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Migration of Polycyclic Aromatic Hydrocarbons (PAHs) from plastic and rubber articles

Final report *on the development of a migration measurement method*

Study conducted on behalf of DG GROW

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Abstract

Polycyclic Aromatic Hydrocarbons (PAHs) constitute a large group of chemically related substances many of which are known carcinogens. To minimise human exposure there are already several pieces of EU legislation which limit their presence in certain food products, as well as in water and ambient air. Under the REACH regulation (EC 1907/2006 Annex XVII, Entry 50), eight priority PAHs have for some time been restricted in extender oils used in tyres. Although not added deliberately to consumer products, PAHs can still be present as impurities. An amendment of the above mentioned legislation (Regulation EU 1272/2013) establishes content limits for the eight PAHs of 0.5 mg kg-1 for plastic and rubber components of toys/childcare articles, and 1 mg kg-1 for all other consumer articles, in direct and prolonged, or short-term repetitive, contact with the skin or oral cavity.

In May 2016 DG JRC and DG GROW signed an Administrative Arrangement (AA 34003) known as the STANPAHs project. The main objective of this contract was for the JRC to provide scientific support in the implementation and potential amendment of the restriction on polycyclic aromatic hydrocarbons, in particular concerning paragraphs 5 and 6 of entry 50 of Annex XVII to the REACH legislation. The main objectives of the project were: a) to gain a better understanding of the migration behaviour of certain PAHs in plastic and rubber components of articles, and b) to develop a reliable methodology to determine PAH migration from these matrices, under conditions simulating, to the best possible extent, dermal contact (including the oral cavity).

This report presents the outcomes of the experimental studies carried out at JRC and the achievements towards fulfilling these objectives.

A set of manufactured polymeric plastic and rubber matrices, to be used as test materials in the project, has been chosen based on criteria such as their frequency of use in articles within the scope of the restriction and the likelihood of the presence of high PAH contents (e.g. due to their content in carbon black or extender oils). Various grades and types of ingredients known to be PAH sources were used in the formulation of the manufactured ad-hoc materials. The test materials included low density polyethylene (LDPE), polystyrene (PS) and polyvinyl chloride (PVC) as plastic matrices, and ethylene-propylene diene monomer (EPDM), natural rubber-butadiene rubber (NR-BR) and silicone as rubber matrices. Moreover, recycled granules (coated and uncoated) originating from end-of-life tyres produced before and after 2010 as well as rubber tiles made of the recycled coated granules were also made available for this study. The content of each of the eight restricted PAHs was measured by using a method developed in-house based on Randall hot extraction, purification by Solid Phase Extraction based on Molecular Imprinted Polymers, and Gas Chromatography Mass Spectrometry determination.

A number of experimental studies were undertaken to generate data and information to improve the knowledge on migration of the target PAHs. Migration parameters operated in the STANPAHs project to estimate migration rates were as follows: dynamic mode at 40°C for 24 hours using a variety of migration media including artificial aqueous simulants, modified biosimulants formulations with lipidic content such as skin surface film liquid (SSFL), and 20% ethanol in water. According to scientific literature the use of 20% ethanol as the migration medium proved to correlate well with human skin absorption. Using these conditions, migration of the target PAHs into artificial sweat (EN1811) and artificial saliva (DIN53160-1) was not detected in any of the materials studied. Moreover none of the plastic polymeric materials led to detectable release of the target PAHs in any of the migration media used in this study (i.e. artificial sweat and saliva, skin surface film liquid (SSFL), and 20% ethanol solution). Similarly the tests with silicone materials did not result in detectable migration. Only the rubber matrices containing Distillate Aromatic Extract (DAE) as extender oil showed detectable migration when using 20% ethanol as the migration solution. In addition, the release of PAHs from

coated recycled rubber granules was lower than from the uncoated granules suggesting that the coating acts as a barrier to chemical migration. According to industrial partners DAE is not used by European industries for manufacturing of parts of articles intended for skin contact. The materials containing DAE, although not representative for marketed products, have been made available to facilitate migration testing method development.

The migration test method using 20% ethanol has been validated in-house and shows good method performance allowing the determination of PAH at trace level. Furthermore it has been considered for an initial inter-laboratory comparison study (ILC) aiming to investigate method applicability and transferability in a variety of laboratories. The within-laboratory precision, expressed as the relative standard deviation for repeatability (RSDr), and the between-laboratory precision, expressed as the relative standard deviation for reproducibility (RSDR) were assessed. In general the RSDR ranged from 28 to 113% and the RSDr from 7 to 23%. It is worth remembering that the level of PAH migration was very close to the quantification limit of the method and therefore this variability can be expected. Similar values have been reported in a recent German study with the participation of 9 laboratories on the migration of PAHs from rubber materials in contact with aqueous ethanol. The fact that better values of RSDr and RSDR were obtained for chrysene and benzo(e)pyrene that had the highest concentrations in the final migration solutions and that the analysis of the control solution used in this exercise showed a good reproducibility (RSDR% <10%), shows the possibility to reduce the variability between laboratories with a revised operating procedure in terms of injection volume and/or elution volume.

In conclusion this report makes available new data and scientific information on the migration behaviour of certain PAHs from selected plastic and rubber polymeric matrices, in support of the European Commission's legal obligation to review the PAHs restriction under REACH. Standard operating procedures for quantification of the content of each of the eight restricted PAHs as well as their migration into 20% ethanol have been developed. Moreover the information gathered in STANPAHs (e.g. literature search), the ad-hoc manufactured materials still available, as well as the JRC in-house analysis method for PAH content could be of great benefit to accelerate the work towards standardisation of PAH content analysis in consumer products that has been recently undertaken by the European Standardisation Committee following a request by DG GROW.

Keywords
EU-PAH, migration, consumer products, plastic, rubber, sweat, saliva.

1. Introduction

The exposure of the general population to hazardous polycyclic aromatic hydrocarbons (PAHs), has since long been recognised to be a matter of concern and there are several pieces of EU legislation which already limit the presence of these substances in certain food products, in water and in ambient air. Exposure of consumers, in particular children, to PAHs via contact with articles has been the subject of considerable public attention over the last years and has generated extensive media coverage and political interest, particularly in Germany. More recently exposure to PAHs in rubber granules used as infill in synthetic sports fields has also been the cause of public concern in a number of EU Member States.

In 2010, the German authorities presented the Annex XV restriction dossier under the scope of REACH [1] containing a risk assessment which supported the need to limit the content of eight PAHs in plastic and rubber parts of articles than can be used by consumers, particularly used by children. These PAHs, have a harmonised classification as carcinogenic IB classification in the Classification Labelling and Packaging regulation (CLP) [2] and namely are: Benzo[a]pyrene (BaP), Benzo[b]fluoranthene (BbFA), Benzo[k]fluoranthene (BkFA) Dibenzo[a,h]anthracene (DBahA), Chrysene (CHR) Benzo[a]anthracene (BaA) Benzo[j]fluoranthene (BjFA) Benzo[e]pyrene(BeP) Benzo[a]pyrene (Carc 1B, Muta 1B Repr 1B) and chrysene (Carc 1B, Muta 2) are also legally classified mutagens.

On the basis of this dossier and following subsequent consultations with stakeholders and an ad-hoc working group on PAHs, the Commission prepared a draft amendment to Annex XVII to the REACH Regulation which led, in December 2013, to the adoption of Regulation (EU) No. 1272/2013, amending entry 50 of Annex XVII and establishing a restriction on the PAH content of plastic and rubber parts of articles, supplied to the general public, that come into direct as well as prolonged or short-term repetitive contact with the human skin or the oral cavity, under normal or reasonably foreseeable conditions of use.

A limit expressed in terms of the PAHs content in the plastic and rubber components of articles was chosen as the most appropriate method for defining the restriction A limit value of 0.5 mg/kg was established for each of the 8 listed PAHs in toys and childcare articles. A limit of 1 mg kg^{-1} was established for all other articles in the scope of the restriction. According to the supporting dossier, the risks are associated to the dermal exposure to PAHs released from articles during normal conditions of handling and currently there is no established conclusion that the restriction should be defined in terms of content, rather than of migration. The knowledge gaps regarding the migration behaviour of PAHs from plastic and rubber and the appropriateness of exploring the possibility of deriving migration based limits for the restriction were however acknowledged in paragraph 8 of entry 50 of Annex XVII to REACH from articles during normal conditions of handling.

In this context JRC was entrusted by DG GROW to gather scientific evidence towards the revision of PAHs restriction described in paragraphs 5 and 6 of entry 50 of Annex XVII to the REACH legislation. DG GROW and JRC signed in May 2016 the Administrative Arrangement 34003, "Development of an analytical method to determine the migration of eight Polycyclic Aromatic Hydrocarbons from plastic and rubber articles (STANPAHs)".

The main objective of the STANPAHs project is to provide support in the implementation and potential amendment of the restriction on polycyclic aromatic hydrocarbons (PAHs). Main objectives of the project are: a) to gather a better understanding of the migration behaviour of certain PAHs in plastic and rubber components of articles and b) to develop a reliable methodology to determine PAH migration from these matrices, under conditions simulating, to the extent possible, dermal contact (including the oral cavity).

The methodology shall be scientifically-based, robust and reproducible, in particular capable of being applied by commercial and service laboratories at an acceptable cost. Furthermore, it shall be developed to a point where it could potentially be taken up as the basis for its harmonisation into a European Standard.

In order to achieve the objectives the following activities were undertaken: 1) scoping study comprising a comprehensive review of recent literature and the experimental design concept 2) selection of rubber and plastic representative materials 3) development and optimization of a migration method 4) in house validation of the migration testing method and 5) an initial collaborative trial. In addition and although not foreseen initially in the project it was considered necessary to develop/optimize a method for the analysis of the content of PAHs in plastic and rubber materials enabling the calculation of the fraction released from the materials.

A project technical committee with participation of EC services, European standardisation bodies, ECHA, industry stakeholders, experts from MS, governmental laboratories and academia, was established. Representatives from the following organisations participated: European Tyre and Rubber Manufacturers Association (ETRMA), International Carbon Black Association (ICBA), Toy Industries of Europe (TIE), the European Rubber Laboratories network (ERRLAB), European Petroleum Refiners Association (CONCAWE), European Plastics Converters (EuPC), Belgian Scientific Institute of Publich Health, Fraunhofer IVV, German Federal Institute for Risk Asssessment (BfR), Netherlands National Institute for Public Health and the Environment (RIVM), Service Commun des Laboratories (SCL Ile de France), Laboratoire de Recherches et de Controle du Caoutchouc et des Plastiques (LRCCP), Deutches Institut fuer Kautschuktechnologie e.V (DIK) Laboratorio Italiano Gomma (CERISIE), HERMES Hansecontrol GmbH, Cabot Peformance Materials Belgium, Conradi+Kaiser GmbH, Decathlon, Exxon Mobil Petroleum and Chemical B.V.B.A, Goodyear Dunlop Tires Operations SA, Pirelli Tyre SpA, Shell.

When necessary and for specific topics experts from other organisations have been contacted. In particular experts from the Swedish Karolinska Institute, Ghent University in Belgium and the US National Institute for Occupational Safety and Health (NIOSH) were consulted on aspects related to, bioelution, microbiome in sweat and skin surface film liquid respectively.

The project technical committee has been actively involved in the discussions and in the decisions made in order to achieve the objectives within the 22 months duration of the project.

In the following sections detailed information of each activity is provided.

1.1 Scoping study and literature review. Short summary.

As the initial phase of the project, a scoping study was carried out with the purpose of framing the activities of the project towards the achievements of the two main objectives of the project: gathering better understanding on the migration behaviour of PAHs from the materials simulating skin contact (including the oral cavity) and developing a robust methodology to determine the release of the eight PAHs that could be applicable in a variety of laboratories and could constitute the basis of a future harmonised migration testing method.

The scoping study consisted on: 1) a systematic review on available information relevant to the development of an analytical method to determine the total content as well as the migration of eight Polycyclic Aromatic Hydrocarbons (PAHs) from plastic and rubber

articles migration of PAHs and 2) setting a broad experimental design towards the achievement of the two objectives.

The systematic review was carried aiming at having most recent literature and relevant standard methods which could give insights into appropriate sample preparation and analytical strategies for the quantification of the migration of the 8 PAHs from plastic and rubber components of consumer products into sweat and saliva , apart from scientific literature it also included searching in the databases of European, national and international standardisation bodies. ISO, EN and DIN.

In summary, the systematic review was based on the identification of relevant sources, selection of appropriate keywords, definition of inclusion and exclusion criteria, and subsequent reading of selected abstracts and followed the objectives:

- Identify official standardized methods for analysis and migration of PAH in relevant aqueous phases and their link to European legislation.
- Describe the state of the art in the scientific literature regarding the migration of PAHs from non-food consumer products to sweat and saliva.
- Identify the relevant formulations of sweat and saliva simulants used in scientific publications.
- Identify relevant migration tests of PAHs into sweat, saliva or a relevant aqueous phase in scientific publications in order to identify the key parameters affecting their extractability.

The extensive review performed revealed that the data available on migration of PAHs from plastic and rubber used in articles for the general public is scarce and most studies point towards the oral bioaccesibility and bioavailability of PAHs present in contaminated environmental soils. Migration methods exist but do not address the PAHs (Table 1). Also, the artificial sweat and saliva simulants are not harmonized across different standardisation bodies and their respective technical committees (Tables 2 and 3). Certified reference materials for the determination of release of PAHs are lacking.

In order to estimate the fraction of contaminant that migrates it is essential to know/determine the PAH content of the material tested however a harmonized method does not exist.

Table 1. Official migration methods from standardisation bodies

Technical Committee	**Title**	**Linked legislation**
CEN/TC 194 - Utensils in contact with food	EN 1186-1:2002 Materials and articles in contact with foodstuffs - Plastics - Part 1: Guide to the selection of conditions and test methods for overall migration	Regulation EU No 10/2011
CEN/TC 194 - Utensils in contact with food	EN 1186-3:2002 Materials and articles in contact with foodstuffs - Plastics - Part 3: Test methods for overall migration into aqueous food simulants by total immersion	Regulation EU No 10/2011
CEN/TC 194 - Utensils in contact with food	EN 1186-5:2002 Materials and articles in contact with foodstuffs - Plastics - Part 5: Test methods for overall migration into aqueous food simulants by cell	Regulation EU No 10/2011
CEN/TC 194 - Utensils in contact with food	EN 1186-7:2002 Materials and articles in contact with foodstuffs - Plastics - Part 7: Test methods for overall migration into aqueous food simulants using a pouch	Regulation EU No 10/2011
CEN/TC 194 - Utensils in contact with food	EN 1186-9:2002 Materials and articles in contact with foodstuffs - Plastics - Part 9: Test methods for overall migration into aqueous food simulants by article filling	Regulation EU No 10/2011
CEN/TC 194 - Utensils in contact with food	EN 1186-12:2002 Materials and articles in contact with foodstuffs - Plastics - Part 12: Test methods for overall migration at low temperatures	Regulation EU No 10/2011
CEN/TC 194 - Utensils in contact with food	EN 1186-13:2002 Materials and articles in contact with foodstuffs - Plastics - Part 13: Test methods for overall migration at high temperatures	Regulation EU No 10/2011
CEN/TC 194 - Utensils in contact with food	EN 13130-1:2004 - Materials and articles in contact with foodstuffs - Plastics substances subject to limitation - Part 1: Guide to test methods for the specific migration of substances from plastics to foods and food simulants and the determination of substances in plastics and the selection of conditions of exposure to food simulants	Regulation EU No 10/2011

CEN/TC 164 - Water supply	EN 15768:2015 - Influence of materials on water intended for human consumption - GC-MS identification of water leachable organic substances	
CEN/TC 164 - Water supply	EN 12873-1:2014 - Influence of materials on water intended for human consumption - Influence due to migration - Part 1: Test method for factory-made products made from or incorporating organic or glassy (porcelain/vitreous enamel) materials	
CEN/TC 164 - Water supply	EN 12873-2:2005 - Influence of materials on water intended for human consumption - Influence due to migration - Part 2: Test method for non-metallic and non-cementitious site-applied materials	
CEN/TC 52 - Safety of toys	EN 71-3:2013+A1:2014 - Migration of certain elements	Directive EC No 48/2009
CEN/TC 248 - Textiles	EN ISO 105-Z06:2000 - Tests for colour fastness - Part Z06: Evaluation of dye and pigment migration	
CEN/TC 309 - Footwear	EN ISO 17701:2016 - Test methods for uppers, lining and insocks - Colour migration	
CEN/TC 249 - Plastics	EN ISO 177:1999 - Determination of migration of plasticizers	
CEN/TC 155 - Plastics piping systems and ducting systems	EN ISO 8795:2001 - Migration assessment - Determination of migration values of plastics pipes and fittings and their joints	
CEN/TC 292 – Characterization of waste	EN 12920:2006+A1:2008 - Methodology for the determination of the leaching behaviour of waste under specified conditions	

CEN/TC 292 - Characterization of waste	EN 12457-1:2002 - Leaching - Compliance test for leaching of granular waste materials and sludges - Part 1: One stage batch test at a liquid to solid ratio of 2 l/kg for materials with high solid content and with particle size below 4 mm (without or with size reduction)	
CEN/TC 216 - Food hygiene	prEN 16889:2015 - Production and dispense of hot beverages from hot beverage appliances - Hygiene requirements, migration test; Text in German and English	
CEN/TC 170 - Ophthalmic optics	EN 16128:2015 - Reference method for the testing of spectacle frames and sunglasses for nickel release	
Child use and care articles	prEN 12868:2015 - Methods for determining the release of N-Nitrosamines and N-Nitrosatable substances from elastomer or rubber teats and soothers	
Sports grounds	DIN 18035-7 : Synthetic turf areas	
Leaching of solid materials	DIN 19529 - Batch test for the examination of the leaching behaviour of inorganic and organic substances at a liquid to solid ratio of 2 l/kg	
Leaching of solid materials	DIN 19527 Leaching of solid materials - Batch test for the examination of the leaching behaviour of organic substances at a liquid to solid ratio of 2 l/kg	
Leaching of solid materials	DIN 19528 - Percolation method for the joint examination of the leaching behaviour of inorganic and organic substances	
	DIN 38414-4 - German standard method for the examination of water, waste water and sludge; sludge and sediments; Determination of Leachability by Water	

Table 2. Sweat simulants composition found in international standards

TC	Title	Composition
Footwear - CEN/TC 309	EN 12801:2000 -Test methods for insoles, lining and insocks - Perspiration resistance	10 g sodium chloride NaCl, 6 g ammonium carbonate (NH4)2CO3 2 g potassium phosphate K2HPO4, Adjust to pH 9 with ammonia hydroxide
Footwear - CEN/TC 309	EN 12801:2000/A1:2001 - Test methods for insoles, lining and insocks - Perspiration resistance	1L grade 3 water containing: 5,0 g l-histidine monohydrochloride monohydrate 5,0 g Sodium chloride, 2,5 g Disodium hydrogen orthophosphate dihydrate The solution is brought to pH 8 with 0,1 M sodium hydroxide solution
Leather - CEN/TC 289	EN ISO 11641:2012 - Tests for colour fastness - Colour fastness to perspiration	Alkaline. 1L grade 3 water containing: 5 g NaCl , 5 g tris(Hydroxymethyl)aminomethane , 0,5 g of Urea , 0,5 g of nitrilotriacetic acid. The solution is brought to pH 8 with 2 M HCl
Textiles - CEN/TC 248	EN ISO 105-B07:2009 - Tests for colour fastness - Part B07: Colour fastness to light of textiles wetted with artificial perspiration	Refers to EN ISO 105-E04
Textiles - CEN/TC 248	EN ISO 105-E04:2013 - Tests for colour fastness - Part E04: Colour fastness to perspiration	Alkaline. 1L grade 3 water containing: 0,5 g of L-histidine monohydrochloride monohydrate, 5 g NaCl, and either: 5 g of Na2HPO4*12H2O or 2,5 g of Na2HPO4*2H2O The solution is brought to pH 8 with 0,1 M NaOH
		Acid 1L grade 3 water containing: 0,5 g of L-histidine monohydrochloride monohydrate, 5 g NaCl , 2,2 g of NaH2PO4*2H2O . The solution is brought to pH 5,5 with 0,1 M NaOH
Textiles - CEN/TC 248	EN 16711-2:2015 - Determination of metal content - Part 2: Determination of metals extracted by acidic artificial perspiration solution	Refers to EN ISO 105-E04

CEN/TC 347	EN 1811:1998+A1:2008- reference test method for release of nickel from products intended to come into direct and prolonged contact with the skin	deionized water containing: 0,5 % (m/m) sodium chloride, 0,1 % (m/m) lactic acid, 0,1 % (m/m) urea, ammonia solution, 1 %
Ophthalmic optics - CEN/TC 170	EN 16128:2015 - Reference method for the testing of spectacle frames and sunglasses for nickel release	deionized water containing: 0,5 % (m/m) sodium chloride, 0,1 % (m/m) lactic acid, 0,1 % (m/m) urea, 1 M and 0,1 M sodium hydroxide solution
Ophthalmic optics - CEN/TC 170	DIN EN ISO 12870 - Spectacle frames - Requirements and test methods	1L grade 3 water containing : 50 g of lactic acid, 100 g of NaCl
Furniture	DIN EN 12720 - Assessment of surface resistance to cold liquids	Refers to EN ISO 105-E04
Protective clothing	DIN EN 14120 - Wrist, palm, knee and elbow protectors for users of roller sports equipment - Requirements and test methods	Refers to EN ISO 105-E04
	DIN 53160-2 - Determination of the colourfastness of articles for common use - Part 2: Test with artificial sweat	deionized water containing: 5 g L^{-1} sodium chloride, 1 g L^{-1} lactic acid, 1 g L^{-1} urea , 1 % (m/m) ammonium hydroxide solution

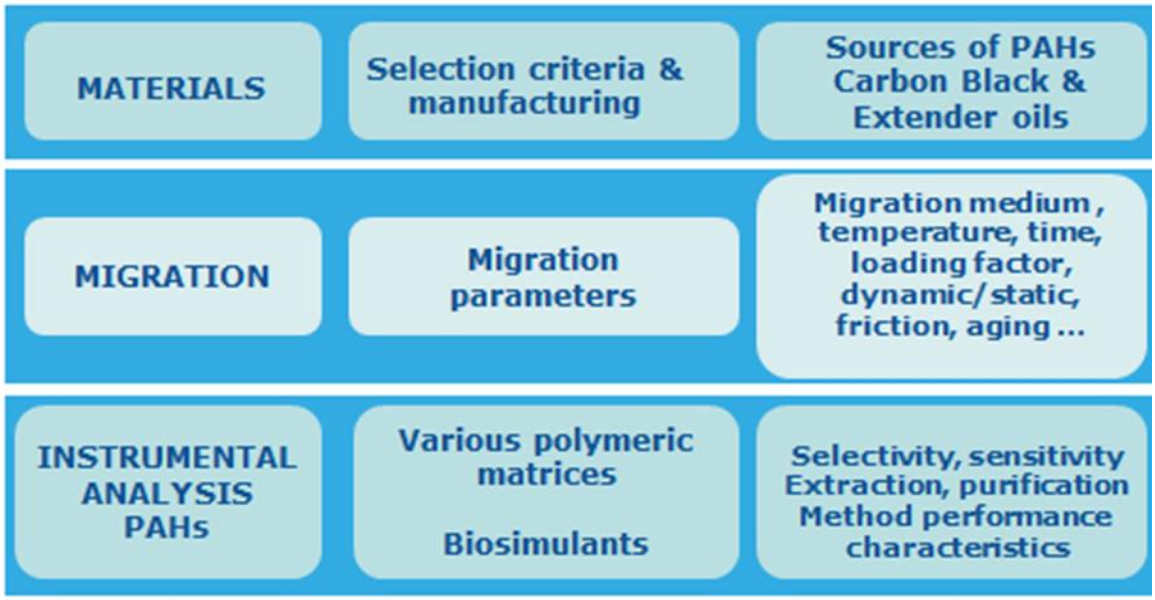
Table 3. Saliva simulants composition found in international standards

TC	Title	Comments	Composition	Linked legislation
	DIN 53160-1 - Determination of the colourfastness of articles for common use - Part 1: Test with artificial saliva		$MgCl_2$ (0,17 g L^{-1}), $CaCl_2$ (0,15 g L^{-1}), K_2HPO_4 (0,76 g L^{-1}), K_2CO_3 (0,53 g L^{-1}), NaCl (0,33 g L^{-1}), KCl (0,75 g L^{-1}) , Adjust to pH 6,8 with HCl	
Child use and care articles-CEN/TC 252	prEN 12868:2015 - Methods for determining the release of N-Nitrosamines and N-Nitrosatable substances from elastomer or rubber teats and soothers	Relevant also for migration	$NaHCO_3$ (4,2 g L^{-1}), NaCl (0,5 g L^{-1}) KCO_3 (0,2 g L^{-1}) , $NaNO_2$ (30 mg L^{-1}) Adjust to pH 9,0 with HCl or NaOH	
Safety of toys - CEN/TC 52	EN 71-12 - N-Nitrosamines and N-nitrosatable substances			Directive EC No 48/2009
Dentistry - CEN/TC 55	EN ISO 10271 - Corrosion test methods for metallic materials		Na_2HPO_4 (4,66 mM), KCl (23,28 mM) NaCl (2,46 mM), NH_4Cl (4,1 mM) Trisodiumcitrate*$2H_2O$ (0,0748mM) lactic cacid (0,78 mM), urea (3,34 mM), uric acid (0,0892 mM) NaOH (0,1 mM), KSCN (2,46 mM)	
Biological evaluation of medical devices - CEN/TC 206	EN ISO 10993-15:2000 - Identification and quantification of degradation products from metals and alloys	It refers to AFNOR NF 91-141, (Biodegradability of dental metal alloys — Standardization of electrochemical test) for the saliva composition	Na_2HPO_4 (0,260 g L^{-1}), NaCl (0,700 g L^{-1}) KSCN (0,330 g L^{-1}), KH_2PO_4 (0,200 g L^{-1}) $NaHCO_3$ (1,500 g L^{-1}), KCl (1,200 g L^{-1})	Proposal for a Regulation of the European Parliament and of the Council on medical devices

2. Experimental design

To fulfil the objectives of the project, initially, a broad experimental design (for details refer to Annex 1) was planned and discussed with the steering committee and with the project technical committee. The refined and agreed version is visualised in Figure 1 and described in this chapter. It aims at providing information on the bioaccesible amount of the PAHs that potentially can migrate from plastic and rubber materials and deposit onto the skin. It has been developed taking into account a compromise between relevant and meaningful research and simplicity and robustness of a migration method intended for potential application in an enforcement or control laboratory if a future legislation would consider the use of a migration based limit.

Figure 1. Main block activities of the STANPAHs project



The main pillars considered in the experimental design are the following:

Materials. A series of ad-hoc manufactured materials containing various amounts of PAHs from known sources have been studied for their migration behaviour. Materials have been selected according to defined criteria including their frequency of use in articles and probability to contain PAHs. PAHs are not intentionally added to the plastic and rubber materials but they are contained in the raw materials used for their manufacturing.

Measurement of migration rates. The following are among the main parameters affecting the chemical release

- Migrating medium
- Surface area to volume ratio or mass of material to volume ratio
- Temperature
- Time
- Dynamic/static mode
- With/without friction
- Aging (not taken into account in the context of the project)

Analytical determination of EU-PAHs, both as total content and as migration rate from the chosen material.

In the following sections these three main phases of the project are illustrated and discussed.

2.1 Selection of reference materials

The selection of representative polymeric materials to be used as reference test materials within the STANPAHs project, one of the milestones of the project, was done in agreement with the project technical committee. A set of polymeric plastic and rubber matrices was chosen based in criteria such as their frequency of use in articles within the scope of the restriction and the likelihood of the presence of high PAH contents (e.g. due to their content in carbon black or extender oils). Various quality grades and types of ingredients known to be PAH sources were used in the formulation of the manufactured materials. The materials were manufactured by industrial partners, represented in the project technical committee, and delivered to JRC. The ad-hoc manufactured materials included low density polyethylene (LDPE), polystyrene (PS), and polyvynil chloride (PVC) as plastic material and ethylene-propylene diene monomer (EPDM), natural rubber-butadiene rubber (NR-BR) and silicone as rubber matrices. Thus twenty plastic and rubber materials containing variable amount of PAHs have been made available for this project. Moreover, recycled granules (coated and uncoated) originated from end-of-life tyres produced after and before 2010 as well as recycled rubber tiles made of the coated granules, although not ad-hoc manufactured, were made also available for this study.

It is important to highlight that some of the above materials (with the highest PAH content) although not representing the market situation were manufactured on purpose to facilitate the method development.

In addition, the raw ingredients used for the manufacturing the reference test material samples carbon black (4 types) and extender oils (2 types) were also provided to JRC. Raw materials used represented high and low content of PAHs and their combination led to reference test materials containing PAHs in a broad range. At a later stage other extender oils, were also received.

Reporting and discussing on aspects of the plastic and rubber manufacturing processes as well as on the variety of polymers and the function and origin of carbon black and extender oils is out of the scope of this section.

2.1.1 Plastic ad-hoc manufactured

Thanks to their versatility, plastics have become key materials in many sectors including consumer products. The selected plastic matrices LDPE, PVC and PS cover a multitude of consumer applications as well as variety in terms of diffusivity characteristics [3-5]. Extender oils are not used in the manufacturing of plastic materials. Two types of carbon black [6] with low and high PAH content were selected as ingredients in the plastic formulation. These were:

1)Carbon Black ELFTEX® TP (CB ETP), a P-type carbon black which is compliant with regulation EU 10/2011, requiring a carbon black with a benzo(a)pyrene level not exceeding 0,250 ppm and suitable for use in food contact materials,

2) Carbon Black N772 having higher PAH level and consisting on larger primary particles and smaller/less branched aggregates which generate less interaction in the plastic matrix. From a theoretical perspective, this could potentially facilitate PAHs to migrate.

Carbon black percentage contained in the materials were set at 2,5% and 40% representing typical carbon black loadings for coloration purposes and for conductive compounds respectively.
In total six plastic materials (sheets of 20 cm width x 20cm lenght x 2 mm thickness) listed in Table 4 were manufactured and provided to JRC.

Table 4. Custom synthesised plastic materials

Manufactured ad hoc plastic materials		
Plastics	**CB type and loading**	**Intended PAH level**
PVC	CB ETP	Low
PVC	CB N772	High
LDPE	CB N772 at 2,5%	High
LDPE	CB ETP at 40%	Low
PS	CB N 772 at 40%	High
PS	CB ETP at 2,5%	Low

2.1.2 Rubber ad-hoc manufactured and raw materials

A great number and variety of products used in everyday life is made from rubber (synthetic or natural), a material that thanks to its properties such as malleability, elasticity and great mechanical resistance has found broad applications in automotive, construction and consumer products. The rubber product it is usually made from a mixture of materials and in most products the base polymer is crosslinked by either chemical or physical links. A rubber product may contain reinforcement.

Carbon black (CB) is extensively used as a reinforcing agent and filler in rubber formulations composing numerous industrial and consumer products [6]. CB may also contain polycyclic aromatic hydrocarbons (PAHs), which can be incorporated into rubber formulations and be present in the end product [7-10]. CB as a compounding ingredient has in its constitution 95 % of elemental carbon in the form of near-spherical particles with major diameters less than 1 μm, generally coalesced into aggregates [6, 11]. It is produced by incomplete burning or thermal decomposition of hydrocarbons. Most of the PAHs present in carbon black are on the surface and are tightly adsorbed [12].

Extender oils which are commonly used in the rubber industry for the production of tyres or parts of tyres contain a relatively high proportion of process oils. These process oils derive from petroleum (crude oil) after the more volatile petrol and heating oil fractions have been removed by distillation. They have in their constitution paraffinic, naphthenic and aromatic compounds of wide range of molecular weight including PAHs. Treated Distillate Aromatic Extract (TDAE) has replaced the Distillate Aromatic Extract (DAE), containing high percentage of PAHs, and is used for the production of rubber products intended for skin contact.

Natural rubber polybutadiene (NR/BR) ethylene - propylene – diene terpolymer (EPDM) and silicone rubber were selected as matrices representative of applications of consumer products (eg. boots, toys, tooling and sport, glasses, rubber bands, etc).

Two types of Carbon Black and two types of extender oils representing low and high content of PAHs were used for the manufacturing of the reference test materials (Table 5).
In particular the CBs used were CB N375 and CB N550 and as extender oils a DAE and TDAE were used. Not all combinations were possible due to compatibility aspects.

Table 5. Selected rubber polymeric matrices representative of articles supplied to the general public

Polymer	Materials combinations
NR-BR (Natural rubber polybutadiene)	CB N375 + TDAE oil CB N375 + DAE oil CB N550 + TDAE oil CB N550 + DAE oil
EPDM (ethylene - propylene – diene terpolymer)	CB N375 + TDAE oil CB N375 + DAE oil CB N550 + TDAE oil CB N550 + DAE oil
PVMQ (silicone rubber)	CB N375 + TDAE oil CB N550 + DAE oil

The materials have been supplied in slabs of 20 cm width x 20cm lenght x 2 mm thickness, for EPDM, NR-BR and silicone.

Also raw materials of carbon black (various types and grades) and extender oils with low and high content of PAH (TDAE and DAE) have been supplied.

Figure 2. Samples of materials as they were received

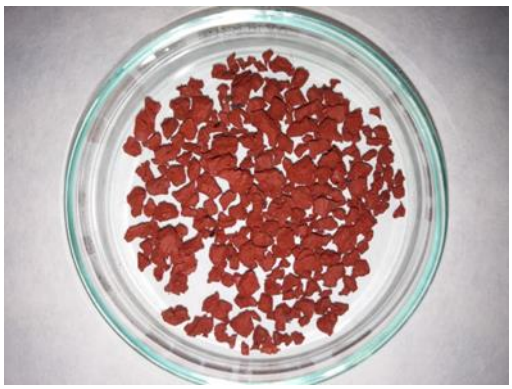
2.1.3 Recycled rubber granules

Usually rubber granules made from end of life tires are present in many synthetic turf fields to enhance the resiliency of these types of materials. However, they might have constituents such as PAHs, metals or volatile organic compounds. Recycled rubber granules, which are considered to be mixtures, do not fall under the scope of the restriction of PAHs under REACH, Entry 50 paragraph 5 and 6, however they were included in this project since little information is available concerning their PAH content of these substances [13-16] but also on their bioaccesibility and bioavailability through exposure especially via oral and dermal contact and the role of coating as potential barrier to the release of chemical substances.

Recycled granules (coated with polyurethane (PU) and uncoated) originated from end-of-life tyres produced after and before 2010 as well as rubber tiles made of recycled coated granules, were made available for this study (Figure 3).

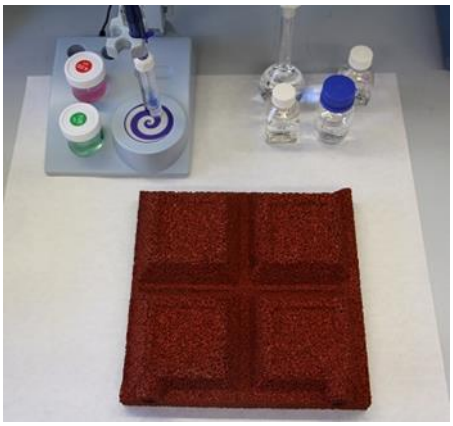
A summary of all materials provided to JRC in the context of this project is found in Table 6.

Figure 3.PU Coated and uncoated granules and tiles samples



PU coated granules

Uncoated granules



(45 x 45) cm tile

Table 6. List of materials received and used in the context of STANPAHs project

Sample description	Provided by
Recycled Granules and Tiles	
Rubber uncoated granulate material, before 2010	Conradi + Kaiser Living Industries GmbH (DE)
Rubber coated granulate material, before 2010	Conradi + Kaiser Living Industries GmbH (DE)
Before 2010 test tile	Conradi + Kaiser Living Industries GmbH (DE)
Rubber uncoated granulate material, after 2010	Conradi + Kaiser Living Industries GmbH (DE)
Rubber coated granulate material, after 2010	Conradi + Kaiser Living Industries GmbH (DE)
After 2010 test tile	Conradi + Kaiser Living Industries GmbH (DE)
Raw Materials	
CB N330	CERISIE (IT)
CB ETP (used in food industry)	Cabot B.V. (NL)
CB N375	Columbian Carbon Europa srl
CB N550	Columbian Carbon Europa srl
CB N772	Columbian Carbon Europa srl
DAE Oil (Tudalen 65)	CERISIE (IT)
TDAE Oil (Vivatec 500)	CERISIE (IT)
CB N375	CERISIE (IT)
CB N550	CERISIE (IT)
Plastic Materials	
PVC plaques containing CB ETP	Polymer Chemie (DE)
PVC plaques containing CB N772	Polymer Chemie (DE)
Resin PS Edistir N2982 PEP2017.4374	Cabot Plastics Belgium
PS N2982 Neat PEP2017.4374	Cabot Plastics Belgium
Plates E4 (2.5% CB ETP in PS Edistir N2982)	Cabot Plastics Belgium
Plates E4 (30% CB ETP in PS Edistir N2982)	Cabot Plastics Belgium
Plates E2 (2.5% CB N772 in PS Edistir N2982)	Cabot Plastics Belgium
Plates E2 (40% CB N772 in PS Edistir N2982)	Cabot Plastics Belgium

Resin LDPE LD653 PEP2017.4374	Cabot Plastics Belgium
LDPE LD653 Neat PEP2017.4374	Cabot Plastics Belgium
Plates E3 (2,5% CB ETP in LDPE LD653)	Cabot Plastics Belgium
Plates E3 (40% CB ETP in LDPE LD653)	Cabot Plastics Belgium
Plates E1 (2,5% CB N772 in LDPE LD653)	Cabot Plastics Belgium
Plates E1 (40% CB N772 in LDPE LD653)	Cabot Plastics Belgium
Rubber Materials	
NR-BR+N375(24,1%)+DAE(2,7%)	CERISIE (IT)
NR-BR+N550(24,1%)+DAE(2,7%)	CERISIE (IT)
NR-BR+N375(24,1%)+TDAE(2,7%)	CERISIE (IT)
NR-BR+N550(24,1%)+TDAE(2,7%)	CERISIE (IT)
EPDM+N375(25,6%)+DAE(2,8%)	CERISIE (IT)
EPDM+N550(25,6%)+DAE(2,8%)	CERISIE (IT)
EPDM+N375(25,6%)+TDAE(2,8%)	CERISIE (IT)
EPDM+N550(25,6%)+TDAE(2,8%)	CERISIE (IT)
Silicone+N375(4,3%)	CERISIE (IT)
Silicone+N550(4,3%)	CERISIE (IT)

2.1.4 Commercial products

Variable amount of the eight target PAHs have been reported in consumer products [1]. A limited number of consumer products were purchased on the retail market and analysed for their PAH content. Technical materials obtained from the JRC central warehouse were used to facilitate the in house method development. More details are described in section 2.2.

Samples investigated are summarized in the Table 7. All the material listed below was brand new, except for the watch wrist band which was a used product. The sample of a car wheel cover was divided into two parts due to the two different materials in its composition, even though the internal part would not be in contact with driver's hands.

Table 7. Commercial samples selected for the study

Sample description	Provided by / Obtained in
Junction for carpets	JRC Central warehouse
Butadiene gloves	
Generic use technical material	
Bicycle handles	Retail market
Swimming pool shoes	
Play pad	
Outdoor playground pad	JRC Technical services
Car wheel cover, external part	Retail market
Car wheel cover, internal part	
House pad	
Inner soles	
Sport inner soles	
Dinosaur toy	
Watch wrist band	
Ball Bladders	Decathlon
Sheet of Neoprene*	

* not used for consumer products production

Figure 4. Commercial products analysed for their EU-PAH content

2.1.5 Extender oils from industries

Various samples of extender oils belonging to the Other Lubricating Base Oils category (OLBO) were provided by industry (Table 8). These oil samples have not been used for the preparation of the manufactured materials, and therefore not reported in Table 6, but have been analysed by JRC, for their content of the eight target PAHs using an in house method.

Table 8. OLBO Extender Oils supplied to JRC

Sample code	**Extender Oil (OLBO)** MC1	**Extender Oil (OLBO)** MC2	**Extender Oil (OLBO)** MC3	**Extender Oil (OLBO/Bitumen)** MC4	**Extender Oil (OLBO)** MC5
CAS No	64741-88-4	64742-52-5	64742-52-5	64742-52-5 64742-54-7 64741-56-6 8052-42-4	64742-54-7 64742-52-5

2.2 Development of a method for the total content determination of EU-PAHs in plastic and rubber materials

Although risks addressed within the scope of the restriction of PAH in consumer articles according to Annex XVII to REACH relate to the dermal doses of PAHs resulting from migration from the rubber and plastics components of articles, the unavailability in 2013 of reliable migration methods for determination of PAHs released from these articles, and due to practical considerations from the point of view of enforcement, the REACH restriction was defined in terms of content, rather than in terms of migration.

A number of methods for the determination of PAHs in a variety of matrices in most cases other than rubber or plastics are available [7-9, 17-19]. While some of these methods are faced with high equipment costs as a main challenge for implementing them in enforcement scenarios, others lack the specificity and capability of determining individual PAH concentrations in plastic and rubber materials which imposes severe limitations on their applicability for these purposes. In addition, some of these methods suffer from outdated protocols, often in the extraction or purification steps which are no longer state of the art.

For this reason an improved method for the determination of the eight EU-PAHs in rubber and plastic materials was developed in the framework of the current project. Existing methodologies were taken as starting point, improving in particular the extraction and the clean-up procedures. Randall hot extraction of the rubber or plastic material with toluene, followed by sample extract clean-up with PAH selective solid phase extraction cartridges, based on molecularly imprinted polymers (MIPs), in combination with gas chromatography-mass spectrometry (GC-MS) analyses in selected ion mode was found to be the optimal method in terms of extraction efficiency, extract purity, and time demands [20].

A detailed standard operating procedure (SOP) of the proposed method can be found in Annex 2.

The method has been used to quantify the amount of the eight target PAHs in the manufactured reference test materials allowing the estimation of the release relative to the content.

2.2.1 Activities undertaken towards the development of an improved methodology for the determination of the 8EU-PAHs in rubber and plastic materials

Based on the analysis of the already existing methods, those parts which were perceived as being particularly worthwhile being improved, were the extraction- and the clean-up procedures. To demonstrate the effective improvement of the measures proposed, a number of comparative measurements were undertaken.

We chose three test materials for this study: soft polyvinylchloride (PVC) containing CB N772 and two natural/butadiene rubber (NR/BR) blends; one containing CB N375 (24.1 %) and distillate aromatic extract (DAE, 2.7 %), and the other containing CB N375 (24.1 %) and treated distillate aromatic extract (TDAE, 2.7 %). These materials were chosen for two reasons: (i) they should be representative of the two polymer groups: plastics and rubber; and (ii) they should be able to account for the impact different PAH concentrations in the same material could have on factors such as the recovery rates. Soft PVC was chosen as the representative of plastic materials as its high phthalate content poses a challenge in the clean-up step. Since singularly available, CB N772 (raw material), as well as treated and untreated distillate aromatic extracts (the same used during the manufacturing process of the NR/BR blends), were separately analysed as well.

2.2.1.1 Extraction

While the extraction method proposed is Randall hot extraction, we also employed ultrasonic extraction for purposes of comparison.

Randall hot extraction

Around 40 mg of the DAE containing NR/BR, or 100 mg of either the TDAE containing NR/BR or the PVC were weighed exactly into cellulose extraction thimbles, which were transferred to the respective extraction cups, adding the isotope labelled internal standard to the interior base of the extraction thimbles, next to the sample material. Then, 95 mL of toluene were added to the extraction cups. The extraction was done with a Velp SER 158 solvent autoextractor set to the highest heating level, using 120 minutes for immersion, 20 minutes for removal, 30 minutes to wash, 7 minutes for recovery, and 15 minutes for cooling, which resulted in a total extraction time of just over 3 hours and a final sample extract volume of approximately 20 mL. Gaskets made of Vaflon were used between the connection funnel solvent and the extraction cup.

Ultrasonic extraction

Around 40 mg of the DAE containing NR/BR or around 100 mg of either the TDAE containing NR/BR or the PVC were weighed exactly into 100 mL Erlenmeyer flasks, to which 95 mL toluene and 20 µL of isotope labelled internal standard (2500 ng mL^{-1} in toluene) were added. The capped flasks were then placed into an ultrasonic bath (800 Watt, 59 KHz, bath area 900 cm^2) for 1 hour at 60 °C.

2.2.1.2 Clean-up/purification

Toluene sample extracts were evaporated to dryness with a rotary evaporator, in a 60 °C water bath and adjusting the vacuum to 90 mbar.

The clean-up procedure used solid phase extraction (SPE) cartridges based on the selective retention of molecularly imprinted polymers (MIPs). Results from this approach were compared against results obtained from the German AfPS method [9], which is based on adsorption chromatography on silica gel.

