative.

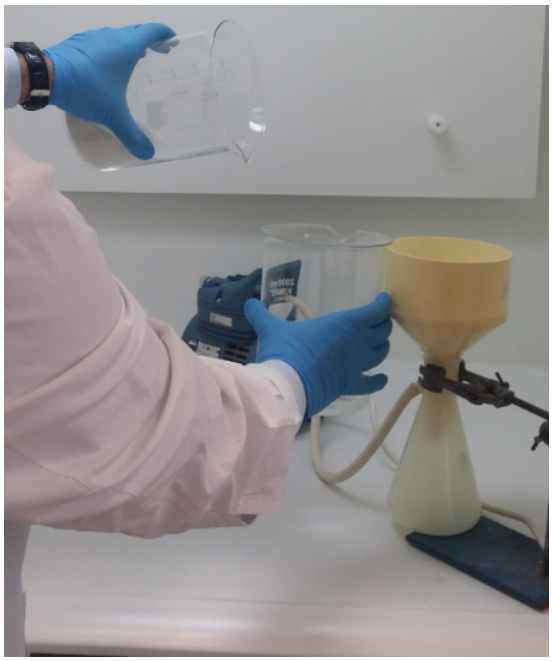


Figure 3.13 Small scale (0.5 kg) lab pilot using Tinopal SWN:talc (25:75) mixture

The first laboratory experiments for which solid Tinopal SWN was used (either pure or as a mixture) demonstrated a decrease of the dustiness after repetitive dumpings. Therefore, it was decided to use grinded Tinopal SWN instead, to increase the dustiness. Sampling was performed in such a way to reflect decrease or increase of dustiness. The outcome of these experiments showed that in case of use of Tinopal SWN-talc mixture, the composition of airborne dust is different from the originally prepared powder mixture, and that the composition of the airborne dust differed from on experiment to the other (not reproducible), which would result in the estimation of inaccurate exposure levels when a 25:75 Tinopal SWN-talc concentration would be assumed for extrapolation. Conclusively, due to the variable particle size distribution, the mixing of Tinopal SWN with other substance (e.g. talc) was not further considered.
Among the several pilot tests performed in the laboratory (inside a laboratory hood and mimicking of the container set up in larger area inside the laboratory), a ‘normal size’ pilot was performed, during which 20 sequential dumping repetitions of 2 kg pure Tinopal SWN was executed. Filter sampling was applied, that demonstrated a range of 4.7 to 9.4 mg Tinopal SWN being collected, which showed a low variability of depositing Tinopal SWN (see Figure 3.14).

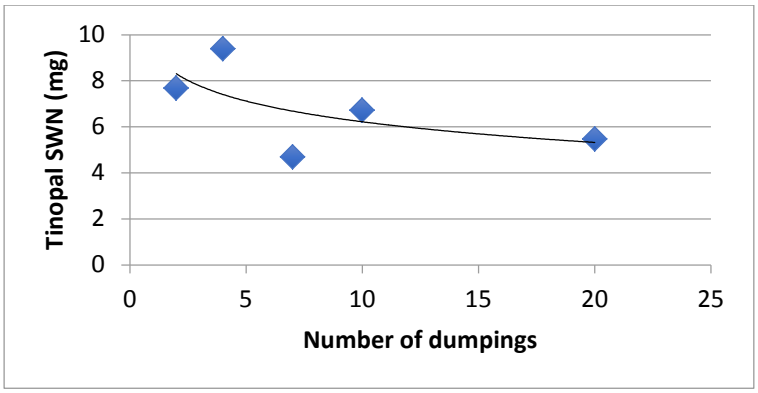


Figure 3.14 Amount of Tinopal SWN on filter versus number of dumpings

However, the volunteer started to feel uncomfortable after the sixth dumping and declared that it is too difficult to proceed. This highlighted the need for replacement of both, the respiratory mask (3M, FFP3 NR D respirator mask) and goggles used. Furthermore, the number of dumpings should be decreased, on the condition that exposure should still be substantial.
When pure Tinopal SWN was used, the dustiness declined after the first dumping and differences were observed between experiments, which was attributed to a non-homogeneous particle size distribution. Also adding 10 % of grinded Tinopal SWN to the total weight did not have any significant effect on the dustiness. Further dumping tests with small amounts of Tinopal SWN (0.5 kg) indicated that after the 11th dumping the amount of airborne Tinopal SWN remained more or less the same, indicating that the dustiness of the powder is more or less stable then.
In addition, the use of Tinopal CBS-X for the dumping powder exposure situation and possibly for the handling contaminated objects exposure situation was also assessed. The results of the dumping pilot with Tinopal CBS-X on laboratory scale demonstrated significantly less formation of airborne dust (e.g. no dust 'cloud' was observed), and therefore its use was considered inappropriate for such an exposure situation and it was discarded (see also paragraph 3.2).
After the tests on laboratory scale, a "normal scale" test was performed during which one batch of 2.5 kg was dumped 20 consecutive times (and thus dumping the 2.5 kg Tinopal SWN from one receiving container into another receiving container and vice versa). During this test filter samples and material samples were collected to measure the dustiness of the Tinopal SWN at the beginning, during and at the end of the dumping exposure situation, to get an idea of the possible variation in dustiness during the repetitive dumping of a certain amount of Tinopal SWN, and to be able to adjust the dustiness of the powder during the exposure situation if the dustiness would not be more or less the same after each dumping experiment. However, after six consecutive dumpings of 2.5 kg the volunteers experienced discomfort again and the test was stopped. The results of the dustiness tests showed low variation in dustiness between T=0 and T=6, and therefore the dustiness was considered being comparable. The dustiness of these samples was relatively lower than the dustiness of the first set of samples, which was possibly caused by differences in moisture level of the powder.
As using 2.5 kg of Tinopal SWN in repetitive mode per dumping trial would still result in the disposal of 2.5*32=80 kg of Tinopal SWN, a pilot test was performed using 1 kg of Tinopal SWN for six consecutive dumpings to assess the potential exposure level when performing this task. The results of this pilot test showed that the potential

dermal exposure levels after 6 consecutive dumpings of 1 kg were substantial and were also higher compared to the other exposure situations (see Figure 3.15). Therefore, it was decided to carry out the dumping exposure situation during the experiments with 1 kg of Tinopal SWN for six consecutive dumpings.

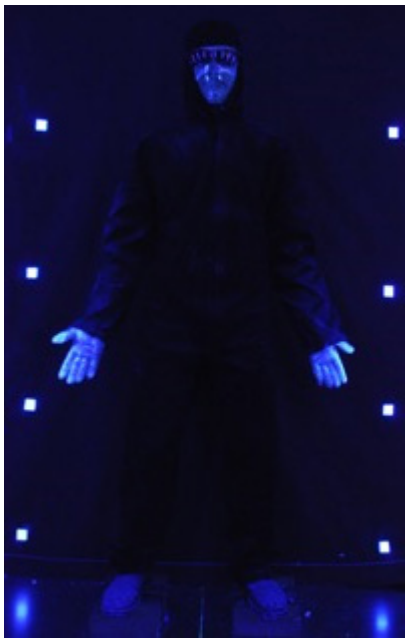


Figure 3.15 Front picture of volunteer after the last pilot test of the dumping exposure situation (six consecutive dumpings of 1 kg), showing the potential dermal exposure level

3.11.1.2 Final protocol

Before the start of the experiment the vessel is filled with 1 kg pure Tinopal SWN (not sieved) by the technical staff (not the volunteer). The volunteer takes the filled vessel from the table with both hands, moves it to the second table and dumps the content in the receiving vessel from a height of approximately 15-25 cm above the opening of the receiving vessel. After emptying, the volunteer waits for 5 seconds (some time for the dust to settle), places the empty vessel back on the first table, picks up the now filled vessel from the second table, moves back to the fist table and repeats dumping of the powder in the empty vessel. This procedure is repeated until in total six consecutive dumpings are performed. The dumping experiments took on average approximately 2 minutes.
Next to each vessel a Büchner funnel equipped with filter paper (Whatman type MN 640 d, 185 mm) connected to a pump operating at a flow of 30 L/min was placed to measure the concentration of Tinopal SWN in the air during the experiments (by means of measuring the total dust fraction). These air measurements were performed to get an idea of the variation of the concentration of dust in the air in the room, as an indication of the variation in potential dermal exposure by means of deposition between the experiments. It should be noted that the aim of collecting these air samples was not to estimate the potential air concentration during the execution of these experiments, for which the inhalable dust fraction would have to be measured instead of the total dust fraction. The filters were weighed before and

after to be able to determine the amount of Tinopal SWN deposited on them (see Figures 3.16 and 3.17).

Figure 3.16 Volunteer during performance of a dumping experiment, dermal exposure measurements by means of the WBD method

Figure 3.17 Volunteer during performance of a dumping experiment, dermal exposure measurements by means of the patch method

3.11.2 Pouring low viscosity liquid (A2) and high viscosity liquid (A3)

3.11.2.1 Optimization of the protocol

The overall set-up for these exposure situations was tested to ensure that performance of these exposure situations would result in detectable dermal exposure levels. The first pilot experiments showed substantial exposure (particularly on the hands), which was anticipated based on the nature of the task executed.
During the optimization of the protocol for the pouring of low viscosity liquid (A2) and high viscosity liquid (A3) exposure situations, particular attention was given to the training of each volunteer in the way of handling the jug (without handles), the narrow

neck containers, and the movement after each repetitive pouring of 1 L from the 5 L containers. The latter was considered especially for the 'patch method', in which the experiments are performed with bare hands, because the jug tended to get slippery after the first few pourings.
Moreover, it was decided to divide the total amount of 10 L into two batches of 5 L. During the first pilot experiment, dripping was observed, as expected, near the receiving vessel and on the workbench. In one case, the volunteer had an accident during filling of the jug, which resulted in contamination of the workbench and floor. Therefore, it was decided to cover the area with an additional layer of cardboard. Throughout the actual experiments, spillages and splashes were observed and reported.
The final protocol for pouring of a low viscosity and a high viscosity liquid remained nearly identical to the concept protocol as presented in the initial work plan.

3.11.2.2 Final protocol

As a result of the findings in the pilot experiments, the following refined procedure was used during the experiments. The volunteer opens the lid of one of the 5 L glass bottles as positioned on the first workbench, using one hand to hold the container and the other hand to unscrew the lid. Then, the volunteer pours 1 L of liquid from the container into the 1 L jug using both hands (the jug is marked with a line at the height of 1 L to be able to estimate the aforementioned volume during pouring). The height of the glass bottle above the jug is kept at approximately 10-15 cm during pouring. The volunteer closes the lid of the container using both hands. Then, the volunteer carries the 1 L jug to the second workbench using both hands and pours the content of the jug into the 10 L receiving vessel (using both hands to pour). After having the content of the jug poured to the open vessel, the volunteer waits a few seconds with the jug above the vessel to empty thoroughly. The procedure is repeated five times until the 5 L container is empty. Then the volunteer repeats the same activities when emptying the second 5 L container and transferring its content to the open vessel using the 1 L jug as described above. See Figures 3.18 and 3.19 for an impression of how the experiments for these exposure situations were performed. The exposure situation had a mean duration of 7 minutes.

Figure 3.18 Volunteer during performance of a pouring experiment, dermal exposure measurements by means of the WBD method

Figure 3.19 Volunteer during performance of a pouring experiment, dermal exposure measurements by means of the patch method

3.11.3 Rolling low viscosity liquid (B1) and high viscosity liquid (B2)

3.11.3.1 Optimization of the protocol

The protocol for the exposure situations “rolling low viscosity liquid” (B1) and “rolling high viscosity liquid” (B2) was also tested during the pilot. The roller was tested both, for its “affinity” to the liquid formulation (whether the material sufficiently absorbed the liquid) and for its uniform distribution on the panel surface. Two rollers were tested (one thick and one thin), and the thin roller was selected since this one performed better (in terms of rolling efficiency and produced exposure.
The frequency of wetting the roller was standardized during the pilot experiments. The volunteers were instructed to avoid leaving the roller “dry”. This was also supervised by the scientific personnel inside the container, who instructed the volunteers to wet the roller if necessary.
Another parameter standardized was the pressure applied by the volunteer on the panel during rolling. As it was observed during the pilot that the volunteers applied different pressures during rolling (as a behavioral characteristic), they were demonstrated how to use the roller (not too tight not too loose), to avoid discrepancies in hand pressure that would be reflected in the subsequent measured dermal exposure levels as much as possible.
Although in the work plan the use of one wooden flat surface panel was considered, it was observed that it was better to use two panels instead (increase of the surface area to be rolled). This decision was based on the observation of the first pilot tests that clearly showed a) “saturation” of the panel surface and sides by the 2 L of Tinopal SWN solution, and b) substantial run-off of the liquid from the one side of the panel. The addition of the second panel improved the situation, since less amount of liquid per panel side was applied. Using two panels also contributed in avoiding extensive spillages that would create accidental contamination.

3.11.3.2 Final protocol

Two dry wooden panels are used for each experiment. The volunteer stands in front of the first wooden panel at a distance of 0.5 m. The volunteer is not involved in the preparation of the liquid or loading procedure of the tray, which is filled by the field scientist with approximately 500 mL of test liquid. This quantity (500 mL) is used to cover one side of the panel (four sides in total). The weight of the clean tray and roller is recorded in the field registration forms, as well as the respective weight of the empty wet tray and wet roller before each new loading. The weight of the test liquid that is loaded each time in the tray is also recorded (for the calculation of the weight of the net applied liquid). The volunteer dips the roller in the tray and spreads the liquid by rolling on the panel surface, while exerting pressure on the panel with the roller, starting from the top and working down to the bottom, moving his body sideward. He rolls each strip at least one time back and forth, so as to cover part of the surface area two or three times with the roller before proceeding to the next strip. After full coverage of the surface of one side of the panel (determined by field scientific team) with 500 mL of the test liquid, the volunteer repeats the rolling at the second panel, standing at the same side, by applying another 500 mL of liquid, with the same technique. Precision work on the corners or bottom is not required. Then the volunteer moves to the other side of the installed panels. The volunteer should not touch any surface other than the roller handle. During this procedure, each time the tray is empty, the volunteer pauses for a few seconds while the field scientist refills the tray and the procedure is continued. See Figures 3.20 and 3.21 for an impression of how the experiments for these exposure situations were performed. The mean duration of the task was 20 minutes.

Figure 3.20 Volunteer during performance of a rolling experiment, dermal exposure measurements by means of the WBD method

Figure 3.21 Volunteer during performance of a rolling experiment, dermal exposure measurements by means of the patch method

3.11.4 Spraying low viscosity liquid (C1) and high viscosity liquid (C2)

3.11.4.1 Optimization of the protocol

The overall set-up as described in the protocol for the exposure situations “spraying low viscosity liquid” (C1) and “spraying high viscosity liquid” (C2) was tested during the pilot. For instance, it was needed to be tested whether the low and high viscosity liquid formulations were viscous enough to remain on the panel surface adequately after spraying. Based on optical observation of the liquid flow on the panel it was verified that the two Tinopal SWN formulations remained on the surface. Furthermore, the most suitable spray pressure, nozzle size, and spray gun settings were determined during the pilot study. With regard to the choice of the spray gun, two high volume-low pressure (HVLP) professional spray guns were tested, of which one was found fit for purpose.
Moreover, the optimal spray pressure was tested outside the container to avoid contamination of the experimental room and container applications, based on the initial work plan in which a pressure of approximately 1.5 bar was proposed. For this reason, a mobile air-compressor was utilized (capacity of 25 L) equipped with a long hose and a maximum operating pressure of 8 bar to cover these requirements. The air compressor was connected to the selected HVLP professional spray gun via the hose. The spray gun was connected to the pump ensuring no loss of pressure. Bubbling tests were performed during the pilot and occasionally during the course of the actual experiments to ensure that all parts were properly connected and a stable spray pressure was maintained during operation.
After testing on the appropriate diffusion of spray mist and applications the pressure was set at 1.8 bar for the low and high viscosity liquid exposure situations.
One of the key parameters tested during the pilot was the efficacy of the sprayer and the avoidance of blocking of the nozzle during spraying (which was of particular concern with regard to the high viscosity liquid). During the pilot the repeatability of spraying efficacy of the sprayer was secured.
As the panels were to be reused, during the pilot the wetness of the panels after the spraying operation was assessed, to avoid that reuse of the panels on the same day

might cause problems, for instance if the liquid did not stay on the surface but would run off and the possibly elevated levels of Tinopal SWN that the volunteer might be exposed to in the course of the following application. Therefore, to be on the safe side it was decided that different sets of panels were used for the two experiments performed on one day. Furthermore, the used panels were thoroughly cleaned and dried at the end of the day.
With regard to the amount used, the use of approximately 2 L for spraying, regardless of viscosity, was confirmed during the pilot. The only deviation from the initial work plan was the number of panels to be sprayed. The first tests were conducted using two panels that were positioned adjacent to each other. Each side of the panel was sprayed with 0.5 L of the liquid. However, due to ergonomic reasons and the nature of the task it was not feasible to use two panels inside the container for spraying. The natural movement of the volunteers was somehow disturbed when two panels were present in the container, as when carrying the spray gun connected to the long hose (and the latter with the air pump) accidents due to entangling with the hose could arise. And as already substantial dermal exposure levels were observed when only one panel was sprayed during the experiments, it was decided to spray one instead of two panels.

3.11.4.2 Final protocol

A dry wooden panel is used for each experiment. The volunteer stands in front of the wooden panel at 0.5 m and receives a pre-tested and calibrated spray gun ready for use from the field scientist. The volunteer is not involved in the preparation of the test formulation or the loading procedure of the canister of the spray gun. The refills of the canister of the spraying liquid are recorded in the field registration forms for each loading. The volunteer sprays the liquid on the panel surface, starting from top and working down to the bottom, with sideward movements. There was no evidence of a difference of performance between the two types of liquids. Therefore, the field scientist regulates the pressure on the air compressor set at 1.8 bar, both for the high and low viscosity liquid formulation. After sufficient coating of one side of the wooden panel (determined optically by field scientific team), the volunteer repeats the spraying at the other side of the same panel. Precision work on the corners or bottom of the panel is not required (to ensure that no unusual working positions or surface shapes are sprayed). The task should not require the subject to touch surfaces other than the spray gun. When the canister is empty, the volunteer returns the spray gun to the field scientist for a refill of the spraying liquid. After refilling the volunteer continues his task. See Figures 3.22 and 3.23 for an impression of how the experiments for these exposure situations were performed. The mean task duration was 20 minutes.

Figure 3.22 Volunteer during performance of a spraying experiment, dermal exposure measurements by means of the WBD method

Figure 3.23 Volunteer during performance of a spraying experiment, dermal exposure measurements by means of the patch method

3.11.5 Manually handling objects immersed in low viscosity liquid (D1) and high viscosity liquid (D2)

3.11.5.1 Optimization of the protocol

The overall set-up for the exposure situations “handling immersed objects using low viscosity liquid” (D1) and “handling immersed objects using high viscosity liquid” (D2) was changed after the first pilot experiments. The initial approach for this exposure situation consisted of the direct transfer of 15 metal cylinders to the opposite rack and hanged on the rack wire after being dipped in a bath. However, following this rationale, too much liquid dripped on the floor, inducing risk of sliding/falling of volunteers as well as drops falling of the cylinders during transfer that predominantly contaminated the lower parts of the body (e.g. legs), which was not considered to be

representative of common practice when performing such tasks. Consequently, it was decided to incorporate an extra mobile rack next to the dipping bath, where the cylinders were hanged after the dipping procedure to lose excess liquid, before the cylinders were transferred and deposited onto the “target” rack. Further, to avoid contamination of the floor, two plastic trays were positioned below the two racks, where the liquid dripping from the cylinders was collected. The trays were covered with paper to ensure a “smooth” landing of droplets in the tray, without them splashing to the sides.
With regard to the hanging of the cylinders on the racks, special emphasis was given to avoid extended “swinging” of the cylinders, which could result in drops of liquids being transferred onto the volunteer’s coverall and other PPE.
As in all exposure situations, the volunteers were instructed to standardize their moves. More specifically, the pattern of use of hands (one hand for dipping the objects in the bath, and two hands for the (un)hanging of the cylinders) was tested to avoid misplacement of hands (which could be easily observed for this). Furthermore, it was observed during the pilot tests that the distance between the stretched hands and torso was not always approximately 40 cm but less, which sometimes resulted in accidental contamination. Thus, particular attention was given to maintain this distance, and before each experiment the volunteers were reminded of this.
The exposure levels as observed during one pilot of the pilot tests (by evaluation of the photographs taken under UV light and by the quantified amount of Tinopal SWN by chemical analysis).
It was decided to reuse the liquid formulation used in these experiments, which was grounded on three main reasons: a) the transfer of the heavy bath out of the container for refilling was cumbersome and could result in contamination of the nearby area, while emptying and refilling the bath inside the container was considered problematic in terms of contamination of the area inside the container, b) the concentration of Tinopal SWN in the liquid formulation was proven to be stable after refilling and use (the bath was covered after the end of trial day to avoid evaporation of solvents and chemicals), and c) reuse of the liquid would avoid large amounts of liquid waste disposal. Samples of the liquid in the bath were occasionally tested during trials, and demonstrated a stable concentration of the solution.
Considering that during each experiment a certain amount of the liquid formulation was removed from the bath due to the dipping procedure, the starting level of the liquid formulation was marked inside the dipping bath (immersion tank containing 20 L of the liquid formulation), and the liquid was replenished with same liquid before the start of each experiment to avoid variation between experiments. Last but not least, the concentration of Tinopal SWN in the solution was frequently assessed to secure its stability among experiments.

3.11.5.2 Final protocol

The volunteer stands in front of the work bench at a distance of 15-25 cm from where the dipping bath is placed. The volunteer picks the first cylinder from the table using one hand, grabbing it at the hook at the side of his body, thus avoiding to extend his hand towards the bath. Holding the cylinder with one finger in the hook, the volunteer dips the cylinder into the bath up to the upper hook of the object for 3 seconds. After dipping, the volunteer lifts the cylinder out of the liquid, but holds the cylinder above the dipping bath to drip for 5 seconds. The volunteer continues to hold the cylinder with one hand from the non-contaminated hook, steps away from the workbench

while turning his body towards the rack next to the dipping bath and hangs it with the non-contaminated hook on this rack. After all 15 cylinders are dipped and hanged on the rack to drip, starting with the cylinder that was dipped first, the volunteer uses both hands to unhang the cylinder from the rack by grabbing it at both hooks and carrying it horizontally to the rack that is placed at the opposite side of the container. During carrying, the volunteer must have his hands stretched in front of his torso at a distance of ca 40 cm to avoid self-contamination. The cylinder is then hanged at the rack keeping consistent the hand he uses. This process is repeated for all 15 cylinders.
After having all cylinders hanged the volunteer uses both hands to unhang the first cylinder from the rack by grabbing it at both hooks and carrying it horizontally back to the workbench to place it in its original position. During this step the volunteer has his hands stretched in front of his torso at a distance of ca 40 cm to avoid self-contamination. After placing the first cylinder on the workbench he repeats this step for the remaining cylinders. The cylinders are placed near the edge of the table with care, to prevent (over)contamination of the sleeves during positioning. The cylinders are put down in a horizontal position one by one, next to one another and left there until the final one is placed. During the placement of cylinders to the edge of table, sleeves are not rolled up or covered to avoid their contamination. See Figures 3.24 and 3.25 for an impression of how the experiments for these exposure situations were performed. The mean duration of the experiment was 10 minutes.

Figure 3.24 Volunteer during performance of an immersion/dipping experiment, dermal exposure measurements by means of the WBD method

Figure 3.25 Volunteer during performance of an immersion/dipping experiment, dermal exposure measurements by means of the patch method

3.11.6 Handling contaminated objects (E)

3.11.6.1 Optimization of the protocol

The handling contaminated objects exposure situation (E) was developed to transferring plates contaminated with Tinopal SWN from one rack to another rack.
The first critical issue to solve was how to uniformly distribute Tinopal SWN on each of the plates and more or less the same amount of Tinopal SWN on each plate to ensure repeatability of the experiments.
Initially the idea was to use an impinger as delivery system for the Tinopal SWN as was also applied by Brouwer et al. (1999). This delivery system should not be fragile and be somehow compact, and therefore two types of impingers were tested in pilot experiments. For the contamination process a ‘contamination box’ was developed (see Figures 3.26 and 3.27). This box was constructed using acrylic glass, and had an opening at one side to be able to place the plates in the box and remove them afterwards. The box was air-tight, and there was an inlet for air at the top, where the outlet of the impinger was connected to a specific nozzle. Furthermore, there was an outlet at the bottom of the box, were a filter or a bottle with water based solution was placed to make sure that excess Tinopal SWN was not blown into the room. Before the start of the pilot experiments, the scientific personnel loaded plates using the impinger with a pump attached to it.
With regard to the type of plates used, glass plates and plexiglass plates were considered. Both types of plates were tested, during which difficulties in the loading process were observed, but foremost Tinopal SWN had less affinity to the surface of the glass plates compared to the plexiglass surface. Even after treatment of the glass plates with hydrochloric acid (as reported by Brouwer et al. (1999)), the affinity of Tinopal SWN to glass plates was not enhanced, and therefore it was decided to proceed with the plexiglass plates.
Furthermore, the optimal composition of the Tinopal SWN to be used for contamination of the plates was tested. The use of non-grinded Tinopal SWN (5 g or 10 g) resulted in an uneven distribution of the substance, that was not reproducible, and with low loading capacity compared to the total amount of Tinopal SWN in the

impinger. Use of sieved Tinopal SWN (at 38 μm) performed better than the non-sieved counterpart, but still resulted in an uneven contamination of the plates (in Table 3.4 indicative results are shown).

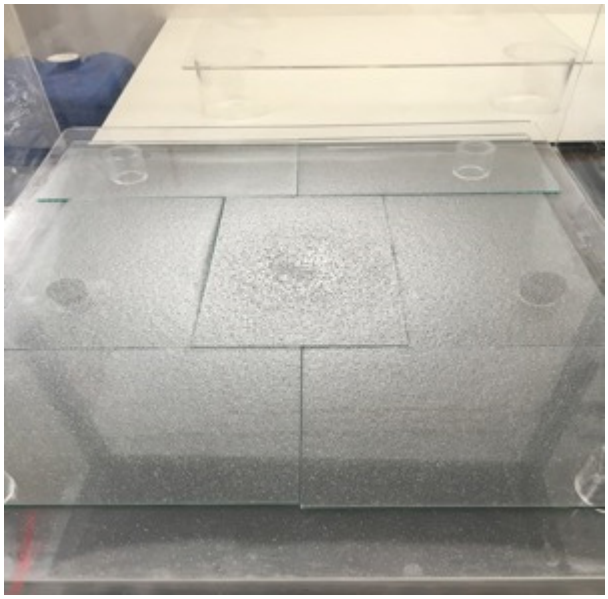


Figure 3.26 Tinopal SWN deposition onto plates inside the plexiglass construction

Figure 3.27 Pilot of handling contaminated objects using the 'contamination box' with Tinopal SWN loaded plates

Table 3.4 Indicative results of pilot testing the procedure for contamination of plates using grinded Tinopal SWN (use of impinger and contamination box for contamination)

Plates *	Tinopal SWN load (mg/cm^2)	Tinopal SWN on plate (g)
Plate 1	1.23	0.46
Plate 2	1.16	0.43
Plate 3	1.90	0.71
Plate 4	2.32	0.87
Plate 5	0.81	0.30
Plate 6	1.02	0.38
Plate 7	6.60	2.48

* Surface area of plates was 375 cm^2

As after all options of loading of the plates in the contamination box did not result in an even and reproducible contamination of the plates, it was proposed to try an alternative approach. The plate was positioned on a balance, and Tinopal SWN was applied to the plate using a sprinkler on which a fine net was mounted. The sprinkler was loaded with a certain quantity of sieved Tinopal SWN (at 38 µm), and with the balance the amount of Tinopal SWN loaded on the plate was measured (see Figure 3.28). The pilot tests with this alternative method showed good results and proved to be less time consuming and ensured repeatability of loading of each plate, and therefore this procedure was chosen. Before each experiment, plates were loaded with 1 g of sieved Tinopal SWN each. And since the racks made to hold the plates had the capacity of 12 plates and in view of reaching a desirable potential dermal exposure level during the experiments, it was decided to handle 12 contaminated plates during each experiment (thus 12 g of Tinopal SWN was used during each experiment in total).

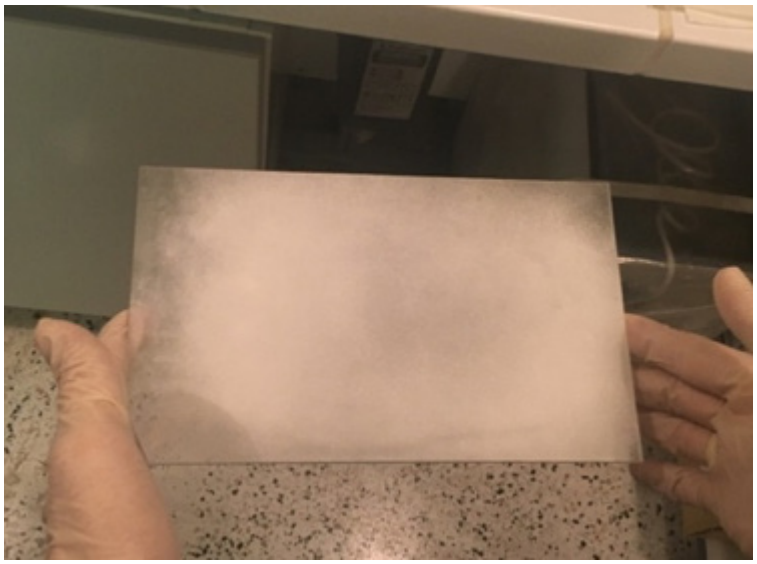


Figure 3.28 Loaded plexiglass plate with 1 g of sieved Tinopal SWN

3.11.6.2 Final protocol

The plates are loaded by the scientific staff before the start of the experiment, and placed in rack 1. The volunteer takes a single plate from rack 1 with both hands (starting from bottom), holds it in a vertical position and gently knocks the plate twice against the bar on the front of the first workbench, and then carries it to the second workbench. During carrying, the volunteer uses both hands to hold the plate horizontally, and must have his hands stretched in front of his torso at a distance of ca 30-40 cm to avoid accidental self-contamination. At the second workbench, the volunteer also knocks the plate twice on the existing bar and then places the plate in an upright position in rack 2 using both hands. This procedure is repeated for each of the 12 plates, with the first plate positioned at the back of rack 2 and subsequent plates in front, respectively. See Figures 3.29 and 3.30 for an impression of how the experiments for this exposure situation were performed. This task's average duration was 3 minutes.

Figure 3.29 Volunteer during performance of a handling contaminated objects experiment, dermal exposure measurements by means of the WBD method

Figure 3.30 Volunteer during performance of a handling contaminated plates experiment, dermal exposure measurements by means of the patch method

4 Validation chemical analysis

4.1 General

The analytical method for the determination of Tinopal SWN in the various sampling matrices as used in the study was developed and validated before the start of the actual experiments. Additional validation experiments were performed after the end of the field trial period. These additional experiments addressed the extension of the linear range of the calibration curve (CC) to ultra-low concentration levels in combination with recovery tests at minimal fortification levels. This allowed for revised and substantially lowered analytical LOQ and method LOQ levels, respectively, which were beneficial for both the analytical method and the presentation of the results of the study, as this approach led to a higher number of samples with an exposure value above LOQ.

4.2 Chemical analytical method

4.2.1 Test substance

Tinopal SWN is a known fluorescent agent. In this study Tinopal SWN was used for the preparation of analytical standard solutions (stock and working standard solutions, calibration standards and quality control solutions). The same substance was used as test substance to prepare the fortification solutions. The test substance (solid form, provided as a powder in a wooden cylindrical vessel by the manufacturer) was stored in a cool and dry place according to the instructions provided by the supplier.

4.2.2 Technique chemical analysis

Tinopal SWN is an organic compound, whose structure bears an extensive conjugation system that renders it fluorophore and consequently is suitable for analysis with fluorescence detector (FD). Therefore, UFLC-FD (Ultra-Fast Liquid Chromatography coupled with Fluorescence Detector) was chosen as analytical technique. In addition, LC-MS was used in preliminary runs to verify and confirm the identity of Tinopal SWN. One clear sharp chromatographic peak is obtained from each sample run, which indicates that the analyte can be identified without any doubt (see Figure 4.1).

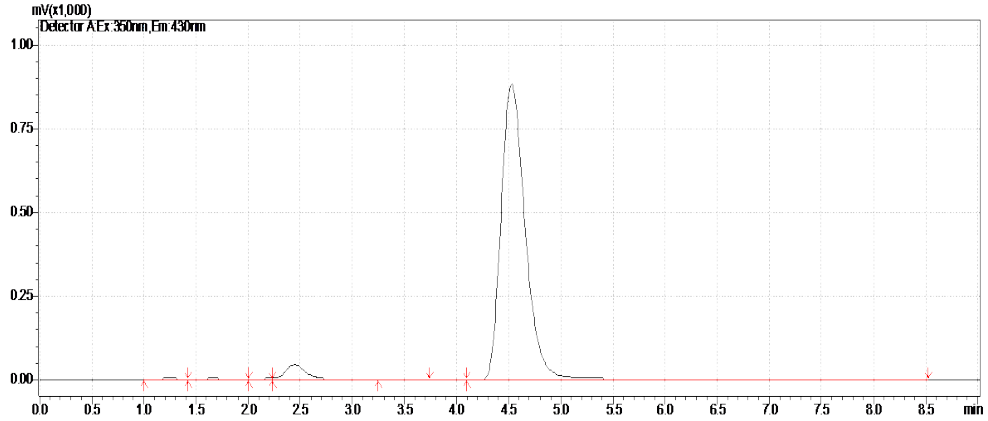


Figure 4.1 Tinopal HPLC-FD chromatogram

4.2.3 Instrumentation chemical analysis

The instrumentation used for the chemical analysis is the UFLC-DAD-FD/MS: A Shimadzu LCMS-2010 EV Liquid Chromatograph Mass Spectrometer equipped with a SIL-20A prominence autosampler, a SPD-M20A diode array detector and a RF-10AXL fluorescence detector. The latter is coupled in series with a mass selective detector equipped with an atmospheric pressure ionization source usable as either ESI or APCI interface. LC-MS solution version 3.0 software is used for data acquisition and data analysis.

4.3 Sample preparation and extraction

For the extraction of the validation samples, the dosimeters were put into screw cap plastic polyethylene pots. For the actual field samples this step was already carried out in the field phase following the procedure for collection of samples while undressing the volunteer.
After removing pots from storage (ambient temperature conditions) the appropriate amount of extraction solvent (methanol) was added in each jar to ensure that they are almost covered by the solvent (at least at ¾ level). The size of the extraction pots and the respective extraction volume is shown in Table A1.2 in Annex 1.
In case of hand contamination for which the hand wash method was used, the extraction was carried out in the field right after the completion of the experiment using 3 x 500 mL aliquots of ethanol/water mixture 1:1 v/v. The three extracts were combined in the field into one pot. Although, in case of hand wash samples, the extraction was already carried out in the field, these ethanol/water extracts were treated the same way as the rest of the samples (shaking in combination with an ultrasonic bath as described below), and were further diluted with HPLC methanol if needed and injected to LC (in most cases using a 1 µL injection volume). Dermal recoveries from hands as well as from forehead are addressed in paragraph 4.5 below.
For all samples except the hand wash samples the extraction solvent was methanol high performance liquid chromatography (HPLC) grade. For all samples the pots containing the added extraction solvent were placed on a platform shaker and were extracted for 30 min in ambient temperature at 180-200 rpm. The rpm value setting

could be modified slightly according to the overall shaker load (weight), in order to ensure stability of the pots placed on it. After the shaking of the pots, the pots were placed in an ultrasonic bath for 2 min to enhance extraction efficiency.
The extracts were transferred into a chromatography vial and injected to LC. In cases where further dilution was required (either for estimated high contaminated samples, for samples that fell over the calibration curve range after the first LC run, or for validation or field spike samples for which the expected concentration could be calculated in advance according to the fortification level and the extraction solvent volume added) this was done with HPLC methanol.
In general, the injection volume was 1 μL. However, in case a sample extract was anticipated to have a very low Tinopal SWN concentration, the injection volume was adjusted to more than 1 μL (usually 2-5 μL), as an equivalent of an indirect condensation of the sample without using rotary evaporation or other concentration techniques that unavoidably pose the risk of having a potential amount loss. This modification in the injection volume was proportionally considered in the algorithm used for the calculation of results.
The amounts and ratios used for the extraction of actual samples and for the validation samples are shown in Tables A1.1 and A1.2 in Annex 1.

4.4 Method validation

4.4.1 Validation parameters and criteria

The Tinopal SWN reference standard was used to prepare fortification solutions for all matrices. Two primary stock solutions of 20.000 μg/mL each were prepared by weighing per each stock 2 g Tinopal SWN into a 100 mL Class A volumetric flask and diluting to volume with HPLC methanol. Primary stock solutions of 10.000 μg/mL were also prepared. Analytical standard solutions, namely substocks, working solutions, calibration solutions, quality control solutions and fortification solutions were prepared and renewed promptly by successive dilutions with methanol. As is described below, addition of matrix extracts was sometimes included during method development and validation. Stability of stock solutions and working solutions was examined and confirmed (see paragraph 4.5). Quality control solutions were freshly made on a weekly basis. All analytical solutions were stored in a freezer and were checked periodically by optical observation of marking on the flasks for identifying any possibility of solvent evaporation (which did not occur).
The quantification method was validated for linearity, accuracy and precision, specificity and sensitivity. Criteria of acceptance for the validation were the following:

- Recovery of Tinopal SWN from spiked matrices between 80-120%;
- Recovery rates (expressed as relative standard deviation (RSD)) lower than 20% as indication for repeatability and reproducibility;
- Regression coefficient higher than 0.99 as indication for linearity.

Calibration curves were constructed to cover the expected range of concentrations for Tinopal SWN as active substance (i.e. minimal and maximal expected Tinopal SWN concentration per sample extract). Calibration curves included up to eight concentration levels per studied curve range, with three data points per concentration level (calibration level replicates).

For the validation procedure, the limit of quantification (LOQ) was the reference starting point for the fortification scheme that was followed for recovery tests. This LOQ, defined as the method LOQ, corresponds to the amount (µg) of the lowest spiking level per dosimeter for which the analyte Tinopal SWN is reliably and reproducibly extracted and quantified with an acceptable recovery (80-120% and RSD<20%). With this approach, the method LOQ values were determined as 0.00011 µg/cm^2 for Tyvek and cotton coverall fabrics, 0.00014 µg/cm^2 for cotton gloves, 0.00104 µg/cm^2 for wipes and 0.029 µg/cm^2 (equivalent to 0.027 µg/mL handwash solution) for hand wash, respectively. On this basis recovery performance was studied at three levels (level 1, 2, 3), corresponding to LOQ, 10xLOQ and 100xLOQ, for which the fortification rates are indicated in Table 4.1.

Table 4.1 Fortification scheme of Tinopal SWN in relation to LOQ of each matrix

Matrix and LOQ$_{(matrix)}$	**Level 1**		**Level 2**		**Level 3**	
	Forti-fication rate	Amount spiked (*µg)*	Fortifi-cation rate	Amount spiked (*µg)*	Fortifi-cation rate	Amount spiked (*µg)*
Tyvek fabric (coverall, patches) *LOQ$_{(tyv)}$=0.00011 µg/cm^2*	LOQ$_{(tyv)}$	0.1	10×LOQ$_{(tyv)}$	1	100×LOQ$_{(tyv)}$	10
Cotton fabric (coverall, patches) *LOQ$_{(cot)}$=0.00011 µg/cm^2*	LOQ$_{(cot)}$	0.1	10×LOQ$_{(cot)}$	1	100×LOQ$_{(cot)}$	10
Cotton glove *LOQ$_{(glo)}$=0.00014 µg/cm^2*	LOQ$_{(glo)}$	0.1	10×LOQ$_{(glo)}$	1	100×LOQ$_{(glo)}$	10
Wipe *LOQ$_{(glo)}$=0.00104 µg/cm^2*	LOQ$_{(wip)}$	0.1	10×LOQ$_{(wip)}$	1	100×LOQ$_{(wip)}$	10
Hand wash solution *LOQ$_{(hws)}$=0.029 µg/cm^2 (0.027 µg/mL)*	LOQ$_{(hws)}$	40	10×LOQ$_{(hws)}$	400	100×LOQ$_{(hws)}$	4000

The recovery efficiency and stability parameters were tested for both the extracts and the dry matrices during the initial validation study, of which the fortification and analysis scheme is presented in Table 4.2. For the fabric dosimeters (i.e. Tyvek fabric, cotton fabric, cotton glove, wipe) the stability study was performed at levels addressed as low/medium/high corresponding to 1000 x LOQ, 10000 x LOQ and 100000 x LOQ, respectively.
The "analytical LOQ" value, addressing the extract concentration that produces a sharp, clear and well-shaped quantifiable peak of analyte, was also considered. Among the various approaches that are found in literature to establish the analytical LOQ, the more conservative approach that considers the analytical LOQ to coincide with the lowest level of the calibration curve was adopted. As the analytical LOQ refers to a solution concentration (Tinopal SWN extract), it is expressed in concentration units, and consequently the analytical LOQ value is not matrix-dependent. It is directly related to the type, specifications and capacity of the analytical instrumentation used, of which the detector performance of the instrument is most important. With regard to the method validated in this study, the analytical LOQ was 0.001 µg/mL.

Table 4.2 Synopsis of validation scheme for determination of recovery from and stability on the matrix or in the extract

Stability testing dry on matrix					**Stability testing extracts**	
Spiking level (day 0)	**Storage dry spike (days)**	**Extrac-tion**	**Ana-lysis**	**Remarks**	**Storage extract (days)**	**Analysis**
Blank	0 days	day 0	day 0	From the derived extract 5 aliquots are kept, of which the 1 is analyzed the same day and the rest 4 are stored for testing the stability of the matrix extract	1 / 3 / 7 days	day 1 / 3 / 7
Low	0 days	day 0	day 0	As above	1 / 3 / 7 days	day 1 / 3 / 7
Me-dium	0 days	day 0	day 0	As above	1 / 3 / 7 days	day 1 / 3 / 7
High	0 days	day 0	day 0	As above	1 / 3 / 7 days	day 1 / 3 / 7
Blank	0 days	day 0	day 0	Discard extract after analysis		
Low	0 days	day 0	day 0	Discard extract after analysis		
Me-dium	0 days	day 0	day 0	Discard extract after analysis		
High	0 days	day 0	day 0	Discard extract after analysis		

Stability testing dry on matrix					Stability testing extracts	
Spiking level (day 0)	**Storage dry spike (days)**	**Extrac-tion**	**Ana-lysis**	**Remarks**	**Storage extract (days)**	**Analysis**
Low	0 days	day 0	day 0	Discard extract after analysis		
Me-dium	0 days	day 0	day 0	Discard extract after analysis		
High	0 days	day 0	day 0	Discard extract after analysis		
Blank	1 day	day 1	day 1	Discard extract after analysis		
Low	1 day	day 1	day 1	Discard extract after analysis		
Me-dium	1 day	day 1	day 1	Discard extract after analysis		
High	1 day	day 1	day 1	Discard extract after analysis		
Blank	3 days	day 3	day 3	Discard extract after analysis		
Low	3 days	day 3	day 3	Discard extract after analysis		
Me-dium	3 days	day 3	day 3	Discard extract after analysis		
High	3 days	day 3	day 3	Discard extract after analysis		
Blank	7 days	day 7	day 7	Discard extract after analysis		
Low	7 days	day 7	day 7	Discard extract after analysis		
Me-dium	7 days	day 7	day 7	Discard extract after analysis		
High	7 days	day 7	day 7	Discard extract after analysis		
Blank	>7 days (until neces-sary)			Control sample		
Low	>7 days (until neces-sary)			Control sample		
Me-dium	>7 days (until neces-sary)			Control sample		

Stability testing dry on matrix					Stability testing extracts	
Spiking level (day 0)	**Storage dry spike (days)**	**Extrac-tion**	**Ana-lysis**	**Remarks**	**Storage extract (days)**	**Analysis**
High	>7 days (until neces-sary)			Control sample		

4.4.2 Linearity

For the quantification of the sample extracts and the investigation of the detector's linearity, three sets of calibration curves were constructed at different points in time during the project, covering in total the range of 0.001 µg/mL to 10 µg/mL. In detail, the first two sets of calibration curves covered the range of 0.01-1 µg/mL and 1-10 µg/mL. The third calibration curve set, as made during the additional validation experiments, covered an even lower range, varying from 0.001 to 0.01 µg/mL. All calibration curve sets exhibited good linearity (R^2 ≥0.997) and factors of slope and intercept were highly comparable between all sets, indicating that they were not significantly affected or shifted throughout duration of the experimental phase of the project (see Figures 4.2, 4.3 and 4.4, and Table 4.3). The data from the calibration curves were not pooled in sum to produce a combined calibration curve, but instead the three curves were used separately and concurrently - at the specific ranges that each one addressed - in order to ensure the best linearity for those respective ranges.
For the Tinopal SWN analyses, the detector showed a linear response at the nominal concentrations of interest. The calibration curves were checked on a daily basis by injection of calibration solutions (QC) and no shift was observed.

Table 4.3 Statistical parameters of first, second and third set of calibration curves

	1st set		2nd set		3rd set	
Range	0.01-1 µg/mL	1-10 µg/mL	0.01-1 µg/mL	1-10 µg/mL	0.001-0.01 µg/mL	0.01-0.1 µg/mL
slope	1660248	1546076	1704240	1674718	1735477	1661819
intercept	8419	67586	7304	28822	0.3568	-1029
R^2	0.9998	0.9997	0.9994	0.9999	0.9975	0.9996

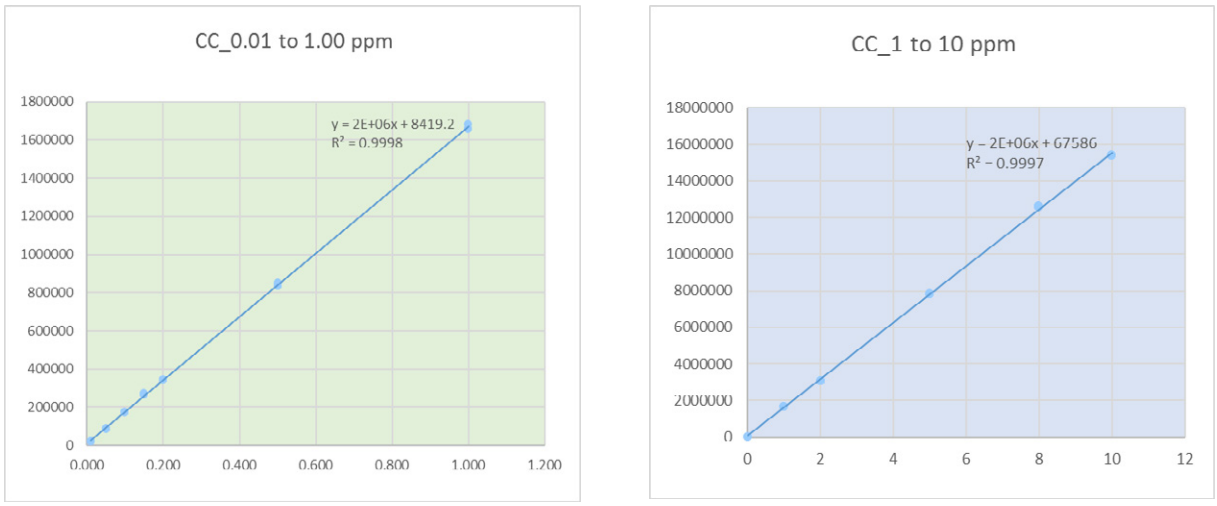


Figure 4.2 First set of calibration curves (15/3/2016)

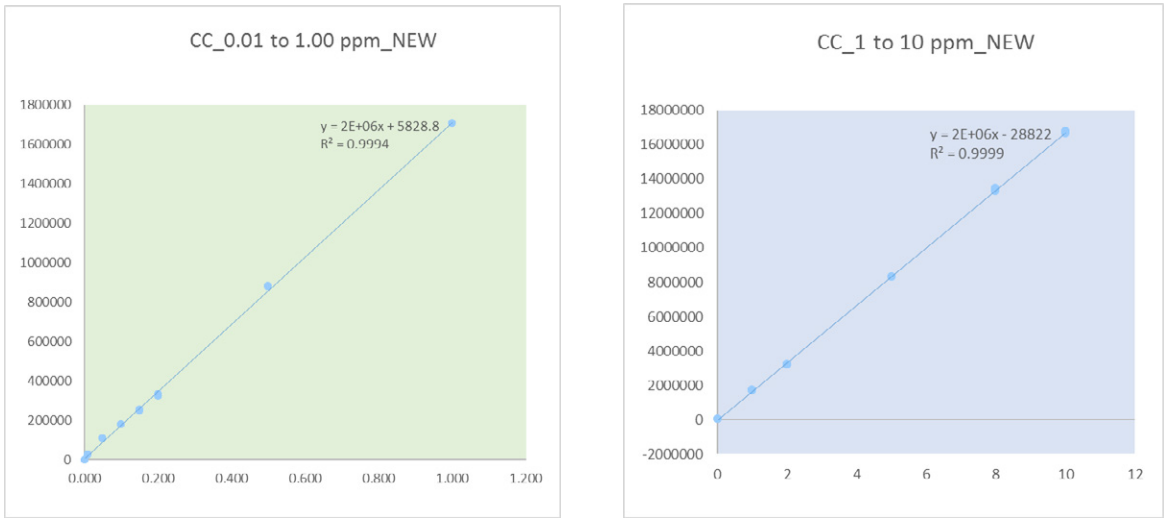


Figure 4.3 Second set of calibration curves (6/12/2016)

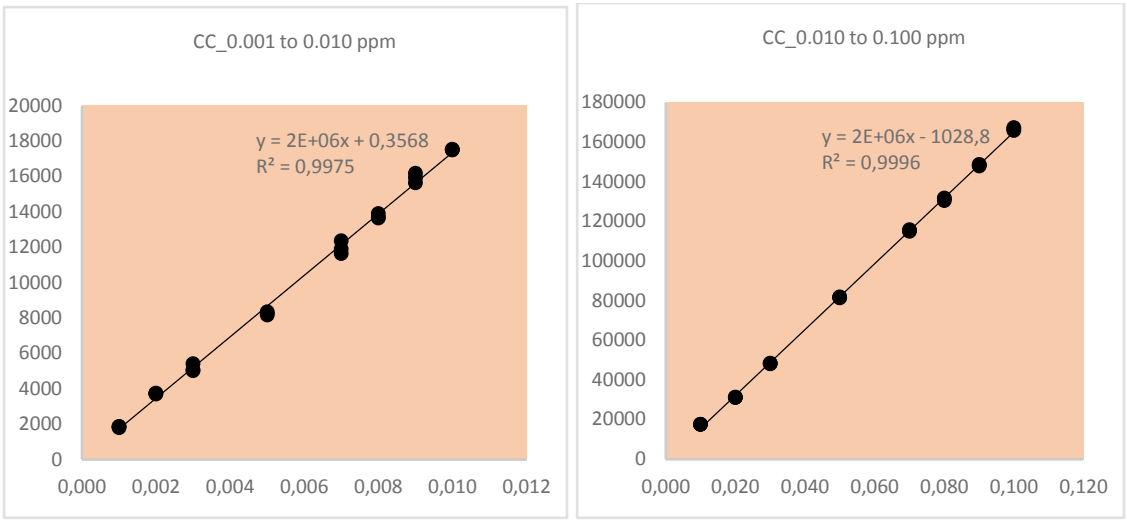


Figure 4.4 Third set of calibration curves (16/04/2018)

4.4.3 Accuracy and precision

For the determination of accuracy and precision of the method the recovery rates were used. Samples of blank control matrices were spiked at four concentration levels (including zero) on the same day and extracted to determine repeatability (based on three replicates).
Similarly, spiking of replicates was performed between days to determine reproducibility. Stability of dry matrices and extracts was also assessed by a detailed scheme as presented in Table 4.2.
The acceptable range for percentage of recovery of all analyses in experiments of exposure measurements is 70-120% according to respective OECD protocols (OECD, 1997), but in this study 80-120% recovery was set as acceptable. Although no certified reference materials were used, a scientifically accepted approach for method validation was applied. The accuracy assessment was based on the recovery values obtained from matrices fortified with the analyte of concern. The average recovery rates per matrix at all levels ranged between 85-101%. In Table 4.5 a detailed presentation of figures per level and per matrix is given, which all fall inside the range of 80-120% (acceptance criterion). Thus, the recovery rates fulfill the requirements for analytical methods to determine residues.
The determined recovery rates per fortification level for Tinopal SWN demonstrate the accuracy of the method. For Tinopal SWN a set of three replicates at three fortification levels was performed per sample matrix. The RSDs from all sample matrices at all fortification levels are used as criteria for repeatability. These RSDs (per level and per matrix) ranged from 1% to 6% (see Table 4.4) demonstrating a very good precision of the method.

Table 4.4 Validation results for the recovery rates

Matrix	Fortification level	Average recovery per level (%) (range)	SD * per level	RSD * per level (%)	Average recovery all levels (%)	SD all levels	RSD all levels (%)
Tyvek	LOQ	85 (81-91)	0.05	6	92	0.06	6
	10 x LOQ	96 (91-102)	0.05	6			
	100 x LOQ	93 (91-95)	0.02	2			
Cotton	LOQ	86 (82-87)	0.04	4	90	0.03	4
	10 x LOQ	93 (93-94)	0.01	1			
	100 x LOQ	90 (89-90)	0.01	1			
Gloves	LOQ	87 (84-92)	0.04	5	93	0.07	7
	10 x LOQ	92 (90-94)	0.02	2			
	100 x LOQ	101(100-102)	0.01	1			

Matrix	Fortification level	Average recovery per level (%) (range)	SD * per level	RSD * per level (%)	Average recovery all levels (%)	SD all levels	RSD all levels (%)
Wipes	LOQ	91 (86-97)	0.05	6	93	0.02	3
	10 x LOQ	93 (92-94)	0.01	1			
	100 x LOQ	94 (92-96)	0.02	2			
Hand wash	LOQ	90 (97-94)	0.04	4	92	0.02	3
	10 x LOQ	92 (90-93)	0.02	2			
	100 x LOQ	93 (92-94)	0.01	2			

* SD = standard deviation, RSD = relative standard deviation

4.4.4 Recovery from dosimeters after spiking with the test formulation

The fortification solutions used for the recovery tests in all levels were analytical solutions of Tinopal SWN in solvent (methanol). The use of this type of fortification solution (i.e. in analytical solvent) was considered the optimum choice to ensure both accuracy in the solution concentration as well as total absorption of the analyte spiked on the matrix (as a result of the complete evaporation of the solvent). It also allowed for keeping the fortification volume at a fixed value of 0.5 mL, regardless of the level of fortification, by preparing the appropriate concentrations of Tinopal SWN solutions, which cannot be done with the actual field solutions due to precipitation issues. However, apart from the above approach and in order to mimic real field conditions as well as to confirm recovery efficiency with the actual test solution as fortification liquid, some additional recovery tests were carried out using the high and low viscosity solutions. In these cases, the lowest spiking volume possible (0.5 mL) was applied. The results as shown in Table 4.5 are in line with the determined recoveries after spiking with analytical solutions (see Table 4.4 above), with average recoveries of 92-98% (RSD <2%).

Table 4.5 Recovery from dosimeters after spiking of test liquid

Matrix	Test liquid *	Test liquid concentra-tion (µg/mL)	Spiking volume (mL)	Fortification amount (µg Tinopal SWN)	Average recovery (%) (range)	SD **	RSD (%) **
Tyvek	LV	2000	0.5	1000	98 (97-98)	0.007	1
Cotton	LV	2000	0.5	1000	94 (92-95)	0.015	2
Gloves	LV	2000	0.5	1000	93 (92-93)	0.007	1
Wipes	LV	2000	0.5	1000	92 (90-94)	0.023	2

* LV = low viscosity liquid
** SD = standard deviation, RSD = relative standard deviation

4.4.5 Dermal recovery results

For the determination of the most efficient way to remove the Tinopal SWN from the hands (hand wash procedure) and the forehead (head wipe procedure) of the volunteers (determination of dermal recovery rates), several tests were performed.
With regard to the hand wash procedure, due to health and safety issues usual laboratory organic solvents such as acetone or methanol could not be used. Therefore, a solution of ethanol/water 1:1 v/v was tested for the hand wash procedure, since Tinopal SWN dissolves very well in this solution. This sampling solution was proved to be very efficient, showing dermal recovery rates of 96 %, 94 % and 95 % in the hand wash samples collected after spiking with an ethanolic solution of Tinopal SWN and the low viscosity and high viscosity test formulations, respectively. Most of the Tinopal SWN seemed to be removed during the first wash, based on optical observation inside the UV-room and the subsequent chemical analysis of the samples (see Table 4.6).
With regard to the wiping procedure, for the extraction of Tinopal SWN, the forearms were considered to serve as a good alternative for the forehead for applying the spiking solution. The spiking solution (20 µL) was applied on the inner side skin underarm of the volunteer (field scientist were the volunteers for this test) and spread along the lower arm (ca 10 cm) with a small glass plate (microscope slide). This piece of glass was also extracted and analyzed in order to be able to determine the amount spiked on the arm exactly and account for the recovery rate. After spreading, the skin of the forearm was allowed to dry for 3 min before hygienic wet wipes were used to remove the active substance from the skin. Spiking the skin with pure Tinopal SWN (powder) was more challenging. Initially, spiking was conducted by putting a minimal amount of Tinopal SWN on the fingertip of the person performing the spiking. This procedure was chosen since application of an accurate and reproducible low amount of dust was not feasible, and application of larger amounts would lead to

“saturation” of the specific small area of the forearm and subsequent low recovery of Tinopal SWN when wipes were applied.

Table 4.6 Dermal recovery from hand wash extracts after spiking hands with test liquid

Hand spiking / extraction	Test liquid	Test liquid concentration (µg/mL)	Spiking volume (mL)	Fortification amount (µg Tinopal SWN)	Recovery in wash extracts (%)			Total recovery (%)
					1st	2nd	3rd	
I	Tinopal SWN solution (in ethanol)	2000	0.2	400	95%	2%	<LOQ	96%
II	Low viscosity liquid	1955	0.2	391	92%	1%	<LOQ	94%
III	High viscosity liquid	2063	0.2	413	93%	1%	<LOQ	95%

To solve the encountered issue of low and possibly not reproducible dust quantities, it was decided to apply an ethanol solution on the arm, let the ethanol evaporate and then apply wipes to recover the analyte Tinopal SWN. This procedure was implemented as a surrogate for spiking dust directly and ensured repeatability of recovery results. Hygienic wet wipes (commercially available baby hankies containing water ethanol solution: wet hankies) proved to be very efficient for removal of Tinopal SWN from the skin by means of wiping, yielding recoveries of 90 %, 93 % and 89 % in the wipe extracts collected after spiking with an ethanolic solution of Tinopal SWN and the low viscosity and high viscosity test formulations, respectively (see Table 4.7).

Table 4.7 Dermal recovery from wipe extracts after spiking forearms with test liquid

Dermal spiking / wiping	Test liquid	Test liquid concentration (µg/mL)	Spiking volume (mL)	Fortifi-cation amount (µg Tinopal SWN)	Recovery on wipe (%)				Total recovery (%)
					1st	2nd	3rd	plate residue	
I	Tinopal SWN solution (in ethanol)	2000	0.02	40	85	4	<LOQ	1	90
II	Low viscosity liquid	1955	0.02	39	88	4	<LOQ	1	93
III	High viscosity liquid	2063	0.02	41	84	4	<LOQ	1	89

4.5 Stability testing

Some fluorescent markers are known to be very instable in solution. Therefore, an important part of the method validation was the performance of stability tests. The stability of the tracer substance on matrices and in extracts was assessed and determined over a period of time that reached a week taking into account the maximum necessary period of storage of samples and extracts after execution of the experiments. From the results as presented in Table 4.8 it is evident that all the dry matrices as well their extracts are stable and provide credible results for at least one week. Based on past experience with Tinopal SWN as a tracer substance it is also assumed that the samples and extracts can be stable over a longer period of time.

Table 4.8 Results of stability testing for all dosimeters (dry matrix and extracts)

Dosimeter	Level	Sample type	Average recovery (range) (%)	SD *	RSD (%) **	Average recovery per level (%)	SD per level	RSD per level (%)
Tyvek	Low	Dry matrix	92 (86-95)	0.03	3	91	0.03	3
		Extract	90 (88-91)	0.02	2			
	Medium	Dry matrix	92 (90-97)	0.03	3	91	0.04	4
		Extract	87 (84-90)	0.03	3			
	High	Dry matrix	92 (85-98)	0.05	5	90	0.05	5
		Extract	87 (93-93)	0.05	6			
Cotton	Low	Dry matrix	96 (94-99)	0.02	2	94	0.03	3
		Extract	90 (90-91)	0.00	0			
	Medium	Dry matrix	95 (89-106)	0.06	6	93	0.06	6
		Extract	89 (88-90)	0.01	1			
	High	Dry matrix	93 (89-98)	0.03	4	92	0.03	3
		Extract	91 (91-92)	0.01	1			
Gloves	Low	Dry matrix	95 (86-100)	0.05	5	96	0.04	4
		Extract	99 97-100)	0.02	2			
	Medium	Dry matrix	94 (88-98)	0.05	5	94	0.04	4
		Extract	94 (92-96)	0.02	2			
	High	Dry matrix	95 (88-100)	0.06	6	93	0.06	6
		Extract	90 (89-91)	0.01	1			
Wipes	Low	Dry matrix	94 (93-96)	0.01	1	93	0.02	2
		Extract	91 (90-91)	0.00	0			
	Medium	Dry matrix	95 (92-96)	0.02	2	94	0.02	2
		Extract	94 (92-95)	0.02	2			
	High	Dry matrix	93 (91-97)	0.02	2	93	0.02	2
		Extract	94 (93-94)	0.00	0			

* SD = standard deviation, RSD = relative standard deviation

The stability of the stock solution (two replicate primary stocks) as well as of an indicative working solution was also tested and found to be valid for at least one month and two weeks, respectively, as shown in the Tables 4.9 and 4.10 below.

Table 4.9 Stability results for stock solutions 1 and 2 *

Time period tested (days) for stock solution 1	Sample	day 1	day 3	day 7	day 21	day 30
Measured concentration (µg/mL), stock solution 1	1	0.51	0.50	0.50	0.50	0.50
	2	0.50	0.50	0.50	0.50	0.50
	3	0.50	0.50	0.50	0.50	0.50
Time period tested (days) for stock solution 2	Sample	day 2	day 4	day 8	day 22	day 31
Measured concentration (µg/mL), stock solution 2	1	0.51	0.50	0.50	0.50	0.50
	2	0.51	0.51	0.50	0.50	0.50
	3	0.50	0.50	0.51	0.50	0.50

* Nominal initial concentration of diluted stock solutions measured at 0.5 µg/mL was 10014 µg/mL for stock solution 1 and 10006 µg/mL for stock solution 2

Table 4.10 Stability results for working standard solution (4 µg/mL)

Day	LC run	Peak area	Retention time (min)	RSD peak area [1]	RSD retention time [2]
day 0	r1	6837304	4.314	0.22%	0.02%
	r2	6865256	4.313		
	r3	6862509	4.315		
	r4	6872541	4.315		
	r5	6875687	4.315		
day 1	r1	6926556	4.338	0.20%	0.01%
	r2	6922476	4.338		
	r3	6955944	4.337		
	r4	6924682	4.338		
	r5	6932518	4.337		
day 4	r1	6958336	4.373	0.32%	0.15%
	r2	6928564	4.368		
	r3	6897907	4.361		
	r4	6943820	4.360		
	r5	6931473	4.357		
day 14	r1	6925124	4.316	0.74%	0.29%
	r2	6903506	4.344		
	r3	6795896	4.345		
	r4	6907892	4.343		
	r5	6892776	4.344		

[1] Overall RSD: 0.60%
[2] Overall RSD: 0.44%

4.6 Matrix effect testing

Matrix-matched standards were prepared by spiking the final extract solutions obtained from treated control samples. Assessment of matrix effect was performed by comparing the chromatographic peaks, area and retention time, between matrix matched- and matrix free analytical standards at lowest concentration/fortification rate and no significant difference was observed. This outcome - i.e. no significant matrix effect - was anticipated and is in line with the laboratory's previous experience over a range of compounds on similar matrices. In conclusion, in the specific matrices the added solvent of choice (methanol) does not seem to co-extract any matrix components under the specific extraction conditions. Supporting this consideration, it is also underlined that the dosimeters used in the study (matrices) were ordered from the manufacturers/providers by request on their specifications - and subsequently tested under UV light in the laboratory - to be free of any potential fluorescent compounds or additives acting as whitening agents.

4.7 Specificity

With regard to the specificity of the method, the use of a fluorescence detector is an advantage in the measurement of specific fluorescent agents in samples. Fluorescence detectors are very specific and selective in comparison to other optical detectors. They are also considered to be the most sensitive among the existing modern HPLC detectors, as it is possible to detect even a presence of a single analyte molecule in the flow cell (typically, fluorescence sensitivity is 10-1000 times higher than that of the UV detector for strong UV absorbing materials). In combination with HPLC separation this leads to a highly selective method.
Well-resolved peaks for Tinopal SWN were obtained in the tested matrices at all fortification levels. No interference from other compounds was observed. In addition, no signal peak values exceeding 10% of the respective lowest fortification level were detected in the blank samples of the tested specimens. This observation demonstrates the specificity of the method.

4.8 Quality control, analysis of samples, storage of samples

As mentioned in the paragraph 4.4.2 on linearity, five to eight calibration levels were utilized for the construction of each respective calibration curve that covered the concentration range of interest (overall range from 0.001 µg/mL to 10 µg/mL). Sample extracts containing analyte levels outside this range were either diluted or injected at a multiplied volume (more than 1 µL) to give a peak with the area that fitted the calibrated range. Control sample extracts, fortified sample extracts and real sample extracts were kept in a refrigerator (-20 °C) until the date of their analysis.
Although stability tests provided assurance for the stability of the dry matrices as well as the extracts for several days, in order to eliminate the possibility that samples may deteriorate after extended periods of time and to ensure credibility of results, special effort was given to perform the extraction and the chemical analysis of samples on the same day as the samples were collected, which was possible for the majority of

the samples, or within a few days later, which in most cases was done the next day and in very few cases the analysis took place a couple of days after extraction.
In the frame of quality control, field recovery samples of sampling matrices were collected and tested on several days during the whole period of performing experiments. Field quality control samples were collected to check stability during sampling, storage and (intra-lab) transport. In addition, the derived results were assessed to check and verify the recovery of Tinopal SWN for the different matrices monitored.
The location of the field spike sampling was in a designated part inside the UV-room, on a clean table set up in such a way that maximum surveillance and minimum contamination was guaranteed.
The field spiking consisted of the following sampling scheme:
- Each experimental day one set of blanks per matrix (in triplicate) was collected.
- Each week one set of field quality control samples spiked at three levels per matrix (Tyvek fabric, cotton fabric, gloves, wipes, hand wash solution) was collected.

The three fortification levels (low (LOQ) / medium (10x LOQ) / high (100x LOQ) corresponded to initial method LOQs (before their enhancement) of 0.11 µg/cm^2 for Tyvek cotton coverall fabric, 0.14 µg/cm^2 for cotton gloves, 1.04 µg/cm² for wipes and 0.029 µg/cm² (equivalent to 0.027 µg/mL) for handwash solution.

The field blanks were intended to reflect possible systemic cross-contaminations during sample preparation and processing. Per monitoring day, a single blank for each dermal exposure matrix (Tyvek fabric, cotton fabric, gloves, hand wash solution, wipes) was spiked. These samples were stored each day after their collection in a clean, cool, dry place (inside a closet, isolated from other samples, chemicals and Tinopal SWN contamination sources) in order to be extracted if necessary. More concrete, the analysis of the blank samples followed a tiered approach: one of the blank sample sets - the one collected the last experimental day of each sampling week - was analyzed. If these results were as expected (showing no/minimal contamination), the rest of the blank sampling sets of that week were not analyzed. However, if the results were not as expected, more than one of the aforementioned blank sampling sets were available to be analyzed, to be able to detect possible contamination incidents or issues related to the handling of samples, extraction, analysis, etc.
For the blanks, representative parts (for instance a single glove, a piece of fabric, an amount of hand wash solution, a wipe) were prepared in the laboratory and were put in an extraction pot (polyethylene vessel) and transferred to the test location. At the test location, the clean designated area of the UV-room was taken care of to remain free of potential contamination throughout the duration of the experiment, but with the same environmental conditions as where the experiments are performed. The PE bottles with the blanks were spiked with analytical solution of Tinopal SWN in methanol at three different concentrations (see above). Pots were left open during the monitoring period. After the monitoring period, the PE pots were closed and treated as dermal exposure samples. Exact duration of exposure was recorded on the respective field forms along with other relevant information. All the analyses from the blank samples showed no contamination (results <LOQ).
For the field fortification of the field spikes a known amount of Tinopal SWN was added to the different matrices. Each spiked sample was fortified in the field location using fresh solutions of known concentration already prepared in the laboratory. For

each new batch of tank mix a sample of solution or solid accordingly (sample in triplicate), was also kept in a bottle or screw cap tube for chemical analysis. The fortification solutions (Tinopal SWN in methanol) were prepared in the laboratory and transferred to the test location by a field scientist.
For all fortification sets throughout the experiments all the field spiked samples were fortified and located in the field spiking area throughout the duration of the respective experiment, thus being exposed to the same environmental conditions as those in the container where the experiments are performed. The spiked matrices were already placed in separate extraction pots, and after spiking the caps of the pots were placed upside-down on the screw opening, but not tightened to allow for exposure to ambient environmental conditions. This procedure minimized the possibility of (unwanted) incidental contamination of spikes.
At the end of the monitoring period the pots were closed and the fortified samples were treated as the field samples. The exact duration of exposure was recorded on the respective field forms along with other relevant information such as fortification amounts, and storage information.
It was foreseen that all experiments for one exposure situation would be executed 'back to back'. This was indeed the case, and as all experiments for one exposure situation were performed in a sequence, no switching between exposure situations and/or experimental set-up was required. The collection of the field spiked samples in the aforementioned scheme allowed for proper "coverage" on the switches between experimental setups.
With regard to the analysis of the field spikes, again a tiered approach was adopted. In principle, the field spike sets collected during each sampling week were analyzed. The derived results showed recoveries that were in line with the expected ones previously obtained from the validation experiments, and confirmed both the stability of the samples (during preparation, sampling, transport and storage- steps) and that the experimental plan adopted was "fit-for-purpose". Results of field spikes are summarized in Table 4.11 and Table 4.12 below.

Table 4.11 Results of field spiked samples for exposure situations with a dusty solid

Matrix	Dumping (A1)		Handling contaminated objects (E)	
	Average recovery (%)	RSD (%) *	Average recovery (%)	RSD (%) *
Cotton	94	6	98	7
Gloves	94	6	98	7
Wipes	94	6	100	6
Hand wash	96	6	96	6

* RSD = relative standard deviation

Table 4.12 Results of field spiked samples for exposure situations of liquid test product

Matrix	EXPOSURE SITUATION															
	Pouring LV (A2)		Pouring HV (A3)		Rolling LV (B1)		Rolling HV (B2)		Spraying LV (C1)		Spraying HV (C2)		Immersion LV (D1)		Immersion HV (D2)	
	Av. rec. (%)	RSD (%)	Av. rec. (%)	RSD (%)	Av. rec. (%)	RSD (%)	Av. rec. (%)	RSD (%)	Av. rec. (%)	RSD (%)	Av. rec. (%)	RSD (%)	Av. rec. (%)	RSD (%)	Av. rec. (%)	RSD (%)
Tyvek	99	5	103	7	97	6	97	7	105	7	102	6	103	6	93	6
Cotton	101	5	102	6	95	6	98	7	105	6	102	6	102	5	94	6
Gloves	101	4	102	5	96	6	100	5	104	6	102	7	102	5	93	5
Hand wash	97	6	99	7	94	5	96	6	99	5	100	7	99	6	90	4
Wipes	99	6	103	7	95	6	98	6	105	7	99	7	101	7	94	6

* Av. rec. = average recovery; RSD = relative standard deviation

5 Fluorescence tool development

5.1 Introduction

This chapter focuses on the development of the fluorescence quantification method as used within the SysDEA project. The possible advantage of a calibrated fluorescence quantification method is that it stands apart from other measuring techniques since it does not rely on chemical analysis of acquired samples, therefore providing a possibility to reduce time and costs of dermal exposure assessments considerably. Although it is not a commonly used method, several exploratory studies have been performed looking into the possibilities of fluorescence as a quantitative measuring technique (Brouwer et al., 2000; Roff et al., 1994). In 1986, Fenske et al. developed the VITAE system, two banks of UV lights were used to illuminate a subject contaminated with a tracer. A linear correlation was found at low concentrations between exposure and measured fluorescence intensity. Over the years other systems were developed, each with its own advantages and limitations. A system that was small enough to be mobile and capable of providing a whole body assessment is, to our knowledge, currently not available in the literature. The goal was to characterize the fluorescence method as developed within the current study, gain insight in the advantages and disadvantages of this method for different exposure situations, and develop a protocol for standardized quantitative fluorescence-based exposure assessment.

5.2 Description of the fluorescence method

The fluorescence method used within the SysDEA project consists of two parts, namely 1) a set-up under UV light used to photograph the fluorescent exposure from the volunteers, which has been discussed in more detail in paragraph 3.6, and 2) the software used to analyze the recorded images and convert measured light intensity into exposure in mass.
This setup was constructed in a room that could be darkened completely, to ensure that no external light would interfere. Several experiments were conducted to verify if the illumination provided by this setup was diffuse enough. The setup was used for the calibration of the software and the optimization of the setup during the pilot phase. At the premises of BPI another setup was built with the same specifications to capture the images used for the SysDEA project.

5.2.1 Stepwise description of the quantification software

When photos are taken of the volunteers, the camera produces two files (the photo and a RAW image file containing more information). The RAW file is used with the program for the analyzing of the photograph. The software used to analyze the RAW format photos taken of volunteers as well as the calibration experiments, operates based on a sequence of steps, as shown in Figure 5.1.

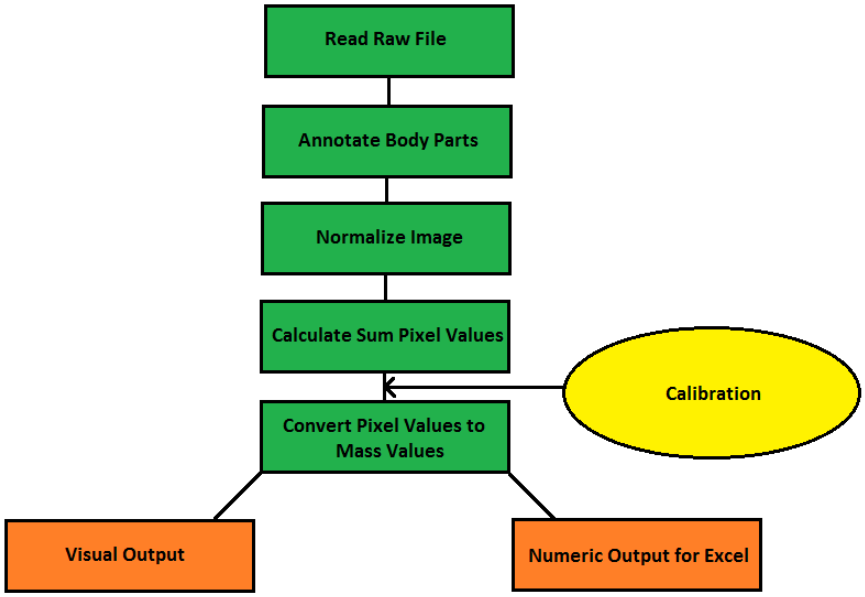


Figure 5.1 Flow chart of fluorescence software tool

5.2.1.1 Step 1: Read RAW file

The tool reads the RAW photograph with the open source tool DCRaw (https://www.cybercom.net/~dcoffin/dcraw/), which converts the camera output into a ppm-file. The ppm file is a large data matrix in which each value represents one optical sensor of the camera. These values are directly derived from the chip and no post-possessing was performed by the camera on these values. The ppm file can be easily processed further by MATLAB (R2014a, Mathworks) in which the tool was developed.

5.2.1.2 Step 2: Annotate body parts by means of User Interface

After converting the camera output to a ppm file, the “annotation tool” is used to indicate where on the photograph the different body parts are located and contextual data, such as the experiment ID, volunteer number, exposure situation, etc., can be added to the photograph. The User Interface (UI) can be seen in Figure 5.2.

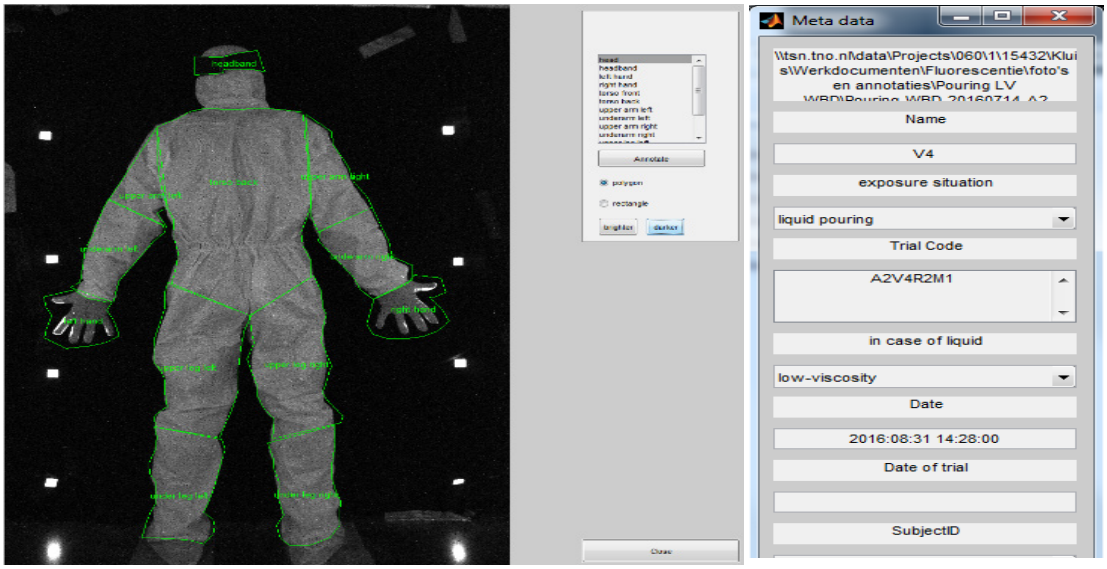


Figure 5.2 User Interface of the annotation tool. Left: Annotation the different body parts. Right: entering so called "meta data" or contextual data

The annotating of photos and the recording of contextual information for the experiments is done by BPI after one series of photographs per type of experiment (e.g. handling of contaminated objects) have been checked for either over- or underexposure (lighting of the tracer on the photograph). After all contextual data has been entered and all body parts are annotated the data and the annotation polygons are stored next to the ppm file as separate files in the working directory.

5.2.1.3 Step 3: Normalization of image (correction)

Before the tool can start calculating exposure data, first the image has to be processed. There are two corrections that must be applied to the ppm file. First, the pixel values must be corrected for the settings of the camera. All photographs were made with a shutter time of either 1/80th, 1/20th or 1/5th second, with 1/20th as the default setting. In special cases, where the 1/20th setting did not yield satisfactory images a different setting was used depending on whether the picture is under- or overexposed. This insures that no overexposure occurs in the photographs used for quantification. However, ideally all photos are made with the same camera settings, so all photos are corrected for the 1/20th shutter time, this was not the case for all photos (especially during powder experiments), a log was kept for photographs where the shutter time deviated.
The second correction that must be applied is the “background correction”. For taking the photographs, two types of consumer brand cameras were used, namely the Canon EOS 700D (at TNO) and the Nikon D90 (at BPI). Each has a Bayer filter on the light sensitive chip that has three color filters arranged in a grid pattern, which relates to the RGB channels. The fluorescence tool only uses the blue channel, since Tinopal SWN emits at 430-436 nm, which falls within the blue range. The blue channel on most consumer brand cameras is sensitive for blue light of a much broader range, resulting in some background noise. Figure 5.3 illustrates this background noise and how it looks after correcting the background noise. In this

figure, the red pixels represent a non-zero value and the blue pixels are pixels with value 0. The left image is the uncorrected background noise correction.

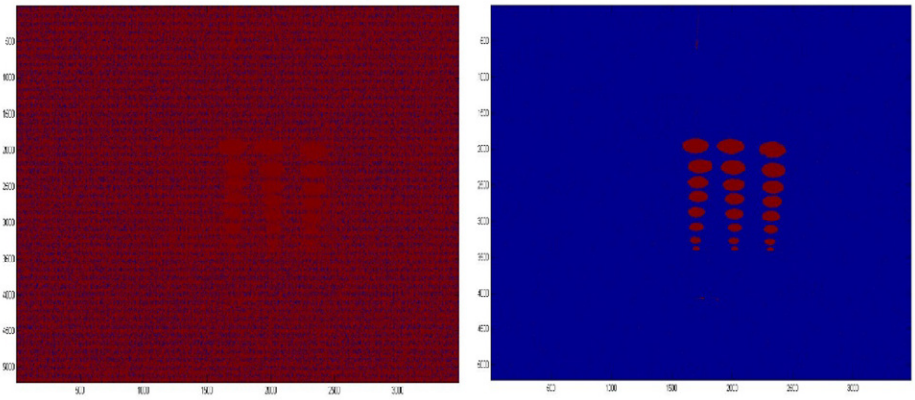


Figure 5.3 Left: uncorrected image, red pixels are non-zero, blue are zero value pixels. Right: same image with background correction applied. To correct for background noise, the pixel value from the background annotation was subtracted from all other annotations

5.2.1.4 Step 4: Calculate sum of pixel values

After the blue channel image is corrected for camera settings and background, the tool sums all pixel values within an annotation (which typically is one side of a particular body part). This is done for all annotations within one photo separately. This summed value is the measure of light intensity emitted by the tracer for the annotated body part.

5.2.1.5 Step 5: Calibration & conversion

To convert measured pixel values to a mass value, experiments were done to determine a linear regression model, tracer mass = a*∑Pixel values + b. These experiments are described below. After all pixels per annotated body part have been summed, these values are correlated to mass concentrations (in µg for the liquid exposure situations, and in mg for the powder exposure situations) per annotated body part with the determined models. Note that pure Tinopal SWN (powder) and Tinopal SWN in solution have different parameters. The tool automatically selects the correct model based on the metadata.

5.2.1.6 Step 6: Output of the tool

The user of the tool can determine the way the output is presented, which can be by means of a visual representation of measured exposure per annotation, the metadata and/or exposure values. The output can be exported to Excel, or a more comprehensive visualization of a single photograph with bar graphs can be made to investigate the quality of the images used. Examples of these outputs are shown in Figure 5.4. The tool can produce these data formats at the same time and for a multitude of photographs.

Name	V1	V1
exposure situation	liquid spraying	liquid spraying
Trial Code	C1_V1_R1_M1	C1_V1_R1_M1
in case of liquid	low-viscosity	low-viscosity
Date	2016:10:05 15:42	2016:10:05 14:47
Date of trial	3-10-2016	3-10-2016
SubjectID	B1	B1
Photo taken before or after activity	after	after
front or backside of volunteer	frontside	backside
comment	-	-
annotation type	human	human
head	NaN	NaN
headband	0,000	0,003
upper arm left	0,007	0,046
upper arm right	0,111	0,597
underarm left	0,106	0,125
underarm right	0,448	0,317
torso front	0,463	NaN
torso back	NaN	1,255
upper leg left	0,149	0,122
upper leg right	0,518	0,118
under leg left	0,163	1,094
under leg right	0,373	0,545
left hand	0,112	0,056
right hand	0,662	0,547

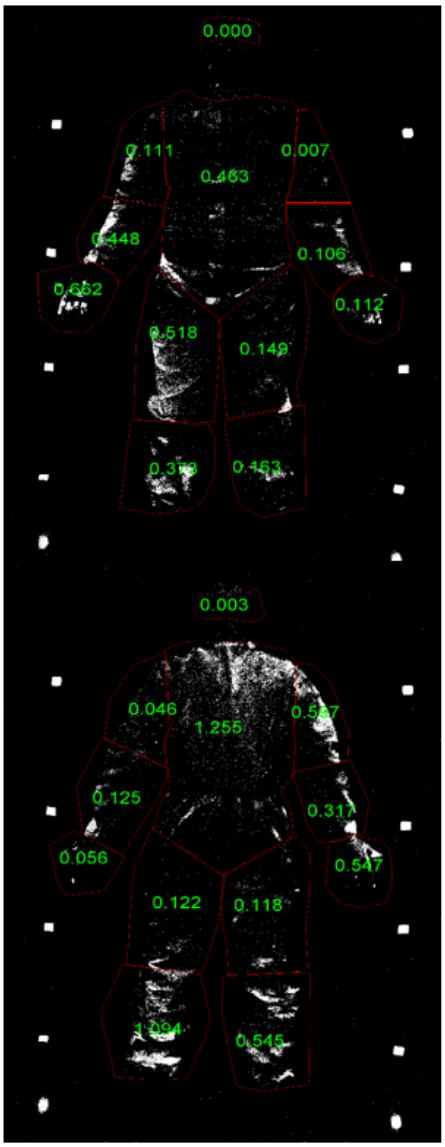


Figure 5.4 Output of the fluorescence analysis tool. Left: the data that is written to excel. Right: the visual output that is stored in the same folder as the input photographs

5.3 Set-up and results calibration experiments

In this paragraph the set-up of the calibration experiments and their results are described. This format was chosen due to the large amount of experiments and results that need to be discussed.
The software relates pixel intensity to an exposure value in micrograms (µg) for liquids and milligrams (mg) for powders. To be able do this, the tool must be calibrated. Various experiments have been performed under controlled circumstances to develop the models the tool uses for the conversion of pixel values to dermal exposure values in µg. All calibration experiments at TNO or BPI were performed as much as possible under the same experimental conditions (e.g. liquid tracer solutions, camera settings) as applied during the main exposure experiments conducted at the premises of BPI.

5.3.1 Fluorescent tracer formulations for calibration experiments

Tinopal SWN was used during the current study as the analytical compound to assess each measuring method. Tinopal SWN is an optical brightener and highly fluorescent under UV light, and can be easily detected using high-performance liquid chromatography (HPLC) methods. An extra advantage that comes with using a fluorescent compound as the analyte for all exposure situations and measurement methods is that a large amount of data is generated that eventually can be used for the development/optimization of the fluorescence method. Furthermore, by means of visual representation, additional information with regard to for instance patterns of exposure becomes available.
Tinopal SWN is being used in either a liquid medium or in its pure form as a powder. When used in a liquid form, solutions were prepared following the standard operating procedure (SOP) developed within this project. The appropriate amount of Tinopal SWN is weighed in a beaker. Acetone (2 mL) is added to dissolve Tinopal SWN and the solution is transferred into a volumetric cylinder. After the Tinopal SWN is dissolved, Triton X-100, glycerol and water are added sequentially in 10:2:20:68 fractions to form the final liquid product. For different concentrations, amounts as presented in Table 5.1 were used.

Table 5.1 Concentrations Tinopal SWN solutions per 20 mL

Concentration Tinopal SWN (gram/Liter)	Tinopal SWN (gram)	Acetone (mL)	Triton-X (mL)	Glycerol (mL)	H20 (mL)
4	0.08	2	0.4	4	13.6
2	0.04	2	0.4	4	13.6
1	0.02	2	0.4	4	13.6
0.5	0.01	2	0.4	4	13.6
0.25	0.005	2	0.4	4	13.6
0.125	0.0025	2	0.4	4	13.6
0.0625	0.00125	2	0.4	4	13.6
0.0312	0.000625	2	0.4	4	13.6
0.0156	0.000312	2	0.4	4	13.6

For all calibration experiments in which pure Tinopal SWN in its powder form was used, grinded Tinopal SWN was used and was passed through a 50 µm sieve. This differs slightly from the SYSDEA SOP, in which a 38 µm sieve was used. Reasons for the deviation of the protocol were that no smaller sieve was available at the TNO location. For all spray experiments the 2 g/L solution was used.
The distance between the camera and the experimental calibration plates, as well as the height of the camera, is identical to the distance and height used between the camera and the volunteers during the experiments.

5.3.2 Angular dependency

In this experiment, the effect of the angle of the contaminated surface relative to the camera on the output intensity of the tracer was tested. If a surface is diffusely illuminated it may still be under an angle relative to the camera, reducing registered intensity according to a cosine law, ideally a contaminated surface is parallel to the

camera. Spots of 2.5 µg Tinopal SWN were applied on a Tyvek surface. The horizontal distance between each spot was calculated so that each spot would be under an angle that was 10 degrees more relative to the camera as the previous spot as can be seen in Figure 5.5.

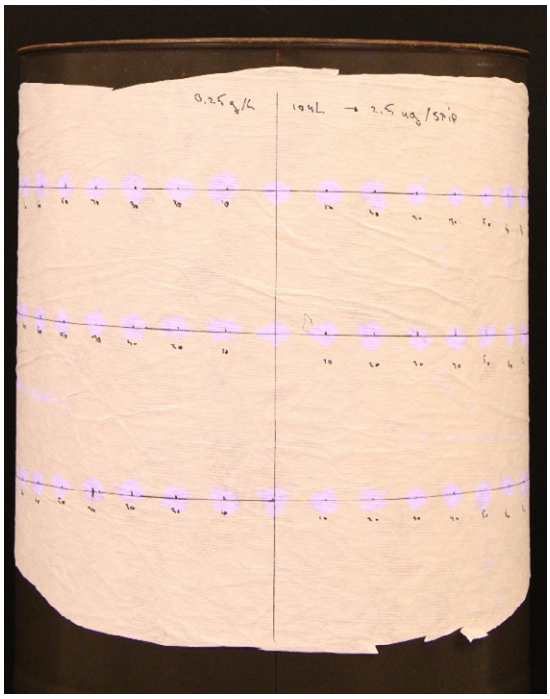


Figure 5.5 Angular dependency experiment. 3 rows of 2.5 µg spots of Tinopal SWN under an angle relative to the camera

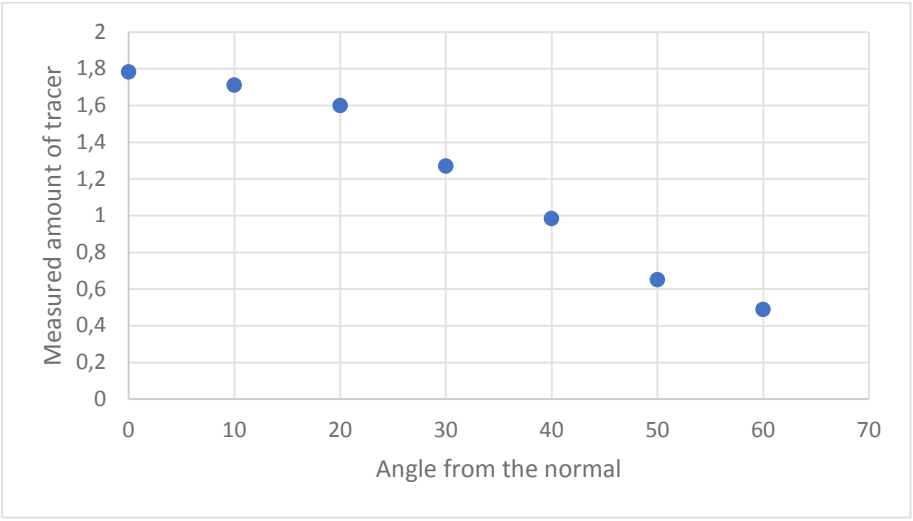


Figure 5.6 Angular dependency experiment, showing intensity scales with based on camera angle

The results show that when a surface is under an angle relative to the camera, the measured intensity decreases. This effect is shown in Figure 5.6.

5.3.3 Applicability domain liquids: varied concentration experiment on HPL plates

The goal of this experiment is to determine the tool's ability to register differences in fluorescence intensity for liquid formulations containing Tinopal SWN, and to see if there is a linear relation between surface area loaded with tracer and registered tracer intensity. A high-pressure laminate (HPL) plate was used as a background to apply spots of predetermined size (5.3 cm^2) with 15 µL tracer formulation, as shown in Figure 5.7. HPL was used as it is a smooth non-fluorescent material that does not absorb any liquids. Spot size was determined by circling a bottle cap and measuring the diameter. The tracer solution was spread out evenly using a needle to make sure the total area of the spots was more or less evenly loaded. The concentration of Tinopal SWN in each row was decreased by half in each following row, starting at 4 gram/Liter down to 0.015 gram/Liter. The HPL plate was photographed under UV light, the plate was located at chest height in the same position as a person would stand. The photo was annotated and analyzed with the fluorescence software tool in MATLAB, to see if a relation between the amount of tracer applied and registered light intensity could be found.
This experiment was repeated with spots that were 2.3 cm^2. By decreasing the surface area of the spots, the maximum loading should be reached sooner when the same amount of tracer is used. This should show up in the analysis and is considered to be an extra step to verify the maximum loading value.

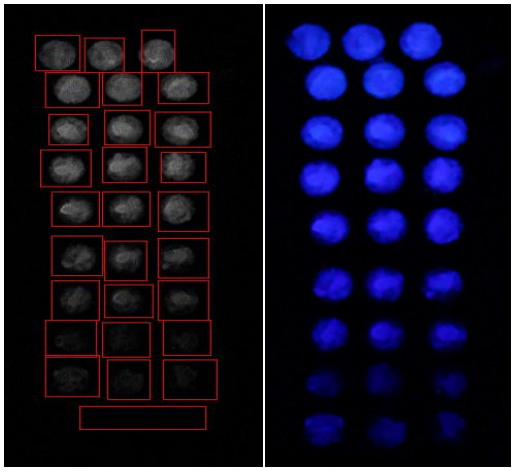


Figure 5.7 Left: spots of tracer liquid on HPL under UV light. Right: associated annotations in software tool

Here are displayed the results of these experiments, the experiments assessed if there was a relation between the loading of surface area and the registered fluorescence intensity. As seen in the graph there is an observed relation between the measured intensity and the amount of tracer per spot.
In Figure 5.8 a clear relation between loading and summed pixel values can be observed. It can be observed that the curve flattens at a concentration of 0.5 g/L. To determine the surface loading the following calculation leads to the maximum surface loading: 15 µL * 0.5 g/L results in 7.5 µg Tinopal in the spots. By dividing this by the surface area of 5.3 cm^2 a maximum loading of 1.4 µg/cm^2 can be assumed.

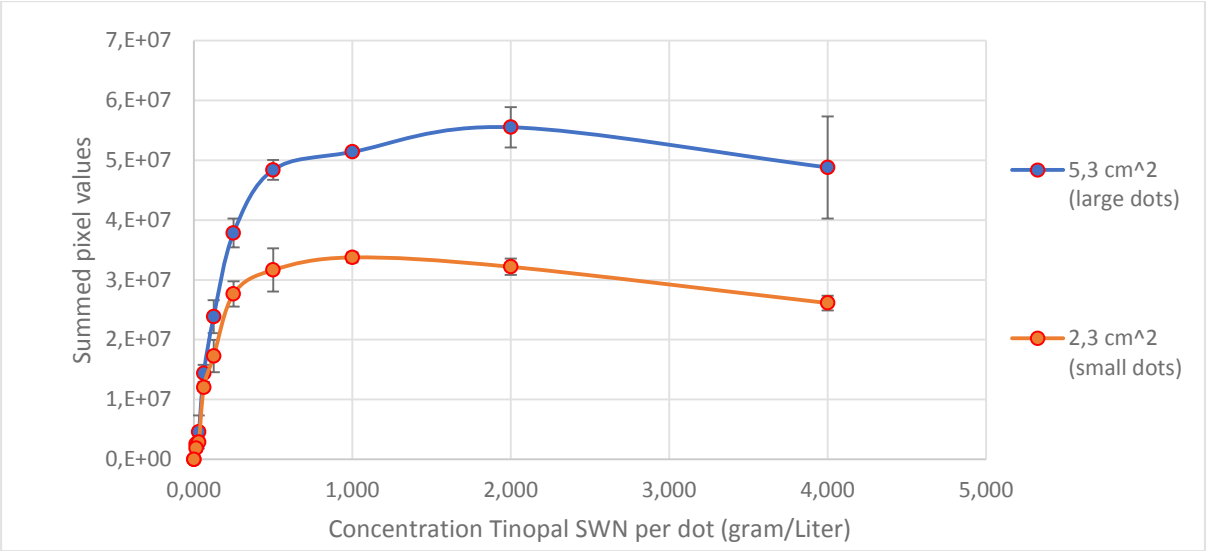


Figure 5.8 Relation concentration of applied Tinopal SWN per dot of liquid formulation and measured intensity (as summed pixel values)

5.3.4 Applicability domain liquids: varied surface area experiment on HPL plates

The goal of this experiment was to find the upper limit of detection of the software (the maximum amount of tracer that the tool can distinguish based on measured light intensity). This was done by changing the surface area, instead of the amount of tracer applied thus verifying earlier experiments determining this upper limit. A HPL plate was used as a background to apply spots of tracer solution containing 30 µg of Tinopal SWN per spot. The radius (r) of the spots was increased by ½ cm each row from r=1.5 cm up to r=4 cm as seen in Figure 5.9. Please note that an extra row with r=2.7 cm was added and the volume of applied tracer solution was adjusted to accommodate for larger surface areas. The tracer solution was spread out using a needle so spots were evenly applied.

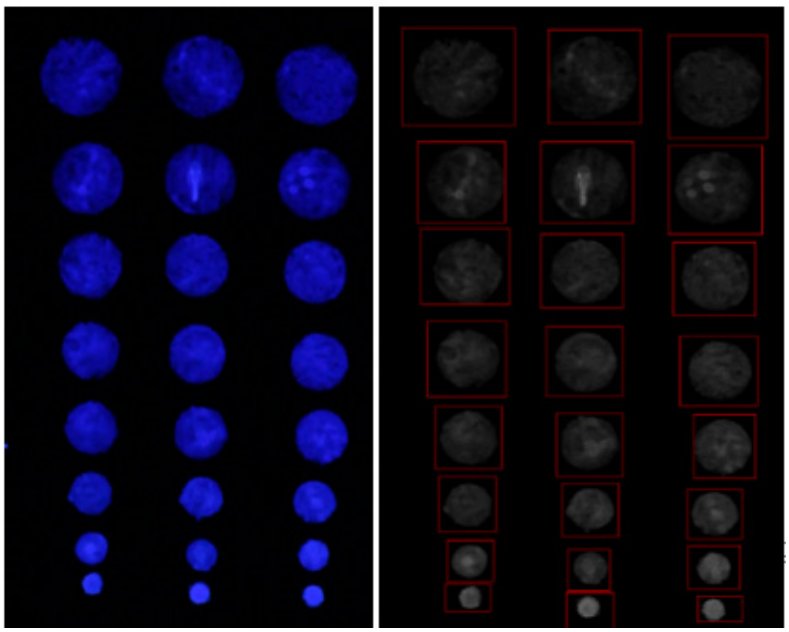


Figure 5.9 Left: Spots of tracer liquid on HPL under UV light applied in three columns. Right: Annotations of these spots in software tool

The HPL plate was photographed under UV light, the plate was located at chest height in the same position as a person would stand, and annotated with the fluorescence software tool and analyzed in MATLAB by examining the relation between the measured pixel values versus the applied amount of Tinopal SWN to see if a similar relation between the loading of the spots and the measured light intensity of the spots could be found. Figure 5.10 displays the results of this experiment. The result for each column is given separately.

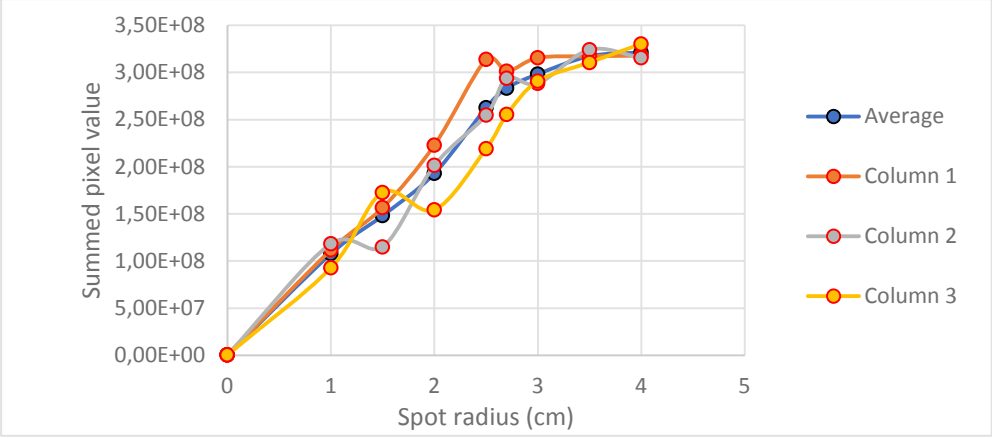


Figure 5.10 Relation between varying surface area using 30 µg Tinopal SWN per spot of liquid formulation and measured intensity (as summed pixel value)

While there is some variance in each set of spots of the same surface area, all colums follow the same trend. Column 1 (one series of spots with decreasing concentration Tinopal SWN) approximates the theoretically expected relation the best. It is indicated that measured intensity returns the same value when the spot

radius increases above 2.5 cm, this corresponds with 1.4 µg/cm^2. These results seem to confirm the upper detection limit of 1.4 µg/cm² established in previous experiments.

5.3.5 Applicability domain liquids: varied concentration experiment on Tyvek

Volunteers performing the exposure experiments at BPI wear Tyvek coveralls during the execution of the exposure situations (scenarios) with the liquid formulations, and therefore it is prudent to also assess if the relation between tracer loading and registered fluorescence intensity when applied on Tyvek material is similar compared to the results from previous experiments performed on HPL. This was determined by applying 15 µL spots of tracer solution of decreasing concentration on a piece of Tyvek cut from a coverall as worn during the execution of the exposure situations. Tinopal SWN concentrations were halved each row ranging from 4 gram/Liter to 0.015 gram/Liter. The Tyvek surface was photographed under UV light, the plate was located at chest height in the same position as a person would stand and annotated and analyzed with the fluorescence software tool in MATLAB (as can be seen in Figure 5.11), to see if a relation between the amount of tracer applied per surface area and measured light intensity could be found, and if this relation is similar to what was found previously.

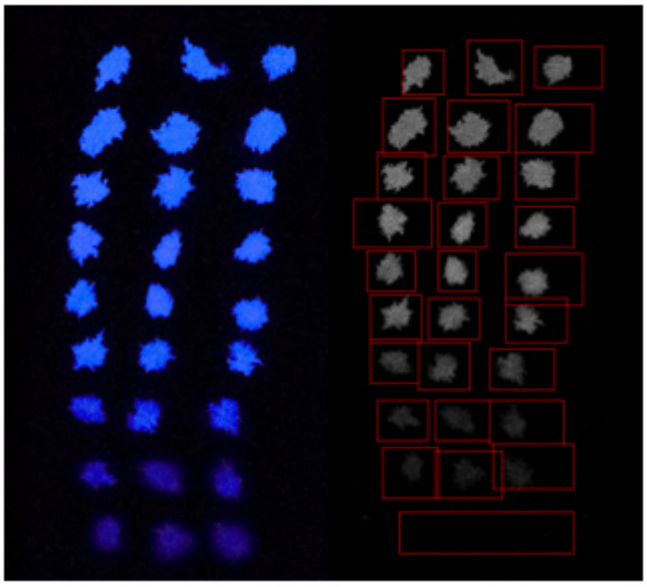


Figure 5.11 Left: spots of tracer liquid on Tyvek under UV light. Right: associated annotations in software tool

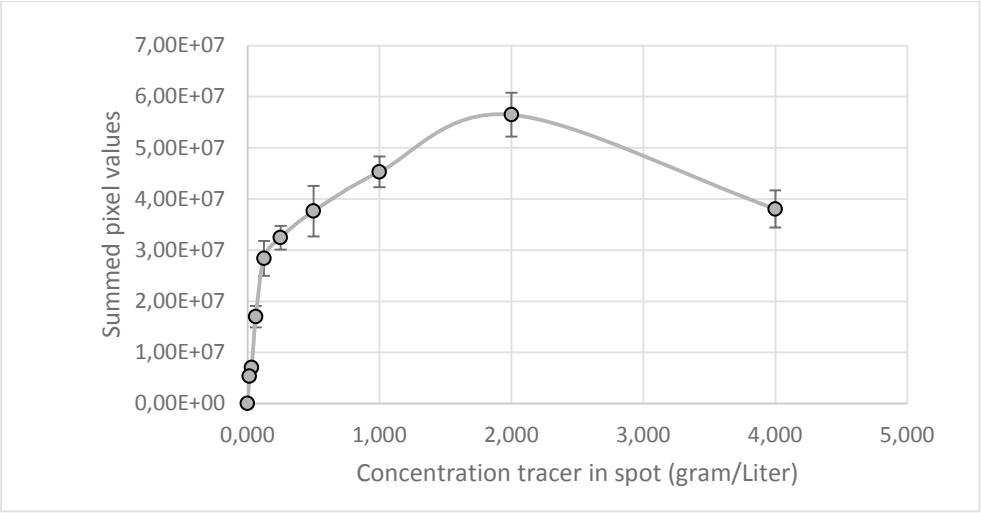


Figure 5.12 Relation between applied concentration of Tinopal SWN in spots of liquid formulation on Tyvek and measured intensity (as summed pixel values)

Shown in Figure 5.12 are the results of this experiment. The relation between the applied Tinopal SWN and the measured intensity seems to follow the same trend as in the previous experiments, there is more variance however. This is likely because the Tyvek slightly absorbs the Tinopal SWN solution and these extra surface interactions likely cause this extra variance.

5.3.6 Applicability domain liquids: assessment of sprays

Goal of this experiment was to assess whether one liquid conversion model can be used for exposure situations with liquid formulations, or whether a separate model should be used for the spray exposure situation. Two experiments were conducted.
12x5 pieces of Tyvek material (30x30 cm) were contaminated using the spray solution with a nebulizer. A fine spray was used to spray the material in triplet using 1, 2, 4, 7 and 10 sprays per patch (15) as can be seen in Figure 5.13.

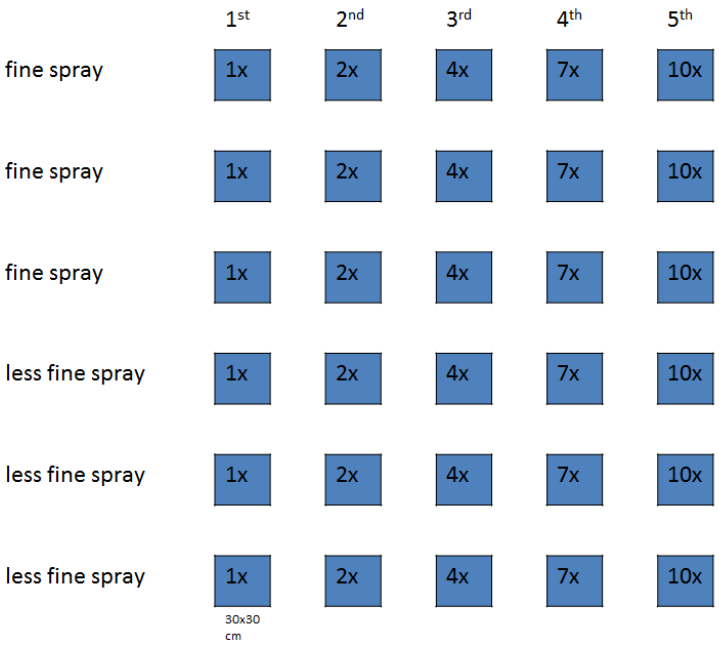


Figure 5.13 Set-up first spray experiment.

This experiment was repeated with a more coarse spray to represent small droplets (instead of a mist) and was conducted for both low and high viscosity liquids. These experiments were conducted at the premises of BPI and the contaminated Tyvek patches were photographed and chemically analyzed to assess the total mass on the material.

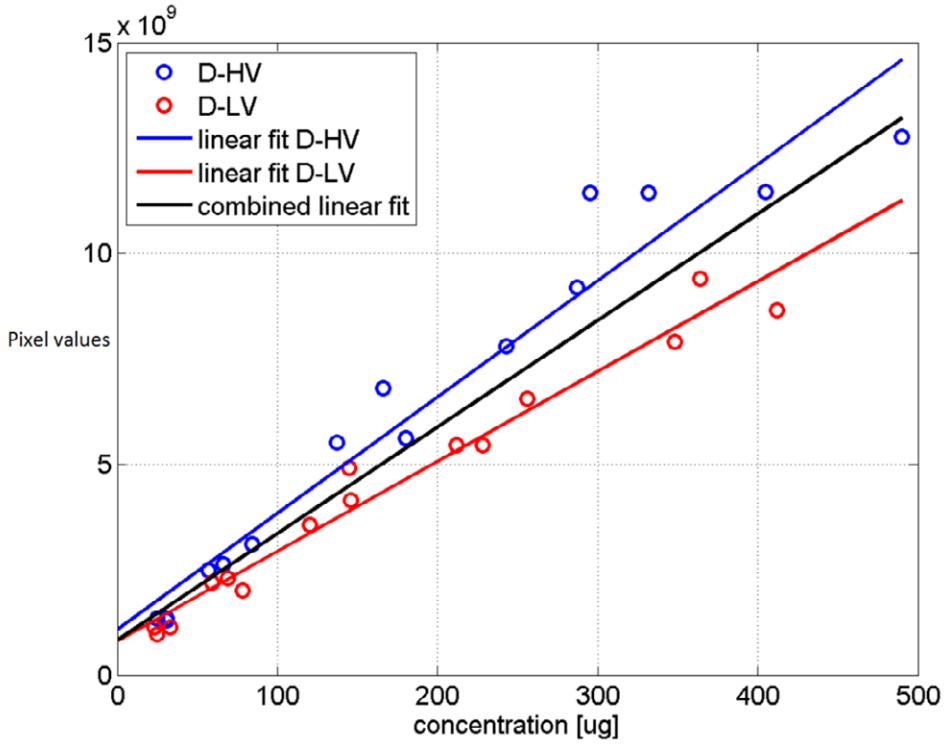


Figure 5.14 Results first spray experiment, showing relation between applied concentration of Tinopal SWN in spray samples and measured intensity (as pixel values)

Results of the spray experiments conducted at BPI are shown in Figure 5.14. Here a linear fit is visible for both LV and HV samples. Two different relations are observed, indicating a difference between using LV or HV. A combination of both models can be used when the viscosity of the material is unknown, the specific models can be used when the viscosity is known. During this experiment, no maximum loading for the spray model could be found suggesting the model can predict at least until 500 µg. This corresponds to 0.56 µg/cm^2.

For the second experiment, petri dishes were covered with a layer of Tyvek and weighed on an analytical scale. The samples were exposed to the tracer solution with a spray bottle and weighed again. All samples were photographed under UV light, the samples were located at chest height in the same position as a person would stand and annotated and analyzed with the fluorescence software tool in MATLAB as can be seen in Figure 5.15.