Solid Phase Extraction with SupelMIPTM cartridges

The dry extract was reconstituted in 1 mL hexane and cleaned-up using solid phase extraction (SPE) cartridges filled with MIPs. The following procedure was used:

conditioning 1 mL cyclohexane, loading of sample, washing with 3x 1 mL cyclohexane, elution with 3x 1 mL ethylacetate, evaporating the ethylacetate extract to dryness with a nitrogen evaporator (heating block set to 40 °C), and reconstituting in 1 mL toluene for GC-MS analysis.

Clean-up with silica gel packed columns

A glass chromatography column (20 cm x 2 cm) was packed with 4 g of previously deactivated silica (Supelco, Washed silica, product code 21342-U), employing the wet packing method, to which 1 cm of anhydrous sodium sulphate was added. Deactivation was achieved by adding 10 % in weight of ultrapure water to the silica and subsequent homogenisation for 1 hour. The column was conditioned with 10 mL petroleum ether. The dry extract was then reconstituted in 1 mL toluene and loaded onto the column. Elution of PAH was achieved with 50 mL petroleum ether. In the next step, the petroleum ether extract was evaporated to dryness with a nitrogen evaporator (heating block set to 40 °C) and reconstituted in 1 mL toluene for GC-MS analysis.

Recovery determination using SupelMIPTM SPE cartridges

To determine the recovery rates, 100 mg of distillate aromatic extract, for which the PAH content had previously been determined, were initially solved in 100 mL hexane. Then 1 mL of this extract was loaded onto the SPE columns and treated as described above. A further 1 mL of hexane extract was evaporated to dryness with a nitrogen evaporator and reconstituted in 1 mL toluene. Both solutions were analysed with GC-MS to determine the ratio of the respective peak areas. Absolute masses loaded onto the cartridges were 18 ng of benzo[a]anthracene (BaA), 90 ng of chrysene (Chr), 40 ng of benzo[b]fluoranthene (BbF), 6 ng of benzo[k]fluoranthen (BkF), 8 ng of benzo[j]fluoranthene (BjF), 100 ng of benzo[e]pyrene (BeP), 25 ng of benzo[a]pyrene (BaP) and 2 ng of dibenzo[a,h]anthracene (DBahA). Recovery determinations were made in quintuplicate.

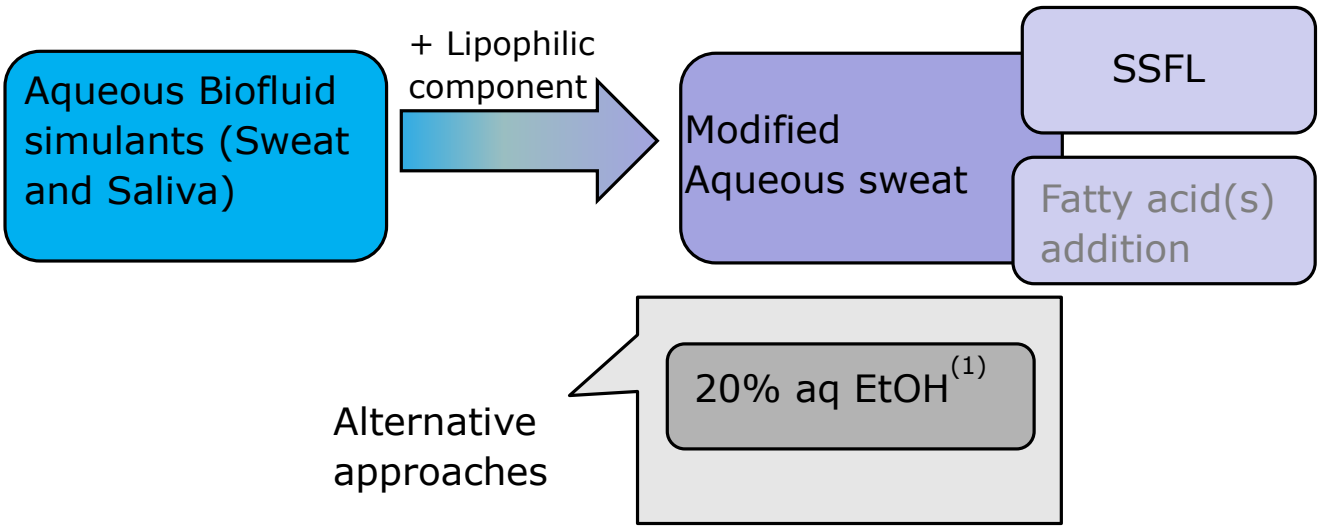
2.2.2 Total content analysis of ad-hoc manufactured materials, raw-materials and consumer articles purchased on the retail market

Applying the method described above and detailed in the standard operating procedure (Annex 2), all ad-manufactured test materials, a number of raw materials and selected consumer articles purchased on the retail market were analysed on their EU-PAH content. The detailed description of the analysed materials can be found under Section 2.1 of this report.

2.3 Selection of simulants for the Development of a method for the evaluation of the release of EU-PAHs from plastic and rubber materials

The choice of migration simulants, as well as the prioritisation that should have been given to the use of different formulations, was presented, discussed and agreed during the first technical meeting of the STANPAHs project held in Brussels on the 3rd of October 2016. The process is visualised in Figure 5 and discussed in the following paragraphs.

Figure 5. Selection of simulants to be used in migration tests



2.3.1 Aqueous biofluid simulants

As the starting point for migration tests, aqueous biofluid simulants, having the formulation described in national and international standards were used.

In particular, as the result of the initial scoping study, the following artificial simulants were selected:

Sweat simulant: standard EN 1811:2011+A1:2015 [21]

0,5 % (*m/m*) sodium chloride, 0,1 % (*m/m*) lactic acid and 0,1 % (*m/m*) urea

Saliva simulant: standard DIN 53160-1: 2010-10 [22]

0,17 g L^{-1} magnesium chloride, 0,15 g L^{-1} calcium chloride, 0,76 g L^{-1} dipotassium carbonate, 0,33 g L^{-1} sodium chloride, 0,75 g L^{-1} potassium chloride and 1 % (*m/m*) hydrochloric acid to be added until a pH value of 6,8 ± 0,1 is achieved.

Nevertheless, the simulants in the above-mentioned formulations are very effective in the evaluation of hydrophilic components release from products in contact with the skin but have some limitations when used for the assessment of hydrophobic chemicals release [7, 23, 24]. In this case, higher migration rates have been obtained when using simulants with higher lipophilic affinity [25-27].

For these reasons, modified simulants formulations were taken into consideration. In addition, as agreed during the first technical meeting of the STANPAHs project, priority was given to the investigation of modification of sweat as it has been considered more relevant for exposure route from the articles in the scope of the restriction.

2.3.2 Modified sweat simulant formulation

The agreed experimental design (outcome of the first technical meeting) entailed a double approach to this end:

a) Standard sweat simulant formulation with added lipophilic content

The objective is to choose the appropriate chemicals to be added to a standard sweat formulation in order to better extract the target PAHs, according to similar properties of these substances, namely the partition coefficients octanol/water (Kow) at estimated/measured physiological concentrations to better mimic the human sweat.

b) Migration into a "skin surrogate" which mimics the skin surface film liquid (SSFL).

The outermost layer of human skin, the stratum corneum, consists of dead cells and is covered with a complex liquid layer of mainly sweat and sebum which are excreted from sweat and sebaceous glands, respectively [28, 29]. The artificial combination of these two components is known as skin surface film liquid (SSFL) [25]. The goal was to reproduce in laboratory this film by mixing an artificial sweat composition to an artificial sebum composition (e.g. by emulsification) [25, 27].

2.3.3 Alternative approaches

Parallel to the modification of aqueous sweat formulation, alternative approaches to test migration of lipophilic substances from products intended to be in contact with the skin emerged from the preliminary scoping study.

a) Migration using a solvent-based approach of 20% EtOH/water

The use of an aqueous sweat simulant in the prediction of dermal exposure is a very common approach. However, as mentioned before, published reports clearly indicate that an aqueous simulant is not suitable for migration testing of lipophilic substances. Data from the literature [30, 31] have suggested that 95% ethanol could be a valid simulant for fat. In fact, the regulation from the European Commission (EU) No 10/2011[32], has set 50% ethanolic simulant as a substitute for milk matrix. Nevertheless, the recent study of Bartsch, N. et al 2016 [33], reports this proportion as an overestimation in the migration analysis of a PAH (BaP) in comparison to the human skin. The authors proved that the use of 20% ethanol at 37 ºC is a valid alternative simulant that mimics the real human skin exposure that results from direct contact with chemicals (PAHs) that migrate from consumer products.

b) Migration into TENAX^{TM} (modified polyphenylene oxide, MPPO)

TENAX^{TM} could be another valuable option of a simulant since there are recent studies that investigated the migration rate of PAHs from rubber materials into skin using lipophilic matrices[8, 34-36]. TENAX^{TM} is commonly used in test methods for the determination of the migration into fatty food simulants from plastic materials and articles [37].

From the broader list of possible migration media, the following were taken into consideration in the context of the STANPAHs project:

- Aqueous sweat simulant according to EN 1811:2011+A1:2015
- Aqueous saliva simulant according to DIN 53160-1
- Artificial Skin Surface Film Liquid (SSFL)
- 20 % aqueous Ethanol

A summary of the materials that were tested in the four different migration simulants is shown in Table 9.

Table 9. Overview of migration tests carried out.

	AQUEOUS BIOFLUID SIMULANTS		MODIFIED AQ SWEAT (SSFL)	ALTERNATIVE APPROACH
	SWEAT EN1811	SALIVA DIN53160-1		20 % EtOH
	RUBBER MATERIALS			
EPDM CB375 DAE				
EPDM CB375 TDAE				
EPDM CB550 DAE				
EPDM CB550 TDAE				
NR/BR CB375 DAE				
NR/BR CB375 TDAE				
NR/BR CB550 DAE				
NR/BR CB550 TDAE				
SILICONE N375				
SILICONE N550				
	PLASTIC MATERIALS			
PVC CB772				
LDPE 40% CB772				
LDPE 2,5% CB772				
PS 40% CB772				
PS 2,5% CB772				
	GRANULES			
Granule PU COATED After 2010				
Granule UNCOATED After 2010				

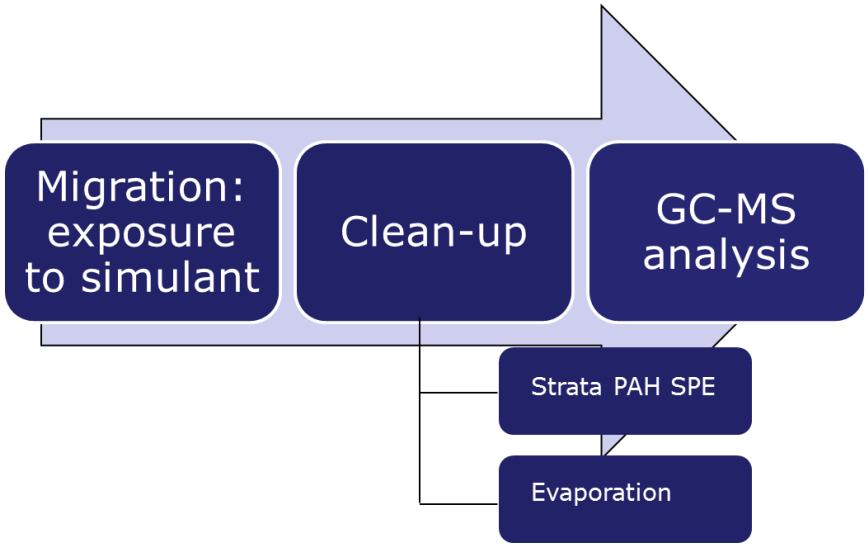
Shaded cells indicate the combination of material/migration medium tested; white cells indicate combinations not subjected to experimental testing. Materials with higher EU-PAH content, per polymer type, are reported in bold.

In addition to the simulants mentioned so far, preliminary migration tests were also performed from a single selected rubber material onto a sebum imbued filter paper strips. Composition of this simulant as well as methodology and results are discussed in the sections 2.4.4 and 3.3.3 and Annex 5 of this report**.**

2.4 Development of a method for the evaluation of the release of EU-PAHs from plastic and rubber materials

Sample of custom synthesised rubber or plastic material was exposed to a solution intended to mimicking the characteristics of the selected body fluid (sweat or saliva). Target compounds (namely the 8 EU PAHs) were then extracted from the simulant through a clean-up procedure and quantified against an internal standard by GC-MS analysis. Figure 6 depicts the steps of the whole migration procedure which are going to be discussed in the following sections.

Figure 6. Steps of migration protocol



2.4.1 Working procedure applied to migration in aqueous biofluid. Preliminary tests with aqueous sweat EN1811 and aqueous saliva DIN 53161-1

During the first technical meeting, some considerations about migration parameters were debated and guidelines to set migration parameters were specified. They are reflected in the bullet points bellow:

- Attention should be given to find the most suitable surface area to volume ratio
- The temperature to be considered should range between 37-40 ºC, higher temperatures might be relevant in specific cases.
- 24 hours was considered as maximum test migration duration. In order to have insights on associated kinetics sampling at given time intervals (e.g. 0.5, 1, 2, 4, 6, 24 h) would be desiderable.
- Dynamic migration (e.g. stirring) should simulate better the reality rather than static migration and it was also discussed whether friction should be considered
- As rubbing or aging effects was concerned, it was the perception of the participants that these aspects seem not to be within the scope of the restriction (short and repetitive use or prolonged contact)

On the basis of what was debated, a preliminary set of migration tests to aqueous biofluid were run on materials containing the highest amount of 8 EU-PAHs applying the conditions reported in Table 10.

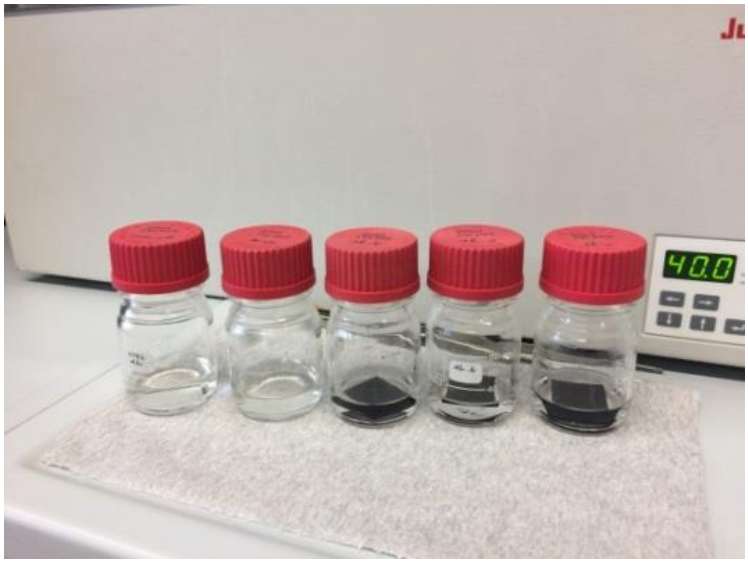
Table 10. Preliminary migration test to aqueous biofluids experimental conditions.

Sample Type	EPDM CB375 DAE	PVC CB772
	NR BR CB375 DAE	LDPE 40% CB772
	Silicone CB375	PS 40% CB772
	Recycled rubber granules PU coated and Uncoated after 2010	
Kind of exposure	Total immersion	
Temperature	40 ºC	
Type of migration	Dynamic (150 rpm)	
Duration	1h, 4h, 24h	
Exposed surface*	3 x 3 cm square shaped test specimen, 2 mm thick (0.2 dm^2 total surface),	
Volume of simulant	20 mL	
Simulants used	Saliva (DIN 53160-1); Sweat (EN 1811:2011+A1:2015)	

* Exposed surface of 1 dm^2/100 mL was chosen on the basis of EN1186-1:2002, which provides guidance on 'The selection of conditions and test methods for overall migration' [38]

Squares of 3 cm x 3 cm were cut from the rubber sheet or plastic plate. Considering the sheet/plate thickness of about 0.2 cm, the total area in contact with the simulant was 0.2 dm^2. The test specimens were then added to 100 mL laboratory glass bottles together with exactly 20 mL of aqueous simulant. This resulted in a final loading factor of 0.2 dm^2 20 mL^{-1}. The laboratory glass bottles were placed in a pre-heated water-bath shaker (150 rpm) at a temperature of 40 °C. Migrations tests were conducted for 1, 4 and 24 hours. Migration experiment was set up so that each migration run was made of one blank solution, acting a negative control which works for excluding PAH contamination, a control solution to guarantee the accuracy (if the recovery of primary standard deviates more than 15 percentage points from 100%, it is advisable to check for a systematic error) and three identical test material sample (Figure 7).

Figure 7. Sample set for a migration run



After the migration step, samples were cooled down to room temperature and a mixture of deuterated internal standard (ISTD) was added to the liquid extract in each of the five bottles. The ISTD is a mix of the corresponding deuterated forms for each of the 8 PAHs except for BjF for which BkF-D_{12} was used.
It is worth underlining the importance of adding the internal standard after the exposure to simulant and immediately before the SPE clean-up. If the internal standard is added at beginning of the migration very low recoveries values are obtained indicating that some interactions adsorption-desorption phenomena among the ISTD and the materials surface likely occur.

Clean-up and solvent change were achieved using Strata PAH solid-phase extraction cartridges, a silica based proprietary sorbent designed to provide high recoveries of polycyclic aromatic hydrocarbons from water. After the solvent was evaporated under nitrogen stream, the residue was reconstituted in the appropriate solvent and subjected to GC-MS analysis.

As already mentioned in the previous section of this report, PAHs are highly lipophilic compounds, therefore it was not surprising to find out that migration of each of the 8 EU PAH into aqueous artificial sweat and saliva was under the instrumental limit of detection.

This made necessary to move towards the use of different simulants which allowed us to develop and optimise the migration protocol.

2.4.2 20% aqueous Ethanol. Set-up of migration step and in-house validation of protocol

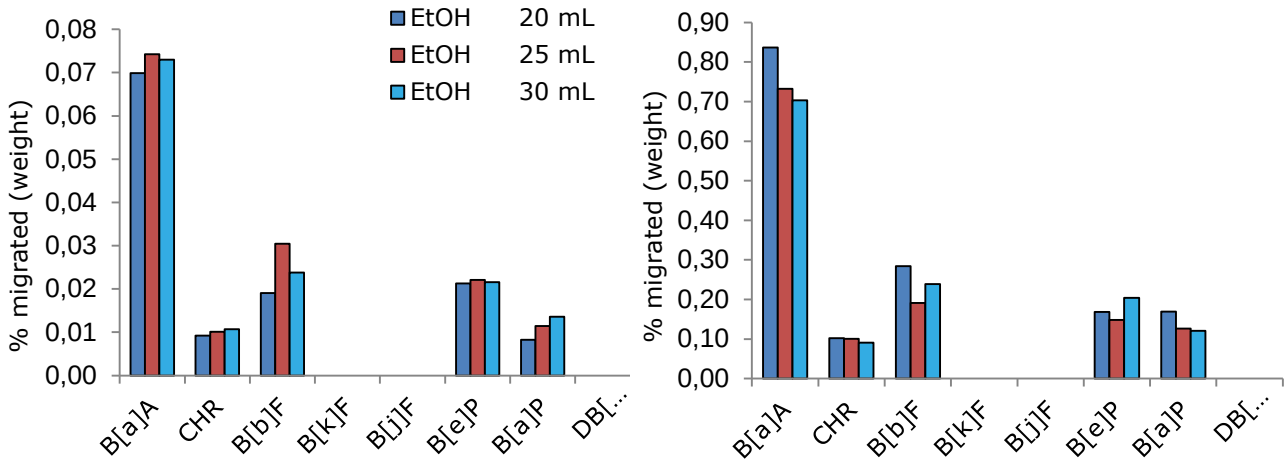
An approach alternative to the modification of aqueous simulants was the use of a 20% aqueous ethanolic solution. This choice was based on the results of an investigation by Bartsch and co-workers [33]. They conducted a study on the migration of PAHs from rubber/plastic containing consumer products into aqueous sweat simulant and aqueous ethanol, with the aim of determining their suitability for human skin penetration assessment. One conclusion of this study was that migration levels obtained with 20% ethanol/water solution at 37 ºC were in excellent agreement with the values measured in real human skin *ex vivo (*Franz Cell chambers assay).

Initially, two custom synthesised materials having the highest EU PAH content of PAHs was selected for migration testing into 20% ethanol under the same conditions already used with aqueous biofluids. The selected materials used to qualitatively assess the release were NR/BR CB375 DAE and EPDM CB375 DAE. Once proved that migration was detectable and quantifiable, migration conditions were set-up in terms of:

- Variation of surface to volume ratio (loading factor)
- Static *vs* Dynamic migration
- Effect of temperature on migration

In order to study the effect of variation of the loading factor, pieces of 0,2 dm^2 of sample material were totally immersed in 20, 25, 30 mL of 20% aq EtOH (40 °C linear shacking) for 1h and 4 h. The PAH released were quantified and the results, expressed as relative migration (wt/total content wt %) plotted and compared (Figure 8).

Figure 8. Results of loading factor variation



NR BR CB375 DAE, 1h

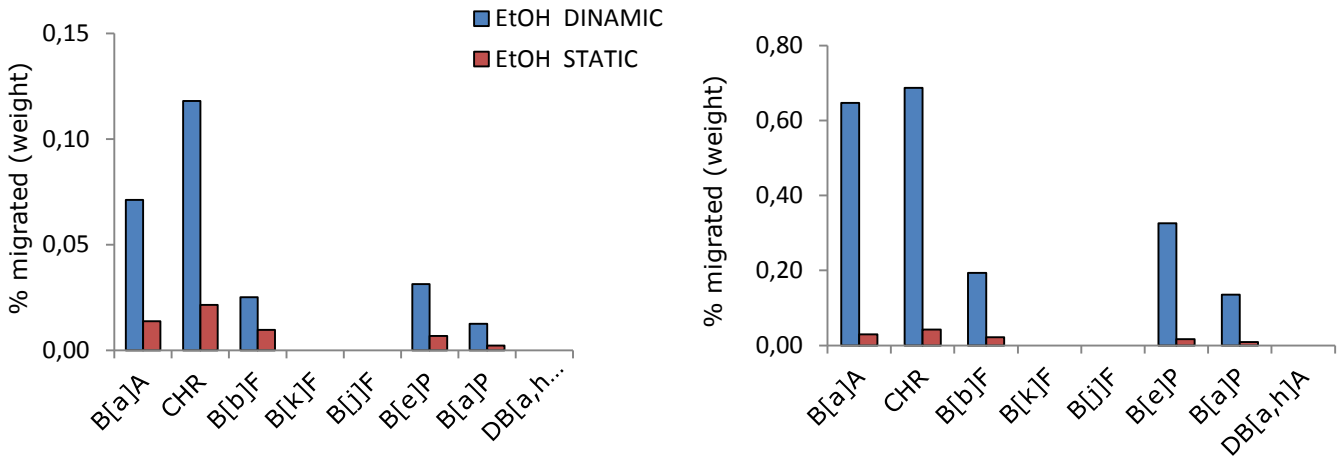
NR BR CB375 DAE 4h

It is worthy to mention that the choice of volume variations had practical constraints due to the need of covering the tested material (as for the lower limit) and to the loading factor of SPE (maximum volume allowed by Strata PAH solid-phase extraction cartridges used in the clean-up step).

As no notable differences were observed, 20 mL was kept as migration volume.

Similarly, the effect of dynamic exposure was tested under the same migration conditions previously applied. EPDM CB375 DAE was subjected to a migration experimental test in 20% aqueous EtOH at 40 °C for 1 and 4 h under static immersion and linear shaking. Results expressed as relative migration (wt/total content wt %) are shown in Figure 9.

Figure 9. Dynamic *vs* static migration



EPDM CB375 DAE, 1h

EPDM CB375 DAE, 4h

Migration increases under dynamic conditions up to a factor of 20. Therefore dynamic condition were selected to be used for testing.

Analogously a migration experiment from EPDM CB375 DAE was set up at 37 °C with the aim of verifying whether a minor change of incubation temperature would affect the migration rate. Since the results were equivalent to the migration rates at 40 °C, it was decided this latter parameter as migration temperature.

The results so far obtained confirmed the best migration conditions which are summarised in Table 11.

Table 11. 20% aq EtOH migration conditions.

Sample Type	All the received plastic and rubber ad-hoc produced materials (15) Recycled rubber granules PU coated and Uncoated after 2010
Kind of exposure	Total immersion
Temperature	40 ºC
Type of migration	Dynamic (150 rpm)
Duration	1h, 4h, 24h
Exposed surface*	3x3 cm square shaped test specimen (0.2 dm^2 total surface), 2 mm thick
Volume of simulant	20 mL
Simulants used	20% aqueous Ethanol

All the received *ad-hoc* materials were triplicate tested (results are discussed in section 3.3.1 of this report) and the method was in-house validated in terms of

- Linearity
- Limit of Detection (LOD)
- Limit of Quantification (LOQ)
- Trueness as Recovery
- Intermediate precision (intra-day repeatability, between-day precision)

Linearity was verified by triplicate injection of the 6 points calibration curve (0-25 ng mL^{-1}). The relative response ratios from the peak areas for each of the calibration standards was calculated ($_{APAH(native)}$ / $_{APAH(deuterated)}$) and their mean values plotted against calibration points concentration.
A regression was then derived by the method of mean square root and with the help of Excel functions:

$y = ax + b$,
a – slope,
b – intercept

Table 12 reports R^2 for each of the 8 PAH.

Table 12. Evaluation of Linearity

	Av Slope	**R^2**	**sd**	**sd%**
BaA	0,1012	0,9974	0,0023	2,28
CHR	0,1064	0,9995	0,0050	4,79
BbF	0,1036	0,9993	0,0050	4,83
BkF	0,0902	0,9998	0,0043	4,72
BjF	0,1367	0,9991	0,0060	4,33
BeP	0,1012	0,9994	0,0048	4,79
BaP	0,0908	0,9994	0,0041	4,48
DBahA	0,1131	0,9977	0,0047	4,09

Range is considered linear for correlation coefficient $R^2 > 0.99$

Limit of detection (LOD) and limit of quantification (LOQ) were calculated separately for each PAH by injecting 6 times the lowest concentration of the calibration curve (0,5 ng mL^{-1}) through the following equation:
LOD=3*sd/slope
LOQ=10*sd/slope
Where
Slope= Slope of the linear regression obtained from the standard calibration curves
sd= Standard deviation of the average of six measurements of the lowest concentration of the calibration curve

Table 13 reports the calculated LOD and LOQ for each of the 8 PAH.

Table 13. Limit of Detection (LOD) and Limit of Quantification (LOQ)

0.5 ng/ml lowest cal point	**av** (ng mL^{-1})	**sd**	**slope**	**LOD** (ng mL^{-1})	**LOQ** (ng/mL^{-1})	**LOQ (ug/kg=ppb)** (Sample weigh 2 g, final volume 1 ml)
BaA	0,049	0,002	0,111	0,1	0,2	0,1
CHR	0,049	0,002	0,108	0,0	0,1	0,1
BbF	0,055	0,002	0,106	0,1	0,2	0,1
BkF	0,050	0,005	0,100	0,2	0,5	0,3
BjF	0,062	0,003	0,132	0,1	0,3	0,1
BeP	0,049	0,002	0,102	0,1	0,2	0,1
BaP	0,047	0,004	0,113	0,1	0,3	0,2
DBahA	0,060	0,008	0,116	0,2	0,6	0,3

Trueness (as recovery): in the absence of reference materials bias can be investigated by spiking and recovery determination. In this work, recovery experiments were performed comparing peak areas of a standard solution at 10 ng mL^{-1} of each PAH before and after a migration cycle and elution through strata PAH column. Recovery rates were determined on 3 replicates at 24 hours migration. Results are reported in Table 14.

Table 14. Recoveries (%) at 24h migration test

Conc 10 ng/mL	**rec 1**	**rec 2**	**rec 3**	**Rec 24h av. %**	**sd**	**RSD**
BaA	92,68	102,55	100,00	98,41	5,12	5,20
CHR	89,52	100,71	95,21	95,15	5,60	5,88
BbF	91,68	93,78	112,51	99,33	11,47	11,54
BkF	83,70	98,97	99,68	94,12	9,03	9,59
BjF	91,26	95,66	96,74	94,56	2,91	3,07
BeP	90,89	94,91	101,31	95,70	5,25	5,49
BaP	93,63	97,78	93,71	95,04	2,37	2,50
DBahA	94,83	98,32	87,48	93,54	5,53	5,92

Good recoveries ranging from 93,5% (Dibenzoanthracene) to 99,3% (Benzo[b]fluoranthene) were obtained.

Intermediate precision: Intra-day repeatability was evaluated at 10 ng mL^{-1} (spike of 8 native standards) for each PAH through analysis of variance of 5 replicates after a 24 h migration cycle and elution through strata PAH column
Between-day reproducibility was evaluated at 10 ng mL^{-1} (spike of standard) for each PAH through analysis of variance of 5 replicates after a 24 h migration cycle and elution through strata PAH column over 3 days. Results are shown in the following Table 15.

Table 15. Repeatability (r) and Reproducibility (R)

	Mean (ng/mL)	RSD(r) d1	RSD(r) d2	RSD(r) d3	RSD(R)
BaA	11,15	1,3	3,7	2,3	4,2
CHR	9,97	1,5	5,3	1,2	3,5
BbF	10,22	1,2	3,5	2,9	3,9
BkF	10,49	2,8	3,5	5,6	4,3
BjF	9,73	2,1	7,1	4,7	7,3
BeP	9,90	1,4	2,7	1,9	7,5
BaP	10,59	3,0	2,0	1,8	9,9
DBahA	10,04	3,3	3,6	7,9	11,4

2.4.3 Aqueous sweat modification: artificial Skin Surface Film Liquid (SSFL)

At the same time of tests done with 20% aqueous EtOH, the modification of aqueous sweat simulant, was also considered [39]. In particular we tried to artificially mimic the complex liquid layer that covers the outermost layer of human skin, (the stratum corneum) which is composed of mainly sweat and sebum excreted, respectively, from sweat and sebaceous glands, [27, 29]. The combination of these two components is known as skin surface film liquid (SSFL) [26].

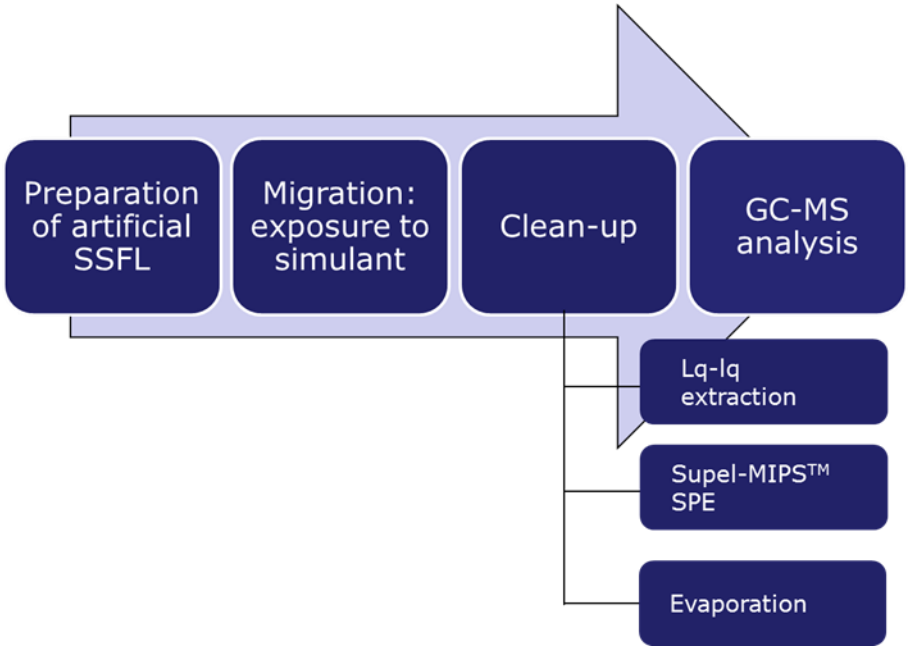
There are two main challenges in preparing comprehensive (sweat and sebum) SSFLs. Firstly, sweat and sebum are both complex mixtures the preparation of which is demanding and laborious, and secondly, the sweat to sebum proportion on human skin is currently not well known [26]. Only a very few studies conducted in the past have reported on SSFL compositions. Stefaniak et al. reviewed the compositions of 45 different formulations of artificial sweat and 18 formulations of artificial sebum [25]. In that study the authors referred to a work conducted by Buckley and Lewis in 1960 [40] on the rusting of metal when in contact with palmar sweat, which reports concentrations of 50 % of each component without however providing the source of evidence. Callewaert and co-workers published a note which focussed on the development of a novel artificial sweat composition to sustain and grow the autochthonous mixed skin axillary microbiome [41]. The three main constituents of their artificial sweat were amino acids, salts and fatty acids originating from methylated human abdominal subcutaneous fat at a concentration of 1,6 %. Another indirect indication on the proportions of sweat and sebum is found in the American ASTM Standard Guide for Evaluating Stain Removal Performance in Home Laundering [42] in which an artificial aqueous mixture of sebum (5 %) and particulate soil is one of the staining materials. However none of these studies provides scientific evidence for the choice of respective sebum concentrations.

For the scope of this project, the artificial SSFL was composed of a mixture of commercially available sebum prepared according to the Spangler procedure [43] (Pickering Laboratories, Mountain View CA, Product Code 1700-0700) suspended in the EN 1811:2011+A1:2015 artificial aqueous sweat [21]. A series of experiment were set to define the best formulation in terms of sebum and emulsifier content needed to obtain an emulsion sufficiently stable to allow migration tests.
As the first attempt, Polysorbate 80 was chosen as emulsifier and a variable amount of amount of molten sebum (0,5-5% in weight) was added to the aqueous sweat base.

As the result of this screening it was found that the best stability was achieved for emulsion containing 3-4% emulsifier and maximum 3% of molten sebum.

First migration run was then set-up according to the procedure designed as follows (Figure 10).

Figure 10. Steps of migration to artificial SSFL



Preparation of the migration medium: The sebum was melted on a heating plate, paying careful attention not to reach temperatures that would result in thermal decomposition. The exact volumes of molten sebum and polysorbate 80 for the corresponding theoretical volume percent concentrations of 0.5 %, 1 %, 2 % sebum and 4% emulsifier were added to the artificial sweat solution pre-heated up to approximately 70 °C. This was followed by immediate and thorough manual shaking for a couple of minutes. The SSFL containing glass containers were subsequently placed on a mechanical horizontal shaker at 300 rpm for 20 minutes.

Migration step: NR BR CB375 DAE was chosen as test sample. Migration test conditions were the same as already described for aqueous biofluids and 20% EtOH both in terms of loading factor (0.2 dm^2 20 mL^{-1}), temperature (40 °C) and quality control/quality assurance (presence of blank and control solutions). Migrations tests were conducted for 1 hour and for 4 hours. After the migration test was terminated, the rubber specimens were removed and the internal standard solution was added to the laboratory glass bottles.

Extraction of PAH from skin surface film liquid and extract clean-up: The planned procedure foresee a step of liquid liquid extraction, followed by purification of the concentrated extract through solid phase extraction (SPE) cartridges filled with molecularly imprinted polymer (MIPs) and GC-MS analysis.

Unfortunately, the presence of emulsifier prevented the target molecules to be extracted from the organic phase.

Further stability experiments were then conducted without the use of the emulsifier, in order to understand if was possible to obtain an emulsion sufficiently stable for testing purposes. Experiments indicated that without emulsifier and with max 2% sebum, the emulsion was stable up to 4h.
Migration from NR BR CB375 DAE was tested in EN 1811:2011+A1:2015 aqueous Sweat containing 0,5%, 1%, 2% sebum (V/V%) for 1 hour and 4 hours under dynamic conditions at 40 °C.

Once proved that migration protocol was suitable for obtaining a detectable and quantifiable migration, additional materials and SSFL compositions were tested (Table 16).

Table 16. Summary of materials tested in SSFL

Material	Conditions
NR BR CB375 DAE	0,1% 0,2%, 0.5%, 1%, 2% sebum; 1h and 4h; 40 °C
EPDM CB375 DAE	0,1% 0,2% 0,5% sebum; 1h; 40 °C
NR BR 550 DAE	0,2% 0,5% sebum; 1h; 40 °C
EPDM 550 DAE	0,2% 0,5% sebum; 1h; 40 °C
Silicone N375	0,5% sebum; 1h; 40 °C
PVC CB772	0,5% sebum; 1h; 40 °C
LDPE 40% CB772	0,5% sebum; 1h; 40 °C
PS 40% CB772	0,5% sebum; 1h; 40 °C

Results are shown and discussed in section 3.3.2 of this report.

2.4.4 Migration of EU-PAHs from NR BR onto sebum imbued filter paper strips

In addition to the migration tests conducted with skin surface film liquid (SSFL) as migration medium, an alternative approach was explored in which paper strips imbued with pure sebum acted as simulant of the lipophilic layer on human skin. In contrast to SSFL where the liquid simulant is continuously wetting the sample material, the imbued strips reflect more the physical contact between the lipophilic skin layer and the product.

The rubber test material used for this study was a natural/butadiene rubber (NR/BR) blend containing 24,1% N375 grade carbon black and 2,7 % distillate aromatic extract (DAE).

Preparation of sebum imbued filter strips: Both the rubber samples and the filter material were cut into squares with a single side area of 0,09 dm^2. The commercially available sebum was melted in a narrow beaker avoiding temperatures thermally decomposing the sebum. The filter paper strips were immersed into the molten sebum containing beaker. Excess of sebum was removed by wiping the filter strips onto a cleansing tissue. Immersion and removal of excess sebum proved to be very important for the homogenous application of sebum on the strips. Importantly all filter strips were imbued with approximately the same amount of sebum.

Migration tests: Rubber samples and blanks each in three replicates were placed on a glass dish and covered each with one of the sebum imbued filter strips of the same size covering its whole area (single side). A second glass dish was placed on the samples paying attention not to move the filter strips from the rubber samples. A weight of approximately 1,5 kg was placed on the upper glass dish assuring tight contact between rubber and filter strips. This setup was transferred into a climatic chamber set at 37 °C and 50 % relative humidity (rH). Migrations tests were conducted for 1 hour, 4 hours and 24 hours.

Extraction of PAH from sebum imbued filter strips and extract clean-up: after the migration test the filter strips were transferred into glass vials and extracted with hexane

on a rotatory shaker for 60 minutes. After the extraction was completed, the filter strips were removed from the vials and the volume of hexane extract was reduced with a sample concentrator under nitrogen flow at a temperature of 40 °C to a volume of approximately 2-3 mL. The concentrated sample extract was cleaned-up using solid phase extraction (SPE) cartridges filled with molecularly imprinted polymer (MIPs). The resulting ethylacetate extract was evaporated to dryness and reconstituted in 1 mL toluene for GC-MS analysis.

Results are shown in section 3.3.3 of this report and a detailed protocol including sample preparation and migration test can be found in Annex 5.

3. Results and discussion

3.1 Evaluation of the total content of EU-PAHs from plastic and rubber materials

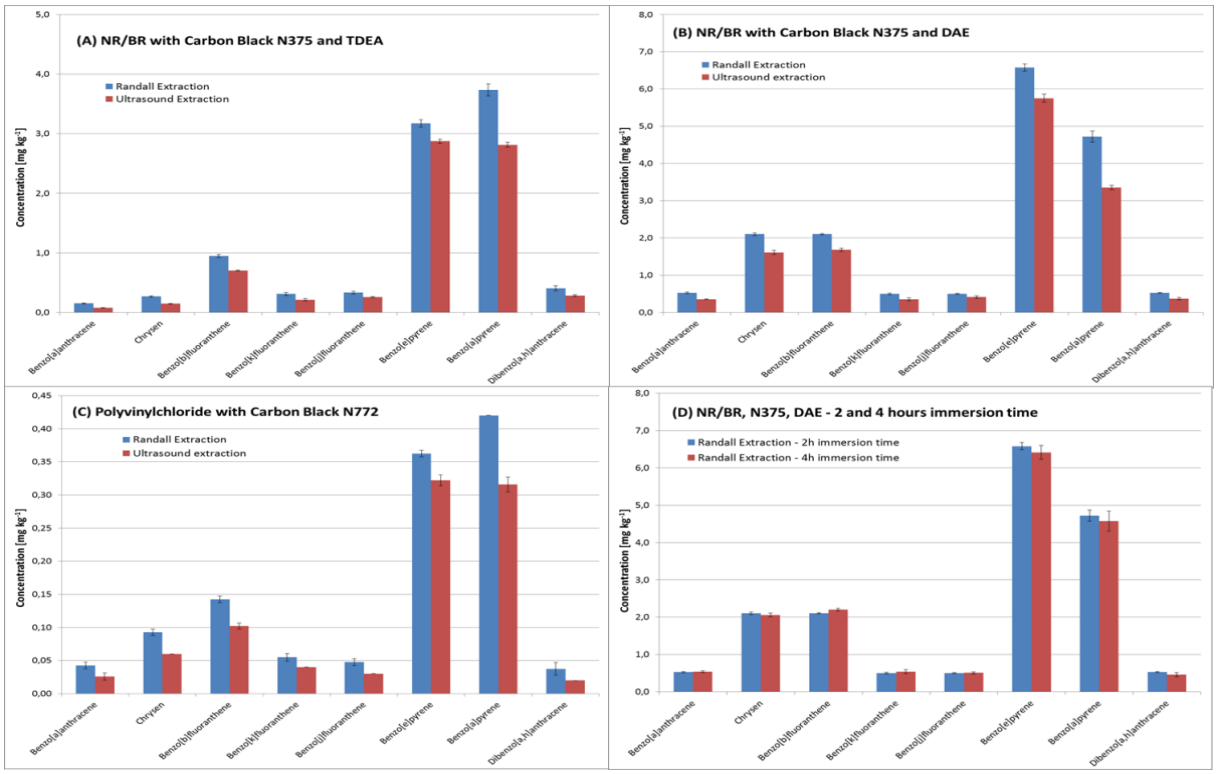
3.1.1 Extraction

The selection of a suitable solvent is crucial for the extraction process as its effectiveness depends on the solvent's polarity and boiling point. Due to the large surface area of carbon black, it has a relatively strong adsorption affinity for PAHs, with extractability becoming optimal at high temperatures. The two most commonly described [7, 9, 17-19] solvents for the extraction of PAHs in various matrices are toluene and hexane/cyclohexane. Hexane has a lower polarity index (close to the polarity index of the highly lipophilic PAHs). However, its boiling point is around 40 °C lower than for toluene. For this reason, toluene was given preference over hexane as extraction solvent.

3.1.1.1 Randall hot extraction compared to ultrasound extraction

Randall hot extraction and ultrasonic extraction were compared with regard to their extraction efficiency for the materials under investigation and the conditions used. With the exception of the extraction technique, all other steps were identical (clean-up with SPE and analysis).

Figure 11. Comparison of PAH extraction from various polymeric materials (A,B,C) and extraction time (D) using Randall hot extraction and ultrasound extraction (A) NR/BR with carbon black N375 and TDEA, (B) NR/BR with carbon black N375 and DEA, (C) soft-PVC. (D) Extraction efficiencies for 2-hour and 4-hour immersion times.



Mass concentrations achieved when extracting with Randall hot extraction were always higher compared to the concentrations obtained with ultrasound extraction (Figure 11). This difference appears to be independent of the PAH content (Figure 11A and B) and the type of material. The contents achieved with ultrasound extraction were between 10 – 40 % lower, depending on the specific PAH, suggesting that ultrasound extraction under these conditions may be less efficient compared to Randall hot extraction. Our extraction

procedure deviated from the AfPS protocol [9] in that the AfPS protocol extracts higher weightings of sample material in lower amounts of solvent. This deviation was necessary to ensure identical extraction conditions in terms of loading factor for the Randall and ultrasound extractions and should have no effect on extraction efficiency. The Randall hot extraction process represents an improvement over the classical Soxhlet extraction technique in that it considerably shortens the extraction time. Compared to the classical Soxhlet method where the condensed solvent is at a temperature below the boiling point, the Randall method has the sample material completely immersed in boiling solvent which provides great time savings as analytes are more soluble in boiling solvent. Other benefits of the hot extraction process include short process paths, low solvent requirements, and a process that is gentler on the extract (due to the shorter extraction period).

3.1.1.2 Completeness of extraction

The Randall hot extraction process can be split into 3 steps: (i) immersion: the thimble is lowered into the boiling solvent, (ii) rinsing/washing: the thimble is raised above the boiling solvent for a period of time until residual extract is removed from the solid material by the condensed solvent, and (iii) recovery: part of the solvent is removed from the extraction cup, concentrating the analytes for further processing. Within the margin of error provided by the standard deviations, no difference in mass concentrations (content) was observed between immersion times of 2h and 4h (Figure 1D) which indicates that the extraction was already complete after immersing for 2h. Hamm and co-authors [7] investigated the extraction time/cycles necessary to obtain complete extraction of PAHs from carbon black with traditional Soxhlet extraction [44, 45] and concluded that 16 hours (320 cycles) with toluene were required. Randall-extraction is known to shorten extraction times by a factor of 4 to 5 when compared to classical Soxhlet extraction. This is equivalent to a total extraction time of 3-4 hours (including all three steps) and confirms the quantitative extraction of all PAHs in our study.