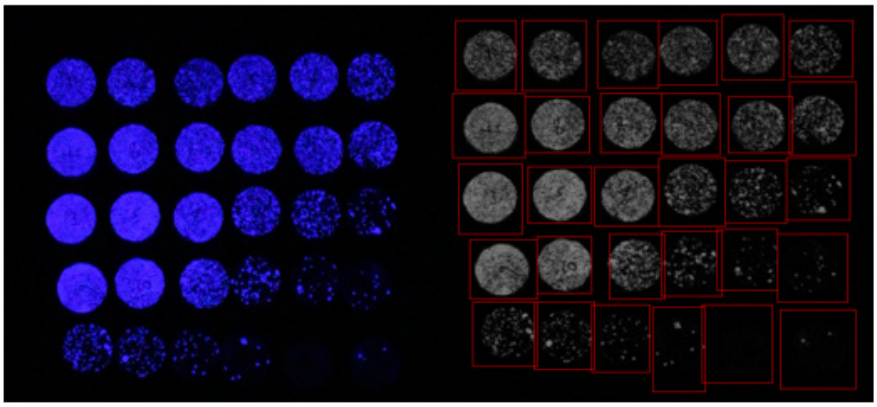


Figure 5.15 Left: Spray samples as seen under UV light. Right: Annotations of spray samples with fluorescence software tool

In the spray experiments the same Tinopal SWN formulation is used as in the liquid experiments. To ensure that the model derived from the results of the calibration experiments for liquids is also suitable for spray exposure situations, a validation of the liquid model was also done on new spray samples. The results of this validation can be seen in Figure 5.16.

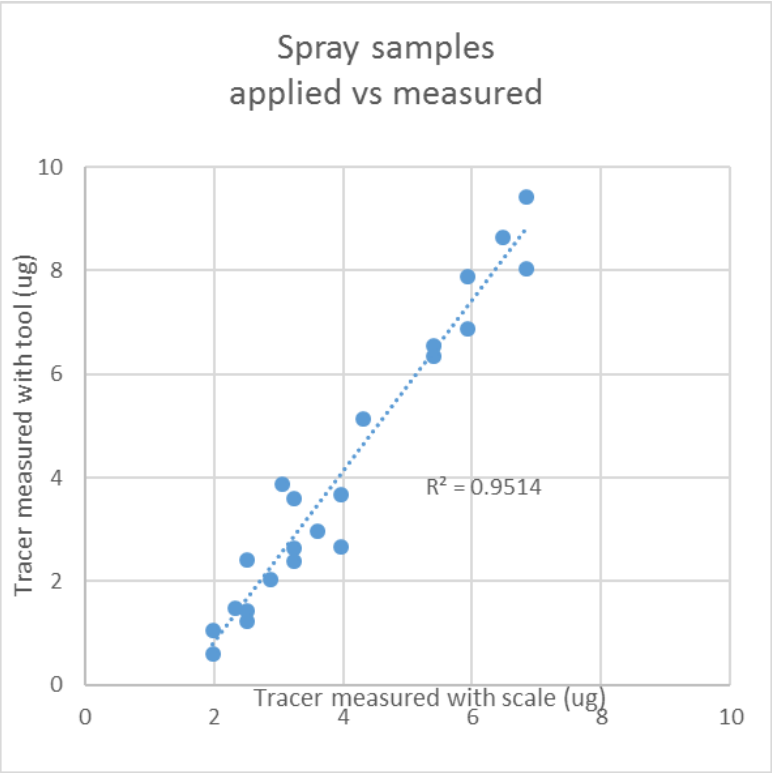


Figure 5.16 Results of validation experiment for the liquid spray model, showing the relation between the measured concentration (with scale) and the predicted concentration (with tool) of Tinopal SWN on spray samples

The results of this spray validation experiment indicate that the liquid model is sufficiently accurate so that it can estimate the amount of tracer in a spray exposure situation. This also means that the applicability domain for sprays is assumed to be similar to that of liquids. However, due to the more diffuse exposure pattern as observed during spray exposure situations (very small droplets that are distributed over more or less the whole body) compared to for instance rolling or pouring exposure situations (larger droplets and/or splashes distributed on particular parts of the body), the upper limit of 1.4 µg/cm² is not expected to be reached often in case of the spray experiments compared to other experiments conducted with liquid formulations.

5.3.7 Applicability domain powders

The goals of the powder experiments are the same as those of the liquid formulation experiments. It is necessary to determine if there is a linear relation between the loading of a surface with powder Tinopal SWN and the registered fluorescence intensity, as well as determining the lower and upper detection limit of the software. The powder experiments were similar in setup as the first liquid experiments. A fixed surface area of 10.7 cm^2 was covered with the material from the coveralls the volunteers were using. This surface was loaded with different amounts of powdered Tinopal SWN and was examined with the tool for a linear correlation between the amount of tracer applied and the registered intensity.
The Tinopal SWN used was grinded and sieved, following the procedure described in paragraph 5.3.1. The petri dishes used were numbered and covered with cotton, the

same material as the coveralls used during the exposure situations with Tinopal SWN powder. They were weighed on an analytical balance and then loaded with Tinopal SWN using a sieve to ensure a homogeneous loading. The loaded petri dishes were weighed again to determine the amount of Tinopal SWN. 40 samples were produced this way. All samples were photographed under UV light, the samples were placed at chest height and in the place a person would stand. Afterwards, the photos were annotated and analyzed with the fluorescence software tool in MATLAB as can be seen in Figure 5.17.

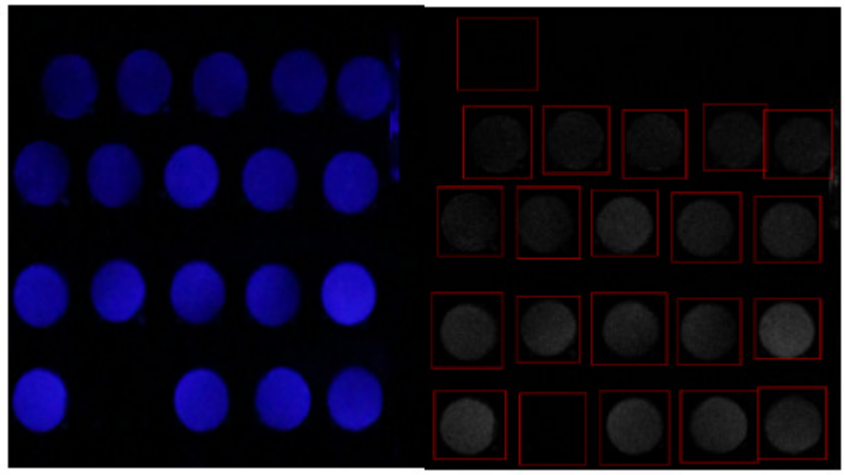


Figure 5.17 Left: Tracer powder samples as seen under UV light. Right: associated annotations of samples as seen with fluorescence software tool

The results of these experiments are presented below in Figure 5.18. A clear relation between the amount of Tinopal SWN on the samples and the measured pixel values can be seen. The results indicate there is a lower detection limit around 2 mg per sample, which correlates to 180 µg/cm^2. Furthermore, no indication of an upper limit has been detected.

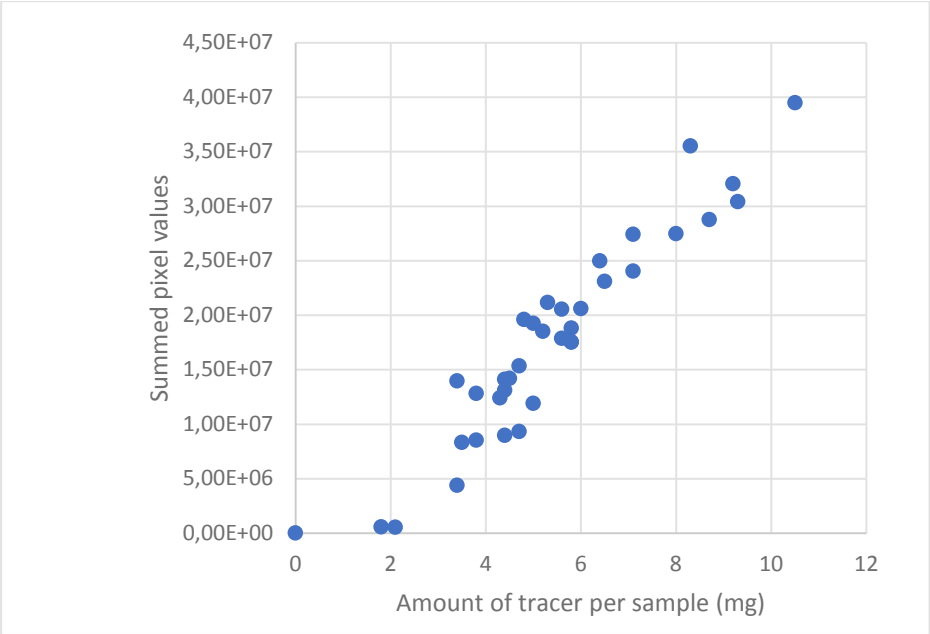


Figure 5.18 Relation between amount of Tinopal SWN (as powder) per sample and the measured intensity (as summed pixel value) per sample

5.4 Determination of models for conversion of light intensity of the tracer into a dermal exposure value representing mass (quantification)

Below, in figure 5.19 the determination of the models used to convert the measured light intensity of the tracer (in pixel values) into a dermal exposure value that represents mass based on the results of the calibration experiments is presented.

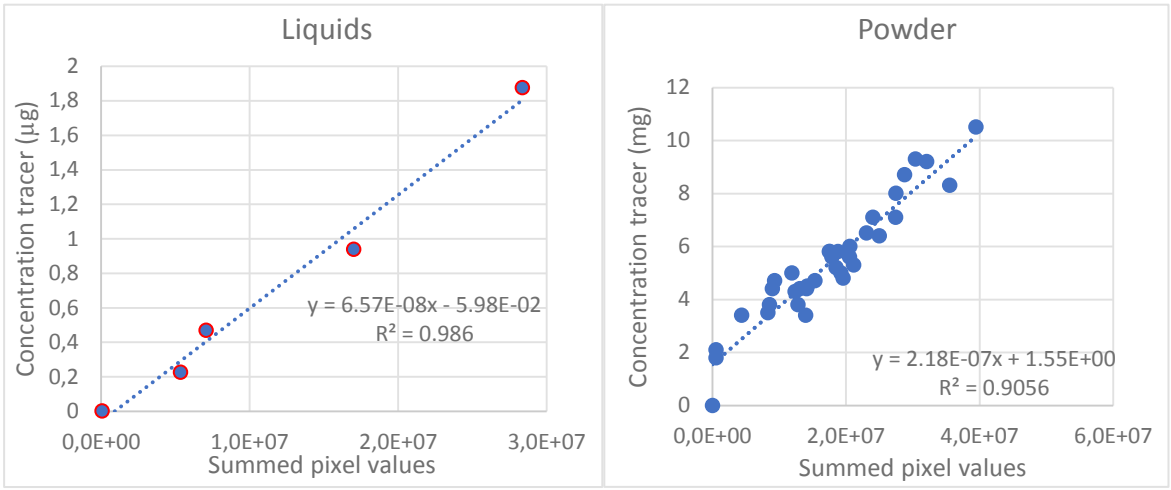


Figure 5.19 Left: The linear part of the calibration curve of liquids on Tyvek, for conversion of the summed pixel values to an amount of Tinopal SWN in µg. Right: Calibration curve for powder on cotton using all powder data, for conversion of the summed pixel values to an amount of Tinopal SWN in mg.

The conversion models are derived from the linear part of the results of both the spot experiments as well as the powder experiments.
By setting the measured pixel values as the X-axis and the applied Tinopal SWN as the Y-axis, the equation of the trendline describes the parameters needed to convert measured intensity into a mass value.
This equation for liquids is as follows:
$[\mu g \text{ tracer}] = 6.57*10^{-8} * [\sum \text{Pixel values}] - 5.97*10^{-2}$

For powders this equation is:
$[mg \text{ tracer}] = 2.18*10^{-7} * [\sum \text{Pixel values}] + 1.55*10^{0}$

These derived models are not yet validated, the validation experiments for these models are described below.

5.5 Internal validation experiments

To validate the models that convert summed pixel values to mass in µg for both, liquids and powders, repeated experiments were conducted with new samples that were not used for the calibration of the models. For liquids, 15 µL drops were applied on a Tyvek surface, tracer concentrations ranged from 2 g/L to 0.015 g/L per row. For the powder samples petri dishes were covered with cutouts of cotton coveralls and weighed before loading with pure, grinded and sieved (50 µm) Tinopal SWN. Loaded samples were weighed again and photographed under UV light and annotated and analyzed with the fluorescence software tool in MATLAB.
The output of the tool is compared with the (known) amount of tracer that was applied during the repeated experiments.

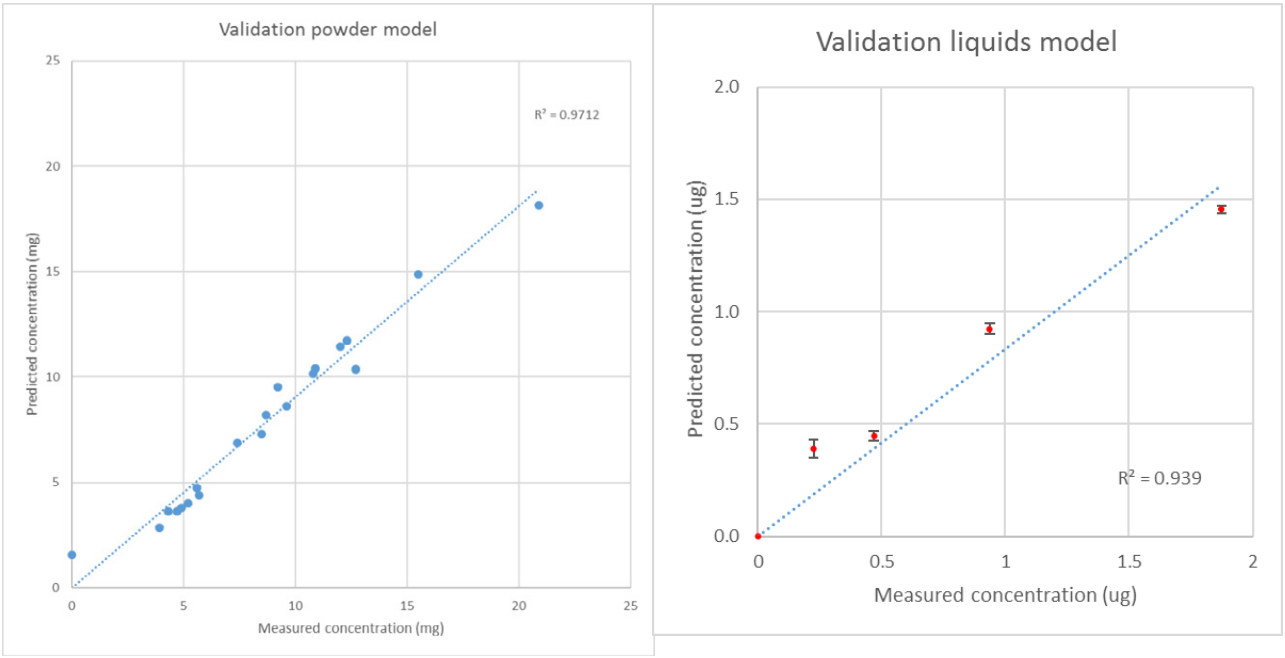


Figure 5.20 Left: Correlation between the applied amount of Tinopal SWN as powder per sample and the predicted concentration by means of the fluorescence tool (validation powder model). Right: Correlation between the applied amount of Tinopal SWN as liquid formulation and the predicted concentration by means of the fluorescence tool (validation liquids model)

The validation of the models derived from the results of the experiments that were performed earlier with powder and liquid samples show that both are sufficiently accurate to measure exposure via fluorescence. Using both models, the tool can fairly estimate powders and liquids with R^2 values of 0.97 and 0.94 respectively, as can be seen in Figure 5.20. For the validation of powders 19 samples were used and for liquids 15 samples were used with five different concentrations and three repeats.

5.6 Preliminary validation fluorescence tool based on comparison with results of chemical analysis

To validate the fluorescence tool in its current form, after the calibration and internal validation steps, a comparison was made between the output of the fluorescence tool and the results of the chemical analysis for the same experiment.

5.6.1 Liquids

In exposure situations in which the liquid tracer formulation was used different exposure patterns were observed. A comparison was made between samples as collected with the whole body dosimetry measurement method during painting a wall with a paint roller and a spray gun, and the output of the fluorescence tool for the same exposure situations, based on the photographs made from volunteers after the experiment.

5.6.1.1 Rolling

For the rolling exposure situation, exposure is often seen on the lower legs and the hand holding the paint roller as is shown in Figure 5.21. This is likely the result of droplets flying from the spinning roller and/or paint leaking from the roller.

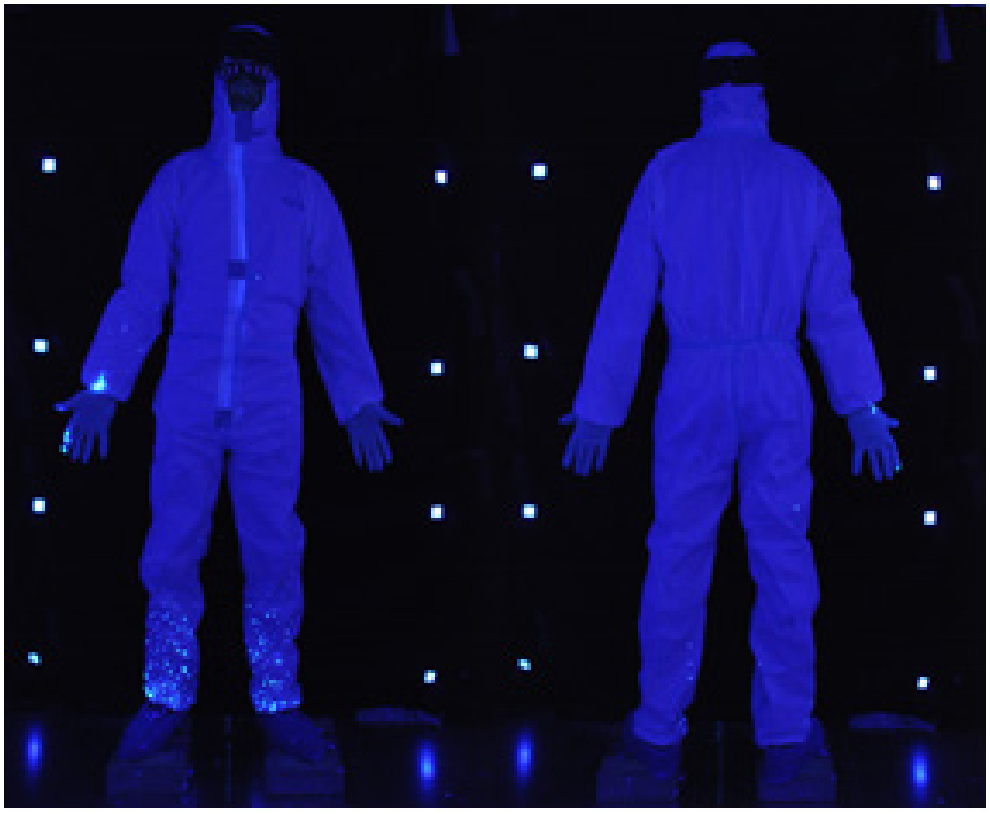


Figure 5.21 Typical exposure pattern during the usage of a paint roller

Table 5.2 Results of the chemical analysis of the coverall and the corresponding output of the fluorescence tool

Part name	Output tool (µg)		Chemical analysis (µg)
	front	back	Total
headband	<LOQ	<LOQ	33.9
upper arm left	0.061	0.063	1.3
upper arm right	0.061	0.063	1.3
underarm left	0.062	0.063	<LOQ
underarm right	2.352	0.071	34.1
torso front	0.091	n/a	3.3
torso back	n/a	0,078	2.4
upper leg left	0.063	0.066	1.3
upper leg right	0.063	0.123	2.3
under leg left	1.077	0.090	48.8
under leg right	1.369	0.069	87
left hand	<LOQ	0.061	1
right hand	0.525	0.325	163.3
Total per side	5.724	1.073	-
Total loading	6.797		380.096

The chemical analysis shows this pattern as well, exposure ranges from 163 µg on the hand holding the paint roller to 1 µg on the other hand, as can be seen in Table 5.2. The tool also detects exposure in a fairly similar pattern, although the tool severely underestimates the amount of tracer on each body part compared to the results of the chemical analysis. The total loading estimated by the tool is 6.78 µg where the total loading determined by the chemical analysis is 380.1 µg indicating that the tool underestimates this exposure situation with a factor ~55.

5.6.1.2 Spraying

The spraying exposure situation shows more diffuse exposure spread out over all the body parts (see Figure 5.22). While the tool underestimates exposure greatly for the rolling exposure situation, it might perform better where exposure is more evenly distributed with less concentrated splashes which the tool is not capable of dealing with.

Figure 5.22 Typical exposure pattern during the usage of a spray gun

Table 5.3 Results of the chemical analysis of the coverall and the corresponding output of the fluorescence tool

Part name	Output tool (µg)		Chemical analysis (µg)
	front	back	Total
headband	0.116	*na*	8
upper arm left	0.171	1.064	40
upper arm right	0.677	10.492	79
underarm left	8.181	0.651	35
underarm right	8.250	12.566	76
torso front	9.490	*na*	114
torso back	*na*	20.628	145
upper leg left	3.667	1.374	48
upper leg right	9.694	1.318	111

Part name	Output tool (µg)		Chemical analysis (µg)
	front	back	Total
under leg left	2.299	7.498	105
under leg right	3.684	7.516	117
left hand	0.312	0.130	16
right hand	0.958	2.042	83
Total per side	47.382	65.395	*na*
Total loading	112.776		978

The results of the chemical analysis also indicate a more diffuse exposure pattern for spraying compared to rolling, ranging from a maximum of 145 µg on the back of the torso to a minimum of 8 µg on the head. Also, the exposure is a factor 3 higher in comparison with the rolling exposure situation, as can be seen in Table 5.3. The tool also detects exposure in a similar pattern, although it again underestimates the amount of tracer on each body part compared to the results of the chemical analysis. The tool estimates a total exposure of 112.78 µg where the chemical analysis shows an exposure of 978 µg which relates to a factor ~9 underestimation of the tool. While the tool still underestimates, the underestimation is significantly lower compared to the rolling exposure situation (which underestimated with a factor of ~55). This implies that the tool performs better when less stacking of Tinopal SWN occurs and when the exposure is relatively low and spread out.

5.6.2 Powders

The task performed in the powder exposure situation described here involves the dumping of 1kg of powder from one container into another. This results in exposure on the hands and a diffuse exposure pattern on the rest of the body as can be seen in Figure 5.23.

Figure 5.23 Exposure distribution dumping of powders

Hand exposure is likely due to the handling of the containers and the diffuse exposure is probably a result from the dust cloud generated by the dumping. Again, the results of the chemical analysis indicate a reasonably diffuse exposure with the hand having the highest level of exposure (see Table 5.4). The chemical analysis

also shows that, even though there is not much fluorescence visible on the photos, high levels of exposure are measured, up to 37 mg. However, the tool has trouble picking up the fluorescence as emitted by the powdered form of the tracer, as is shown in the results. Most exposures as estimated by the tool are below the detection limit of the tool.

Table 5.4 Output tool and the chemical analysis of the same experiment

Part name	Output tool (µg)		Chemical analysis (µg)
	front	back	Total
headband	2.360	<LOQ	194
upper arm left	8.940	1.596	548
upper arm right	13.699	1.903	1017
underarm left	<LOQ	<LOQ	2954
underarm right	<LOQ	<LOQ	2444
torso front	<LOQ	*na*	2104
torso back	*na*	<LOQ	1445
upper leg left	<LOQ	<LOQ	732
upper leg right	<LOQ	<LOQ	1048
under leg left	<LOQ	<LOQ	1550
under leg right	<LOQ	<LOQ	1569
left hand	<LOQ	<LOQ	8803
right hand	<LOQ	<LOQ	13206
Total per side	24.999	3.499	-
Total loading	28.498		37613

5.7 Limitations of the current tool

The tool in its current form underestimates exposure by numerous factors depending on the exposure situation and is not accurate in generating quantitative data based solely on the measured fluorescent intensity (determined by the calibration experiments). For the exposure situations with liquid formulations the tool severely underestimates exposures, up to a factor 55, and in case of powder exposure situations this factor is even higher. In many cases within the powder exposure situations, the output of the tool is below LOQ, while the chemical analysis shows clear exposure to those body parts. It is expected that the inaccuracy of the tool is caused by a combination of multiple factors. For instance, a photograph's RAW file contains no data regarding the angles in the surface. This means that the step from a 3D person to a 2D photograph results in loss of information, as was demonstrated in the calibration phase.
Furthermore, using a consumer market camera means that there are red, green, and blue filters on the light sensitive chip. While this filter does generate a "Blue channel", the range of wavelengths picked up by the camera is broad. This results in difficulties separating tracer fluorescence from other light sources, such as background fluorescence of Tyvek coveralls or general "noise" that is inherent to the camera system. It must also be noted that for the calibration experiments a Canon EOS 700D was used as the imaging device, and for the experiments at BPI a Nikon 90D camera was used. While these cameras are nearly identical with regard to image quality,

their Bayer filters are slightly different, which means the light intensity of the tracer might differ depending on the camera used, which can influence the estimated exposure derived from the tool.

5.8 Statistical fit model development

Since we were not able to calibrate the fluorescence model using different experiments to find a relationship between Tinopal SWN emitted light and amount of Tinopal SWN, the next step to derive a predictive model was to statistically fit a model on a training data set which consists of 25 % of the chemical analysis data and their related photographs. First a random selection of 25 % of the experiments was made including both whole body and patch methods. For this implementation it was decided to exclude the hands since preliminary results showed that this would not be feasible as can be expected regarding the upper limit of detection discovered in the experiments and the high exposures on the hand for most of the experiments. Body exposures are relatively lower compared to hand exposures which might be beneficial for the development of a quantitative model, however it must be noted that this will limit the applicability domain of the model towards body exposure only (excluding the hands).
For this implementation, multiple steps and models have been developed using a statistical fit approach to see how well the model fits for certain situations. Models were fitted on the entire selection of the training set (with powders and liquids separated) to see if a 'one size fits all' model was possible over all the situations for each state of the substance. Here the data of experiments of spraying LV and HV liquid are taken together. In the next exercise all models were plotted per exposure situation, where also the exposure situations with LV and HV liquid were separated, as well as the exposure situations with powder. When a model for a specific situation (either HV or LV liquid) performs well, a separate model was developed for that exposure situation, where LV and HV liquid are both included (due to the feasibility of using a general model for all viscosity ranges), and the performance of this model was evaluated as well.
For each model, the related fit curve was noted with the R^2 value representing the correlation between the pixel values and the exposure in µg. For all the models, the estimated exposure was calculated on the remaining dataset without the training dataset.
Figure 5.24 shows a linear and second order polynomial best fit curves derived on the dataset for all experiments conducted with the liquid products. For the use of the model, the second order polynomial was chosen for comparison with the chemical analysis.

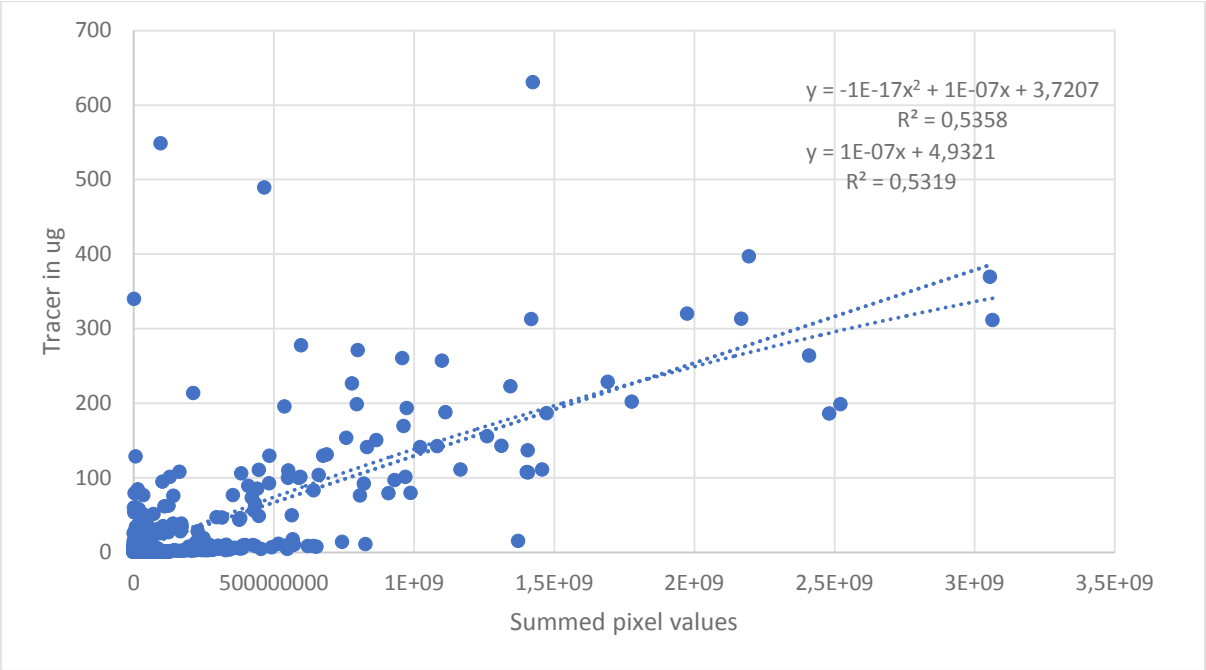


Figure 5.24 Linear and second order statistical fit for overall model for exposure situations with liquids

The overall liquid model was used to convert the total pixel values of all exposure situations. Figure 5.25 shows the model estimates compared with the measured exposure values. As can be seen, the overall model predicts reasonably well for some data points, however a tendency for underestimation is visible as well as a large variation. A moderate correlation of 0.66 has been found between the model estimates and the measured exposure values.

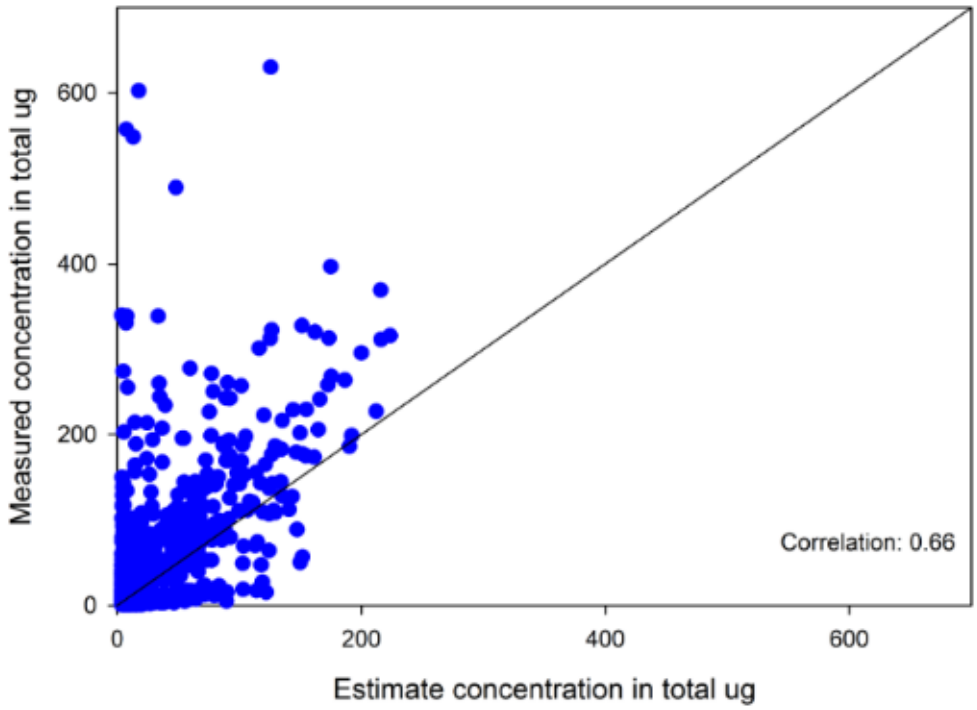


Figure 5.25 Estimated concentrations (based on model) versus measured concentration (based on chemical analysis) for all liquid exposure situations

Preliminary results indicated that the calibration of a model for the powder scenarios was not feasible as no correlation between the pixel values and the measured values existed. Therefore this attempt was disregarded. Next, a model was created for each of the exposure situations for exposure situations involving liquids as can be seen in Figure 5.26. As can be observed, the models vary depending on the exposure situations where spraying appears to have the best fitted relationship between pixel values and measured exposure values. These results also show that the spraying exposure situation data points were the deciding points on how the fitted 'total' model for liquids is generated. Based on these models the spray model was investigated further.

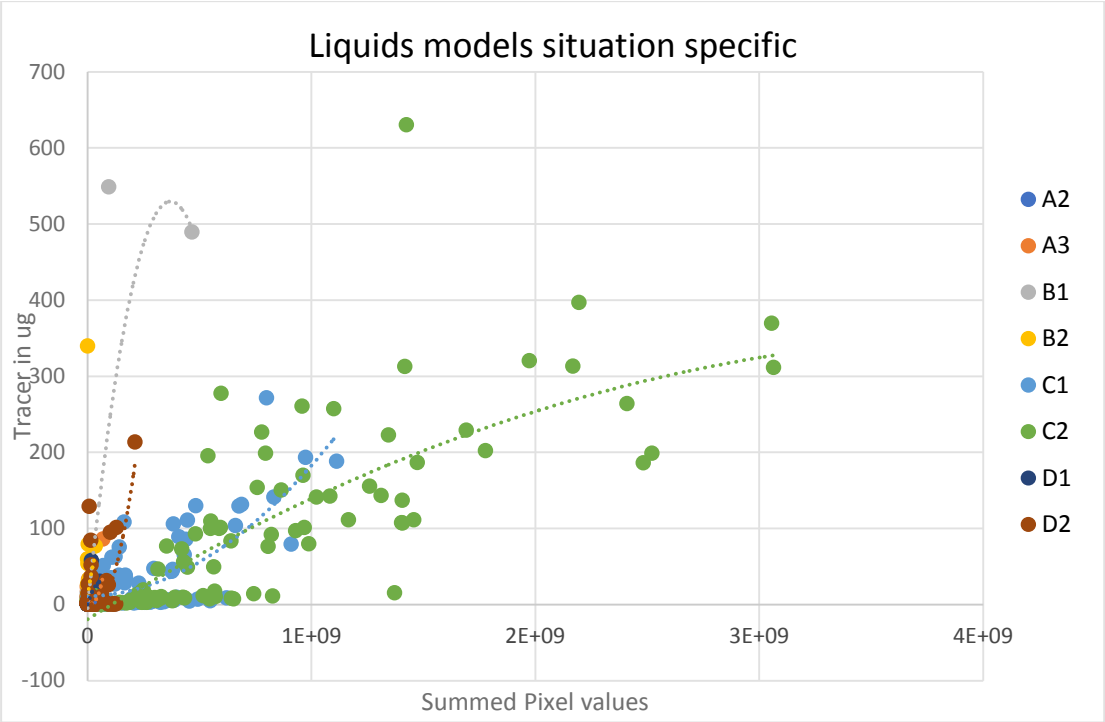


Figure 5.26 Exposure situation specific statistical fit of models, separate for exposure situations with LV and HV

Two separate models were developed for both the spraying LV and HV exposure situations separately. Due to the perspective of a predictive model for spray exposure situations it was decided to develop a model for generic spray exposure situations regardless of the viscosity. For the development of this model, the training dataset of C1 and C2 were combined in one training data set. Figure 5.27 shows the calibration of the general spray model which has an R^2 value of 6.3.

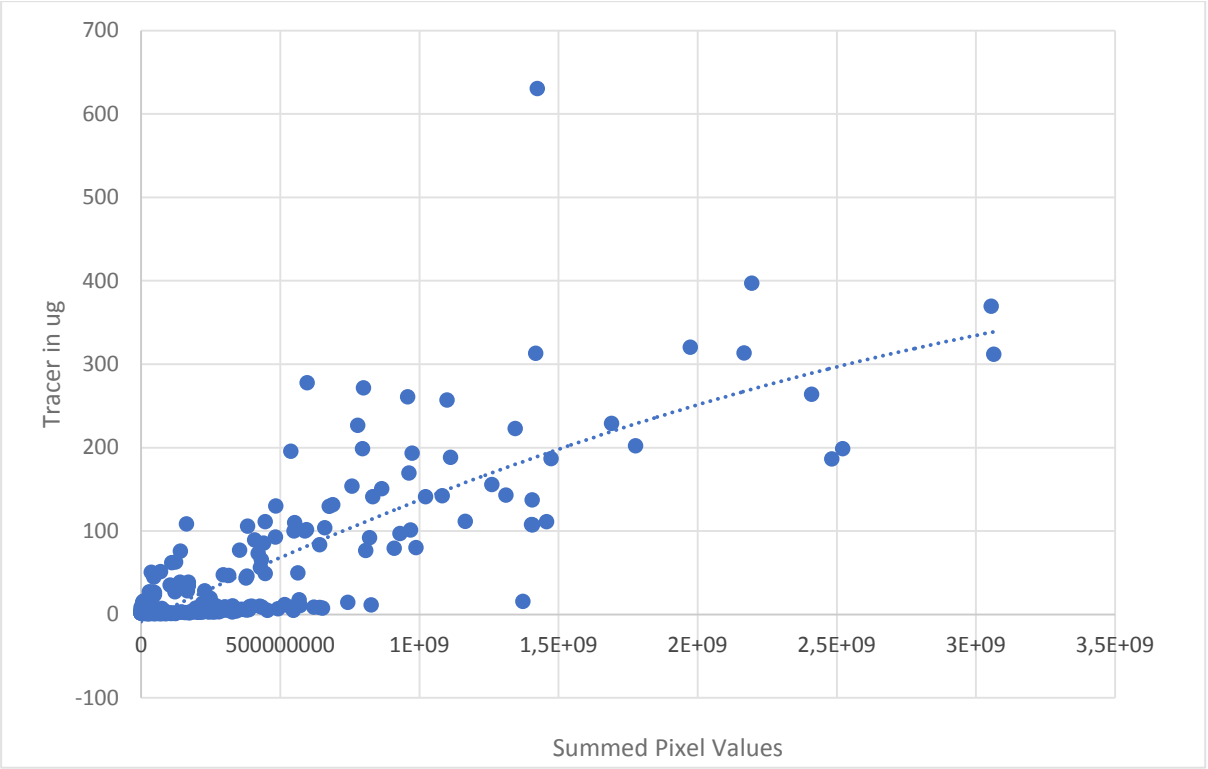


Figure 5.27 Statistical fit of model for spraying exposure situation, based on a combination of the training data sets for spraying LV and spraying HV

The spray model was used on the entire dataset for the exposure situations spraying LV and spraying HV liquid. Figure 5.28 shows the comparison between the model estimates and the measured values for the entire exposure situation spraying. A correlation of 0.76 was found.

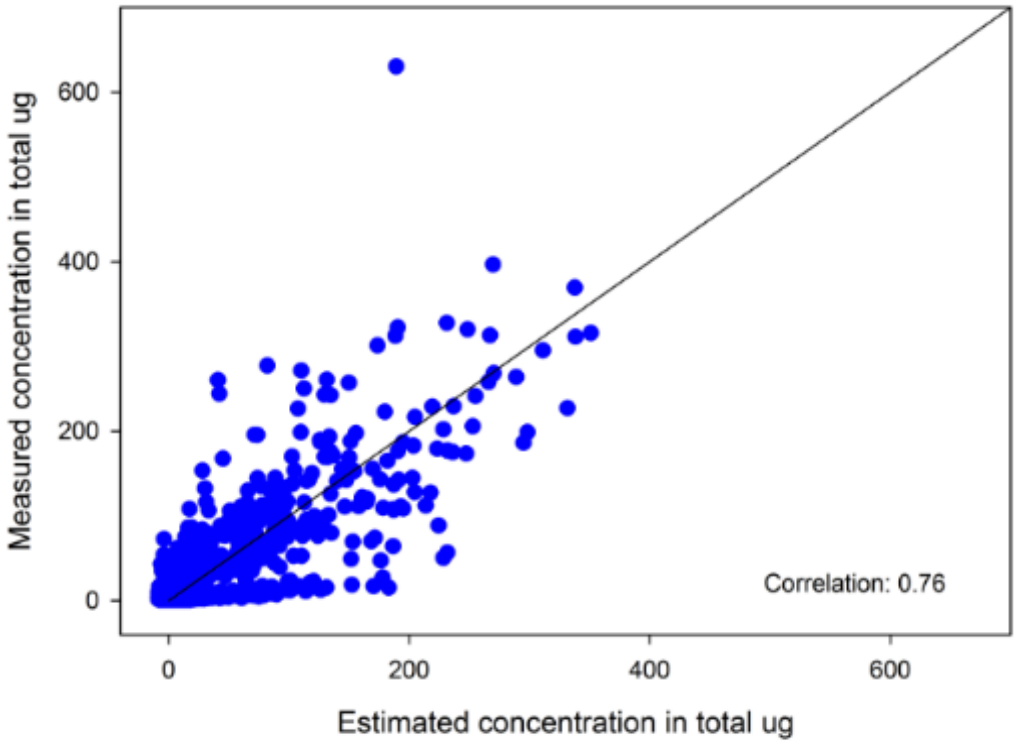


Figure 5.28 Estimated concentrations (based on model) versus measured concentration (based on chemical analysis) for exposure situation spraying as a whole

6 Experimental work

6.1 Logistics during the experiments

The logistics during the experimental phase included the sequence of tasks to be performed by both, the volunteers and the scientific staff in the course of an experiment, including dressing of the volunteer, taking photographs before the experiment, execution of the experiment, taking photographs after the experiment, undressing of the volunteer, collection of samples during undressing, showering of the volunteer, extraction and analysis of the samples. Below a brief summary is given, the details can be found in the respective SOPs (see Annex 1).

The division of tasks was done in such a way to prevent contamination of the UV-room and the volunteers. The dressing and undressing was performed in a designated area opposite to the containers, that was closed with walls and a roof top to protect the volunteer from bad weather conditions. This area was big enough to fit three tables, a clothing rack and a sink. The dressing was performed by the volunteer. Each volunteer also has an own set of PPE. The undressing was performed with the help of a member of the scientific team. The PPE and the matrices used for the experiments were kept in a portable closet with appropriate labelling outside the dressing area to prevent any contamination.

After dressing, the volunteer entered the UV-room for the “before” photographs. This process took about 3 minutes. A field scientist was responsible for the right body posture and the stickers that were put on the coverall in case there were tiny parts that fluorescence. When the field scientist was sure for the above, he took the photographs and guided the volunteer back outside.

When this part was concluded, the actual experiment, considering one of the exposure situations, was performed. The duration varied for each exposure situation. All the experiments were recorded with a camera that was placed inside the experimental room at a stationary point. A field scientist was always present inside the container to make sure that all experiments operated smoothly. When the experiment was concluded, the volunteer left the experimental room, took off his mask and goggles with the help of the technical assistant, and put on protective plastic covers on the boots before entering the UV-room, where another field scientist waited for him to take the “after” photographs. This field scientist had to make sure that every contaminated part of the coverall was visible to the camera (e.g. the part that is visible to the camera based on the orientation of the volunteer to UV lamps). The latter was accomplished after careful investigation of the volunteer and extremely careful stretching of the fabric if required, with minimum contact with gloves to avoid transfer of Tinopal SWN from coverall to glove, simple instructions for positioning.

The UV-room was big enough to also fit a table for the field spikes and blanks. This was performed every day during the actual experiment by a member of the scientific team.

The undressing of the volunteer after the experiment took about 20 minutes. One field scientist was responsible for the cutting of the parts or the collection of the patches as well as performing the hand wash, wiping of the forehead, and collection of the gloves and headband. The same cutting scheme was used for both Tyvek and cotton coveralls. All dosimeters were put directly in plastic pots that were closed tight to prevent any spillage and were also used for extraction. These pots were placed in the undressing area beforehand by the technical assistant.

While undressing, the technical assistant cleaned the container where the experiment took place, as well as the mask and the goggles, to prepare for the next experiment.
After this, the volunteers took a shower for about 5 minutes, while a field scientist transferred the dosimeters in the lab for extraction and analysis. The extraction took about 30 minutes. When this stage was completed, the samples were transferred for analysis, which took about 15 minutes.

The scientific team was responsible for preparing as much as possible before the experiment to save time by labeling the pots, use standardized forms, set the camera, pre-cut patches and headbands.

6.2 Overview of the planning of the experimental work

For each exposure situation, the dates on which the experiments were performed are shown in Table 6.1.

Table 6.1 Complete planning of experimental work

Week		Monday	Tuesday	Wednesday	Thursday	Friday
27 / 1	Date Exp.*	4 July A2V1R1M1 A2V1R1M2	5 July A2V2R1M1 A2V2R1M2	6 July A2V3R1M1 **	7 July A2V3R1M2	8 July A2V4R1M1 A2V4R1M2
28 / 2	Date Exp.	11 July A2V1R2M1 A2V1R2M2	12 July A2V2R2M1 A2V2R2M2	13 July A2V3R2M1 A2V3R2M2	14 July A2V4R2M1 A2V4R2M2	15 July -
29 / 3	Date Exp.	18 July -	19 July A2V2R3M1 A2V2R3M2	20 July A2V1R3M1 A2V1R3M2	21 July A2V4R3M1 A2V4R3M2	22 July -
32 / 4	Date Exp.	8 August A2V1R4M1 A2V1R4M2	9 August A2V3R3M1 A2V3R3M2	10 August A2V4R4M1 A2V4R4M2	11 August -	12 August -

Week		Monday	Tuesday	Wednesday	Thursday	Friday
33 / 5	Date Exp.	15 August -	16 August A2V2R4M1 A2V2R4M2	17 August A2V3R4M1 A2V3R4M1	18 August -	19 August -
34 / 6	Date Exp.	5 September A3V2R1M1 A3V2R1M2	6 September A3V4R1M1 A3V4R1M2	7 September A3V3R1M1 A3V3R1M2	8 September A3V1R1M1 A3V1R1M2	9 September -
35 / 7	Date Exp.	12 September A3V1R2M1 A3V1R2M2	13 September A3V2R2M1 A3V2R2M2	14 September A3V3R2M1 A3V3R2M2	15 September A3V4R2M1 A3V4R2M2	16 September -
36 / 8	Date Exp.	19 September A3V1R3M1 A3V1R3M2	20 September -	21 September A3V3R3M1 A3V3R3M2	22 September A3V4R3M1 A3V4R3M2	23 September A3V2R3M1 A3V2R3M2
37 / 9	Date Exp.	26 September A3V1R4M1 A3V1R4M2	27 September A3V2R4M1 A3V2R4M2	28 September A3V3R4M1 A3V3R4M2	29 September A3V4R4M1 A3V4R4M2	30 September -
38 / 10	Date Exp.	3 October C1V1R1M1 C1V1R1M2	4 October C1V2R1M1 C1V2R1M2	5 October C1V3R1M1 C1V3R1M2	6 October C1V4R1M1 C1V4R1M2	7 October -
39 / 11	Date Exp.	10 October C1V2R2M1 C1V2R2M2	11 October C1V1R2M1 C1V1R2M2	12 October C1V4R2M1 C1V4R2M2	13 October C1V3R2M1 C1V3R2M2	14 October -
40 / 12	Date Exp.	17 October C1V1R3M1	18 October C1V2R3M1	19 October C1V3R3M1 C1V3R3M2	20 October C1V4R3M1	21 October -

Week		Monday	Tuesday	Wednesday	Thursday	Friday
		C1V1R3M2	C1V2R3M2		C1V4R3M2	
41 / 13	Date Exp.	24 October C1V1R4M1 C1V1R4M2	25 October C1V2R4M1 C1V2R4M2	26 October C1V3R4M1 C1V3R4M2	27 October C1V4R4M1 C1V4R4M2	28 October -
42 / 14	Date Exp.	31 October C2V2R1M1 C2V2R1M2	1 November C2V1R1M1 C2V1R1M2	2 November C2V3R1M1 C2V3R1M2	3 November C2V4R1M1 C2V4R1M2	4 November -
43 / 15	Date Exp.	7 November C2V1R2M1 C2V1R2M2	8 November C2V2R2M1 C2V2R2M2	9 November C2V3R2M1 C2V3R2M2	10 November C2V4R2M1 C2V4R2M2	11 November -
44 / 16	Date Exp.	14 November C2V1R3M1 C2V1R3M2	15 November C2V2R3M1 C2V2R3M2	16 November C2V3R3M1 C2V3R3M2	17 November C2V4R3M1 C2V4R3M2	18 November -
45 / 17	Date Exp.	21 November C2V1R4M1 C2V1R4M2	22 November C2V2R4M1 C2V2R4M2	23 November C2V3R4M1 C2V3R4M2	24 November C2V4R4M1 C2V4R4M2	25 November -
46 / 18	Date Exp.	28 November D1V1R1M1 D1V1R1M2	29 November D1V2R1M1 D1V2R1M2	30 November D1V3R1M1 D1V3R1M2	1 December D1V4R1M1 D1V4R1M2	2 December -
47/19	Date Exp.	5 December D1V1R2M1 D1V1R2M	6 December D1V4R2M1 D1V4R2	7 December -	8 December D1V2R2M1 D1V2R2M	9 December D1V3R2M1 D1V3R2M2

Week		Monday	Tuesday	Wednesday	Thursday	Friday
		2	M2		2	
50/20	Date Exp.	12 December D1V2R3M1 D1V2R3M2	13 December D1V3R3M1 D1V3R3M2	14 December D1V1R3M1 D1V1R3M2	15 December D1V4R3M1 D1V4R3M2	16 December -
51/21	Date Exp.	19 December D1V1R4M1 D1V1R4M2	20 December D1V2R4M1 D1V2R4M2	21 December D1V3R4M1 D1V3R4M2	22 December D1V4R4M1 D1V4R4M2	23 December -
52/22	Date Exp.	26 December -	27 December D2V1R1M1 D2V1R1M2 D2V2R1M1 D2V2R1M2	28 December D2V3R1M1 D2V3R1M2 D2V4R1M1 D2V4R1M2	29 December -	30 December -
01/23	Date Exp.	2 January -	3 January -	4 January -	5 January -	6 January -
02/24	Date Exp.	9 January D2V1R2M1 D2V1R2M2	10 January D2V2R2M1 D2V2R2M2	11 January D2V3R2M1 D2V3R2M2	12 January D2V4R2M1 D2V4R2M2	13 January -
03/25	Date Exp.	16 January D2V1R3M1 D2V1R3M2	17 January D2V2R3M1 D2V2R3M2	18 January D2V3R3M1 D2V3R3M2	19 January D2V4R3M1 D2V4R3M2	20 January -
04/26	Date Exp.	23 January D2V1R4M1 D2V1R4M2	24 January D2V2R4M1 D2V2R4M2	25 January D2V3R4M1 D2V3R4M2	26 January D2V4R4M1 D2V4R4M2	27 January -

Week		Monday	Tuesday	Wednesday	Thursday	Friday
05/27	Date Exp.	6 February B1V4R1M1 B1V4R1M2	7 February B1V2R1M1 B1V2R1M2	8 February B1V3R1M1 B1V3R1M2	9 February B1V1R1M1 B1V1R1M2	10 February -
06 / 28	Date Exp.	13 February B1V1R2M1 B1V1R2M2	14 February B1V2R2M1 B1V2R2M2	15 February B1V3R2M1 B1V3R2M2	16 February B1V4R2M1 B1V4R2M2	17 February -
07 / 29	Date Exp.	20 February B1V1R3M1 B1V1R3M2	21 February B1V2R3M1 B1V2R3M2	22 February B1V3R3M1 B1V3R3M2	23 February B1V4R3M1 B1V4R3M2	24 February -
08 / 30	Date Exp.	27 February -	28 February B1V2R4M1 B1V2R4M2	1 March B1V3R4M1 B1V3R4M2	2 March B1V4R4M1 B1V4R4M2	3 March B1V1R4M1 B1V1R4M2
09 / 31	Date Exp.	6 March B2V1R1M1 B2V1R1M2	7 March B2V2R1M1 B2V2R1M2	8 March B2V3R1M1 B2V3R1M2	9 March B2V4R1M1 B2V4R1M2	10 March -
10 / 32	Date Exp.	13 March B2V1R2M1 B2V1R2M2	14 March B2V2R2M1 B2V2R2M2	15 March B2V3R2M1 B2V3R2M2	16 March B2V4R2M1 B2V4R2M2	17 March -
11 / 33	Date Exp.	20 March B2V1R3M1 B2V1R3M2	21 March B2V2R3M1 B2V2R3M2	22 March B2V3R3M1 B2V3R3M2	23 March B2V4R3M1 B2V4R3M2	24 March -
12 / 34	Date Exp.	27 March B2V1R4M1 B2V1R4M2	28 March B2V2R4M1 B2V2R4M2	29 March B2V3R4M1 B2V3R4M2	30 March B2V4R4M1 B2V4R4M2	31 March -
13 / 35	Date Exp.	3 April EV1R1M2	4 April EV2R1M2	5 April EV3R1M1 EV3R1M2	6 April EV4R1M1 EV4R1M2	7 April -

Week		Monday	Tuesday	Wednesday	Thursday	Friday
14 / 36	Date Exp.	10 April -	11 April EV1R1M1 EV2R1M1	12 April -	13 April -	14 April -
15 / 37	Date Exp.	24 April EV1R2M1 EV1R2M2	25 April EV2R2M1 EV2R2M2	26 April EV3R2M1 EV3R2M2	27 April EV4R2M1 EV4R2M2	28 April -
16 / 38	Date Exp.	1 May -	2 May EV2R3M1 EV2R3M2	3 May EV3R3M1 EV3R3M2	4 May EV4R3M1 EV4R3M2	5 May EV1R3M1 EV1R3M2
17 / 39	Date Exp.	8 May EV1R4M1 EV1R4M2	9 May EV2R4M1 EV2R4M2	10 May EV3R4M1 EV3R4M2	11 May EV4R4M1 EV4R4M2	12 May -
18 / 40	Date Exp.	15 May A1V1R1M1 A1V1R1M2	16 May A1V2R1M1 A1V2R1M2	17 May A1V4R1M1 A1V4R1M2	18 May -	19 May A1V3R1M1 A1V3R1M2
19 / 41	Date Exp.	22 May A1V1R2M1 A1V1R2M2	23 May A1V2R2M1 A1V2R2M2	24 May A1V3R2M1 A1V3R2M2	25 May A1V4R2M1 A1V4R2M2	26 May -
20 / 42	Date Exp.	29 May A1V1R3M1 A1V1R3M2	30 May A1V2R3M1 A1V2R3M2	31 May A1V3R3M1 A1V3R3M2	1 June A1V4R3M1 A1V4R3M2	2 June A1V1R4M1 A1V1R4M2
21 / 43	Date Exp.	5 June -	6 June A1V2R4M1 A1V2R4M2	7 June A1V3R4M1 A1V3R4M2	8 June A1V4R4M1 A1V4R4M2	9 June -

* General legend for coding of experiments: A1 = dumping, A2 = pouring LV, A3 = pouring HV, B1 = Rolling LV, B2 = Rolling HV, C = Spraying LV, C2 = Spraying HV, D1 = Immersion/dipping LV, D2 = Immersion/dipping HV, E = Handling contaminated objects; V1-V4 = volunteer 1 – volunteer 4; R1-R4 = repetition 1 – repetition 4; M1 = whole body (coverall) / headband / gloves, M2 = patches / head wipe / hand wash

** not possible to perform second experiment of volunteer 3 on 06/07/2016 as originally scheduled due to a temporary malfunction of the camera, problem solved

6.3 Details of experiments

6.3.1 Characteristics of the volunteers

In Table 6.2 the characteristics of the volunteers are presented. Volunteers 1, 2 and 3 were right-handed while volunteer 4 was left-handed. All volunteers had more or less the same height (approximately 1.75 meters), and none of them performed any of these tasks on a regular (professional) basis before the start of the experiments.