3.1.1.3 Estimation of trueness

In the absence of a certified reference material, trueness - defined as the closeness of agreement between a test result and a reference value - could not be directly determined. However, the content of distilled aromatic extract (2,7 %) and carbon black (24,1 %) were known for the NR/BR blends and each of these two ingredients were separately available and could be analysed with regard to their PAH content. Assuming that carbon black and the distilled aromatic extract were the only sources of PAHs, the theoretical final mass-concentration for the test-material could be determined from

$$c(\text{PAH, mg kg-1}) = c(\text{PAH}_{\text{CarbonBlack}}, \text{mg kg-1}) \times \text{Content in rubber [\%]} + c(\text{PAH}_{\text{DAE/TDAE}}, \text{mg kg-1}) \times \text{Content in rubber [\%]}, \text{[9]}$$

where $c(\text{PAH}_{\text{CarbonBlack}})$ is the individual PAH concentration in the carbon black (N375) and $c(\text{PAH}_{\text{DAE/TDAE}})$ the individual PAH concentration in the treated/untreated distilled aromatic extract, respectively. These concentrations are multiplied with their respective relative contents. Results are generally in good agreement (Table 17) with only benzo[a]pyrene showing a higher discrepancy between the theoretical and the measured values for both NR/BR blends. Unfortunately, these results cannot be used for a fully quantitative trueness evaluation of the method as the values provided by the manufacturer for the content of carbon black and distilled aromatic extract can only be considered semi-quantitative estimates.

Table 17. Theoretical and measured mass-concentrations of PAHs in NR/BR blends

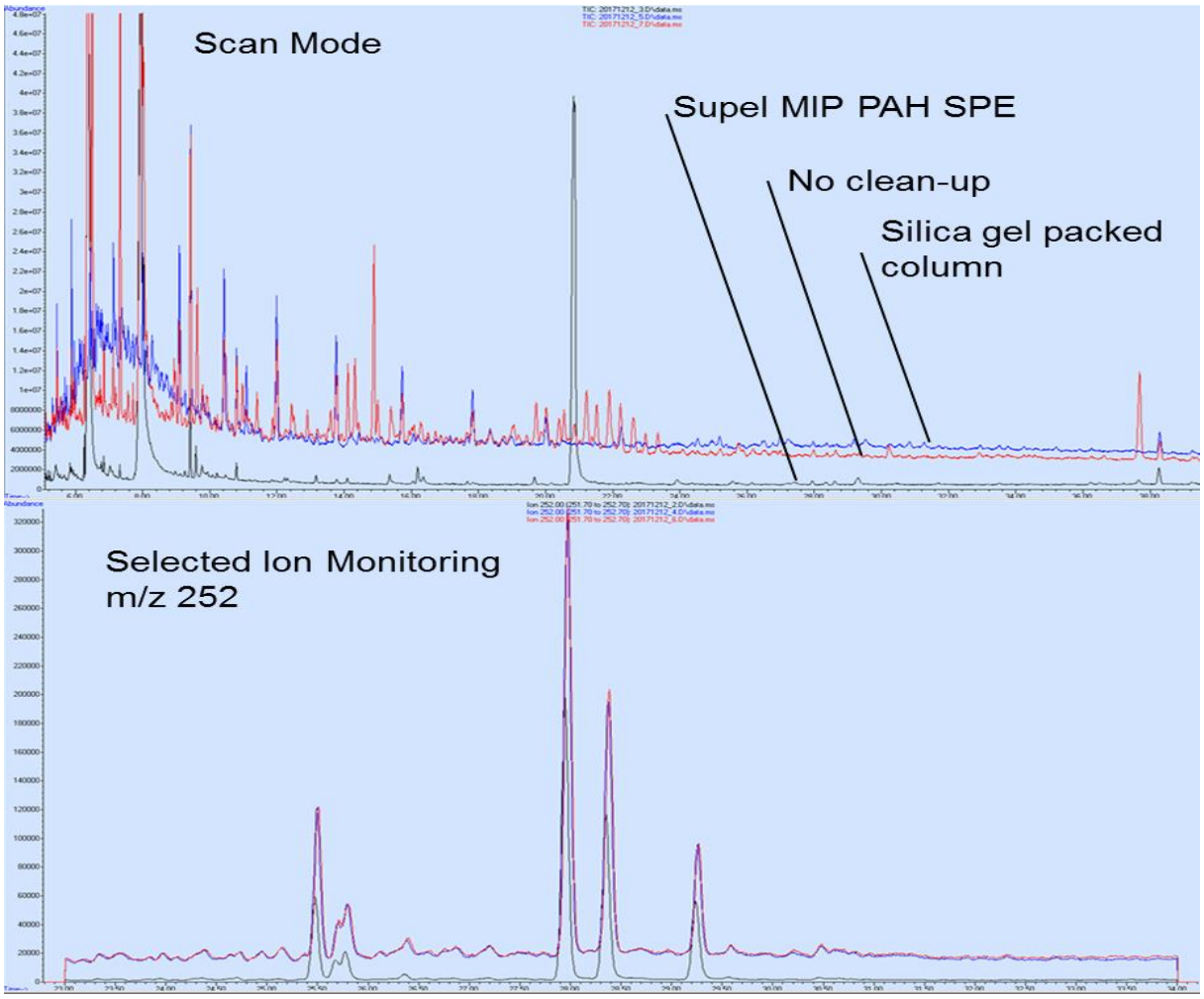
	Mass Concentration [mg kg^{-1}]						
	Carbon black N375	**Treated Distillate Aromatic Extract (TDAE)**	**Distillate Aromatic Extract (DAE)[1]**	**NR/BR, N375, TDAE**		**NR/BR, N375, DAE**	
				Th	**Meas**	**Th**	**Meas**
BaA	0,8	0,16	18,3	0,2	0,2	0,7	0,5
CHR	1,3	0,57	88,0	0,3	0,3	2,7	2,1
BbF	4,9	0,28	40,1	1,2	0,9	2,3	2,1
BkF	1,7	0,1	6,2	0,4	0,3	0,6	0,5
BjF	2,0	0,04	8,4	0,5	0,3	0,7	0,5
BeP	14,7	1,16	101,3	3,6	3,2	6,3	6,6
BaP	20,2	0,21	25,4	4,9	3,7	5,5	4,7
DBahA	1,4	0	2,2	0,3	0,4	0,4	0,5

[1] Not available on the market. Used only for the purpose of this research project

3.1.2 Purification

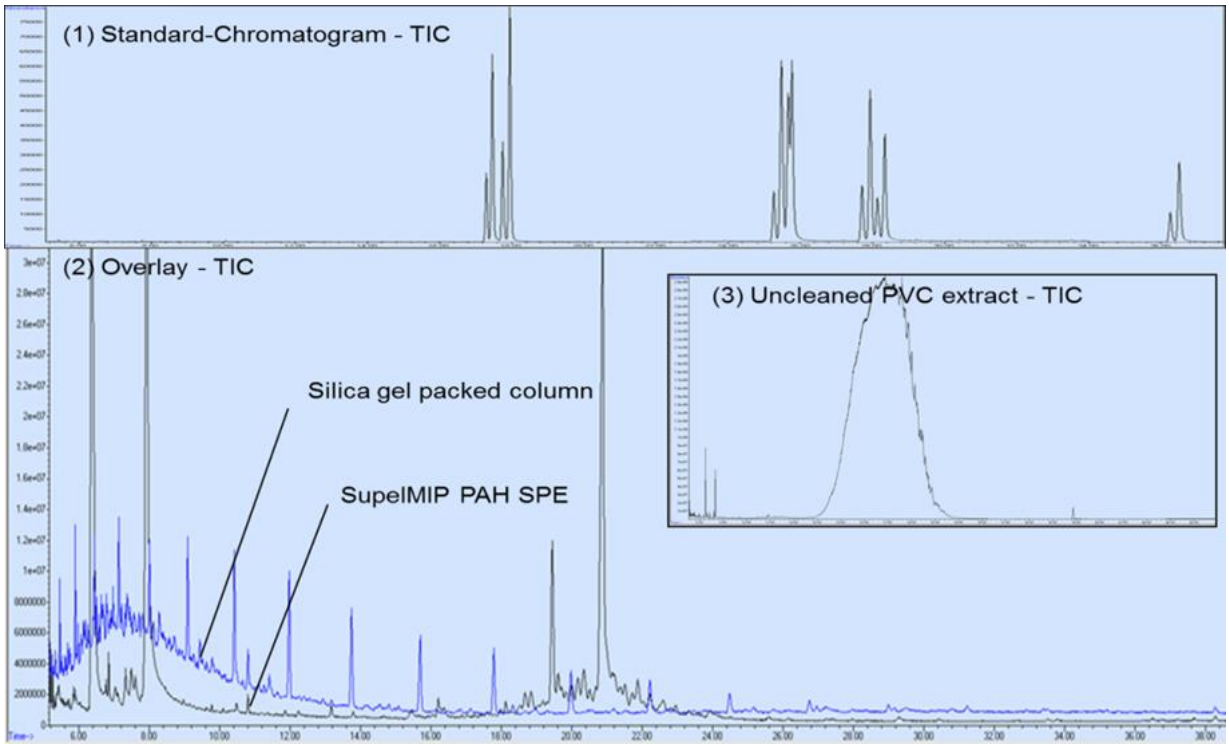
Rubber and plastic materials are complex matrices and during the extraction process a number of undesired substances such as monomers, aliphatic hydrocarbons, and aromatic hydrocarbons (other than PAHs), as well as plasticizer-additives are extracted together with the desired analytes. These contaminations lead to higher detection/quantification limits, accelerated dirtying of the mass-spectrometer, and - in the presence of phthalates - may render determination of the correct analyte outright impossible. For this reason, sample extracts undergo a clean-up before being injected into the GC-MS system. Many existing methods either forego this clean-up process entirely [19] or rely on a normal phase silica clean-up using SPE-cartridges [17] or silica-gel packed glass columns ,[7-9, 18]. In this work, we tested a new SPE-phase, based on molecularly imprinted polymer (MIP) technology, on extracts obtained through Randall hot extraction on both the NR/BR blend and the phthalate containing soft-PVC. MIPs are highly cross-linked polymer phases that have pre-determined selectivity for a single analyte or a group of structurally related analytes. To the best of our knowledge, these SPE cartridges have so far only been used for the extraction and analysis of PAHs in olive oil environmental water samples [46] and from tea leaves [47]. Having extracted the same amount (35 mg) of rubber for all three samples allows for direct comparisons of the areas below the different peaks in the obtained chromatograms (Figure 12). Clearly, the silica-gel packed column did not remove most of the undesired contaminants. Baselines from silica gel purified and unpurified extracts show similar total ion currents. Silica-gel primarily retains polar compounds which appear to be absent in the sample extract. In particular, the aliphatic hydrocarbons eluted in the first part of the chromatogram were not removed from the sample extract. The extract purified with the MIP-SPE columns shows a much cleaner chromatogram, indicated by the lower total ion current baseline. A dominant peak elutes around 21 mins deriving from the release of an additive from the frits used in the SPE-columns. However, the retention time of this peak did not interfere with the retention times of the analytes. Also in selected ion monitoring mode (lower part of Figure 12), the MIP-SPE baseline is lower compared to the other two.

Figure 12 Comparison clean-up efficiency NR/BR extract. Overlaid chromatograms of non-purified NR/BR extract, with MIP-SPE columns cleaned NR/BR extracts and with silica-gel packed columns purified NR/BR extracts in Scan mode (upper) and selected ion monitoring mode (lower). Sample weigh-in around 40 mg



Large amounts of phthalates were extracted together with the PAHs, which, even in selected ion monitoring mode, resulted in a relatively strong increase in the baseline signal which in turn needed to be removed before injection (Figure 13). Both the MIP-SPE and the silica-gel packed columns successfully removed almost quantitatively the more polar phthalates from the sample extract. By overlaying a standard chromatogram (panel 1 in Figure 13) onto the sample extract chromatogram (panel 2 in Figure 13), we demonstrate that the eight priority PAHs and their respective deuterated forms do not co-elute with impurities released from the frits of the SPE columns.

Figure 13. Soft-PVC extracts: (1) Standard chromatogram on same retention time scale as overlaid sample extract chromatograms in panel (2); (2)overlaid chromatograms of MIP-SPE column cleaned extract and silica-gel packed column purified extract ; (3) non purified sample extract.



3.1.2.1 Recovery experiments of SupelMIP™ SPE cartridges

Depending on the type of compound, PAH recoveries ranged from 51 – 95 % (Table 18).

Table 18. Recovery rates for priority PAHs purified on SupelMIPTM SPE cartridges

Compound	Absolute mass loaded on column [ng]	Average recovery [%]	RSD [%] n=5	Recovery of spiked olive oil[1] [%]
BaA	18	59	4,3	65
CHR	90	84	3,5	70
BbF	40	94	2,6	82
BkF	6	89	10,8	84
BjF	8	51	9,3	n/a
BeP	100	95	1,4	n/a
BaP	25	93	3,5	87
DBahA	2	91	3,2	82

[1] From Supelco application note 192

The lowest recoveries were found for benzo[a]anthracene and benzo[j]fluoranthene with recoveries of 59 % and 51 %, respectively. A similarly low recovery rate for benzo[a]anthracene has been reported in an application note for the extraction and analysis of PAHs in olive oils using SupelMIPTM SPE and can likely be attributed to the design and intrinsic functioning of MIPs, which are a class of highly cross linked polymer-based molecular recognition elements engineered to bind one specific target compound or a class of structurally related compounds. The MIP material is designed with cavities that are sterically and chemically complementary to the target analytes [48]. Compared to most other PAHs investigated in this study, benzo[a]anthracene has a relatively small molecular structure and might therefore be retained less efficiently. Also benzo[j]fluoranthene exhibited a relatively low recovery rate which can be attributed both to the relatively low absolute amount loaded onto the SPE cartridge and non-

baseline separation from benzo[k]fluoranthene which added uncertainty to the integration procedure (cf., RSD column in Table 15). All other recovery rates were found to be above 84 %.

The simple usage-protocol (fast), low amount of required solvents, commercial availability (no need to pack the column), and, above all, high selectivity resulting in lower baselines (i.e., lower detection limits), highlight the superiority of the MIP-based solid phase extraction procedure with regard to traditional, silica-based purification methodologies.

3.2 Total content analysis of ad-hoc manufactured materials, raw-materials and consumer articles purchased on the retail market

Applying the method described in section 2.2 and detailed in the standard operating procedure (Annex 2), all ad-manufactured test materials, a number of raw materials and selected consumer articles purchased on the retail market were analysed on their total EU-PAH content. The detailed description of the analysed materials can be found under Section 2.1.

3.2.1 Total EU-PAH content in ad-hoc manufactured test materials and raw materials

Table 19 reports the individual EU-PAH content of the ad-hoc manufactured test-materials and the raw-materials (carbon blacks and extender oils) supplied by industry partners.

The type and amount of carbon blacks and extender oils used in each of these materials is directly reflected in their PAH content. Having availability of plastic and rubber materials containing various levels of PAHs was exactly the purpose of using ad-hoc manufactured materials in this project for the migration studies.

Table 19. Total EU-PAH content of the ad-hoc manufactured test-materials and the raw-materials (carbon blacks and extender oils) supplied by industry partners. All concentration are expressed as mg kg^{-1}.

Sample	Sample Source	BaA	CHR	BbF	BkF	BjF	BeP	BaP	DBahA	Σ 8-EU PAHs
Carbon Black Elftex TP	*Cabot*	nd	nd	nd	nd	nd	nd	nd	nd	nd
LDPE 2,5% CB N772	*Cabot*	0,02	0,04	0,11	0,03	0,04	0,2	0,29	0,03	0,88
LDPE 40% CB N772	*Cabot*	0,32	0,80	1,67	0,66	0,55	4,95	4,91	0,39	14,25
LDPE 2,5% CB Elftex	*Cabot*	nd	nd	nd	nd	nd	nd	nd	nd	nd
LDPE 40% CB Elftex	*Cabot*	nd	nd	nd	nd	nd	nd	nd	nd	nd
PS 2,5% CBN772	*Cabot*	0,21	nd	0,09	0,03	0,03	0,28	0,31	0,02	0,97
PS 40% CB N772	*Cabot*	0,24	0,42	1,43	0,64	0,44	4,48	4,52	0,28	12,45
PS 2,5% CB Elftex	*Cabot*	nd	nd	nd	nd	nd	nd	nd	nd	nd
PS 30% CB Elftex	*Cabot*	nd	nd	nd	nd	nd	nd	nd	nd	nd
PVC with CB Elftex	*Polymer Chemie*	nd	nd	nd	nd	nd	nd	nd	nd	nd
PVC with CB N772	*Polymer Chemie*	0,03	0,06	0,13	0,05	0,04	0,37	0,40	0,05	1,13
Carbon Black N772	*Columbian Carbon Europe*	1,73	0,45	6,54	2,28	1,77	16,45	18,06	1,60	64,66
Carbon Black N330	*Cerisie*	0,08	0,19	0,44	0,13	0,22	5,52	6,38	0,36	13,32
Carbon Black N375	*Cerisie*	0,78	1,28	4,89	1,74	1,95	14,69	20,16	1,37	46,86
Carbon Black N550	*Cerisie*	0,59	0,67	0,94	0,68	0,53	0,63	0,91	0,09	5,04
Treated Distillate aromatic extract (**TDAE)**	*Cerisie*	0,16	0,57	0,28	0,10	0,04	1,16	0,21	nd	2,52
Distillate aromatic extract (**DAE)**	*Cerisie*	18,3	88,0	40,1	6,2	8,4	101,3	25,4	2,2	289,9
EPDM CB550 (25,6%), TDAE (2,8%)	*Cerisie*	0,11	0,02	0,18	0,13	0,13	0,11	0,18	nd	0,86

Sample	Sample Source	BaA	CHR	BbF	BkF	BjF	BeP	BaP	DBahA	Σ 8-EU PAHs
EPDM CB550 (25,6%), DAE (2,8%)	*Cerisie*	0,63	2,14	1,30	0,29	0,43	2,47	0,66	0,08	8,00
EPDM CB375 (25,6%), TDAE (2,8%)	*Cerisie*	0,07	0,14	0,82	0,29	0,40	2,68	3,72	0,32	8,44
EPDM CB375 (25,6%), DAE (2,8%)	*Cerisie*	0,65	1,93	2,12	0,42	0,89	6,43	4,40	0,21	17,05
NR/BR CB550 (25,6%), TDAE (2,8%)	*Cerisie*	0,09	0,10	0,15	0,10	0,11	0,09	0,11	nd	0,70
NR/BR CB550 (25,6%), DAE (2,8%)	*Cerisie*	0,55	2,12	1,17	0,25	0,40	2,38	0,59	0,03	7,49
NR/BR CB375 (25,6%), TDAE (2,8%)	*Cerisie*	0,06	0,12	0,75	0,27	0,30	2,36	3,05	0,27	7,18
NR/BR CB375 (25,6%), DAE (2,8%)	*Cerisie*	0,68	2,09	2,10	0,57	0,75	7,15	4,72	0,83	19,57
Silicone CB550 (4,3%)	*Cerisie*	0,02	0,03	0,02	0,01	0,01	0,03	0,01	0,04	0,17
Silicone CB375 (4,3%)	*Cerisie*	0,03	0,05	0,07	0,03	0,03	0,23	0,13	0,05	0,62
Recycled rubber granules Uncoated, <2010	*Conradi & Kaiser*	0,29	0,41	0,42	0,24	0,15	1,51	1,49	0,15	4,66
Recycled rubber granules Uncoated, >2010	*Conradi & Kaiser*	0,06	0,15	0,22	0,05	0,08	1,38	1,24	0,12	3,30
Recycled rubber granules Coated, <2010	*Conradi & Kaiser*	0,22	0,45	0,43	0,15	0,15	1,60	1,30	0,17	4,47
Recycled rubber granules Coated, >2010	*Conradi & Kaiser*	0,05	0,15	0,30	0,24	0,10	1,18	1,09	0,12	3,23
Recycled rubber granules Tiles, <2010	*Conradi & Kaiser*	0,32	0,49	0,38	0,18	0,15	1,29	1,13	0,12	4,06
Recycled rubber granules Tiles, >2010	*Conradi & Kaiser*	0,05	0,10	0,19	0,07	0,06	1,08	0,95	0,08	2,58
Extender Oil (OLBO) MC1-CAS 64741-88-4	*Concawe*	0,04	0,23	0,16	nd	0,03	0,39	0,11	0,03	0,99
Extender Oil (OLBO) MC2-CAS 64742-52-2	*Concawe*	nd	0,18	nd	nd	nd	0,02	nd	nd	0,20
Extender Oil (OLBO) MC3-CAS 64742-52-5	*Concawe*	nd	nd	nd	0,06	nd	0,16	nd	0,37	0,59
Extender Oil (OLBO) MC4-Mixture of CAS[1]	*Concawe*	nd	0,1	0,07	0,12	nd	0,76	0,07	0,22	1,34

Sample	Sample Source	BaA	CHR	BbF	BkF	BjF	BeP	BaP	DBahA	Σ 8-EU PAHs
Extender Oil (OLBO) MC5-CAS 64742-54-7, 64742-52-5	*Concawe*	nd	nd	nd	nd	nd	0,09	nd	0,14	0,23
Neoprene	*Decathlon*	3,99	14,55	3,05	nd	0,64	9,66	1,85	0,56	34,3

Absolute LOQ ranging between 0,6 and 3,6 ng mL^{-1}. Relative LOQ depends on the sample weight (for details please refer to Annex 2, point 15.1).

Nd. Not detected

3.2.2 Total EU-PAH content in consumer articles purchased on the retail market

The selection of the screened materials was random with no particular criterion except that they were made (or contained) plastic and rubber parts. This limited set of materials, acquired from the North Italian retail market, cannot be considered representative of consumer products available in the European market. Results of the EU-PAH content analyses cover a wide range in terms of measured PAHs concentrations as shown in Table 20.

Table 20. EU-PAH content of articles purchased from the retail market [mg kg-1the eight regulated PAHs determined by GC-MS in real samples. All concentration are expressed as mg kg^{-1}

Sample	BEP	BAP	CHR	BBF	BKF	BJF	BAA	DbA	Σ 8-EU PAHs
Car driving wheel cover (internal part)	135,1	26,2	72,6	40,6	10,5	11,5	22,2	12,5	331,2
Car driving wheel cover (external part)	15,4	5,1	28,2	5,5	1,9	2,0	13,7	1,0	72,7
Outdoor playground pad	4,7	1,7	1,9	1,5	0,6	0,8	1,1	nd	12,1
House pad	3,4	1,5	1,9	1,6	0,7	0,7	1,5	0,1	11,4
Junction for carpets	2,2	1,1	0,9	0,8	0,2	0,3	1,0	0,1	6,6
Sport inner soles	0,01	nd	0,02	nd	0,01	nd	0,01	nd	0,04
Gloves (butadiene)	0,02	0,01	nd	nd	nd	nd	nd	nd	0,03
Dinosaur toy	0,01	nd	0.01	nd	nd	nd	nd	nd	0,02
Watch wrist band	nd	nd	nd	nd	nd	nd	nd	nd	-
Swimming pool shoes	nd	nd	nd	nd	nd	nd	nd	nd	-

Nd. Not detected

3.3 Evaluation of the release of EU-PAHs from plastic and rubber materials

As already mentioned earlier in this document, migration of each of the 8 EU REACH PAH into aqueous artificial sweat and saliva was under the instrumental limit of detection. In the following sections, results of migration into 20% aqueous Ethanol as well as into artificial Skin Surface Film Liquid and onto sebum imbued filter paper strips are discussed.

3.3.1 20% aqueous Ethanol

All the available custom synthesised materials, plus PU coated and uncoated granule produced after 2010 were tested, in triplicate, at three different exposure times (1 hour, 4 hours and 24 hours). Testing conditions are described in section 2.3.2.

Qualitative results are reported in Table 21.

Table 21. Tested materials and qualitative evaluation of PAHs release

Rubber Matrix		Migration detected (Y/N)
NR BR	CB375 DAE	Y
	CB375 TDAE	N
	CB550 DAE	Y
	CB 550 TDAE	N
EPDM	CB375 DAE	Y
	CB375 TDAE	N
	CB550 DAE	Y
	CB 550 TDAE	N
Silicone	CB375	N
	CB550	N
Plastic Matrix		Migration detected (Y/N)
LDPE	2,5% CB772	N
	40% CB772	N
PS	2,5% CB772	N
	40% CB772	N
Soft PVC	CB772	N

Test Conditions: Dynamic exposure to 20 mL of 20% EtOH; 40 °C; 1h, 4h, 24h,
Tested surface: 0,2 dm^2, squared samples (2,0 cm x 2,0cm, 2mm thick)

From a qualitative evaluation of PAH release, some consideration can already be done. PAHs were not detected in cleaned-up extract coming from migration tests of plastic matrices differently from what observed for some rubber materials. In particular,

migration from rubbers seems to be related to the type of extender oil used in their formulation since no release was observed from rubber matrices containing treated distilled aromatic extract (TDAE). Furthermore, the fact that no release was detected from both Silicones suggests that extender oil, which is not contained in silicone matrices, has the major impact on determining PAHs release.

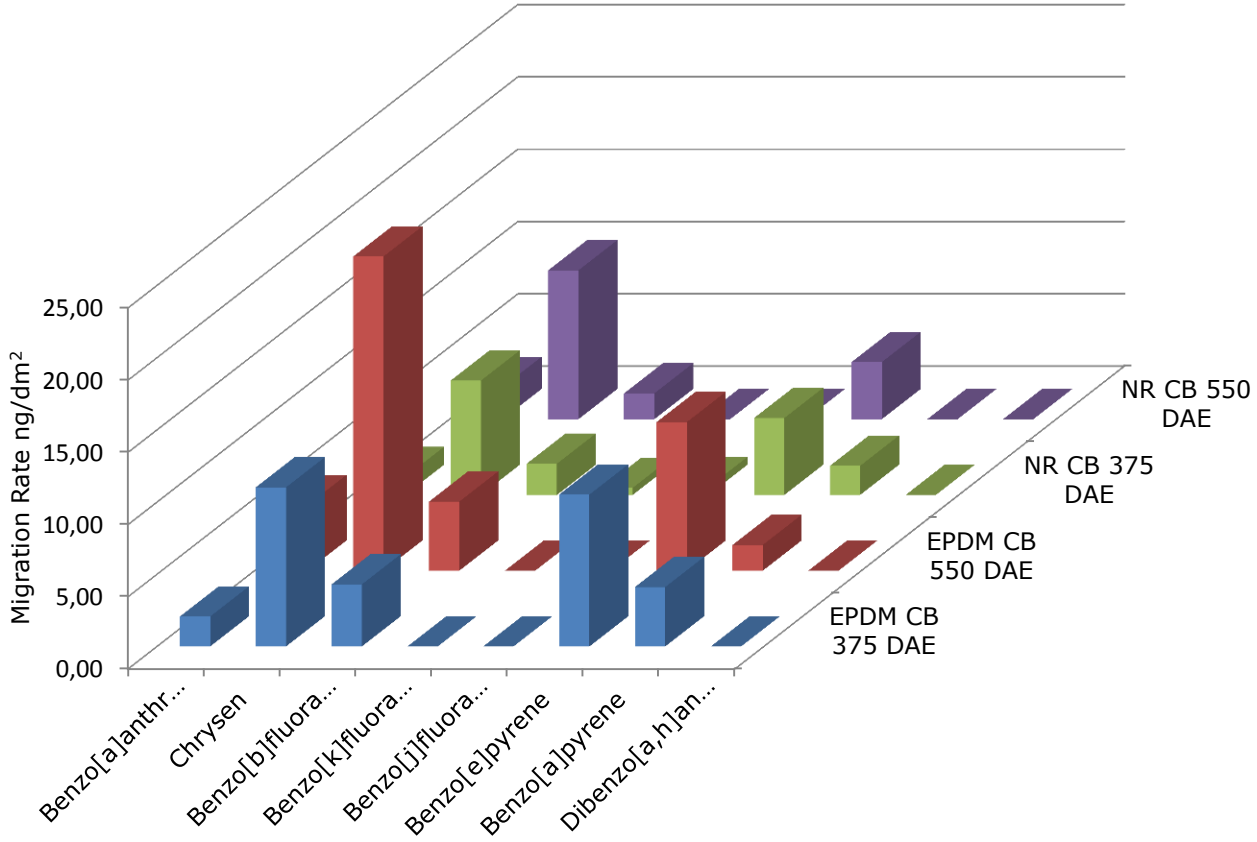
Quantitative results of 24 hours migration experiments, expressed both as migration values *per* surface (ng dm^{-2}) and as relative migration per content (wt/total wt %) are summarised in Table 22. For those materials which proved to release PAHs, the migration values (expressed per surface and as relative migration per content) are shown in Figure 14 and Figure 15 respectively.

Table 22. Results of 8-EU PAHs release in 20% aq EtOH as simulant (*per* surface and as relative migration in weight), 24 hours exposure, dynamic, 40 °C.

	B[a]A				CHR				B[b]F				B[k]F			
	EPDM 375 DAE	EPDM 550 DAE	NR BR 375 DAE	NR BR 550 DAE	EPDM 375 DAE	EPDM 550 DAE	NR BR 375 DAE	NR BR 550 DAE	EPDM 375 DAE	EPDM 550 DAE	NR BR 375 DAE	NR BR 550 DAE	EPDM 375 DAE	EPDM 550 DAE	NR BR 375 DAE	NR BR 550 DAE
Av (ng/dm^2)	**2,07**	**4,54**	**1,24**	**2,22**	**11,02**	**21,74**	**7,92**	**10,30**	**4,29**	**4,78**	**2,16**	**1,79**	nd	nd	**0,49**	nd
sd	0,49	0,26	0,38	0,34	2,30	0,91	0,72	1,72	1,61	1,37	0,82	0,37			0,85	
Av (µg/Kg)	**0,20**	**0,40**	**0,11**	**0,19**	**1,06**	**1,95**	**0,72**	**0,86**	**0,41**	**0,43**	**0,20**	**0,15**	nd	nd	**0,05**	nd
sd	0,05	0,02	0,04	0,03	0,22	0,23	0,09	0,14	0,15	0,16	0,08	0,03			0,08	
W/Total W %	**0,031**	**0,064**	**0,017**	**0,034**	**0,055**	**0,091**	**0,034**	**0,041**	**0,020**	**0,033**	**0,009**	**0,013**	nd	nd	**0,008**	nd
	B[j]F				**B[e]P**				**B[a]P**				**DB[a,h]A**			
	EPDM 375 DAE	EPDM 550 DAE	NR BR 375 DAE	NR BR 550 DAE	EPDM 375 DAE	EPDM 550 DAE	NR BR 375 DAE	NR BR 550 DAE	EPDM 375 DAE	EPDM 550 DAE	NR BR 375 DAE	NR BR 550 DAE	EPDM 375 DAE	EPDM 550 DAE	NR BR 375 DAE	NR BR 550 DAE
Av (ng/dm^2)	nd	nd	**0,54**	nd	**10,51**	**10,29**	**5,33**	**3,95**	**4,11**	**1,78**	**2,02**	nd	nd	nd	nd	nd
sd			0,93		1,96	2,18	0,60	0,60	1,98	0,30	0,56					
Av (µg/Kg)	nd	nd	**0,05**	nd	**1,02**	**0,93**	**0,48**	**0,33**	**0,40**	**0,16**	**0,18**	nd	nd	nd	nd	nd
sd			0,09		0,19	0,27	0,07	0,05	0,19	0,04	0,06					
W/Total W %	nd	nd	**0,007**	nd	**0,016**	**0,038**	**0,007**	**0,014**	**0,009**	**0,024**	**0,004**	nd	nd	nd	nd	nd

Average of 3 measurements. LOQ ranging between 0.1 and 0.6 ng mL^{-1}

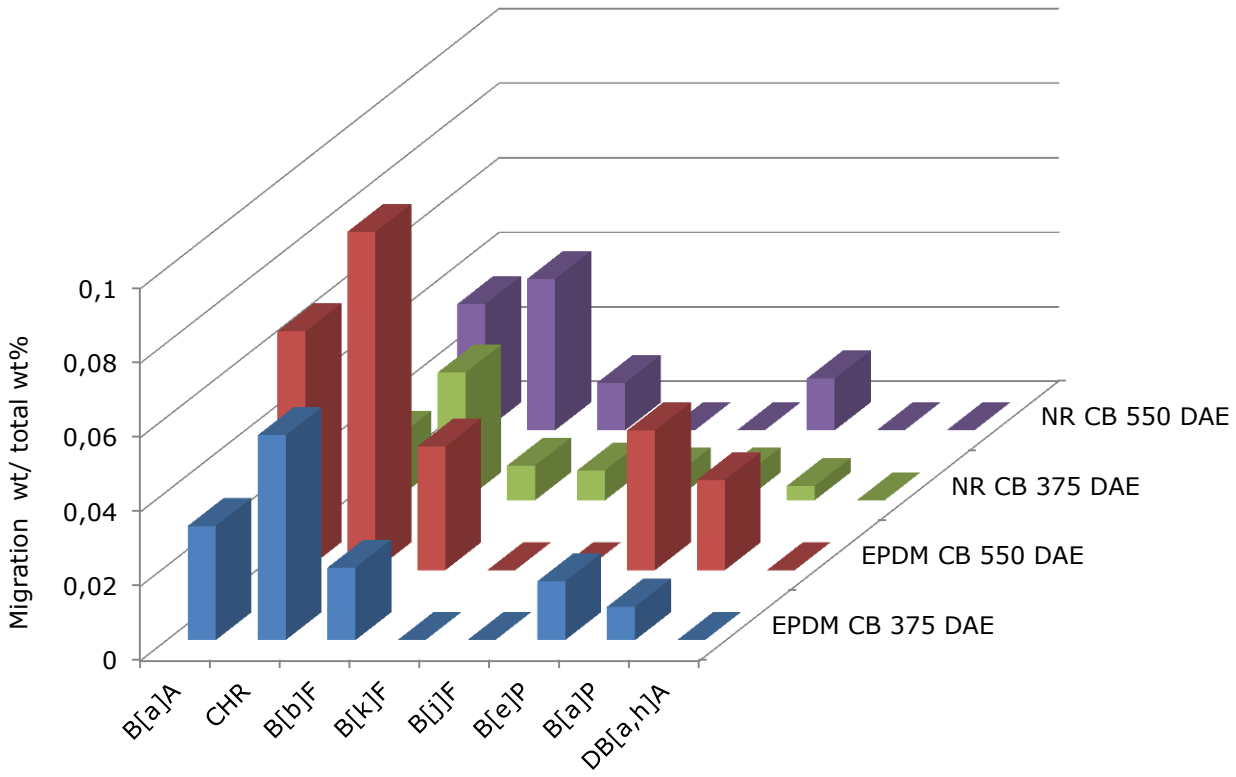
Figure 14. Migration per surface, into 20% aqueous EtOH at 24 hours 40 °C, dynamic mode,.



Migration values measured range from 0 to 21,74 ng dm^{-2}. The highest migration rate is observed for chrysene, 21,74 ng dm^{-2}, and benzo[e]pyrene, 10,51 ng dm^{-2}, which are respectively, the target molecule with lowest molecular weight and the one with higher total content in all the four materials. To interpret these results it must be considered that migration of chemical substances is a diffusion process which can be described by diffusion mathematics derived from Fick's Law. The diffusion process is a function of time, temperature, thickness of the material, amount of chemical in the material (elastomers are permeable materials) and the partition-coefficient/distribution-coefficient. Another important factor that controls the migration process is the mobility of the chemical in the specific matrix (in this case rubber) and depends on the size and shape of the molecule [49]. Most of these parameters such as temperature and material thickness are constant in the migrations test conducted in this study. The amount of migrated PAHs therefore primarily depends on the balance between their total content in the rubber specimen and their molecular structure.

The importance of total content measurement on the measured release of PAHs is more clear when migration is expressed as relative migration, calculated by normalising the absolute migrated PAH masses to their respective total contents in the samples (Figure 15).

Figure 15. Relative migration into 20% aqueous EtOH at 24 hours, 40 °C dynamic mode,.

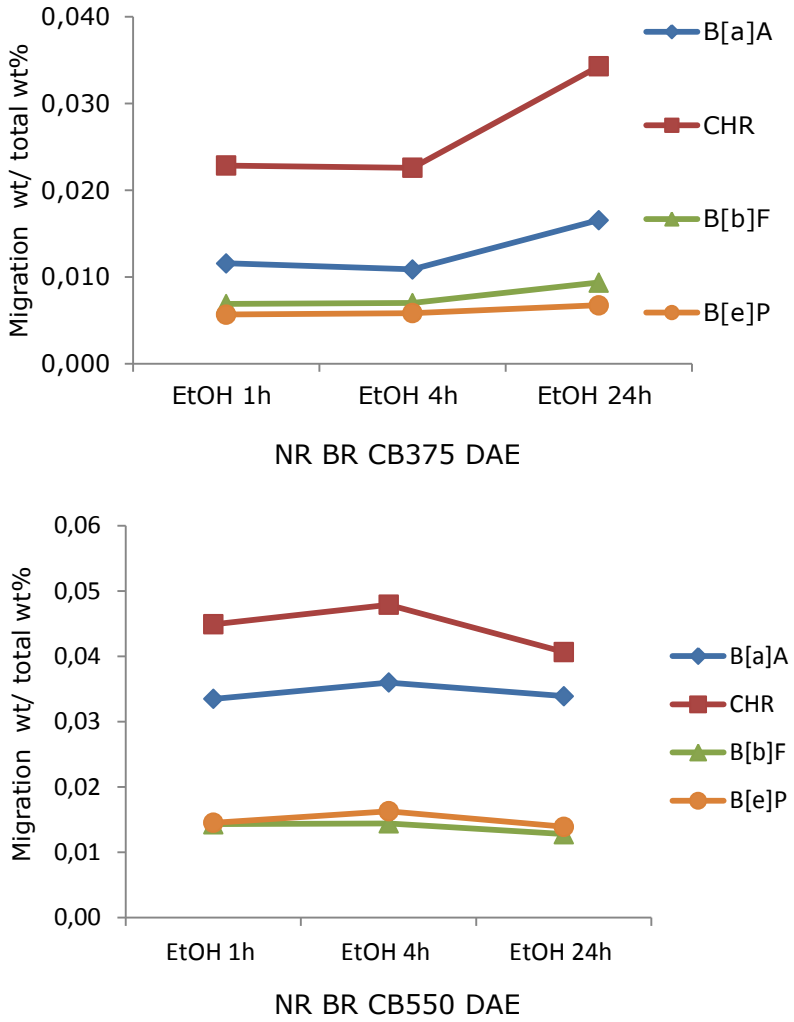


Relative migrations range from 0 to 0.09%, corresponding the maximum measured value to Chrysene. Despite the lower total content, the relative migration of benzo[a]anthracene, having the lowest molecular weight therefore the highest mobility, is higher when compared with the relative migration of benzo[e]pyrene (highest total content).

Another remark can be done by comparing the release of PAHs from the same material containing different carbon black. Despite materials containing CB550 shows lower total content in PAHs than the same material blended with CB375, migration is lower from matrices containing the latter. An interpretation could be that carbon blacks have the capacity of re-adsorbing PAHs already released (e.g. from extender oils). This effect would be actually enhanced by the higher surface area of CB 375, resulting in a lower effective release of PAHs.

As mentioned earlier in this document, migration was assessed at 1 hour, 4 hours and 24 hours and this allowed to gain some insights on the migration behaviour as a function of time. In the following Figure 16, relative migrations from NR BR CB375 and CB550, as examples, are plotted at 1 hour, 4 hours and 24 hours.

Figure 16. Release/Time dependency



From the analysis of NR BR CB375 DAE material it seems that the relative amount of PAHs that is released over the time is increasing, and the trend suggests that the system is tending to reach a plateau. Nevertheless, when the relative release is higher, as it is in the case of the analogous material containing CB550, it can be noticed that after a certain time there is a decrease of release of PAH, as if the system have a tendency to reach a release-re-uptake equilibrium. This means that time at which the release of a certain PAH is at his maximum, and the release-re-uptake equilibrium is reached, depends on a series of factors such as the mobility of the target molecule, its total content and the type of matrix.

The tendency of the system to reach release re-uptake equilibrium is even more evident when migration data of granules (Table 23) are plotted against time (Figure 17). In this case, the relative migration (ranging from 0 up to 2.7% for PU coated and to 8.6% for uncoated in the worst cases) is much higher if compared to NR BR and EPDM materials because of the larger surface in contact with the simulant.

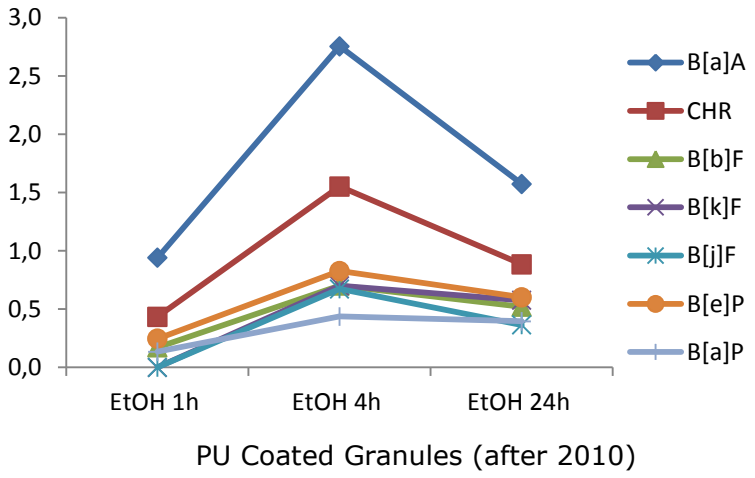
Table 23. Results of 8-EU PAHs release from PU coated and uncoated granules in 20% aqueous EtOH as simulant (*per* weight and as relative migration in weight), 1 hour, 4 hours and 24 hours exposure, dynamic, 40 °C

	Migration Rate µg/Kg			Relative Migration wt/total wt %			TOTAL CONTENT (mg/Kg)
PU coated granules	**EtOH 1h**	**EtOH 4h**	**EtOH 24h**	**EtOH 1h**	**EtOH 4h**	**EtOH 24h**	
BaA	0,47	1,38	0,79	0,94	2,75	1,57	0,05
CHR	0,65	2,33	1,33	0,43	1,55	0,88	0,15
BbF	0,52	2,10	1,56	0,17	0,70	0,52	0,3
BkF	0,00	1,68	1,38	0,00	0,70	0,58	0,24
BjF	0,00	0,67	0,36	0,00	0,67	0,36	0,1
BeP	2,91	9,75	7,11	0,25	0,83	0,60	1,18
BaP	1,47	4,77	4,32	0,14	0,44	0,40	1,09
DBahA	0,00	0,00	0,00	0,00	0,00	0,00	0,12

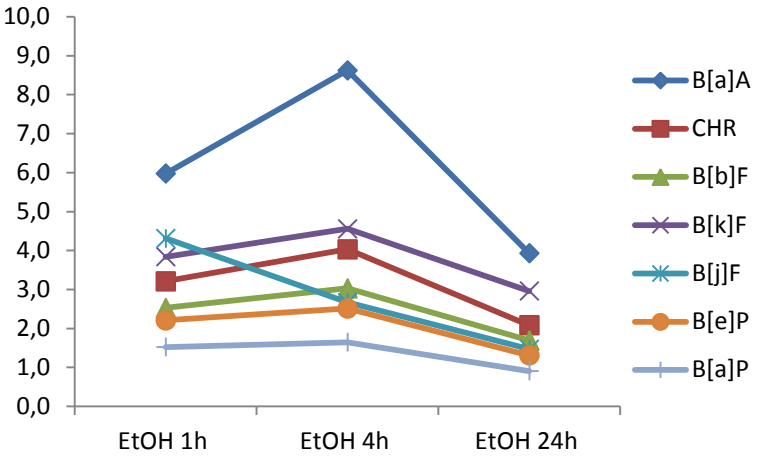
	Migration Rate µg/Kg			Relative Migration wt/total wt %			TOTAL CONTENT (mg/Kg)
Uncoated granules	**EtOH 1h**	**EtOH 4h**	**EtOH 24h**	**EtOH 1h**	**EtOH 4h**	**EtOH 24h**	
BaA	3,58	5,18	2,36	5,97	8,63	3,93	0,06
CHR	4,82	6,05	3,12	3,21	4,03	2,08	0,15
BbF	5,57	6,67	3,72	2,53	3,03	1,69	0,22
BkF	1,92	2,28	1,48	3,84	4,56	2,96	0,05
BjF	3,45	2,14	1,18	4,31	2,67	1,47	0,08
BeP	30,48	34,67	18,07	2,21	2,51	1,31	1,38
BaP	18,87	20,42	11,23	1,52	1,65	0,91	1,24
DBahA	1,57	1,87	0,00	1,31	1,56	0,00	0,12

Average of 3 measurements

Figure 17. Release over time, PU coated and uncoated granules (after 2010)



PU Coated Granules (after 2010)



Uncoated granules (after 20101)

It is also worth noticing that despite the total content is similar in PU coated and uncoated granules, migration from uncoated is up to 3 times higher, thus proving the efficacy of PU coating to act as a barrier.

3.3.2 Skin Surface Film Liquid

Plastic matrices containing the highest total content of the 8-EU PAHs and rubber matrices which proved to release PAHs when immersed in 20% EtOH were exposed to different SSFL formulations (refer to section 2.4.3). Testing conditions and qualitative results are summarised in Table 24.

Table 24. Tested materials and qualitative evaluation of PAHs release

Material	Conditions	Migration detected (Y/N)
NR BR CB375 DAE	0,1% 0,2%, 0.5%, 1%, 2% sebum; 1h and 4h; 40 °C	Y
EPDM CB375 DAE	0,1% 0,2% 0,5% sebum; 1h; 40 °C	Y
NR BR CB550 DAE	0,2% 0,5% sebum; 1h; 40 °C	Y
EPDM CB550 DAE	0,2% 0,5% sebum; 1h; 40 °C	Y
Silicone N375	0,5% sebum; 1h; 40 °C	N
Plastic Matrix		Migration detected (Y/N)
PVC CB772	0,5% sebum; 1h; 40 °C	N
LDPE 40% CB772	0,5% sebum; 1h; 40 °C	N
PS 40% CB772	0,5% sebum; 1h; 40 °C	N

In line with what already observed when using 20% aqueous EtOH solution as simulant, PAHs were not released from neither plastic matrices nor silicone, while it was possible to detect and quantify PAHs from NR BR and EPDM.

Migration rate *per* surface from NR BR CB375 DAE is plotted against percentage of sebum concentration (v/v) in sweat EN 1811 at 1 hour and 4 hours in Figure 18 and 19 respectively.

Figure 18. Migration rates (ng dm^{-2}) of 8 EU-PAHs from NR BR CB375 DAE to SSFL (0,1, 0,2, 0,5, 1 and 2% sebum in EN 1811 sweat) at 1h.

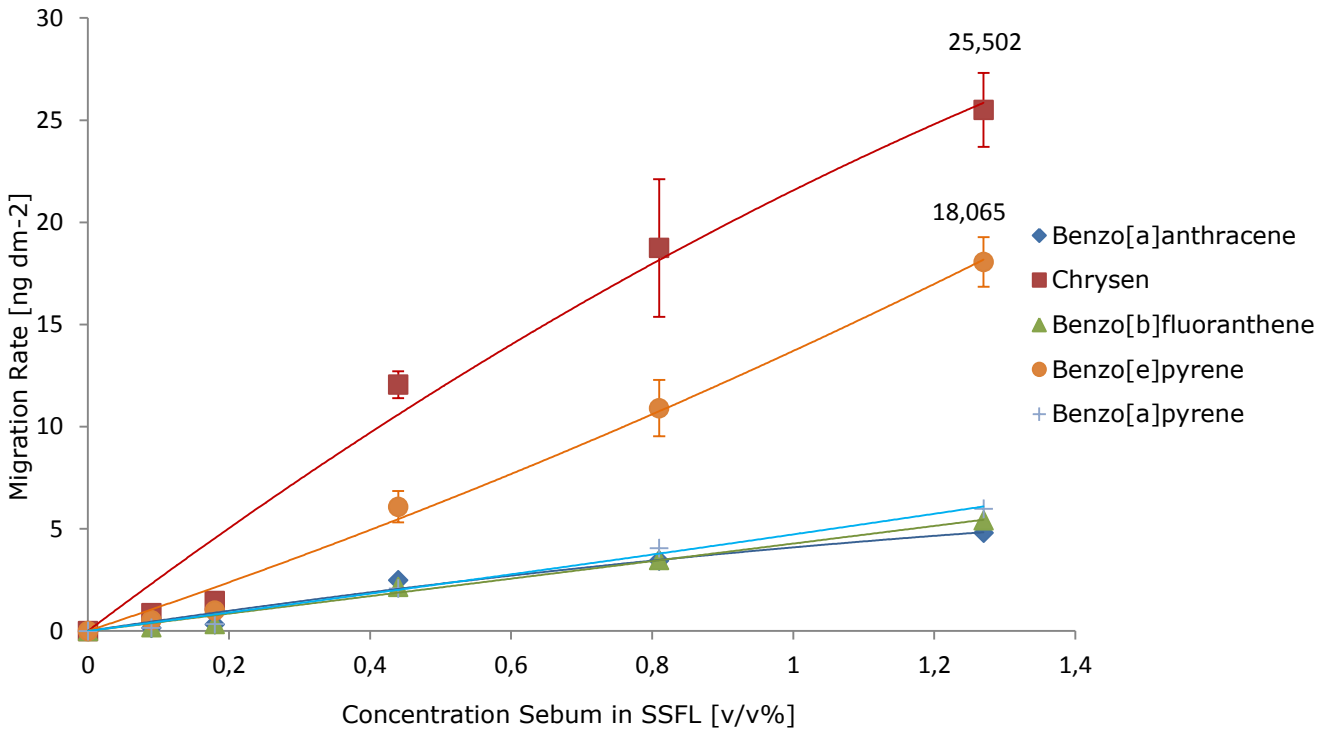
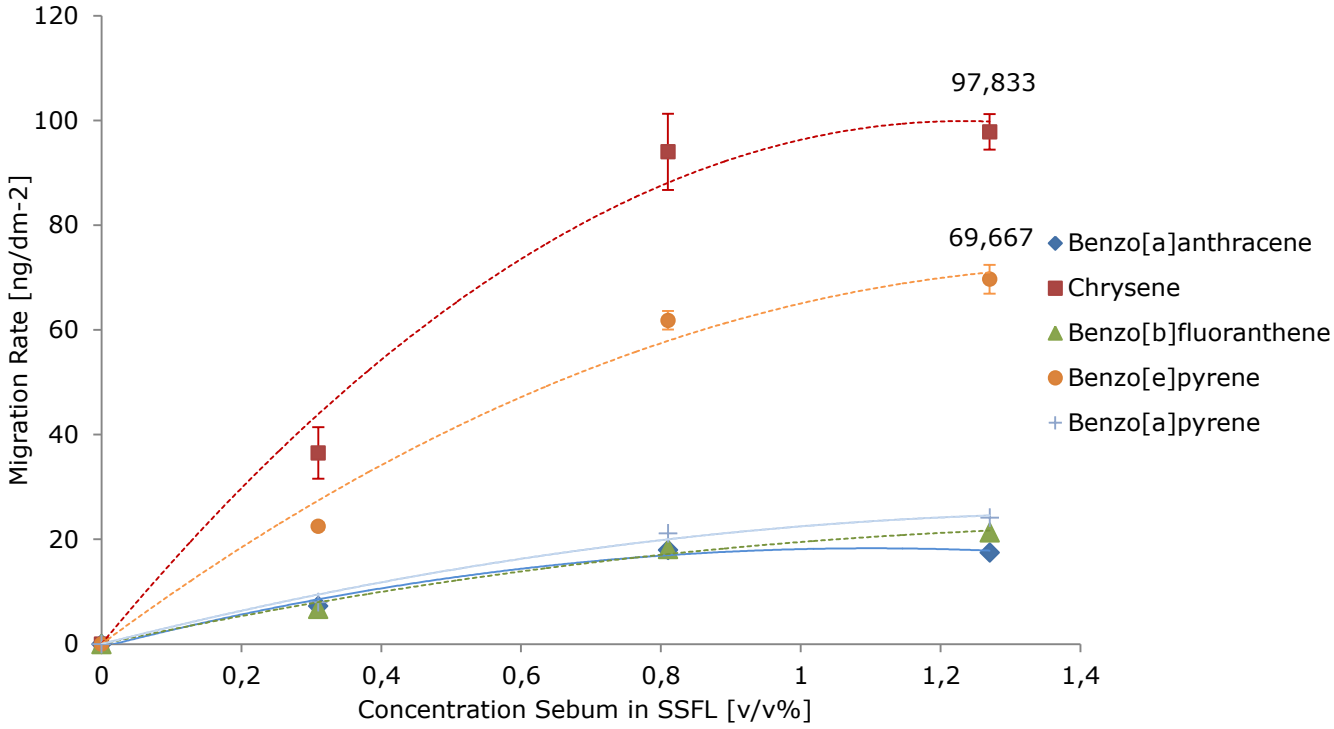


Figure 19. Migration rates (ng dm^{-2}) of 8 EU-PAHs from NR BR CB375 DAE to SSFL (0,1, 0,2, 0,5, 1 and 2% sebum in EN 1811 sweat) at 4 hours.



From the analysis of figures, it can be observes that migration to SSFL increases with migration time and with the sebum content, proving the significance of the presence of the lipophilic component in extracting target molecules.

The migration values into SSFL were compared to those obtained when using 20% aqueous Ethanol as simulant Figure 20 and 21 show the relative migration of the 8 EU PAHs from NR BR 375 DAE and EPDM 375 DAE, respectively, to SSFL (0,1, 0,2 and 0,5% in sebum) and 20% aqueous EtOH.

Figure 20. Comparison of relative migration of 8 EU-PAHs from NR BR CB375 DAE to 20% aq EtOH, 0,1% sebum SSFL, 0,2% sebum SSFL and 0,5% sebum SSFL

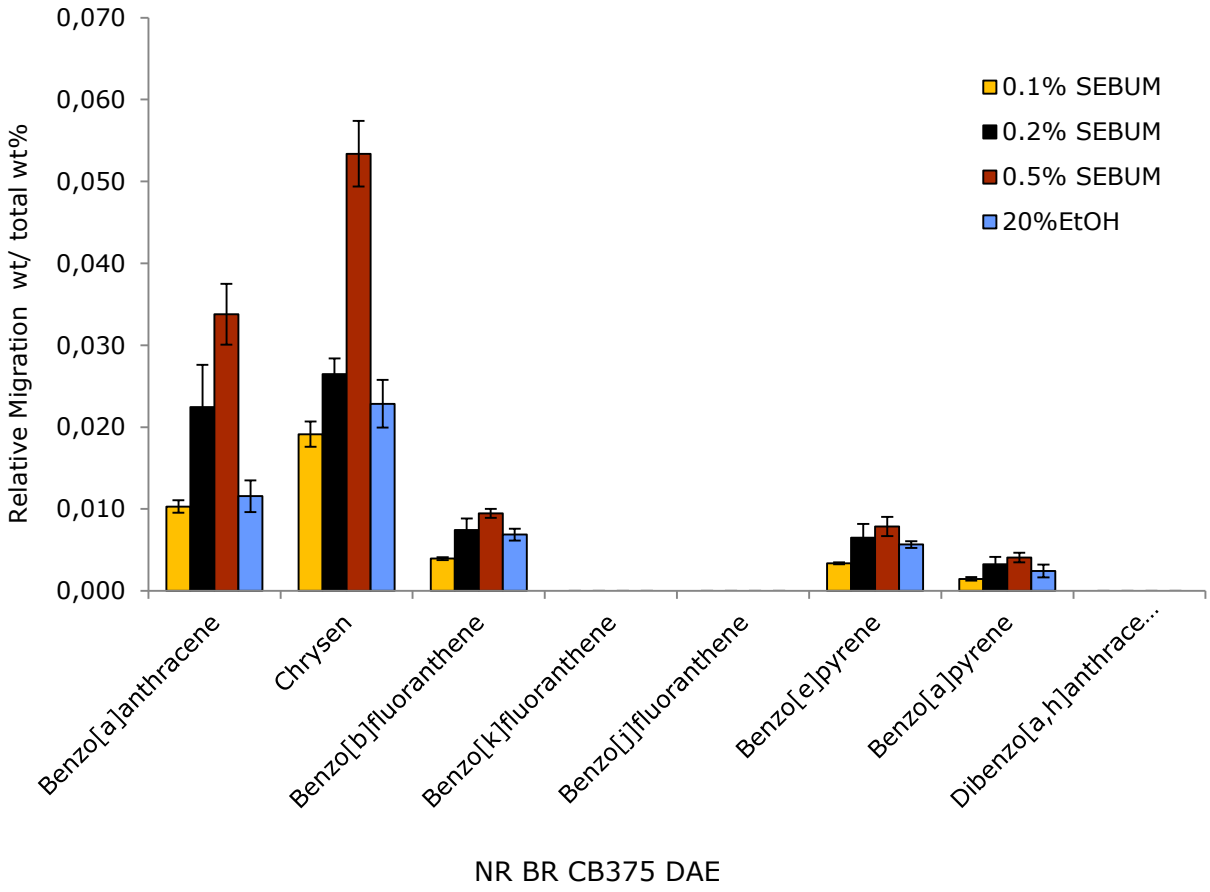
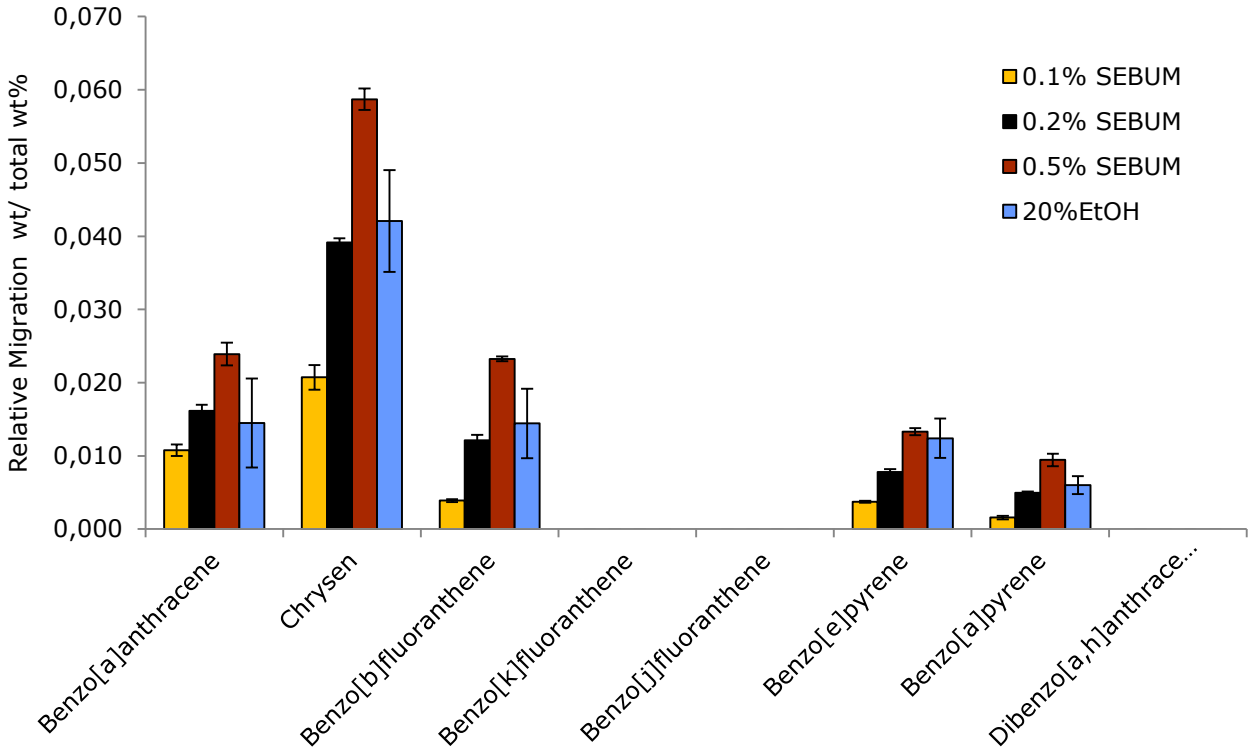


Figure 21. Comparison of relative migration of 8 EU-PAHs from EPDM CB375 DAE to 20% aq EtOH, 0,1% sebum SSFL, 0,2% sebum SSFL and 0,5% sebum SSFL



SSFL containing 0,1-0,2% sebum in EN 1811 Sweat showed good agreement with data obtained when 20% EtOH was used as simulant, which in turn, showed good agreement with human skin [39]. This fact suggests that using around 0.2 % sebum containing SSFL as a migration simulant is a reasonable proxy for real skin conditions relating to body parts with an average concentration of sebaceous glands [50], such as in the real skin originating from female abdomen, which is used in the Franz Cell chamber studies conducted by Bartsch et al. [33]. Sebum is an oily secretion produced by sebaceous glands, unevenly distributed in all areas of the body - with the exception of the palms and foot soles - for waterproofing purposes[51, 52]. Although there are no sebaceous glands in the skin of the palms or palmar surface of the fingers, sebum flows from the dorsal part of the hands to the palm and is also transferred when palms touch other skin surfaces of the body (e.g. the face) [40, 53]. It can therefore be assumed that the amount of sebum on the hands, and /or fingers, is comparable to other parts of the body which have an average-to-high distribution of sebaceous glands.

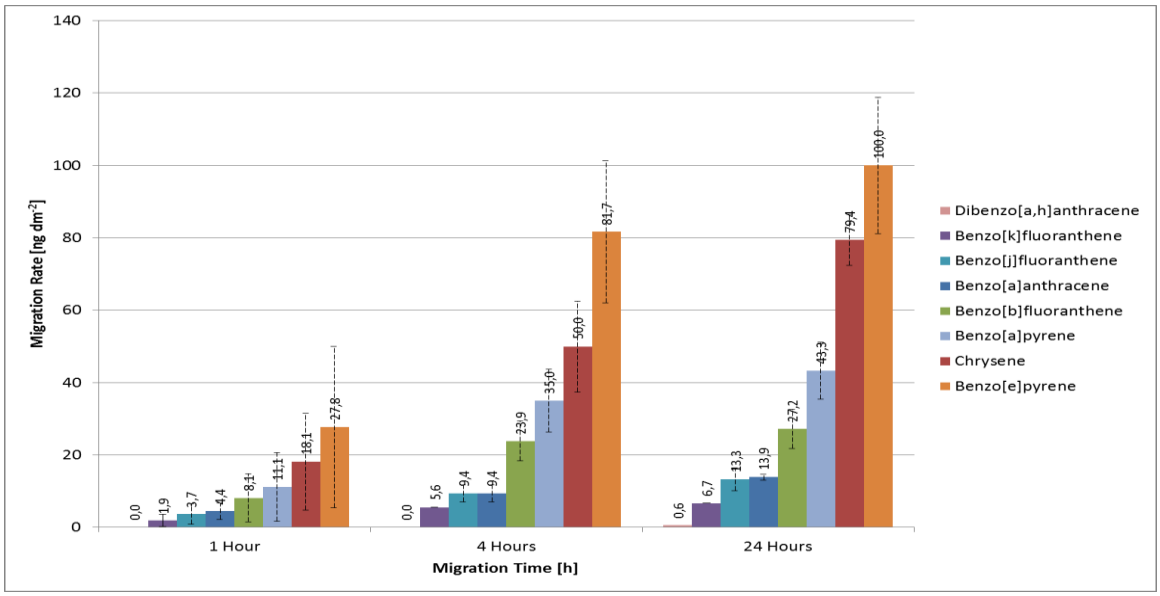
It is known that organic solvents can interact with polymeric matrices to cause their swelling, which can possibly have an impact on the migration behaviour of any contained PAHs. An advantage of using skin surface film liquid (SSFL) over these organic solvent based simulants is that being solvent-free it does not chemically interact with the polymeric matrices thus preventing their deformation and swelling.

On the other hand, its preparation is laborious, and could potentially lead to errors when measuring PAHs release

3.3.3 Sebum imbued filter paper strips

Figure 22 shows the migration rates of the 8 EU-PAHs from the rubber sample material into the sebum imbued filter paper at 1 hour, 4 hours and 24 hours. As expected, the migration rates increase with time. Remarkably the highest migration rate is obtained for benzo[e]pyrene, followed by chrysene. Migration tests done with SSFL (section 3.3.2) showed a reversed order of the migration rates for these two compounds. This indicates that the aspect of the molecules mobility seems to have a lower impact on the overall diffusion process in the strip tests compared to the use of SSFL as migration medium.

Figure 22. Migration rates at 1 hours, 4 hours and 24 hours



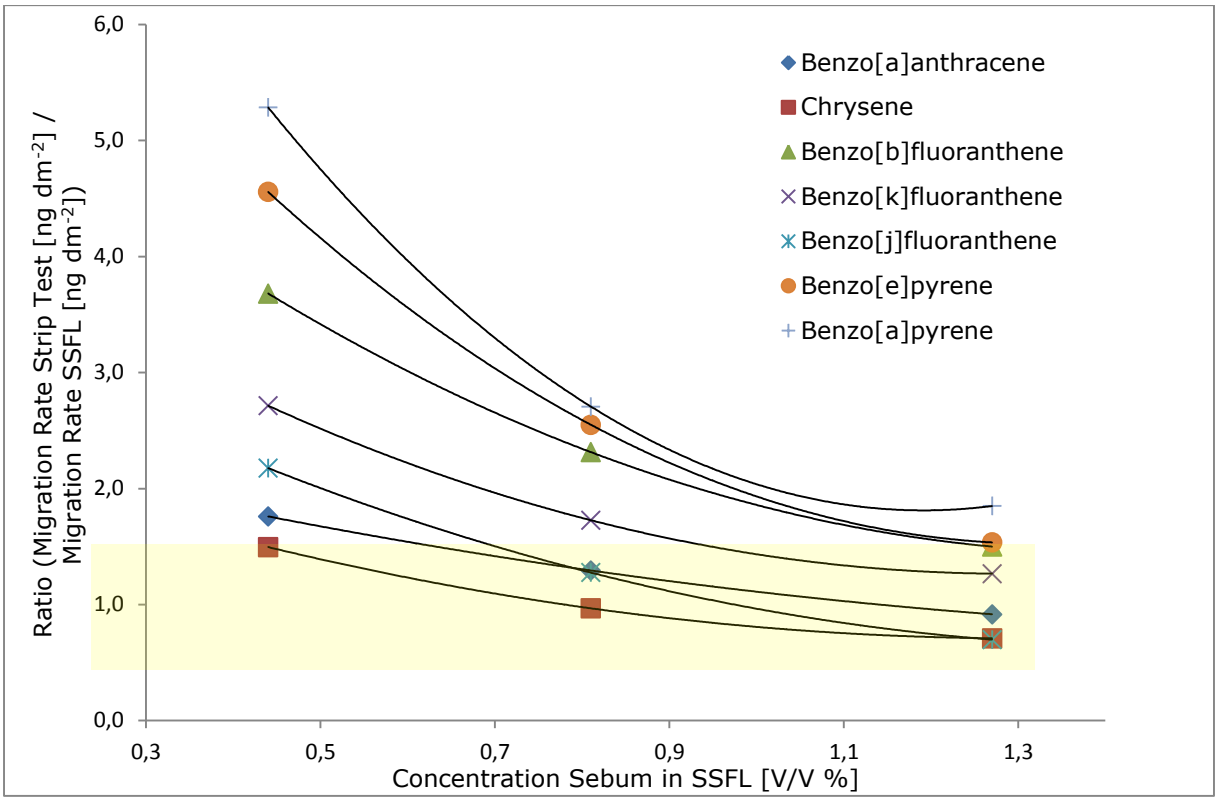
Columns 2 and 3 of the Table 25 report the migration rates of the investigated PAHs from the rubber material into the sebum imbued filter paper after 1 and 4 hours and compares them against the migration rates obtained when using SSFL with various percentages of sebum as migration simulant.

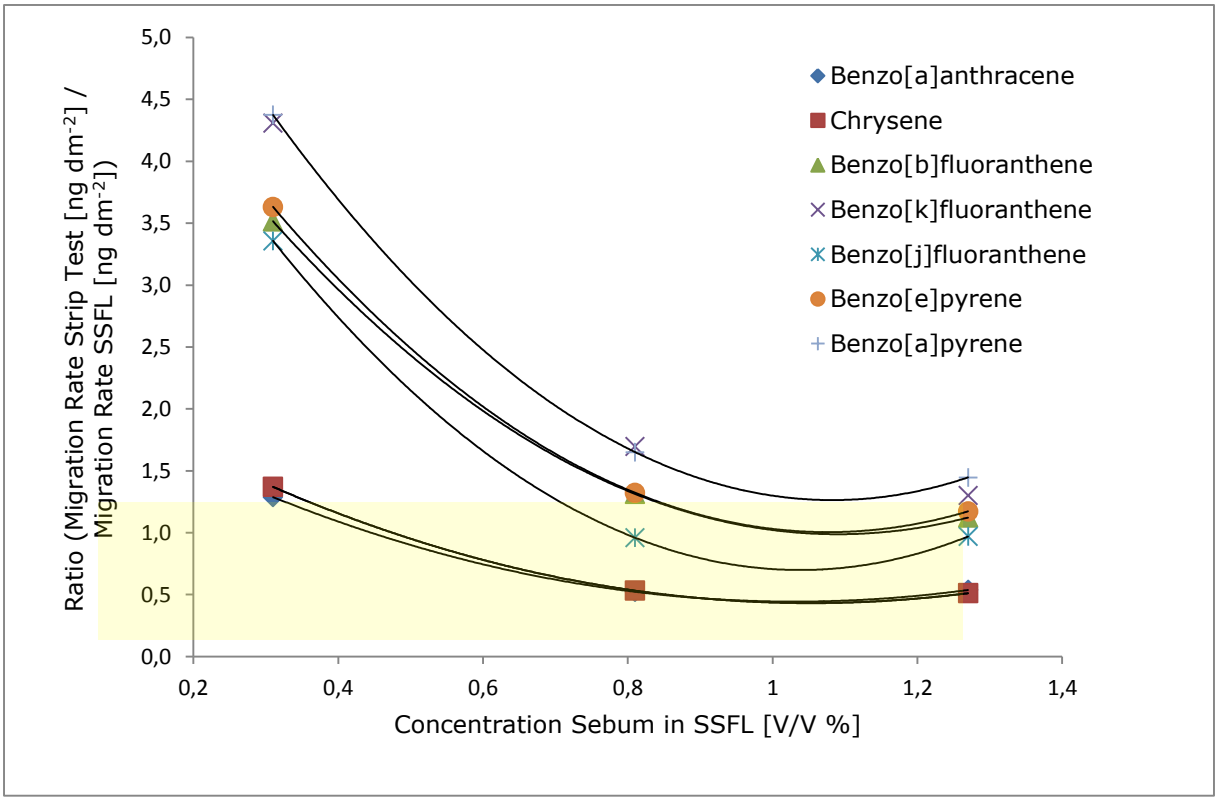
Table 25. Migration rates determined with sebum imbued strips and with SSFL at 1 hour and at 4 hours

	Migration Rates [ng dm^{-2}]							
	Strip-test		SSFL					
	1 hour	4 hours	0.44 % 1 hour	0.81 % 1 hour	1.27 % 1 hour	0.31 % 4 hours	0.81 % 4 hours	1.27 % 4 hours
BaA	4,4	9,4	2,5	3,4	4,8	7,3	18,0	17,5
CHR	18,1	50,0	12,1	18,7	25,5	36,5	94	97,8
BbF	8,1	23,9	2,2	3,5	5,4	6,8	18,2	21,3
BkF	1,9	5,6	0,7	1,1	1,5	1,3	3,3	4,3
BjF	3,7	9,4	1,7	2,9	5,3	2,8	9,8	9,7
BeP	27,8	81,7	6,1	10,9	18,1	22,5	61,8	69,7
BaP	11,1	35,0	2,1	4,1	6,0	8,0	21,2	24,2
DBahA	0,0	0,0	0,1	0,3	0,4	0	1,0	1,0

Figure 23 illustrates the ratios of the migration rates determined using filter paper strips and at various concentrations of sebum in skin surface film liquid. In the part highlighted in yellow the ratio is around 1, when migration rates for both migration media are similar.

Figure 23. Migration rates ratios of strip-tests and SSFL migration experiments at 1 hour (top) and 4 hours (bottom)





It appears that the migration rates are generally in the same order of magnitude and that the migration rates determined with the stripe test match best with skin surface film liquid containing around 1% of sebum.

Although imbued with pure sebum, the migration rates determined with filter-strips are in the same order of magnitude when compared with the migration rates obtained when using SSFL as migration simulant. A possible explanation for this unexpected finding is that the sebum on the filter strips is not homogenously and continuously in contact with the sample material over the whole surface, resulting thus in a migration rate that is lower than expected.

Although highly interesting from a research perspective, the approach of using sebum imbued filter-strips for alternative migration experiments was not considered further in this project due to the laborious and complex preparation procedure of the homogenously sebum-imbued strips that could difficult its standardisation.

4. Inter-laboratory study on the migration of EU-PAHs from plastic and rubber materials into 20% aqueous Ethanol

The objective of this preliminary inter-laboratory comparison was to obtain information on the method's applicability and transferability in a variety of laboratories as well as on its analytical performance.

The method under evaluation aims at determining the migration of the 8 EU-PAHs from rubber and plastic samples by total immersion into a simulant (20% ethanol).

The scope of this study was not to assess the individual performance of laboratories but to evaluate the analytical method proposed.

The exercise was launched in August 2017 and in the same month samples were prepared and tested for homogeneity.

A practical training took place at the premises of JRC in Ispra in October 19th.

The test materials, the instructions and the shipping kit were sent to participants at the end of October/beginning of November and the deadline to report results was set to the 15th of January 2018.

Twenty-one laboratories took part in this study, more than expected at the beginning of the project.

Not all participants were experienced in the migration of PAHs from rubber and plastic materials. Some had a lot of experience on migration in general (for instance from food contact materials), others had no experience on migration at all.

Many perform as routine analysis the determination of PAHs total content from different consumer products.

4.1 Test materials and sample preparation

Three samples were selected for the intel-laboratory comparison, 2 rubber and 1 plastic material:

- EPDM N375 DAE (Sample 1)
- NRBR N375 DAE (Sample 2)
- Soft PVC N772 (Sample 3)

EPDM and NRBR samples were chosen because of their high content in PAHs (See Chapter 2.2) while as PVC sample was selected for its low level of PAHs.

Samples have been manufactured ad hoc by industry as described in Chapter 2 and came as (20x20)cm sheets, 2mm thick (rubber) or (6x6)cm plates, 2mm thick (plastic).

Three (3*3) cm test specimens were cut from each material, making an exposed surface of 0,204 dm^2 for each of them. The weight varied from 2,2 to 2,7 grams depending on the material.

The cut test samples were packed in aluminium foil to avoid contamination. Each package contained 5 specimens (three were necessary to perform the analyses foreseen in the collaborative trial and the other two had to be considered as spare samples).

Ten specimens of each material were randomly selected and used for the homogeneity study and the rest were coded and prepared to be sent to the participants.

4.2 Homogeneity assessment

The homogeneity of samples was assessed for the total content of each of the 8 REACH PAHs in the three samples.

Homogeneity evaluation was carried out according to the test established in ISO 13528:2015 [39].

Ten randomly selected test specimens for each sample were analysed in duplicate in random order under repeatability conditions. Results are reported in Annex 2.

Homogeneity data were then examined visually for pathologies. No trends or non-random distribution of differences between duplicates test results were observed.

Homogeneity testing requires the computation of the following parameters: the analytical precision SD (analytical) (= repeatability standard deviation for one test portion, with-in sample) and the heterogeneity standard deviation SD (samples) (= standard deviation across the test portions for one particular sample, between samples).

In order to test for homogeneity according to ISO 13528 a target standard deviation SD (target) is required, against which to compare the resulting heterogeneity standard deviation SD (samples).

As the precision of the method was unknown, the homogeneity results and their statistical evaluation were obtained using the target standard deviation SD (target) calculated on the basis of the Horwitz equation [54].

In Table 26 are reported the homogeneity assessment for each PAH in the three samples.

Table 26. Samples' homogeneity evaluation

Unit	Measurand	Mean	SD analytical	SD sample	SD target	Homogeneity check (ISO 13528 section B.2.3)
µg/kg	BaA	741,95	30,01	0,00	124,14	Ok
	CHR	1507,79	170,95	157,69	226,75	Ok
	BbF	2175,11	91,48	37,58	309,55	Ok
	BkF	541,72	50,33	24,15	95,04	Ok
	BjF	1220,82	64,31	0,00	189,52	Ok
	BeP	7498,11	296,73	164,48	885,75	Ok
	BaP	4990,18	291,82	96,05	626,74	Ok
	DBahA	512,88	24,65	41,63	90,72	Ok
µg/kg	BaA	612,21	21,20	17,42	105,44	Ok
	CHR	1895,67	51,.73	42,99	275,42	Ok
	BbF	1725,81	50,95	57,80	254,31	Ok
	BkF	523,89	36,95	41,37	92,37	Ok
	BjF	635,74	32,37	26,70	108,87	Ok
	BeP	5878.88	224,49	0,00	720,37	Ok
	BaP	3852,59	74,38	187,59	503,08	Ok
	DBahA	506,02	93,16	0,00	89,69	Ok
µg/kg	BaA	52,28	5,49	5,05	13,04	Ok
	CHR	108,08	15,12	9,88	24,17	Ok
	BbF	163,75	9,68	6,65	34,39	Ok
	BkF	62,82	6,26	2,33	15,24	Ok
	BjF	66,12	2,88	5,26	15,92	Ok
	BeP	520,68	50,06	17,34	91,89	Ok
	BaP	430,69	28,84	26,77	78,21	Ok
	DBahA	40,32	3,30	3,44	10,46	Ok

According to the test for absence of significant heterogeneity described in ISO 13528 section B.2.3, no significant heterogeneity could be identified in EPDM, NRBR and PVC samples; therefore they could be considered homogeneous for all considered PAHs and then suitable for the inter-comparison study.

4.3 Sample distribution

Samples were dispatched from mid-October to the beginning-November 2017 to 21 participants, listed in Table 27.

Table 27. Participants in the interlaboratory comparison (ILC) study.

Research Institute/Company/University	Country	Hands on training
Scientific Institute of Public Health	BE	
The Danish Veterinary and Food Administration (FVST)	DK	x
Service Commun des Laboratoires (SCL - Ile de France - Site de MASSY)	FR	x
Laboratoire de Recherches et de Contrôle du Caoutchouc et des Plastiques (LRCCP)	FR	x
Laboratoire National de métrologie et d'essais	FR	x
TUV Rheinland LGA Products GmbH	DE	
Bureau Veritas Consumer Product Services Germany GmbH	DE	x
Prüfinstitut Hansecontrol GmbH	DE	
Landesuntersuchungsanstalt Dresden	DE	
Chemisches und Veterinäruntersuchungsamt - Ostwestfalen-Lippe (CVUA-OWL)	DE	x
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit	DE	
Deutsches Institut fuer Kautschuktechnologie e.V.	DE	
MAS | Münster Analytical Solutions GmbH	DE	x
Fraunhofer IVV	DE	x
Biochemical Institute for Environmental Carcinogens Prof. Dr. G. Grimmer-Foundation	DE	
The Dublin Public Analyst's Laboratory	IE	x
Pirelli Tyre SpA	IT	x
LIG - CERISIE	IT	x
European Commission-Joint Research Centre	IT	x
Food and Consumer Product Safety Authority (NVWA)	NL	x
Fera Science	UK	x

Each participant received:

- 5 specimens of each of three polymeric types, named Material 1, Material 2 and Material 3 (EPDM N375 DAE, NRBR N375 DAE and soft PVC N772)
- Mixture of 8 native standards and a mixture of 7 isotopes labelled standards (internal standard) at 25 µg/ml
- Glass bottles for migration test
- Solid phase extraction cartridges
- GC column
- Standard Operating Procedure (see Annex 3)
- Calculation Excel File to report results
- Presentation of the procedure gave during the hands-on training
- Standard chromatogram

Laboratories were requested to analyse three replicates for each sample and to strictly follow the Standard Operating Procedure provided. Sample analysis had to be conducted in repeatability conditions and results had to be reported by January 15th 2018, in the Excel templates provided by the JRC. By January 23rd 2018, 19 out of 21 laboratories reported their results.

A hands-on practical training intended specifically for technical staff executing the migration tests was organised on the 19th October by the JRC in order to present the methodology to follow in the execution of the trial. Fourteen laboratories took part in the training.

The standard operating procedure to be followed was presented and the migration method was carried out step by step together with participants.

The instrumental analysis was described in details and chromatograms were deeply analysed.

4.4 Results of collaborative trial

The results were received from 20 out of 21 laboratories to which samples had been sent. One laboratory was excluded from the statistical evaluation of results due to some difficulties in the detection of selected PAHs (high limits of detection).

Participants were asked to strictly follow the SOP of the method. Each sample was analysed in triplicate under repeatability conditions, i.e. within a short interval of time and by the same operator in the same laboratory using the same equipment.

About 1500 results were collected and statistically evaluated (19 laboratories * 3 samples * 3 replicates * 8 PAHs).

The results were treated with a validated commercial software [42].

Consensus value, repeatability and reproducibility of the method were calculated following the rules laid down in ISO 5725 Part 5 [20].

The robust method proposed in this section of ISO 5725 allows elaborating data without eliminating any. The algorithm proposed calculates results by assigning different weights to the data coming from each laboratory in a way that they are not influenced much by data of low quality.

Participants provided their results in the templates developed by the JRC.

The original data, the mean values and the standard deviation, corresponding to each PAH, sample and laboratory are reported in Tables in Annex 6.

In Annex 6 are also reported the Kernel density estimation for each PAH in the three samples, Mandel's h and k statistics and the graphical results of the statistical evaluation.

Some participants sent remarks and their comments are inserted in Annex 6.

In the absence of appropriate collaborative trial data, the precision of the method was calculated using the robust reproducibility standard deviation (R) for all measurands.

Data were treated in terms of migration per surface (ng/dm2) and migration per weight (µg/kg).

In Tables 28 and 29 are summarized the ILC results expressed as migration per surface and migration per weight respectively.

Table 28. Results of the collaborative trial expressed as migration per sample surface

ISO 5725-5	Sample	X_{pt} (ng/dm^2)	$U(x_{pt})$ (ng/dm^2)	R (ng/dm^2)	r (ng/dm^2)	RSD_R (%)	RSD_r (%)	N. Labs
BaA	EPDM	0,94	0,34	0,74	0,16	78,4	17,	19
	NRBR	0,42	0,22	0,48	0,08	112,8	17,5	19
	PVC	0		0	0			19
CHR	EPDM	4,42	0,76	1,71	0,42	38,5	9,5	19
	NRBR	2,88	0,58	1,28	0,36	44,3	12,4	19
	PVC	0		0	0			19
BbF	EPDM	1,36	0,48	1,05	0,14	77,1	9,9	19
	NRBR	0,88	0,40	0,88	0,18	99,7	20,7	19
	PVC	0		0	0			19
BeP	EPDM	4,65	0,58	1,31	0,35	28,0	7,6	19
	NRBR	3,07	0,62	1,37	0,35	44,6	11,3	19
	PVC	0		0	0			19
BaP	EPDM	1,18	0,40	0,88	0,21	74,4	17,8	19

	NRBR	0,67	0,34	0,73	0,11	108,8	16,3	19
	PVC	0		0	0			19
BkF/BjF/ DBahA	EPDM	0		0	0			19
	NRBR	0		0	0			19
	PVC	0		0	0			19

Table 29. Results of the collaborative trial expressed as migration per sample weight

ISO 5725-5	Sample	X_{pt} (µg/kg)	$U(x_{pt})$ (µg/kg)	R (µg/kg)	r (µg/kg)	RSD_R (%)	RSD_r (%)	HORRAT	N. Labs
BaA	EPDM	0,09	0,04	0,07	0,01	79,3	16,3	1,23	19
	NRBR	0,04	0,02	0,04	0,01	113,7	16,2	1,54	19
	PVC	0		0	0				19
CHR	EPDM	0,41	0,08	0,16	0,04	38,2	10,6	0,74	19
	NRBR	0,26	0,06	0,12	0,04	44,8	14,3	0,81	19
	PVC	0		0	0				19
BbF	EPDM	0,13	0,04	0,10	0,01	77,9	10,8	1,26	19
	NRBR	0,08	0,04	0,08	0,02	100,0	23,3	1,51	19
	PVC	0		0	0				19
BeP	EPDM	0,43	0,06	0,13	0,03	29,7	7,4	0,58	19
	NRBR	0,28	0,06	0,12	0,04	45,1	13,9	0,82	19
	PVC	0		0	0				19
BaP	EPDM	0,11	0,04	0,08	0,02	74,8	16,7	1,19	19
	NRBR	0,06	0,04	0,07	0,01	109,3	19,3	1,8	19
	PVC	0		0	0				19
BkF/BjF/ DBahA	EPDM	0		0	0				19
	NRBR	0		0	0				19
	PVC	0		0	0				19

In the Tables above are reported the robust average (consensus value), the uncertainty (calculated as 2*standard error), the reproducibility and repeatability, the standard deviation of the reproducibility and repeatability and the number of laboratories that provided results.

The method performance characteristics assessed were the within-laboratory precision, expressed as the relative standard deviation for repeatability (RSDr), and the between-laboratory precision, expressed as the relative standard deviation for reproducibility (RSDR).

Considering results related to the in migration per surface, RSDR ranged from 28% for Benzo(e)pyrene in Sample 1 (EPDM) to 112% for Benzo(a)anthracene in Sample 2 (NRBR)

As it can be observed, RSDR and RSDr values for the migration per weight don't differ from the ones derived for the migration per surface.

Even though the collaborative trial was not a proficiency test aimed to establish laboratory performances, the performances of the participating laboratories were evaluated in terms of z-scores according to ISO 13528.

The z-scores compared the participants' deviation from the assigned value with the standard deviation obtained from the precision experiment.

The usual interpretation of z-scores considers values above 3.0 or below -3.0 as unsatisfactory and above 2.0 or below -2.0 as questionable. The z-scores per laboratory, sample and PAH are shown in Tables 30 and 31, whereas the graphical presentation is reported in Annex 6. In both cases, the absolute values are highlighted in red when higher than 3 and in yellow if higher than 2 but lower than 3.

Table 30. z-scores per laboratory, sample and PAH expressed as migration per surface

ng/dm² ISO 5725-5	Sample 1 (EPDM N375 DAE)					Sample 2 (NRBR N375 DAE)				
Laboratory	**BAA**	**CHR**	**BBF**	**BEP**	**BAP**	**BAA**	**CHR**	**BBF**	**BEP**	**BAP**
LAB01	0.15	0.37	0.28	0.49	0.29	-0.33	-0.27	-0.39	-0.02	-0.37
LAB02	0.65	0.10	0.19	0.00	0.66	0.64	0.46	0.30	-0.08	0.72
LAB03	0.91	0.76	1.39	-0.05	0.09	-0.47	-0.70	-0.06	-1.07	-0.92
LAB04	-0.21	-0.14	-0.13	-0.49	-0.02	0.26	-0.16	0.10	-0.31	0.10
LAB05	-1.28	0.33	-1.30	-2.32	-1.34	-0.89	0.43	-1.00	-1.41	-0.92
LAB06	-0.01	-0.46	0.31	0.04	0.22	0.27	0.13	0.49	0.00	0.27
LAB07	1.28	-1.14	1.87	0.91	3.47	3.32	-1.08	7.24	1.23	4.69
LAB08	2.82	4.31	2.86	6.72	3.43	3.40	4.21	2.29	4.23	3.45
LAB09	0.78	9.18	-1.30	1.27	0.71	-0.89	7.60	-1.00	0.89	-0.92
LAB10	-0.36	-1.62	0.52	-0.83	-0.20	1.18	-1.27	1.49	0.13	0.88
LAB11	-0.40	-0.66	-0.29	-0.99	-0.40	-0.29	-0.59	-0.02	-0.78	-0.21
LAB12	-1.28	0.03	-0.33	-0.58	-1.34	-0.89	0.11	-1.00	-0.39	-0.92
LAB14	-0.46	-0.68	-0.08	-0.53	-0.32	0.26	-0.39	0.37	-0.47	0.27
LAB15	0.95	-0.29	0.00	-0.12	0.14	1.32	-0.33	0.04	0.16	0.47
LAB17	0.03	-0.25	-0.03	-1.06	0.06	-0.89	-0.47	0.02	-0.89	0.03
LAB18	-1.28	-2.59	-1.30	-0.47	-1.34	-0.89	-2.25	-1.00	-1.07	-0.92
LAB19	-1.28	0.60	-1.30	1.96	-1.34	-0.89	1.12	-1.00	1.70	-0.92
LAB20	0.34	0.93	0.08	0.31	0.68	-0.72	0.65	-1.00	0.21	-0.92
LAB21	-0.03	0.51	0.27	0.64	0.49	0.21	0.88	0.70	0.88	1.29

Table 31. z-scores per laboratory, sample and PAH expressed as migration per weight.