Table 6.2 Characteristics of volunteers

	Volunteer 1	**Volunteer 2**	**Volunteer 3**	**Volunteer 4**
Experience	Experienced in field studies with regard to pesticides' applications (under open field conditions). Limited experience in professional activities related to the exposure situations conducted within the project.	Partly experienced in professional activities related to some of the exposure situations conducted within the project (e.g. rolling, dumping), but not in all.	Limited experience in activities related to the exposure situations. His performance was entirely based on the training given before the experiments by the BPI team.	Partly experienced in professional activities related to some of the exposure situations conducted within the project.
Height	175 cm	175 cm	170 cm	175 cm
Dominant hand	Right	Right	Right	Left

6.3.2 Characteristics of experiments

In Table 6.3 an overview of the characteristics of the experiments as performed for each of the exposure situations is given. In general, the average duration of the experiments varied between 2 minutes (for dumping of powder) and 20 minutes (for spraying and rolling).

Table 6.3 Characteristics of experiments for each of the exposure situations

Exposure situation	Formulation	Amount	Surface area / repeats	Average duration (min-max)	Remarks
Dumping (A1)	Pure Tinopal SWN (no treatment)	1 kg	6 times dumping of 1 kg	1.9 (1-2) minutes	No remarks
Pouring (A2, A3)	Low viscosity or high viscosity formulation containing 2 gram Tinopal SWN/L	10 L	10 times pouring of 1 L	LV: 6.1 (5-8) minutes HV: 5.6 (5-7) minutes	Slippery jugs during use. Standardization of hand to open the lid (pilot). During an experiment of volunteer 2 high exposure was observed as 2-3 drops fell on the left hand
Rolling (B1, B2)	Low viscosity or high viscosity formulation containing 2 gram Tinopal SWN/L	~2 L	12 m²	LV: 25.8 (20-35) minutes HV: 22.2 (17-30) minutes	Where high exposure occurred (compared to other experiments within this exposure situation), it was attributed to accidental cross contamination (e.g. hand on patch).
Spraying (C1, C2)	Low viscosity or high viscosity formulation containing 2 gram Tinopal SWN/L	~2 L	6 m²	LV: 17.4 (14-21) minutes HV: 16.1 (13-32) minutes	No remarks
handling immersed objects (D1,	Low viscosity or high	Vessel containing 20 L	Dipping and handling	LV: 8.1 (7-10) minutes	No remarks

Exposure situation	Formulation	Amount	Surface area / repeats	Average duration (min-max)	Remarks
D2)	viscosity formulation containing 2 gram Tinopal SWN/L	formulation	15 cylinders	HV: 7.8 (7-10) minutes	
Handling contaminated objects (E)	Pure Tinopal SWN (sieved)	12 g (1 g per plate)	Handling 12 plates	3.1 (2-6) minutes	No remarks

6.3.3 Remarks with regard to the performance of the experiments

Slight irritations to hands after experiments in which the 'patch method' (including hand wash) was applied were observed in a limited number of experiments for some volunteers. After washing of the hands the irritation was minimized and considered negligible. In case a temporary malfunction (of equipment, e.g. camera) was observed, the experiment was performed after resolving the shortcoming.
Below, remarks (if any) with regard to the execution of the experiments for each of the exposure situations are given.

6.3.3.1 Dumping powder (A1)

During the conduct of the experiments no significant problems were encountered.

As described in the protocol, the concentration of Tinopal SWN in the air was measured during the dumping exposure situation experiments taking into account a) the flow of the pumps of 30 L/min under standard operating conditions of the pump, b) the average duration of the task (2 min), and c) the amount of Tinopal SWN that was deposited on the filters (based on the difference of the weight of the filter before and after the experiment). The average air concentration of Tinopal SWN during the dumping experiments was 145.1 mg/m^3, but varied considerably (range 24-275 mg/m^3) (see Table 6.4).

Table 6.4 Overview of measured air concentrations of Tinopal SWN during dumping experiments

Experiment	Date of experiment	Concentration filter A (mg/m³)	Concentration filter B (mg/m³)	Average air concen-tration (mg/m³)
A1_V1_R1_M1	15-5-2017	130.0	152.2	141.1
A1_V1_R1_M2	15-5-2017	142.0	131.0	136.5
A1_V1_R2_M1	16-5-2017	237.7	148.8	193.3
A1_V1_R2_M2	16-5-2017	190.2	202.3	196.3
A1_V1_R3_M1	17-5-2017	183.3	110.0	146.7

Experiment	Date of experiment	Concentration filter A (mg/m³)	Concentration filter B (mg/m³)	Average air concen-tration (mg/m³)
A1_V1_R3_M2	17-5-2017	116.7	134.2	125.4
A1_V1_R4_M1	19-5-2017	53.2	146.7	99.9
A1_V1_R4_M2	19-5-2017	112.3	24.0	68.2
A1_V2_R1_M1	22-5-2017	267.0	241.0	254.0
A1_V2_R1_M2	22-5-2017	25.0	110.5	67.8
A1_V2_R2_M1	23-5-2017	90.3	110.8	100.6
A1_V2_R2_M2	23-5-2017	111.7	147.3	129.5
A1_V2_R3_M1	24-5-2017	90.2	100.8	95.5
A1_V2_R3_M2	24-5-2017	62.2	87.7	74.9
A1_V2_R4_M1	25-5-2017	96.0	120.5	108.3
A1_V2_R4_M2	25-5-2017	36.3	276.3	156.3
A1_V3_R1_M1	29-5-2017	127.2	130.0	128.6
A1_V3_R1_M2	29-5-2017	82.5	131.8	107.2
A1_V3_R2_M1	30-5-2017	158.8	232.5	195.7
A1_V3_R2_M2	30-5-2017	133.7	179.2	156.4
A1_V3_R3_M1	31-5-2017	279.7	270.0	274.8
A1_V3_R3_M2	31-5-2017	48.8	116.3	82.6
A1_V3_R4_M1	1-6-2017	80.3	89.8	85.1
A1_V3_R4_M2	1-6-2017	199.0	208.3	203.7
A1_V4_R1_M1	2-6-2017	149.0	178.2	163.6
A1_V4_R1_M2	2-6-2017	180.0	138.8	159.4
A1_V4_R2_M1	6-6-2017	201.7	248.5	225.1
A1_V4_R2_M2	6-6-2017	110.3	93.3	101.8
A1_V4_R3_M1	7-6-2017	72.3	190.3	131.3
A1_V4_R3_M2	7-6-2017	338.3	141.8	240.1
A1_V4_R4_M1	8-6-2017	176.0	159.3	167.7
A1_V4_R4_M2	8-6-2017	110.3	144.8	127.6
AM	**-**	**137.3**	**153.0**	**145.1**
SD		**73.5**	**57.3**	**54.9**
GM		**117.9**	**141.0**	**135.5**
Min		**25.0**	**24.0**	**67.8**
Max		**338.3**	**276.3**	**274.8**

In Figure 6.1 the parallel measured air concentrations during each of the 32 dumping experiments are plotted. In general more or less the same concentrations were measured at both sides of the container, where the dumping took place. However, this figure also shows the relative large variation in measured air concentrations, for which no clear pattern could be distinguished.

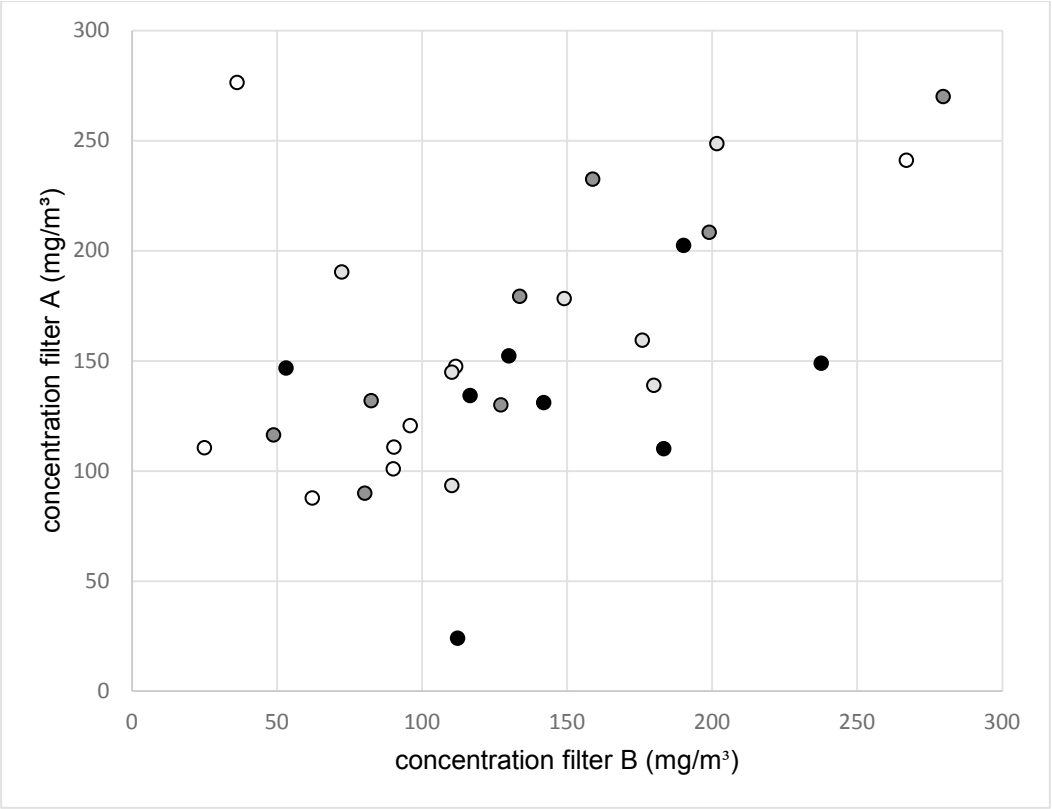


Figure 6.1 Overview of parallel measured air concentrations of Tinopal SWN during dumping experiments (V1 = black, V2 = white, V3 = dark grey and V4 = light grey)

6.3.3.2 Pouring LV (A2) and HV (A3)

During pouring no significant problems were encountered. Difficulties (such as the use of a slippery jug), and standardization of movements (e.g. selection of hand to open the lid) were counteracted during the respective pilot. Pouring LV when applying the “WBD method” (coverall / headband / gloves) for V2 (A2V2R4M1) during the fourth replicate demonstrated significantly high exposure compared to other experiments, as 2 - 3 drops (at the seventh pouring) fell onto the left hand while filling the jug.

6.3.3.3 Rolling LV (B1) and HV (B2)

No significant problems were encountered during the conduct of the experiments. Where high exposure occurred (compared to other experiments within this exposure situation), it was attributed to accidental cross-contamination (e.g. hand on patch).

6.3.3.4 Spraying LV (C1) and HV (C2)

No significant problems were encountered during the conduct of the experiments.

6.3.3.5 Handling objects immersed in LV (D1) and HV (D2)

No significant problems were encountered during the conduct of the experiments.

6.3.3.6 Handling contaminated objects (E)

No significant problems were encountered during the conduct of the experiments.

7 Statistical analysis and evaluation of the results

7.1 Statistical analysis

Data entered in different field forms, for which per exposure situation a database was created in Excel, were collected and merged into a single database, which was statistically analyzed using SAS (SAS version 9.4; SAS Institute Inc.).
The main focus of the statistical analysis lay on the comparison of the measurement methods and to generate results that can be used for further standardization of dermal exposure assessment. Relevant descriptive statistics of measured dermal exposure values (in µg/body part or µg/cm²) were assessed for each measurement method and exposure situation. As the data was lognormally distributed, also the geometric mean (GM) and geometric standard deviation (GSD) were calculated. Furthermore, patterns of exposure per body part were explored for each exposure situation to give insight in the different exposure patterns per task.
If residue levels were less than LOQ for a body part, a value of ½*LOQ of that particular body part was assigned (for more details, see paragraph 7.2).
To get a sense of the levels of exposure that were measured during each exposure situation, as well as to get an idea of the differences in observed exposure levels between the different measurement methods, various overviews in the form of boxplots were made in Sigmaplot 12.5. The boxplots as presented in this report show the lower ten percent of the distribution (dots), 10th percentile (whisker), 25th percentile, 50th percentile (median), 75th percentile, 90th percentile (whisker) and upper 10 percent of the distribution (dots). An example of a boxplot is presented in Figure 7.1. Please note that all figures are presented on a log-scale.

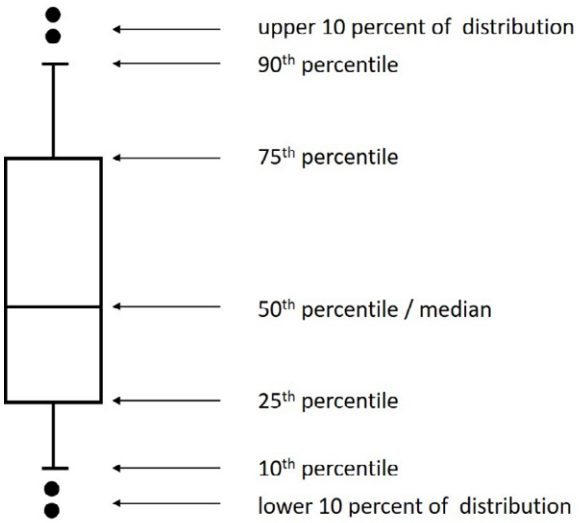


Figure 7.1 Example of a box plot

To be able to compare the concentrations on the patches with the concentrations on the coveralls, the concentrations as measured on the patches were extrapolated to a concentration for the respective body part using the determined surface area of that

particular body part of the Tyvek (in case of Tyvek patches) or cotton (in case of cotton patches) coverall (for more details see, paragraph 7.3).
In addition, the weight fraction of Tinopal SWN in the powders as used for exposure situations A1 (dumping powder) and E (handling contaminated objects) (pure Tinopal SWN, 100 %) were normalized to represent the weight fraction of Tinopal SWN in the liquids, which had a concentration of 0.2 % w/w (for more details, see paragraph 7.4.2).

Furthermore, variability in the measured dermal exposure distributions was investigated by means of regression analysis, taking into account between- and within-person variability, between- and within-exposure situation variability, and between- and within-measurement method variability (for more details, see paragraph 7.5). To do so, first a regression model was fitted to determine the significant fixed effects and to identify the random components within the experiments (and model). These random components form the basis for the estimation of the between and within person variation. Since the experiments were designed to make sure that all possible combinations of exposure situation (including product) and (combination of) measurement method(s) were repeated four times by each of the four volunteers, the random components are considered to be nested within these combinations of factors. This allowed for a very elaborate estimation of the within and between person variation.
Determinants of exposure were explored by mixed effect analysis of variance in order to account for correlations between measurements of the same repeat, and of the same occasion. Here, occasion is defined as a combination of subject, exposure task and product. Method, exposure task and product were introduced as fixed effects, while occasion, repeat and within-repeat variation were introduced as a random effects. The mixed-effect models are specified by the following expression:

$$Y_{ijk} = f(method, exposure\ task, product)_{ijk} + \omega_i + \rho_{ij} + e_{ijk}$$

for i = 1, . . ., O (occasions), j = 1, . . ., 4 (repetitions of the i^{th} occasion) and k = 1, 2 measurements within a repeat. In this formula, Y_{ijk} is the log-transformed exposure level, *f(method, exposure task, product)* is the expected value for the exposure task and product at occasion i and the method at measurement k, ω_i is the random effect of the i^{th} occasion, ρ_{ij} is the random effect of the j^{th} repeat within the i^{th} occasion, and e_{ijk} is the random effect of the k^{th} measurement of the j^{th} repeat at the i^{th} occasion. It is assumed that the random effects are each normally distributed and mutually independent, with zero means and between-occasion ($_{bo}\sigma^2$), between repeat ($_{br}\sigma^2$) and within-repeat ($_{wr}\sigma^2$) variances. The *between-person* variance is taken to be the same as the between-occasion variance, because the difference between the subjects may differ over the various methods and exposure tasks. The *within-person* variance is the sum of the between-repeat and within-repeat variance.
To determine the significant fixed effects for the model, the starting point was a complete model consisting of the three main fixed effects (namely exposure situation, which was divided into product and specific task, and measurement method) and their six possible pairwise interactions. Next to these main fixed effects and interactions, the random components formed the basis of the complete model. Then, one-by-one the non-significant effects were eliminated from the model, resulting in the final model. Note that an interaction is considered significant if its p-value is smaller than 0.05.

To determine the within and between person variation, the random components were determined for the final model. Depending on its origin, each random component contributes to either the within or the between person variation. Combining all these contributions resulted in the within and between person variation for the final model.
Since the dermal exposure values showed to be lognormally distributed, the regression analysis was performed on lognormally transformed exposure values. To be able to discriminate between high and low viscous liquids as well as to discriminate between liquids and powder, the exposure situations involving liquids were analyzed first, after which the data set as a whole, including all exposure situations, was analyzed.

7.2 Choice of approach followed for the LOQ determination

Based on the recommendations from the OECD with regard to handling dermal exposure samples below the limit of detection of the analytical technique, it was decided to assign 1/2*LOQ values to samples with a concentration below the LOQ, and use these values in the data analysis (OECD, 1997). This approach has been used before in case of presenting dermal exposure data (e.g. Mäkinen et al., 2003).

7.3 Surface areas of body parts

In paragraph 3.4.3 a description is given of how the surface area of the different parts of the Tyvek and cotton coveralls was determined. Table 7.1 shows the surface areas of the different body parts that were taken into account in this study. All patches were 100 cm², the surface area of the forehead that was wiped was approximately 96 cm², the surface area of the headbands was 414 cm², and the surface area of the hands as considered was 1380 cm² (the sum of two gloves). The surface areas of the Tyvek and cotton coveralls were used to extrapolate the measured amounts of Tinopal SWN on the Tyvek and cotton patches to a dermal exposure value for the whole body part. Exposure data from patches were extrapolated from the size of the patch to the respective size of the corresponding body parts from the WBD method. For example, for the upper left arm the extrapolation was calculated as follows: Exposure patch / 100 * 2088 in case of Tyvek material, and Exposure patch / 100 * 1185 in case of cotton material.

Table 7.1 Overview of the considered surface areas of the body parts for both combinations of measurement methods

Dosimeter	WBD method (cm²) (Tyvek coverall / gloves / headband)	WBD method (cm²) (cotton coverall / gloves / headband)	Patch method (cm²) (patches * / hand wash / head wipe)
Head	414		96
Upper arms left	2088	1185	100
Upper arms right	2088	1185	100
Forearms left	2194	1579	100
Forearms right	2194	1579	100
Torso front	9825	5709	100
Torso back	7841	4927	100
Upper legs left	4027	2785	100
Upper legs right	4027	2785	100
Lower legs left	4929	3169	100
Lower legs right	4929	3169	100
Glove left	690	690	
Glove right	690	690	
Hand wash			1380

* Patches were the same size for both Tyvek (liquid exposure situations) and cotton (powder exposure situations)

7.4 Differences observed between volunteers

Volunteer 1 and volunteer 4 demonstrated most frequently the highest measured exposure values (see Table 7.2). Of these two, volunteer 1 has been involved in field trials prior to SysDEA and is considered the most professional among the volunteers, however, the nature of the previous trials differ compared to the tasks performed in the current study. The high maximum exposures of volunteer 4 are attributed to his willingness and “lack of fear” to perform tasks as portrayed during trials. Both of them, although having different professional activities, converge in a number of maximum exposure values. Volunteer 3 exhibited a “mixed behaviour”, which is reflected in the measured exposure values. Volunteer 3 appears only three times in the list of maximum exposure values and is regarded to be the most careful among the volunteers.

Hence, characteristics inherent to behaviour and the interpretation of each the particular exposure situation by each of the volunteers seems to have shaped their attitude during the performance of the tasks and influenced exposure.

Table 7.2 Maximum exposure per exposure situation and to which volunteer these exposure values are attributed to

Exposure situation *	Method	Exposure (µg)	Volunteer
A1: Dumping powder	Coverall	175.4	V4
A1: Dumping powder	Patches	166.5	V2
A2: Pouring LV	Coverall	57964	V2
A2: Pouring LV	Patches	11445.5	V3
A3: Pouring HV	Coverall	4163.7	V4
A3: Pouring HV	Patches	7059.8	V2
B1: Rolling LV	Coverall	3942.3	V3
B1: Rolling LV	Patches	2953.3	V4
B2: Rolling HV	Coverall	1961.5	V4
B2: Rolling HV	Patches	1208.4	V4
C1: Spraying LV	Coverall	1255.5	V4
C1: Spraying LV	Patches	2708	V4
C2: Spraying HV	Coverall	2659.8	V1
C2: Spraying HV	Patches	6589.6	V4
D1: Manually handling objects immersed in LV	Coverall	2701.8	V1
D1: Manually handling objects immersed in LV	Patches	1144.6	V2
D2: Manually handling objects immersed in HV	Coverall	2851.2	V1
D2: Manually handling objects immersed in HV	Patches	5740	V1
E: Handling objects contaminated with powder	Coverall	573.5	V3
E: Handling objects contaminated with powder	Patches	300.9	V1

* LV = low viscosity liquid; HV = high viscosity liquid

7.5 Descriptive statistics

7.5.1 Overview of exposure levels per exposure situation and comparison of measurement methods

To be able to compare the exposure situations where liquids were involved with exposure situations where solids were involved, the results of the exposure situations with liquid formulations (concentration 2 g Tinopal SWN/L, 0.2 %) were at first normalized towards the concentration Tinopal SWN in the powder as applied during the exposure situations with dusty solids (100 %), by multiplying with a factor of 500.

In general, these normalized results indicated that the dermal exposure values measured during exposure situations with liquid formulations exceed those of exposure situations with dusty solids. However, because in the analysis ½ LOQ values were assigned to samples of body parts <LOQ, it must be taken into account that these values are extrapolated as well, leading to high “minimum” dermal exposure values for the liquid exposure situations.
Therefore, to reduce the influence of the values <LOQ on the normalized results, instead of normalizing the measured exposure values for the exposure situations with liquids towards the concentration of Tinopal in the powder (100 %), the measured exposure values of the exposure situations with a dusty solid were normalized to match the concentration of Tinopal in the liquid formulations (0.2 %) by correcting with a factor 500. This correction is based on the w/w fraction and not the w/v fraction. Since the same material was used for both powder and liquid exposure situations, and the same materials were analysed, the density of the material was not taken into account when normalizing the pure powders to match the concentration of Tinopal SWN used in the liquid formulations. The results as presented in this report are all based on the normalized values for the exposure situations with a dusty solid.

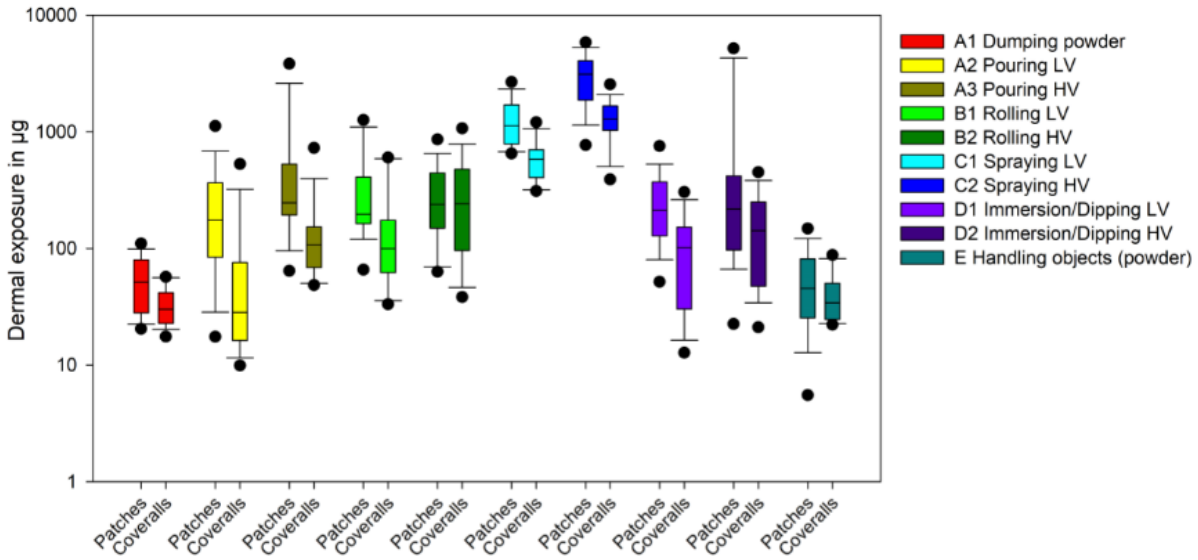


Figure 7.2 Total dermal body exposure levels (in µg) of all body parts combined (excluding hands and head) per exposure situation, differentiation between measurement methods, normalized for exposure situations with powder towards a concentration of 2 g/kg, exposure levels of the patches have been extrapolated based on the surface area of the corresponding part of the coverall

A subset was created where the total body exposure was displayed, thus without the hands and head, to be able to compare using either a coverall or patches for measuring dermal exposure of the body. These results are shown in Figure 7.2. Table 7.3 shows summary statistics for the measured total body exposure (in µg) for both the patch method (patches) and the WBD method (coverall), thus excluding exposure on the hands and head for each of the exposure situations. In general the total body exposure values measured with patches are higher than the total body exposure values measured with a coverall. After normalization, the exposure

situations with powder in general resulted in the lowest body exposure (GM = 30.9 µg for dumping and GM = 36.1 µg for handling contaminated objects in case of measuring dermal exposure with coveralls, and GM = 48.7 µg for dumping and 42.3 µg for handling contaminated objects in case of patches). For the coveralls, spraying HV liquid led to the highest exposure (GM = 1,208 µg), while spraying LV liquid led to a significant lower body exposure (GM = 569.4 µg). For the patches, spraying HV liquid led to the highest exposure (GM = 2,724 µg) and also high exposure for spraying LV liquid (GM = 1,193 µg), although the latter was much lower compared to HV liquid). Other exposure situations with liquids, such as handling immersed objects, rolling and pouring, showed relatively similar body exposures between HV and LV liquids, but were lower compared to spraying.

Table 7.3 Summary descriptive statistics for total body exposure (excluding hands and head) in µg per exposure situation and measurement method

Exposure situation	Method	AM	GM	GSD	Min	P10	P25	P50	P75	P90	Max
A1	Coverall	32.8	30.9	1.4	17.5	21.5	23.1	30.1	41.7	55.9	56.9
A1	Patches	55.7	48.7	1.7	20.5	23.3	28.2	51.8	78.9	94.6	110.2
A2	Coverall	78.2	37.7	3.0	9.9	12.3	16.6	28.3	69.9	233.9	529.1
A2	Patches	252.0	157.4	2.9	17.5	33.1	86.8	174.6	327.7	496.0	1119.3
A3	Coverall	156.8	117.9	2.0	48.5	51.0	71.3	107.5	152.4	257.6	727.8
A3	Patches	655.9	348.6	2.9	64.2	109.5	198.2	246.7	527.1	2081.3	3824.0
B1	Coverall	184.9	120.3	2.5	33.2	36.9	66.8	100.1	162.7	583.0	601.6
B1	Patches	345.3	254.1	2.1	65.7	143.1	164.3	196.5	389.8	1033.4	1262.3
B2	Coverall	311.7	210.6	2.6	38.3	49.9	108.5	242.4	425.0	663.8	1070.4
B2	Patches	308.8	244.3	2.1	63.1	72.1	154.5	238.7	431.1	565.7	859.7
C1	Coverall	612.6	569.4	1.5	310.6	321.4	441.9	583.6	696.0	1004.0	1206.4
C1	Patches	1307.6	1192.6	1.5	651.7	684.6	788.3	1125.6	1660.8	2177.6	2667.2
C2	Coverall	1317.6	1208.0	1.6	390.6	552.3	1049.2	1289.4	1625.0	1891.0	2551.5
C2	Patches	3059.1	2724.0	1.7	769.3	1300.2	1910.1	3120.4	4040.9	5035.8	5836.4
D1	Coverall	108.6	74.4	2.7	12.8	17.9	33.2	101.8	149.7	243.7	304.7
D1	Patches	261.0	210.9	2.0	51.7	92.5	134.2	212.9	372.0	430.9	753.9
D2	Coverall	169.4	117.6	2.6	21.1	40.0	48.4	142.1	249.9	354.3	449.9
D2	Patches	798.8	259.4	4.2	22.5	85.3	98.1	217.3	406.0	3919.7	5182.2
E	Coverall	39.9	36.1	1.5	22.2	22.8	25.3	34.3	46.5	79.2	87.8
E	Patches	55.5	42.3	2.3	5.5	15.9	26.3	45.4	78.1	110.5	147.8

AM= arithmetic mean, GM= geometric mean, GSD= geometric standard deviation, Min= minimum, P75= 75th percentile, P90= 90th percentile, Max= maximum
Normalized for exposure situations with powder towards concentration of 2 g/kg; exposure levels of the patches have been extrapolated based on the surface area of the corresponding part of the coverall

When comparing exposure measured using patches by exposure measured using coveralls (both interception methods), exposures measured on patches are on average higher compared to exposures measured on the coverall, although the difference varies depending on the exposure situation. Pouring showed the highest difference and rolling showed the least difference. This might be attributed to the difference in exposure patterns for these exposure situations. Spraying seems to

result in a more evenly distributed exposure pattern over the body parts (and patches), while dermal exposure during pouring is mainly based on the occurrence of splashes and/or spills. In addition, when a splash deposits on a patch, this will result in a relatively higher extrapolated exposure value of the body part as a whole compared to a more evenly distributed exposure pattern as is the case for spraying. In addition, patches are placed on locations where exposure to the body parts is most likely to occur. When the amount measured on the patch is extrapolated to the body part as a whole, areas of that body part that are less likely prone to exposure (e.g. inner parts of the upper arms or legs) are also included. This likely contributes to the higher exposure values found on patches compared to coveralls.

Hand exposure was measured with two different methods, namely hand wash (removal) versus gloves (interception). The measured exposure on the hands based on these two methods is shown in Figure 7.3 for each of the exposure situations. For the comparison, the exposure values of the two gloves were summed.

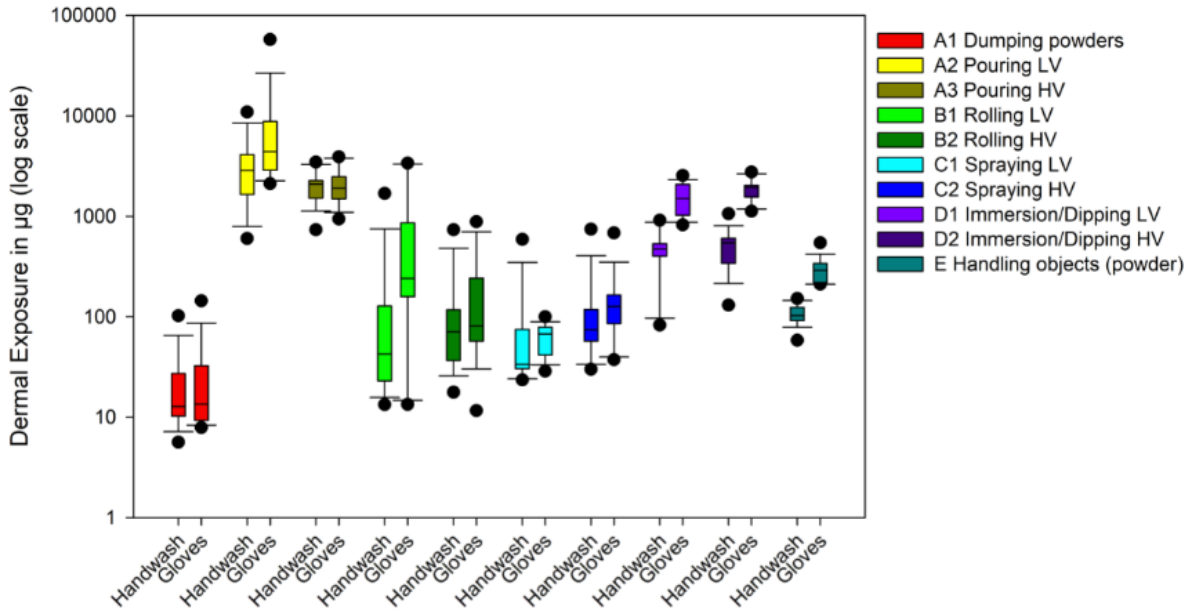


Figure 7.3 Hand exposure (in µg) per exposure situation measured by means of hand wash (removal) and cotton gloves (interception), normalized for exposure situations with powder towards a concentration of 2 g/kg

Table 7.4 shows the total hand exposure for both measurement methods per exposure situation. Hand exposure was the highest during pouring of liquids (GM= 5.515 µg for gloves and GM= 2,746 µg for hand wash) and the lowest during dumping of powders (GM= 18.2 µg for gloves and GM= 16.4 µg for hand wash). For the exposure situations in which liquid formulations are applied, the measured exposure values varied depending on the type of liquid used. Especially for the pouring exposure situation the difference seems high (GM= 5,515 µg for low viscosity liquid compared to GM= 1,986 µg for high viscosity liquid). However, the difference in measured hand exposure between the two types of liquid formulation does not appear to be consistent over all the exposure situations, as for example spraying and handling immersed objects exposure on the hands is on average higher when a high viscosity liquid is applied.

When measuring dermal hand exposure by means of gloves is compared with measuring dermal hand exposure by means of a hand wash, exposure values found on gloves are on average higher compared to exposure values found in the hand wash solution, although the difference varies depending on the exposure situation. The exposure situations for which the highest difference between the measurement methods seems to have occurred was rolling and immersion, while during spraying and pouring HV liquids, the measurement methods seem to show the lowest difference.

Table 7.4 Summary descriptive statistics for total hand exposure in µg per exposure situation and measurement method

Exposure situation	Method	AM	GM	GSD	Min	P10	P25	P50	P75	P90	Max
A1	Gloves	27.7	18.2	2.3	7.9	8.6	9.7	13.6	29.5	61.9	144.5
A1	Hand wash	22.7	16.4	2.1	5.7	7.9	10.8	12.8	24.4	49.4	102.4
A2	Gloves	8782.8	5515.3	2.3	2111.2	2332.4	3206.0	4423.4	8325.4	13530.8	57423.8
A2	Hand wash	3546.6	2746.8	2.1	602.5	875.2	1738.1	2872.8	4015.3	7465.3	10913.9
A3	Gloves	2143.1	1985.7	1.5	942.3	1165.2	1524.7	1908.1	2476.4	3729.3	3907.8
A3	Hand wash	2019.1	1904.6	1.4	735.9	1302.7	1534.4	2097.6	2254.5	3224.1	3456.6
B1	Gloves	815.3	279.8	5.3	13.4	15.3	160.9	242.1	735.6	3288.3	3380.5
B1	Hand wash	178.8	60.9	3.6	13.4	16.8	25.6	42.6	110.0	352.6	1689.2
B2	Gloves	202.1	110.3	3.1	11.6	38.3	58.6	81.6	243.7	626.4	882.9
B2	Hand wash	127.5	75.0	2.6	17.8	29.3	37.3	71.2	117.0	371.3	735.7
C1	Gloves	63.3	59.6	1.5	28.8	35.3	43.3	67.4	78.9	84.6	100.2
C1	Hand wash	94.0	54.1	2.5	23.6	24.5	30.6	33.7	73.7	245.8	589.8
C2	Gloves	150.5	115.0	2.0	37.4	40.9	85.9	127.1	158.4	208.6	683.4
C2	Hand wash	132.1	87.8	2.3	30.1	35.3	59.0	74.6	110.6	261.6	745.8
D1	Gloves	1555.3	1464.9	1.4	823.0	903.2	1064.3	1509.7	2068.7	2216.4	2545.6
D1	Hand wash	485.0	418.2	1.9	82.9	103.3	414.8	474.3	527.8	859.1	912.4
D2	Gloves	1873.0	1821.7	1.3	1122.8	1212.0	1568.6	1951.5	2047.0	2604.8	2761.5
D2	Hand wash	516.7	468.0	1.6	131.0	251.6	368.0	547.5	601.8	701.6	1062.7
E	Gloves	296.7	287.0	1.3	211.3	211.3	222.3	290.4	335.0	368.0	545.0
E	Hand wash	108.0	105.5	1.3	58.5	87.7	92.8	103.1	124.2	142.8	152.3

AM = arithmetic mean, GM = geometric mean, GSD = geometric standard deviation, Min = minimum, P75 = 75th percentile, P90 = 90th percentile, Max = maximum
Normalized for exposure situations with powder towards concentration of 2 g/kg; exposure levels of the hand wash have been extrapolated based on the surface area of the corresponding part of the gloves

For measuring exposure on the (fore)head, a head wipe (removal) and a headband (interception) were used. Figure 7.4 and Table 7.5 show the measured exposure values when using these two methods for the different exposure situations. It can be observed that, on average, head exposure was higher when measuring (fore)head exposure using head wipes (with the exception of spraying high viscosity liquids). Generally, head exposures were highest during rolling (11.8 µg using wipes and

7.4 µg using a headband) and spraying HV liquids (11.3 µg using a headband and 9.3 µg using wipes), and lowest during handling immersed objects.
This difference between the two measurement methods is probably at least partly caused by the extrapolation of the surface area of the wipe to the surface area of the headband, which was done to make the exposure values from both methods more comparable. As it is generally assumed that the front of the head is more exposed than the back of the head, this surface area of the forehead that was wiped may have been relatively more exposed than the surface area of the headband.

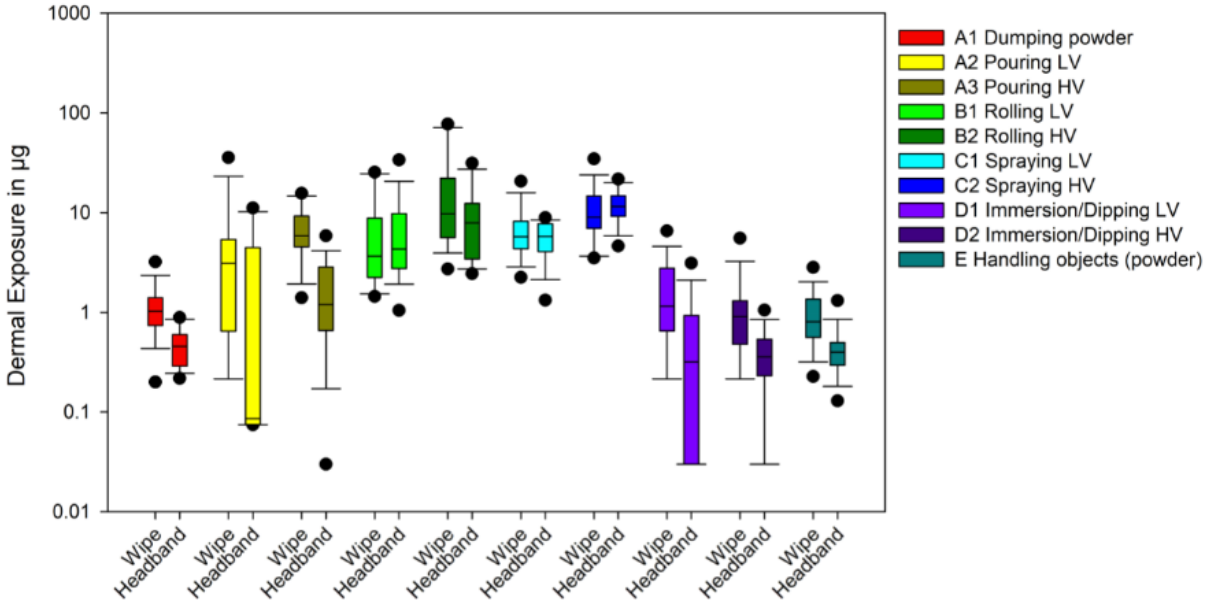


Figure 7.4 Head exposure (in µg) per exposure situation measured by means of head wipe (removal) and headband (interception), normalized for exposure situations with powder towards a concentration of 2 g/kg

Table 7.5 Summary descriptive statistics for head exposure in µg per exposure situation and measurement method

Exposure situation	Method	AM	GM	GSD	Min	P10	P25	P50	P75	P90	Max
A1	Headband	0.5	0.4	1.5	0.2	0.3	0.3	0.5	0.6	0.8	0.9
A1	Wipe	1.2	1.0	1.9	0.2	0.5	0.7	1.0	1.4	2.0	3.2
A2	Headband	2.4	0.4	8.1	0.1	0.1	0.1	0.1	4.4	9.8	11.1
A2	Wipe	5.7	2.2	4.6	0.2	0.2	0.7	3.1	5.0	17.8	35.7
A3	Headband	1.7	1.0	3.6	0.03	0.2	0.7	1.2	2.7	3.4	5.9
A3	Wipe	7.2	6.1	1.9	1.4	2.2	4.5	5.9	9.2	14.4	15.6
B1	Headband	7.7	5.3	2.4	1.0	2.3	3.1	4.3	9.8	15.0	33.9
B1	Wipe	6.9	4.5	2.5	1.4	1.6	2.3	3.7	8.1	24.3	25.4
B2	Headband	9.7	7.4	2.1	2.4	2.9	3.5	7.9	11.3	25.6	31.4
B2	Wipe	19.0	11.8	2.6	2.7	4.5	5.6	9.7	22.1	68.4	77.2
C1	Headband	5.7	5.1	1.7	1.3	2.5	4.1	5.8	7.7	8.2	8.9
C1	Wipe	7.1	6.1	1.7	2.2	3.1	4.4	5.7	8.2	13.8	20.6
C2	Headband	12.1	11.3	1.5	4.6	6.4	9.4	11.6	14.6	19.3	21.7
C2	Wipe	11.3	9.3	1.9	3.5	3.7	7.0	9.0	14.4	19.3	34.7
D1	Headband	0.6	0.2	6.0	0.03	0.03	0.03	0.3	0.9	1.7	3.1
D1	Wipe	1.7	1.1	2.8	0.2	0.2	0.7	1.2	2.6	3.7	6.6
D2	Headband	0.4	0.3	2.7	0.03	0.03	0.2	0.4	0.5	0.8	1.1
D2	Wipe	1.2	0.8	2.4	0.2	0.2	0.5	0.9	1.3	2.3	5.5
E	Headband	0.5	0.4	1.7	0.1	0.2	0.3	0.4	0.5	0.7	1.3
E	Wipe	1.0	0.8	1.9	0.2	0.4	0.6	0.8	1.3	1.7	2.8

AM = arithmetic mean, GM = geometric mean, GSD = geometric standard deviation, Min = minimum, P75 = 75th percentile, P90 = 90th percentile, Max = maximum
Normalized for exposure situations with powder towards concentration of 2 g/kg; exposure levels of head wipe have been extrapolated based on the surface area of the corresponding part of the headband

The difference in measured exposure levels when using patches compared to using coveralls to measure dermal exposure is further investigated by means of a comparison for three individual body parts (torso front, right forearm and right lower leg), to see if the observed differences between the patch method and the WBD method are consistent over the individual body parts as well. These body parts were chosen to represent differences in expected exposure patterns and exposure levels as well as exposure situations where exposures are generally above LOQ. Figure 7.5, 7.6 and 7.7 show the comparison between the patch method and the WBD method for torso front, the right forearm and the lower right leg, respectively.

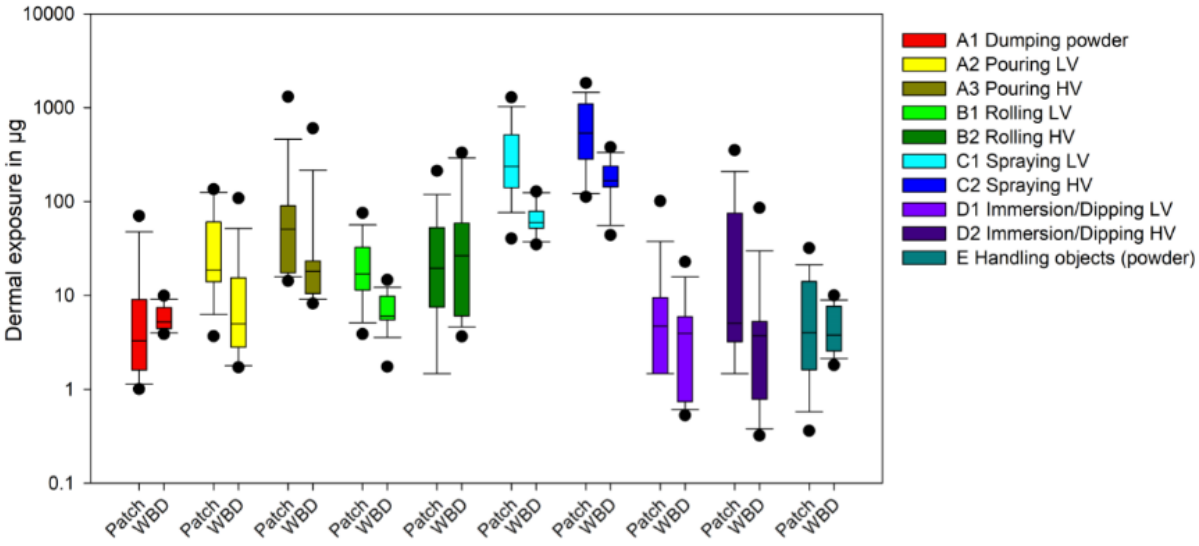


Figure 7.5 Torso front exposure (in µg) per exposure situation measured by means of a patch or a part of a coverall (WBD), normalized for exposure situations with powder towards a concentration of 2 g/kg, exposure levels of the patches have been extrapolated based on the surface area of the corresponding part of the coverall

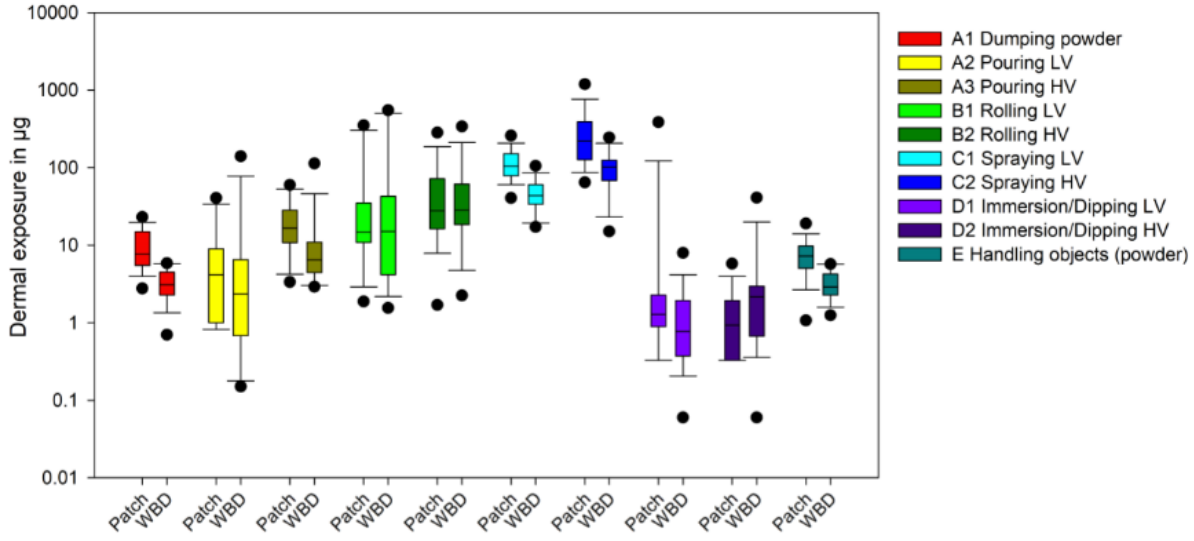


Figure 7.6 Right forearm exposure (in µg) per exposure situation measured by means of a patch or a part of a coverall (WBD), normalized for exposure situations with powder towards a concentration of 2 g/kg, exposure levels of the patches have been extrapolated based on the surface area of the corresponding part of the coverall

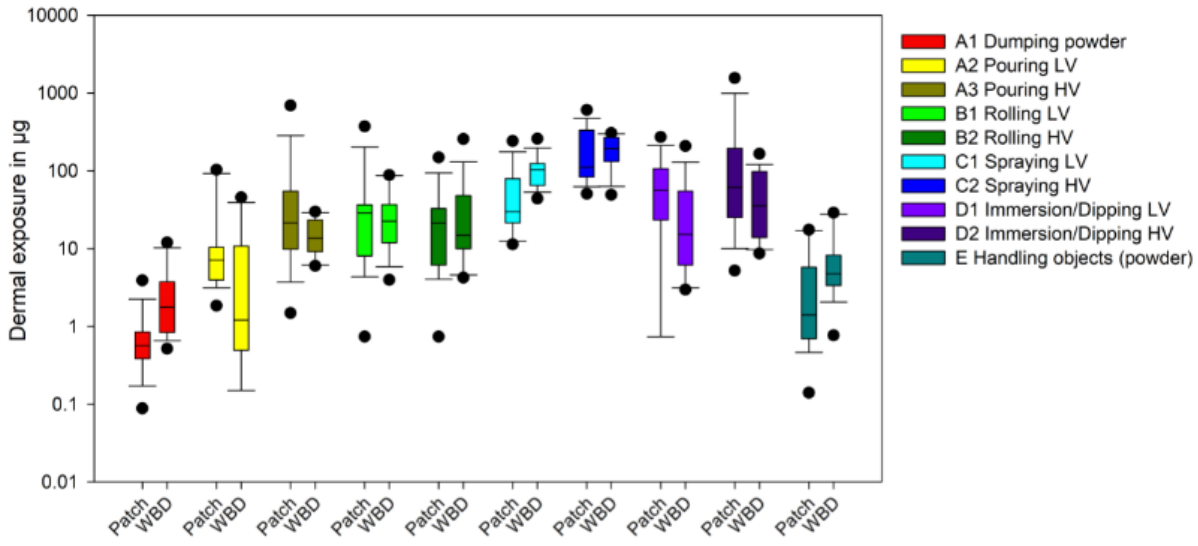


Figure 7.7 Right lower leg exposure (in µg) per exposure situation measured by means of a patch or a part of a coverall (WBD), normalized for exposure situations with powder towards a concentration of 2 g/kg, exposure levels of the patches have been extrapolated based on the surface area of the corresponding part of the coverall

For both the torso front and the right forearm, exposures values measured by means of patches are generally higher compared to exposure values measured by means of coveralls, which is in line with the comparison of the two measurement methods for total body exposure. However, in case of the right lower leg an opposite trend is observed for most of the exposure situations (exposure values measured by means of coveralls are higher compared to exposure values measured by means of patches). Even in case of spraying, which is the exposure situation for which the most evenly distributed exposure pattern over the different body parts is observed, the WBD method resulted in slightly higher exposure values compared to the patch method. This difference could be attributed to the placement of the patches on the lower legs. As can be seen in Figure 7.8, in general mainly the lower part of the lower legs are exposed during spraying, while the patches were placed on the upper part of the lower leg, due to which the levels as measured on the patches as placed on the lower legs most probably result in an underestimation of the exposure levels for this particular body part. The same pattern is observed for the exposure situations handling immersed objects and rolling, during which droplets / splashes are mainly situated at the lower part of the lower legs. There is no indication that patches are misplaced for other body parts based on the photographs. It must be noted that these observations were made on the samples which are above the LOQ. The extrapolation of <LOQ exposures found on patches results in higher minimum exposure values compared to <LOQ exposures found on the coveralls.

Figure 7.8 Two photographs of the exposure pattern to Tinopal SWN after spraying (taken with UV light), indicating the influence of the placement of the patches on the lower legs on the measured exposure value

7.5.2 Comparison of the fluorescence method with other measurement methods for spraying

In chapter 5 is described how a predictive model was developed to estimate dermal exposure values based on the measured fluorescence (pixel values) for the spraying exposure situations. This predictive model was used to estimate dermal body exposure values (hand and head exposure were excluded from the predictive model) for the spray experiments based on the pictures taken before and after the spray experiments, to be able to compare this dermal measurement method with the other measurement methods for body exposure (patches and coverall). Table 7.6 shows the summary descriptive statistics for the estimated total body exposure based on fluorescence for spraying of LV and HV liquid, which are estimated for exposure situations where coveralls and patches are used. Please note that during the annotation process either body parts as a whole (from the coverall) or patches on a particular body part were annotated, and therefore exposure is estimated separately. Due to the unreliable performance of the developed models for the other exposure situations, they were not taken into account for the comparison with the other measurement methods.

As can be seen in Table 7.6, the estimated dermal body exposure for the coveralls is higher compared to the estimated dermal body exposure for the patches. However, this comparison should actually not be directly made, since it should be noted that the estimates for patches represent the surface area of the patch itself, which is not extrapolated to reflect the surface area of the body part as a whole. The estimated values were not extrapolated, since the model was calibrated on “fixed” surfaces representing the body part or patch. Since this resulted in a second order equation, extrapolation of the pixel values found on a patch towards either a pixel value for the surface area of the body part as a whole or an exposure value for the body part as a whole would most probably result in unreliable exposure estimates, which has not been validated. Furthermore, as the model was intended to predict the exposure within the annotation boundaries, recalculation of the estimated patch exposure to represent the surface of the respective body part would introduce an extra uncertainty in the model, which already is relatively uncertain. Therefore, the comparison is made separately for patches and coveralls.

Table 7.6 Summary descriptive statistics of the estimated total body exposure (in µg) based on fluorescence (in site) for the spraying exposure situation

Exposure situation	Fluorescence in combination with measurement method	AM	GM	GSD	Min	P75	P90	Max
C1: spraying LV	Body parts from experiments with coverall	437.1	314.8	2.9	-16.4	741.7	957	1057.1
C2: spraying HV	Body parts from experiments with coverall	1257.3	1011.1	2.4	93.2	1545.2	2033.1	2166.5
C1: spraying LV	Body parts from experiments with patches	195.4	163.3	2.0	37.8	268.1	310	387.5
C2: spraying HV	Body parts from experiments with patches	402.7	321.9	2.4	18.9	516	664.4	834.7

AM = arithmetic mean, GM = geometric mean, GSD = geometric standard deviation, Min = minimum, P75 = 75^{th} percentile, P90 = 90^{th} percentile, Max = maximum

Table 7.7 shows the comparison of the GMs of the predicted dermal exposure based on the fluorescence tool and reported dermal exposure based on the chemical analysis of either parts of the coverall or the patches for the two spraying exposure situations. The total dermal body exposure based on chemical analysis of the samples compared to the estimation based on the fluorescence tool is higher for both patches and the coverall. For both comparisons in general a higher exposure is found during spraying HV liquid compared to spraying LV liquid. When for now the chemical analysis would be considered the “gold standard”, the fluorescence tool seems to underestimate total body exposure as measured on a body as a whole (both coverall but severely patches).

Table 7.7 Comparison between total body dermal exposure based on chemical analysis and estimated total body dermal exposure based on the fluorescence tool (in µg) for spraying of LV and HV liquid using patches and coveralls

Exposure situation	Fluorescence in combination with measurement method	GM (min - max)	
		Based on chemical analysis (reported)	Based on fluorescence tool (estimated)
C1: spraying LV	Body parts from experiments with coverall	569 (311-1206)	315 (-16-1057)
C2: spraying HV	Body parts from experiments with coverall	1208 (391-2552)	1011 (93-2167)
C1: spraying LV	Body parts from experiments with patches	1193 (652-2667)	163 (38-388)
C2: spraying HV	Body parts from experiments with patches	2724 (769-5836)	322 (19-835)

GM = geometric mean, min = minimum, max = maximum

7.5.3 Exposure patterns

In this paragraph the exposure patterns for each of the exposure situations are presented. The figures on the left represents the average, minimum and maximum contribution of the measured exposure of each of the body parts (excluding the hands) to the total dermal exposure (body and head) per group of experiments per exposure situation, discriminating between the “patch method” (patches, hand wash and head wipe) and the “WBD method” (coverall, gloves and headband). On the right the total exposure on both the hands and the body (all body parts summed together) are shown. For the exposure patterns that represent the patch method, exposure values measured on the patches of each of the body parts have first been extrapolated to resembling surface areas of the coverall used in the WBD method before the total dermal exposure and the percentages were calculated. Furthermore, one of the volunteers (volunteer 4) was left-handed, while the other three volunteers were all right-handed. As a difference in dominant hand is assumed to influence the observed exposure pattern, and this difference could thus ‘contaminate’ the average exposure pattern, the body parts were switched for the left-handed volunteer to mimic a right-handed volunteer for all relevant body parts (hands, arms and legs), thus not only the hands, since being left-handed might also affect your stance during each task.

Please note that the color coding as applied for indicating the relative exposure for each of the body parts is based on the average percentage for that particular body part. In addition, for each of the exposure situations representative ‘after’

photographs were selected for both measurement methods, to be able to relate this graphical representation of the exposure pattern to an observed exposure pattern.

For most exposure situations, such as pouring, manually handling immersed objects and handling contaminated objects, the majority of the exposure was found on the hands. For pouring, the majority of the body exposure was allocated to the torso and the non-dominant forearm during pouring of LV liquid. During pouring of HV liquid, exposure also occurred on the upper legs when measuring with patches. For manually handling immersed objects (both for LV and HV liquid) the majority of the exposure occurred on both lower legs, and slightly on the front torso for both the patch method and the WBD method. For handling of contaminated objects, the body exposures were more or less equally distributed over all body parts, with slightly increased values for the torso and forearms (Patch method) and torso and lower legs (WBD method). When looking at exposure on the body, in some cases exposure was observed on the dominant forearm torso and lower legs (both left and right) during rolling. Furthermore, a large difference can be observed for both using LV and HV and measuring exposure using the WBD method and Patch method. During rolling with LV liquids, for the WBD method hands contributed to 63% to the total exposure when using LV liquid compared to ~38% when using HV liquid. When looking at the patch method, the hand exposure contributed ~25% to the total exposure for both LV and HV liquid. For spraying, most exposure values were found on the body contributing >90% for all liquid types and measurement methods. Here, the torso (both front and back) received the majority of the exposure, followed by the lower legs (WBD method) and lower arms (patch method and HV liquid). Similar observations can be made for the dumping scenario, where between 63% and 73% of the exposure is allocated to the body for the WBD method and the patch method, respectively.

In case of using gloves to measure hand exposure (WBD method) it is possible to differentiate exposure levels between hands. The exposure patterns as shown in Figures 7.9-7.34 below show a clear difference in exposure between the non-dominant and dominant hand for all exposure situations except spraying and handling of contaminated objects. It is also shown that for hand exposure differences between exposure of the non-dominant and dominant hand differ for each scenario. This can be contributed to where the dominant hand is used for. For example, during rolling the dominant hand (right hand) is higher exposed compared to the non-dominant hand, as volunteers hold the roller in their dominant hand. However, for pouring and manually handling immersed objects the non-dominant hand is higher exposed, likely due to the volunteer using the non-dominant hand on more exposed surfaces (e.g. for immersed objects the dominant hand holds the object while immersing the object and the non-dominant hand touches the object on a surface that was immersed). For handling of contaminated objects, no distinction can be made in the level of exposure for both hands because the volunteer uses both hands to handle the contaminated plates. For the legs, the stance of the volunteer does not seem to have an impact on whether the leg on the non-dominant side (left) or the dominant side (right) of the body is higher exposed.

Furthermore, in addition to the average exposure patterns for each exposure task a photograph under UV light was included to show the exposure patterns as found on the volunteers. It must be noted that for the experiments with patches, the volunteers

hold their hands in the opposite direction compared to the experiments with a coverall. This was done to make sure that the patches were kept straight, and to reduce angles in the patches by the positioning of the arms as can also be seen in the photographs. On these photographs the exposure patterns are less visible under UV light for the exposure situations with powder compared to the exposure situations with liquids. It seems that Tinopal SWN emits less brightly in the powder form. Furthermore, as is also described in paragraph 7.5.1, it can be observed from the photographs taken after spraying, manually handling immersed objects and (to a lesser extent) rolling that exposure levels as measured on the lower legs by means of patches might be underestimated, since the exposure seems to occur mainly on the lower part of the lower legs (due to the larger droplets in case of handling of immersed objects or smaller scattered droplets in case of rolling). In case of rolling small dots are observed on both the lower legs and the forearms, which seem to be randomly scattered over the surface. These observations indicate that using patches to measure dermal exposure may introduce some uncertainty in case of exposure situations during which (random) splashes may occur.

7.5.3.1 A1: Dumping powder

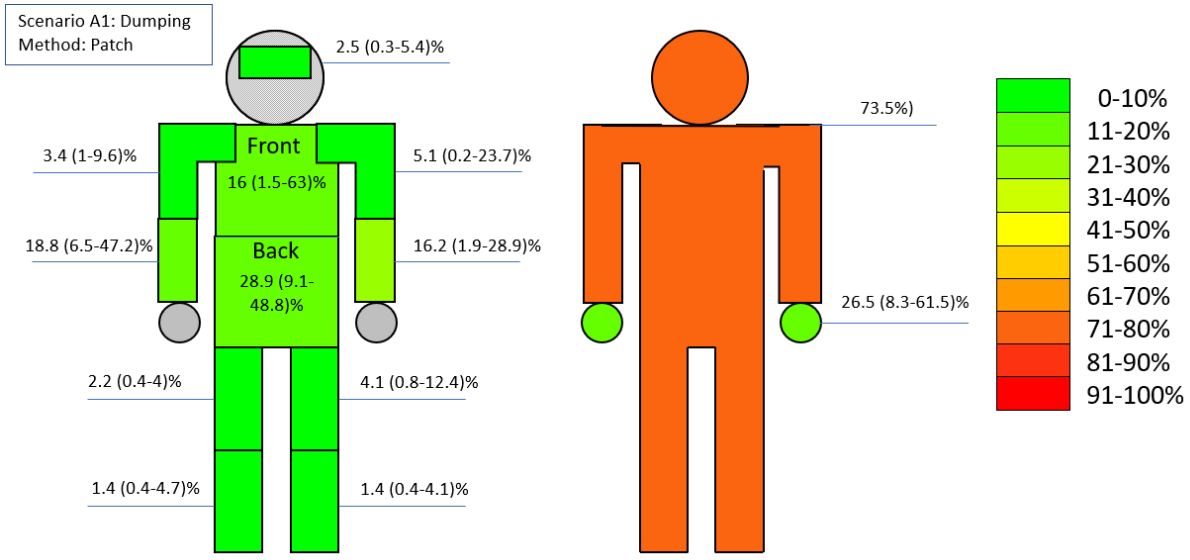


Figure 7.9 Percentage exposure coverage per body part for dumping powders using the patch method (patches, hand wash, head wipe). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

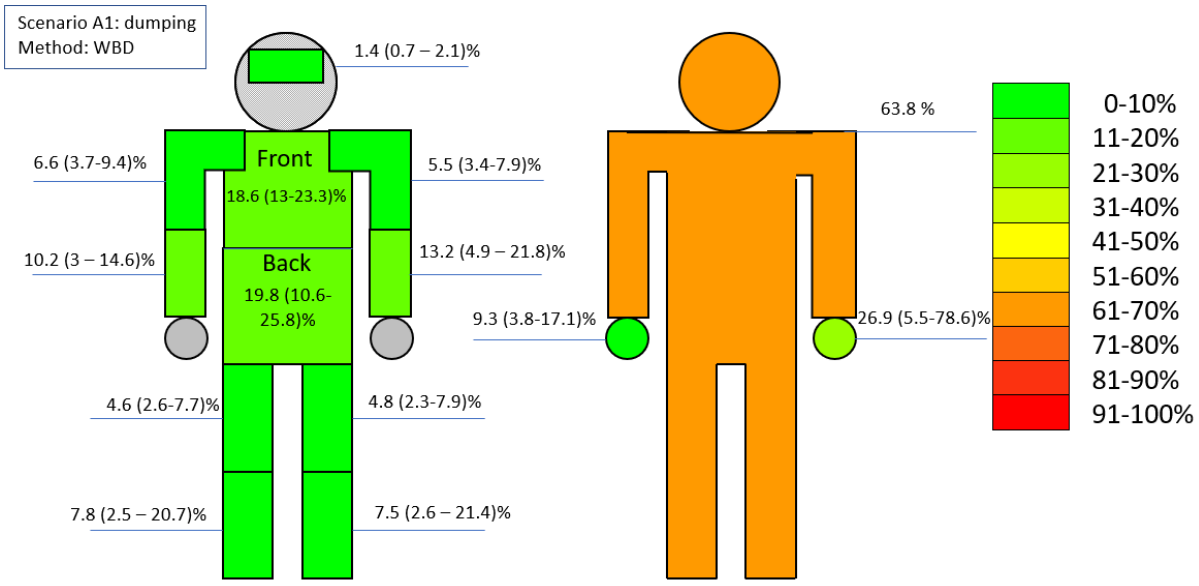


Figure 7.10 Percentage exposure coverage per body part for dumping powders using the WBD method (coverall, gloves, headband). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

Figure 7.11 Typical exposure pattern from volunteers under UV light after dumping powder (left: WBD method, right: patch method)

7.5.3.2 A2: Pouring LV liquid

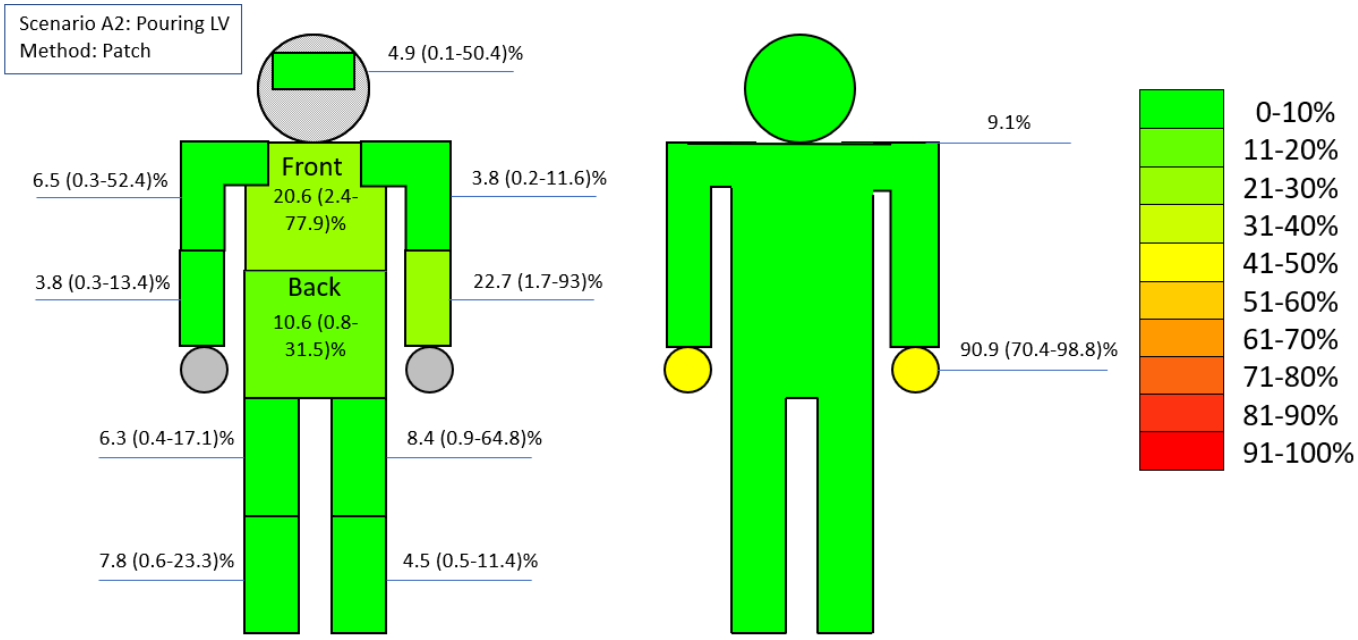


Figure 7.12 Percentage exposure coverage per body part for pouring LV liquid using the patch method (patches, hand wash, head wipe). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

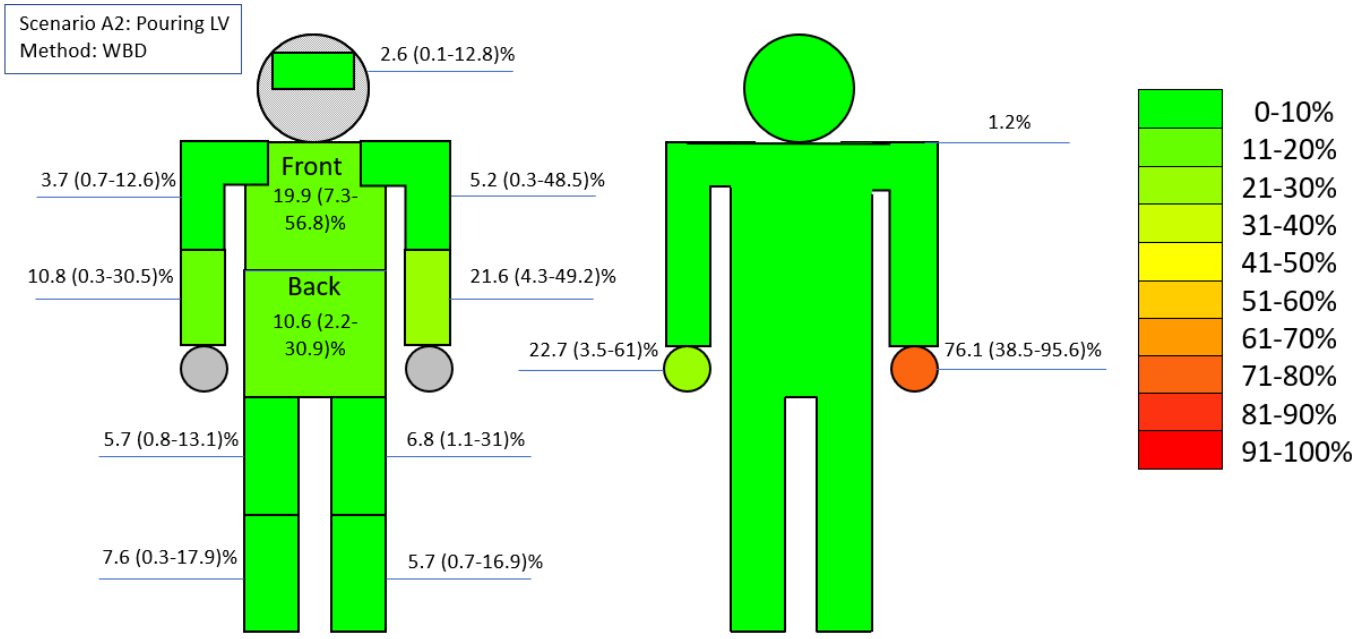


Figure 7.13 Percentage exposure coverage per body part for pouring LV liquid using the WBD method (coverall, gloves, headband). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

7.5.3.3 A3: Pouring HV liquid

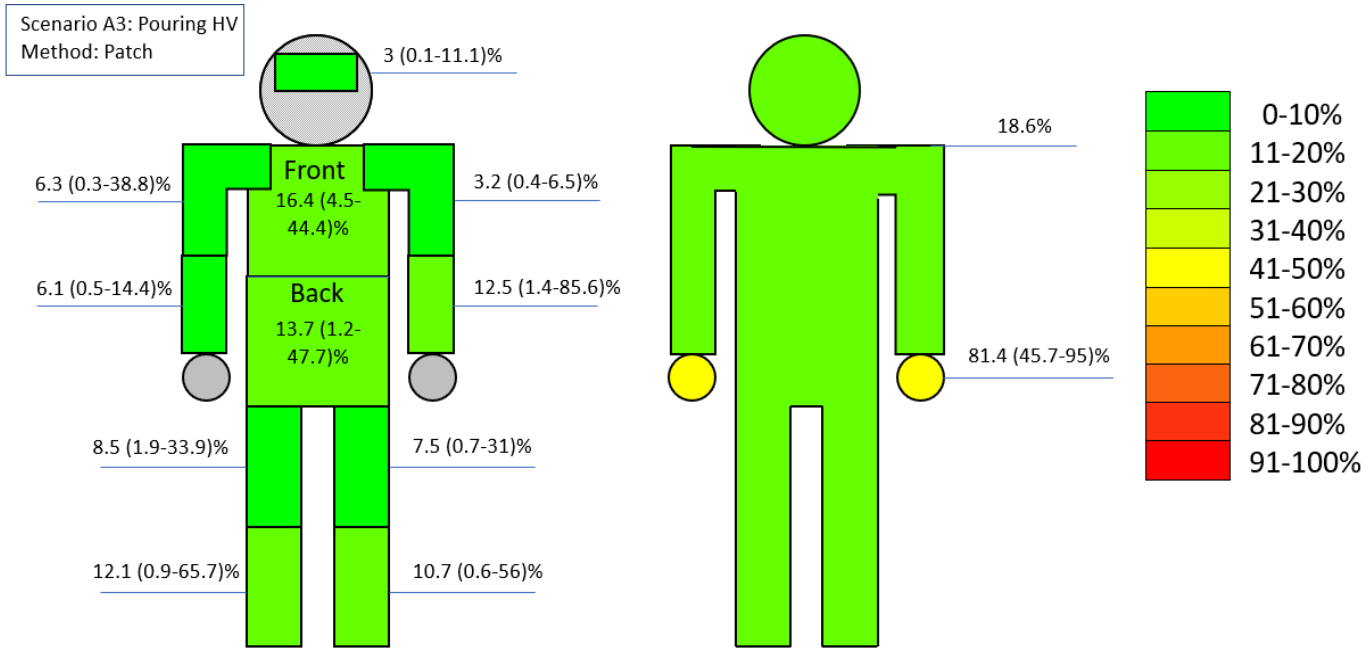


Figure 7.14 Percentage exposure coverage per body part for pouring HV liquid using the patch method (patches, hand wash, head wipe). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

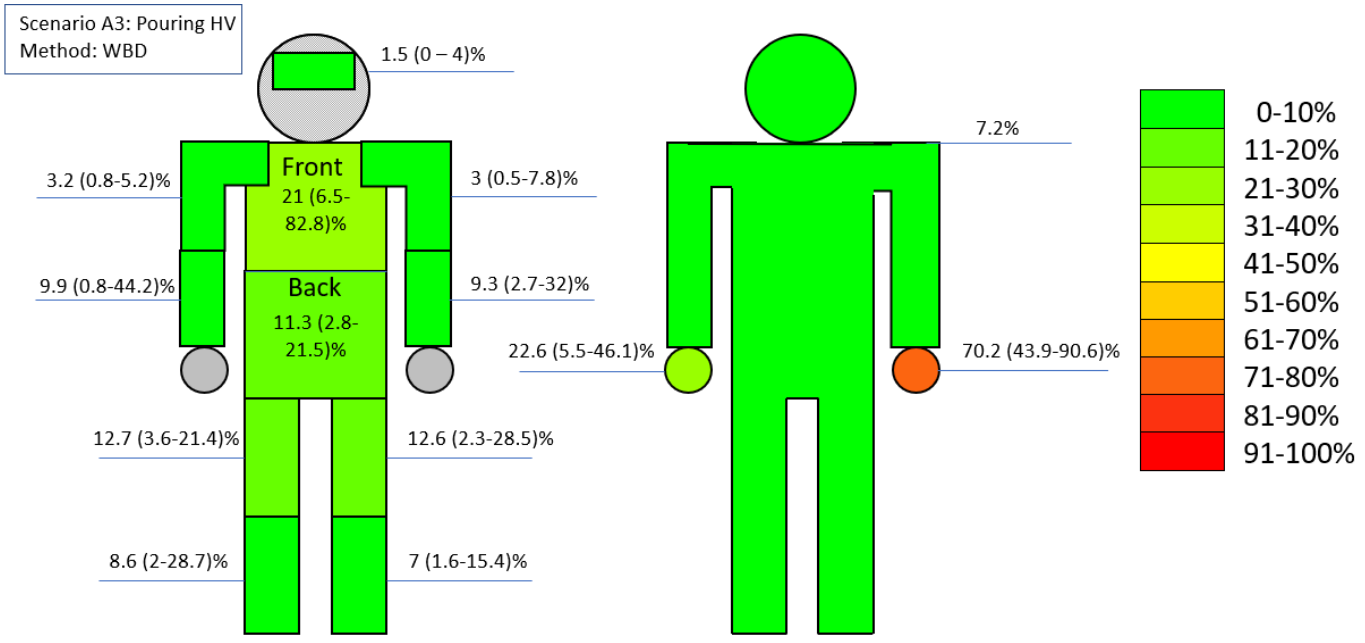


Figure 7.15 Percentage exposure coverage per body part for pouring HV liquid using the WBD method (coverall, gloves, headband). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

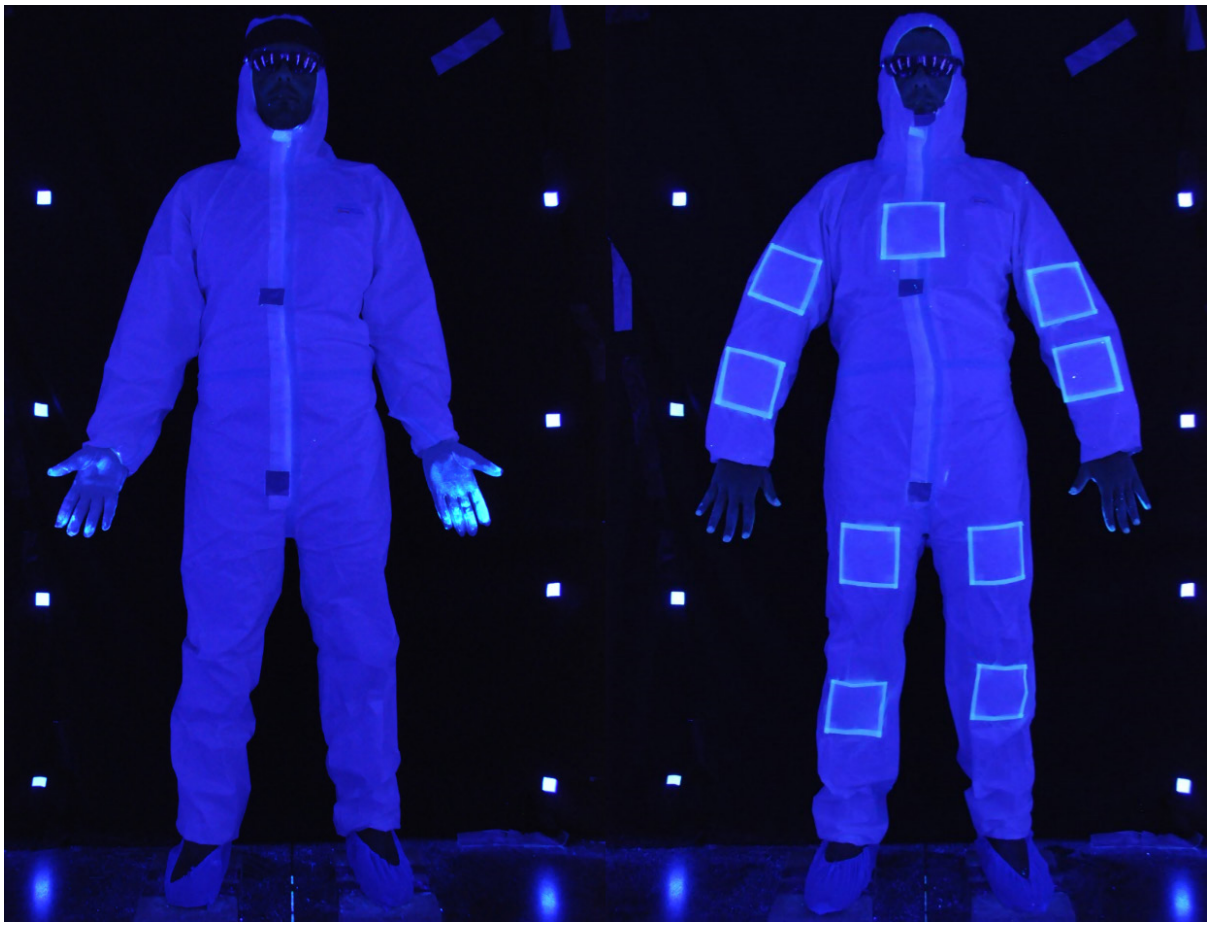


Figure 7.16 Typical exposure pattern from volunteers under UV light after pouring liquids (left: WBD method, right: patch method)

7.5.3.4 B1: Rolling LV liquid

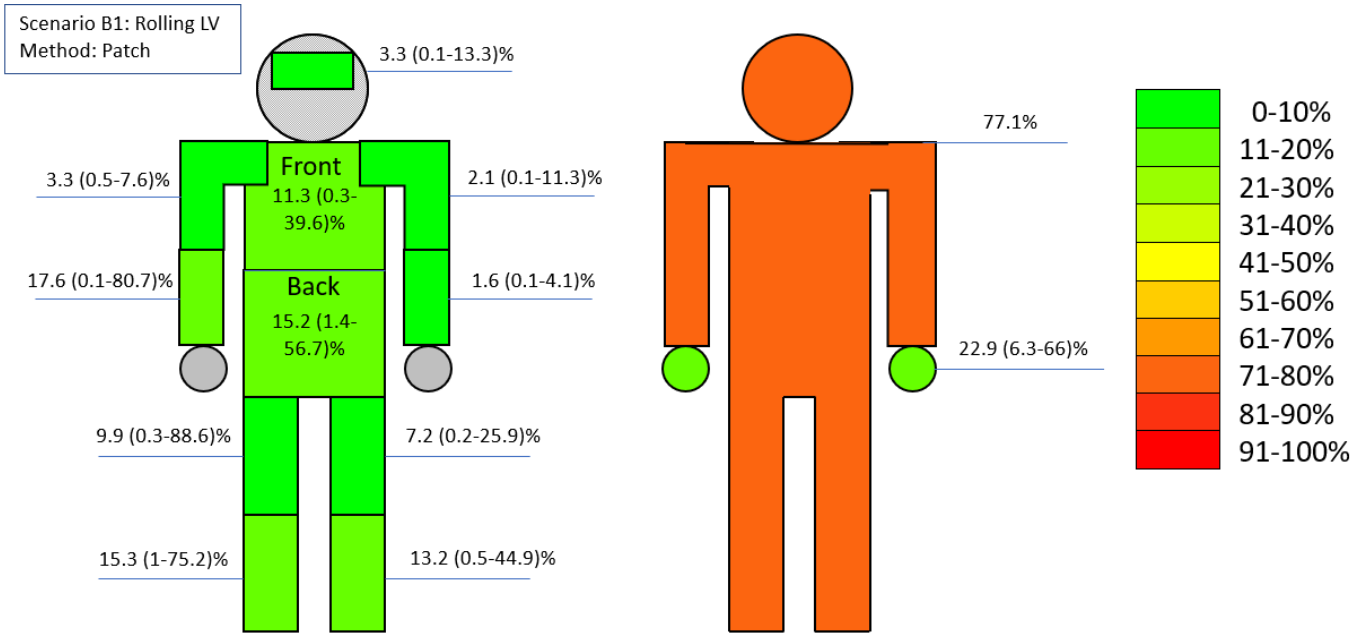


Figure 7.17 Percentage exposure coverage per body part for rolling LV liquid using the patch method (patches, hand wash, head wipe). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

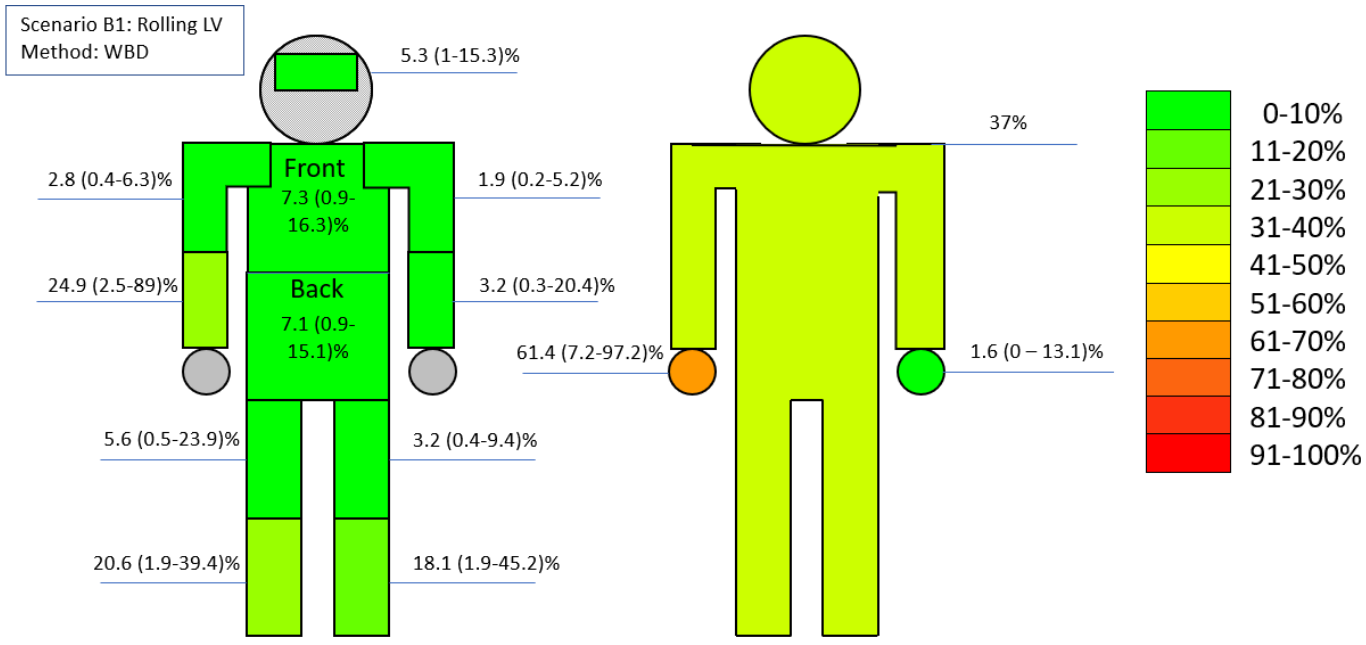


Figure 7.18 Percentage exposure coverage per body part for rolling LV liquid using the WBD method (coverall, gloves, headband). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

7.5.3.5 B2: Rolling HV liquid

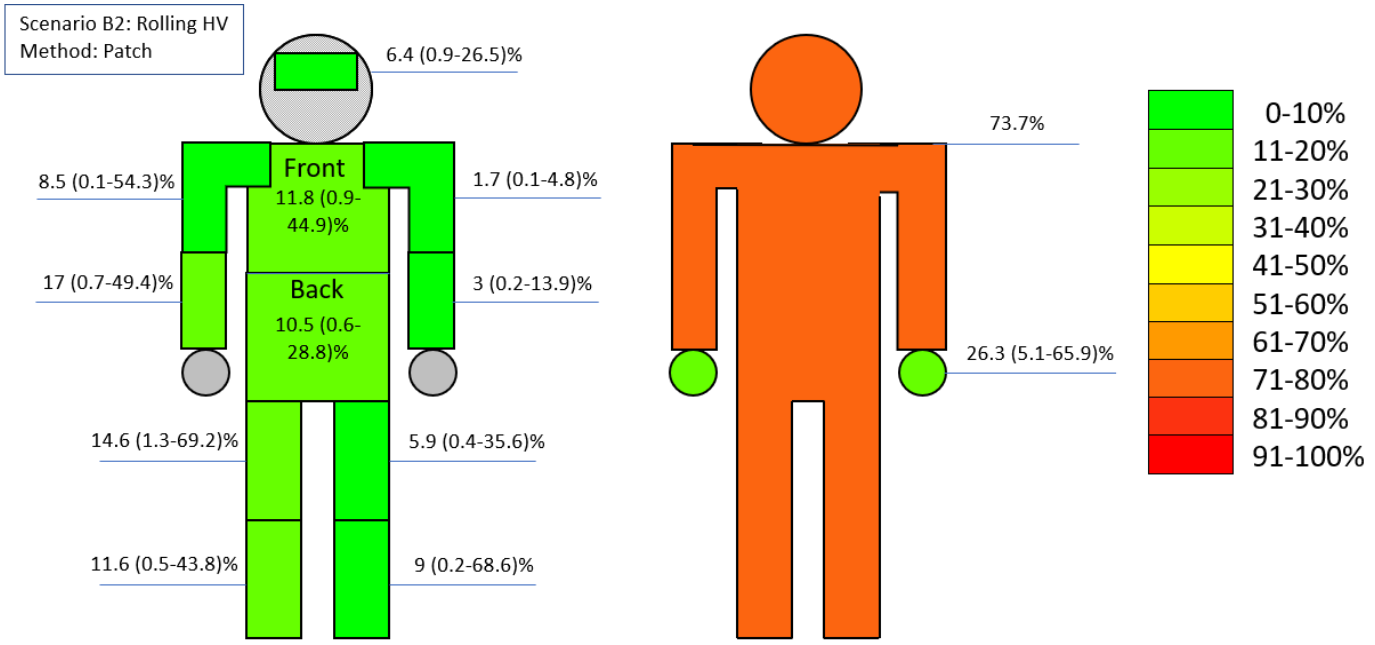


Figure 7.19 Percentage exposure coverage per body part for rolling HV liquid using the patch method (patches, hand wash, head wipe). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

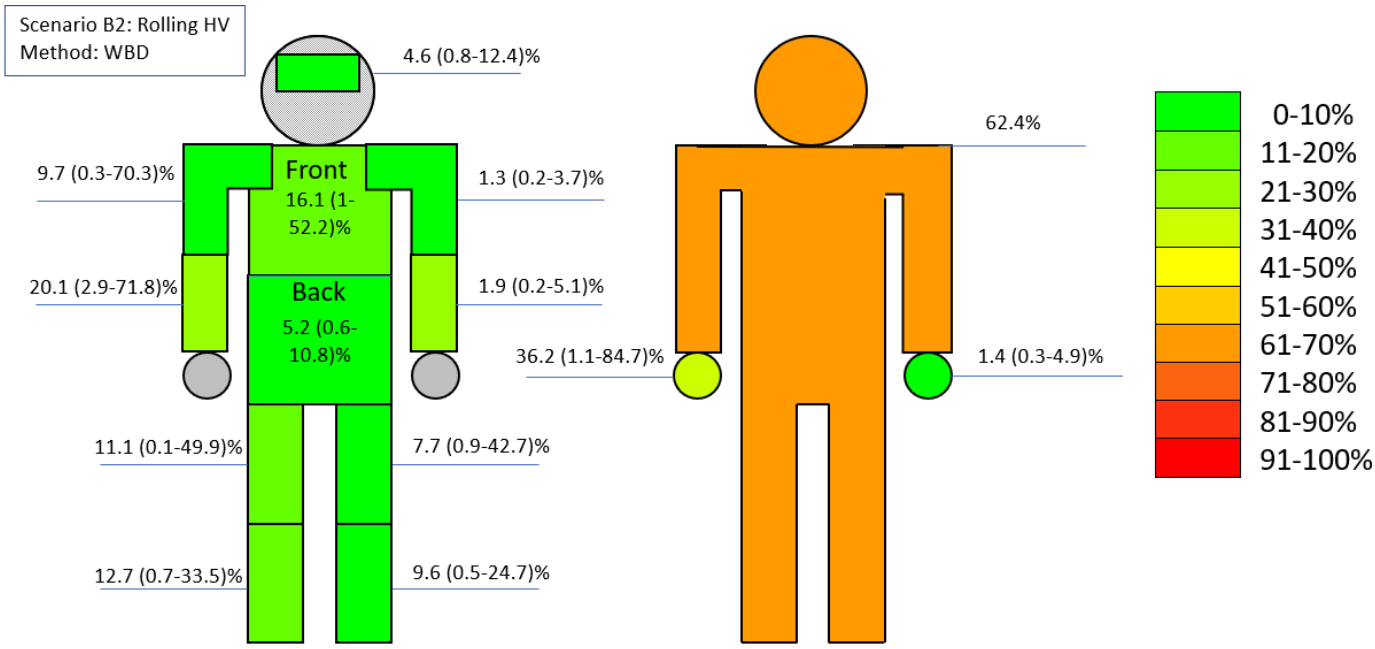


Figure 7.20 Percentage exposure coverage per body part for rolling HV liquid using the WBD method (coverall, gloves, headband). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

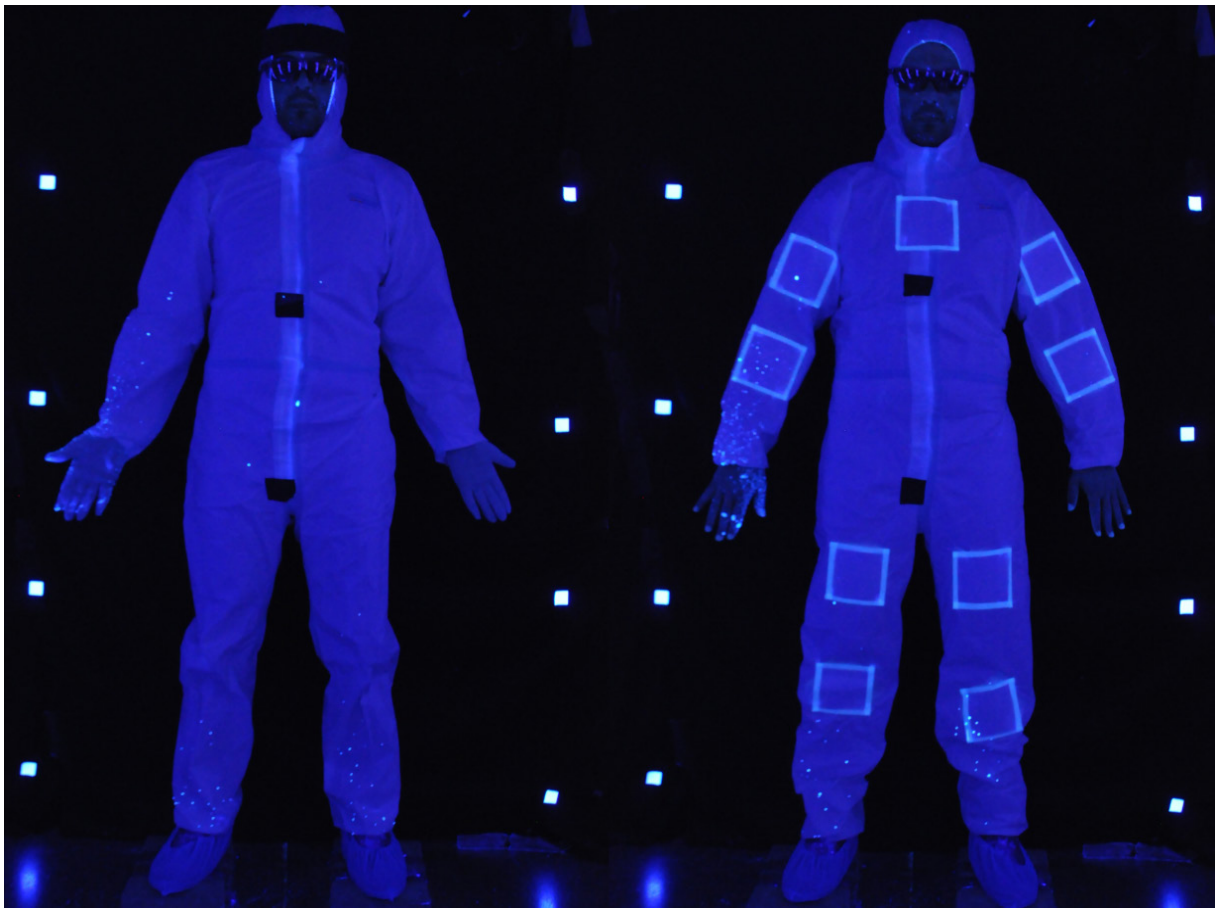


Figure 7.21 Typical exposure pattern from volunteers under UV light after rolling liquids (left: WBD method, right: patch method)

7.5.3.6 C1: Spraying LV liquid

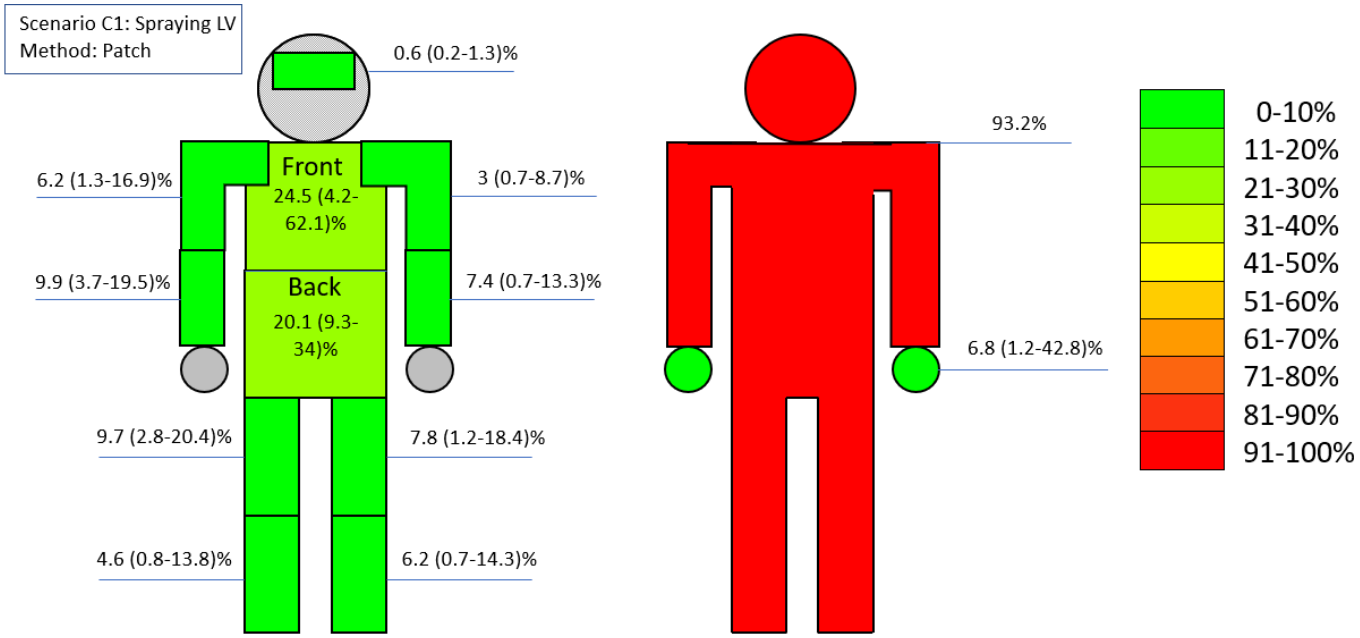


Figure 7.22 Percentage exposure coverage per body part for spraying LV liquid using the patch method (patches, hand wash, head wipe). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

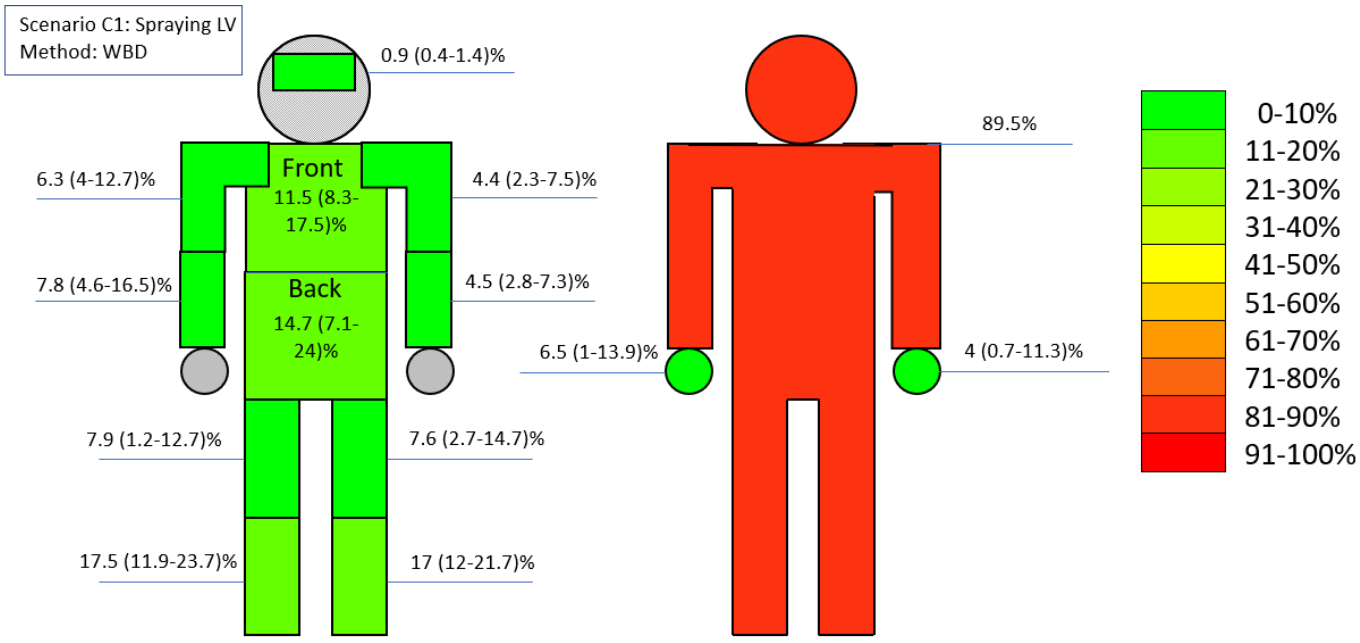


Figure 7.23 Percentage exposure coverage per body part for spraying LV liquid using the WBD method (coverall, gloves, headband). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

7.5.3.7 C2: Spraying HV liquid

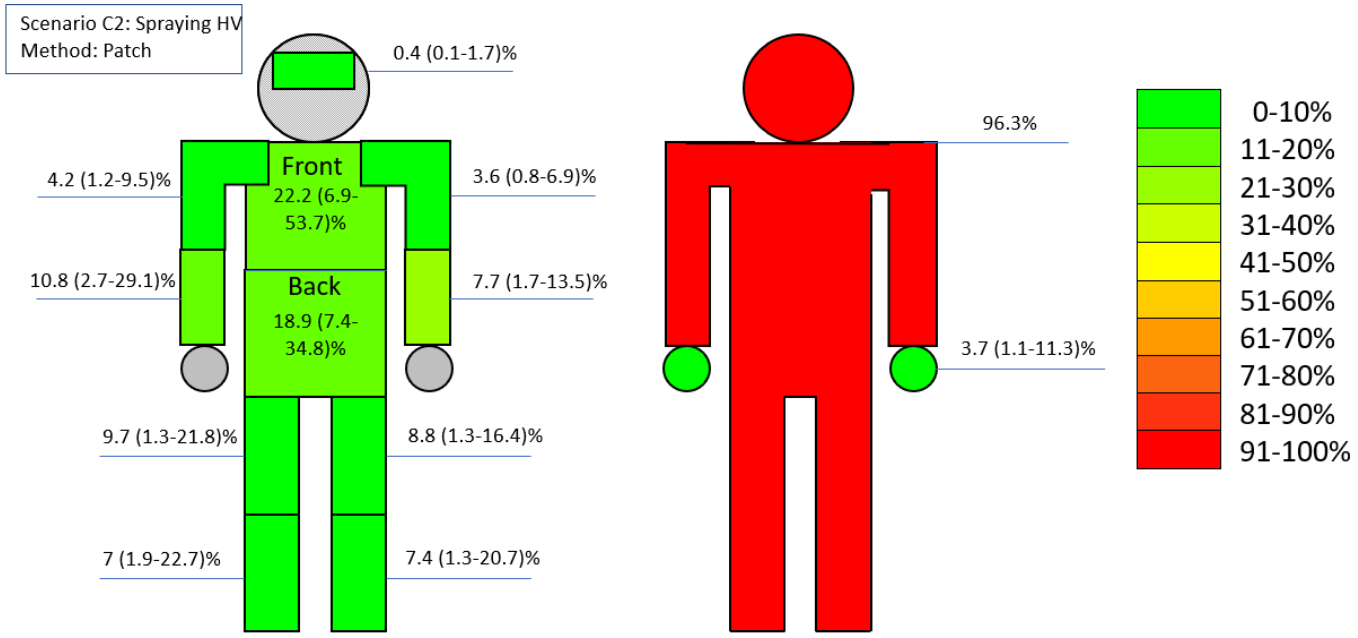


Figure 7.24 Percentage exposure coverage per body part for spraying HV liquid using the patch method (patches, hand wash, head wipe). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

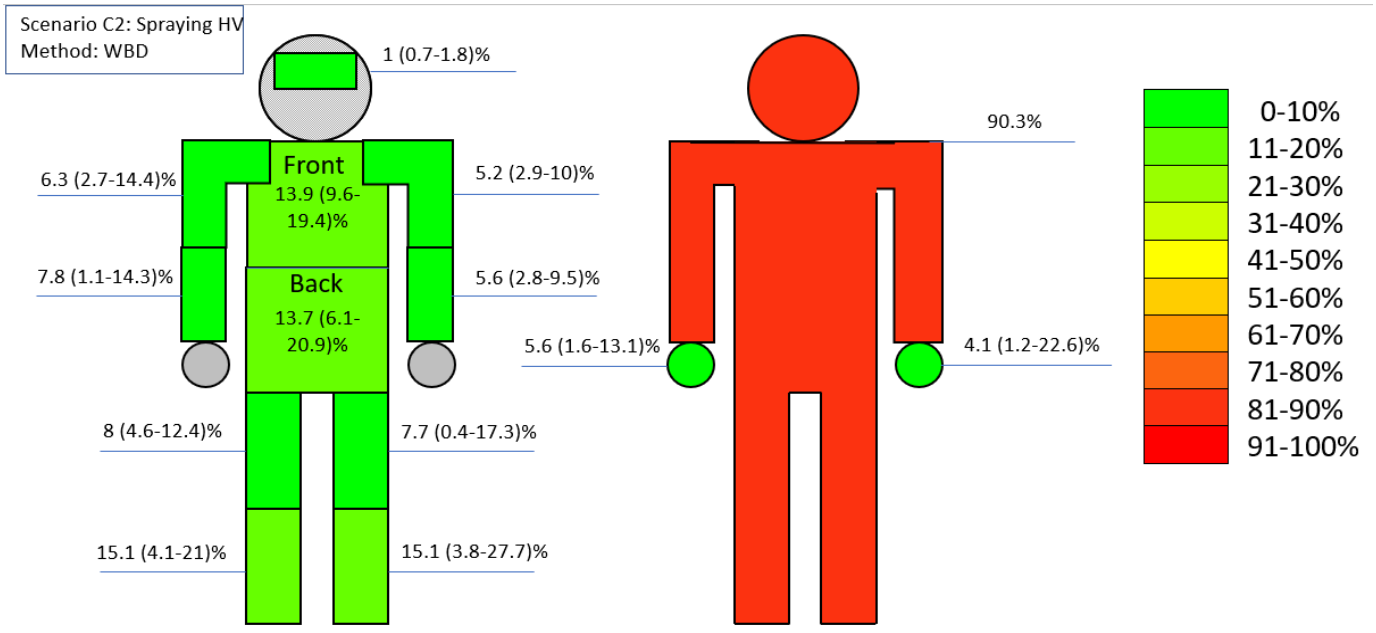


Figure 7.25 Percentage exposure coverage per body part for spraying HV liquid using the WBD method (coverall, gloves, headband). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

Figure 7.26 Typical exposure pattern from volunteers under UV light after spraying liquids (left: WBD method, right: patch method)

7.5.3.8 D1: Handling of objects immersed in LV liquid

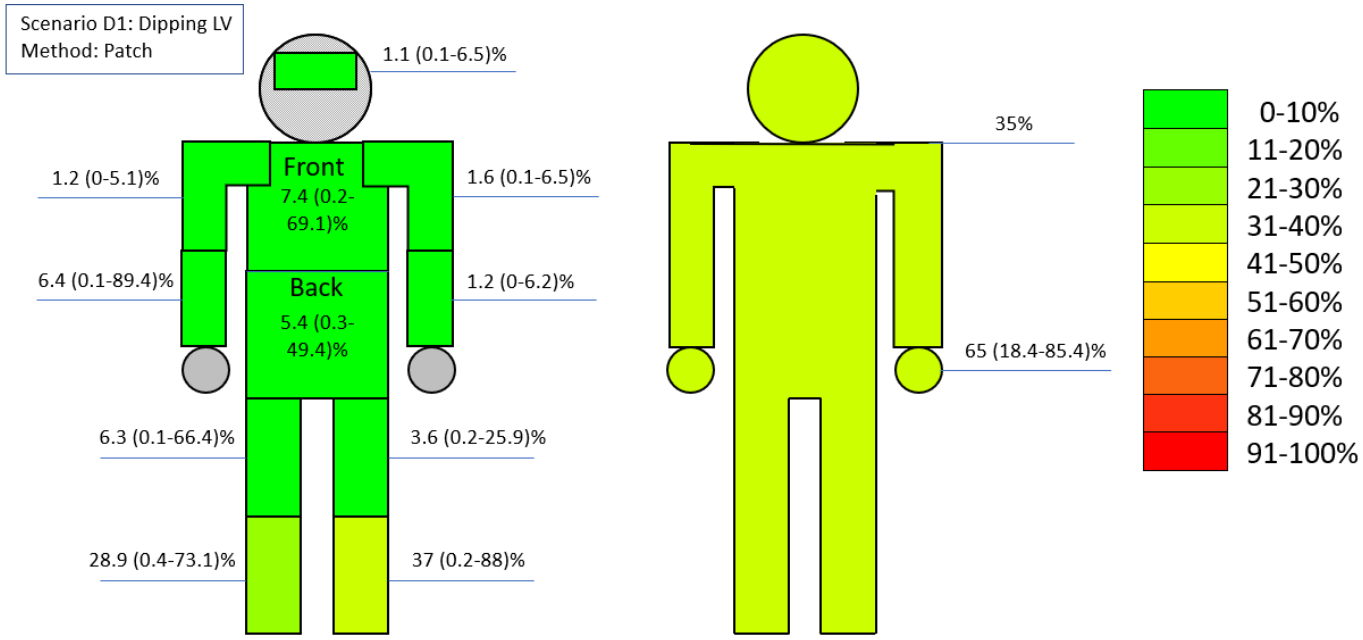


Figure 7.27 Percentage exposure coverage per body part for handling objects immersed in LV liquid using the patch method (patches, hand wash, head wipe). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

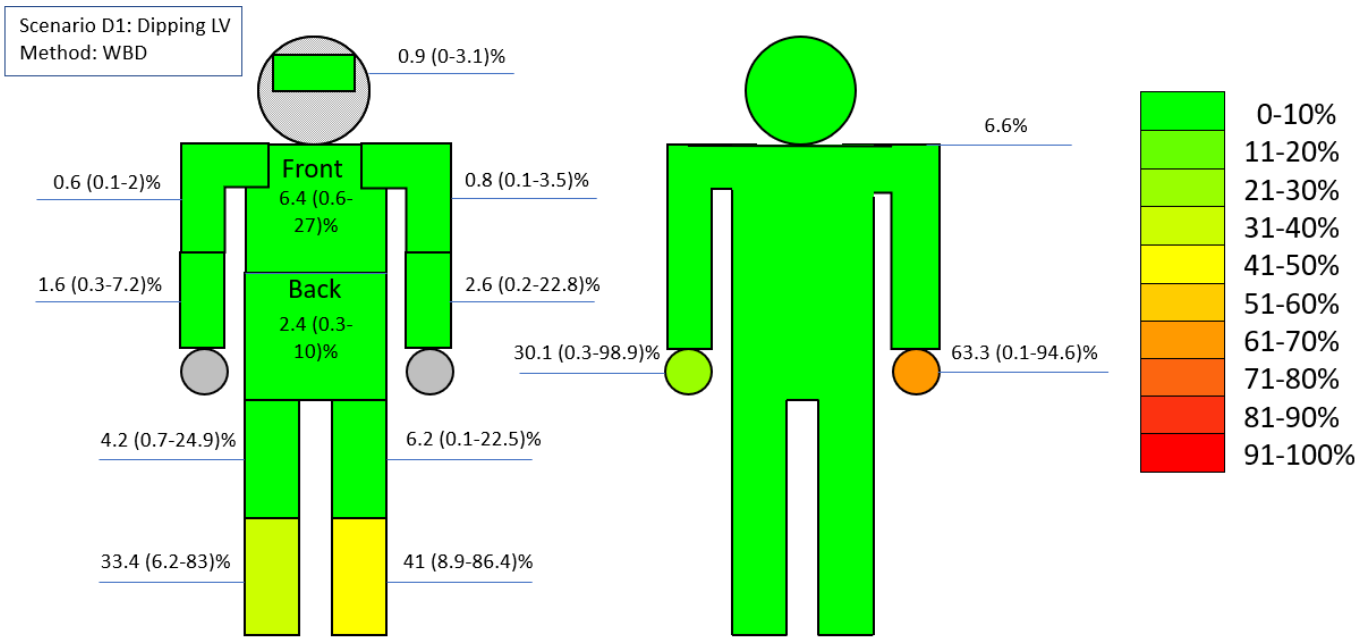


Figure 7.28 Percentage exposure coverage per body part for handling objects immersed in LV liquid using the WBD method (coverall, gloves, headband). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