µg/kg ISO 5725-5	Sample 1 (EPDM N375 DAE)					Sample 2 (NRBR N375 DAE)				
Laboratory	**BAA**	**CHR**	**BBF**	**BEP**	**BAP**	**BAA**	**CHR**	**BBF**	**BEP**	**BAP**
LAB01	0.12	0.34	0.26	0.39	0.26	-0.33	-0.26	-0.38	-0.02	-0.37
LAB02	0.68	0.20	0.24	0.09	0.71	0.60	0.42	0.30	-0.11	0.70
LAB03	0.94	0.87	1.47	0.03	0.15	-0.48	-0.74	-0.07	-1.09	-0.92
LAB04	-0.22	-0.16	-0.13	-0.49	-0.03	0.25	-0.18	0.10	-0.33	0.10
LAB05	-1.25	0.24	-1.28	-2.23	-1.34	-0.88	0.44	-1.00	-1.39	-0.92
LAB06	-0.04	-0.49	0.28	-0.03	0.20	0.25	0.11	0.49	-0.02	0.26
LAB07	1.34	-1.08	1.99	1.03	3.67	3.29	-1.07	7.35	1.21	4.70
LAB08	2.77	4.42	2.86	6.35	3.44	3.31	4.11	2.28	4.12	3.40
LAB09	0.82	9.75	-1.28	1.34	0.79	-0.88	7.36	-1.00	0.82	-0.92
LAB10	-0.38	-1.65	0.48	-0.85	-0.23	1.19	-1.24	1.53	0.16	0.91
LAB11	-0.41	-0.68	-0.30	-0.97	-0.41	-0.30	-0.61	-0.03	-0.79	-0.22
LAB12	-1.25	0.10	-0.30	-0.49	-1.34	-0.88	0.19	-1.00	-0.33	-0.92
LAB14	-0.48	-0.74	-0.12	-0.61	-0.36	0.24	-0.41	0.36	-0.49	0.25
LAB15	0.91	-0.28	0.01	-0.13	0.14	1.37	-0.27	0.08	0.23	0.51
LAB17	0.03	-0.23	-0.03	-0.99	0.06	-0.88	-0.51	0.00	-0.92	0.01
LAB18	-1.25	-2.61	-1.28	-0.50	-1.34	-0.88	-2.23	-1.00	-1.05	-0.92
LAB19	-1.25	0.59	-1.28	1.78	-1.34	-0.88	1.06	-1.00	1.62	-0.92
LAB20	0.28	0.85	0.04	0.18	0.62	-0.71	0.73	-1.00	0.28	-0.92
LAB21	-0.06	0.48	0.25	0.52	0.46	0.20	0.86	0.70	0.87	1.29

As it can be observed in Tables 28 and 29, the values for RSD_R were quite high; it was then decided to verify if identifying outliers could help in improving these values.

The Mandel's h and k statistics were applied to identify visual outliers. Mandel's h detects deviation of the results of a single laboratory from the overall mean while Mandel's k detects deviation regarding within each laboratory test results.
Some laboratories could be considered suspect outliers (see 5.5.2 and 5.6.2 in Annex 6) and for this reason as a comparison, results, in terms of assigned values and precision parameters, were also calculated using Part-2 of the standard ISO 5725 [55].
In ISO 5725-2 some tests are performed to identify statistical outliers and stragglers. Of course, the elimination of some data has an impact on the calculated standard mean values and standard deviations for repeatability and reproducibility.
Results are analysed first with Cochran's test to identify exceeding intra-laboratory standard deviations. Grubbs' test is successively applied for the outlier identification of individual test results and laboratory mean values.
In Tables 32 and 33 are reported the results of the statistical evaluation according to the requirements of both parts of ISO 5725 of Chrysene and Benzo(e)pyrene in Sample 1 (EPDM) in terms of migration per surface and per weight respectively. These two compounds presented the highest migration rates values and the best reproducibility and repeatability in terms of migration per surface and weight (calculated according to ISO Part 5).
A good agreement was observed among results calculated with ISO 5725 Part 5 and 2 and the two approaches can be considered substantially equivalent.

Table 32. Comparison of results calculated with Parts 2 and 5 of ISO 5725 (migration per surface).

Sample	**CHR-EPDM**		**BEP-EPDM**	
ng/dm²	**ISO 5725-5**	**ISO 5725-2**	**ISO 5725-5**	**ISO 5725-2**
X_{pt} (ng/dm²)	4,42	3,98	4,65	4,41
$U(x_{pt})$(ng/dm²)	0,76	0,76	0,58	0,54
R (ng/dm²)	1,71	1,65	1,31	1,11
r (ng/dm²)	0,42	0,65	0,35	0,29
RSD_R (%)	38,5%	41,3%	28,0%	25,1%
RSD_r (%)	9,5%	16,2%	7,6%	6,5%
N. Labs	19	17	19	16

Table 33. Comparison of results calculated with Parts 2 and 5 of ISO 5725 (migration per weight).

Sample	**CHR-EPDM**		**BEP-EPDM**	
µg/kg	**ISO 5725-5**	**ISO 5725-2**	**ISO 5725-5**	**ISO 5725-2**
X_{pt} (µg/kg)	0,41	0,37	0,43	0,41
$U(x_{pt})$(µg/kg)	0,08	0,08	0,06	0,06
R (µg/kg)	0,16	0,15	0,13	0,11
r (µg/kg)	0,04	0,06	0,03	0,03
RSD_R (%)	38,2%	41,5%	29,7%	26,4%
RSD_r (%)	10,6%	17,2%	7,4%	6,7%
HORRAT	0,74	0,79	0,58	0,51
N. Labs	19	17	19	16

In addition participants were asked to apply the proposed method to analyse a control solution at 10 ng/ml of each PAH. These results were treated with ISO 5725 Part 5 and successively with Part 2.

As shown in Table 34 and 35, RSD_R values improved until less than 10%.

No significant differences between the two ISO 5725 approaches were noticed also in this case.

Repeatability was not calculated because analysis was done in single measurement.

Table 34. Results of the collaborative trial for 10 ng/ml control solution.

ISO 5725-5	X_{pt} (ng/ml)	$U(x_{pt})$(ng/ml)	R (ng/ml)	RSD_R (%)	N. Labs
BaA	9,67	0,38	0,83	8,5%	19
CHR	9,81	0,44	0,96	9,7%	19
BbF	9,74	0,36	0,79	8,1%	19
BkF	9,96	0,38	0,84	8,3%	19
BjF	9,79	0,42	0,91	9,3%	19
BeP	9,77	0,52	1,12	11,4%	19
BaP	9,66	0,36	0,76	7,8%	19
DBahA	9,39	0,46	1,00	10,6%	19

Table 35. Results of the collaborative trial for 10 ng/ml control solution.

ISO 5725-2	X_{pt} (ng/ml)	$U(x_{pt})$(ng/ml)	R (ng/ml)	RSD_R (%)	N. Labs
BaA	9,59	0,34	0,71	7,4%	18
CHR	9,75	0,40	0,85	8,6%	18
BbF	9,64	0,34	0,72	7,4%	18
BkF	9,89	0,34	0,72	7,2%	18
BjF	9,78	0,46	0,96	9,8%	18
BeP	9,67	0,44	0,94	9,7%	18
BaP	9,53	0,36	0,77	8,0%	18
DBahA	9,30	0,40	0,83	8,9%	18

The better reproducibility obtained analysing the control solution can be due to the fact that its concentration is 10 times the highest PAH concentration extracted from the migration media (BEP in Sample 1) that corresponds to about 1 ng/ml, a value slightly above the limits of quantification of laboratories. Quantifying a substance in the proximity of quantification could be risky because, as it can be observed from the results obtained, the quantification cannot be enough accurate, thus leading to high reproducibility values.
In 2016 a restricted study on the migration of PAHs from rubber materials into aqueous ethanol was organised in Germany by the Bundesinstitut für Risikobewertung (BfR) [56]. Ten laboratories took part in the trial; some of them also participated in the present exercise.
The experimental conditions were similar to the ones applied by the ILC organised by the JRC, but obviously samples were different in terms of composition, raw materials, shape and PAH content.
Nevertheless a comparison between the two studies can be made comparing RSD_R and RSD_r values obtained.
As it can be seen in Table 36, values for RSD_R and RSD_r calculated for four different samples can be considered similar in JRC and BfR studies. Also in this case results are reported as migration per surface and migration per weight.

Table 36. BfR study results of BeP.

Sample	Analyt	X_{pt} (ng/cm^2)	$U(x_{pt})$ (ng/cm^2)	R (ng/cm^2)	r (ng/cm^2)	RSD_R (%)	RSD_r (%)	N. Labs
Sample A	BEP	0,24	0,12	0,11	0,05	44,8	21,7	6
Sample B		0,63	0,37	0,22	0,12	34,7	19,7	7
Sample C		1,59	0,84	0,7	0,39	44,2	24,6	7
Sample D		0,42	0,25	0,22	0,07	52,3	16,4	7
Sample	**Analyt**	**X_{pt} (ng/mg)**	**$U(x_{pt})$ (ng/mg)**	**R (ng/mg)**	**r (ng/mg)**	**RSD_R (%)**	**RSD_r (%)**	**N. Labs**
Sample A	BEP	0,0024	0,0012	0,0011	0,0007	45,4	28,3	6
Sample B		0,0063	0,0031	0,0022	0,0013	34,9	19,8	7

Sample C	0,0169	0,0099	0,0071	0,0037	42,3	21,7	7
Sample D	0,0042	0,0026	0,0020	0,0008	47,9	18,8	7

In conclusion, the method performance characteristics assessed to verify the analytical performance of the proposed method were the within-laboratory precision, expressed as the relative standard deviation for repeatability (RSD_r), and the between-laboratory precision, expressed as the relative standard deviation for reproducibility (RSD_R).
In general the between Lab Variability (RSD_R %) ranged from 28 to 113% and the with-in Lab Variability (RSD_r %) from 7 to 23%.
These values were in accordance with the outcomes of a similar precedent study on the migration of PAHs from rubber materials in contact with aqueous ethanol.
The challenge of this exercise was to develop a method sensitive enough to determine the potential small fraction of PAHs that could migrate from rubber and plastic materials into an aqueous simulant.
In some cases the concentrations of PAHs released from samples could be lower than the quantification limit of the method. Quantification in the proximity of the LOQ values leads often to high repeatability and reproducibility values.
In fact better values of RSD_r % and RSD_R % were obtained for Chrysene and Benzo(e)pyrene that had the highest concentrations in the final migration solutions.
Moreover, if the concentration of PAHs further increases (case of control solutions with PAHs concentration ten times higher the ones of the highest PAHs in the migration solutions) RSD_R% values unequivocally improves (<10%).
In order to achieve better reproducibility values, the final volume of the solution to be instrumentally analysed could be decreased from the proposed 1 ml to 100 or 200 µl.
The concentrations of the PAHs would so be higher than the LOQs leading to a more precise quantification and consequently improving the reproducibility of the method.

5. Conclusions

The work carried out by JRC on request of DG GROW (Administrative Arrangement 34003) has made available new data and scientific information on the migration behaviour of certain PAHs from selected plastic and rubber polymeric matrices. The results will support the European Commission's legal obligation to review the PAHs restriction under REACH (Annex XVII, entry 50, paragraph 5 and 6), and will be of benefit to other ongoing EC activities on PAHs (such as mandate M556 to the European standardisation committee).

Experimental studies on content and migration of the eight carcinogenic PAHs targeted in this study have been performed on a variety of polymeric plastic and rubber materials, manufactured ad-hoc, with known origin and content of PAHs. Various grades and types of ingredients known to be PAH sources were used in the formulation of the manufactured materials. Moreover, recycled granules (coated and uncoated) originating from end-of-life tyres produced before and after 2010 were also made available for this study. Each of the eight PAHs were quantified by applying a method developed in-house based on Randall hot extraction, followed by purification with Solid Phase Extraction using molecular imprinted polymers cartridges (MIPs), and GC-MS in selected ion mode. The maximum amount of PAHs in the test materials, expressed as the sum of the eight target PAHs, was about 20 mg Kg^{-1} in rubber, 15 mg Kg^{-1} in plastic materials and 5 mg Kg-1 in the recycled rubber granules. Rubber materials with the highest content of PAHs were prepared with distillate aromatic oils in order to facilitate the migration testing method development. According to industrial partners the use of distillate aromatic extracts (DAE) is not representative of European manufacturing practice for component of articles intended to be in contact with the skin. Other categories of extender oils less rich in PAHs are used instead, such as treated distillate oil (TDAE).

The release of the target set of PAHs in migration media of various compositions was investigated. In addition to aqueous artificial sweat and saliva simulants (described in EN1811 and DIN53160-1 respectively), skin surface film liquid (sweat plus sebum), and 20% ethanol (described in literature as good model for human absorption simulation) were considered relevant biosimulants for this research study. The migration tests were carried out by total immersion (0.2 dm^2 surface area of test sample in 20 mL) in dynamic mode at 40 ºC. The amount of PAHs released after 24 hours was measured. Overall, under these conditions very low amounts of PAHs were released, frequently under the quantification limit of the method. The absolute quantification limit was in the range 0.2-0.6 ng mL-1 depending on the specific substance detected.

None of the plastic polymeric materials led to detectable release of the target PAHs in any of the migration media used in this study. Similarly the tests with silicone rubber materials did not result in detectable migration in any of the biosimulants. Only the rubber matrices containing DAE as extender oil showed detectable migration when using 20% ethanol as migration solution.

Migration of PAHs from rubbers seems to be related to the type of extender oil used in their manufacturing process since no release was observed from rubber matrices containing treated distilled aromatic extract (TDAE). Furthermore, the fact that no release was detected in tests with silicones suggests that extender oil, which is not contained in silicone matrices, has the main impact on the PAHs release. Qualitatively it appears that PAHs contained in the extender oils migrate more easily than those in the carbon black component of the rubbers.

The migration test method into 20% ethanol in water has been validated in-house and showed good method performance. The absolute quantification limit varied in the 0.2-0.6 ng mL^{-1} range depending on the specific substance being detected. Between-day reproducibility over 3 days varied from 3.5% (chrysene) to 11.5% (dibenzoanthracene), and recoveries in the range 93,5% (dibenzoanthracene) to 99,3% (benzo[b]fluoranthene) were obtained.

Chrysene and benzo[e]pyrene are the substances that were released in higher amounts in the migration experiments with 20% ethanol, indicating that for similar conditions the relative migration of PAHs depends on the molecular weight of each substance, as well as the content and the matrix. The amount of PAHs released into 20% ethanol at 24 hours, expressed as a function of the surface contact area, ranged from 0 to 21.74 ng dm^{-2}. The highest migration rates correspond to chrysene, with the lowest molecular weight, followed by benzo[e]pyrene with highest total content in the four rubber materials. The amount of PAHs measured in the alcoholic solution can also be calculated relative to the total content in the material and varies from 0 to 0.09%, with the maximum value corresponding to chrysene which exhibits the higher mobility. In the same set of experiments, carried out with the rubber samples containing DAE, higher amounts of PAHs were generally released from ethylene-propylene diene monomer (EPDM) compared to natural rubber/butadiene rubber blend samples, independent of the type of carbon black in the formulation.

In addition, the release of PAHs in experimental tests with coated recycled rubber granules was two to three times lower than the released amount measured in tests using uncoated granules giving an indication that the coating acts as a barrier to the chemical migration of the target substances. Although the content of PAHs was lower compared to the other polymeric matrices, the relative migration expressed in mass percentage was higher. The migration was not calculated in terms of surface contact area due to the difficulty of measuring the surface area of the weighed granules but it may be assumed that in these experiments there is high surface contact area with the migration medium that would facilitate the release of the substances. Moreover the exact composition of the granules was unknown.

Experimental tests with skin surface film liquid, carried out to investigate the influence of fatty constituents in the bio simulants, have revealed that PAH migration to SSFL increases with migration time and with the sebum content in the SSFL, proving the significance of the presence of the lipophilic component in the release of the target lipophilic substances. SSFL containing 0,1-0,2% sebum in EN 1811 Sweat showed good agreement with data obtained when 20% EtOH was used as simulant, which in turn, as reported in literature, showed good agreement with human skin *ex vivo tests* carried out with the Franz Cell. The use of 20% EtOH as migration medium was further considered towards validation and standardisation due to the above mentioned correlations and the simplicity and lower experimental costs associated with the use of 20% EtOH

The method developed for determining the migration of the eight restricted PAHs from rubber and plastic samples by total immersion into a simulant (20% ethanol) was subjected to an initial interlaboratory comparison study with the aim of investigating the transferability of the method to other laboratories. Twenty-one laboratories from seven European countries took part in this study. Not all participants were experienced in the migration of PAHs from rubber and plastic materials. Some were experts on migration methods in general (for instance from food contact materials) while others had less general expertise but routinely perform the determination of PAHs total content from different consumer products. Three samples, two rubber and one plastic, whose homogeneity was previously checked, were sent to the laboratories along with a detailed standard operating procedure and calculation sheets. The within-laboratory precision, expressed as the relative standard deviation for repeatability (RSDr %) varied from 7 to 23%, and the between-laboratory precision, expressed as the relative standard deviation for reproducibility. (RSDR %) ranged from 28 to 113%. This variability is expected when determination close to the quantification limit of the method is necessary (in this specific case due to the small extent of the chemical release). Similar variability has been found in a recent German study, with the participation of nine laboratories, on the migration of PAHs from rubber materials in contact with 20% ethanol. Variability of the method could be reduced by revising the injection volume and/or elution volume of the standard operating procedure. It might then be considered as a good basis for a harmonised

method after appropriate validation. The applicability to other lipophilic substances has not been part of this study, however the considerations made in the experimental design and the methodological approach of this research study could be useful in an inception phase.

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List of abbreviations and definitions

BaA	Benzo[a]anthracene
BaP	Benzo[a]pyrene
BAuA	Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (German Federal Institute for Occupational Safety and Health)
BbFA	Benzo[b]fluoranthene
BeP	Benzo[e]pyrene
BfR	Bundesinstitut für Risikobewertung (German Federal Institute for Risk Assessment)
BjFA	Benzo[j]fluoranthene
BkFA	Benzo[k]fluoranthene
CEN	European Committee for Standardisation
CHR	Chrysene
DBahA	Dibenzo[a,h]anthracene
DIN	Deutsches Institut für Normung
EPA	Environmental Protection Agency
ECHA	European Chemicals Agency
EPA-PAHs	16 PAHs selected by the United States Environmental Agency to characterise environnemental PAH pollution
EU-PAHs	8 PAHs listed in entry 50 of annex XVII to Reg. (EC) 1907/2006
FOREhST	Fed ORganic Estimation human Simulation Test
GC-MS	Gas chromatography coupled with mass spectrometry
HPLC-UV/FLD	High pressure liquid chromatography coupled with Ultraviolet and fluorescence detectors
ISTDs	Deuterated Internal Standards
ISO	International Organization for Standardization
LOD	Limit Of Detection
LOQ	Limit Of Quantification
NIOSH	US National Institute for Occupational Safety and Health
PAHs	Polycyclic Aromatic Hydrocarbons
PAH-6	6 PAHs common to both the EPA-PAH and EU-PAH lists
PCA	Polycyclic Aromatics
PU	Polyurethane
REACH	Regulation (EC) 1906/2007 on the Registration, Evaluation, Authorisation and restriction of Chemicals
SPME	Solid Phase Microextraction SPME
UAE	Ultrasound assisted extraction

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ANNEX 1

STANPAHs: MIGRATION OF PAHs FROM PLASTIC AND RUBBER MATERIALS INTENDED FOR THE GENERAL PUBLIC

EXPERIMENTAL DESIGN

Migration of PAHs from materials intended for the general public

JRC.F2

Background:

Regulation (EU) No. 1272/2013 amending Annex XVII to REACH restricts the content of 8 Polyaromatic Hydrocarbons (PAHs) all with a harmonised classification as carcinogenic 1B. Information on potential release of such substances and suitable testing methods is pursuit to decide on eventual revision of the restriction to be based on migration limits.

The research project has two goals:

- Better understanding of the migration behaviour of certain PAHs in selected materials
- Develop a reliable methodology to determine PAH migration from these matrices, under conditions simulating, to the extent possible, dermal contact

Target PAHs are substances with high lipophilicity, the so far reported migration rates that have been obtained using aqueous simulants can underestimate the migration under real skin contact conditions.

The experimental design illustrated below aims at providing information on the bioaccesible amount of the PAHs that potentially can deposit onto the skin. It is developed taking into account a compromise between relevant and meaningful research and simplicity and robustness of a migration method intended for potential application in an enforcement or control laboratory if in a future legislation would consider migration instead of content.

Materials

A series of **ad-hoc manufactured materials** containing various amounts of PAHs from known sources will be studied for their migration behaviour Materials have been selected according to defined criteria including their frequency of use in articles and probability to contain PAHs. PAHs are not intentionally added to the materials rather are contained in the raw materials.

Measurement of migration rates

The following are among the main parameters affecting the chemical release

- **Migrating medium**
- **Surface area to volume ratio or mass of material to volume ratio**
- **Temperature**
- **Time**
- **Dynamic/static mode**
- **With/without friction**
- **aging**

While all parameters are important the focus in this project refers **to the migration medium.** The following migration media will be considered:

1) Standardized Sweat simulant according to EN 1811. It generally recognized that aqueous simulant is not suitable for migration testing of lipophilic substances but will serve in this study as reference sweat composition.

2) Sweat EN 1811 with added lipophilic content (one of the following two to be selected)

The two following media intend to simulate the lipophilic nature of the skin yet having a simplified approach since the inclusion of lipophilic compounds in a primarily aqueous environment (sweat) could create some difficulties for the preparation of the migrarion testing media. The first approach includes the addition of some fatty acids that have

been found in the human sweat analysis, to the EN1811 reference sweat. The second approach foresees the addition of some lipids of the sebum. The identification of the most suitable compounds and concentrations is of outmost importance. Additionally the need to test both approaches or to select just one should be considered due to the limited resources.

2a) Sweat EN 1811 plus fatty acids

Identify the appropriate chemicals to be added to the standard sweat formulation in order to better extract the target PAH at estimated/measured physiological concentrations to better mimic the human sweat. Selection of a set of compounds will be done according to similarity of partition coefficients octanol/water (K_{ow}) to those of the target PAHs. (see Table 1).Suggested lipids will be palmitic, myristic, oleic and linoleic.

2b) Artificial skin surface film liquid (Sweat formulation plus fatty acids plus sebum)

According to Sterfaniak et al the artificial SSFL can be prepared by adding an artificial sweat composition to a mixture of an artificial sebum composition and mix them together. This is possible either by emulsification or, alternatively, by immersing a coated filter of the sebum composition into the artificial sweat, in order to better simulate the skin surface

Callewaert et al Journal of MICROBIOLOGICAL Methods 103 (2014) 6-8 have reported an artificial sweat containing hydrolysed fatty acids 16.4 ml/L, squalene 2mL/L and cholesterol 0.8 mL/L, that could be

3) Ethanolic solution at 20%

The regulation from the European Commission (EU) No 10/2011 [32], has set 50% ethanolic simulant as a substitute for milk matrix. Nevertheless, a recent study (Bartsch, N. et al Journal of occupation and environmental hygiene vol 13 , 12, 2016) , reports this proportion as an overestimation in the migration analysis of a PAH (BaP) in comparison to the human skin. The authors proved that the use of 20% ethanol at 37 ºC is a valid alternative simulant that mimics the real human skin exposure that results from direct contact with chemicals (PAHs) that migrate from consumer products

Rest of parameters

In order to enhance comparability of results, the rest of parameters affecting the migration process will be aligned as much as possible to existing standards or guidelines or reported studies

In terms of **loading factor** (material surface area or mass to fluid volume ratio), initially we intend to start about 10 cm^2/50mL or 20cm^2/25mL. It might change depending if we can quantify or not the migrating amounts.

I would opt to have migration kinetics information but maximum migration experimental **time** would be set at 24 hours (time series analyses at fixed intervals)

Concerning **temperature** 37 C could be considered as an appropriate value but I would opt for 40C since a variety of temperatures in the range 32-40 have been reported by the literature and the various standards of guidelines. I would not expect big variations from 37 to 40 C while the preparation of the sweat with lipids could be easier at higher temperature.

Although not a priority for this project, ideally higher temperatures should be tested (in the 60-80 C range) if relevant for specific scenario (e.g could be found in interior of cars, in the floors of playgrounds). Values much higher will not be meaningful since skin contact will be discontinued due to burn.

The migration would be carried out using an orbital shaker, thus bringing some movement to the migration solution and simulating in a simple way a **dynamic system**

In order to simulate some friction and pressure and to facilitate the immersion of the test materials the use of stainless steel beads is foreseen

Aging will not be considered

Table 1 Suggested substances to be included into to the sweat simulant formulation and respective partitioning coefficients, K_{ow}.

Substance	Log K_{ow}	Substance	Log K_{ow}	C in sweat	Wt% in sebum
PAH		**Fatty acids**			
Benzo[a]pyrene (BaP)	6.34[1]	Decanoic acid (capric)	3.92[4]	0.01-0.1 µg/ml	
Benzo[a]anthracene (BaA)	5.61[1]	Dodecanoic acid (lauric)	4.97[4]		
Chrysene (CHR)	5.73[1]	Tetradecanoic acid (myristic)	6.02[4]		6.9[7]
Benzo[b]fluoranthene (BbFA)	6.50[2]	Hexadecanoic acid (palmitic)	7.08[4]		25.3[7]
Benzo[k]fluoranthene (BkFA)	6.63[2]	Octadecanoic acid (stearic)	8.13[4]		2.9[7]
Dibenzo[a,h]anthracene (DBAhA)	7.22[2]	Eicosanoic acid (behenic)	9.19[4]		
Benzo[e]pyrene (BeP)	6.58[2]	Docasanoic acid (behenic)	10.24[4]		
Benzo[j]fluoranthene (BjFA)	6.44[3]	cis-9-Octadecenoic acid (oleic)	7.74[5]		1.9[7]
		(9Z,12Z)-9,12-Octadecadienoic acid (linoleic)	7.05[5]		0.5[7]
		Ceramides			
		C2-Ceramide (N-acetylsphingosine)	6.14[4,6]		
		Sphingosine	5.94[4,6]		
		C2-C14	from 6.14 to 12.48[4,6]		
		Liposoluble vitamins			
		Vitamin E	12.2[7, 8]		traces

(1) W.-N. Hung et al., Journal of Hazardous Materials 279 (2014) 197–202; (2) Environmental Toxicology and Chemistry, Vol. 35 (6), (2016) 1371–1377; (3) PAHs and Related Aromatic Hydrocarbons, Handbook of Physical-chemical properties and environmental fate for organic chemicals, Volume I Introduction and Hydrocarbons, Mackay D., Shiu W., Ma K.C., Lee S., 2nd Ed., Taylor&Francis, 2006; (4) Takemura,T. et al., Lipids in eccrine sweat, British Journal of Dermatology, 120 (1989) 43-47; (5) ©2000 - 2012 U.S. Environmental Protection Agency for EPI Suite™; (6) S. Nybond et al., Biochimica et Biophysica Acta 1718 (2005) 61–66; (7) Stefaniak, AB., International Journal of Cosmetic Science 32 (2010), 347–355; (8) https://pubchem.ncbi.nlm.nih.gov/.

ANNEX 2

STANDARD OPERATING PROCEDURE

TOTAL CONTENT DETERMINATION OF EU-PAHs IN PLASTIC AND RUBBER MATERIALS

Contents

STANDARD OPERATING PROCEDURE FOR THE TOTAL CONTENT DETERMINATION OF 'EU-PAHs' IN PLASTIC AND RUBBER MATERIALS

Method : STANDARD OPERATING PROCEDURE FOR THE TOTAL CONTENT DETERMINATION OF 'EU-PAHs' IN PLASTIC AND RUBBER MATERIALS

Analytes : Benzo[a]anthracene (CAS # 56-55-3)
Chrysene (CAS # 218-01-9)
Benzo[b]fluoranthene (CAS # 205-99-2)
Benzo[k]fluoranthene (CAS # 207-08-9)
Benzo[j]fluoranthene (CAS # 205-82-3)
Benzo[e]pyrene (CAS # 192-97-2)
Benzo[a]pyrene (CAS # 50-32-8)
Dibenzo[a,h]anthracene (CAS # 53-70-3)

Matrix : Rubber and Plastic Materials

Foreword

This document was prepared by the Joint Research Centre of the European Commission in Directorate F.2 (Health, Consumers & Reference Materials) in the framework of the administrative arrangement No. 34003 established between the Joint Research Centre and DG Grow (D.1) in the field of the migration of eight PAHs from plastic and rubber materials.

1. Scope

This method is suitable for the quantitative determination of benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene, benzo[e]pyrene, benzo[a]pyrene and dibenzo[a,h]anthracene in rubber and plastic materials by gas chromatography coupled with mass spectrometry (GC-MS).

The above listed polycyclic aromatic hydrocarbons have all been classified as substances presumed to have carcinogenic potential for humans (Category 1B) according to the CLP criteria.

2. Terms and Definitions

CLP	Classification, Labelling and Packaging. The CLP Regulation ensures that the hazards presented by chemicals are clearly communicated to workers and consumers in the European Union through classification and labelling of chemicals.
GC-MS	Gas chromatograph coupled to mass-spectrometer
BaA	Benzo[a]anthracene
Chr	Chrysene
BbF	Benzo[a]fluoranthene
BkF	Benzo[a]fluoranthene
BjF	Benzo[j]fluoranthene
BeP	Benzo[e]pyrene
BaP	Benzo[a]pyrene
DBahA	Dibenzo[a,h]anthracene
BaA-d12	Benzo[a]anthracene (deuterium labelled)
Chr-d12	Chrysene (deuterium labelled)
BbF-d12	Benzo[a]fluoranthene (deuterium labelled)
BkF-12	Benzo[a]fluoranthene (deuterium labelled)
BjF-d12	Benzo[j]fluoranthene (deuterium labelled)
BeP-d12	Benzo[e]pyrene (deuterium labelled)
BaP-d12	Benzo[a]pyrene (deuterium labelled)
DBahA-d14	Dibenzo[a,h]anthracene (deuterium labelled)
PAH	Polycyclic Aromatic Hydrocarbons

3. Method Summary

3.1 The rubber or plastic material is reduced to small pieces and weighed into extraction thimbles.

3.2 A solution containing isotope-labelled internal standards is spiked onto the extraction thimble.

3.3 The material is extracted with toluene using a Randall Auto Extractor

3.4 Extracts are cleaned-up using a solid phase extraction cartridge filled with molecularly imprinted polymer. The collected eluent is analysed by gas chromatography-mass spectrometry (GC-MS).

4. Safety and Environmental Precautions

CAUTION: The analysed polycyclic aromatic hydrocarbons are human carcinogens. Precaution shall be taken to avoid exposure.

All solutions should be handled in an adequately ventilated fume hood, glove box or equivalent.

The laboratory shall establish procedures for disposal of solutions containing PAHs.

4.1 Take routine safety and environmental precautions, as in any chemical laboratory activity.

4.2 Care should be taken to avoid inhalation or oral or dermal exposure to harmful chemicals. Use a chemical fume hood, and wear an appropriate laboratory coat, gloves and safety goggles when preparing or handling undiluted materials, standard solutions or material extracts.

5. Apparatus and Equipment

Usual laboratory apparatus, in particular:

5.1 Analytical balance capable of measurement to at least four decimal places

5.2 Pipettes and tips capable of accurately dispensing volumes 10-1000 µL

5.3 Volumetric pipette or equivalent, 20 mL

5.4 Volumetric flasks (10 mL, 20 mL)

5.5 Solid phase extraction manifold

5.6 Solid Phase Extraction cartridges with molecularly imprinted polymers sorbent (Supelco, SupelMIP PAHs, 50mg/3mL), product Code 52773-U

5.7 Solvent Autoextractor (Velp Scientifica, Model SER158, or equivalent).

5.8 Rotary evaporator with suitable flasks

5.9 GC-MS system in single-ion monitoring detection mode. The gas chromatograph must be configured to perform splitless injections on a capillary column.

5.10 Column: Restek, RXi-PAH, 30m, 0.25mm ID, 0.10 µm df, Product Code 49318. Or equivalent.

5.11 PTFE Disc Filters, 0.45 µm, 25 mm diameter

5.12 Glass vials, 20 mL (Supelco product code 27199, or equivalent)

6. Reagents and Supplies

6.1 All reagents shall at least be of analytical reagent grade unless otherwise noted.

6.2 Toluene

6.3 Dichloromethane

6.4 Ethylacetate

6.5 Hexane

6.6 Cyclohexane

6.7 Standard Mixture (Native Standards)
Special 1mL all 8 PAHs containing concentrated (50 µg/mL, in toluene) standard solutions were purchased through LabService Analitica s.r.l. (Anzola dell'Emilia, Italy) Reference: ML309M050TOEC

6.8 Internal Standard Mixture (Isotope labelled)
Special 1mL 7 deuterated PAHs containing concentrated (50 µg/mL, in toluene) internal standard solutions were purchased through LabService Analitica s.r.l. (Anzola dell'Emilia, Italy). Reference: ML310M050TOEH

7. Preparation of Glassware

Clean and dry glassware to avoid contamination. PAHs have low aqueous solubility. Rinse glassware additionally with toluene or hexane.

8. Standard Solutions

The blow described preparation procedure for standard solutions covers the linear range of the mass-spectrometer used for the preparation of this procedure (Agilent 5975C). The linear response range of mass-spectrometers other than the one used in this study might be different.

8.1 Primary Standards

8.1.1 Primary Native Standards (2500 ng mL^{-1})

8.1.1.1 Pipette 500 µL of the native standard mixture (6.7) in a 10 mL volumetric flask.

8.1.1.2 Dilute to the mark with toluene.

8.1.1.3 Label and store in refrigerator (4 °C) at dark.

8.1.2 Primary Isotope Labelled Standards (2500 ng mL^{-1}) – *Internal Standard*

8.1.2.1 Pipette 500 µL of the native standard mixture (6.8) in a 10 mL volumetric flask.

8.1.2.2 Dilute to the mark with toluene.

8.1.2.3 Label and store in refrigerator (4 °C) at dark.

8.2 Preparation of Working Standard Solutions

The calibration curve should cover a concentrations range from 0-200 ng mL^{-1} and the internal standard should be prepared at a concentration of 50 ng mL^{-1}.

		STD	**Internal Standard** *(final conc. 50 ng/mL)*
Calibration Level	Final Concentration [ng mL^{-1}]	Volume of 2500 ng/mL solution [8.1.1] in 10 mL volumetric flask [µL]	Volume of 2500 ng mL^{-1} [8.1.2] solution in 10 mL volumetric flask [µL]
1	5	20	200
2	10	40	200
3	50	200	200
4	100	400	200
5	200	800	200

9. Control Solution

(Native Standards: 100 ng mL^{-1}, Isotope Labelled Standards: 50 ng mL^{-1})

Known amounts of native and isotope labelled standards are spiked into toluene. This solution is worked off in the same way (concentration with Rotavapor, SPE clean-up) as the sample extracts.
The purpose of this solution is to identify errors in the preparation of standard solutions or major errors in any other procedural step.
Satisfactory recovery-rates of this solution should fall within 85-100 %.

9.1 Pipette 40 µL of the primary native standard mixture (8.1.1) in a 20 mL volumetric flask.

9.2 Pipette 20 µL of the primary isotope labelled standard (internal standard) mixture (8.1.2) into the same 20 mL volumetric flask as in 9.1.

9.3 Bring volumetric flask to volume with toluene.

10. Sample Preparation

10.1 Shredding of sample material

Shred the sample material as much as possible. Only small (d<1 mm) sample pieces should be weighed into the extraction thimble.

10.2 Weigh sample into extraction thimble.

The amount of weighed sample material depends on the PAH content of the material. It should be chosen in such a way that the final concentration falls within the calibration curve (0-200 ng mL^{-1}).

In most cases the content is unknown. The following indicative weighing recommendations can be followed for a first estimate:

Material	Weighing [mg]
Natural Rubber	50
Ethylene propylene diene monomer (EPDM-rubber)	100
Silicone-Rubber	50
Polyvinyl chloride	100
Polystyrene	100
Polyethylene	100

If the chemical composition of the sample-material is unknown, it is recommended to weigh 100 mg sample material. Alternatively the polymeric material could be first characterised with e.g. FTIR or IR.

10.3 Spiking of internal standard on extraction thimble

Spike 20 µL of the primary isotope labelled standard solution (8.1.2) directly on the interior bottom of the extraction thimble close to the sample material.

10.4 Addition of extraction solvent

Add 95 mL toluene and a few glass boiling chips to the extraction cup.

10.5 Sample extraction with autoextractor

Samples are extracted with a solvent autoextractor (5.7) based on the Randall extraction principle. The Randall Hot Extraction process according to Randall consists of three steps: boiling, rinsing, and evaporation. Benefits of the hot extraction process include short process paths, low solvent requirements, short extraction periods and due to the short extraction period, hot extraction is gentle on the extract.

Parameters set for the autoextractor[1] are detailed below:

Parameter	Value
Solvent (toluene) volume	95 mL[2]
Immersion time	120 minutes
Removing time	15 minutes
Washing time	30 minutes
Recovery time	7 minutes
Cooling Time	15 minutes
Total extraction time	**3 hours, 7 minutes**

[1] Parameters may change depending of the extractor model
[2] Highest heating level required

After the extraction procedure the residual extract-volume should be below 20 mL.

11. Sample extracts concentration with rotary evaporator

Note: Before proceeding, please read notes under 11.1.

Evaporate the toluene-extract to dryness.

Note: The conditions of the rotary evaporator are to be set at 60 C ° with a vacuum pressure of 90 mbar (approximately 10-15 mins).

11.1 Potential need of additional sample treatment before sample clean-up or concentration with rotary evaporator

Especially when analysing plastic materials (rubber materials are typically not affected), some undesired and disturbing substances are sometimes extracted together with the PAHs during the hot sample extraction.

Polyethylene and Polystyrene:
Small portions of polymer and/or fine black-carbon (if material is black) particles may precipitate (or may form a film on the rotating flask) during the concentration step with the rotary evaporator.
This can be avoided by filtering the approximately 20 mL of extract (10.5) obtained after the hot sample extraction, through a 0.45 µm PTFE disc-filter (5.11) before proceeding with concentrating on the rotary evaporator.

12. Sample clean-up

Clean-up using SupelMIP PAHs solid-phase extraction columns (5.6) Molecularly imprinted polymers (MIPs) are a class of highly cross-linked polymer-based molecular based recognition elements engineered to bind one target or a class of structurally related target compounds with high selectivity. This sorbent specifically binds PAH and thus allows the removal of most other aliphatic and aromatic hydrocarbons from the extract.

12.1 Conditioning, loading and elution procedure.

12.1.1 Condition SPE with 1 mL cyclohexane.

12.1.2 Dissolve dry extract (in round-bottom flask after rotary evaporation) in 2x 1 mL (total volume 2 mL) hexane and transfer each aliquot on SPE cartridge.

12.1.3 Let extract soak into sorbent bed.

12.1.4 Wash with 2 mL cyclohexane.

12.1.5 Elute analytes with 3x 1 mL ethylacetate and collect eluate in 20 mL vial (5.12). Apply a gentle vacuum (-0.4 bar or -12 in Hg for 5-10 seconds) between each wash step.

12.2 Evaporate ethylacetate to dryness with nitrogen evaporator system (set heating block at 40°C). Duration approx. 15 minutes.

12.3 Dissolve dry extracts in 1 mL toluene.
This extract is injected in the GC-MS system.

13. Sample Analysis

The method for quantifying the PAHs in rubber and plastic materials involves GC-MS. The analytes are resolved from other potentially interfering substances on a GC column. Comparison of the area ratios (native analyte to isotope-labelled analyte) of the unknowns with the area ratios (native analyte to isotope-labelled analyte) of the known standard concentrations yields the concentration of the analytes.

13.1 GC-MS operating conditions: example

GC column	Restek, Rxi-PAH, 30 m, 0.25 mmID, 0.10 µm df (Cat#49318) or equivalent.
Injector temperature	300 °C
Mode	Constant flow
Flow rate	1.75 mL/min
Injection	1 µL pulsed splitless
Column temperature	60 °C for 1 min 40 °C/min to 200 °C 2.5 °C/min to 300 °C 30 °C/min to 320 °C for 5 min
Run time	49,5 mins
Transfer line temperature	320 °C
MS source	300 °C
Ionization mode	Electron ionization

Ion traces

Group 1 (Start Time: 0 mins)	**(Masses [m/z], Dwell time [ms])** In bold the MW masses
Benzo[a]anthracene-d12 Benzo[a]anthracene Chrysene-d12 Chrysene	(114, 45), (120, 45), (**228**, 45), (229, 45), (**240**, 45), (241, 45)
Group 2 (Start Time: 23 mins)	**(Masses [m/z], Dwell time [ms])** In bold the MW masses
Benzo[b]fluorantrhene-d12 Benzo[b]fluorantrhene Benzo[k]fluoranthene-d12 Benzo[k]fluoranthene Benzo[j]fluoranthene Benzo[e]pyrene-d12 Benzo[e]pyrene Benzo[a]pyrene-d12 Benzo[a]pyrene	(125, 40), (126, 40), (132, 40), (**252**, 40), (253, 40), (**264**, 40), (265, 40)
Group 3 (Start Time: 34 mins)	**(Masses [m/z], Dwell time [ms])** In bold the MW masses
Dibenzo[a,h]anthracene-d14 Dibenzo[a,h]anthracene	(139, 45), (146, 45), (**278**, 45), (279, 45), (**292**, 45), (293, 45)

<u>Note:</u> The operating parameters may have to be adjusted to the instrument, the used column and the resolution of the chromatographic peaks.

13.2 General analytical information

13.2.1 For the conditions described here, the expected sequence of elution will be Benzo[a]anthracene-d12, benzo[a]anthracene, chrysene-d12, chrysene, benzo[b]fluoranthene-d12, benzo[b]fluoranthene, benzo[k]fluoranthene-d12, benzo[k]fluoranthene, benzo[j]fluoranthene, benzo[e]pyrene-d12, benzo[e]pyrene, benzo[a]pyrene-d12, benzo[a]pyrene, dibenzo[a,h]anthracene-d14, dibenzo[a,h]anthracene.

See chromatogram in Annex 1.

13.2.2 Differences in e.g. temperature, gas flow rate and the age of the column can be expected to alter retention times

13.2.3 The sequence of determination of PAHs will be in accordance with individual laboratory practice. This section gives an example.

13.2.4 Inject pure toluene to check for contamination

13.2.5 Inject a blank extraction solution to check for contamination in the extraction system.
This sample covers all steps starting from (10.2) including extraction and SPE clean-up but without any sample material being weighed into the extraction thimble.

13.2.6 Inject the calibration standards, the control solution (9.) and the samples.

13.2.7 Record the peak areas of all labelled (deuterated) and not labelled substances

Important: Integrate peak areas in **selected ion mode (SIM)** using the molecular weight mass for each compound.

13.2.8 Calculate the relative response ratios of all PAHs ($A_{PAH(native)}$ / $A_{PAH(deuterated)}$) for each standard solution.

13.2.9 Plot graph of the concentration of each of the PAHs (x-axis) against the area ratios (y-axis).

13.2.10 The intercept should not be statistically significantly different from zero.

13.2.11 The calibration curve should be linear over the entire standard range for all PAHs (if the linear regression is less than 0.99, the calibration should be repeated).

13.2.12 Inject the quality controls (9) and samples, and determine the peak areas.

13.2.13 The peak ratios obtained for all test portions must fall within the working range of the calibration curve; otherwise the concentrations of the test portion solutions should be adjusted (lower weighing).

See Annex 1 for representative standard chromatogram

14. Data Analysis and Calculations

14.1 Calculate the relative response ratios from the peak areas for each of the calibration standards

$RF(PAH) = (A_{PAH(native)} / A_{PAH(deuterated)})$

Where RF is the relative response ration, $A_{PAH(native)}$ is the peak area of each single native PAH (in selected ion mode and $A_{PAH(deuterated)}$ is the peak area of each single isotope labelled PAH (in selected ion mode.

Selected ions to be used for area integration:

Compound	**Ion [m/z]**
Benzo[a]anthracene-d12	240
Benzo[a]anthracene	228
Chrysene-d12	240
Chrysene	228
Benzo[b]fluorantrhene-d12	264
Benzo[b]fluorantrhene	252
Benzo[k]fluoranthene-d12	264
Benzo[k]fluoranthene	252
Benzo[j]fluoranthene	252
Benzo[e]pyrene-d12	264
Benzo[e]pyrene	252
Benzo[a]pyrene-d12	264
Benzo[a]pyrene	252
Dibenzo[a,h]anthracene-d14	292
Dibenzo[a,h]anthracene	278

14.2 Plot a graph of the relative response factors $A_{PAH(native)}$ / $A_{PAH(deuterated)}$ (y-axis), versus concentration (x-axis) for each PAH and for each standard solution. Calculate the linear regression (Y=a+bx) from these data, and use both the slope (b) and the intercept (a) of the linear regression.

14.3 The content of the single PAHs (mg PAH / kg rubber or plastic) is determined from the calculated relative response ratios of each PAH in the test sample, the slope and the intercept obtained from the appropriate calibration curves (one curve for each of the 8 PAHs):

$$\text{Concentration [mg kg}^{-1}\text{]} = \frac{(Y - a)}{b \times SW}$$

Y = relative response ratio ($A_{PAH(native)}$ / $A_{PAH(deuterated)}$)
a = intercept of the linear regression obtained from the standard calibration curves
b = Slope of the linear regression obtained from the standard calibration curves
SW = Sample weight in mg

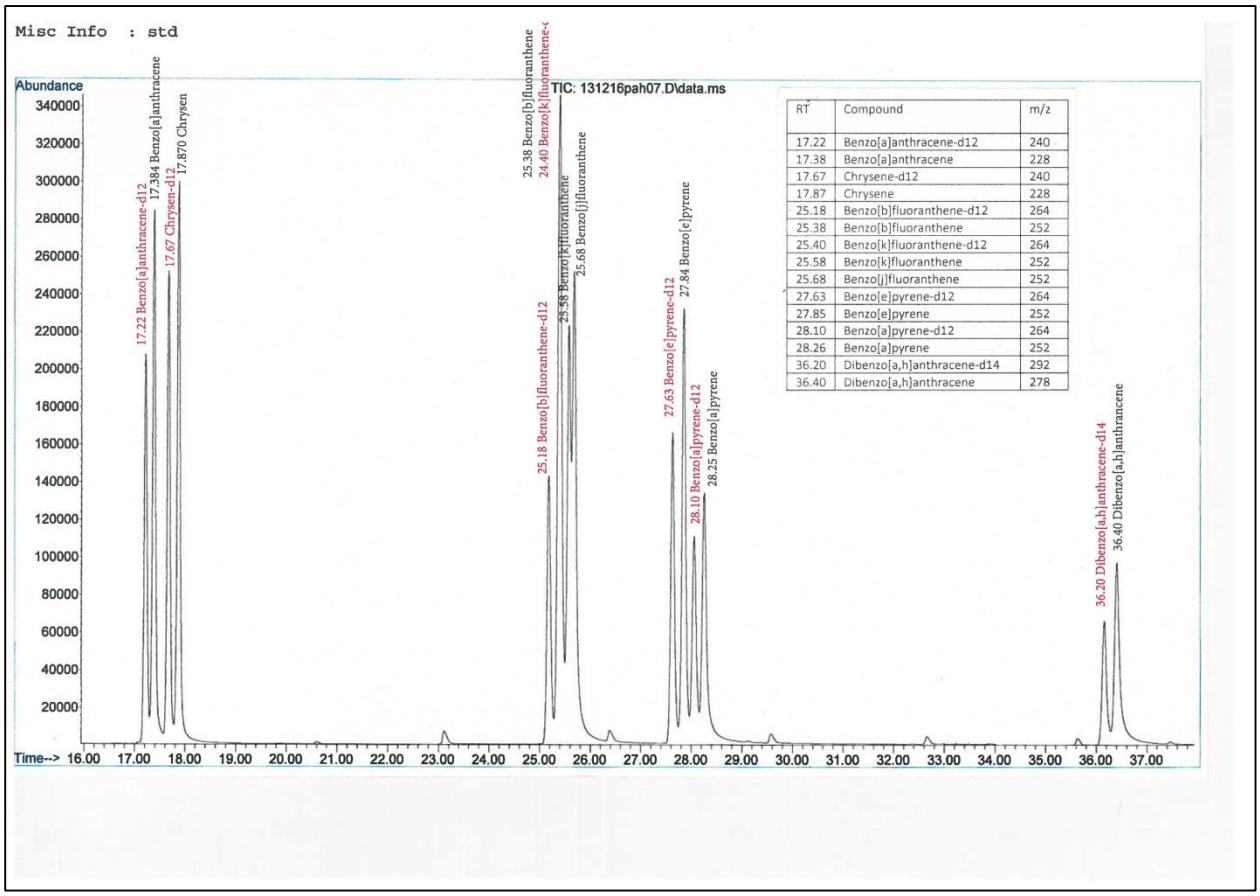
15. Method Performance Specifications

15.1 Limit of quantification (LoQ)

Compound	Absolute LoQ[1] [ng mL^{-1}]	Relative LoQ (500 mg of sample) [mg kg^{-1}]	Relative LoQ (100 mg of sample) [mg kg^{-1}]	Relative LoQ (20 mg of sample) [mg kg^{-1}]
Benzo[a]anthracene	0.8	0.002	0.008	0.040
Chrysene	0.8	0.002	0.008	0.040
Benzo[b]fluoranthene	3.6	0.007	0.036	0.180
Benzo[k]fluoranthene	0.9	0.002	0.009	0.045
Benzo[j]fluoranthene	0.8	0.002	0.008	0.040
Benzo[e]pyrene	0.8	0.002	0.008	0.040
Benzo[a]pyrene	1.1	0.002	0.011	0.055
Dibenzo[a,h]anthracene	0.6	0.001	0.006	0.030

[1] Signal-to-noise ratio 10:1 (European Pharmacopoeia. European Directorate for the Quality of Medicines. Strasbourg 2007).

Annex 1. Representative chromatogram of a standard solution (100 ng mL^{-1})



RT	Compound	m/z
17.22	Benzo[a]anthracene-d12	240
17.38	Benzo[a]anthracene	228
17.67	Chrysene-d12	240
17.87	Chrysene	228
25.18	Benzo[b]fluoranthene-d12	264
25.38	Benzo[b]fluoranthene	252
25.40	Benzo[k]fluoranthene-d12	264
25.58	Benzo[k]fluoranthene	252
25.68	Benzo[j]fluoranthene	252
27.63	Benzo[e]pyrene-d12	264
27.85	Benzo[e]pyrene	252
28.10	Benzo[a]pyrene-d12	264
28.26	Benzo[a]pyrene	252
36.20	Dibenzo[a,h]anthracene-d14	292
36.40	Dibenzo[a,h]anthracene	278

Annex 2. Recovery SupelMIP PAHs SPE columns

Recovery experiments were performed using a PAH containing extender oil, comparing peak areas before loading the column with peak areas after elution from the column. Recovery rates were determined on 6 replicates.

Compound	Absolute mass loaded on column [ng]	Average recovery [%]	STDEV% (6 replicates)
Benzo[a]anthracene	18	59	4.3
Chrysene	90	84	3.5
Benzo[b]fluoranthene	40	94	2.6
Benzo[k]fluoranthene	6	89	10.8
Benzo[j]fluoranthene	8	51	9.3
Benzo[e]pyrene	100	95	1.4
Benzo[a]pyrene	25	93	3.5
Dibenzo[a,h]anthracene	2	91	3.2

ANNEX 3

STANDARD OPERATING PROCEDURE

DETERMINATION OF MIGRATION OF EU-PAHs FROM RUBBER AND PLASTIC MATERIALS INTO 20% AQUEOUS ETHANOL

Contents

STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF MIGRATION OF EU-PAHs FROM RUBBER AND PLASTIC MATERIALS

Method: STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF MIGRATION OF EU-PAHs FROM RUBBER AND PLASTIC MATERIALS

Analytes: Benzo[a]anthracene (CAS # 56-55-3)
Chrysene (CAS # 218-01-9)
Benzo[b]fluoranthene (CAS # 205-99-2)
Benzo[k]fluoranthene (CAS # 207-08-9)
Benzo[j]fluoranthene (CAS # 205-82-3)
Benzo[e]pyrene (CAS # 192-97-2)
Benzo[a]pyrene (CAS # 50-32-8)
Dibenzo[a,h]anthracene (CAS # 53-70-3)

Matrix: Rubber and Plastic Materials

Foreword

This document was prepared by the Joint Research Centre of the European Commission in Directorate F.2 (Health, Consumers & Reference Materials) as an analytical method standard operating procedure (SOP) for measuring the migration of eight selected polycyclic aromatic hydrocarbons from rubber and plastic materials to a solution intended to mimicking the characteristics of the outermost layer of the skin.

1. Scope and principle

This method is suitable for the detection and quantification of the eight polycyclic aromatic hydrocarbons (PAHs) reported in the following table that might migrate from in rubber and plastic materials into a 20% Ethanol aqueous solution, by gas chromatography coupled with mass spectrometry (GC-MS).

Analyte	CLP Classification
Benzo[a]anthracene	CARC 1B
Chrysene	CARC 1B
	MUTA 2
Benzo[b]fluoranthene	CARC 1B
Benzo[k]fluoranthene	CARC 1B
Benzo[j]fluoranthene	CARC 1B
Benzo[e]pyrene	CARC 1B
Benzo[a]pyrene	CARC 1B
	MUTA 2
Dibenzo[a,h]anthracene	CARC 1B

The overall migration of 8 EU-PAHs from the sample is determined by total immersion in the simulant and are expressed both as nanograms of the single PAH per square decimetre of the surface of the sample and as nanograms of the single PAH per weight of the sample.

Overall migration is determined as the mean of three measures on separate test specimens.

2. Terms and Definitions

CLP	Classification, Labelling and Packaging. The CLP Regulation ensures that the hazards presented by chemicals are clearly communicated to workers and consumers in the European Union through classification and labelling of chemicals.
GC-MS	Gas chromatograph coupled to mass-spectrometer
BaA	Benzo[a]anthracene
Chr	Chrysene
BbF	Benzo[b]fluoranthene
BkF	Benzo[k]fluoranthene
BjF	Benzo[j]fluoranthene
BeP	Benzo[e]pyrene
BaP	Benzo[a]pyrene
DBahA	Dibenzo[a,h]anthracene
BaA-d12	Benzo[a]anthracene (deuterium labelled)
Chr-d12	Chrysene (deuterium labelled)
BbF-d12	Benzo[a]fluoranthene (deuterium labelled)
BkF-12	Benzo[a]fluoranthene (deuterium labelled)
BjF-d12	Benzo[j]fluoranthene (deuterium labelled)
BeP-d12	Benzo[e]pyrene (deuterium labelled)
BaP-d12	Benzo[a]pyrene (deuterium labelled)
DBahA-d14	Dibenzo[a,h]anthracene (deuterium labelled)
PAH	Polycyclic Aromatic Hydrocarbons

3. Safety and Environmental Precautions

CAUTION: The analysed polycyclic aromatic hydrocarbons are human carcinogens. Precaution shall be taken to avoid exposure.

All solutions should be handled in an adequately ventilated fume hood, glove box or equivalent.

The laboratory shall establish procedures for disposal of solutions containing PAHs.

3.1 Take routine safety and environmental precautions, as in any chemical laboratory activity.

3.2 Care should be taken to avoid inhalation or oral or dermal exposure to harmful chemicals. Use a chemical fume hood, and wear an appropriate laboratory coat, gloves and safety goggles when preparing or handling undiluted materials, standard solutions or material extracts.

4. Apparatus and Equipment

Beside the standard laboratory glassware the following items are needed:

4.1. Cutting tool (scissors or sharp knife or other suitable device).

4.2. Tweezers, stainless steel, blunt nosed.

4.3. Analytical balance capable of measurement to at least four decimal places.

4.4. Pipettes and tips capable of accurately dispensing volumes 10-1000 µL.

4.5. 100 mL bottles with wide neck and screw cap, or equivalent.

4.6. 10 mL vial

4.7. Shaking water bath (linear shaking, speed range 20-200 rpm, temperature range 20- 99.9 °C, 15 mm shacking stroke).

4.8. Solid phase extraction manifold.

4.9. Solid phase extraction cartridges (Phenomenex, Starta PAH, 1.5 g/6 mL).

4.10. Techne dry block sample concentrator or equivalent.

4.11. GC-MS system in single-ion monitoring detection mode. The gas chromatograph must be configured to perform splitless injections on a capillary column.

4.12. Column: Restek, RXi-PAH, 30m, 0.25mm ID, 0.10 µm df, Product Code 49318.

NOTE: Use clean and dry glassware in order to avoid contamination. PAHs have low aqueous solubility but tend to stick on glass. Rinse glassware additionally with toluene after use.

5. Reagents and Supplies

All solvents shall be "**pesticide & GC residue"** grade unless otherwise noted.

5.1 Toluene

5.2 Dichloromethane

5.3 Ethanol (99.8%)

5.4 Methanol

5.5 Acetonitrile

5.6 MilliQ Water

5.7 50 µg/mL 8 native PAHs standards in Toluene (Benzo[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[j]fluoranthene, Benzo[e]pyrene, Benzo[a]pyrene, Dibenzo[a,h]anthracene) REF: ML309M025TOEC, custom synthesised by Lab Service Analytica s.r.l.

5.8 50 µg /mL 7 isotope Labelled PAHs standards in Toluene (Benzo[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[e]pyrene Benzo[a]pyrene, Dibenzo[a,h]anthracene) REF: ML310M025TOEH, custom synthesised by Lab Service Analytica s.r.l.

6. Preparation of working solutions

Dependig on the expected level of release of PAHs, working range was divided in two calibration ranges:

a. 0 – 200 ng/mL WORKING RANGE a (HIGH RANGE)
b. 0 - 25 ng/mL WORKING RANGE b (LOW RANGE)

In the following paragraphs, working instructions are given for both working range.

6.1 20% aqueous Ethanol

1L of 20% aqueous Ethanol solution is prepared as follows:

6.1.1 Measure 200 mL of absolute EtOH (99.8%) in a 250 mL cylinder.

6.1.2 Pour the solvent into a 1000 mL volumetric flask.

6.1.3 Dilute to the mark with MilliQ water.

6.2 Standard Solutions

Two different "standards" are used in this procedure, namely "Native standards" and "Isotope Labelled Standards". The first is used as standard for quality control and the latter as an internal standard.

Two different set of dilutions are made out of the same vial of commercial standard mixture [5.7 and 5.8]:

1. STANDARDS SOLUTIONS FOR MIGRATION (IN CH_3CN. PRIMARY NATIVE STANDARD AND ISOTOPE LABELLED STANDARD)

2. STANDARDS SOLUTIONS FOR CALIBRATION CURVE (IN TOLUENE. PRIMARY NATIVE STANDARD AND ISOTOPE LABELLED STANDARD USED TO BUILD THE CALIBRATION CURVE ACCORDING TO TABLE 1)

Preparation of Standard solution for Migration Experiment (in CH_3CN)_

WORKING RANGE a

a. 6.2.1 Primary Native Standards (2500 ng/mL)

a. 6.2.1.1 Pipette 500 µL of the native standard mixture [5.7] in a 10 mL volumetric flask.

a. 6.2.1.2 Dilute to the mark with acetonitrile.

a. 6.2.1.3 Label and store in refrigerator (4 °C) at dark.

a. 6.2.2 Primary Isotope Labelled Standards (2500 ng/mL)

a. 6.2.2.1 Pipette 500 µL of the native standard mixture [5.8] in a 10 mL volumetric flask.

a. 6.2.2.2 Dilute to the mark with acetonitrile.

a. 6.2.2.3 Label and store in refrigerator (4 °C) at dark.

WORKING RANGE b

b. 6.2.1 Native Standards in CH_3CN for migration (500 ng/mL)

b. 6.2.1.1 Pipette 100 µL of the commercial native standard mixture [5.7 CODE ML309] in a 10 mL volumetric flask.

b. 6.2.1.2 Add acetonitrile to bring flask to volume.

b. 6.2.1.3 Label and store in refrigerator (4 °C) at dark.

b. 6.2.2 Isotope Labelled Standards (Internal Standard) in CH3CN for migration (500 ng/mL)

b. 6.2.2.1 Pipette 100 µL of the commercial isotope labelled standard mixture [5.8 CODE ML310] in a 10 mL volumetric flask.

b. 6.2.2.2 Add acetonitrile to bring flask to volume.

b. 6.2.2.3 Label and use to build the calibration curve as reported in Table 1

6.2.3 Preparation of calibration Standard Solutions (in Toluene)

WORKING RANGE a

The calibration curve should cover a concentrations range from 0-200 ng/mL with the internal standard at a concentration of 50 ng/mL. The aforementioned range covers the linear range of the mass-spectrometer used for the preparation of this procedure (Agilent 5975C). The linear response range of mass-spectrometers other than the one used in this study might be different.

		STD	**Internal Standard** *(final Conc. 50 ng/mL)*
Calibration Level	Final Concentration [ng/mL]	Volume of 2500 ng/mL solution [as for a. 6.2.1 but dilute with Toluene] in 10 mL volumetric flask [µL]	Volume of 2500 ng/mL [as for a. 6.2.2 but dilute with Toluene] solution in 10 mL volumetric flask [µL]
1	5	20	200
2	10	40	200
3	50	200	200
4	100	400	200
5	200	800	200

WORKING RANGE b

The calibration curve should cover a concentrations range from 0-25 ng/mL with the internal standard at a concentration of 10 ng/mL. The aforementioned limit falls into the linear range of the mass-spectrometer used for the preparation of this procedure (Agilent 5975C). The linear response range of mass-spectrometers other than the one used in this study might be different.

b. 6.2.3 Native Standards in Toluene for calibration (2500 ng/mL and 250 ng/mL)

2500 ng/mL dilution:

b. 6.2.3.1 Pipette 500 µL of the commercial native standard mixture [5.7, CODE ML309] in a 10 mL volumetric flask.

b. 6.2.3.2 Add toluene to bring flask to volume.

b. 6.2.3.3 Label and use an aliquot to build the calibration curve as reported in Table 1, and an aliquot to achieve the next calibration dilution.

250 ng/mL dilution:

b. 6.2.3.4 Pipette 1 mL of the 2500 ng/mL standard [b. 6.2.3.2] in a 10 mL volumetric flask

b. 6.2.3.5 Add toluene to bring flask to volume.

b. 6.2.3.6 Label and use to build the calibration curve as reported in Table 1

b. 6.2.4 Isotope Labelled Standards (Internal Standard) in Toluene for calibration (2500 ng/mL)

b. 6.2.4.1 Pipette 500 µL of the commercial isotope labelled standard (internal standard) mixture [5.8, CODE ML310] in a 10 mL volumetric flask.

b. 6.2.4.2 Add toluene to bring flask to volume.

b. 6.2.4.3 Label and use to build the calibration curve as reported in Table 1

Table 1. Calibration curve

			COMPOSITION		
			Native Standards		**Internal Standard** *(Final Conc. 10 ng/mL)*
Calibration Level	Final Concentration [ng/mL]	Final Volume [mL]	Volume of 2500 ng/mL [b. 6.2.3.1-b .6.2.3.2] [µL]	Volume of 250 ng/mL [b. 6.2.3.4 –b. 6.2.3.5][µL]	Volume of 2500 ng/mL [b. 6.2.4] [µL]
1	0.5	10		20	40
2	1	10		40	40
3	5	10	20		40
4	10	10	40		40
5	25	10	100		40

7. Samples

The present procedure is suitable for testing the plastic/rubber article in its ready-for-use state. In the following section, general guidelines on how to prepare representative specimen from the article are given.

7.1 Definition of sample area

Sampling is achieved by cutting representative areas from the article to be tested.

Only the surfaces that come into direct contact with the skin can be taken into account for sampling.

It is advisable to select the the areas of the article having the thinnest material cross-section.

7.2 Determination of sample area

Regularly, about 0.2 dm^2 test specimens are used.

The determination of the specimen dimensions has to be done carefully. To facilitate this operation, it is advisable to cut square-shaped sections. In this case, the total surface is calculated as the sum of 6 individual faces.
On the other hand, when the cut test specimen has irregular shape, its thickness has to be determined with a calliper at three different places at 10 mm from the outer margin and in the middle and to consider the average of the four measurements for the calculation of the surface.

8. Method Summary

8.1 Preparation of three test specimens *per* material, of about 0.2 dm^2 each.

8.2 Dynamic exposure to simulant (20% aqueous Ethanol) by total immersion, at 40 °C.

8.3 Removal of test specimen, addition of internal standard and elution through solid-phase extraction cartridge.

8.4 Evaporation of the eluted fractions under stream of nitrogen.

8.5 Dissolution of the residue in toluene and analysis by GS-MS.

9. Migration description

Each migration experiment involves 5 test bottles:

3 containing three different test specimens of the same material [8.1]

1 Control solution [9.2]

1 Blank solution [9.3]

9.1 Preparation of test specimens

(20 mL Simulant, test specimen)

Before preparing test specimens, remove any surface contamination from the sample by gently wiping it with a lint free fabric, or by brushing with a soft brush. Avoid washing the sample with water or any other solvent. Three different test specimens are necessary for each migration experiment.

9.1.1 Cut each test specimen in a way that its total surface is about 0.2 dm^2.

As an example, from a rubber sheet 20cm*20cm*0.2cm (length*width*thickness) use a scissor to cut each test specimen 3 cm * 3 cm using the rule.

9.1.2 Repeat this procedure for all test specimens.

9.1.3 Weigh each specimen, record the weight and transfer it into a 100 mL bottles with wide neck and screw cap [4.5] using tweezers [4.2].

9.1.4 Add 20 mL of 20% aq EtOH freshly prepared as reported in [6.1] in the same bottle as 9.1.3.

9.2 Preparation of Control Solution

WORKING RANGE a

(20 mL Simulant, Native Standards: 100 ng/mL after evaporation and re-dissolution in Toluene)

Known amounts of native standard [a. 6.2.1] is spiked into 20% aq EtOH. This solution is submitted to the same procedural steps as the specimen. The purpose of this solution is to ensure the accuracy.

a. 9.2.1 Add 20 mL of 20% aqueous EtOH [6.1] into a 100 mL bottles with wide neck and screw cap [4.5].

a. 9.2.2 Pipette 40 µL of the primary native standard mixture [a. 6.2.1] into the same bottle as in a. 9.2.1.

WORKING RANGE b

(20 mL Simulant, Native Standards: 10 ng/mL after evaporation and re-dissolution in Toluene)

Known amount of native standards [b. 6.2.1] is spiked into 20% aq EtOH. This solution undergoes to the same procedural steps as the specimen. The purpose of this solution is to ensure the accuracy (if the recovery of primary standard deviates more than 15 percentage points from 100%, it is advisable to check for a systematic error).

b. 9.2.1 Add 20 mL of 20% aqueous EtOH [5.1] into a 100 mL bottles with wide neck and screw cap [4.4].

b. 9.2.2 Pipette 20 µL of the primary native standard mixture [b. 6.2.1] into the same bottle as in b. 9.2.1.

9.3 Preparation of Blank solution

WORKING RANGE a and b

(20 mL Simulant)

This represents a negative control which works for excluding PAH contamination

9.3.1 Add 20 mL of 20% aqueous EtOH [6.1] into a 100 mL bottles with wide neck and screw cap

9.4 Migration protocol

WORKING RANGE a and b

9.4.1 Set the shaking water bath at 120 rpm and let it reach the temperature equilibrate at 40 °C.

9.4.3 Transfer the bottles prepared as in [9.1], [9.2] and [9.3] into the shaking water bath and let them shake for the migration time established (1h, 4h or 24h).

9.4.4 Remove bottles from the water shaking bath and allow them to cool down at room temperature.

9.4.5 Clamping test specimen with tweezers, rinse it with 5 mL of 20% aq EtOH prepared as reported in [6.1], then remove it from the bottle.

9.4.5 To each of the five bottles, **pipette 20 µL of the primary isotope labelled standard mixture (internal standard) prepared as in [a. 6.2.2] OR [b. 6.2.2].**

10. Sample clean-up and evaporation

Clean-up is achieved using Strata PAH solid-phase extraction cartridges [4.8], a silica based proprietary sorbent designed to provide high recoveries of polycyclic aromatic hydrocarbons from water.

Set the manifold pressure between 5 and 10 psi (-5 and -10 inches of mercury for vacuum) unless otherwise noted.

10.1 Condition SPE cartridge with 2x6 mL DCM, 2x6 mL MeOH, 2x6 mL deionised H_2O.

10.2 Load the extract and let it soak into the sorbent bed

10.3 Wash with 3 mL MeOH.

10.3 Let the sorbent dry for 10 minutes under a pressure of 20 psi (-15 inHg).

10.4 Elute PAH with 6 mL DCM and collect eluate in 10 mL vial [4.6].

10.5 Set the temperature of dry block sample concentrator [4.10] at 40 °C and evaporate dichloromethane to dryness under a steady stream of nitrogen (approximately 15 minutes).

10.6 Dissolve dry extracts in 1 mL toluene. This extract is injected in the GC-MS system.

11. Sample Analysis

The method for quantifying the PAHs in rubber and plastic materials involves GC-MS. The analytes are resolved from other potentially interfering substances on a GC column. Comparison of the area ratios (native analyte to isotope-labelled analyte) of the unknowns with the area ratios (native analyte to isotope-labelled analyte) of the known standard concentrations yields the concentration of the analytes.

11.1 GC-MS operating conditions: example

GC column	Restek, Rxi-PAH, 30 m, 0.25 mmID, 0.10 µm df (Cat#49318) or equivalent.
Injector temperature	300 °C
Mode	Constant flow
Flow rate	1.75 mL/min
Injection	1 µL pulsed splitless
Column temperature	60 °C for 1 min 40 °C/min to 200 °C 2.5 °C/min to 300 °C 30 °C/min to 320 °C for 5 min
Run time	49,5 mins
Transfer line temperature	320 °C
MS source	300 °C
Ionization mode	Electron ionization

Ion traces

Group 1 (Start Time: 0 mins)	**(Masses [m/z], Dwell time [ms])** In bold the MW masses
Benzo[a]anthracene-d12 Benzo[a]anthracene Chrysene-d12 Chrysene	(114, 45), (120, 45), (**228**, 45), (229, 45), (**240**, 45), (241, 45)
Group 2 (Start Time: 23 mins)	**(Masses [m/z], Dwell time [ms])** In bold the MW masses
Benzo[b]fluorantrhene-d12 Benzo[b]fluorantrhene Benzo[k]fluoranthene-d12 Benzo[k]fluoranthene Benzo[j]fluoranthene Benzo[e]pyrene-d12 Benzo[e]pyrene Benzo[a]pyrene-d12 Benzo[a]pyrene	(125, 40), (126, 40), (132, 40), (**252**, 40), (253, 40), (**264**, 40), (265, 40)
Group 3 (Start Time: 34 mins)	**(Masses [m/z], Dwell time [ms])** In bold the MW masses
Dibenzo[a,h]anthracene-d14 Dibenzo[a,h]anthracene	(139, 45), (146, 45), (**278**, 45), (279, 45), (**292**, 45), (293, 45)

Note: The operating parameters may have to be adjusted to the instrument and column conditions and the resolution of the chromatographic peaks.

11.2 General analytical information

11.2.1 For the conditions described here, the expected sequence of elution will be Benzo[a]anthracene-d12, Benzo[a]anthracene, Chrysene-d12, Chrysene, Benzo[b]fluoranthene-d12, Benzo[b]fluoranthene, Benzo[k]fluoranthene-d12, Benzo[k]fluoranthene, Benzo[j]fluoranthene, Benzo[e]pyrene-d12, Benzo[e]pyrene, Benzo[a]pyrene-d12, Benzo[a]pyrene, Dibenzo[a,h]anthracene-d14, Dibenzo[a,h]anthracene.

11.2.2 Differences in e.g. temperature, gas flow rate and the age of the column can be expected to alter retention times

11.2.3 The sequence of determination of PAHs will be in accordance with individual laboratory practice. This section gives an example.

11.2.4 Inject pure toluene to check for contamination

11.2.6 Inject the calibration standards, the control solution, the blank solution and the samples.

11.2.7 Record the peak areas of all labelled (deuterated) and not labelled substances

Important: Integrate peak areas in selected ion mode (SIM) using the molecular weight mass for each compound.

11.2.8 Calculate the relative response ratios of all PAHs ($A_{PAH(native)}$ / $A_{PAH(deuterated)}$) for each standard solution.

11.2.9 Plot graph of the concentration of each of the PAHs (x-axis) against the area ratios (y-axis).

11.2.10 The intercept should not be statistically significantly different from zero.

11.2.11 The calibration curve should be linear over the entire standard range for all PAHs (if the linear regression is less than 0.99, the calibration should be repeated).

See Annex 1 for representative standard chromatogram

12. Data Analysis and Calculations

12.1 Calculate the relative response ratios from the peak areas for each of the calibration standards

$RF(PAH) = (A_{PAH(native)} / A_{PAH(deuterated)})$

Where RF is the relative response ratio, $A_{PAH(native)}$ is the peak area of each single native PAH (in selected ion mode and $A_{PAH(deuterated)}$ is the peak area of each single isotope labelled PAH (in selected ion mode.

Selected ions to be used for area integration:

Compound	Ion [m/z]
Benzo[a]anthracene-d12	240
Benzo[a]anthracene	228
Chrysene-d12	240
Chrysene	228
Benzo[b]fluorantrhene-d12	264
Benzo[b]fluorantrhene	252
Benzo[k]fluoranthene-d12	264
Benzo[k]fluoranthene	252
Benzo[j]fluoranthene	252
Benzo[e]pyrene-d12	264
Benzo[e]pyrene	252
Benzo[a]pyrene-d12	264
Benzo[a]pyrene	252
Dibenzo[a,h]anthracene-d14	292
Dibenzo[a,h]anthracene	278

12.2 Plot a graph of the relative response factors $A_{PAH(native)}$ / $A_{PAH(deuterated)}$ (y-axis), versus concentration (x-axis) for each PAH and for each standard solution. Set the intercept to zero. Calculate the linear regression (Y= bX) from these data, and use the slope (b) to calculate the concentration in ng/mL.

12.3 The migration of the single PAHs is expressed both as ng PAH/dm^2 rubber or plastic and µg PAH /Kg rubber or plastic) is determined from the calculated relative response ratios of each PAH in the test sample and the slope from the appropriate calibration curves (one curve for each of the 8 PAHs):

$$\text{Concentration [µg /kg]} = \frac{(Y)}{b \times SW}$$

$$\text{Concentration [ng /dm}^2\text{]} = \frac{(Y)}{b \times S}$$

Y = relative response ratio ($A_{PAH(native)}$ / $A_{PAH(deuterated)}$)

b = Slope of the linear regression obtained from the standard calibration curves

SW = specimen weight in g

S = surface of specimen in dm^2

13. Method Performance Specifications

13.1 Limit of quantification (LOQ) and limit of detection (LOD)

The limit of quantification is calculated separately for each PAH by injecting 6 times the lowest concentration of the calibration curve through the following equation:

LOD=3*sd/slope

LOQ=10*sd/slope

Slope= Slope of the linear regression obtained from the standard calibration curves

sd= Standard deviation of the average of six measurements of the lowest concentration of the calibration curve

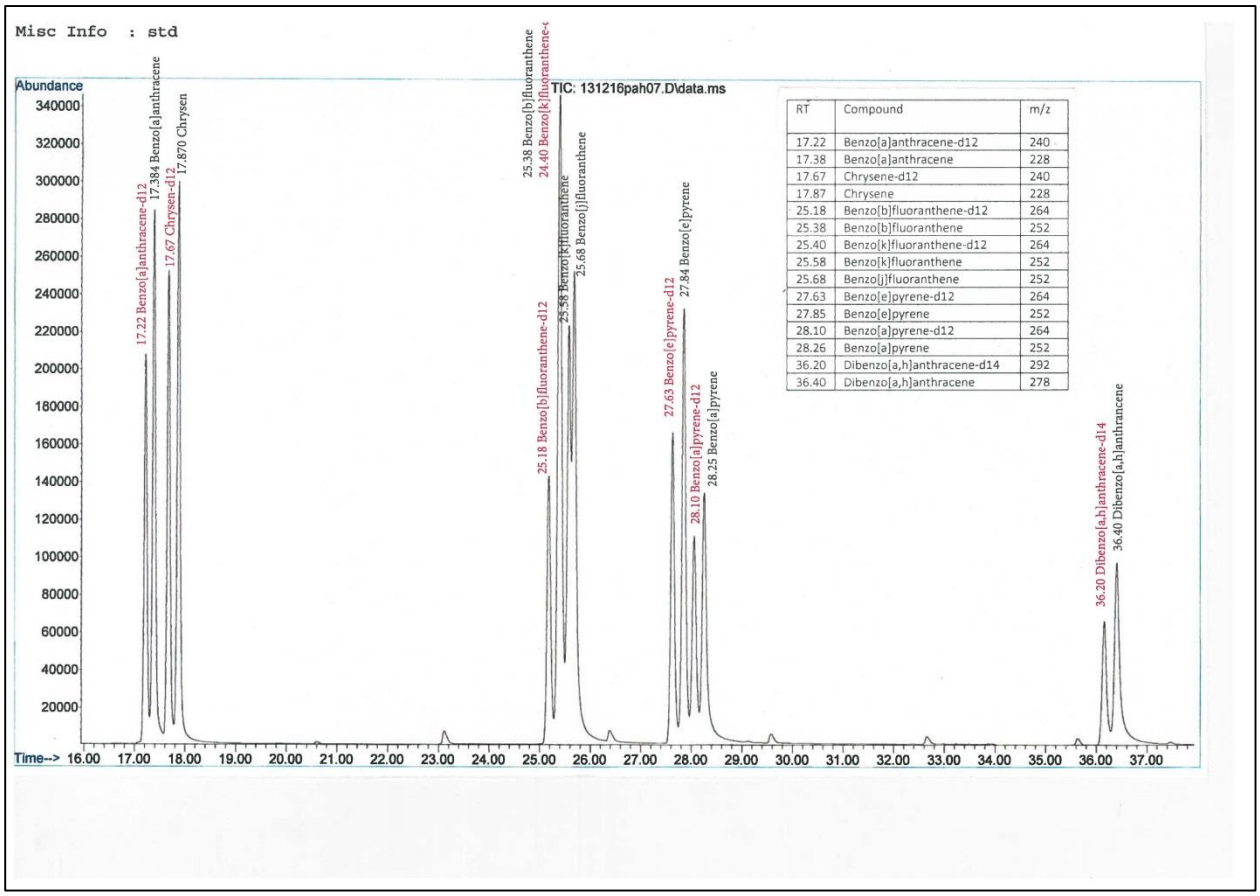
Compound	LOD (ng/mL)	LOQ (ng/mL)	LOQ (ug/kg=ppb) (Sample weigh 2 g, final volume 1 ml)
Benzo[a]anthracene	0.1	0.5	0.2
Chrysene	0.1	0.2	0.1
Bnzo[b]fluoranthene	0.1	0.3	0.2
Benzo[k]fluoranthene	0.3	0.9	0.5
Benzo[j]fluoranthene	0.2	0.7	0.4
Benzo[e]pyrene	0.2	0.8	0.4
Benzo[a]pyrene	0.3	0.9	0.5
Dibenzo[a,h]anthracene	0.3	0.9	0.4

LOQ and LOD for 0-200 ng/mL working range (a)

Compound	LOD (ng/mL)	LOQ (ng/mL)	LOQ (ug/kg=ppb) (Sample weigh 2 g, final volume 1 ml)
Benzo[a]anthracene	0,1	0,2	0,1
Chrysene	0,0	0,1	0,1
Benzo[b]fluoranthene	0,1	0,2	0,1
Benzo[k]fluoranthene	0,2	0,5	0,3
Benzo[j]fluoranthene	0,1	0,3	0,1
Benzo[e]pyrene	0,1	0,2	0,1
Benzo[a]pyrene	0,1	0,3	0,2
Dibenzo[a,h]anthracene	0,2	0,6	0,3

LOQ and LOD for 0-25 ng/mL working range (b)

Annex 1. Representative chromatogram of a standard solution (100 ng/mL)



RT	Compound	m/z
17.22	Benzo[a]anthracene-d12	240
17.38	Benzo[a]anthracene	228
17.67	Chrysene-d12	240
17.87	Chrysene	228
25.18	Benzo[b]fluoranthene-d12	264
25.38	Benzo[b]fluoranthene	252
25.40	Benzo[k]fluoranthene-d12	264
25.58	Benzo[k]fluoranthene	252
25.68	Benzo[j]fluoranthene	252
27.63	Benzo[e]pyrene-d12	264
27.85	Benzo[e]pyrene	252
28.10	Benzo[a]pyrene-d12	264
28.26	Benzo[a]pyrene	252
36.20	Dibenzo[a,h]anthracene-d14	292
36.40	Dibenzo[a,h]anthracene	278

Annex 2. Recovery Strata PAHs SPE columns

Recovery experiments were performed comparing peak areas of a standard solution at 100 ng/mL (Working range a) or 10 ng/mL (working range b) of each PAH before and after a migration cycle and elution through strata PAH column. Recovery rates were determined on 3 replicates at 1, 4 and 24 hours migration.

1 hour Migration **Working Range a**	Average recovery [%]	STDEV% (3 replicates)
Benzo[a]anthracene	90.6	12.2
Chrysene	78.5	9.0
Benzo[b]fluoranthene	99.4	13.2
Benzo[k]fluoranthene	80.1	10.4
Benzo[j]fluoranthene	92.0	11.7
Benzo[e]pyrene	93.3	12.1
Benzo[a]pyrene	86.7	10.5
Dibenzo[a,h]anthracene	74.2	14.9

4 hours Migration **Working Range a**	Average recovery [%]	STDEV% (3 replicates)
Benzo[a]anthracene	100.9	7.2
Chrysene	81.8	10.5
Benzo[b]fluoranthene	113.5	3.0
Benzo[k]fluoranthene	83.7	8.1
Benzo[j]fluoranthene	100.2	10.7
Benzo[e]pyrene	105.2	4.0
Benzo[a]pyrene	93.3	8.9
Dibenzo[a,h]anthracene	70.6	2.3

24 hours Migration **Working Range a**	Average recovery [%]	STDEV% (3 replicates)
Benzo[a]anthracene	106.2	15.1
Chrysene	97.6	12.9
Benzo[b]fluoranthene	113.6	17.9
Benzo[k]fluoranthene	97.8	17.7
Benzo[j]fluoranthene	110.8	17.3
Benzo[e]pyrene	105.7	16.4
Benzo[a]pyrene	104.5	18.3

24 hours Migration **Working Range b**	Average recovery [%]	STDEV% (3 replicates)
Benzo[a]anthracene	98,41	5,20
Chrysene	95,15	5,88
Benzo[b]fluoranthene	99,33	11,54
Benzo[k]fluoranthene	94,12	9,59
Benzo[j]fluoranthene	94,56	3,07
Benzo[e]pyrene	95,70	5,49
Benzo[a]pyrene	95,04	2,50

ANNEX 4

MIGRATION TEST PROTOCOL USING ARTIFICAL SKIN SURFACE FILM LIQUID (SSFL) AS MIGRATION MEDIUM

1. Preparation of sample material

Cut sample to shapes (rectangles) of 3cm x 3cm (=0.2 dm^2).

a= 3 cm

h= 0.2 cm

A = ((3cm x 3cm) x 2) + (3cm x 0.2 cm)x4 = 20 cm^2 = 0.2 dm^2

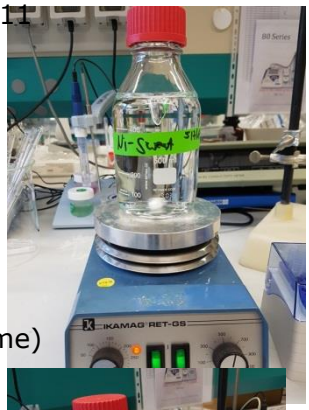
Amount of required sample materials:
3 replicates of blank
3 replicates for each sebum concentration

Example: 0% Sebum (blank), 0.5% sebum, 1% Sebum, 2% Sebum
→ required materials: 4x3 =12

2. Weigh the sample pieces and note weight in lab-book

3. Preparation of Sebum Suspensions

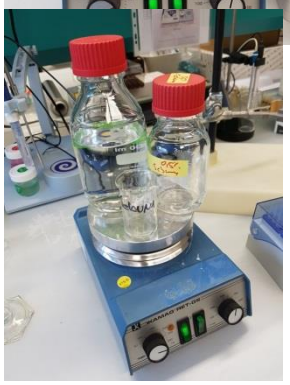
3.1 Prepare Sweat Component (Ni-sweat) – According to CEN EN1811:2011



- Add approximately 400 mL of Milli-Q water to a 500 mL glass bottle
- Weigh 0.5g of urea and add to glass bottle while stirring
- Weigh 2.5g of sodium chloride and add to glass bottle while stirring
- Add 415 µL of lactic acid to glass bottle while stirring
- Immerse pH electrode into bottle
- Slowly add 1M NaOH solution until pH 5,5 is reached
- Slowly add 0.1M NaOH until pH 6,5 is reached
- Bring glass bottle to volume (500 mL)

3.2 Melt sebum on hot plate (don't leave it on very hot plate for a long time)

3.3 Heat up sweat (3.1) to 70 °C (on hot plate)

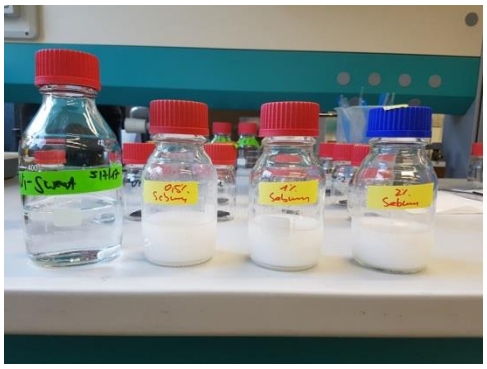


3.4 Prepare suspensions (in 250 mL glass bottles)

Concentration Sebum (V/V)	
0%	Only Ni-Sweat (3.1)
0.5%	0.5 mL molten sebum in 100 mL sweat (3.1)
1%	1.0 mL molten sebum in 100 mL sweat (3.1)
2%	2.0 mL molten sebum in 100 mL sweat (3.1)

Shake manually very thoroughly for a few minutes

Place 250 mL bottles on mechanical shaker for 20 minutes at strongest shake-intensity.



4. Determination of effective sebum concentration

Given the limited stability of the SSFL emulsions and the variability of the sebum concentrations of each new preparation, the effective sebum concentration needs to be determined just before each batch of migration tests.

4.1 Weigh empty petri-dishes (d=6 cm) → 3 replicates for blank (aqueous sweat) and three blanks for each sebum-concentration[3].

4.2 Transfer exactly 10 mL of the freshly prepared SSFL-suspensions and 10 mL of the aqueous sweat solution into the previously (3.1) weighed petri-dishes (d = 6 cm).

4.3 For the evaporation of the aqueous phase, place petri-dished for 6-7 hours in an oven at 100 °C.

4.4 Once the aqueous phase has evaporated, remove petri-dishes from oven and cool down to room-temperature.

4.5 Weigh petri-dishes.

4.6 Subtract average weight of petri-dishes with only aqueous sweat (salt residues) from the average weight of the petri-dishes containing the sebum-emulsions.

4.7 Convert sebum-mass into sebum-volume by applying a density value for sebum of 0.867 g mL^{-1}.

<u>Practical example for the calculation of the effective sebum volume-% of a SSFL-suspension:</u>

Average weight petri-dishes containing aqueous sweat (after drying) : 75,3 mg
Average weight petri-dishes containing SSFL suspension (after drying) : 145.7 mg
Difference of the above weights (= effective weight sebum without salts) : 70.4 mg

Concentration mass content sebum = 70.4 mg sebum in 10 mL suspension
= 0.704 g sebum / 100 mL suspension

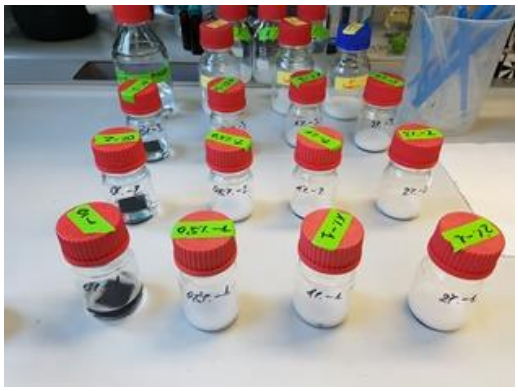
V(Sebum) = m(Sebum) / 0.867 g mL^{-1} = 0.81 mL

Volume-% (Sebum) = 0.81 % (V/V)

5. Start migration tests

- Add sample material (plastic, rubber) to 100 mL glass bottles
- Add 20 mL of each sebum suspension to the respective 100 mL glass bottles (graduated cylinder)
- For the 0% sebum migration test, add 20 mL of nickel sweat (3.1)

[3] If only one sebum concentration is prepared (e.g. 0.5% sebum), a total of 6 petri-dishes are required (3 aqueous sweat + 3 sebum emulsions).



5.1 Control solution

Add 20 mL of 1% sebum suspension to 100 mL glass bottle (DO NOT ADD SAMPLE MATERIAL TO THIS BOTTLE)

- Add all bottles to rotary shaker bath (140 rpm) previously heated up to 40°C
- Leave shaking for desired time (e.g. 1h, 4h or 24 h)

6. Stop migration test

- Remove sample material with tweezers from bottles
- Add 20 µL of internal standard (in MeCN, 2500 ng/mL) to 100 mL glass bottles
- Mix for a few seconds

6.1 Control solution

In addition to the internal standard solution add 40 µL of the non-labelled standard solution (in MeCN, 2500 ng/mL).