7.5.3.9 D2: Immersion/dipping HV liquid

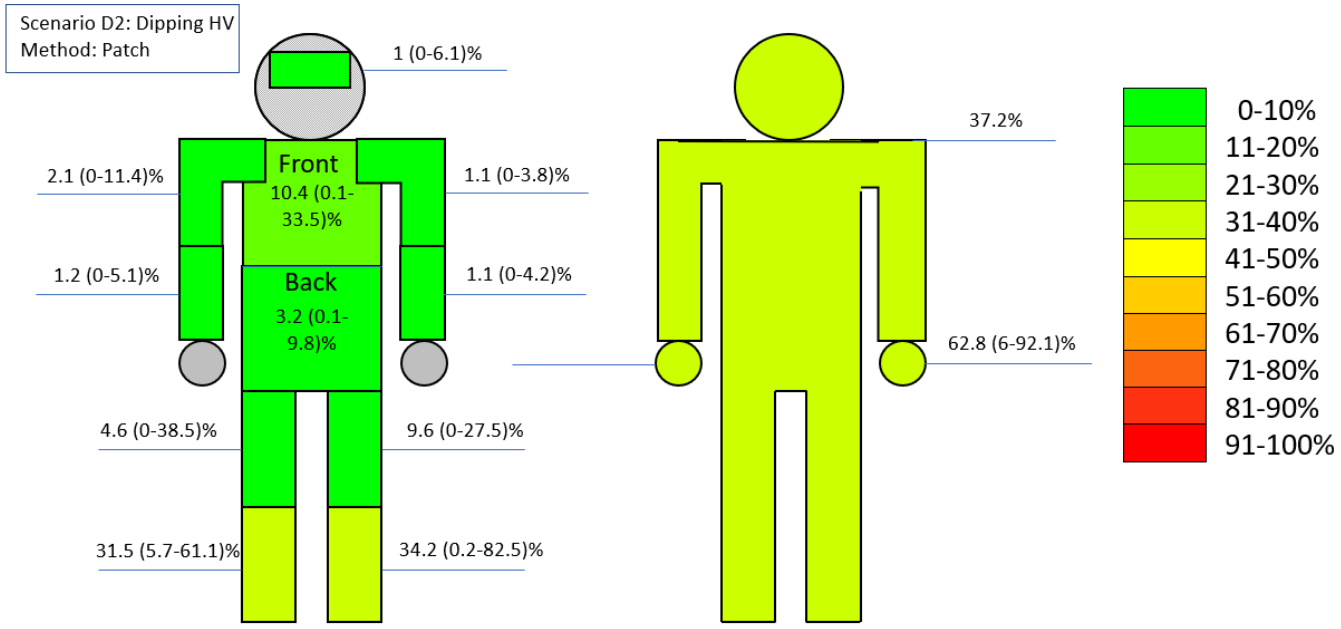


Figure 7.29 Percentage exposure coverage per body part for handling objects immersed in HV liquid using the patch method (patches, hand wash, head wipe). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

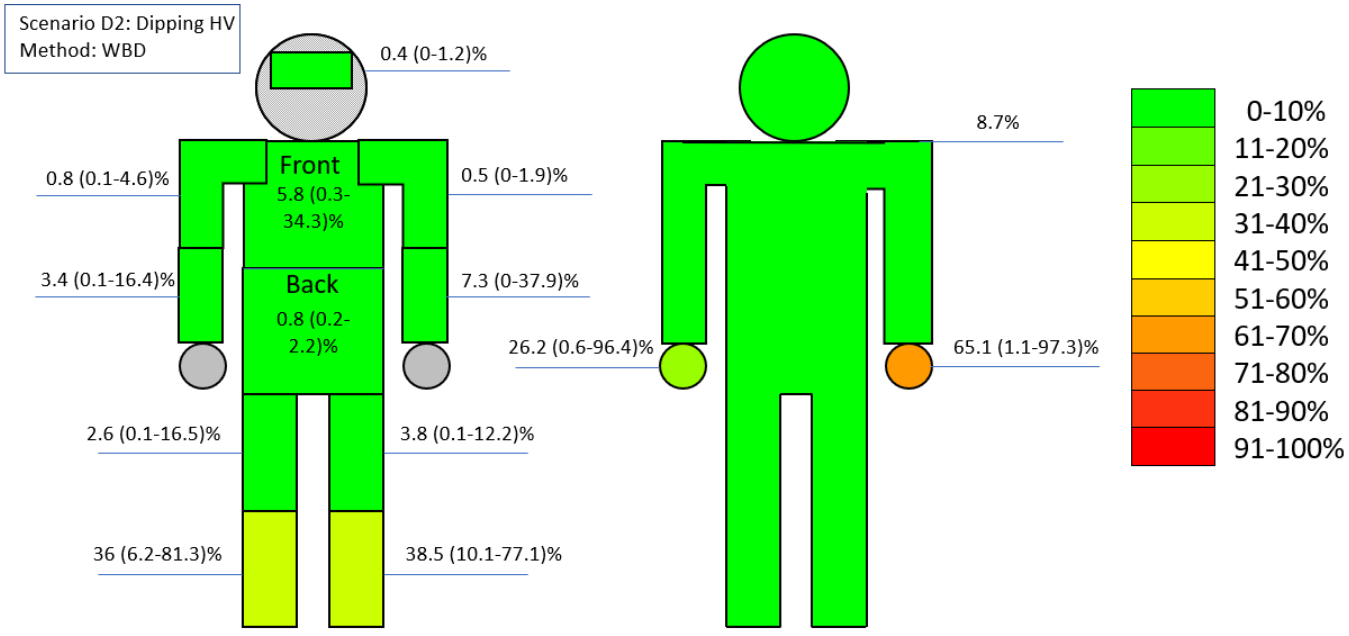


Figure 7.30 Percentage exposure coverage per body part for handling objects immersed in HV liquid using the WBD method (coverall, gloves, headband). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

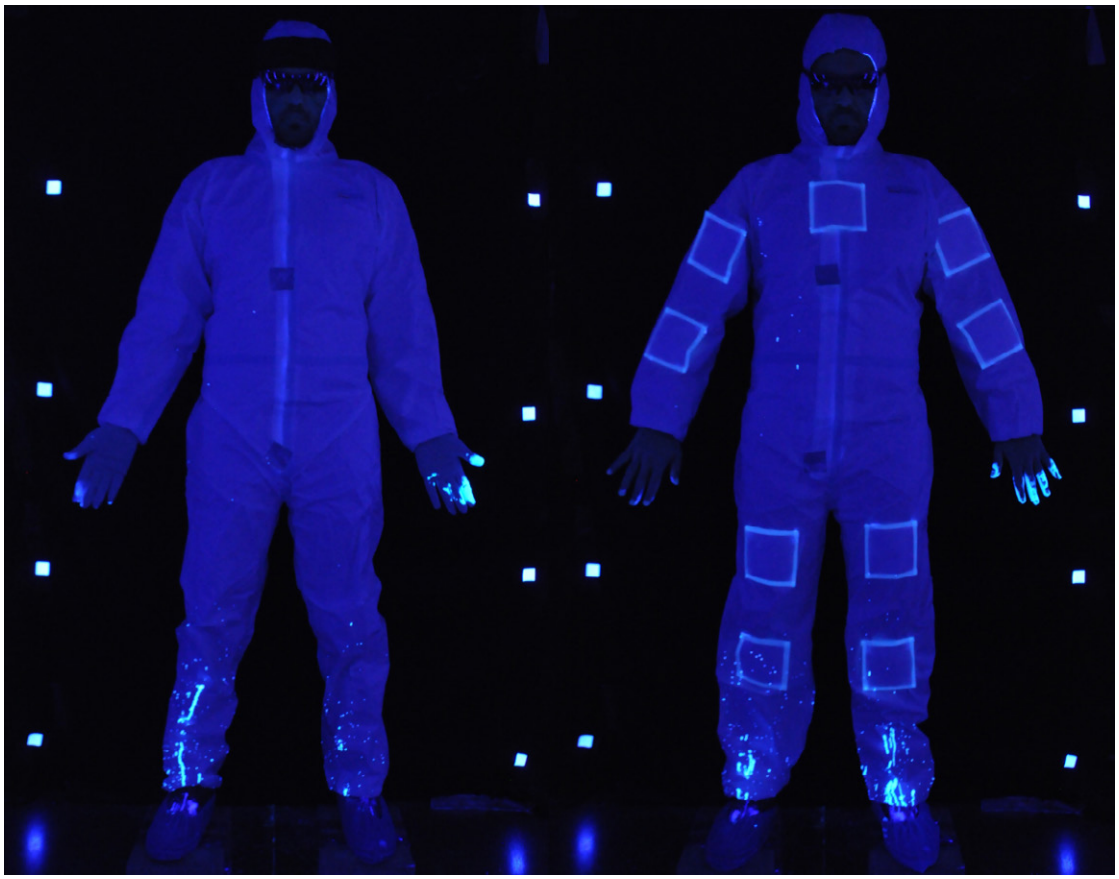


Figure 7.31 Typical exposure pattern from volunteers under UV light after immersion/dipping of objects in liquids (left: WBD method, right: patch method)

7.5.3.10 E. Handling of contaminated objects

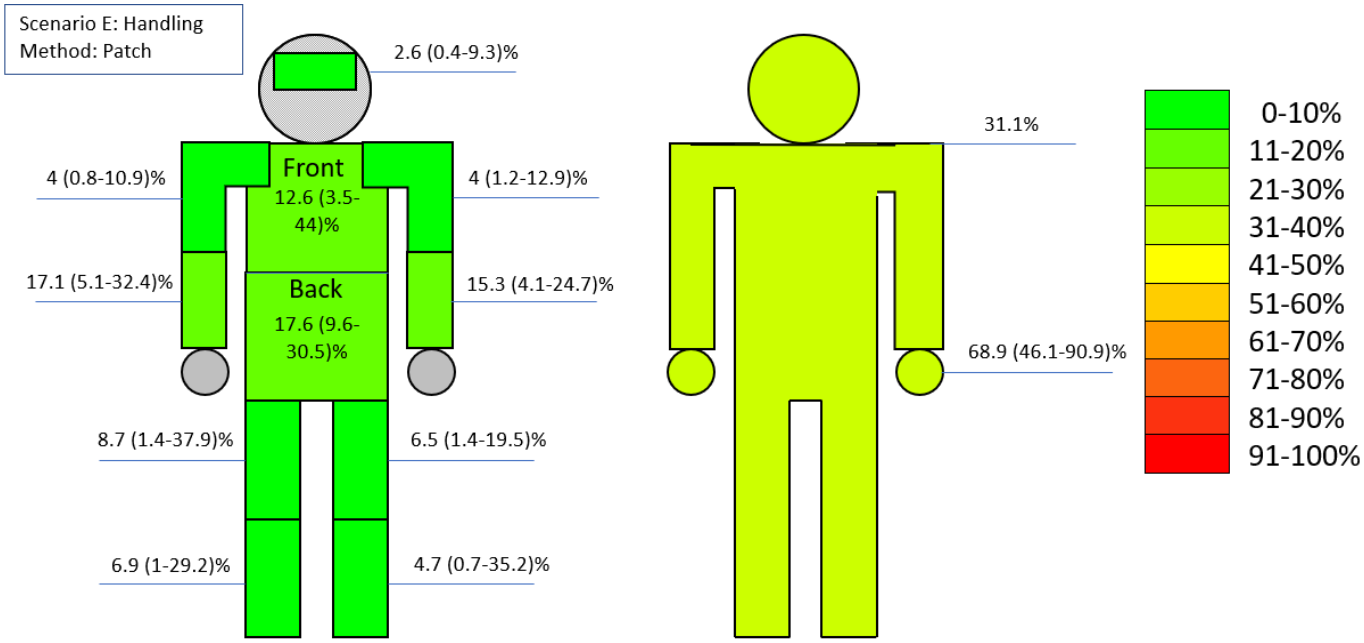


Figure 7.32 Percentage exposure coverage per body part for handling contaminated objects using the patch method (patches, hand wash, head wipe). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

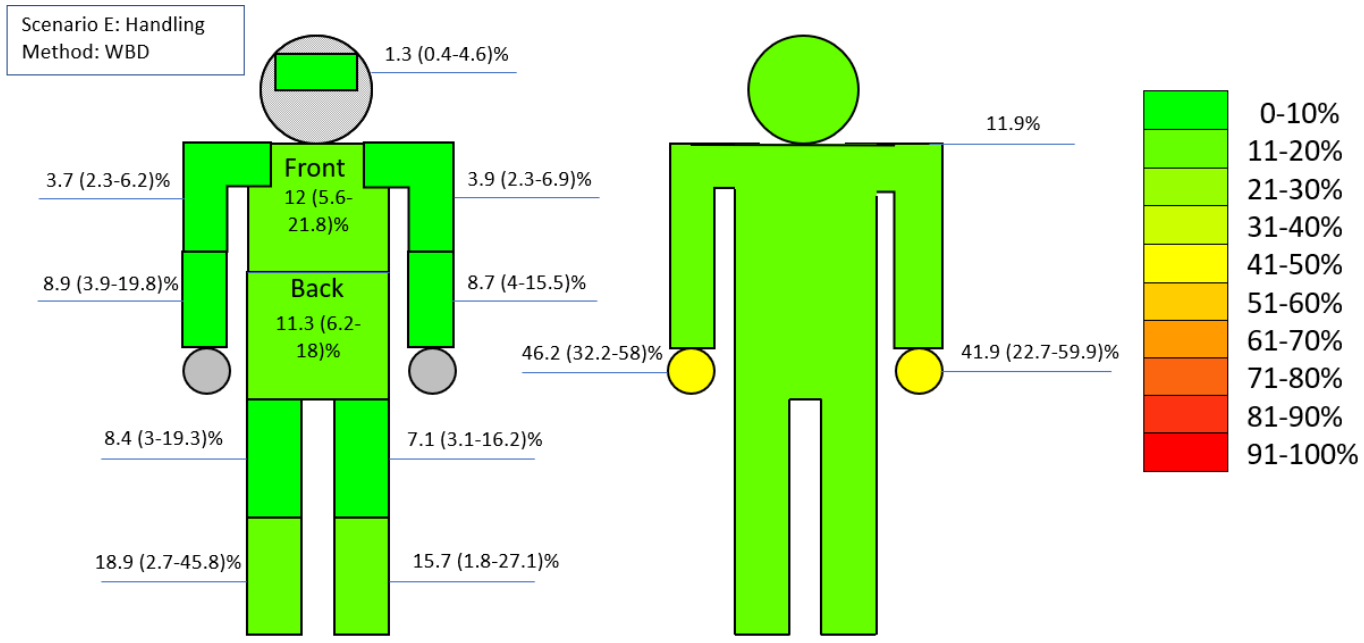


Figure 7.33 Percentage exposure coverage per body part for handling contaminated objects using the WBD method (coverall, gloves, headband). Left: separate for all body parts and head (excluding hands); right: body exposure (summed for all body parts and head) and hand exposure.

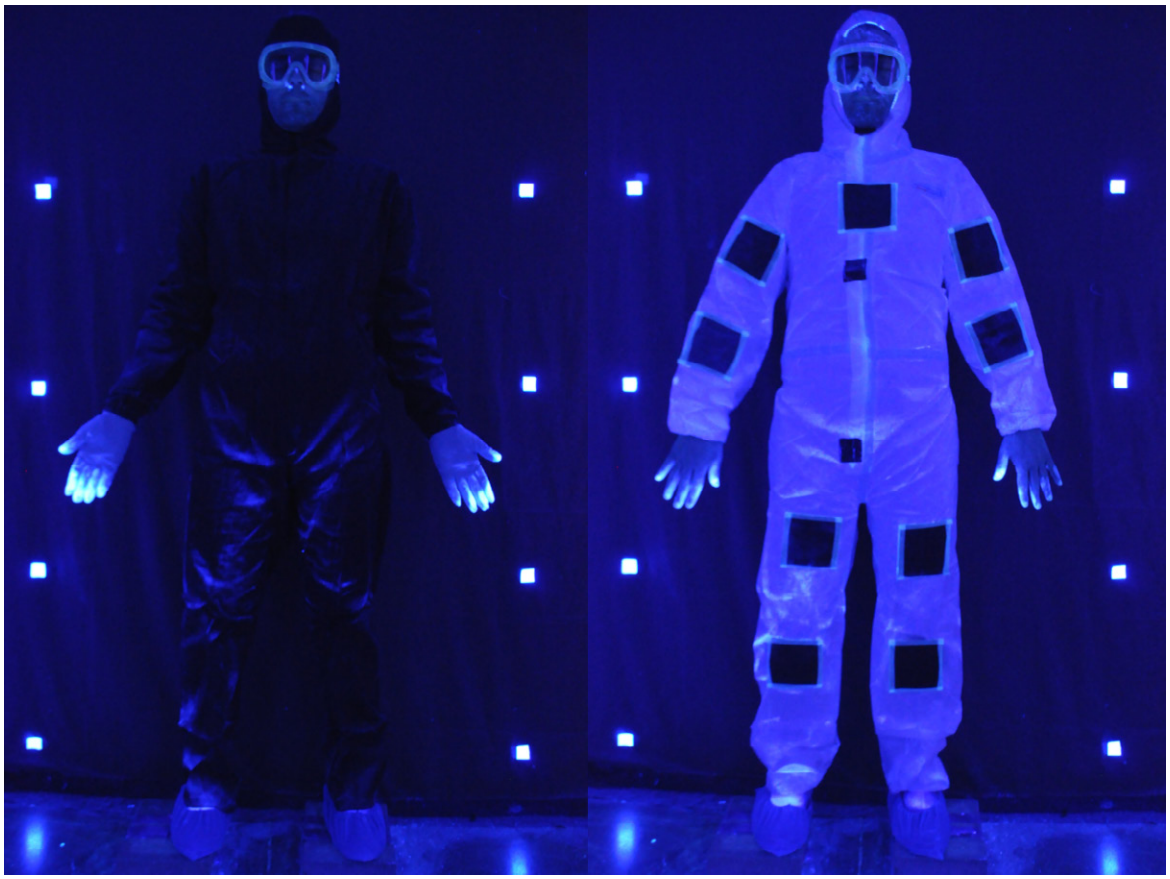


Figure 7.34 Typical exposure pattern from volunteers under UV light after handling of contaminated objects (left: WBD method, right: patch method)

7.6 Regression analysis

7.6.1 Analysis of the liquid exposure tasks

First of all, it should be mentioned that the results of one of the experiments for pouring low viscosity liquid (namely A2V2R4M1) was omitted from the dataset, since during this experiment liquid dripped directly on the glove of the volunteer (see also Table 6.3). This resulted in an extremely high exposure value (around six times higher than the other experiments), and therefore it would have influenced the regression analysis in a disproportionate way.
The first analysis focused on the exposure situations with liquids, to be able to also take into account the possible effect of type of liquid on the measured dermal exposure. Models have been constructed for each of the objective dermal exposure outcomes: body exposure, head exposure, and hand exposure. Furthermore, the analysis is based on an extrapolation towards the whole surface area of the coverall of the respective body part in case of patches, as well as the normalized concentrations in case of exposure situations with powders. The ingredients for these models were the factors measurement method (patch method (patches, hand wash, head wipes) and WBD method (coverall, gloves, headband), exposure task (pouring, rolling, spraying and handling immersed objects)), product (LV or HV) and their pairwise cross-terms, i.e. interactions. In all of the models, the factors measurement

method, exposure task and product were found to have significant effects. This means that Method 1 (WBD method: coverall, gloves and headband) resulted in significantly different values than Method 2 (Patch method: patches, hand wash and wipes). Also, exposure situations in which high viscosity liquid was applied were significantly different from exposure situations in which low viscosity liquid was applied. And the different exposure tasks (i.e. pouring, rolling, spraying and handling immersed objects) were also found to be significantly different. Moreover, the interaction between measurement method and exposure task was found to be significant for all models. This means that the effect of measurement method (the difference between Method 1 and Method 2) is different for the various exposure tasks. Furthermore, for the outcome body exposure, the interaction between product and measurement method was significant, whereas for the other three outcomes, the interaction between product and exposure task was significant.

Table 7.8 Overview of the significant effects for each of the three models

Factor \ outcome *	Body	Head	Hands
Measurement method	✓	✓	✓
Exposure task	✓	✓	✓
Product	✓	✓	✓
Measurement method X Exposure task	✓	✓	✓
Measurement method X Product			
Exposure task X Product	✓		✓

* Significant effects for each of the four models are indicated by a ✓. The interactions, or cross-terms, are indicated by an 'X' in between the factor names.

An overview of the significant effects for all three models is shown in Table 7.8. These models, including the workings of interactions, are explained in more detail below. In the figures that are shown below, the 'lsd (5%)' bar indicates the least significant difference (least significant difference at a 5% detection level). In case a parameter does not have a significant effect on the measured exposure values, the difference is within the lsd bar. If a parameter does have a significant effect the difference is larger than the lsd bar.

7.6.1.1 Body exposure (excluding hands and head)

Figure 7.35 shows a difference in exposure between the exposure tasks and the different measurement methods. The patch method shows no significant differences in exposure between handling immersed objects (indicated as 'dipping' in the figures below), pouring and rolling, while the WBD method shows a statistically significant difference in exposure between pouring on one hand and handling immersed objects or rolling on the other hand. The WBD method does not show a difference in exposure between handling immersed objects and rolling. The results for both measurement methods indicate that spraying differs from the other exposure tasks. In general, for body exposure, the calculated factor differs in the patch method and the WBD method between a factor 1.6 and a factor 3.8 depending on the exposure task, where pouring liquids shows the largest difference and rolling liquids the smallest difference. These results indicate that when patches are used to measure body exposure, significantly higher dermal exposure values are measured compared to when a coverall is used. These differences appear to be larger in exposure tasks

where splashes are likely to occur, such as handling immersed objects and pouring, and seem to be smaller when a more uniform exposure pattern is expected, like during rolling and spraying.

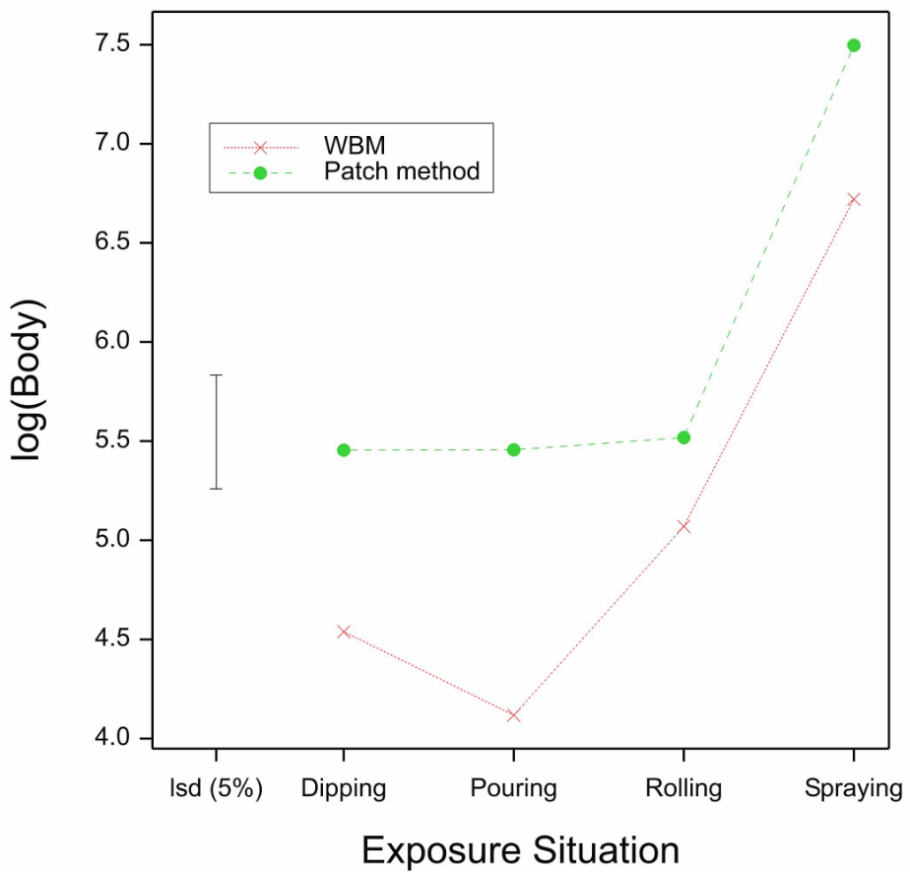


Figure 7.35 Interaction between measurement method and exposure task for the outcome body exposure (excluding hands and head)

Figure 7.36 shows that generally, using HV liquids led to a higher exposure. However, there was only a significant difference observed for pouring liquids.

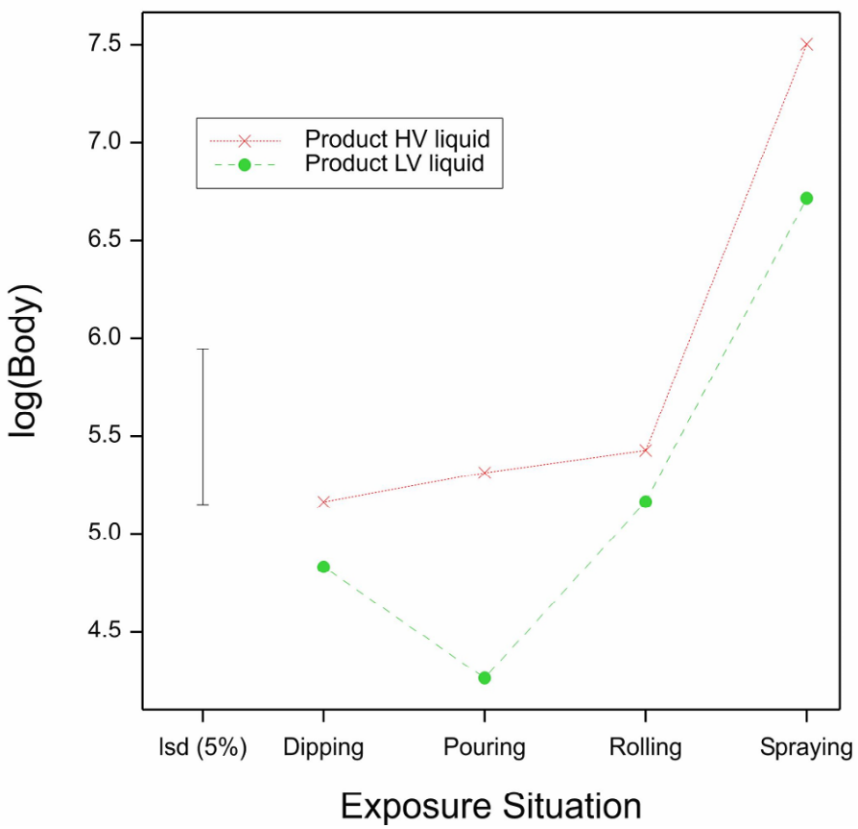


Figure 7.36 Interaction between measurement method and product (LV and HV) for the outcome body exposure (excluding hands and head)

7.6.1.2 Hand exposure (gloves and hand wash)

In Figure 7.37, the methods gloves (WBD) and hand wash (Patch method) for the various exposure situations are compared. For pouring and spraying, the methods do not differ significantly in the results for hands, while they do differ significantly for handling immersed objects and rolling. For pouring and spraying, the factor difference between gloves and hand wash are relatively low (0.75 and 0.8 respectively), while the difference for handling immersed objects and rolling is higher, where the hand wash results in exposure of a factor 0.27 and 0.38 lower compared to using gloves as measurement method.

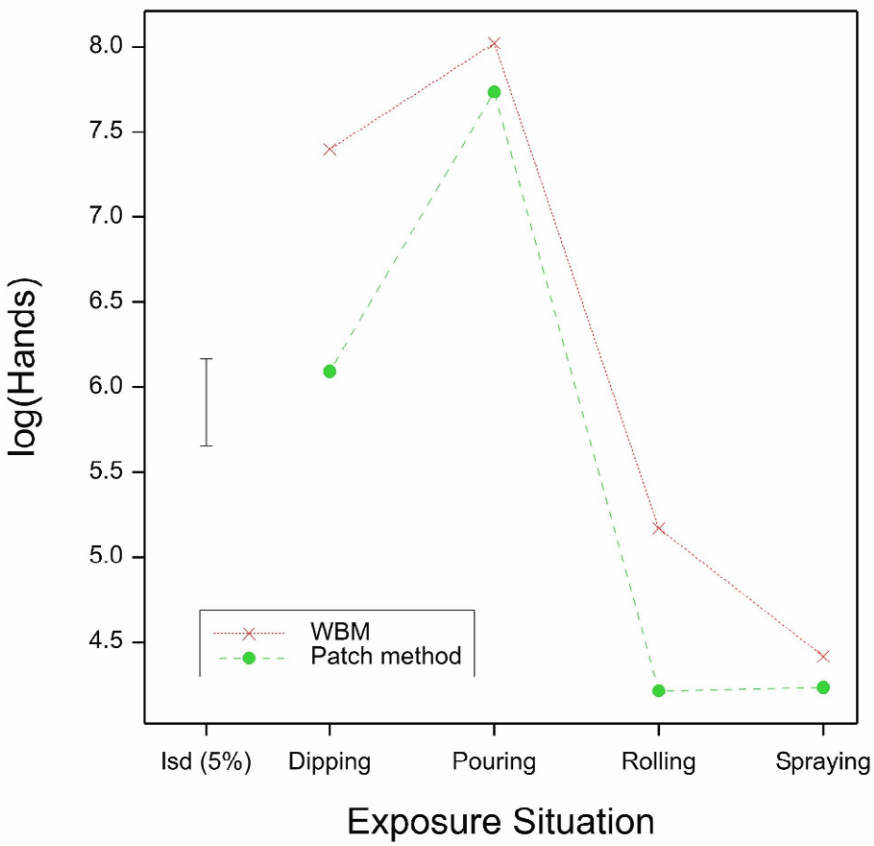


Figure 7.37 Interaction between measurement methods (gloves and hand wash) and exposure tasks for the outcome hand exposure

The product by exposure interaction shown in Figure 7.38 shows no statistically significant difference between low viscosity liquids and high viscosity liquids for all exposure scenarios. However, a difference can be observed between handling immersed objects and pouring on the one hand and rolling and spraying on the other hand. For handling immersed objects and spraying, exposure values are a factor 0.9 and 0.6 lower for low viscosity liquid, while exposure values for low viscosity liquid are a factor of 1.8 and 1.4 higher for pouring and rolling compared to exposure values for high viscosity liquid.

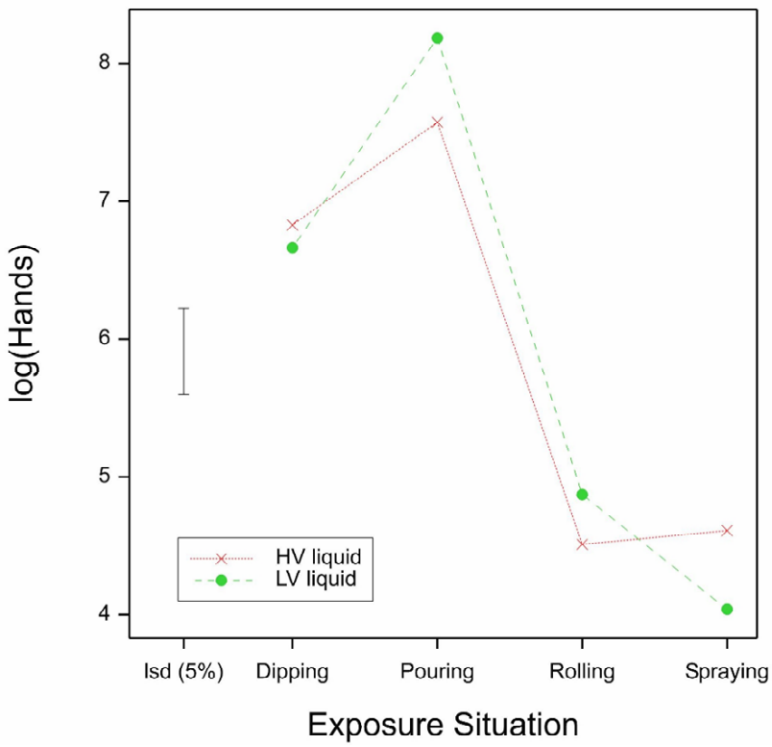


Figure 7.38 Interaction between product (high and low viscosity liquids) and exposure task for the outcome hand exposure

7.6.1.3 Head exposure (headband and wipes)

From Figure 7.39 it is observed that using wipes (patch method) results in significant higher exposures for handling immersed objects and pouring when compared to using a headband. No significant differences are observed for rolling and spraying. This is also apparent when looking at the factor differences between the different methods. These differ between 0.99 to 3.8, where the difference is the lowest for spraying liquids and the highest difference is shown for pouring liquids. Handling immersed objects and rolling resulted in a factor difference of 2.5 and 1.6 respectively. These high differences might possibly be explained by the extrapolation of the wiped surface of the forehead to the headband area, where this extrapolation assumes homogenous exposure (and therefore also exposure on the back of the head) which may not be the case.

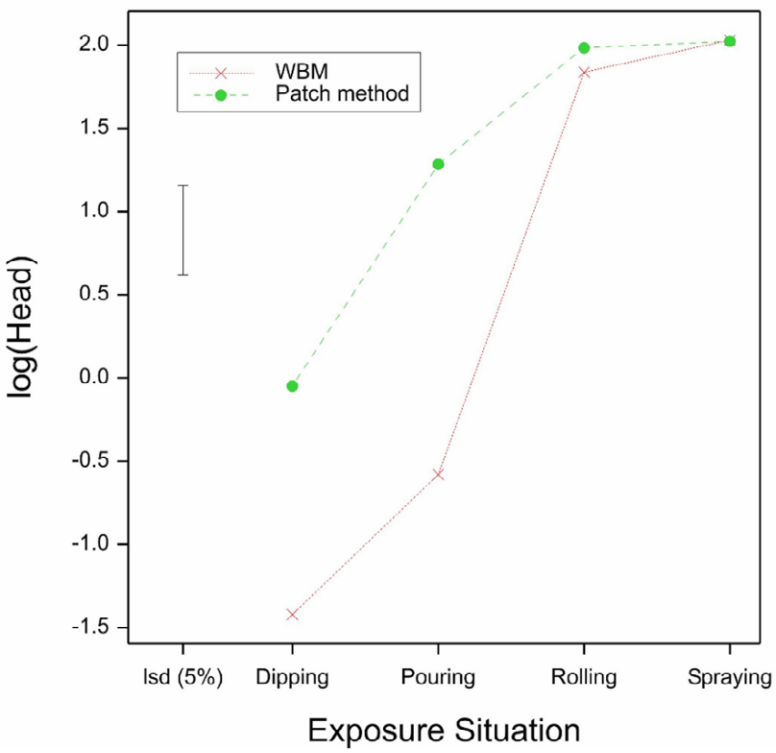


Figure 7.39 Interaction between measuring method (headband and wipes) and exposure task for the outcome head exposure

Figure 7.40 shows a much more substantial difference between spraying and rolling for high viscosity liquid compared with the difference for low viscosity liquid. There is no statistically significant difference among the four mean values for handling immersed objects and pouring and high and low viscosity liquids. There is no difference between rolling and spraying for low viscosity liquid and also no such difference for high viscosity liquid. However, high and low viscosity liquids do differ for rolling and spraying. For each exposure situation, there appears no significant difference in exposure between low viscosity and high viscosity liquid for handling immersed objects and pouring, while a significant difference was found between low viscosity and high viscosity liquid during rolling and spraying.

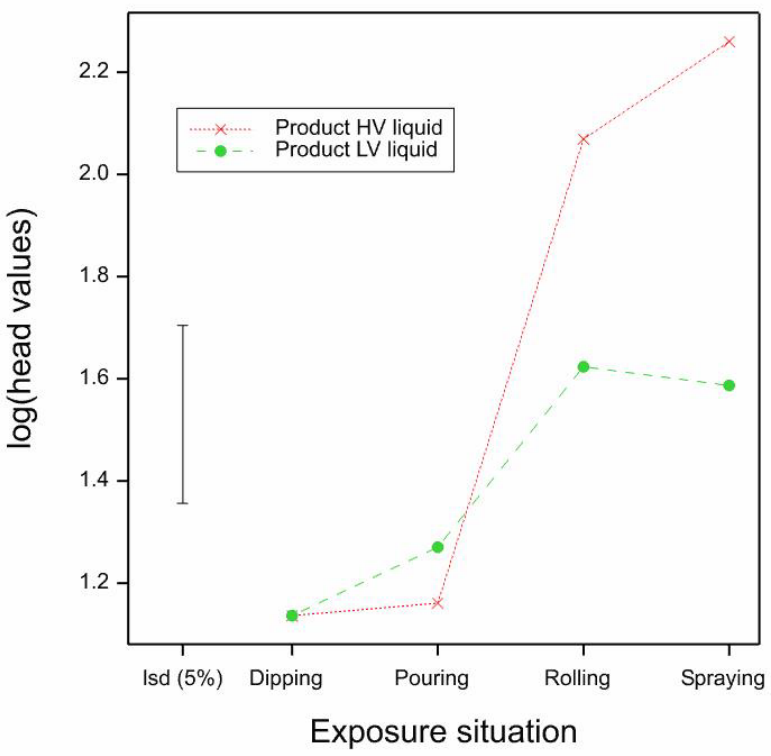


Figure 7.40 Interaction between product (LV and HV) and exposure task for the outcome head exposure

7.6.1.4 Within and between person variation

One of the objectives of this study is to determine the within and between persons' variation. In Table 7.9 an overview of these variation coefficients per outcome is presented.

Table 7.9 Within and between person variation coefficients expressed as relative standard deviations

	Body	**Head**	**Hands**	**Total**
Within person variation coefficient	81.1 %	108.2 %	78.9 %	59.5 %
Between person variation coefficient	30.1 %	0.0 %	30.2 %	23.1 %

These coefficients are presented as relative standard deviations and should be interpreted the following way. The variation around the expected (modelled) value due to either within person variation or between person variation is the presented coefficient multiplied by the expected value. For example, for body the variation due to within person variation is 81.1 % of the expected value and due to between variation this is 30.1 % of the GM. It can be seen that the within person variation is 2-3 times larger than the between person variation. Furthermore, it can be seen that head exposure has the largest within person (and lowest between person) variation. Note that the absolute variation increases as the expected value increases since the variation is a percentage of the expected value. This is common for values on a logarithmic scale.

7.6.2 Analysis of all exposure situations

In order to combine the two exposure situations with powders with the exposure situations with liquids, instead of the two separate factors exposure task and product the “superfactor” (as in combination of factors) is introduced, which is the merger of product and exposure task, resulting in all exposure situations (including powders) and viscosities in one Figure.

7.6.2.1 Body exposure (excluding hands and head)

In Figure 7.41 the same superfactor was applied for when analyzing the data for body exposure. The results show no significant difference for the exposure situations during which powders are used, although for dumping the difference in measured exposure between the patch method and the WBD method is higher (factor 1.6) compared to handling contaminated objects (factor 1.17).

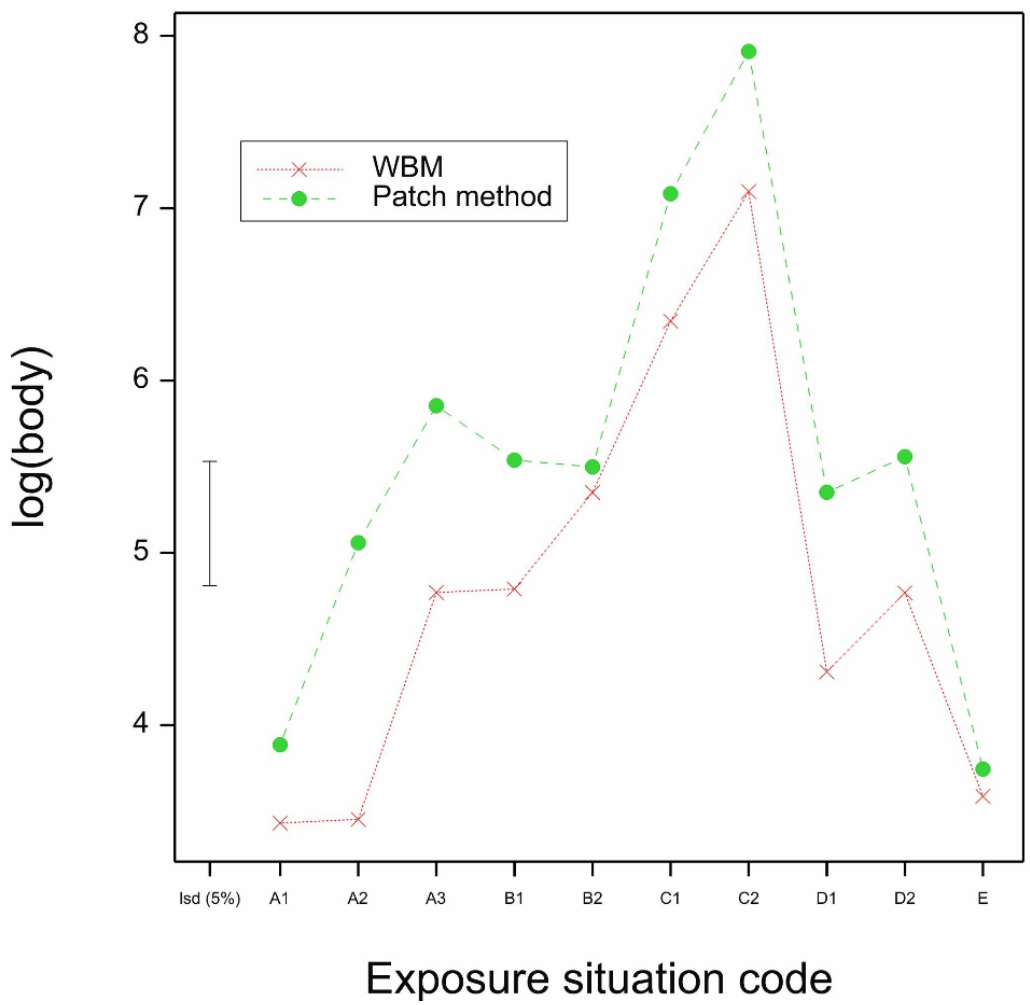


Figure 7.41 Interaction between measurement method and superfactor exposure situation for the outcome dermal exposure body

7.6.2.2 Hand exposure

Figure 7.42 shows no significant difference in exposure of the hands for the exposure situation dumping, where the hand wash method results in a factor 0.9 less exposure compared to use of gloves. For handling of contaminated objects, a significant difference was found between using gloves and using a hand wash, with the hand wash technique resulting in a factor 0.37 lower exposure values compared to gloves.

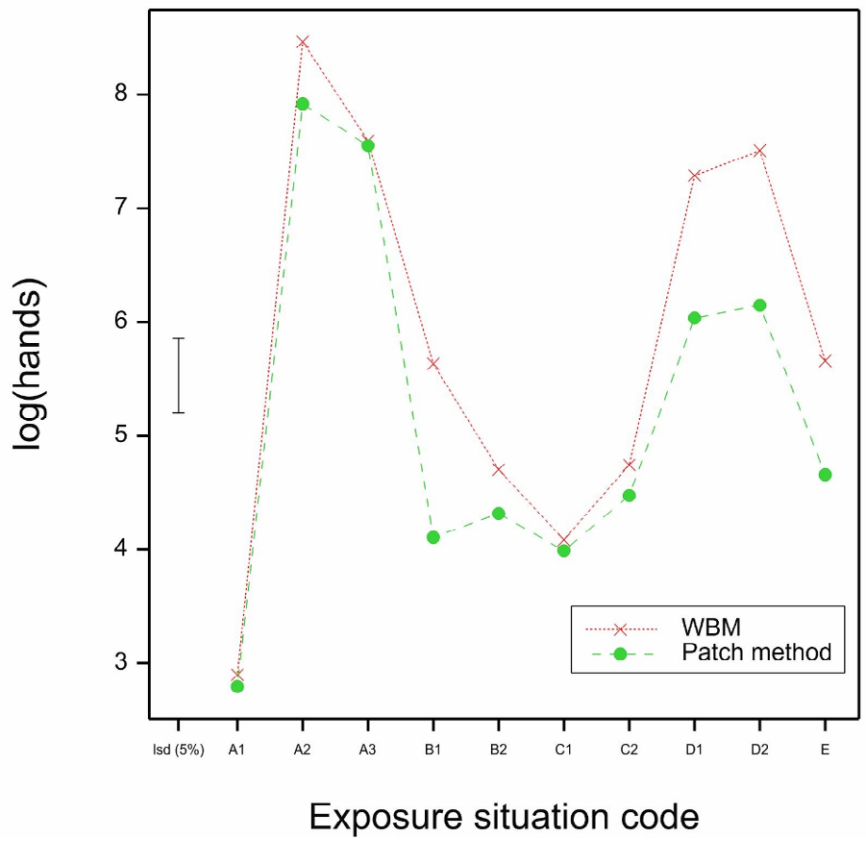


Figure 7.42 Interaction between measurement method and the 'superfactor' exposure situation for the outcome dermal exposure to the hands

7.6.2.3 Head exposure

Figure 7.43 shows a significant difference in head exposure between using a head band and using head wipes for measuring (fore)head exposure. For dumping, using wipes resulted in a factor 2.3 higher exposure compared to using the headband. For handling of contaminated objects, using wipes resulted in a factor 2.1 higher exposure compared to using the headband.

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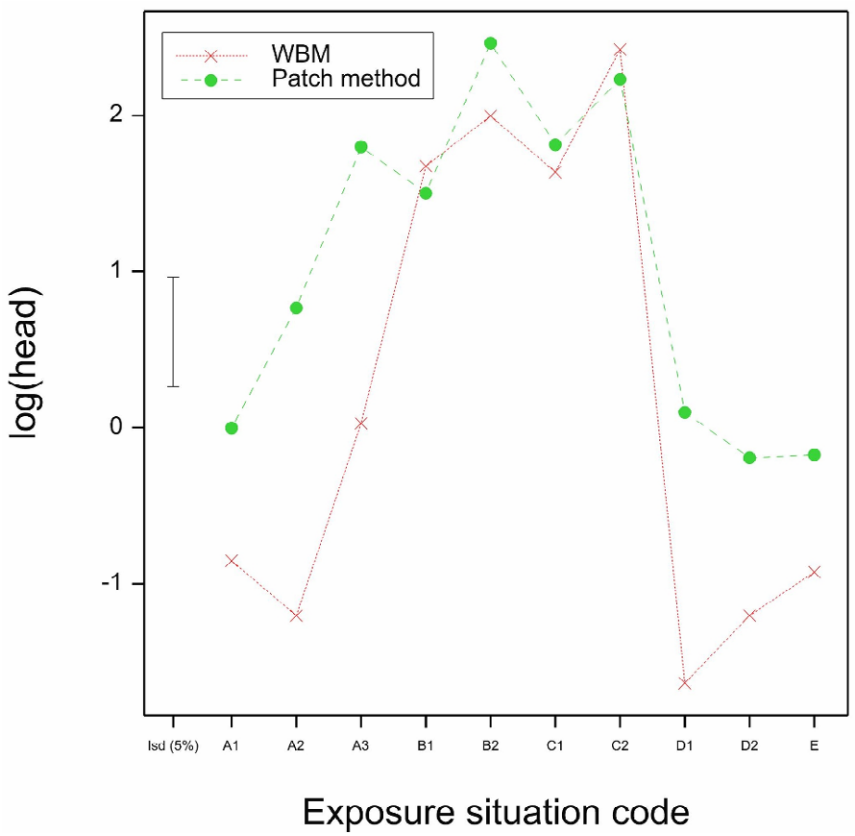

Figure 7.43 Interaction between measurement method and the 'superfactor' exposure situation for the outcome dermal exposure to the head

7.6.2.4 Within and between person variation

As before, the within and between person variation coefficients were calculated. These are shown in Table 7.10. The same trends can be seen as before: the within person variation is 2-3 times larger than the between person variation. And the outcome body/hand exposure has the largest between person variation coefficient. Head exposure has the lowest between person variation coefficient.

Table 7.10 Within and between person variation coefficients expressed as relative standard deviations

	Body	Head	Hands	Total
Within person variation coefficient	75.0 %	98.9 %	75.0 %	56.0 %
Between person variation coefficient	32.5 %	0.0 %	25.0 %	22.0 %

8 Discussion

The overall objective of the current study was to generate scientific knowledge that forms the basis for an improvement and a standardization of measurement methods for dermal exposure to chemicals at the workplace.
By performing well-designed experiments to be able to perform tasks in a reproducible way and thereby reducing the variability in outcomes due to variation in behavior and environmental conditions, the data gathered were used to investigate the following specific objectives:

- The advantages and disadvantages of each of the measurement methods.
- The differences in measured dermal exposure outcomes between different measurement techniques.
- Identification of the best method to be applied for specific tasks.
- To which extend the results of the different measurement methods can be converted to one another by means of conversion factors.

To answer the research goals as stated above, a detailed study plan was developed to make sure that tasks could be performed in a standardized and reproducible way. Well-designed experiments were conducted to reduce the variability in outcomes due to variation in behavior and environmental conditions. For each exposure task, detailed protocols were established which enabled standardized working conditions, including ventilation rate, the amount of substance handled, surface area treated. Furthermore, the execution of the task was standardized by describing the execution of the task in detail in standard operating procedures and by providing clear instructions to the volunteers, to ensure that volunteers performed the tasks in a similar way, to reduce the chance of accidental cross-contamination, and reduce the number of determinants that could influence the exposure values. This allowed for a ‘clean’ comparison of the different measurement methods by reducing as much as possible unintended exposure due to accidents or cross contamination. Based on the results of the experiments it is concluded that this study design was successful. The results of only one out of 320 experiments were excluded from the regression analysis. That was due to accidental spillage on the hands, which occurred during a pouring experiment, and which resulted in an exposure value that was considered to be an outlier. The results of the other experiments showed no signs of cross-contamination or accidents (such as accidentally touching a treated surface, which was not intended to happen during the task), which were also not observed for any of the other experiments. Furthermore, the performance of pilot trials for each exposure situation was considered very useful. During the pilot trials, unforeseen issues, such as the discomfort of volunteers during dumping of powder or the liquid dripping of the immersed objects when these were moved, led to additional improvements of the way the tasks were carried out by the volunteers. The experiments were set-up to ensure a considerable level of exposure under practical experimental conditions. A downside to this set-up might be that the results may be difficult to compare with exposure measurements collected under “real-life” working conditions, since it is expected that for instance a lot more accidental exposure will occur in normal working conditions, or in practice often a sequence of tasks will be performed by workers, that could all lead to a certain level of dermal exposure and/or could result in transfer of the substance from a body surface to another surface.

Kromhout & Vermeulen (2001) reported that in case of dermal exposure both between- and within-person variance are (often) substantial. In the current study the within-worker variance appeared to be higher than the between worker variance, which indicates that the standardization of the tasks and the experimental conditions has probably reduced the between person variability. In advance it was expected that the volunteers may show a “training effect”, resulting in reduced exposure levels after performing the same tasks for several times, and perhaps even getting more experienced in the execution of the experiments during the course of the project. And although a possible training effect cannot be completely excluded, in general the data does not show clear trends with regard to measured exposure values (either increase or reduction) for the repeated experiments.
Some limitations of the study design need to be considered. Firstly, not all possible measurement methods have been investigated in the current study. While a clear distinction has been made between interception and removal methods, and also an in situ method was applied, removal methods such as tape stripping and rinsing were not included. Secondly, only a limited number of tasks that are considered relevant with regard to dermal exposure could be included in the study design. However, in view of the REACH and the biocides regulations, it is assumed that the most relevant tasks were considered in this study. Moreover, although spraying of powders is also considered a relevant scenario with regard to dermal exposure, it was not feasible to include this in the study design.

8.1 Comparison, advantages and disadvantages of measurement methods

8.1.1 Patches and coverall

Some exposure situations, such as pouring liquids and handling immersed objects, resulted in (very) low levels of exposure on the body. Rolling of liquids resulted in more body exposure, but instead of a homogenous exposure pattern showed the occurrence of splashes and the formation of droplets that landed on certain parts of the body. The body exposure levels as measured for the exposure situations spraying, dumping of powders and handling of contaminated objects were higher, and showed a more homogenous exposure pattern. For measuring body exposure, using patches resulted in significantly higher exposure values compared to using coveralls for all exposure situations with liquids, with the exception of rolling (where exposures on patches were also higher, but the difference was not statistically significant). For powders, no significant difference was found although based on comparison of the GMs patches indicatively showed higher exposure values compared to the coverall.
The higher exposure values as measured with patches might be caused by the extrapolation that is always involved in this method. In this study, the exposure found on the patches was extrapolated to the respective coverall surfaces, assuming that exposure is evenly distributed on the whole body segment. However, the patches are intentionally placed at parts of the body that are (expected to be) most exposed, thus the even exptrapolation to the entire body segement (including surfaces that are less likely exposed such as the inner arms or legs and the back of arms and legs) might eventually be conservative, resulting in a slight overestimation of values measured with patches (*vide infra* for a more distinct discussion). It should be noted that in this

study these findings are based on extrapolation to surface areas of the coverall, which are significantly larger than the respective body surfaces. If exposure measured with patches is instead extrapolated to actual body surfaces, the resulting values will decrease and may become lower than values that have been measured with coveralls.

8.1.1.1 Advantages and disadvantages of use of patches and coveralls

Soutar et al. (2000) described the advantages and disadvantages of using either patches or a coverall to measure dermal body exposure. They mention that patches only estimate the amount of a substance deposited on a particular area, where extrapolation to body surface could either lead to an underestimation should droplets miss the patch, or overestimation when a splash lands on the patch (Soutar et al., 2000). Furthermore, when using patches for measuring body exposure, a uniform distribution of contamination over the body parts is assumed (Behroozy, 2013; Fenske, 1990). In this study the fluorescence photographs show that a uniform distribution of contamination over body parts cannot be assumed for most of the tasks investigated in this study, with the exception of spraying liquids, dumping powder and to some extend rolling liquids. This suggests that using patches to measure dermal exposure for exposure situations where a uniform exposure is not observed (such as pouring, handling immersed objects and handling of contaminated objects) may lead to increased uncertainty in the exposure values due to possible overestimation (splashes landing on the patches) or underestimation (splashes missing the patches) when the exposure value as measured on the patch is being extrapolated to the body part as a whole.
Interception methods as using patches or a coverall are considered the most common measurement methods to assess dermal body exposure. Removal techniques such as wiping and/or tape stripping might be applicable for other sections of the body (for which the skin is generally not covered, such as the hands, forearms and head), but they are not considered feasible to determine body exposures, since in practice it is not considered feasible to use them on clothing. Wiping and/or tape stripping is mainly applicable on bare skin and thus measuring ‘actual’ exposure. The advantage of measuring dermal exposure using a coverall is that by including the whole body surface area in the measurement, no assumptions with regard to uniformly distributed exposure need to be made. Furthermore accidental splashes or droplets are captured by this method, which may be missed or may result in (very) high exposure values when patches are used. Furthermore, there is no need for extrapolation to the surface area of a particular body part, which in case of exposure values below LOQ may result in large minimum exposure values (depending on the LOQ value and how values below LOQ are processed). A disadvantage of using a coverall is that due to the often large size of the samples, a relatively large amount of liquid (solvent) is needed for the extraction of the chemical, which is expected to result in a more expensive analysis. Furthermore, Behroozy (2013) states that patches are usually backed by a protective (impermeable) layer, such as aluminum or in this study paper, which prevents leaking of chemicals through the patch, and therefore is less likely to result in underestimation of potential exposure. Depending on the material of the coverall, such a layer is lacking, and therefore chemicals may leak through the coverall and can thus be not measured.

8.1.1.2 Differences in exposure outcomes of patches and coveralls

The results of the current study showed that the difference between exposure measured using patches and exposure measured using coveralls is lower for exposure situations where a uniform distribution of exposure is observed, such as spraying liquids and to a lesser extend dumping powder and handling of objects contaminated with powder. Furthermore, a previous study made an effort to compare using patches with using a coverall covering the whole body (Tannahill et al., 1996), where both methods were used for measuring dermal exposure in different sectors such as pest control, timber treatment and the paint sector. They found that exposure values during pest control and timber treatment were a factor 2 higher for patches compared to exposure on coveralls, which is in line with our general difference of a factor between 1.6 and 3.8 depending on the exposure task. However, they also found 40% less exposure on patches compared to the coverall when spraying anti-fouling paint (Tannahill et al., 1996), which was in disagreement with current results for spraying (factor 2.2 higher exposure on patches compared to coveralls). The differences between the studies are possibly explained by the use of a standardized protocol in the current study versus “real-life” working conditions as were present in the study of Tannahil et al. (1996). Like in the current study, in that study exposure mainly came from overspray, although it was reported that direct exposure due to accidentally touching the sprayed surface led to exposure of different body parts, which did not occur in the current study. This kind of accidental exposure moments might be more frequently “missed” by the patches due to the placement of the patches on the body. Also, mixing of paint was taken into account in the previous study, during which splashes sometimes occurred (Tannahil et al., 1996), while this task was not included in the current study. In addition, for all exposure scenarios in reality, more handlings are taken into account for each exposure situation, compared to the exposure situations in the current study. For example, for rolling and brushing, in reality, workers would fill their paint trays themselves, as well as dispose of the trays, which was in the current study done by the technician, and not the volunteers.
In addition, the difference in exposure for the WBD method and the patch method were compared for three individual body parts, namely the torso front, the right (in our study, dominant) forearm and the right lower leg (considered to be the dominant side of the body). When considering the torso front and the right forearm, exposure values found on the patches were higher compared to exposure values found on the coveralls, which is decisive when comparing the patch method with the WBD method for the body as a whole. When comparing the exposure on the lower right legs, it must be noted that higher exposures were found on the coveralls compared to patches. Tannahil et al. (1996) reported that the lower legs showed the best agreement, which (when looking at the fluorescent photographs) seems to be in disagreement with the current study. For exposure situations where a dermal exposure pattern in the form of larger droplets/splashes is observed, these droplets/splashes seem to occur most frequently beneath the position of the patch on the lower legs as applied in the current study. This may explain why exposure values as measured with the coveralls are generally higher for the lower right legs compared to exposure values as measured with the patches. When comparing the placement of the patches between the two studies, it appears that the patches in the current study were placed higher (just below the knee) compared to the study of Tannahil et al. (1996), where the patches were placed on the shin. Tannahil et al. (1996) found that the patch on the front torso showed the least agreement with the coverall method

and suggested to move the patch towards the center of the torso for a better agreement. When looking at the fluorescence photographs, generally there is no evidence that shows that the center of the torso is more exposed compared to the (slightly higher) position where the patch is placed. However, during the pouring of liquids, there were some occasions where small dots of Tinopal SWN were visible on the center of the torso (as well as a large smear, which is likely caused by cross-contamination).

8.1.1.3 Identification of the preferred measurement method per exposure situation

With regard to measuring body exposure, there is no clear preference for using either the WBD method or the patch method. The choice of which measurement method to apply in a certain study or a certain situation mainly depends on the goal of that specific measurement campaign. Researchers should take into account the extrapolation based on the assumption of a uniform distribution of exposure for that body part when patches are used, which could results in higher exposure values compared to when coveralls are used, depending on the exposure pattern that is expected. When one is mainly interested in worst-case (conservative) dermal exposure estimates, and/or when homogenous exposure patterns are expected (e.g. during dumping of powders or spraying of liquids) the patch method is considered to be a useful method. When using patches, it should be taken into account that the placement of the patches (depending on the expected exposure patterns) may also have a large influence on the measured exposure. When a more precise dermal exposure assessment is required and/or dermal exposure is not expected to be uniformly distributed (e.g. during pouring, rolling, immersion of objects), the WBD method is considered to be more suitable. Using a fluorescence method (in situ), even if this is only applied during the preparatory phase, is considered to be a useful tool to determine the most suitable measurement strategy, as this will give valuable insight on the exposure patterns for a certain task, activity or scenario. A more simplified set-up compared to the set-up used in the current study could be used for this purpose, e.g. by placing two UV lights in front of the person when evaluating the exposure, either by visual inspection or by taking a photograph.

8.1.1.4 Conversion factors between the WBD method and the patch method

For body exposure, it was explored if it was possible to derive one or more conversion factors based on the results for the two measurement methods. However, it was found that factor differences in exposure between the patch method and the WBD method varied greatly between the different exposure situations (ranging from 1.2 for handling contaminated objects to 3.8 for pouring). It was also observed that the difference in measured exposure values between the patch method and WBD method also depended on the type of liquid used (for exposure situations in which a high viscosity liquid was used the exposure values as measured on patches were on average a factor ~2 higher compared to the WBD method. In addition, the patch method always requires extrapolation to a reference surface, which can either be the body surface or the surface of work clothing or protective clothing. The differences in the sizes of the respective reference surfaces chosen in different studies additionally affect the final results of such measurements. Due to the variability of these factor differences when comparing the patch method with the WBD method with regard to

both exposure situation and viscosity of the liquid applied, there is too much uncertainty to reliably establish a generally applicable conversion factor.

8.1.2 Gloves and hand wash

8.1.2.1 Advantages and disadvantages of use of gloves and hand wash

Dermal exposure as measured with gloves represents potential dermal exposure, whereas dermal exposure measured by means of a hand wash represents actual dermal exposure. The advantages of using gloves as well as a hand wash procedure to measure hand exposure is that both methods are relatively simple to apply with relatively low costs involved, although performing a hand wash technique requires a bit more effort compared to gloves, both from the technician collecting the samples and the person who's hand exposure is being measured. Gloves, especially cotton gloves, generally result in higher measured hand exposure due to the absorbance of the substance by the material (higher loading capacity than bare skin), which is considered to be a relative worst-case measurement approach. In case of washing bare hands after performance of a task, maximum loading of the skin can be reached, and chemicals might be transferred more easily from the hands to other (body) surfaces. With regard to the disadvantages of both methods, Marquart et al. (2002) stated that in case of hand wash, some wash solutions may influence the skin, making it more penetrable, which could influence the measured concentration (as well as the well-being of the person washing his/her hands). Also, the hand wash technique is of limited use when the substance is highly volatile (which also applies for gloves) or is absorbed rapidly through the skin (Behroozy, 2013). Although no specific penetration study of Tinopal SWN through the skin was conducted, as this was not within the scope of the project, taking into account the relatively short duration of the experiments and immediate collection of samples after the experiments, the acceptable recovery of Tinopal SWN from the skin by both the hand wash and head wipe method, the sequence of experiments of volunteers per day (first the combination of measurement methods in which the skin is completely covered, and second the combination of measurement methods that involves bare skin), the observations from checking the volunteers in the UV-room after each experiment, and in general a week in between trial days (which would allow for any Tinopal SWN to be removed from the skin if present), penetration of Tinopal SWN to the skin is not assumed to have affected the results of this study.

8.1.2.2 Differences in exposure outcomes of gloves and hand wash

In case of exposure situations with liquids, wearing gloves resulted in significant higher exposure values compared to the hand wash method during rolling liquids and handling objects that were immersed in liquid. Although the difference was not statistically significant, based on the exposure distribution hand exposure values measured with gloves also seem higher for spraying liquids and pouring liquids. In case of exposure situations with powders, wearing gloves resulted in a significant difference compared to the hand wash method for handling of contaminated objects, but not for dumping powders. These findings are in line with the findings of Gorman et al. (2014), who compared measuring hand exposure with an interception method (gloves) with a removal method (wipes). They found that using gloves resulted in significant higher exposures compared to wiping the hands. Although they did not

compare a hand wash method with a glove method, as used in the SysDEA project, it is expected that they would have found more or less similar outcomes, since Gorman et al. (2014) did compare rinsing the hands with wiping the hands, and found that wiping the hands led to higher exposure values compared to rinsing the hands. Although not investigated, based on the results of the test to find the most efficient washing technique, it is assumed that rinsing would have resulted in a lower removal efficiency compared to washing.

The amount of substance as measured on the hands (mainly) depended on the type of task that was performed. For example, hand exposure values during pouring liquids, rolling liquids and handling of objects immersed in liquids were higher compared to spraying liquids and dumping powder. This is most likely caused by the nature of the tasks performed during each of the exposure situations. E.g. during pouring liquids in the standardized way as performed during the study, the rest of the body is less exposed than the hands because the most contaminated surfaces are only in contact with the hands (contamination of the surface of the jugs and/or containers, possible spills on the hands during pouring, etc.). During these tasks, a large part of the exposure is absorbed into the matrix of the gloves (an interception method that is intended to measure all exposure potentially reaching the skin), and therefore it is assumed that the contaminant layer is transferred to other surfaces (or body parts) during execution of this task when gloves are worn compared to execution of this task with bare hands after which the hands are washed (a removal method intended to measure the actual exposure). When bare hands are highly exposed, it is likely that the contaminant layer is at least partially transferred from the skin by (in case of extremely high exposures) dripping/falling off or (accidental) touching other surfaces, such as equipment or other body parts, for instance due to repositioning of the hands. Therefore, it is assumed that using an interception method for measuring hand exposure most likely results in a higher exposure compared to the “actual” hand exposure when using a removal method.

To investigate whether the dermal hand exposure values as found in the current study are comparable with hand exposures found within the literature, the results of the current study were compared with the results of other studies that reported hand exposure values. In the study of Marquart et al. (2017), studies were selected and matched based on task(s) performed. Studies in which workers wore protective gloves or in which multiple tasks represented the same exposure measurement (e.g. mixing and spraying in one measurement) were excluded. To be able to put the exposure values more into perspective, the concentration of the product and the sampling time were taken into account as well. An overview of this comparison is shown in Table 8.1. With regard to gloves, comparisons could be made for dumping, rolling/brushing (both LV and HV liquids), spraying (both HV and LV liquids) and manually handling objects immersed/dipped in HV liquids. With regard to hand wash, fewer comparable exposure situations were available, namely dumping powders, pouring LV liquid and spraying LV liquid. For dumping measurements with gloves, the exposure values found in the study from De Haan (1998) were about twice as high compared to the values found in the current study. However, the amount of powder handled (filling drums with 10 kg pesticides), the exposure duration and the concentration were higher compared to our study. It is expected that within the current study, exposure values would be higher when the same conditions were met (taking into account the concentration and amount handled). Furthermore, in the

current study the volunteers only handled drums pre-filled with 1 kg of powder, while in the study of De Haan (1998) the worker also cut open the cardboard box, with less standardized working conditions, which might have influenced the exposure values found in that study.
For rolling and/or brushing, three studies were identified with similar tasks (RISKOFDERM, 2003; Gijsbers et al., 2004; Garrod et al., 2000). One was graffiti removal by means of spreading/rolling/brushing, which resulted in a range of hand exposures between 210,000 and 720,000 µg, with a concentration of 11 % of the active substance (solvent) in the product handled and the task duration was 27 minutes. Compared to the study of RISKOFDERM (2003) the exposure values as found in the current study (with a range of 13-3381µg) were lower, even when the concentration difference is taken into account (286 - 74,782 µg when normalized to 11 %). For both studies, the sampling times were similar (~25 minutes in the current study and ~27 minutes in RISKOFDERM (2003)). The higher exposures in RISKOFDERM (2003) might be explained by the experimental design of the task in the current study, where the volunteer held the roller constantly in his dominant hand and the tray was filled by technical staff rather than by himself.
When looking at HV liquid rolling, the study of Gijsbers et al. (2004) showed an exposure range of 190-33,000 µg during rolling and/or brushing of paint compared to 12-883 µg found in the current study during rolling of HV liquids (or 48-3,532 µg when these results are normalized to the 2 % concentration used in the study of Gijsbers et al. (2004)). Sampling time was higher in that study (74 min) compared to the current study (22 min), which might partially explain the higher exposures found in that study. Furthermore, it is expected that exposure is lower in the current study due to the standardization of the rolling task, as exposure from handling the product or filling the tray might result in higher exposures during rolling.
For the other exposure situations, like handling immersed objects and spraying, when taking into account concentration differences and the experimental nature of the current study exposures seem to be in line, or lower, compared to what was found in the literature.

8.1.2.3 Identification of the preferred measurement method per exposure situation

Taking into account the considerations mentioned above, both techniques have their advantages and disadvantages. Therefore, it is suggested to choose the most useful measuring technique based on the goal of the measurement study. When measuring dermal exposure for e.g. REACH dossiers, for which a conservative/worst-case exposure assessment is preferred, using the glove method might be the better option, while in case the measurements need to represent actual exposure, a removal technique like hand wash might be the better option. Unlike for measuring dermal body exposure, the exposure pattern is generally not taken into account when deciding the best strategy for measuring dermal hand exposure. However, when a differentiation between hands is to be made, using gloves would be the preferred option, since a hand wash procedure does not allow for differentiation between hands. If one would like to measure actual exposure of the individual hands, rinsing, wiping, or tape stripping would be better options, but these were not included in this study.

Table 8.1 Comparison of exposure ranges for all tasks and measurement techniques used for hand exposures between the current study and other studies taking into account the different concentrations and sampling time

SYSDEA study						**Studies found in the literature**				
Exposure situation	**Method**	**Concen-tration**	**Duration (min)**	**GM (µg)**	**Range (µg)**	**Exposure situation**	**Concentration**	**Duration (min)**	**GM (µg)**	**Range (µg)**
A1	Gloves	0.5 %*	2	18.2	8-145	Filling drums with 10 kg pesticides (de Haan, 1998)	5-25%	18	30	9-1,070
A2	Gloves	0.5 %	6	5515	2111-57424	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
A3	Gloves	0.5 %	6	1986	942-3908	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
B1	Gloves	0.5 %	26	280	13-3381	Gravity removal by spreading solvents (RISKOFDERM Deliverable 42)	11%	27	440,000	210,000 - 720,000
B2	Gloves	0.5 %	22	110	12-883	Rolling and/or brushing paint (Gijsbers et al., 2004)	2% (0.4-3.2%)	74	2,800	190-33,000
						Consumer painting (Garrod et al., 2000)	Normalized to represent product	96-148	mean 2,505,000	200-8,760,000
C1	Gloves	0.5 %	16	60	29-100	Spraying crops using lance (Hughes et al., 2008)	0.005%	20	110	14-428
						High volume spraying using lance (Machera et al., 2002)	Normalized to represent product	12-19	mean 6495	920-20,830
C2	Gloves	0.5 %	17	115	37-683	Spray painting (Delgado et al., 2004)	2.7% (1.2-5.0%)	16	808	20-6,520
D1	Gloves	0.5 %	8	1465	823-	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*

SYSDEA study						Studies found in the literature				
Exposure situation	**Method**	**Concen-tration**	**Duration (min)**	**GM (µg)**	**Range (µg)**	**Exposure situation**	**Concentration**	**Duration (min)**	**GM (µg)**	**Range (µg)**
					2547					
D2	Gloves	0.5 %	8	1822	1123-2762	Dipping objects in NMP baths carried out for stripping and decontaminating (RISKOFDERM Deliverable 42)	~25% (up to 90%)	53	25000	60-6,650,000
E	Gloves	0.5 %*	3	287	211-545	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
A1	Hand wash	0.5 %*	2	16	6-102	Handling, cutting & dumping bag in mixer (~281 kg handled) (RISKOFDERM Deliverable 40)	85%	6	47,000	21,000-122,000
A2	Hand wash	0.5 %	6	2747	603-10914	Pouring product in disinfectant tank (Preller et al., 1999)	9.5%	2	21,700	*n/a*
A3	Hand wash	0.5 %	6	1905	736-3457	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
B1	Hand wash	0.5 %	26	61	13-1689	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
B2	Hand wash	0.5 %	22	75	18-736	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
C1	Hand wash	0.5 %	16	54	24-590	Disinfectant spraying (Preller et al., 1999)	0.14-0.36%	38	6,090	190-27,200
						Disinfectant spraying (Snippe et al., 2001)	0.03	3.9	163	<50-1,898
C2	Hand wash	0.5 %	17	88	30-746	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
D1	Hand	0.5 %	8	418	83-912	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*

SYSDEA study						Studies found in the literature				
Exposure situation	**Method**	**Concen-tration**	**Duration (min)**	**GM (µg)**	**Range (µg)**	**Exposure situation**	**Concentration**	**Duration (min)**	**GM (µg)**	**Range (µg)**
	wash									
D2	Hand wash	0.5 %	8	468	131-1063	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
E	Hand wash	0.5 %*	3	106	59-152	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*

* Concentration for these scenarios were recalculated from 100% pure product to 0.5% w/w to be comparable with the liquid exposure situations

8.1.2.4 Conversion factors between gloves and hand wash

In a validation study of the dermal module of ECETOC TRA, Marquart et al. (2017) collated a large amount of dermal hand exposure measurement studies in a database. Within this database, a distinction was made between interception methods (gloves and patches) and removal methods (wipes, hand washes, tape strips), since all these methods were reported in the measurement studies. They found that the use of interception methods on average resulted in a factor 6 higher exposure values compared to removal methods, which is considerably higher than the factor that was derived in the current study, where measuring with gloves resulted in a factor 1.2 (for spraying) 3.7 (for handling immersed objects) higher exposure depending on the exposure situation. Like in the current study, Marquart et al. (2017) found that there were large differences between studies and between different experiments within a study. The authors suggested that, based on the results from the studies they evaluated, interception techniques probably overestimate real exposure levels and concluded that based on the wide variability of factor differences between interception and removal techniques, derivation of a general conversion factor between the two principles of dermal sampling is unlikely to be feasible. The results of the current study underpin this conclusion, and therefore it is not considered appropriate to derive a conversion factor based on the results for interception by gloves and removal by hand wash as investigated in this study, since the uncertainty around that factor would be too high to reliably estimate dermal exposure for one method based on the results of the other method.

8.1.3 Headband and head wipe

For head exposure, the results suggest that wipes (removal technique) resulted in higher exposure values compared to using the headband (interception technique) for all exposure situations except spraying and rolling liquids, for which no significant difference was observed between the two methods. The extrapolation of the surface area wiped to represent the headband assumes homogenous exposure, and when doing this, the assumption is made that exposure also occurs on the back of the head, which might not be the case.

For the exposure situations where exposure to the forehead was observed, either the volunteer worked a part of the time above shoulder height (during spraying liquids and rolling liquids), or visible dust or aerosol (overspray) formation was observed (during spraying liquids, dumping powder and handling contaminated objects) which resulted in a more homogeneous exposure pattern that also reached the forehead.

Although related to hand exposure instead of head exposure, Gorman et al. (2014) compared wiping with use of gloves during deposition and immersion experiments under standardized conditions, and found significantly higher exposures on the gloves, which is different from the results of the current study. However, due to the difference in exposure between the hands and the head, this comparison is not considered very relevant.

When taking into account the effect of extrapolating head wipes to the headband surface area, there are no clear advantages or disadvantages over either method when measuring exposure situations such as rolling and spraying, since no

significant difference was found between the two measurement methods. When measuring other exposure situations, where head exposure is not expected to be uniform, it should be taken into account that using wipes could result in higher exposures.

8.1.4 Comparing fluorescence predictions with results from chemical analysis

When comparing the exposure values estimated with the fluorescence model developed for spraying scenarios for either patches or a coverall, it was found that the model slightly underestimated exposure compared to measuring with a coverall in combination with chemical analysis of the coverall. However, when comparing the model outputs with the measurements using patches, the model greatly underestimated the exposure. Since using the fluorescence method in its current form is relatively expensive (buying UV lights, proper camera) and time consuming (building and calibrating UV light setup), and the fact that in case one would like to use the fluorescence method in an actual work situation a tracer would have to be introduced in the process / work environment to be able to 'visualize' dermal exposure, the fluorescence method in its current form has no advantages over the more traditional dermal exposure measurement methods, especially in case of workplace exposure studies. While using a fluorescence method could be less expensive as no chemical analysis is required, and could gather a lot of information about for instance the distribution of exposure over the body (which would be valuable information for instance for instruction of workers and/or targeted preventive measures), this technique needs to be improved with regard to accuracy and applicability domain, before it can be used in the field. This is further discussed in section 8.3.

8.1.5 Difference in exposure when using low or high viscosity liquid formulations

As mentioned before, for the exposure situations manually handling objects immersed/dipped in liquids and rolling liquids the viscosity of the liquids did not show a significant effect on the total exposures. However, for pouring and spraying a significant difference was observed between LV and HV liquid formulations: pouring LV liquid resulted in significant higher exposure values than pouring HV liquid, and spraying LV liquid resulted in lower exposure values compared to spraying HV liquid. In case of pouring, this observed difference was expected as LV liquids tend to drip down slightly faster from the container and jug than HV liquids, which increases the chance that a volunteer comes into contact with liquid on the outside of the container and/or jug if it gets contaminated. Furthermore, it is expected that more splashes occur during pouring LV liquid compared to pouring HV liquid.
The significant higher exposure when spraying HV liquid compared to LV liquid is more difficult to explain. Usually, the spray nozzle and airflow are adjusted based on the type of liquid that is being sprayed, which might result in a higher use rate during spraying of HV liquids such as paint. This was not the case in the current study but might be the case in worker situations where higher viscose liquids are sprayed. In the current study, the exposure duration and the amount used during spraying were kept the same during the spraying experiments with both types of liquid. This suggests that the behaviour of the aerosols that are formed during spraying of either

LV of HV liquid formulations might play a role in the observed differences, although this was not further investigated.

8.1.6 Quantification of fluorescence method

With regard to the fluorescence method, TNO investigated different options to quantify dermal exposure (the amount of tracer on body surfaces). The initial idea was to use the SMF 2 surface monitoring light probe (STS Ltd), a portable direct reading spectrofluorometer that can analyze spots of 3.14 cm^2, for the in situ quantification of the deposited mass on the skin in combination with by visual localization of the fluorescence and a standardized sampling scheme based on the geo-statistical sampling scheme as proposed by Wheeler & Warren (2002), to map the distribution of the contamination over the coverall, in which more measurements are allocated to areas of high exposure. However, in practice the use of the purchased probe was not considered very user friendly, and the method was also found to be very labor intensive. Furthermore, this probe could only be applied on flat surfaces for a correct reading, which made it impossible to use the probe directly on a coverall or patches when these are still on a volunteer. However, taking off the coverall and placing it on a flat surface for sampling with the probe was considered not feasible, since this would involve a high risk of cross-contamination when handling the coverall and possible transfer of the tracer from the coverall to the flat surface, which in turn would influence the results of the chemical analysis of the different body parts after use of the probe. Therefore, this technique was discarded for use within this study. Instead, it was decided to use direct quantification of the amount of tracer on the basis of high resolution images by means of quantification software. An advantage of this method compared to the probe was that the whole coverall could be quantified instead of spots on the coverall or patches when using the probe in combination with extrapolation to a large surface area, and the possibility of cross contamination was negligible as pictures from the front and the back of the volunteer wearing the coverall or patches were immediately taken and processed after the experiment, with very limited contact with the coverall or patches in between.
During the development of the fluorescence method several observations were made, which are described below. While the concept of a quantitative whole-body fluorescent measuring technique is considered feasible, the hardware in combination with the tracers' characteristics and the concentration of the tracer in the formulations as used in the current study were not optimal. However, the approach of the development of the fluorescence measuring technique was performed in a systematic way, which makes it possible to plan for future improvements of the hardware and methodology.

8.1.7 Hardware

8.1.7.1 Lighting

The fluorescence method used in the current study has a thoroughly tested lighting setup. This ensures that the volunteer is illuminated by light that comes from a light source that approximates an 180° sphere, which provides the most optimal distribution of UV light on the volunteer. In other studies different lighting setups were applied, with advantages and disadvantages for each lighting. The VITAE system

(Fenske et al., 1986) and the system used in the study of Bierman et al. (1998) used only two sets of lamps directly next to the camera. This is a fast and easy way to build a lighting setup, and makes it useful as a portable setup. However, it only resulted in diffuse lighting on flat surfaces and its usability decreased when non-flat surfaces such as exposed workers are used. The FIVES system (Roff, 1994) uses a different and more complex array of lamps, where strip lights are used to construct a dodecahedron where there is room at the center for the volunteer to stand. This setup is better at providing diffuse illumination than the setup developed within the current project, because it provides actual all-round illumination instead of relying on reflective surfaces as the current setup does. However, the size of the dodecahedron ensures that only the upper torso and arms can be properly illuminated and measured, while the current setup can light up the entire body of the volunteer. Moreover, due to the construction of the FIVES system, the setup is stationary and cannot be used in measuring campaigns at for instance companies. A small measuring area is also a limitation of the VITAE system and the system used by Bierman et al. (1998). They are more suited to only measure exposure on the hands. The current setup is (to our knowledge) the only one that is suitable to measure the entire body, and can be setup at workplaces.

8.1.7.2 Imaging

To capture images of volunteers wearing coveralls contaminated with Tinopal, consumer market cameras were used, namely the Canon EOS 700D at TNO and the NIKON 90D at BPI. These cameras are relatively cheap and they are easy to use and install. Even though the use of a different camera brands during the calibration experiments introduced extra uncertainty with regard to interpretation of these result, ultimately, this did not influence the development of the model, since the model was developed based on $1/4^{th}$ of the experimental chemical analysis data, for which the photographs were taken using the same camera. However, compared to the cameras used in the other systems, the use of these cameras is slightly limited, which comes with some disadvantages. For instance, consumer market cameras are equipped with color filters on their light sensitive chips. This made it difficult to separate tracer fluorescence from other light sources, such as background fluorescence of Tyvek coveralls or general “noise” that is inherent to the camera system. These filters are normally used to provide the camera with color information by dividing all optical sensors into a sensor that detects either red, blue or green light. The colors in a photograph are constructed based on the relation between these differently colored sensors. Because Tinopal emits light of a very specific color blue (430-436 nm), it is not useful to capture additional colors during measurements, as this will cause a large amount of background noise. Furthermore, the filters of the used cameras stop detecting light of a wavelength shorter than 400 nm. Because 430 nm is close to this limit, signal loss might occur when using these cameras (although this was not quantified). Within the studies of Roff (1994), Bierman et al. (1998) and Fenske et al. (1986) different cameras than the ones used in the SysDEA project were used. These studies used a monochrome light sensitive chip combined with bandpass filters to ensure as much as possible that only the light emitted by the fluorescent tracer is picked up by the camera. This reduced the background noise considerably and provides cleaner images. Another interesting feature that only the FIVES system uses is in combination with a diffuse light source, the usage of a point source to capture images. Taking two pictures immediately after each other, one with

diffuse lighting and one with the light coming from one bright point source above the camera, gives information about the orientation of contaminated surfaces in relation to the camera. This information can be used to correct for the loss of information that occurs when taking a 2D picture of a 3D surface. This lessens the artifacts that occur because of wrinkles/creases in clothing and other curved surfaces.

8.1.8 Tracer

Often, studies did not provide a reasoning for their choice of tracer, but their choice may depend on solubility in certain matrixes or availability might be the determining factor. For the current study, multiple substances were tried and it was found that Tinopal SWN was the easiest to wash off the volunteers. No real up or down sides were seen in using different Tinopal variants with regard to the fluorescence part.
In the previously named studies, Tinopal was only used when dissolved in other products such as paints or pesticides (Bierman et al, 1998; Roff, 1994, Schneider et al., 2000). It is not known if their methods can also be used to measure both powder and liquid exposures on a whole body level. In the study (Brouwer et al., 1999) a modified version of the VITAE system was used to look at powder exposures on hands. The setup explored in this study indicates that using Tinopal SWN in powder form is not suitable for a fluorescence model. It is not known when changing the type of tracer, if the use in a powder form improves the model results. This should be explored in future research. In the current study, the main limitation of the tool is expected to be the concentration of Tinopal SWN in the liquid formulation, since the calibration experiments showed a clear upper limit of detection from the tool which is exceeded by the concentration used in our study design. Experiments with lower concentrations should be useful to determine the full effect of this detection limit.

8.1.9 Validation

In the current study the generation of exposure on samples used for the calibration experiments was well controlled and defined. This ensured that there was a broad base of material to assess the functionality of the tool resulting in a thorough assessment and eventual validation. Previous studies in which a fluorescence method was developed often suffered from a limited number of available samples, or used an approach to generate exposure that was not very consistent. For instance, the calibration curve used in the study described by Roff (1997) was generated by applying amounts of Tinopal to the forearm of a volunteer (Roff, 1997). Next to the increase of Tinopal concentrations, this also measured possible effects that skin-color and curvature of the arm have on the measured intensity (Roff, 1997).
In the current study all samples that were used for the calibration were made so that the only variable that changed through all the experiments was the amount of tracer. This minimizes uncertainties and variability due to unknown variables.
This consistency in the makeup of samples also helped to identify other artifacts inherent to the fluorescence method as used in the current study, such as angle dependency and the effects caused by the different surfaces used (e.g. HPL vs Tyvek).

8.1.10 Usage of fluorescence data in current study

The data generated during this study with the fluorescence tool could have added value compared to more established methods such as the whole body, patches or hand wash measurement methods that generate exposure data by means of chemical analysis of collected samples. These methods lack information about the exact exposure pattern, for instance diffuse exposure on the whole body versus concentrated exposure on parts of the body. The photographs made in conjunction with the chemical analysis of parts of a coverall or patches give an opportunity to visually detect potential validity problems with extrapolation of loading on patches to loading of a body part as a whole. The fluorescence tool could be used to quantify these exposure patterns. For instance, it could give visual output with percentages of the total exposure per body part. This has been done previously as well by Galea et al. (2013), who investigated a fluorescence imaging technique which would 'flag' exposed areas and calculate the area exposed (while not quantifying the exposure). In principle, their setup and idea was comparable, and like our setup Galea et al. (2013) concluded that the fluorescence technique was not sensitive enough to give accurate predictions of exposed surface areas. They concluded that a fluorescence method should for now be used qualitatively, as has ultimately been done in the current study as well. Although it is assumed that a refinement of this method, taking into account the new insights reported in this study, a more quantitative exposure estimate is possible.

UV photographs in combination with Tinopal SWN can also be used for educational purposes, workers could be shown how their work methods result in certain exposure patterns. The visual information provided by fluorescence photographs in a pre-pilot could also be used to determine what measuring strategy to use during studies, or indicate a need for placement of patches in different ways (when found that exposure often misses the patches during an experiment).

8.2 Future research

Based on the results of the current study, some insights with regard to future research can be formulated.

Firstly, while the fluorescence model proved not applicable within the current study due to the above mentioned insights regarding the model, it is expected that this model can be improved when the limitations are taken into account. Proposed improvements include experiments using a monochrome light sensor with a narrow bandpass filter to investigate whether this improves the image quality by reducing background noise or other lighting interference without having to manually correct for this which introduces uncertainty in the estimates. Furthermore, the lack of depth perception by the current setup could be corrected by also using a point light source in conjunction with the diffuse lighting source. The difference in measured intensity between a point source lighting and a diffuse light source could be used to correct for surface curvature as seen in the FIVES method by (Roff, 1994). Another possibility is to spread the coverall on a flat surface after each experiment before taking a photograph. This does introduce a risk of cross-contamination when coveralls are removed from the volunteer as a whole. It should be investigated whether lowering

the concentration of tracer in the solution increases the upper limit of detection. Lastly, it can be investigated what effect different tracers have on the model outcomes. For powders it was shown that a suitable model could not be calibrated, this might be different when using different tracers.

Secondly, although relevant tasks and dermal exposure measurement methods were selected, the number of tasks and measurement methods explored in the current study is still limited and could be expanded. For example, tape-stripping and rinsing of the hands were not included in this study, although these are also commonly used methods, especially tape stripping, to measure dermal exposure of the hands.

Thirdly, the current exposure situations were heavily standardized, which served its purpose in enabling a ‘clean’ comparison between different measurement methods. In future research it would be useful to vary some of the determinants that were now fixed (like amount of product used, drop/pour height, spray pressure) to investigate how these influence either exposure patterns or measured exposure values, and whether this would influence the choice of the best suited measurement method for a certain exposure scenario. For dermal exposure modelling purposes, information regarding the determination of the maximum loading of the hands is considered important but is still lacking. Having an idea of the maximum loading of the hands would also give input to put the observed differences between interception methods and removal methods into perspective, as for bare hands it is expected that an upper limit of exposure could be reached (indicating maximum loading) while for gloves it is expected that this upper limit would be much higher due to the absorption of the materials used for measuring hand exposure (like cotton gloves).

Lastly, the materials used for the coveralls, gloves and patches were the same throughout the experiments, depending on whether exposure was measured for liquids or for powders. Using different materials for interception might also result in different exposure values, depending on the matrix used in combination with the product that is used. Additional information on the performance of other materials might be valuable when determining which measurement strategy to use during a certain measurement campaign.

9 Conclusions

Taking into account the objectives of the study it can be concluded that:

- When comparing measuring dermal body exposure by means of patches or a coverall, using patches resulted in significant higher exposure values compared to using a coverall for all liquid exposure situations. For powder exposure situations, using patches resulted in generally higher exposures, but these values were not significantly different.

- When comparing measuring dermal exposure using gloves (interception) or a hand wash technique (removal), using gloves led to significant higher exposures for handling immersed objects and rolling. During pouring and spraying, the exposure values on the gloves were slightly higher, but the difference was not significant.

- When comparing measuring dermal exposure using an interception technique (headband) or a removal technique (wipes), wipes seemed to result in higher exposure values. Both methods were suitable when measuring spraying or rolling activities, or activities where a uniform exposure distribution is expected. For other activities, it needs to be taken into account that using wipes possibly results in higher exposure values. Especially when extrapolation to a representative surface occurs.

- When taking into account the different exposure tasks performed in the current study, using the fluorescence method and the chemical analysis showed that especially spraying and dumping (and to a lesser extend rolling and handling of contaminated objects) result in a more uniformly distributed exposure pattern. Exposure patterns are less uniformly distributed for exposure tasks such as pouring and handling immersed objects.

- When taking into account the exposure to the head, it can be concluded that measuring head exposure might not be relevant for each exposure situation. Especially situations where a mist, dusty solids or overhead working is expected, head exposure needs to be taken into account. For other exposure situations, head exposure seems less relevant.

- It is concluded that no ‘golden standard’ with regard to a preferred measurement method for dermal exposure can be identified from the different methods as investigated in the current study. However, some considerations with regard to the choice of dermal measurement method can be given. For instance, a coverall seems more suitable than using patches for measuring body exposure in case of exposure situations in which a non-uniform distribution of exposure is expected. Furthermore, removal methods like a hand wash are a good way to measure actual hand exposure, while an interception method like gloves generally results in higher exposure values, which are assumed to resemble potential hand exposure resulting in a more worst-case exposure estimate.

- It has not been discussed in this report how far or in what way the data and results generated in this study can be utilised for regulatory purposes. This is part of following reports prepared by BAuA.

- The current fluorescence method is not suitable for estimating dermal exposure values solely on fluorescence photographs. However, using a fluorescence technique can be an excellent source for material for companies to train their workers regarding safe work practices by visually showing the contamination resulting from their task. Furthermore, use of a fluorescence method as applied in this study results in valuable information about the distribution of exposure over the body (exposure pattern), which can be used to define the best suited measurement strategy for a measurement campaign.

10 Recommendations

Based on the findings in the current study, some recommendations can be given with regard to the different measurement methods as investigated in this study.

When measuring body exposure, the use of patches is assumed to be suitable for tasks where a uniform distribution of body exposure is expected and/or when 'worst-case' exposure estimates are considered appropriate. Coveralls are considered more suitable to measure dermal exposure for tasks where no uniform distribution of body exposure is expected, such as tasks during which dermal exposure is caused by 'random' splashes occurring or droplets being formed, such as pouring liquids, rolling liquids and handling of immersed objects.
Lastly, it is not recommended to use a conversion factor to recalculate body exposure values based on measurements with a coverall to exposure values based on measurements with patches, or vice versa.

For hand exposure, homogeneity in exposure patterns is generally not an issue as in many cases the whole hand (or hands) is being included in the sample collection. Interception methods such as using gloves are expected to result in higher exposure values compared to removal methods such washing of hands, as they represent potential and actual dermal hand exposure, respectively. This should thus be taken into account when deciding on which measurement method to use during a specific measurement campaign, based on the goal of that campaign. When a hand wash method is used, the possible absorbance of the substance by the skin and/or adherence of the substance to the skin needs to be taken into account, as well as the duration of the tasks. The longer the duration of the measurement, the higher the chance of transfer from the hands to other surfaces, cross-contamination and absorbance of the substance through the skin. Therefore, for shift-based measurements or longer task-based measurements it is recommended to use gloves to measure hand exposure.

Regarding head exposure, exposure assessors should indicate whether head exposure is relevant to take into account when measuring dermal exposure. The results of the current study indicate that head exposure is likely to occur during situations in which exposure is more or less uniformly distributed (like during spraying, dumping powder and handling objects contaminated with powder) and situations where the worker is (partially) working above his/her head (like rolling).

Use of a fluorescence method as applied in this study results in valuable information about the distribution of exposure over the body (exposure pattern), which can be used to define the best suited measurement strategy to be applied in a measurement campaign.

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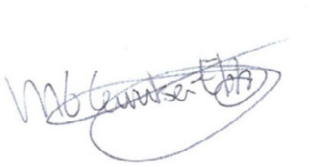
13 Signature

Zeist, September 2018 TNO



M. Rennen
Research Manager

R. Gerritsen
Project manager

Annex 1 Protocols and Standard Operating Procedures (SOPs)

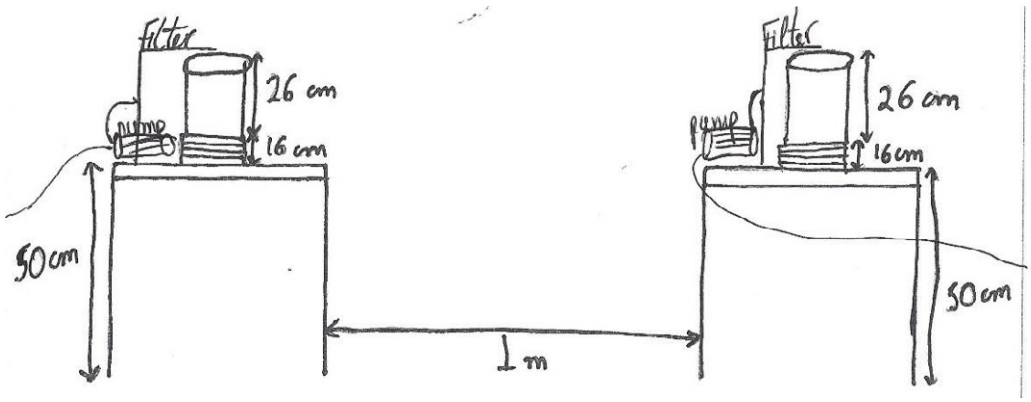
Protocol dumping (A1)

Product/s:	A dusty solid powder containing pure Tinopal SWN (1 kg).
Number of objects handled for this exposure situation:	*Two (2) open bins*. The first bin, before the trial and after loading with Tinopal SWN, is covered with filter paper to avoid contamination. The filling-loading of the first vessel-bin has to be made beforehand by the scientific team without any involvement of the volunteer.
Size and description of objects and equipment:	*Two (2) open bins ("vessel")* with 20L capacity and dimensions 26cm height and 26cm opening *(see photo)*. *Two (2) small workbenches* with dimensions 50cm length and 50 cm height (*see photo*). *Two (2) Büchner funnels* equipped with filter paper (Whatman type MN 640 d, 185 mm) *Two (2) identical bricks* upon which the vessels are positioned-supported. *Two (2) pumps connected to Büchner funnels.*
Placement of objects and equipment	The vessels are put on the bricks of the tables before the trial. The distance between the tables is 1m. Right next to each vessel a Büchner funnel equipped with filter paper is placed at the same height with the rim of the vessel.
Protective measures and setup for limiting contamination of workplace	The walls of the test room are covered with thin plastic film. The floor is protected with plastic film and layers of disposable carton paper (non- slippery type). The door of the test room remains closed throughout the application.
Description of work for the exposure situation:	The volunteer takes the filled vessel from the table with both hands and dumps all the content in the receiving vessel from a height of approximately 15-25cm above the rim (opening). After emptying, he waits for 5 seconds (dust to settle), places the empty vessel back to the table, picks again the now filled vessel, moves back and repeats dumping to the empty vessel. The procedure is recurrent (in total six (6) consecutive dumpings). The pumps operate throughout the experiment.
Task completion point ("finish line"):	The task is considered to be completed when the six successive dumpings will be performed following the procedure described above.

Observations:	• Time of task completion (start and end time) • Estimated dropping height • Generation of dust cloud and exposure of the volunteer to it (optical estimation of cloud maximum height and direction). • Breaks for accidents such as spilling some of the vessel's powder outside the receiving vessel etc. • Contamination of the workbench and the container from cloud dust and/or powder

(a)

(b)



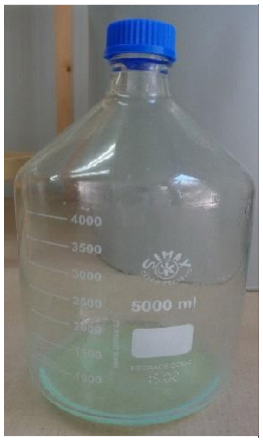
c)

Photos: (a) vessel-bin, (b) dumping set up, (c) table

Protocol pouring (A2 and A3)

Product/s:	Low viscosity liquid (LV) High viscosity liquid (HV)
Number of objects handled for this exposure situation:	*Two (2) 5-liter narrow neck containers (glass bottle with screw cap)* *One (1) 1 liter jug without handles*
Amount of liquid handled for this exposure situation:	10 liters of HV or LV liquid (Tinopal SWN 2g/L)
Size and description of objects and equipment:	*Narrow neck containers:* 5L each (see photo), with an opening diameter of ~40 mm *Jug without handles:* cylinder shaped, diameter approx. 15cm *One (1) open vessel:* 10L capacity, dimensions height 0.25 m, opening diameter approx. 0.25 m *Two (2) workbenches* with dimensions 70 cm width, 100 cm length, 100 cm height
Placement of objects and equipment	The two glass bottles filled with the liquid (HV or LV) and the jug are placed on first workbench. The open vessel (receiving container) is placed on the other workbench. The two workbenches are located at 3 m distance from each other.
Protective measures and setup for limiting contamination of workplace	The floor of the test room is protected with plastic film and layers of disposable carton paper (non- slippery type) to retain any potential contamination from accidental dripping of the liquid used in the trial.

Description of work for the exposure situation:	The volunteer opens the lid of one of the 5 L glass bottles, using one hand to hold the container and the other hand to unscrew the lid. Then he pours 1 L of content from the container into the 1 L jug using both hands (the jug is marked with a marker line at the height of 1 L for the volunteer to be able to estimate the volume during pouring). The height of the glass bottle above the jug during pouring is kept at approximately 10- 15 cm. The volunteer closes the lid of the container using both hands. Then the volunteer carries the 1 L jug to the second workbench using both hands and pours the content of the jug into the 10 L receiving vessel (using both hands to pour). After having the content of the jug poured to the open vessel, the volunteer waits a few seconds with the jug above the vessel to empty thoroughly. The procedure is repeated 5 times until the 5 L container is empty. Then the volunteer repeats the same task for emptying the second 5L container and transfer its content to the open vessel using the 1L jug as described above.
Task completion point ("finish line"):	The task is completed when the volunteer has transferred all the liquid from the 2 narrow neck glass bottles (i.e. totally 10 L liquid) to the open vessel following the transfer procedure described using the jug for pouring. The respective duration of this procedure is recorded.
Observations:	Time of task completion (start and end time) Breaks for technical problems / trouble shooting (i.e. fixing protective equipment) Contamination of the bottles / screw cap Incidental dripping on the floor Time and record of cross-contamination incidents such as spillages or splashes, any accidental dripping of the liquid from the from the bottles or the jug, cross-contamination by the volunteer (hand to face/body contact), other cross-contamination, etc.



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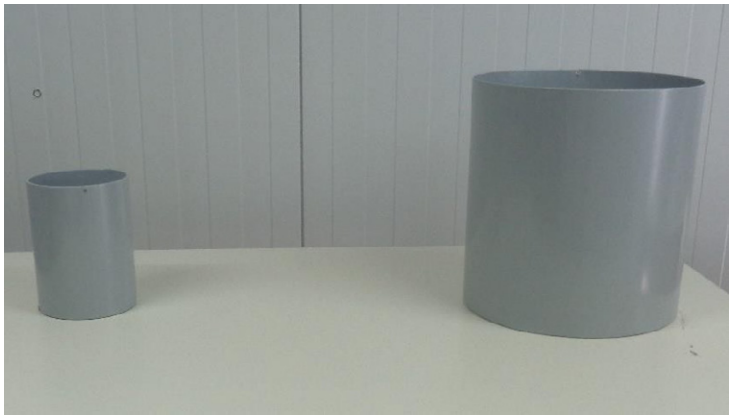

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Photos: (a) Glass bottles of 5L (b) workbench (c) jug and open vessel

Protocol rolling (B1 and B2)

Product/s:	Low viscosity liquid (LV) High viscosity liquid (HV)
Number of objects handled for this exposure situation:	*Two (2) wooden flat surface panels* (see description below) *One (1) short-handle round edged polyester roller One (1) plastic tray that fills with the liquid*
Amount of liquid handled for this exposure situation:	2 liters of HV or LV liquid (Tinopal SWN 2g/L)
Size and description of objects and equipment:	*One (1) short-handle round edged polyester roller* width ˜15 cm, diameter ˜4 cm suitable for professional use (wall painting) (see photo A). A new roller is used for each experiment. Two (2) wooden panels with dimensions of 190 cm height, 160 cm width that stand upright (vertically) on two base stands each (see photo B). One (1) table with short legs and wheels dimensions 50 cm length, 50 cm width, 50 cm height (see photo C) One (1) floor type scales (balance) to weigh the roller and the tray (see photo D)
Placement of objects and equipment	The roller and the tray are placed on the table between the two panels that are concurrently inside the container (see graph below). The volunteer stands at a distance of approximately 0.5 m from the wooden panel. The balance is placed on the other side of the container to avoid any contamination with test liquid.
Protective measures and setup for limiting contamination of workplace	The floor of the test room is protected with layers of disposable carton paper (non- slippery type) since contamination from dripping of the panel is anticipated. The walls of the test room (container) are also covered with plastic film to protect from accidental spillage or contamination.
Description of work for the exposure situation:	Two dry wooden panels are used for each experiment. The volunteer stands in front of the first wooden panel at 0.5 m. The volunteer is not involved in the mixing and loading procedure of the tray, that is filled by the field scientist with approximately 500 mL of test liquid. This quantity (500 mL) is used to cover one side of a panel. The weight of the clean tray and roller is recorded in the field registration forms, as well as the respective weight of the empty wet tray and wet roller before each new loading. The weight of the test liquid that is loaded every time in the tray is also recorded (for the calculation of the weight of the net liquid). The volunteer dips the roller in the tray and spreads the

	liquid by rolling on the panel surface, while exerting pressure on the panel with the roller, starting from the top and working down to the bottom, moving his body sideward. He rolls each strip at least one time back and forth, so as to cover part of the surface area two or three times with the roller before proceeding to the next strip. After full coverage of the surface of one side of the panel (determined by field scientific team) with 500 mL of the test liquid, the volunteer repeats the rolling at the second panel, standing at the same side, by applying another 500 mL of liquid, with the same technique. Precision work on the corners or bottom is not required. Then the volunteer moves to the other side of the installed panels. The volunteer should not touch any surface other than the roller handle. During the above task procedure, each time the tray is emptied, the volunteer pauses and the field scientist refills the tray with liquid recording the weight of both the wetted roller inside the tray plus the added liquid as described above. After refilling the volunteer continues his task. This happens for 4 times and all times are recorded in the field registration forms. When he finishes the 4^{th} aliquot of the 500 mL, the volunteer rolls all four sides of the two panels as described above with the used roller, without any more liquid. A final value of the sum of tray and roller (almost dry now) is also recorded along with the ending time of the task and other environmental parameters (temperature, humidity). Although the field scientists are responsible to determine when the whole area of the wooden panel is fully covered with liquid, the volunteer is trained to observe/confirm which area of the surface has been covered. Wooden panels are reusable, so they are left to dry outside the test room in the open air after each experiment until dripping of the liquid has stopped. The panel is cleaned with paper and ethanol-water solution.
Task completion point (“finish line”):	The task is considered to be completed when all sides of the wooden panels are completely covered with liquid. The respective duration of rolling (exposure duration) is recorded as mentioned above and the amount of used test liquid is determined by weighing. For the procedure described above and the quantities used for the specific treated areas it is anticipated that no liquid remains in the tray (however in case it remains the amount is weighted and recorded).

Observations:	• Time of task completion (start and end time) • Amount of liquid used/remained in tray • Breaks for refilling the tray • Breaks for technical problems/trouble shooting (i.e. fixing protective equipment) • Contamination of the roller • Incidental touching of surfaces by the volunteer • Direction of rolling • Temperature and humidity • Time and description of accidental contamination incidents: e.g. touching the wooden panel, splashes, dripping of the panel, cross-contamination by the volunteer (hand to face/body contact), other cross contamination, etc.

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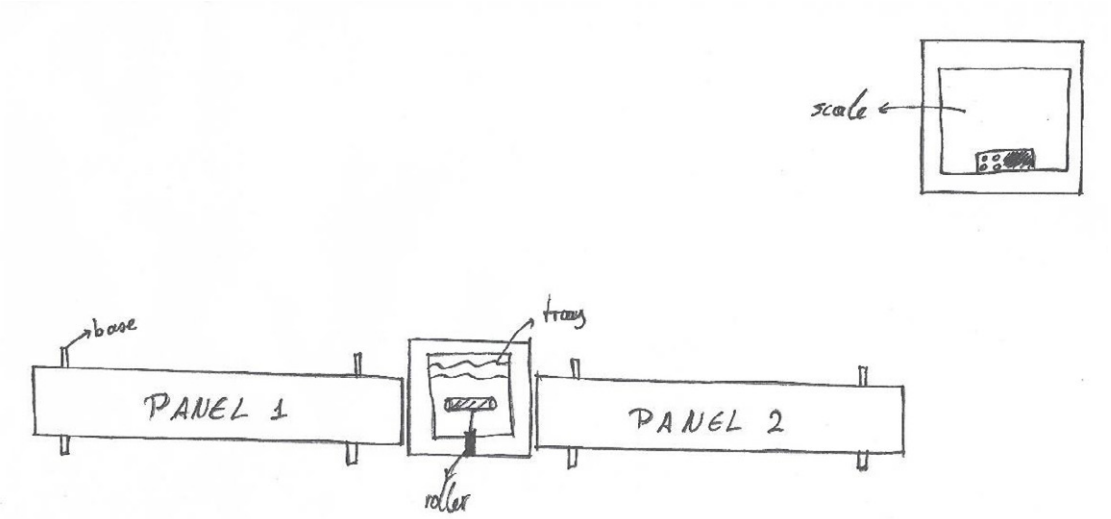

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Photos: (a) Roller with tray, (b) wooden panel, (c) balance for weighing roller and tray, (d) table, (e) Schematic representation of the experimental setup

Protocol spraying (C1 and C2)

Product/s:	Low viscosity liquid (LV) High viscosity liquid (HV)
Number of objects handled for this exposure situation:	*One (1) HVLP spray gun* connected to mobile air compressor device is used to apply LV or HV liquid *One (1) wooden flat surface panel* (see description below)
Amount of liquid handled for this exposure situation:	2 L of HV or LV liquid (Tinopal SWN 2g/L)
Size and description of objects and equipment:	*1 mobile air compressor device* of 25 L tank capacity and maximum pressure of 8 bar with long hose (length ~ 7m, diameter 8mm). The air compressor is powered by usual AC electric plug. *One spray gun* for professional use (metal type) with usual type hose fit adaptors. The spray gun is connected to the compressor via the hose and has a plastic canister of 500 mL capacity. The canister is loaded with the spray liquid from the top having a wide screw cap that seals tight to retain standard pressure during spraying. The compressor device is put on the floor on a stable place but also has a fit option for adding wheels that allow free movement if required. One wooden panel with dimensions of 190 cm height, 160 cm width that stands upright (vertically) on two base stands
Placement of objects and equipment	The air compressor is placed at approximately 2 m distance from the wooden panel while the operator stands in front of the panel at approximately 0.5 m.
Protective measures and setup for limiting contamination of workplace	The floor of the test room is protected with plastic film and layers of disposable carton paper (non- slippery type) since contamination from dripping of the spraying panel is anticipated. The ventilation system is running throughout the duration of the experiment to ensure proper ventilation of the test room against the generated spray cloud. The door of the test room remains closed throughout the application. The walls of the test room (container) are also covered with a plastic film to protect from accidental spillage and contamination.

Description of work for the exposure situation:	A dry wooden panel is used for each experiment. The volunteer stands in front of the wooden panel at 0.5 m and receives a pre-tested and calibrated spray gun ready for use from the field scientist. The volunteer is not involved in the mixing/loading procedure of the canister of the spray gun. The canister refills of the spraying liquid are recorded in the field registration forms for each loading. The volunteer sprays the liquid on the panel surface, starting from top and working down to the bottom, with sideward movements. The field scientist regulates the pressure on the air compressor set at 1.8 bar. After sufficient coating of one side of the wooden panel (determined optically by field scientific team), the volunteer repeats the spraying at the other side of the same panel. Precision work on the corners or bottom of the panel is not required (to ensure that no unusual working positions or surface shapes are sprayed). The activity should not require the subject to touch surfaces other than the spray gun. When the canister is empty, the volunteer gives the spray gun to the field scientist for refilling with the spraying liquid. After refilling the volunteer continues his task. The spray gun after the end of the experiment is cleaned by the field scientific team according to the cleaning procedures of the equipment that apply for the project. Wooden panels are reusable so they are left to dry outside the test room in the open air after each experiment and when dripping of the liquid has stopped. The cleaning procedure that applies includes wiping with paper and ethanol-water solution. Although the field scientists are responsible to determine when the whole surface of the wooden panel is sprayed the volunteer will be trained in order to be able to observe/confirm which area of the surface has been covered.
Task completion point ("finish line"):	The task is completed when the indicated volume of 2 liter of liquid is consumed for spraying the whole surface of the wooden panel on both sides. The respective duration of spraying (exposure duration) is recorded.

Observations:	• Time of task completion (start and end time) • Breaks for refilling the canister • Breaks for technical problems/trouble shooting (i.e. fixing protective equipment) • Contamination of the sprayer • Incidental touching of surfaces by the volunteer • Direction of spraying • Time and record of cross-contamination incidents such as touching the wooden panel or the hose, splashes, dripping of the panel, cross-contamination by the volunteer (hand to face/body contact), other cross-contamination, etc.