7. Liquid-liquid extraction

7.1 With the auxiliary of a Pasteur pipette transfer the 20 mL of the 100 mL glass bottle into a 100 mL separatory funnel. Leave pipette in 100 mL glass bottle

7.2 Add 10 mL of hexane to the 100 mL glass bottle. Wash bottle and pipette and transfer the 10 mL of hexane to separatory funnel

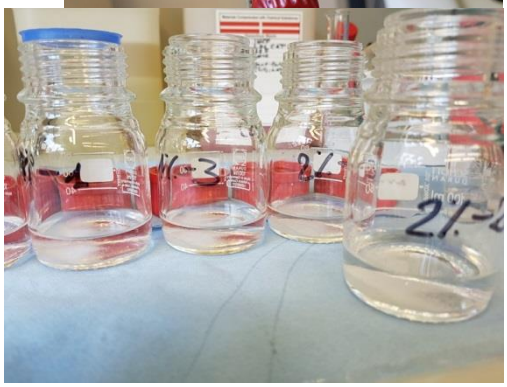
7.3 Shake thoroughly for 30 second

7.4 Wait for separation of phases

7.5 Take off-as much of the UPPER phase as possible and transfer to a new 100 mL glass bottle

7.6 Add another 10 mL (total 20 mL) of hexane directly into the separatory funnel

7.7 Shake thoroughly for 30 seconds



7.8 Discard LOWER phase through valve and transfer hexane phase into the 100 mL glass bottle (6.5)

7.9 Add approximately 1g of anhydrous sodium sulphate to each bottle to dry hexane phase

7.10 Shake gently for a few seconds

7.11 Transfer the approximately 20 mL of hexane from the 100 mL glass bottle (which contains sodium sulphate) to a 20 mL glass vial

7.12 Wash 100 mL glass bottle (which contains sodium sulphate) with approximately 1 mL of hexane and transfer that 1 mL to 20 mL glass vial (7.11)

7.13 Reduce volume of hexane in 20 mL glass vial at 40 °C under nitrogen flow to a volume of approximately 2 mL

8. Clean-up[4]

8.1 Condition SupelMIP SPE-PAH (Supelco) with 1 mL Cylcohexane

8.2 Load sample (approx. 2 mL left in glass vials (7.13)) on SPE column

8.3 Wash with 3 mL cyclohexane

8.4 Elute PAH with 3x 1mL ethylacetate into new 20 mL glass vial

9. Evaporate to dryness

Evaporate 3 mL ethylacetate-extract to dryness (under nitrogen flow, at 40°C)

10. Reconstitute in 1 mL toluene

11. Inject in GC-MS

Material

Artificial Sebum: Pickering Laboratories, Product Code 1700-0700

SPE-cartridges: Supelco, SupelMIP SPE-PAH, 50 mg/3 mL, Product Code 52773-U

[4] For details refer to SPE cartridges instructions of use

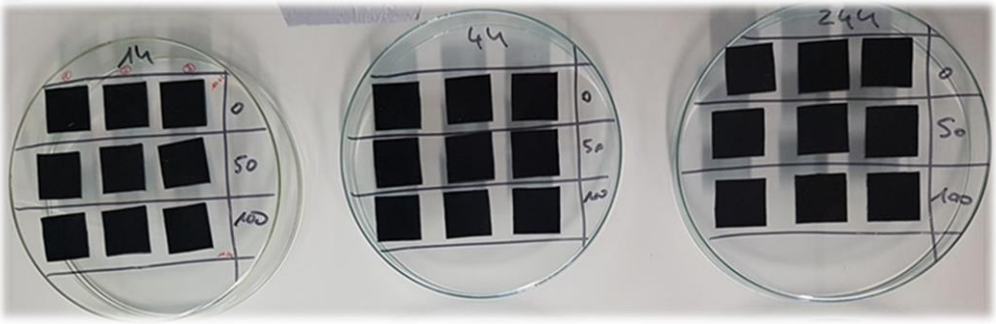
ANNEX 5

MIGRATION TEST PROTOCOL USING SEBUM IMBUED STRIPS AS MIGRATION MEDIUM

1. Preparation of sample material

Cut out rectangles of both the sample materials and filter-papers (3cm x 3cm =0.09 dm^2).

a	=	3 cm
A	=	3 cm x 3 cm = 9 cm^2 = 0.09 dm^2



2. Weigh the sample material and filter paper rectangles

3. Imbue filter-papers with sebum

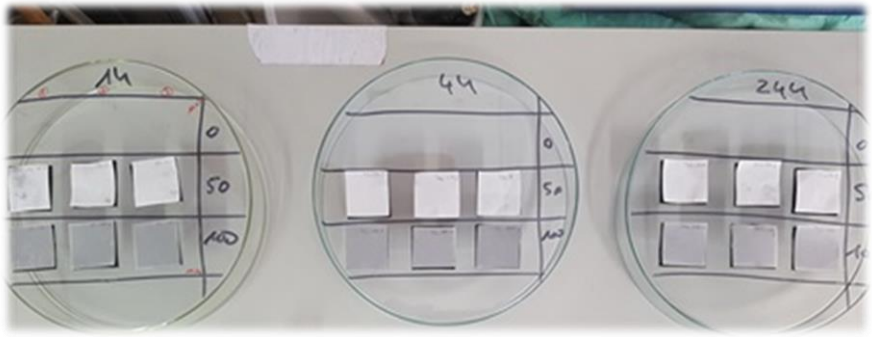
- melt sebum in a relatively narrow beaker
- imbue filter-paper with molten sebum
- wipe excess of sebum from filter-paper with a cleansing tissue (the homogenous distribution of sebum on the filter paper is very important)

- weigh imbued filter paper (the absorbed amount of sebum should be approximately the same for all filter-papers). If not, repeat the preparation until the masses are

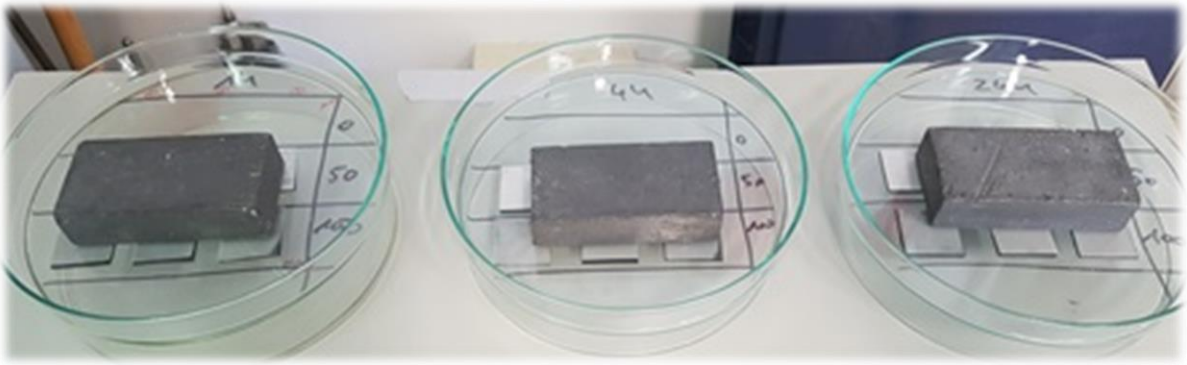
more or less the same.

4. Cover rubber/plastic sample with sebum-imbued filter paper

Place imbued filter papers on the sample-materials. They should perfectly match in size.

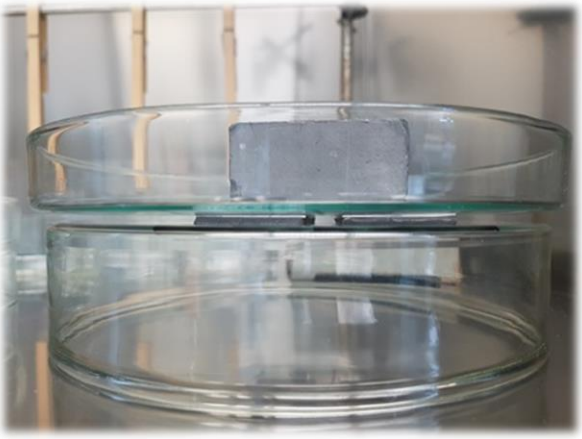


5. Place glass pane on samples/filter papers

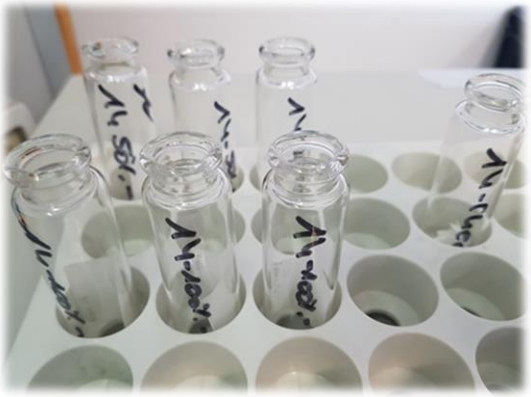


Place a weight (approx. 1,5 kg) on top of the glass pane to optimise the contact between the sebum imbued filter paper and the sample material.

6. Transfer to exposure chamber at 37°C for the desired time of migration

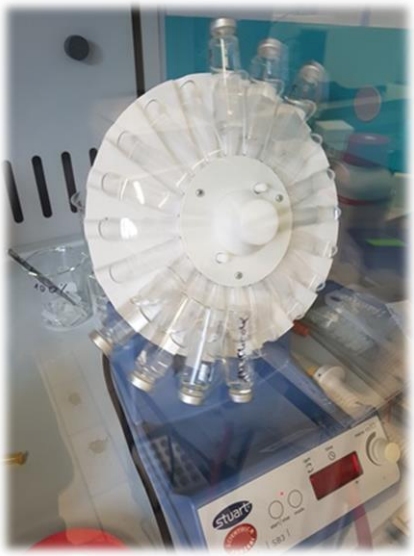


7. End of migration



- remove filter-paper from rubber-plastic sample and transfer in 20 mL glass vial
- one blank filter paper is spiked with 40 µL standard solution (c=2500 ng mL^{-1})
- add 15 mL of hexane to all vials
- add 20 µL of internal standard (c=2500 ng mL^{-1} in toluene) to each vial
- shake for 60 minutes
- remove filter paper from glass vial
- evaporate to approximately 2-3 mL under nitrogen flow (approx. 40 mins)
-

8. Clean-up



- condition SupelMIP SPE-PAH (Supelco) with 1 mL cylcohexane
- load sample (approx. 2 mL left in glass vials) on SPE column
- wash with 3 mL cyclohexane
- elute PAH with 3x 1mL ethylacetate into new 20 mL glass vial
-

9. Evaporate to dryness

Evaporate 3 mL ethylacetate-extract to dryness (under nitrogen flow, at 40°C)

10. Reconstitute in 1 mL toluene

11. Inject in GC-MS

Materials

Artificial Sebum: Pickering Laboratories, Product Code 1700-0700

SPE-cartridges: Supelco, SupelMIP SPE-PAH, 50 mg/3 mL, Product Code 52773-U

Filter-paper circles : Schleicher & Schuell, 589^3 Blue ribbon, d=125mm, Ref.NO. 300211

ANNEX 6

INTER-LABORATORY STUDY ON THE MIGRATION OF THE 8 RESTRICTED PAHS UNDER REACH (ENTRY 50 OF ANNEX XVII) FROM PLASTIC AND RUBBER MATERIALS INTO 20% ETHANOL

5.1 Announcement letter
5.2 Accompanying letter
5.3 Hands-on training agenda
5.4 Homogeneity results
5.5 Statistics in terms of migration per surface (ng/dm^2)-ISO 5725-5
5.6 Statistics in terms of migration per weight (µg/kg)-ISO 5725-5
5.7 Statistics of 10 ng/ml control solution-ISO 5725-5
5.8 Participant's comments

5.1 Announcement letter

Limited Interlaboratory study on the migration of the 8 restricted PAHs under REACH (Entry 50 of Annex XVII) from plastic and rubber materials into 20% Ethanol

Fields marked with * are mandatory.

Dear colleague,

You are receiving this registration form as a follow-up of our recent contact concerning the potential interest of your laboratory in participating in a limited inter-laboratory comparison study foreseen in the context of the STANPAHs project.

The aim of this study is not to evaluate performance of laboratories but to evaluate the analytical method.
The method under evaluation aims at determining the migration of the 8 EU-PAHs from rubber and plastic samples by total immersion into the simulant (20% ethanol).

Method Summary
- Dynamic exposure to simulant (20% ethanol) by total immersion, at 40°C for 24 hours
- Removal of test specimen, addition of internal standard and clean-up through SPE-cartridge
- Evaporation of solvent under nitrogen stream
- Dissolution of residue in toluene and analysis by GC-MS

Specific instrumentation required for the execution of the migration test:
- GC-MS system in single ion monitoring mode
- Analytical balance
- Shaking water bath (temperature adjustable to 40°C)
- Solid phase extraction (SPE) manifold
- Sample concentrator (e.g.Techne dri block)

Timeframe of the exercise:
- Hands-on training session at JRC Ispra (Italy) on 19 October from 8:30 till 16:00
- Dispatch of samples: second half of October. Participants will be asked to return a sample receipt to the organiser
- Deadline for reporting of results: 3 months after the dispatch of the samples

Participation is on voluntary basis. Confidentiality of data is granted. Each participant laboratory will receive the standard operation procedure (SOP) to be used and the test samples to be analysed. Up to 15 migration tests including blanks and controls are foreseen. Mixtures of deuterated and native standards, sufficient solid phase extraction (SPE) cartridges for purification, a specific GC column for PAH separation and appropriate extraction bottles will be provided by JRC.

Please provide here below the address to which you want the samples to be shipped:

* Name of Research Institute/Company/University

* Department

* Address/City

* Postal code

* Country

- Austria
- Belgium
- Bulgaria
- Croatia
- Cyprus
- Czech Republic
- Denmark
- Estonia
- Finland
- France
- Germany
- Greece
- Hungary
- Ireland
- Italy
- Latvia
- Lithuania
- Luxembourg
- Malta
- Netherlands
- Poland
- Portugal
- Romania
- Slovak Republic
- Slovenia
- Spain
- Sweden
- United Kingdom

* Name of the contact person

Email-Address

* Telephone number (DHL requirement)

A one-day hands-on training, specifically intended for technical staff executing the migration tests, is offered on the 19th of October 2017 at the Joint Research Centre in Ispra (Italy). The JRC intends to cover travel and subsistence expenses (according to existing rules) but please wait to receive the official invitation to make the travel arrangements.

* Will a technician participate at the hands-on training session scheduled on 19 October 2017 at JRC Ispra site in Italy?

- Yes
- No

* Please provide first and second name of the technician, who will follow the hands-on training:

* Please provide the email-address of the technician, who will follow the hands-on training.

Please use the below text in case you have any comments.

Please note:

In order to ensure the smooth and effective planning/execution of this hands-on training and collaborative study (e.g. purchase of materials) we need to know how many persons/laboratories will participate. By sending this completed form you commit to your participation in the study and your timely delivery of results.

5.2 Accompanying letter

Dear Colleague,

Your parcel has been shipped today; please reply to this e-mail to acknowledge the receipt once it will be delivered to your laboratory and you have checked the content.
In the package you will find:

- ✓ 5 specimens of 3 different materials, named Material 1, Material 2 and Material 3. You will perform a triplicate analysis; please consider the two remaining specimens for your convenience.
- ✓ 2 vials (1 mL each) labelled as ML309 and ML310: ML309 contains the mixture of 8 native standards while ML310 contains the mixture of 7 isotope labelled standards (internal standard). They serve both as migration standards and standard to build the calibration curve. Please follow the SOP for their dilutions.
- ✓ 11 migration bottles (100 mL with wide neck and screw cap)
- ✓ 1 box of solid phase extraction cartridges (Phenomenex, Strata PAH, 0.5 g/6 mL, 30 cartridges)
- ✓ 1 GC column (Restek, RXi-PAH, 30m, 0.25mm ID, 0.10 µm df)

Attached to this mail:

- ✓ Revised SOP
- ✓ Calculation Excel File
- ✓ Revised presentation of the procedure
- ✓ Standard chromatogram

Please, consider ONLY this last version of SOP and presentation as the concentration points of calibration curve, as well as the concentration of migration standards have been changed.
Please do not hesitate to contact us for any further clarification.

Kind regards,
Team STANPAHs

5.3 Hands-on training agenda

STANPAHs project.
Hands-on training session on migration of PAHs into 20% EtOH from plastic and rubber materials

19 October 2017

08:30-10:00	**Welcome** **Overview of the STANPAHs project and the ILC** **Introduction to the method** **Sample preparation** **Migration experimental set-up and start of the experiments**
10:15-10:30	Coffee break
10:30-12:00	**GC-MS analysis and data evaluation of a ready migration batch**
12:00-13:00	Buffet lunch building 28 F and group photo
13:00-16:00	**SPE work-up, evaporation and analysis** **Questions and Answer related activities**
16:00-16:15	**Transfer to airport**

5.4 Homogeneity results

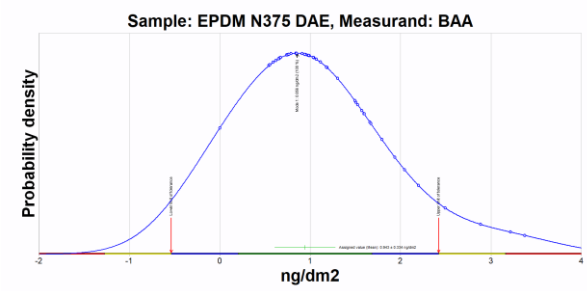
Sample 1 EPDM N375 DAE									
Pack	**Portion**	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
1	A	751.35	1403.38	2279.14	432.00	1231.62	7892.40	5572.69	506.86
	B	724.66	1643.19	2127.59	564.01	1155.40	7543.50	4833.15	520.81
2	A	763.57	1963.22	2152.73	519.86	1192.65	7635.16	4846.10	528.06
	B	793.46	1752.32	2298.04	610.16	1275.24	7745.10	5323.77	488.10
3	A	743.92	1754.41	2283.31	589.80	1265.56	7715.10	5101.31	489.51
	B	755.91	1552.11	2214.93	651.69	1194.01	7574.68	5218.65	455.18
4	A	769.25	1370.26	2307.99	536.47	1314.01	7944.09	5424.85	610.44
	B	751.38	1290.30	2226.50	600.93	1237.70	7636.00	5205.39	607.85
5	A	714.62	1361.12	2127.31	503.65	1212.63	7259.08	4817.35	468.30
	B	741.90	1535.31	2154.17	539.97	1144.45	7351.81	4902.67	498.95
6	A	756.40	1832.88	2127.08	502.75	1231.72	7389.66	4929.99	458.49
	B	656.24	1412.30	1896.88	439.02	1026.43	6494.16	4076.04	519.11
7	A	786.20	1497.35	2307.15	572.26	1317.59	8053.78	5200.72	520.00
	B	715.39	1442.49	2103.85	526.19	1214.03	7320.75	4869.75	482.30
8	A	744.08	1596.70	2084.69	516.05	1200.61	7242.44	4880.27	491.28
	B	745.60	1682.39	2143.95	497.52	1221.41	7262.40	4866.33	459.06
9	A	741.39	1617.13	2230.94	604.12	1271.63	7701.59	5027.72	-
	B	739.34	1152.48	2134.92	510.10	1237.47	7340.00	4888.90	-
10	A	716.18	1169.02	2182.95	548.74	1234.53	7521.87	4921.08	574.15
	B	728.12	1127.51	2118.10	569.13	1237.78	7338.70	4896.86	549.73
Average (ug/kg)		741.95	1507.79	2175.11	541.72	1220.82	7498.11	4990.18	512.68
SD (ug/kg)		29.37	229.74	98.52	55.55	63.58	337.16	306.42	47.28
RSD (%)		3.96	15.24	4.53	10.25	5.21	4.50	6.14	9.22

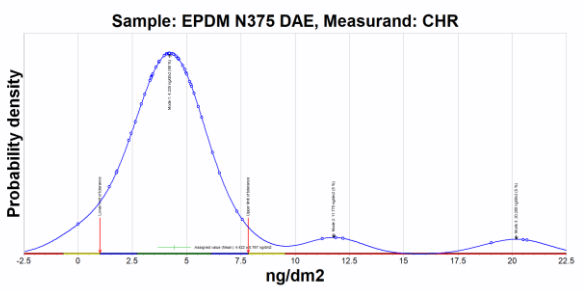
Sample 2 NRBR N375 DAE									
Pack	**Portion**	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
1	A	645.58	1969.49	1786.15	528.00	605.57	6239.38	3832.08	473.70
	B	621.74	1877.26	1648.90	464.41	575.20	5740.26	3865.86	426.03
2	A	579.88	1817.25	1652.42	565.20	606.02	5707.85	3776.68	447.69
	B	577.50	1866.13	1655.73	494.41	562.27	5781.09	3650.65	415.72
3	A	609.83	1905.73	1659.29	498.11	602.03	5738.51	3738.10	433.13
	B	654.05	1811.33	1760.24	503.06	647.37	6225.23	3901.88	686.21
4	A	585.20	1851.86	1688.22	486.41	653.71	5810.33	3592.20	576.76
	B	576.56	1843.92	1688.66	483.55	646.73	5614.70	3655.88	451.06
5	A	599.15	1959.22	1686.75	473.76	647.20	5781.36	3791.67	425.68
	B	605.61	1885.06	1699.02	563.03	611.11	5716.47	3695.26	576.87
6	A	630.32	1906.22	1755.70	559.54	668.87	6193.71	3853.03	639.30
	B	594.87	1799.24	1691.33	471.43	649.66	5827.66	3679.12	547.22
7	A	619.73	1880.02	1767.61	527.03	680.69	6140.37	3868.50	638.76
	B	584.12	1848.57	1664.16	502.66	607.78	5708.35	3875.94	455.29
8	A	592.36	1871.03	1683.69	491.66	605.01	5714.02	3770.97	426.28
	B	621.42	1941.84	1766.28	513.86	692.78	5927.31	3899.30	504.38
9	A	638.68	2033.34	1898.50	660.92	695.24	-	4368.66	-
	B	662.14	2021.98	1920.15	655.36	717.52	-	4309.45	-
10	A	598.08	1860.53	1709.54	499.19	634.40	6018.70	3991.37	514.24
	B	647.32	1963.32	1733.82	536.17	605.59	5934.47	3935.16	470.05
Average (ug/kg)		612.21	1895.67	1725.81	523.89	635.74	5878.88	3852.59	506.02
SD (ug/kg)		27.15	66.53	75.90	54.65	41.51	201.38	197.15	85.29
RSD (%)		4.43	3.51	4.40	10.43	6.53	3.43	5.12	16.85

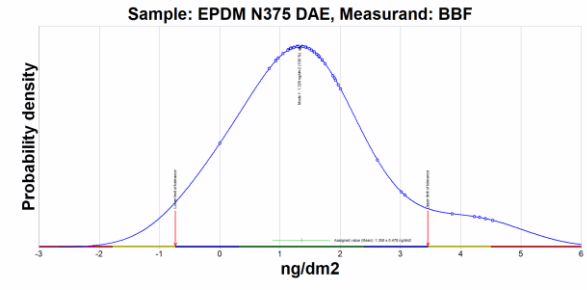
Sample 3 PVC N772									
Pack	**Portion**	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
1	A	46.66	91.90	153.53	56.32	66.39	511.87	412.07	44.59
	B	44.25	86.26	154.39	66.29	69.75	490.31	408.67	39.12
2	A	46.31	95.89	169.10	74.90	67.57	528.48	423.98	47.76
	B	43.89	88.40	155.00	61.41	62.06	494.66	412.77	35.97
3	A	44.49	90.08	163.61	66.45	62.07	492.12	411.98	35.69
	B	43.81	89.31	159.53	67.87	61.98	490.47	411.03	36.18
4	A	46.47	110.29	151.23	59.40	63.36	489.37	399.50	40.56
	B	54.50	109.33	155.76	49.76	58.72	498.89	417.95	41.05
5	A	55.10	113.84	163.87	63.88	63.44	500.49	435.42	39.39
	B	54.30	110.34	161.37	61.54	68.47	509.95	429.22	40.07
6	A	52.66	-	163.31	64.11	67.26	506.54	439.64	36.67
	B	54.69	-	162.02	69.80	65.37	512.65	436.32	35.29
7	A	59.85	114.41	175.92	67.02	79.65	586.36	491.23	50.00
	B	70.29	114.12	199.56	72.17	80.97	610.27	544.40	49.45
8	A	54.10	113.17	157.65	58.49	66.73	515.74	420.31	43.66
	B	56.55	91.69	164.46	60.43	67.73	480.80	378.40	38.20
9	A	58.27	113.62	184.86	69.70	67.36	697.49	495.73	40.00
	B	54.44	108.58	152.90	52.05	63.23	483.34	389.29	40.62
10	A	42.55	-	162.48	54.83	56.50	494.29	420.93	37.58
	B	62.34	-	164.40	59.96	63.85	519.44	434.97	33.63
Average (ug/kg)		52.28	102.58	163.75	62.82	66.12	520.68	430.69	40.27
SD (ug/kg)		7.37	11.29	11.65	6.65	5.88	52.82	38.87	4.69
RSD (%)		14.09	11.01	7.11	10.59	8.89	10.15	9.02	11.64

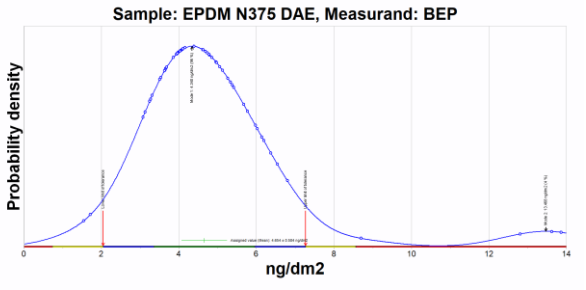
5.5 Statistics in terms of migration per surface (ng/dm^2)-ISO 5725-5

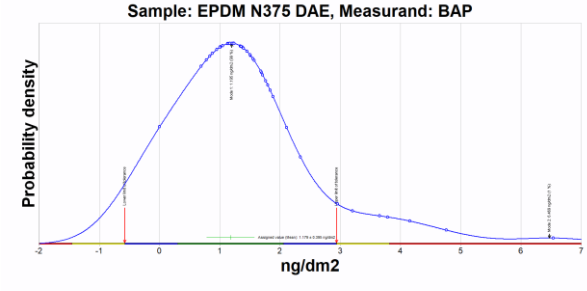
5.5.1 Kernel's density

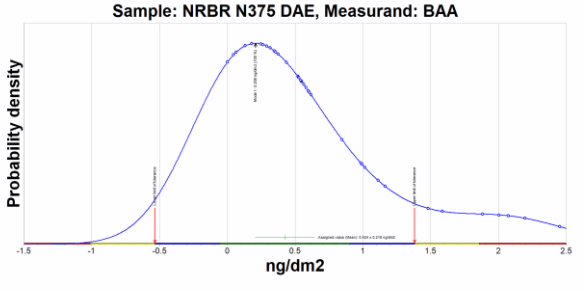


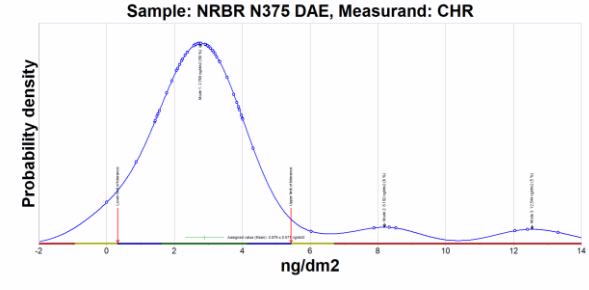


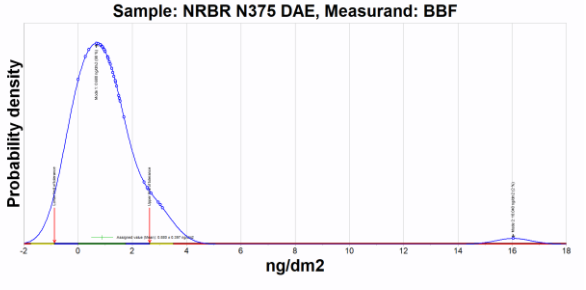


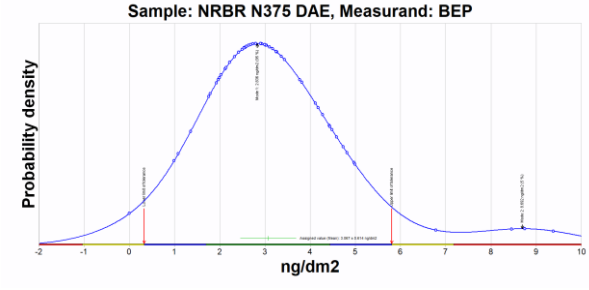


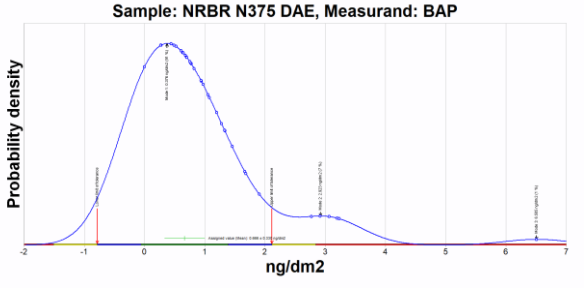




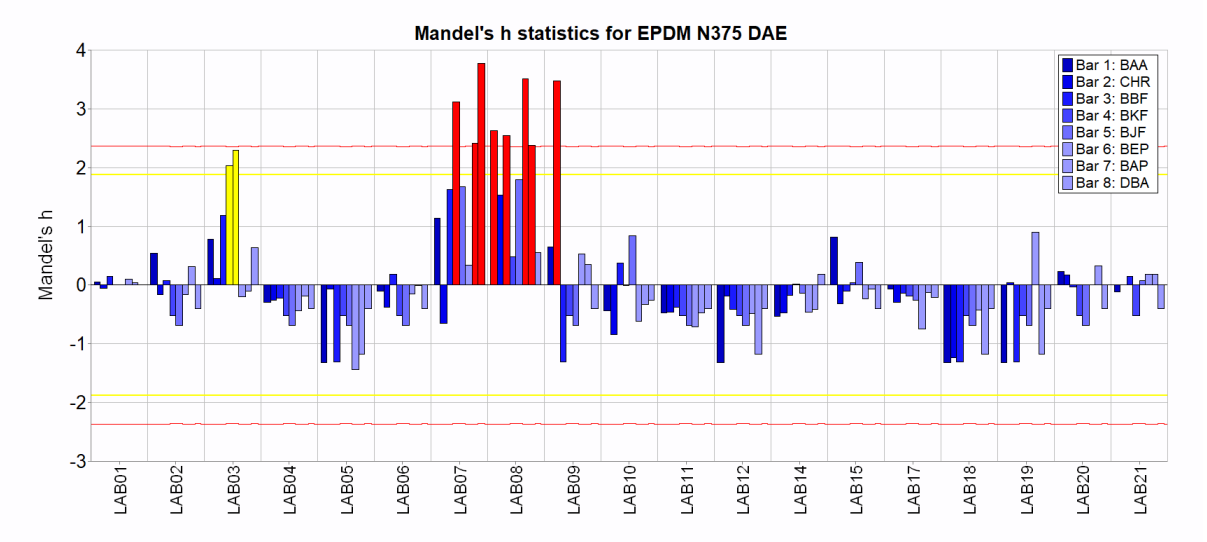


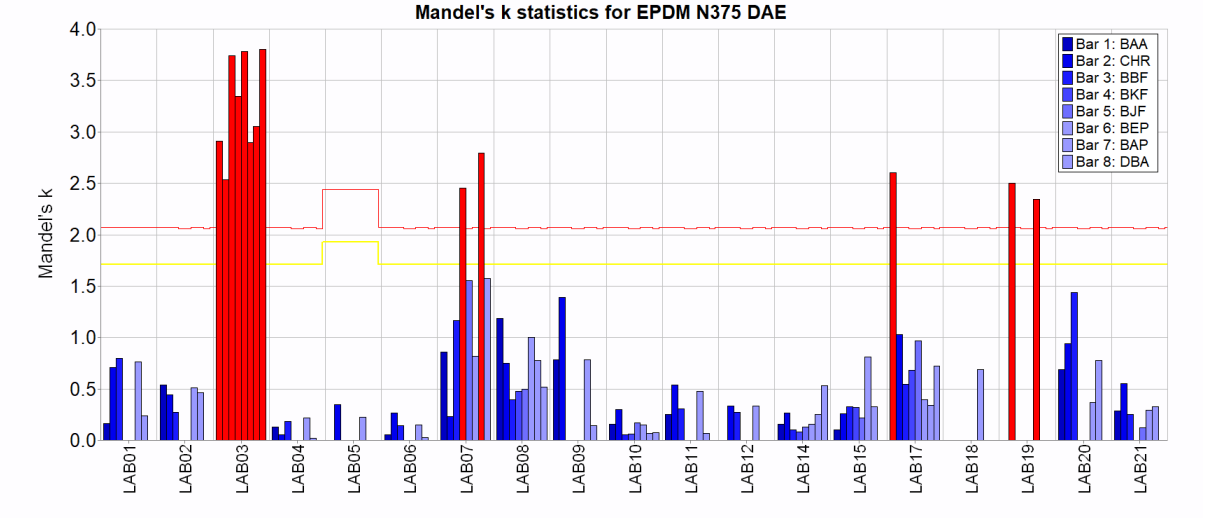


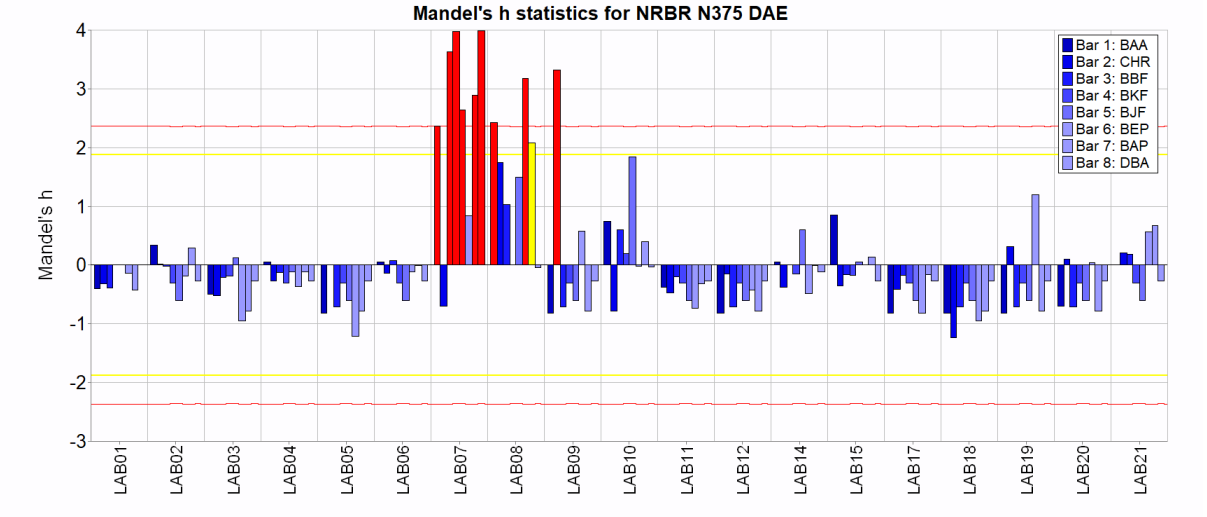


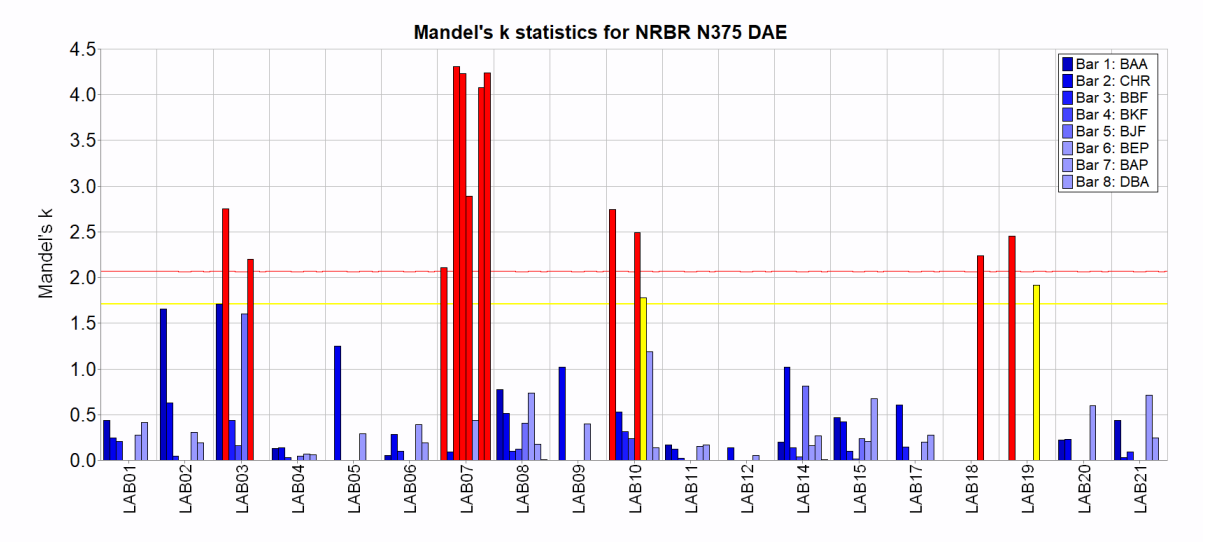


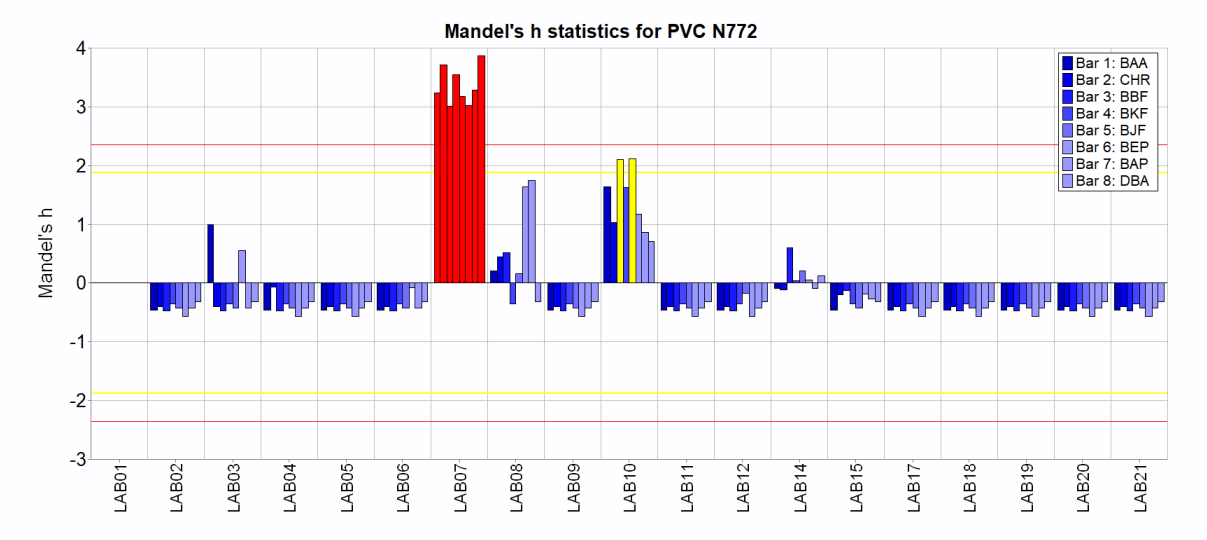
5.5.2 Mandels'h and k statistics

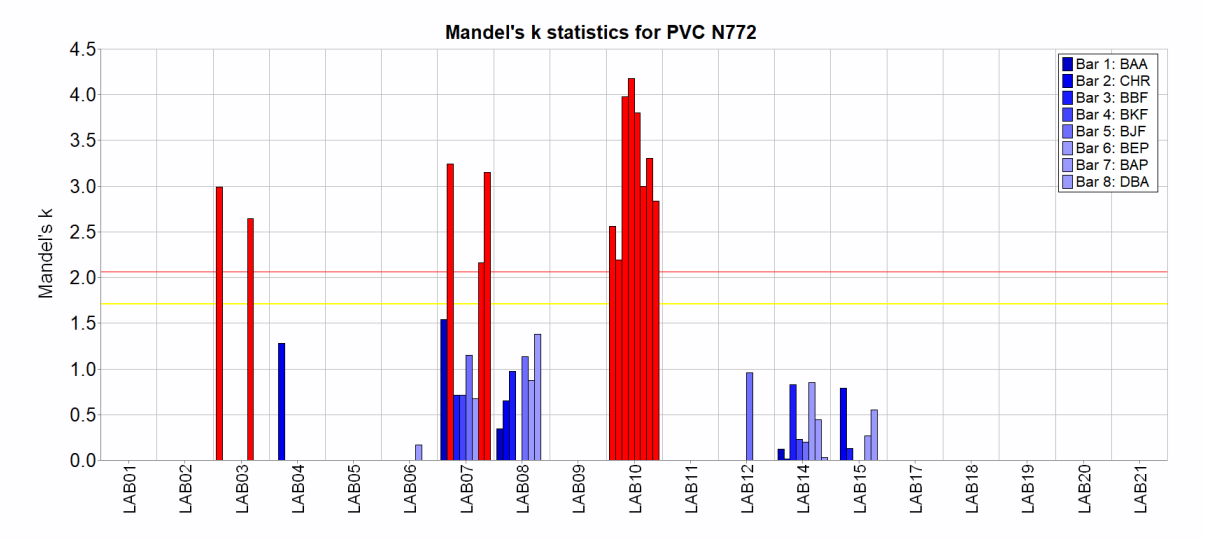







Mandel's k statistics for NRBR N375 DAE
Mandel's k
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Bar 1: BAA
Bar 2: CHR
Bar 3: BBF
Bar 4: BKF
Bar 5: BJF
Bar 6: BEP
Bar 7: BAP
Bar 8: DBA
LAB01
LAB02
LAB03
LAB04
LAB05
LAB06
LAB07
LAB08
LAB09
LAB10
LAB11
LAB12
LAB14
LAB15
LAB17
LAB18
LAB19
LAB20
LAB21


Mandel's h statistics for PVC N772
Mandel's h
4
3
2
1
0
-1
-2
-3
Bar 1: BAA
Bar 2: CHR
Bar 3: BBF
Bar 4: BKF
Bar 5: BJF
Bar 6: BEP
Bar 7: BAP
Bar 8: DBA
LAB01
LAB02
LAB03
LAB04
LAB05
LAB06
LAB07
LAB08
LAB09
LAB10
LAB11
LAB12
LAB14
LAB15
LAB17
LAB18
LAB19
LAB20
LAB21