Photos: (a) mobile air compressor, (b) spray gun with canister, (c) wooden panel

Protocol handling immersed objects (D1 and D2)

Product/s:	Low viscosity liquid (constituents are mentioned in respective SOP). The liquid is reusable, and the level in the immersion tank is replenished. High viscosity liquid (constituents are mentioned in respective SOP). The liquid is reusable, and the level in the immersion tank is replenished. Temperature during trials is maintained constant at 21-23 °C using air condition.
Number of objects handled for this exposure situation: **Nr. of volunteers** **Total nr. of tests**	15 4 64
Size and description of objects and equipment:	*Set of 15 metal cylinders* with diameter ~8 cm, length ~20 cm, ~1.2 kg of smooth material. Each cylinder has an open hook (~8 cm) on both ends (see Figure A) that is large enough to allow carrying with at least one finger, and to hang onto a wire fitted on a mobile rack (see Figure B). *Single rectangular shaped container* with dimensions: ~40 cm base length, ~25 cm base width, 30 cm height *Two small metal clothing type racks with wheels (forming the* 1st and 2nd small *mobile rack) fitted with a horizontal metal slim bar where the cylinders are hanged after dipping for draining.* Dimensions: 1.50 m high and 1 m long each. *A big metal clothing type rack* with wheels (3rd mobile rack) fitted with a horizontal metal slim bar at ~1.6 m height onto which the 15 immersed objects can be hanged after the first rack. Dimensions of rack: 1.80 m high, 2 m length. *Two identical plastic trays* with dimensions 60 cm length, 35 cm width and 4 cm depth, placed below the first and second small rack to collect the liquid from dripping. *One workbench* with dimensions 100 cm height, 70 cm width and 100 cm height, onto which the cylinders and the dipping bath are put (see Figure C). The workbench is covered with plastic film and layers of disposable carton paper prior to the experiment to prevent any contamination of the workbench surface generated from dripping of test product during the conduction of the exposure situation task.

Placement of objects and equipment	The dipping bath should be at chest height of the volunteer. To ensure the same working height, an adjustable platform can be used for volunteers to stand on. The dipping bath is filled with the test liquid up to the height of 30 cm from its bottom. The outer side of the dipping bath is placed approximately 20 cm beyond the workbench edge. The 15 cylinders are placed on the workbench next to the dipping bath (see Figure C), horizontally (lying) on the workbench next to each other with the hook pointing to the volunteer. The first small mobile rack is placed 0.5 meters on the right side of the workbench (see setting in respective Figure g). Below the rack, a tray is placed to collect the liquid product dripping from the cylinders after immersing them in the bath. The second small mobile rack is placed adjacent to the 1st mobile rack (again a tray is positioned below the rack). The 3rd, big metal mobile rack is positioned at a 3 m distance opposite to the workbench (see overall setting in the respective Figure g).
	 **Figure 1** Plastic film and layers of disposable carton paper
Protective measures and setup for limiting contamination of workplace and ensure safety of volunteer	The floor is protected with plastic film and layers of disposable carton paper (non-slippery type, see Figure 1 above) since contamination from dripping is anticipated. This will be removed and replaced with fresh materials at the end of each test. Since the liquid is reusable, the container is covered with aluminum foil after each trial to prevent evaporation.

Description of work for the exposure situation:	The volunteer stands in front of the work bench at a distance of 15-25 cm from where the dipping bath is placed. The volunteer picks the first cylinder from the table using one hand to pick it up, gripping it from the hook that is at the side his body, thus avoiding to extend his hand towards the bath. Holding the cylinder with a finger in the hook the volunteer dips it into the bath (up to the upper hook of the object) for 3 seconds. Immediately after dipping, the volunteer lifts the cylinder out of the liquid, but holds the cylinder above the dipping bath to drip for 5 seconds. The volunteer continues to hold the cylinder with one hand from the non-contaminated hook, steps away from the workbench while turning his body towards the first rack and hangs it on the first (small) rack from the non-contaminated hook. After the dipping and hanging to drip for all 15 cylinders (to both small racks), starting with the cylinder that was dipped first, the volunteer uses both hands to unhang the cylinder from the respective small rack by grabbing it at both hooks and carrying it horizontally to the big metal rack that is placed at the opposite side. During carrying the volunteer must have his carrying hands stretched in front of his torso at a distance ca. 40 cm to avoid self-contamination from dripping. The cylinder is then hanged at the rack keeping consistent the hand he uses. This process is repeated for all 15 cylinders. After having all cylinders hanged the volunteer uses both hands to unhang the first cylinder from the rack by grabbing it at both hooks and carrying it horizontally back to the workbench to place it in its original position. During this step (carrying back) the volunteer has his both hands stretched in front of his torso at a distance of ca. 40 cm to avoid self-contamination from dripping (even to his boots). After placing the 1st cylinder on the workbench he repeats this step for the remaining cylinders. The cylinders are placed near the edge of the table with care, to prevent (over)contamination of the sleeves during positioning. The cylinders are put down in a horizontal position one by one, next to one another and left there until the final one is placed. During the placement of cylinders to the edge of table, sleeves are not rolled up or covered to avoid their contamination.
Task completion point (“finish line”):	Placement of the 15 cylinders on the rack and back on the workbench. Time of exposure duration is recorded (separate time registration for three stages, i.e. dipping of the cylinders and hanging them to drip, hanging of the cylinders on a rack after transport over short distance, placement of the cylinders back on the workbench after transport over short distance) as well as change of hands during the exposure situation.

Observation:	Time of task completion, frequency of touching immersed objects and replacement of hands, occurrence of splashes, occurrence of dripping during transport, other forms of cross-contamination, ease of handling/carrying cylinders with time progressing, use of dominant or non-dominant hand. Measurement of the amount of liquid in the bath before and after the experiment with a measurement mark inside the bath and use of a volumetric cylinder to fill up the liquid up to the mark and thus concurrently estimate the amount of liquid used. For cleaning of items (especially after their reuse and if during the trial contamination is observed) the existing cleaning SOP describes the procedure.

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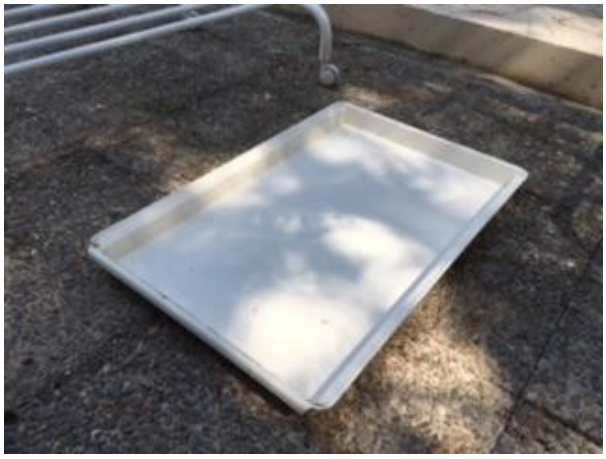

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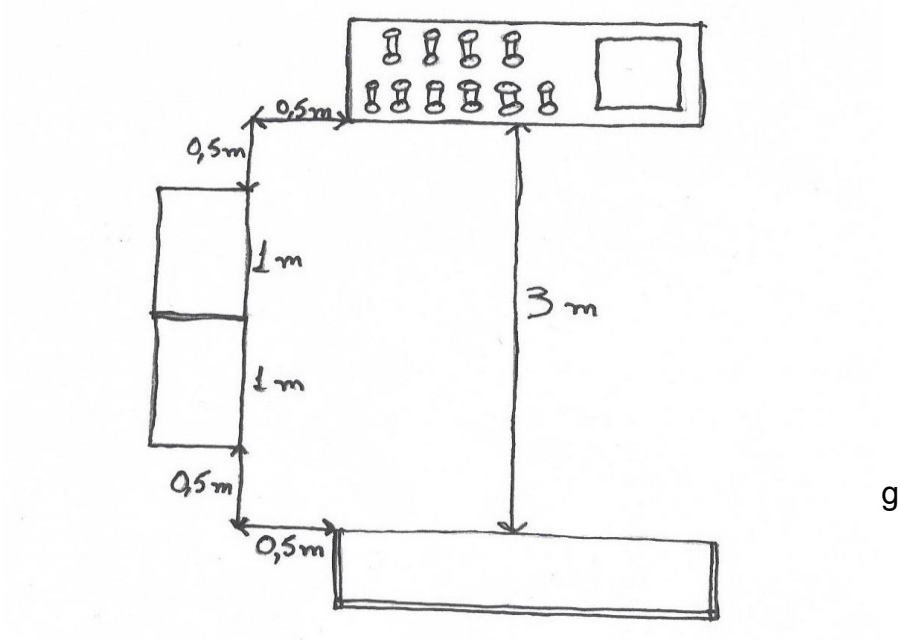

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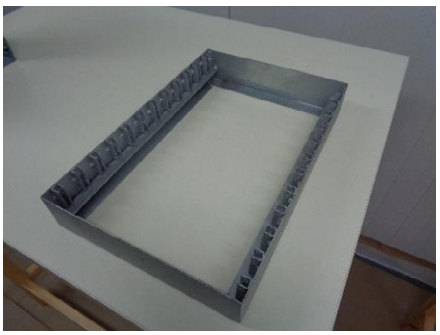
Figures (a) Metal cylinder (b) T Workbench (d) Container filled with immersion dipping liquid (e) First and second rack placed adjacent to each other, for hanging cylinders (f) Plastic tray (g) Schematic representation hird big rack for hanging cylinders (c)

Protocol handling of contaminated objects (E)

Product/s:	*Dusty solid, powder Tinopal SWN* (sieved at 38 µm).
Number of objects handled for this exposure situation:	*Twelve (12) Plexiglas plates* with dimensions of 25 x 15 cm loaded/contaminated with sieved Tinopal SWN powder. The plates have to be loaded (contaminated) beforehand by the scientific team without any involvement of the volunteer (standard loading procedure described in a separate document). Briefly, the standard loading procedure consists of pre-weighing each Plexiglas plate with 1g of sieved Tinopal SWN. The latter confirms the substantial loading and subsequent handling of the plates, in a reproducible way.
Size and description of objects and equipment:	*One (1) metal rack* with twelve positions for placing the plates allowing them to be inserted horizontally. The dimensions of the rack are 26 cm width, 15 cm depth, 39.5 cm height. The distance between neighboring plates is 2.5 cm (*rack 1*). *One (1) metal rack* with twelve positions for the plates to stand vertical with minimal contact with the rack. The dimensions of the rack are 26 cm width, 39.5 cm length, 6 cm height. The distance between neighboring plates is 2.5 cm (*rack 2*). *Two (2) workbenches* with dimensions 70 cm length, 40 cm width, 100 cm height. Each workbench has a bar (30 cm) at the front (along its 40-cm dimension).
Placement of objects and equipment	The workbenches are placed at 3 m distance from one another. At the 1st workbench, the plates are standing horizontally on rack 1. Rack 2 is placed at the centre of 2nd workbench.
Protective measures and setup for limiting contamination of workplace	The walls of the test room are covered with thin plastic film. The floor of the test room is protected with plastic film and layers of disposable carton paper (non-slippery type). The door of the test room remains closed throughout the application.

Description of work for the exposure situation:	The volunteer takes a single plate from rack 1 with both hands (starting from bottom), then knocks it gently (in a vertical position, two knocks) against the bar on the front of the workbench 1, then carries it to the workbench 2. During carrying, the volunteer uses both hands to hold the plate horizontally. In the same context, the volunteer must have his hands stretched in front of his torso at a distance ca 30-40 cm to avoid accidental self- contamination (during carrying). At workbench 2, the plate is also similarly knocked by the volunteer on the existing bar (two knocks) and then it is positioned down upright on a rack shelve (2nd rack) using both hands. This procedure is repeated for each one of the 12 plates, with the 1st plate positioned at the back of rack 2 and subsequent plates in front, respectively. Therefore, trial starts from plate 1, ending to plate 12.
Task completion point ("finish line"):	The task is considered to be completed when all 12 plates are transferred by the volunteer from rack 1 to rack 2 following the procedure described above.
Observations:	• Time of task completion (start and end time) • Breaks for unforeseeable events • Way of handling the plates by the volunteer • Possible contact of the plates with other parts of the body of the volunteer (for instance holding it by accident to his/her belly). • Contamination of the workbench or the racks from dust coming from the plates and/or possible cross-contamination of the volunteer.

(a)



(b)

(c)

Figures (a) rack 1, (b) rack 2, (c) workbench (the two workbenches are identical)

Extraction procedure for analysis of the samples (SOP)

The dosimeters (samples) are put into screw cap plastic polyethylene pots. For the actual field samples this step is already carried out in the field phase following the procedure of sampling while undressing the volunteer.

After removing pots from storage (ambient temperature conditions) the appropriate amount of extraction solvent is added in each jar so as to ensure that they are almost covered by the solvent (at least at ¾ level). The size of the extraction pots and the respective volume is shown in Table A1.1.
For the hand contamination using the hand wash method the extraction is carried out in the field right after the completion of the trial using 3 x 500 mL aliquots of ethanol/water mixture 1:1 v/v. The extracts are combined in the field into one pot.

For all samples the extraction solvent is methanol HPLC grade. For the hand wash samples the extraction has been already carried out in the field (the hand wash samples are already extracts). However, the ethanol/water extract is treated the same way as the rest of the samples (shaking, ultrasonic bath described below), it is further diluted with HPLC methanol and injected to LC (1 µL). For all samples the pots containing the extraction solvent added are placed on a platform shaker and are extracted for 30 min in ambient temperature at 180-200 rpm (rpm may vary according to the overall shaker load to ensure stability of the pots placed on it). After extraction, the pots are placed in an ultrasonic bath for 2 min.

The extracts are transferred into a chromatography vial and injected to LC. In case further dilution is required (either for estimated high contaminated samples or for samples that fall over the calibration curve range after the first LC run, or for samples of validation that expected concentration can be calculated according to the fortification level and the extraction solvent volume added) this is done with HPLC methanol (see Table A1.2).

In case a sample extract is anticipated to have a very low Tinopal SWN concentration, the injection volume can be adjusted to more than 1 µL, as an equivalent of an indirect condensation (concentration) of the sample without using rotary evaporation or other techniques. This modification in the injection volume is considered in the algorithm for the calculation of results.

Table A1.1 Amounts and ratios used for extraction of samples during the experimental field phase

Sample	Extraction volume* mL	Extraction pot used
Upper legs (n = 2), lower legs (n = 2), upper arms (n = 2), lower arms (n = 2)	600	Pot of 1000 mL
Torso front, torso back	1000	Pot of 3600 mL (barrel shaped)
Gloves (left and right separately), headband, wipes (wet hankies, n=3 all combined in one pot)	300	Pot of 500 mL
Hand wash extract (n=3, extracts are combined in one sample)	500	Pot of 1000 mL (triplicate)
Patches (n=10)	150	Pot of 250 mL

** volumes can be adjusted according to contamination observed in UV for dilution purposes*

Table A1.2: Amounts and ratios used for extraction of samples during the validation phase

Matrix	Dosi-meter	Amount Tinopal SWN (in µg)	Extrac-tion volume (in mL)	Extrac-tion pot used (in mL)	Expected concen-tration in extract for 100% recovery (in ppm)	Dilution(s) factor	Expected concen-tration in diluted extract for 100% recovery (in ppm)
Coverall (Cotton or Tyvek)	Fabric (30 x30 cm²)	100			0.33	0.200	0.067
		1000	300	500	3.33	0.020	0.067
		10000			33.3	0.002	0.067
Glove	1 glove	100			0.33	0.200	0.067
		1000	300	500	3.33	0.020	0.067
		10000			33.3	0.002	0.067
Wipe (hankie)	1 wipe	100			0.33	0.200	0.067
		1000	300	500	3.33	0.020	0.067
		10000			33.3	0.002	0.067

Cleaning of equipment, surfaces and extraction pots (SOP)

Cleaning procedure is necessary to be carried out after each experiment (and before the first experiment of a daily or weekly sequence) or after any observed contamination incident to ensure that all the locations, the equipment and the surfaces involved in the trials are free from contamination with Tinopal SWN (or any other in a for the cleaning of all the objects and surfaces that are used during the experimental phases of the project. The cleaning procedure is carried out only by members of the scientific team (and never by the volunteer) and regards both the field part (test location/containers) and the analytical part (laboratory area and equipment).

For the cleaning procedure, the means and actions indicated in the following table apply:

Object/area contaminate		**Cleaning action and solution(s) used**	**Check cleaning status**	**Remarks**
Test room area and surfaces	Walls	Contamination not anticipated due to protection measures (see remarks). In case cleaning is required wipe thoroughly with filter paper wetted with ethanol/water 1:1 v/v at least twice. If contamination residues remain use cotton paper sheets with acetone to repeat wiping.	Use portable UV lamp to examine	Walls are protected by disposable nylon film and therefore are not expected to be contaminated. In case however such incident occurs or is observed during checks due to possible uncovered spot or tearing of film (check with UV lamp prior and after experiment for this purpose) remove and discard nylon film first, then apply cleaning action as described in respective column.

Object/area contaminate		Cleaning action and solution(s) used	Check cleaning status	Remarks
	Floor	Contamination not anticipated due to protection measures (see remarks). In case cleaning is required then wipe floor thoroughly with filter paper wetted with ethanol/water 1:1 v/v at least twice. If contamination residues remain use cotton paper sheets with acetone to repeat wiping.		Floor is protected by disposable carton paper and nylon film underneath therefore is not expected to be contaminated. Carton paper is discarded if contaminated from dripping (in case of minor contamination which is estimated to cause no danger for cross contamination for the next trial the scientific team decides accordingly if the carton paper should remain in place or not). If after the removal of paper and/or nylon floor contamination is observed during checks due to possible uncovered spot or tearing of film (check with UV lamp prior and after experiment for this purpose) apply cleaning action as described in respective column.
Test room equipment	Work-benches, small tables	Wipe first with filter paper wetted with acetone and then ethanol/water 1/1 v/v. Repeat wiping with baby wipes (hankies)	Use portable UV lamp to examine	

Object/area contaminate		Cleaning action and solution(s) used	Check cleaning status	Remarks
	Bins, jugs, receiving vessels, glass bottles for putting Tinopal SWN containing liquids (high- or low viscosity)	Wash empty containers with water in sink, then rinse with acetone. Finally clean with a mixture of ethanol/water 1:1 (triple rinsing). The outside of the vessels is wiped first with filter paper wetted with ethanol then wiping is repeated with baby wipes (hankies). The receiving vessel for the powder dumping trial is cleaned only with regard to its outside surface.	No Tinopal SWN residues are anticipated after this cleaning process.	Most important is to ensure that the outside of the vessels is clean to avoid cross contamination from trial to trial as the volunteer touches the outside surface only.
	Roller	Roller is discarded after use and a new one is used in each trial	No Tinopal SWN residues are anticipated after this cleaning process	
	Tray (for wooden panels roller)	Wash with ethanol/water 1/1 v/v. Allow to dry outside, then wipe once with filter paper wetted with ethanol/water 1/1 v/v to remove bulk contamination	See remark	Since the panel absorbs liquid it is not anticipated to have an absolute clean panel, however as long as it does not drip and it dry it can be reused
	Stands for panels	Wipe first with filter paper wetted with ethanol/water 1/1 v/v. Repeat wiping with baby wipes (hankies)		

Object/area contaminate		Cleaning action and solution(s) used	Check cleaning status	Remarks
	Spray gun (and air compressor / hose)	Wipe first with filter paper wetted with acetone and then ethanol/water 1/1 v/v. Repeat wiping with baby wipes (hankies)		
	Metal cylinders	Wash with water in sink, then rinse cylinders with acetone. Repeat cleaning with a mixture of ethanol/water 1:1 and wipe surface with baby wipes (hankies).	Use portable UV lamp to examine	Special attentions should be given to the hooks. If cylinders surfaces are dry but not absolutely clean this does not create a problem.
	Metal rack for cylinders	Wipe first with filter paper wetted with acetone and then ethanol/water 1/1 v/v. Repeat wiping with baby wipes	Use portable UV lamp to examine	
	Metal racks for Plexiglas plates	Wipe first with filter paper wetted with acetone and then ethanol/water 1/1 v/v. Repeat wiping with baby wipes (hankies)	Use portable UV lamp to examine	
	Plexiglas plates	Wipe with dry cotton paper sheets to remove dust. Then rinse plates with methanol. Finally, wipe with cotton sheet papers wetted with ethanol/water 1/1 v/v.	See remark	Indicative test with UV lamp showed efficiency of cleaning, no need to check each time

Object/area contaminate		Cleaning action and solution(s) used	Check cleaning status	Remarks
	Plexiglas box	Wipe with dry cotton paper sheets to remove dust. Then wipe with cotton sheet papers wetted with ethanol/water 1/1 v/v.	See remark	Indicative test with UV lamp showed efficiency of cleaning, no need to check each time
Laboratory areas and surfaces	Hood surface and benches	Wipe first with filter paper wetted with acetone and then ethanol/water 1/1 v/v. Repeat wiping with baby wipes (hankies)		Indicative test with UV lamp showed efficiency of cleaning, no need to check each time
Laboratory equipment	Extraction pots	Wash empty containers with water in sink, then rinse with acetone. Finally wash with methanol (triple rinsing).	See remark	Test of methanol washing after this process (LC run) showed no contamination residues.
	Laboratory glassware (pipettes, beakers, glass cylinders)	Rinse with tap water, acetone and distilled water.		Applies to glassware that are only used for Tinopal SWN trials (i.e. involving HV and LV) liquids

Loading of the plates (SOP)

The field scientist and the technical assistant ensure that the area is devoid of previous contamination. For the loading of plates, sieved (38 µm) Tinopal SWN is used. A sprinkler (175 µm hole diameter) with a tightly attached net (60 µm hole diameter) is loaded with approximately 3 g of sieved Tinopal SWN (see photos below). The technical assistant positions the plexiglass plate onto the balance and eliminates the weight indication (tare). Then, he picks up the sprinkler, holds it tightly and checks the whole setup before loading. Consequently, he lifts the sprinkler above the plate and balance (approximately 20-30 cm), gently knocks the sprinkler (with circular movements) to distribute Tinopal SWN onto the plate uniformly. The time needed to distribute Tinopal SWN per plate is about 1 min. The end of loading is verified when the weight of Tinopal SWN on the plate is 1 g (the weight is accurately recorded after the Tinopal SWN dust is left to settle for 1 min, see loaded plate below).

Sprinkler	Sprinkler with net
	
1 g loaded plexiglass plate	
	

Figure 1 Set up of sprinkler, net, and plate

Photography part inside the UV room (SOP)

A. <u>The volunteer enters the UV room in the frame of the following "occasions":</u>

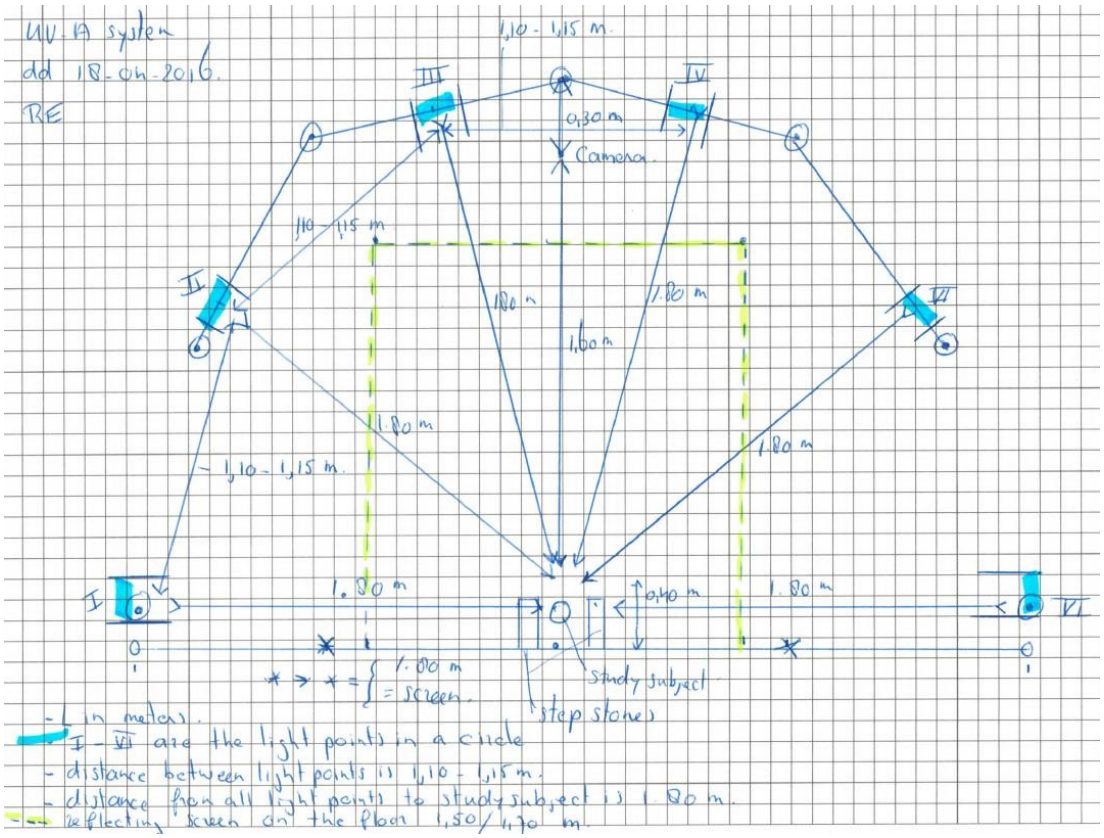
a. As soon as he has carried out the "dressing" part i.e. wearing the coverall and the relevant personal protection equipment (PPE), before the start of the exposure task, in order for the field scientists to take the "before" photographs.

b. After the end of the exposure task and before the sampling of the dosimeters in order for the scientific to take the "after" photographs.

c. After the undressing of the dosimeters, but before taking off the inner clothing (underwear) in order for the scientific team to have an optical check for possible contamination that has penetrated the volunteer's PPE (optical observation under UV without taking photos).

B. <u>UV room set up (distances, heights, angles, background fabric/s, other technical details, photographs)</u>

The UV room contains 6 pairs of UV lamps (12 lamps TLD36/T8 PHILIPS Black light 36W 120cm G-13). The relative positioning of the lamps and the overall UV room setup is in agreement with the diagram below.

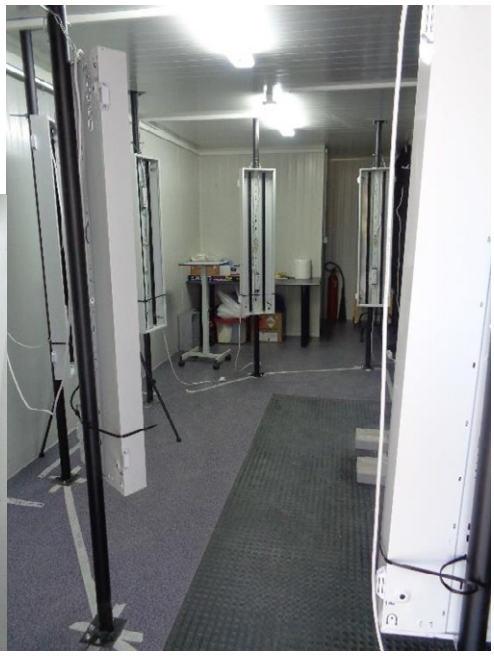


Black or dark grey fabric is used to cover the wall of the container opposite to the camera serving as photographic background.

The distance between the camera and the position of the volunteer is 180 cm

The distance between the UV lights to the wall with the background fabric (where the volunteer is standing) is: From black cloth (wall):

- I & VI = 139 cm
- II & V=53cm
- III & IV = 240 cm

C. Camera settings

- ISO 3200 (twice as much light)
- F: 8 (twice as much light, still sufficient depth of field)
- Shutter time: 1/80, 1/20, 1/5

D. Before entering the UV room

Before entering the UV room the volunteer removes the boots covers (this is done by a member of the scientific team) to ensure that no contamination from the outdoor area is transferred inside the UV room. The mask and the protective goggles are also removed from the volunteer's face by a member of the scientific team.

E. After entering the UV room the following sequence of actions is followed:

Sequence of actions	**Occasion**	**Action**	**UV lamps ***	**Remarks**
1	a	The volunteer is instructed to step on the stepping stones	UV lamps OFF	Volunteer facing the camera
2	a	In case of Tyvek coverall the adhesive stripe in the zipper and the one at the neck (that illuminate under UV light) are removed or cut off respectively	UV lamps OFF	This is done by a member of the scientific team

Sequence of actions	Occasion	Action	UV lamps *	Remarks
3	a	A member of the scientific team puts a pair of UV glasses on the volunteer.	UV lamps OFF	Refer to dressing/undressing SOP
4	a	The spots on the coverall that illuminate under UV are covered by dark colored adhesive tape (grey)	UV lamps ON	This is done by the assigned member of the scientific team
5	a	Volunteer stands with arms at the side of the body, with some room between the sides and the arms. Palms of hands facing the camera for the whole body pictures and reverse for the patches pictures.	UV lamps OFF	Arms angle to body around 150-210 degrees
6	a	Preview of picture frame, zoom and image focus in laptop screen	Check with UV lamps OFF first then Check again with UV lamps ON	Preview done by the assigned member of the scientific team. During this step it is also checked / concluded whether any contamination exists in the coverall or gloves (accidental case, if affirmative then replacement of coverall / gloves takes place)

Sequence of actions	Occasion	Action	UV lamps *	Remarks
7	a	Three photographs “front side” are taken (computer – camera connection	UV lamps ON	Note: Autobracketing applies
8	a	Volunteer turns around with his back on the camera lens for the “back pictures”. Arms at the side of the body, with some room between the sides and the arms. Palms of hands facing the container wall (i.e. the background cover)	UV lamps ON	Arms angle to body around 150-210 degrees
9	a	Three photographs “back side” are taken (computer – camera connection	UV lamps ON	Note: Autobracketing applies
10	a	Optical evaluation of the set of photographs taken	UV lamps OFF	This is done by the assigned member of the scientific team, repeat of actions 5-9 if required
11	a	Volunteer is instructed to leave the room	UV lamps OFF	UV goggles are removed by the assigned member of the scientific team (protective googles and mask are provided for occasion II as described in the respective SOP for dressing)

Sequence of actions	Occasion	Action	UV lamps *	Remarks
12	b	Apply (=repeat) the steps corresponding to the sequence of actions 1, 3, and 5-11 as described above in the present table	UV lamps functioning accordingly as described in the respective sequence of actions	As described in the respective sequence of actions
13	c	Apply (=repeat) the steps corresponding to the sequence of actions 1, 3 and 5-11 as described above in the present table	UV lamps functioning accordingly as described in the respective sequence of actions	As described in the respective sequence of actions

* if “off” then the room lights are on

F. <u>After the end of the photography part</u>

The photographs are copied/transferred electronically to a portable USB storage device (stick or portable hdd) and given to the assigned scientific team member for further process using the software (measurement and file storage or upload).

Extraction process from hands (washing) (SOP)

The field scientist helps the volunteer to perform the hand wash extraction procedure. During this procedure, the extraction of Tinopal SWN from the hands of the volunteer is performed in triplicate with 500 mL of ethanol/water 1:1 v/v solution (three aliquots of 500 mL corresponding to one sample).

The hand wash solution is prepared in the laboratory the same or the previous day of the trial by a member of the scientific team and is carried in the field location (area of sample collection) in the screw cap polyethylene pots (three pots of 1000 mL size capacity) which are labelled accordingly. Following the sequence of the samples collection described in the respective standard operation procedure and when the collection of the hand wash extract is due, the volunteer puts his hands inside a plastic bag (dimension approximately 30x20 cm) and a field scientist pours carefully the content of the first pot (hand wash solution 500 mL) into the bag.

Care is needed to avoid any spillage outside the bag. Another scientist holds the plastic bag from its bottom so that the volunteer can wash his hands for 30 seconds inside the wash solution. A clock with indication of seconds is placed on the wall of the sampling room for this purpose. This second field scientist also lifts the bag a little, to make sure that the volunteer can conveniently have his hands in the wash solution and ensure that it covers most of the hands area in the bags. The volunteer is instructed to wash his hands by rubbing his hands and fingers to each other thoroughly as well as to rub his fingertips and nails for 5-10 seconds during each wash.

The above process is repeated three times and the extracts are stored in separate pots.

Waste management (SOP)

Disposal
A. Organic solvents - mixtures
B. Contaminated solid waste
C. Laboratory glass waste

A. Disposal of organic solvents and their mixtures
IMPORTANT NOTE:
✓ The segregation of halogenated and non-halogenated solvents is imperative. Solvent mixtures are considered contaminated even at concentrations of halogenated solvents as low as 1000 ppm.

Procedure
1. Do not mix halogenated and non-halogenated solvents together but only segregate them. Collect only non-halogenated solvents in adequate non-halogenated waste solvent containers.
2. Do not mix oil with waste solvents.
3. Do not pour acid, sludge, grit, glass, plastic, paper, or inorganic chemicals into the waste solvents.
4. Complete the required information by listing the full names of solvent(s) used and affix the information to the Flammable Liquid Disposal Tag.
5. Affix the tags to the appropriate containers.
6. Tighten all caps before shipping.
7. Come in contact with BPI's waste management committee to inform about the waste.
8. Place the containers in the designated area of BPI's premises for pickup by the waste management company.

SYSDEA case
Solvents and liquid chemicals used: acetone, methanol, ethanol, glycerol, (none is halogenated), therefore only containers for non-halogenated solvents will be used. As soon as these are filled, the procedure described above will be adapted/followed.

Glycerol is regarded as a safe material for which no special handling precautions are required. However, it is flammable. Small amounts of glycerol may be flushed down the sink unless local rules prohibit this. Glycerol is viscous, if the liquid is not all washed away, a small quantity may remain in the sink that can pose a fire risk. Therefore, enough water should run off to ensure removal of glycerol.

Disposal of LV and HV liquid
(mixture of acetone, glycerol, water and Triton-X 100, materials used together)
LV and HV liquid should be disposed of. Such disposal should be conducted in a separate plastic container (20-25 L, capacity). In order to avoid contamination of the trials area, this transfer should be performed in the outside area of the container using a double layer of carton paper (in case of accidental spillage the quantity will be absorbed by the paper). This container should be labelled with all

the names of the chemicals (acetone, glycerol, water and Triton-X 100) and sent (as above) to the designated area of the BPI's premises for pick- up.

B. Disposal of non-regulated contaminated solid waste

Purpose

This procedure ensures that non-regulated solid wastes are diverted from landfill and disposed of properly.

Scope

This disposal procedure applies to non-regulated solid wastes such as contaminated silica gel (Tinopal SWN and Talc can apply to this category).

Background

Certain wastes, although not regulated as hazardous, are not permitted at the landfill. These wastes, classified as non-regulated (NR) waste, must be diverted from the solid waste stream and disposed of properly in a secured landfill.

Procedure

1. Collect solid waste (a mixture of Tinopal SWN, Talc or substances alone) in a thick plastic bag. Ensure there are no leaks. Double-bag waste in a thick clear garbage bag.
2. Each bag must not weigh more than 10 kg. Ensure that there is no liquid in the bag.
3. Package the bag in a heavy cardboard box. Tape box to seal.
4. Affix a Non-Regulated Contaminated Solid Waste tag (Yellow).
5. Indicate waste type on the tag.
6. Come in contact with BPI's waste management committee to inform about the waste.
7. Store the box in the designated chemical waste area of the BPI's premises.
8. Contact the Environmental Services Facility/waste management company.

C. Disposal of laboratory glass waste

Purpose

This procedure specifies the method for proper disposal of glass waste to ensure the safety of disposal workers and to avoid contamination of the environment (by remnants in the glass).

Scope

This process applies to disposal of glass that is uncontaminated or contaminated by hazardous chemicals. Glass waste includes the following:

1. Glass bottles
2. Pipettes
3. Other glassware

Background

The disposal of contaminated glassware waste to landfills is prohibited. At present, laboratory glass cannot be recycled. Lab glass is decontaminated

according to this procedure, accumulated as a sub-solid waste stream in the lab and collected by the waste management company.

Procedure

Only approved containers may be used for these wastes. These containers must not be used for any other purpose.

Approved glass waste containers

Yellow containers currently utilized in the lab. All containers must be clearly labelled as "Glass Waste Only". If uncontaminated, treat as regular glass waste.

- If the glass container was previously contaminated with the following hazardous materials:

1. Hazardous chemicals: safely empty container, decontaminate or neutralize as necessary, triple rinse, dry and dispose of as regular glass waste,
2. Risk Group 1 or Risk Group 2 biohazardous materials: decontaminate empty containers with bleach, by autoclaving, or by using other approved methods. Then treat as regular glass waste.

In general, for the disposal of glass waste follow the steps below:

1. Decontaminate safely as required.
2. Clean thoroughly of residues, including organic vapors and chemicals
   - Leave bottles of organic solvents in a fume hood for at least one day
   - Rinse other reagent bottles well with cold water
3. Remove all bottle caps.
4. Remove or deface all labels and hazard warnings.
5. Place in Glass Waste containers.
6. Once glass waste container is 3/4 full, tie bag closed, ensuring that no glass objects protrude past the top of the container.
7. Attach a label to the bag indicating the laboratory.
8. Take the container to the building's designated area for waste pick-up.

NOTE: NO sharps (e.g. needles, blades, syringes) and glass vials/jars containing chemicals or other hazardous materials can be disposed of in the glass waste container.

Sample collection (including dressing and undressing) (SOP)

Relevant monitoring clothing (dosimeters)

For the Whole-body dosimeter method
- Tyvek coverall with hood (1-piece), for exposure situations with liquids. The hood is not considered as a dosimeter for these experiments but as personal protection equipment (PPE) only.
- Cotton coverall with hood (1-piece), for exposure situations with powders. The hood is not considered as a dosimeter for these experiments but as personal protection equipment (PPE) only.

For the Patch method
- Tyvek patches will be attached on a Tyvek coverall with hood (1-piece), for exposure situations with liquids. The patches are the only clothing dosimeters in this case. They are square pieces of Tyvek fabric 12x12 cm that are placed inside a foil or paper of 14x14 cm whose edges are folded at 1cm round the perimeter of the patch to allow for net patch area of 10x10cm. In the same way cotton patches are made and used for exposure situations with powder. Cotton patches are attached to Tyvek coveralls.

Other clothing (underwear) and PPE

- Black colored cotton T-shirt and long johns (pants).
- Latex gloves worn underneath the cotton gloves (exception: When bare hands are measured via “hand wash method” no latex gloves are worn).
- Mask for respiratory protection.
- Protective goggles
- Protective UV goggles
- Boots
- Covers for boots

Relevant combinations according to measurement methods

1. Tyvek coverall / cotton coverall (body) + cotton gloves (hands) + cotton headband (head)
2. Tyvek / cotton patches placed on Tyvek coverall (body) + hand wash (hands) + forehead wipe (head)

Dressing the volunteer

Before the start of the trial (experiment), the scientific team provides all the dosimeters to the volunteer. Dressing is done in a place free from possible contamination. Black cotton pants and a black T-shirt (either long or short sleeved) are worn as underwear (see paragraph B). The volunteer puts on the underwear, the coverall and the gloves (latex underneath the cotton ones) on his own after instruction of the scientific team, and after has been confirmed that the volunteer skin of the volunteer is not contaminated with tracer (to be visually checked in the UV room), in a clean, covered designated area, which is located near the test rooms (“sampling room”).

The volunteers are provided with the dosimeters that fit them best (S, M, L, and XL). If necessary, the length of the arms or legs of the coverall is adjusted to fit the arms and legs respectively by cutting off a piece of fabric as much as needed, to prevent for instance that the sleeves cover the hands. This is anticipated to mainly occur for the cotton coverall.

The elastics at the legs of the Tyvek coverall are cut off by a field scientist and discarded in order to allow for easy removal of the coverall at the undressing step.

The start and the end time of the procedure is documented on the relevant field form.

With regard to the 'Whole body dosimeter (WBD) method' (combination 1), the following procedure is used:

- For each experiment a full set of monitoring clothing and PPE must be available in place, in the right sizes.
- Adhesive strips on the Tyvek coverall are removed (or cut according to their place and type) by a member of the scientific team.
- Tape is put on Tyvek coverall on the parts that light up.
- The volunteer puts on Tyvek/cotton coverall over the undergarment, and closes it. Since boots are worn, the leg pieces of the coverall should be worn over the boots.
- The coverall zipper must be well zipped up to the neck.
- The headband is fixed by a member of the scientific team around the head of the volunteer with a safety pin. When the volunteer uses respiratory protective equipment, the headband should be worn over the respirator in such a way that the respirator remains fully functional.
- The volunteer puts on protective latex gloves and monitoring cotton gloves. Care must be taken that the coverall is placed over the sleeves (ends) of the cotton monitoring gloves.
- Protective goggles are worn inside the hood. A member of the scientific team puts the goggles on the volunteer.
- The mask is worn over the hood to allow for easy removal. A member of the scientific team puts the mask on the volunteer.

With regard to the 'patch method' (combination 2), the following procedure is used:

- For each experiment a full set of Tyvek coverall, patches and PPE must be available in place, in the right sizes.
- Adhesive strips on the Tyvek coverall are removed (or cut according to their place and type) by a member of the scientific team.
- Tape is put on Tyvek coverall on the parts that light up. The volunteer puts on Tyvek coverall over the undergarment, and closes it. Since boots are worn, the leg pieces of the coverall should be worn over the boots.
- The coverall zipper must be well zipped up to the neck.
- Protective goggles are worn inside the hood. A member of the scientific team puts the goggles on the volunteer.
- The mask is worn over the hood to allow for easy removal. A member of the scientific team puts the mask on the volunteer.
- The patches are placed on the Tyvek coverall by the scientific team after putting on the coverall using duplex tape in 10 locations as follows (also shown in Figure A4.1):

- In the middle of the forearms (between wrist and elbow) (n=2)
- In the middle of the upper arms (between elbow and shoulder) (n=2)
- In the middle of the upper legs (mid-thigh) (n=2)
- In the middle of the lower legs (between ankle and knee) (n=2)
- In the middle of the torso, upper half (n=1)
- In the middle of the back, upper half (n=1)

Especially with regard to the placement of the patches on the arms, but also for the legs, it is important that these are placed at the front of the arms (not at the sides), directed in the same direction of the torso, to make sure that these are completely visible on the pictures with the UV set-up. Instructing the volunteers to turn their thumbs towards the body during photography is mandatory here.

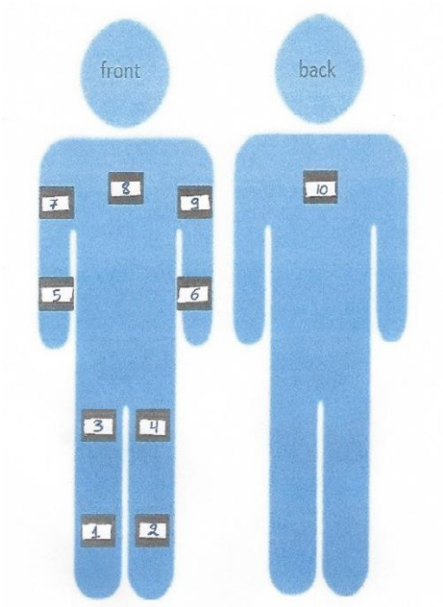


Figure A4.1 Placing of patches

With regard to the 'before' fluorescence measurement (pictures with UV set-up):
After dressing, the scientific team visually checks the volunteer for possible contamination of the monitoring clothing in the UV room (if contamination is observed, that dosimeter is replaced by a clean one) and photographs are taken (front and back sides of the volunteer, with hands in one position). For taking the photographs the following protocol is used:

- The field scientist checks the settings of the camera, checks the position of the camera and checks the position of the UV armatures to see if they have not been changed. The reference points should be within the frame of the picture.
- Before entering the UV room, a field scientist removes the mask and the protective googles
- When the volunteer enters the room, a field scientist puts on the protective UV goggles.
- The field scientists themselves also put on protective UV goggles
- The volunteer is instructed to stand on the stepping stones, with his face towards the camera, and his hands besides his body (not touching his body) with his thumbs turned inwards

- The volunteer is instructed to stand completely still for the duration of the photographs being taken
- One field scientist is standing beside the laptop and one field scientist is standing next to the light switches. If the field scientist indicated that he is ready, the UV light is switched on and the normal light is switched off. The volunteer is again instructed to stand completely still for the duration of the photographs being taken, and the first set of photographs (of the front) are taken.
- The volunteer is asked to turn, with his back facing the camera, and stand on the stepping stones again, without changing the position of his hands (thumb still directed inwards).
- The volunteer is again instructed to stand completely still for the duration of the photographs being taken, and the second set of photographs (of the back) are taken.
- Afterwards, the normal light is switched on again and the UV light is switched off.
- The field scientist operating the laptop briefly visually checks the three photographs. If these are okay, the volunteer and field scientist leave the UV room.

Protective UV-goggles are worn during taking the pictures before and after the activity. The latter are preferably worn over the hood and underneath the headband, to make sure that it can be easily changed. These are placed on the volunteer by a field scientist in the UV room before the photographs are taken and the UV lamps are still off, and also removed by field scientist after the photographs are taken and the UV lamps are off again. After the first set of photographs are taken (before the activity), a field scientist replaces the protective UV- goggles with the protective goggles as worn during the activity.

Undressing the volunteer

After the end of the activity and the procedure of photographing conducted under UV light, the scientific team removes the coverall from the volunteer in a clean, covered designated area, which is located near the test rooms (“sampling room”). Samples collected are put directly into the extraction pots, and therefore all extractions pots for all samples (coverall/patches, headband and gloves, hand wash and wipes) are available on a bench inside the “sampling room” and labelled accordingly. The mask and the goggles of the volunteer are removed by a field scientist before entering the UV room, while the protective UV goggles that the volunteer wears inside the UV room are removed before exiting the UV room by the respective field scientist.
The start and the end time of the procedure is documented on the relevant field form.

The following procedure (sequence of actions) is followed:

With regard to the ‘after’ fluorescence measurement (pictures with UV set-up):
See above (same procedure as ‘before’ fluorescence measurement).

With regard to the ‘WBD method’ (combination 1):

- In general: The field scientist puts on a pair of clean gloves (disposable latex gloves) between each step, and cleans the scissor blades and the tweezer blades with wipes wetted with ethanol between each step.
- The field scientist removes the headband from the volunteer's head and puts it in the respective labelled pot and cuts the hood off (hood is rejected as it is not a monitored dosimeter).
- The field scientist removes the gloves from the volunteer by pulling them inside out (by pulling the wrist part of the gloves over the fingers) and put them in the designated extraction pots (separate pot for each glove).
- The field scientist cuts off and discards the elastics from the Tyvek coverall at the forearms.
- The field scientist removes the volunteer's boots. A steady object near the volunteer (i.e. chair or table) is available for the latter to hold in order to remain stable
- The field scientist cuts off the lower legs parts, after first making an incision (cutting mark) at knee level of the volunteer.
- The filed scientist makes an incision (cutting mark) at elbow level of the volunteer.
- The field scientist unzips the coverall and removes it from the volunteer while placing it on a hanger. During this step a second field scientist is helping him (i.e. one field scientist holds the hanger while the other one pulls the lower part of the coverall off the volunteer's hips). A steady object near the volunteer (i.e. chair or table) is available for the latter to hold in order to remain stable. During the removal of the coverall, the field scientist touches the coverall as little as possible (on as little different places as possible), making sure that no parts of the coverall touch other parts of the coverall, as well as that the coverall does not touch the ground.
- The field scientist hangs the coverall on a wardrobe hanger.
- The field scientist cuts the pieces of the coverall on the wardrobe while another field scientist gives him the respective pots to put the samples in, based on the sequence as indicated in Table A1.3 and Figure A4.2.

Table A1.3 Order of dosimeters removal and cutting lines for 'WBD method' (combination 1)

Sequence (order)	**Samples**	**Cutting line**
a	Headband	*Not applicable* *(just remove pin and place sample in the pot)*
b	Right Glove	*Not applicable*
c	Left Glove	*Not applicable*
d	Lower leg right	cut straight on knee height (on the volunteer)
e	Lower leg left	cut straight on knee height (on the volunteer)
f	Forearm right	cut straight on elbow height (at incision)
g	Forearm left	cut straight on elbow height (at incision)
h	Upper arm right	cut straight across the shoulder stitch
i	Upper arm left	cut straight across the shoulder stitch
j	Upper leg right	cut diagonally down from hip to groin

Sequence (order)	Samples	Cutting line
k	Upper leg left	cut diagonally down from hip to groin
l	Front torso	cut at the side/stitch, dividing it into Torso front and Torso back
m	Back torso	

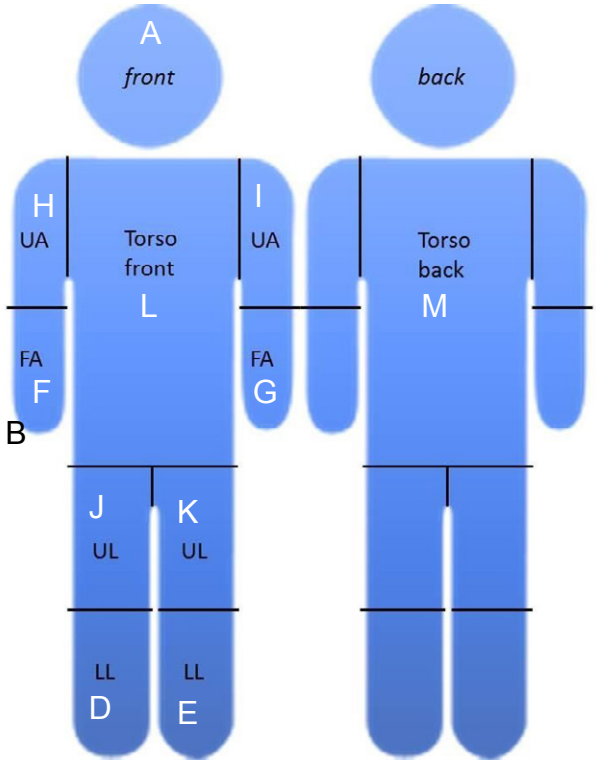


Figure A4.2 Sectioning of coveralls

With regard to the 'patch method' (combination 2):

- In general: The field scientist puts on a pair of clean gloves (disposable latex gloves) between each step, and cleans the tweezer blades with wipes wetted with ethanol between each step of removing the patches.
- The field scientist wipes the forehead of the volunteer with an alcohol pre-wetted wipe and puts the wipe into the designated pot. This is done three times (three wipes) and all three wipes are put in the same pot. For the wiping procedure, the wipe is folded in two for the first wiping and the field scientist starts from the right side of the forehead to the left, applying normal hand pressure with a continuous movement. After this, the wipe is folded again in two leaving the clean part outside and the same wiping procedure is repeated. Again, the wipe is folded in two leaving the clean part outside and the wiping is repeated the same way for the last time.
- Each patch is removed by a field scientist (in a specific order that is shown in Table A1.4 below) from the foil or paper "frame" attached on the coverall with the use of two tweezers. The patch is put directly into the pot using the tweezers. Care is needed to match the patch with the respective pot that corresponds to the designated body part.

- NB1: The order of removing the patches is chosen to make sure that as little cross- contamination between the body parts as possible (from down to up)
- NB2: The field scientists instruct the volunteer to hold his hands at the side of his body but slightly away from the body, making sure that his hand do not touch anything with his hands when the patches are removed, and keep an eye on this during the removal of the patches as well
- The field scientist helps the volunteer to perform the “hand wash procedure” holding a plastic bag where the volunteer puts his hands to wash for 30 seconds (a clock with seconds’ indication is placed on the wall of the sample room) with ethanol/water 1:1 v/v poured into the bag by another field scientist. This second field scientist lifts the bag a little, to make sure that the volunteer can more easily put his hands in the wash solution and the wash solution covers most of the hands in the bags. The volunteer is instructed to wash his hands by rubbing his hands and fingers to each other thoroughly. Furthermore, the volunteer is specifically instructed to rub his fingertips and nails for 5- 10 seconds within the 30 seconds time frame during each wash. This process is repeated three times and the extracts are stored in separate pots.

Table A1.4 Sequence of collection of samples for the ‘patch method’ (combination 2)

Sequence (order)	Samples collected
1	Head wipe
2	Patch - Lower leg right
3	Patch - Lower leg left
4	Patch - Upper leg right
5	Patch - Upper leg left
6	Patch - Forearm right
7	Patch - Forearm left
8	Patch - Upper arm right
9	Patch - Upper arm left
10	Patch - Front torso
11	Patch - Back torso
12	Hand wash

For ‘check’ for possible contamination of the volunteer with UV set-up:
After the undressing of the volunteer with the collection of samples, the skin and underwear of the volunteer is checked with the UV set-up for possible contamination. If contamination is observed, this is registered on the field form.

See above (procedure for ‘before’ fluorescence measurement) for a description of the use of the protective UV goggles.

After this, the volunteer puts on his own clothes.

The collected samples are transported to the lab for extraction and analysis.

Preparation of low and high viscosity liquids (SOP)

1. Tinopal SWN Concentration 2g/L
2. For 2 L preparation see points 2 and 3 below:
3. Low Viscosity (LV): 4 g Tinopal SWN, 200 mL acetone, 40 mL Triton, 400 mL Glycerol, 1360 mL H_2O.
4. High Viscosity (HV): 4 g Tinopal SWN, 200 mL acetone, 40 mL Triton, 800 mL Glycerol, 960 mL H_2O.

Procedure

Tinopal SWN is sieved at 38 μm for 20 min and then used without further treatment for the liquids preparation procedure*. In this context, the calculated appropriate amount is weighed in a small beaker (e.g. 20 mL). This amount is transferred to a 5 L beaker by decanting and with the aid of a spatula. Then, 200 mL of Acetone (pro analysis) are placed into a volumetric cylinder. Consequently, residues of non-transferred Tinopal SWN are dissolved in low volume of acetone (from 200 mL) inside the small beaker, and then all Tinopal SWN is dissolved in acetone under continuous stirring (in the large beaker). After dissolving, Triton, Glycerol and water are added sequentially (under continuous stirring) to form the final liquid product.

* Tinopal SWN can also be used in its non-sieved form since we did not evidence differential behavior in the prepared liquid

Annex 2 Medical ethical approval

SysDEA
MEDICAL ETHICAL APPROVAL FORM FOR THE USE OF CHEMICAL SUBSTANCES

This page is to be read by the physician of the institute and signed prior the start of the pilot and experimental phase of the project. This document exists also in version translated in the native language of the physician, i.e. Greek.

BPI and TNO are conducting a study for Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (BAuA) using Tinopal SWN in solid form (powder in mixture with talc or in liquid dispersion). The purpose of the study is to evaluate the amount of chemical that the study subject is exposed to during the conduction of the following tasks/exposure scenarios:

- Transfer – dumping (dusty solid)
- Transfer – pouring (low and high viscosity liquids)
- Spreading – rolling (low and high viscosity liquids)
- Spraying – surface spraying (low and high viscosity liquids)
- Immersion / dipping – manually handling of immersed objects (low and high viscosity liquids)
- Handling of contaminated objects (dusty solids)

The product that will be used as a surrogate for exposure estimation during different tasks (exposure situations) is a fluorescent tracer substance commercially used as optical brightener for detergents & cleaners and is commercially available in Greece. As the physician of the Institute, I affirm that the volunteers will be exposed to no greater risk than someone would encounter during a normal application of a respective/equivalent product for these professionally carried out tasks.

I have been informed about the chemical substance that will be used for the aforementioned tasks and aims, the dermal sampling methods that will be applied and what is expected from me in that respect.

By agreeing to use the specific substance in the study, the following will be carried out:

- The volunteers will be provided with a coverall, gloves, a headband and dosimeter fabric patches to use for the trial purposes. Along with these, necessary protective equipment (e.g. mask) will be provided. These will be worn by the volunteers for the period of time that they will be being monitored and these will be taken with the appropriate method by the field team for analysis after the experiment.
- Volunteers will be asked to wash their hands in a specified amount of rinsing solution to measure the exposure to hands.
- They will have their forehead wiped with a cotton wipe ("wet hankies" containing ethanol) to measure exposure to the face.
- They will have to follow the field scientific personnel instructions regarding safety and personal hygiene before, during and after each experiment they are participating in.

In addition to this consent form, I have received a copy and got explanations on the Material Safety Data Sheet (MSDS) of product used (Tinopal), and its label, and the MSDS of rest of chemicals considered in this project. I was also given the opportunity to ask questions and I am satisfied with the answers received. I understand that this study will result in no increased health risk.

Name of Physician: NIKOLAOS PSARRAS MD

Signature:

Date and Place: Kifissia, 8-2-2016

Name of BPI Study Scientist in charge: Dr. Koustantinos Kasiotis

Signature:

Date and Place: Kifissia, 8-2-2016