Mandel's k statistics for PVC N772
Mandel's k
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Bar 1: BAA
Bar 2: CHR
Bar 3: BBF
Bar 4: BKF
Bar 5: BJF
Bar 6: BEP
Bar 7: BAP
Bar 8: DBA
LAB01
LAB02
LAB03
LAB04
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LAB07
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LAB11
LAB12
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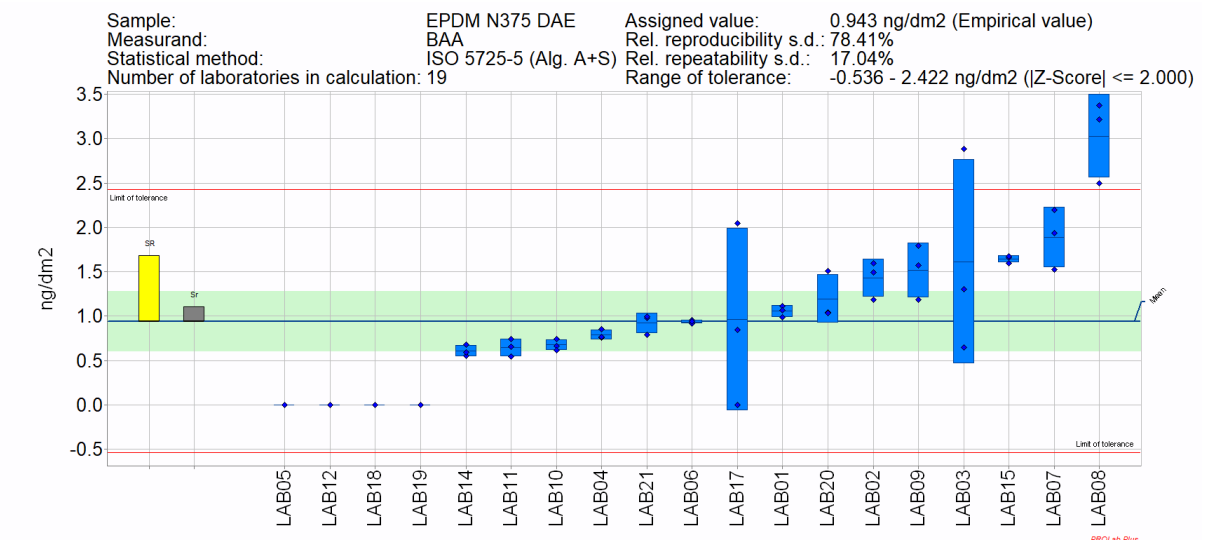
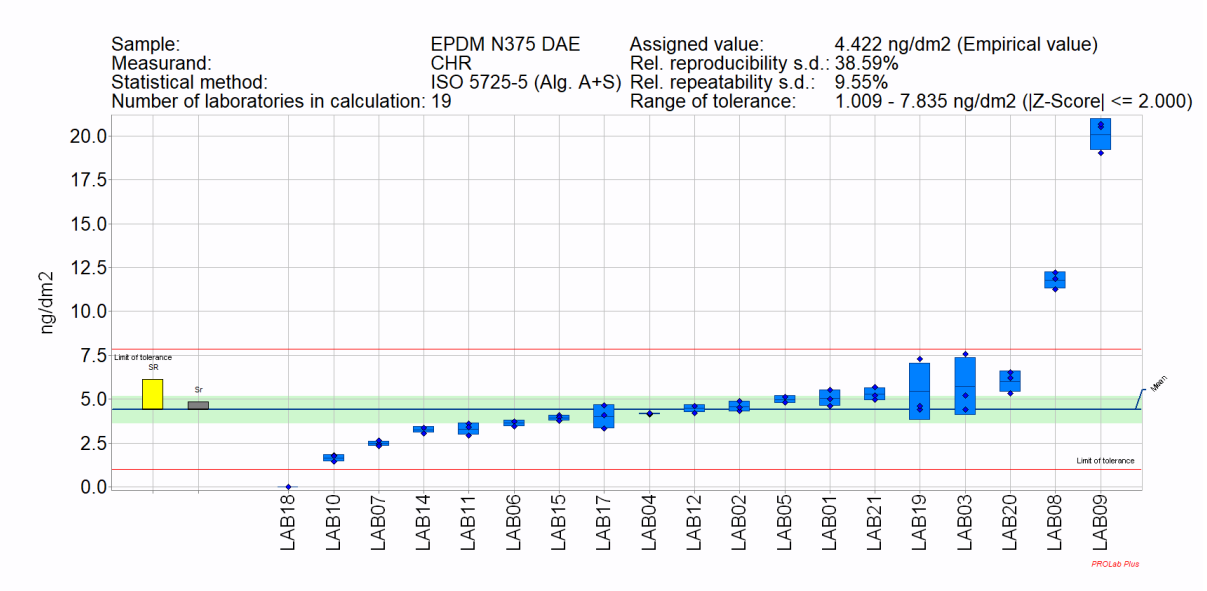
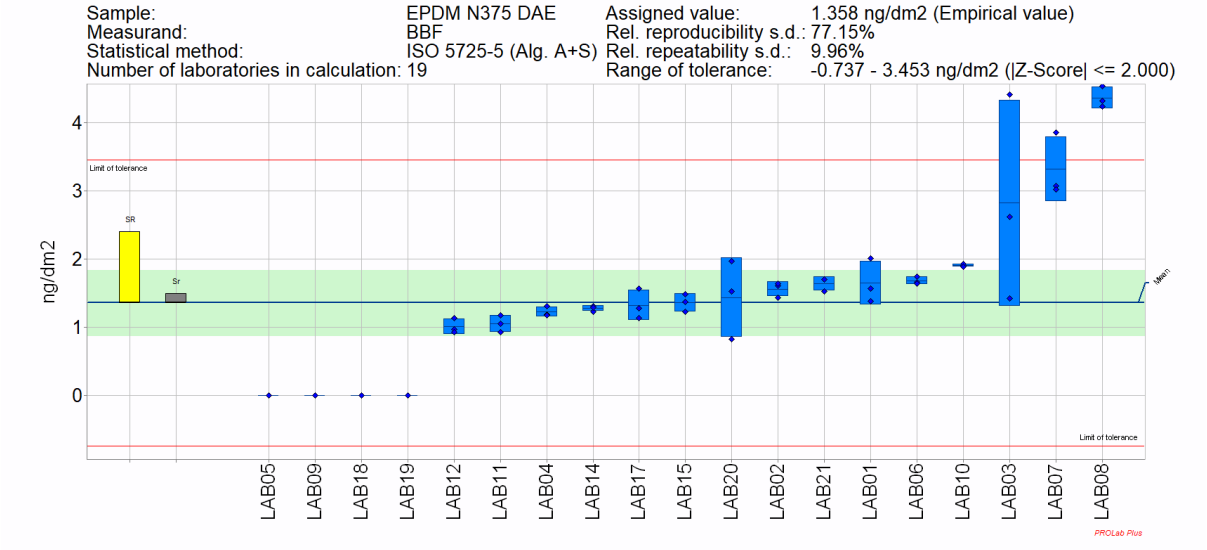
LAB19
LAB20
LAB21

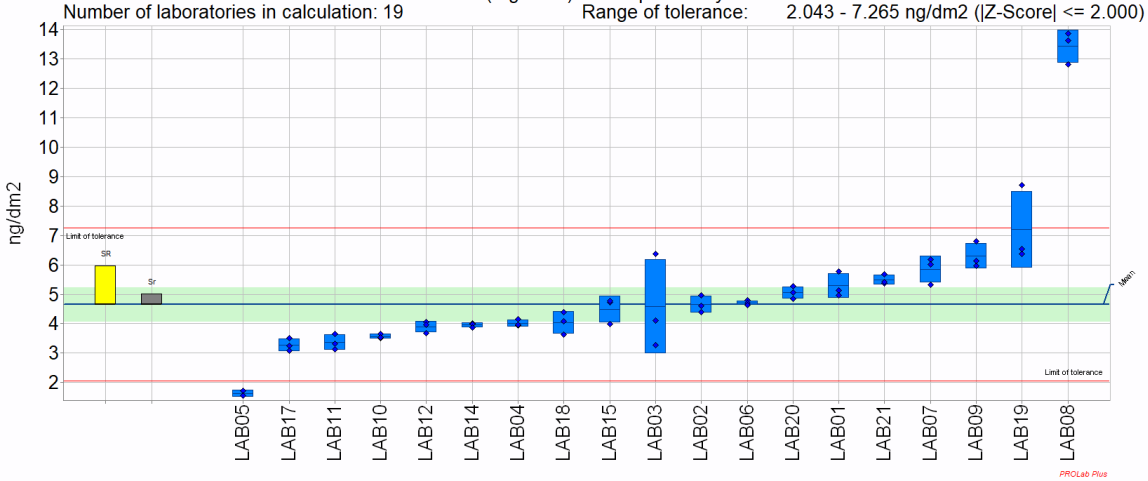
5.5.3 Inter-laboratory study results



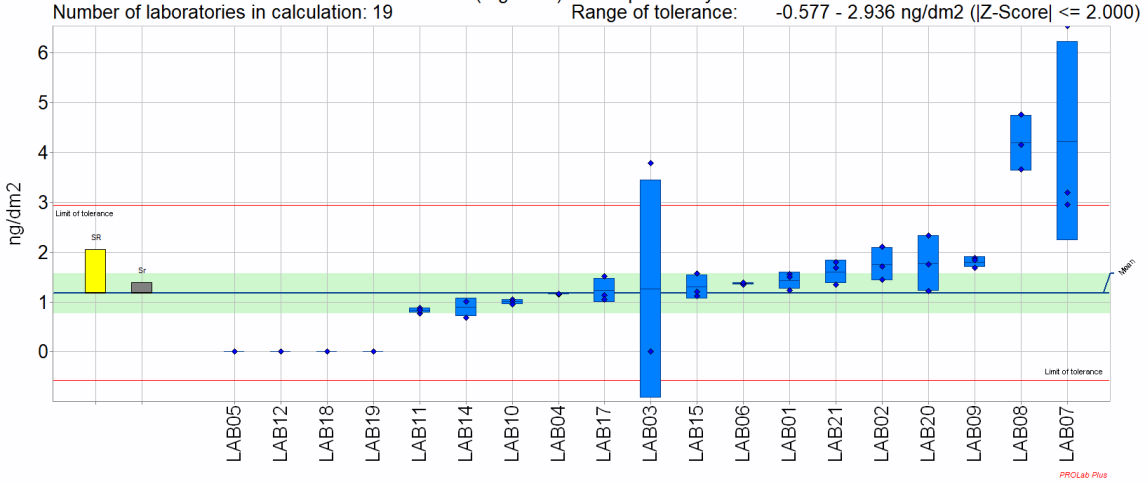




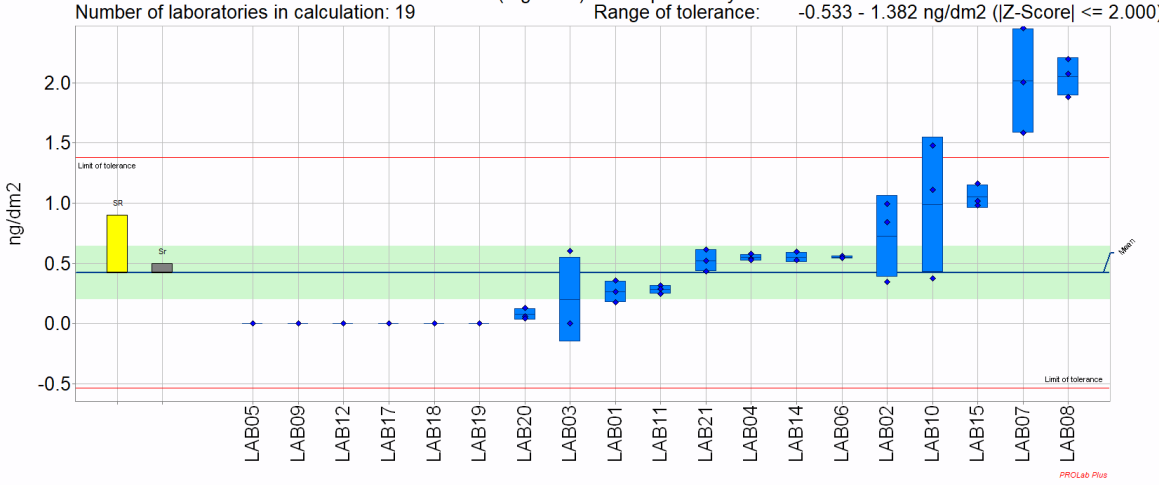
Sample:	EPDM N375 DAE	Assigned value:	4.654 ng/dm2 (Empirical value)
Measurand:	BEP	Rel. reproducibility s.d.:	28.05%
Statistical method:	ISO 5725-5 (Alg. A+S)	Rel. repeatability s.d.:	7.61%
Number of laboratories in calculation: 19		Range of tolerance:	2.043 - 7.265 ng/dm2 (|Z-Score| <= 2.000)



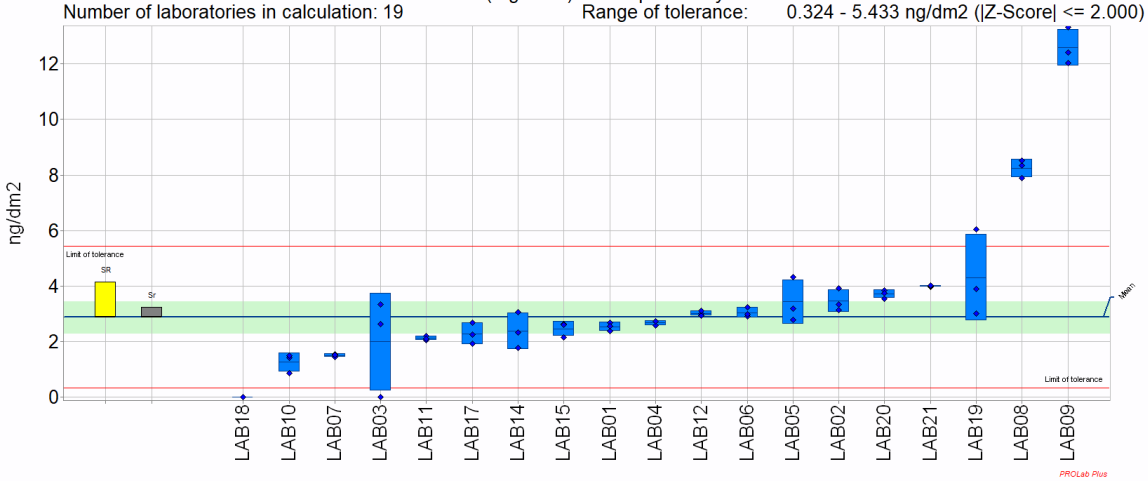
Sample:	EPDM N375 DAE	Assigned value:	1.179 ng/dm2 (Empirical value)
Measurand:	BAP	Rel. reproducibility s.d.:	74.47%
Statistical method:	ISO 5725-5 (Alg. A+S)	Rel. repeatability s.d.:	17.87%
Number of laboratories in calculation: 19		Range of tolerance:	-0.577 - 2.936 ng/dm2 (|Z-Score| <= 2.000)



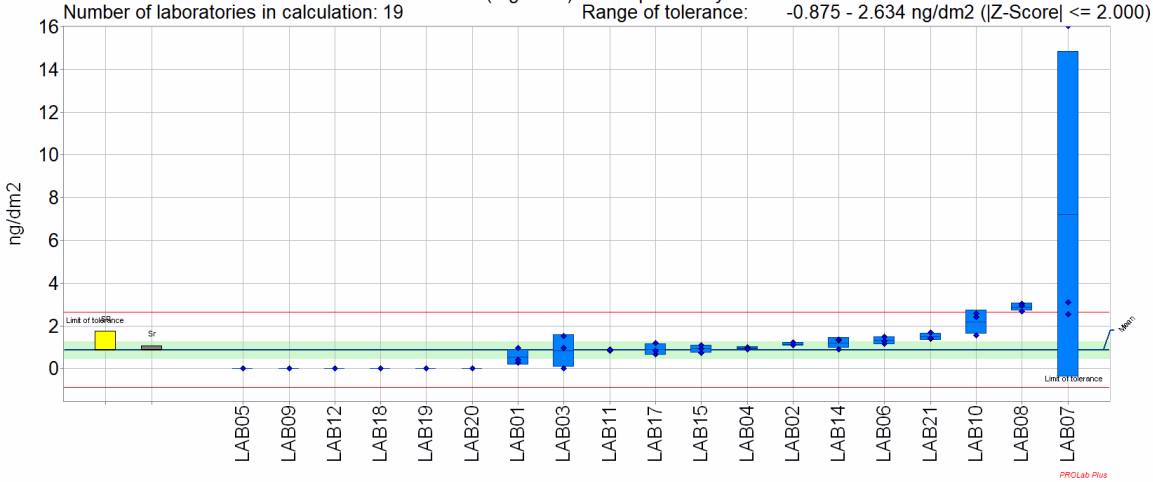
Sample:	NRBR N375 DAE	Assigned value:	0.424 ng/dm2 (Empirical value)
Measurand:	BAA	Rel. reproducibility s.d.:	112.86%
Statistical method:	ISO 5725-5 (Alg. A+S)	Rel. repeatability s.d.:	17.58%
Number of laboratories in calculation: 19		Range of tolerance:	-0.533 - 1.382 ng/dm2 (|Z-Score| <= 2.000)



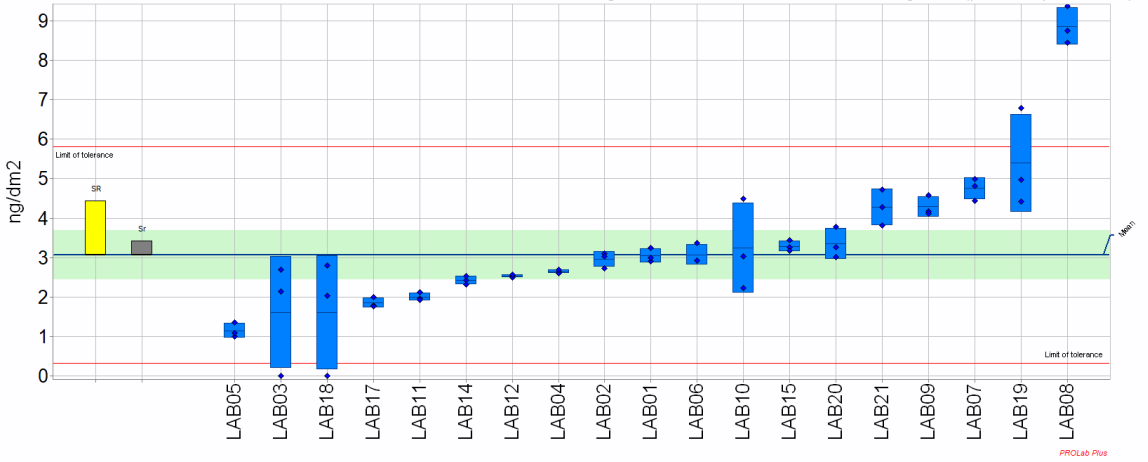
Sample:	NRBR N375 DAE	Assigned value:	2.879 ng/dm2 (Empirical value)
Measurand:	CHR	Rel. reproducibility s.d.:	44.37%
Statistical method:	ISO 5725-5 (Alg. A+S)	Rel. repeatability s.d.:	12.41%
Number of laboratories in calculation:	19	Range of tolerance:	0.324 - 5.433 ng/dm2 (|Z-Score| <= 2.000)

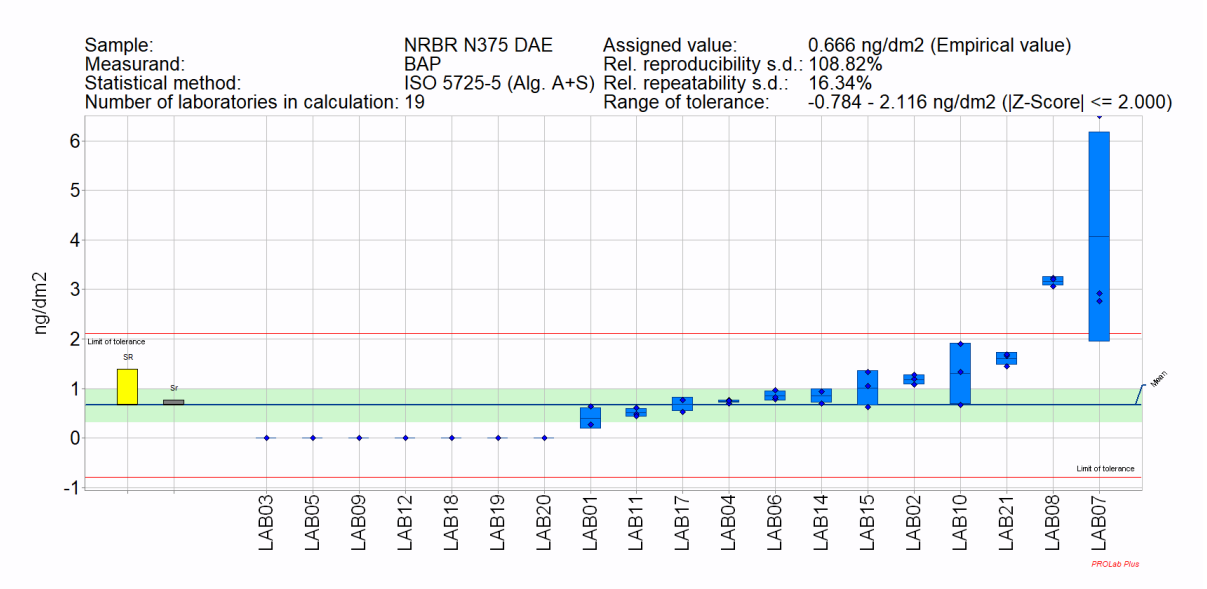


Sample:	NRBR N375 DAE	Assigned value:	0.880 ng/dm2 (Empirical value)
Measurand:	BBF	Rel. reproducibility s.d.:	99.72%
Statistical method:	ISO 5725-5 (Alg. A+S)	Rel. repeatability s.d.:	20.73%
Number of laboratories in calculation:	19	Range of tolerance:	-0.875 - 2.634 ng/dm2 (|Z-Score| <= 2.000)

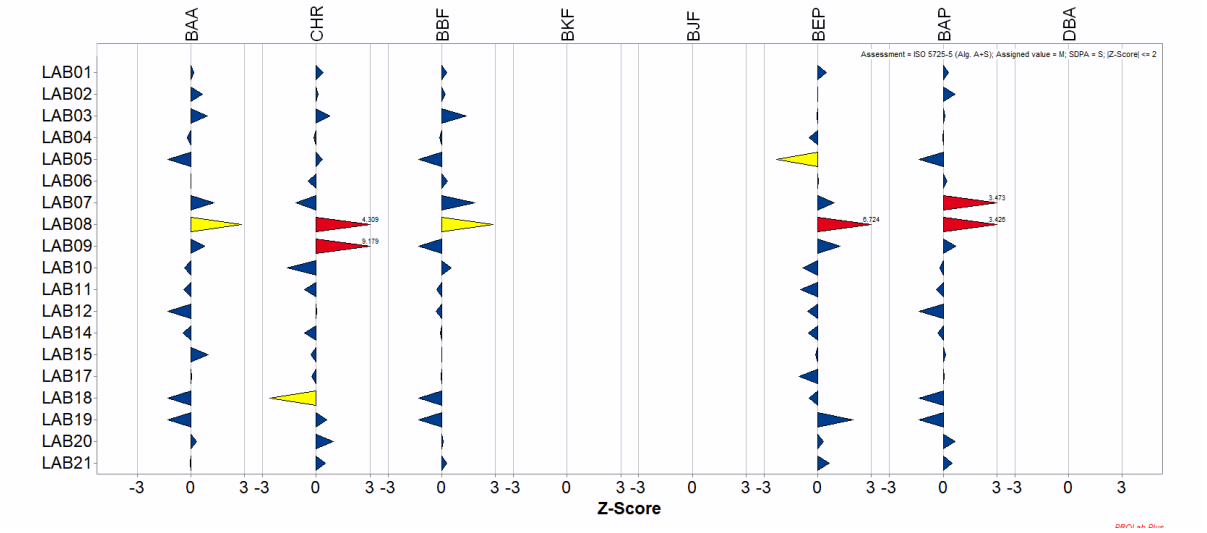


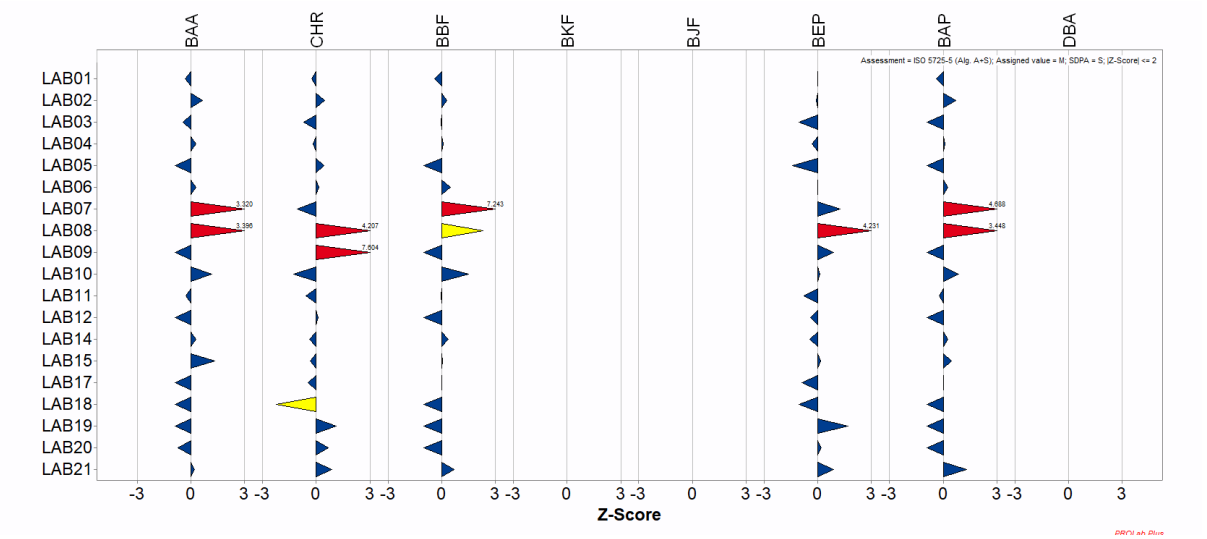
Sample:	NRBR N375 DAE	Assigned value:	3.067 ng/dm2 (Empirical value)
Measurand:	BEP	Rel. reproducibility s.d.:	44.64%
Statistical method:	ISO 5725-5 (Alg. A+S)	Rel. repeatability s.d.:	11.36%
Number of laboratories in calculation:	19	Range of tolerance:	0.329 - 5.805 ng/dm2 (|Z-Score| <= 2.000)




Sample: NRBR N375 DAE
Measurand: BAP
Statistical method: ISO 5725-5 (Alg. A+S)
Number of laboratories in calculation: 19
Assigned value: 0.666 ng/dm2 (Empirical value)
Rel. reproducibility s.d.: 108.82%
Rel. repeatability s.d.: 16.34%
Range of tolerance: -0.784 - 2.116 ng/dm2 (|Z-Score| <= 2.000)
ng/dm2
Limit of tolerance
SR
Sr
Limit of tolerance
LAB03 LAB05 LAB09 LAB12 LAB18 LAB19 LAB20 LAB01 LAB11 LAB17 LAB04 LAB06 LAB14 LAB15 LAB02 LAB10 LAB21 LAB08 LAB07
PROLab Plus

5.5.4 Z-scores


BAA CHR BBF BKF BJF BEP BAP DBA
Assessment = ISO 5725-5 (Alg. A+S); Assigned value = M; SDPA = S; |Z-Score| <= 2
LAB01 LAB02 LAB03 LAB04 LAB05 LAB06 LAB07 LAB08 LAB09 LAB10 LAB11 LAB12 LAB14 LAB15 LAB17 LAB18 LAB19 LAB20 LAB21
4,309
6,179
6,724
3,473
3,426
-3 0 3
Z-Score


BAA CHR BBF BKF BJF BEP BAP DBA
Assessment = ISO 5725-5 (Alg. A+S); Assigned value = M; SDPA = S; |Z-Score| <= 2
LAB01 LAB02 LAB03 LAB04 LAB05 LAB06 LAB07 LAB08 LAB09 LAB10 LAB11 LAB12 LAB14 LAB15 LAB17 LAB18 LAB19 LAB20 LAB21
3,120
3,396
4,207
7,604
7,243
4,231
4,668
3,448
-3 0 3
Z-Score

5.5.5 Table (A, B, C): Original values, means and standard deviations

Table A-Sample 1-Original values

Sample 1 (EPDM N375 DAE)								
ng/dm^2	BAA	CHR	BBF	BKF	BJF	BEP	BAP	DBA
LAB01	1.11	5.53	2.01	<	<	5.77	1.55	<
	0.98	4.61	1.38	<	<	5.13	1.50	<
	1.07	5.01	1.57	<	<	4.97	1.24	<
LAB02	1.19	4.90	1.61	0.00	0.00	4.39	1.45	0.00
	1.60	4.34	1.64	0.00	0.00	4.61	1.71	0.00
	1.49	4.51	1.44	0.00	0.00	4.96	2.11	0.00
LAB03	1.30	4.40	2.62	1.08	1.83	4.11	0.00	0.00
	0.65	5.19	1.42	0.00	0.00	3.27	0.00	0.00
	2.89	7.56	4.41	2.71	3.38	6.37	3.79	3.08
LAB04	0.85	4.14	1.19	0.00	0.00	3.93	1.17	0.00
	0.76	4.17	1.18	0.00	0.00	4.16	1.15	0.00
	0.77	4.21	1.31	0.00	0.00	3.96	1.17	0.00
LAB05	-	-	-	-	-	-	-	-
	0.00	5.14	0.00	0.00	0.00	1.71	0.00	0.00
	0.00	4.82	0.00	0.00	0.00	1.54	0.00	0.00
LAB06	0.91	3.73	1.75	0.00	0.00	4.79	1.38	0.00
	0.95	3.73	1.66	0.00	0.00	4.69	1.35	0.00
	0.94	3.43	1.64	0.00	0.00	4.63	1.40	0.00
LAB07	2.20	2.45	3.86	2.95	2.16	6.19	6.54	4.97
	1.94	2.63	3.07	1.26	0.86	6.02	3.20	3.61
	1.53	2.34	3.02	1.18	1.10	5.33	2.95	3.82
LAB08	2.49	11.25	4.31	0.71	1.19	13.87	4.15	0.82
	3.37	12.21	4.23	0.33	1.61	13.62	4.76	0.80
	3.22	11.86	4.53	0.45	1.54	12.80	3.65	1.23
LAB09	1.57	19.05	0.00	0.00	0.00	5.97	1.69	0.00
	1.79	20.51	0.00	0.00	0.00	6.80	1.88	0.00
	1.18	20.70	0.00	0.00	0.00	6.14	1.84	0.00
LAB10	0.74	1.78	1.90	0.27	0.92	3.66	1.05	0.17
	0.67	1.75	1.92	0.27	0.95	3.51	1.01	0.16
	0.62	1.43	1.88	0.22	0.80	3.53	0.95	0.10
LAB11	0.74	3.59	1.17	0.00	0.00	3.65	0.88	0.00
	0.66	3.39	1.05	0.00	0.00	3.31	0.78	0.00
	0.54	2.91	0.93	0.00	0.00	3.13	0.83	0.00
LAB12	0.00	4.60	1.14	0.00	0.00	4.05	0.00	0.00
	0.00	4.60	0.97	0.00	0.00	3.69	0.00	0.00
	0.00	4.22	0.93	0.00	0.00	3.95	0.00	0.00
LAB14	0.55	3.38	1.23	0.30	0.25	4.02	1.01	0.85
	0.68	3.07	1.31	0.27	0.34	3.99	1.00	0.52
	0.59	3.36	1.29	0.23	0.36	3.86	0.69	0.35
LAB15	1.66	4.08	1.49	0.41	0.64	4.73	1.57	0.00
	1.60	3.96	1.37	0.26	0.52	4.77	1.13	0.00
	1.67	3.75	1.23	0.16	0.71	3.97	1.21	0.00
LAB17	0.84	4.64	1.56	0.48	0.75	3.51	1.51	0.58
	2.05	4.07	1.13	0.00	0.00	3.25	1.14	0.00
	0.00	3.31	1.28	0.00	0.00	3.07	1.05	0.00
LAB18	0.00	0.00	0.00	0.00	0.00	4.09	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	3.63	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	4.39	0.00	0.00
LAB19	0.00	7.31	0.00	0.00	0.00	8.70	0.00	0.00
	0.00	4.41	0.00	0.00	0.00	6.37	0.00	0.00
	0.00	4.60	0.00	0.00	0.00	6.54	0.00	0.00
LAB20	1.04	5.33	0.82	0.00	0.00	4.85	1.23	0.00
	1.03	6.52	1.53	0.00	0.00	5.26	2.34	0.00
	1.51	6.19	1.97	0.00	0.00	5.06	1.76	0.00
LAB21	0.98	5.69	1.70	0.00	0.50	5.67	1.80	0.00
	0.99	4.98	1.52	0.00	0.40	5.38	1.34	0.00
	0.79	5.22	1.70	0.00	0.42	5.41	1.68	0.00

Table B-Sample 1-Mean values

Average - Sample 1 (EPDM N375 DAE)								
ng/dm²	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
LAB01	1.06	5.05	1.65	-	-	5.29	1.43	-
LAB02	1.43	4.58	1.56	0.00	0.00	4.65	1.76	0.00
LAB03	1.61	5.72	2.82	1.26	1.73	4.58	1.26	1.03
LAB04	0.79	4.18	1.23	0.00	0.00	4.02	1.16	0.00
LAB05	0.00	4.98	0.00	0.00	0.00	1.63	0.00	0.00
LAB06	0.94	3.63	1.68	0.00	0.00	4.70	1.37	0.00
LAB07	1.89	2.47	3.32	1.80	1.37	5.85	4.23	4.13
LAB08	3.03	11.78	4.36	0.50	1.45	13.43	4.19	0.95
LAB09	1.52	20.09	0.00	0.00	0.00	6.31	1.80	0.00
LAB10	0.68	1.66	1.90	0.25	0.89	3.57	1.00	0.15
LAB11	0.65	3.30	1.05	0.00	0.00	3.36	0.83	0.00
LAB12	0.00	4.47	1.01	0.00	0.00	3.90	0.00	0.00
LAB14	0.61	3.27	1.28	0.27	0.32	3.96	0.90	0.57
LAB15	1.64	3.93	1.36	0.28	0.63	4.49	1.30	0.00
LAB17	0.96	4.00	1.32	0.16	0.25	3.28	1.23	0.19
LAB18	0.00	0.00	0.00	0.00	0.00	4.04	0.00	0.00
LAB19	0.00	5.44	0.00	0.00	0.00	7.21	0.00	0.00
LAB20	1.19	6.01	1.44	0.00	0.00	5.06	1.78	0.00
LAB21	0.92	5.30	1.64	0.00	0.44	5.49	1.61	0.00

Table C-Sample 1-Standard deviations

Standard Deviation - Sample 1 (EPDM N375 DAE)								
ng/dm²	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
LAB01	0.07	0.46	0.32	-	-	0.42	0.17	-
LAB02	0.21	0.29	0.11	0.00	0.00	0.29	0.33	0.00
LAB03	1.15	1.65	1.50	1.36	1.69	1.61	2.19	1.78
LAB04	0.05	0.04	0.07	0.00	0.00	0.12	0.01	0.00
LAB05	0.00	0.23	0.00	0.00	0.00	0.12	0.00	0.00
LAB06	0.02	0.17	0.06	0.00	0.00	0.08	0.02	0.00
LAB07	0.34	0.15	0.47	1.00	0.69	0.46	2.00	0.74
LAB08	0.47	0.49	0.16	0.19	0.22	0.56	0.55	0.24
LAB09	0.31	0.90	0.00	0.00	0.00	0.44	0.10	0.00
LAB10	0.06	0.19	0.02	0.03	0.08	0.08	0.05	0.04
LAB11	0.10	0.35	0.12	0.00	0.00	0.26	0.05	0.00
LAB12	0.00	0.22	0.11	0.00	0.00	0.19	0.00	0.00
LAB14	0.06	0.17	0.04	0.03	0.06	0.09	0.18	0.25
LAB15	0.04	0.17	0.13	0.13	0.10	0.45	0.24	0.00
LAB17	1.03	0.67	0.22	0.28	0.43	0.22	0.24	0.34
LAB18	0.00	0.00	0.00	0.00	0.00	0.38	0.00	0.00
LAB19	0.00	1.62	0.00	0.00	0.00	1.30	0.00	0.00
LAB20	0.27	0.61	0.58	0.00	0.00	0.21	0.56	0.00
LAB21	0.11	0.36	0.10	0.00	0.05	0.16	0.23	0.00

Table A-Sample 2-Original values

Sample 2 (NRBR N375 DAE)								
ng/dm^2	BAA	CHR	BBF	BKF	BJF	BEP	BAP	DBA
LAB01	0.36	2.68	0.27	<	<	3.25	0.64	<
	0.18	2.37	0.39	<	<	2.90	0.27	<
	0.26	2.56	0.96	<	<	2.99	0.27	<
LAB02	0.35	3.92	1.11	0.00	0.00	3.10	1.08	0.00
	0.85	3.15	1.09	0.00	0.00	3.03	1.20	0.00
	0.99	3.33	1.24	0.00	0.00	2.73	1.28	0.00
LAB03	0.61	2.62	0.97	0.00	0.85	2.69	0.00	0.00
	0.00	3.33	1.52	0.57	0.39	2.14	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB04	0.58	2.72	1.00	0.00	0.29	2.64	0.77	0.00
	0.54	2.57	1.00	0.00	0.27	2.60	0.70	0.00
	0.53	2.72	0.89	0.00	0.27	2.69	0.75	0.00
LAB05	0.00	3.19	0.00	0.00	0.00	0.99	0.00	0.00
	0.00	4.32	0.00	0.00	0.00	1.36	0.00	0.00
	0.00	2.78	0.00	0.00	0.00	1.08	0.00	0.00
LAB06	0.56	3.25	1.50	0.00	0.00	3.36	0.97	0.00
	0.55	2.90	1.15	0.00	0.00	2.93	0.78	0.00
	0.54	2.97	1.28	0.00	0.00	2.93	0.83	0.00
LAB07	2.00	1.55	3.11	1.83	1.68	4.82	2.92	3.50
	1.59	1.43	2.54	1.62	1.17	4.44	2.77	2.30
	2.45	1.51	16.04	16.97	2.68	4.99	6.50	24.36
LAB08	2.07	8.34	3.03	0.24	1.07	8.75	3.23	0.52
	1.88	8.53	2.95	0.75	1.28	9.38	3.20	0.58
	2.20	7.89	2.69	0.49	1.23	8.45	3.06	0.56
LAB09	0.00	13.31	0.00	0.00	0.00	4.58	0.00	0.00
	0.00	12.05	0.00	0.00	0.00	4.18	0.00	0.00
	0.00	12.41	0.00	0.00	0.00	4.11	0.00	0.00
LAB10	0.38	0.87	1.55	0.23	0.63	2.22	0.68	0.11
	1.48	1.42	2.43	1.11	1.73	3.03	1.33	0.71
	1.11	1.48	2.57	1.03	1.82	4.48	1.91	0.89
LAB11	0.32	2.21	0.85	0.00	0.00	1.97	0.49	0.00
	0.29	2.06	0.83	0.00	0.00	1.92	0.44	0.00
	0.25	2.09	0.90	0.00	0.00	2.12	0.61	0.00
LAB12	0.00	3.01	0.00	0.00	0.00	2.54	0.00	0.00
	0.00	2.93	0.00	0.00	0.00	2.49	0.00	0.00
	0.00	3.11	0.00	0.00	0.00	2.56	0.00	0.00
LAB14	0.60	1.77	0.91	0.25	0.58	2.42	0.70	0.38
	0.53	2.32	1.32	0.31	0.54	2.32	0.94	0.38
	0.53	3.06	1.36	0.16	0.93	2.53	0.94	0.35
LAB15	0.98	2.62	0.92	0.23	0.44	3.26	1.33	0.00
	1.02	2.15	0.74	0.17	0.32	3.17	1.06	0.00
	1.16	2.61	1.09	0.21	0.37	3.43	0.63	0.00
LAB17	0.00	1.92	1.19	0.00	0.00	1.79	0.78	0.00
	0.00	2.24	0.68	0.00	0.00	1.76	0.52	0.00
	0.00	2.69	0.82	0.00	0.00	1.99	0.77	0.00
LAB18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	2.80	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	2.03	0.00	0.00
LAB19	0.00	6.04	0.00	0.00	0.00	6.78	0.00	0.00
	0.00	3.00	0.00	0.00	0.00	4.97	0.00	0.00
	0.00	3.89	0.00	0.00	0.00	4.43	0.00	0.00
LAB20	0.06	3.84	0.00	0.00	0.00	3.77	0.00	0.00
	0.13	3.55	0.00	0.00	0.00	3.26	0.00	0.00
	0.05	3.75	0.00	0.00	0.00	3.02	0.00	0.00
LAB21	0.52	3.97	1.38	0.00	0.00	3.81	1.45	0.00
	0.44	4.01	1.42	0.00	0.00	4.73	1.67	0.00
	0.62	4.01	1.68	0.00	0.00	4.27	1.68	0.00

Table B-Sample 2-Mean values

Average - Sample 2 (NRBR N375 DAE)								
ng/dm^2	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
LAB01	0.27	2.54	0.54	-	-	3.05	0.40	-
LAB02	0.73	3.46	1.15	0.00	0.00	2.95	1.18	0.00
LAB03	0.20	1.99	0.83	0.19	0.41	1.61	0.00	0.00
LAB04	0.55	2.67	0.96	0.00	0.28	2.64	0.74	0.00
LAB05	0.00	3.43	0.00	0.00	0.00	1.14	0.00	0.00
LAB06	0.55	3.04	1.31	0.00	0.00	3.07	0.86	0.00
LAB07	2.01	1.50	7.23	6.80	1.84	4.75	4.06	10.05
LAB08	2.05	8.25	2.89	0.49	1.20	8.86	3.16	0.55
LAB09	0.00	12.59	0.00	0.00	0.00	4.29	0.00	0.00
LAB10	0.99	1.26	2.18	0.79	1.39	3.24	1.31	0.57
LAB11	0.28	2.12	0.86	0.00	0.00	2.00	0.51	0.00
LAB12	0.00	3.02	0.00	0.00	0.00	2.53	0.00	0.00
LAB14	0.55	2.38	1.20	0.24	0.68	2.43	0.86	0.37
LAB15	1.05	2.46	0.92	0.20	0.38	3.29	1.01	0.00
LAB17	0.00	2.28	0.90	0.00	0.00	1.85	0.69	0.00
LAB18	0.00	0.00	0.00	0.00	0.00	1.61	0.00	0.00
LAB19	0.00	4.31	0.00	0.00	0.00	5.39	0.00	0.00
LAB20	0.08	3.71	0.00	0.00	0.00	3.35	0.00	0.00
LAB21	0.52	4.00	1.49	0.00	0.00	4.27	1.60	0.00

Table C-Sample 2-Standard deviations

Standard Deviation - Sample 2 (NRBR N375 DAE)								
ng/dm^2	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
LAB01	0.09	0.16	0.37	-	-	0.18	0.21	-
LAB02	0.34	0.40	0.08	0.00	0.00	0.20	0.10	0.00
LAB03	0.35	1.76	0.77	0.33	0.42	1.42	0.00	0.00
LAB04	0.03	0.09	0.06	0.00	0.01	0.05	0.03	0.00
LAB05	0.00	0.80	0.00	0.00	0.00	0.19	0.00	0.00
LAB06	0.01	0.18	0.17	0.00	0.00	0.25	0.10	0.00
LAB07	0.43	0.06	7.63	8.80	0.77	0.28	2.12	12.41
LAB08	0.16	0.33	0.18	0.25	0.11	0.47	0.09	0.03
LAB09	0.00	0.65	0.00	0.00	0.00	0.26	0.00	0.00
LAB10	0.56	0.34	0.55	0.49	0.66	1.15	0.62	0.41
LAB11	0.04	0.08	0.03	0.00	0.00	0.10	0.09	0.00
LAB12	0.00	0.09	0.00	0.00	0.00	0.03	0.00	0.00
LAB14	0.04	0.65	0.25	0.08	0.21	0.11	0.14	0.02
LAB15	0.10	0.27	0.18	0.04	0.06	0.13	0.35	0.00
LAB17	0.00	0.39	0.26	0.00	0.00	0.13	0.14	0.00
LAB18	0.00	0.00	0.00	0.00	0.00	1.44	0.00	0.00
LAB19	0.00	1.56	0.00	0.00	0.00	1.23	0.00	0.00
LAB20	0.05	0.15	0.00	0.00	0.00	0.39	0.00	0.00
LAB21	0.09	0.02	0.17	0.00	0.00	0.46	0.13	0.00

Table A-Sample 3-Original values

Sample 3 (PVC N772)								
ng/dm^2	BAA	CHR	BBF	BKF	BJF	BEP	BAP	DBA
LAB01	<	<	<	<	<	<	<	<
	<	<	<	<	<	<	<	<
	<	<	<	<	<	<	<	<
LAB02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB03	1.11	0.00	0.00	0.00	0.00	0.92	0.00	0.00
	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.71	0.00	0.00
LAB04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.44	0.00	0.00	0.00	0.00	0.00	0.00
LAB05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB06	0.00	0.00	0.00	0.00	0.00	0.22	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.22	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.27	0.00	0.00
LAB07	0.92	1.78	1.79	1.22	1.24	1.59	1.70	2.66
	1.51	1.17	1.86	1.39	1.03	1.77	1.90	2.53
	1.13	2.45	1.67	1.25	1.37	1.83	1.38	3.73
LAB08	0.29	0.50	0.59	0.00	0.27	1.07	1.07	0.00
	0.19	0.35	0.57	0.00	0.32	1.22	1.07	0.00
	0.17	0.25	0.35	0.00	0.00	0.90	0.78	0.00
LAB09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB10	1.18	1.11	1.88	1.22	1.48	1.42	1.00	1.39
	0.63	0.49	1.24	0.55	0.71	0.75	0.53	0.57
	0.20	0.27	0.81	0.19	0.36	0.35	0.20	0.24
LAB11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB12	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB14	0.14	0.13	0.54	0.14	0.24	0.42	0.14	0.31
	0.13	0.12	0.43	0.09	0.20	0.35	0.21	0.32
	0.09	0.12	0.66	0.15	0.19	0.12	0.10	0.31
LAB15	0.00	0.00	0.18	0.00	0.00	0.12	0.13	0.00
	0.00	0.00	0.19	0.00	0.00	0.20	0.09	0.00
	0.00	0.27	0.15	0.00	0.00	0.21	0.00	0.00
LAB17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table B-Sample 3-Mean values

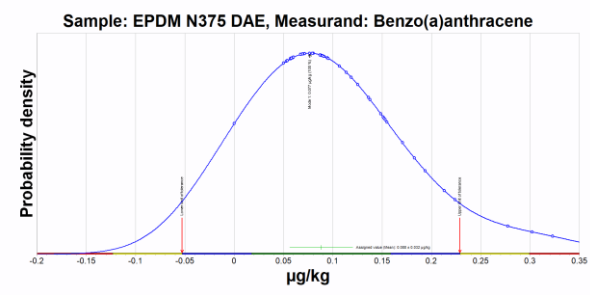
Average - Sample 3 (PVC N772)								
ng/dm^2	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
LAB01	-	-	-	-	-	-	-	-
LAB02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB03	0.47	0.00	0.00	0.00	0.00	0.54	0.00	0.00
LAB04	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00
LAB05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB06	0.00	0.00	0.00	0.00	0.00	0.23	0.00	0.00
LAB07	1.19	1.80	1.77	1.29	1.21	1.73	1.66	2.97
LAB08	0.22	0.37	0.50	0.00	0.20	1.06	0.97	0.00
LAB09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB10	0.67	0.63	1.31	0.65	0.85	0.84	0.58	0.73
LAB11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB12	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
LAB14	0.12	0.12	0.55	0.13	0.21	0.30	0.15	0.31
LAB15	0.00	0.09	0.17	0.00	0.00	0.18	0.07	0.00
LAB17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

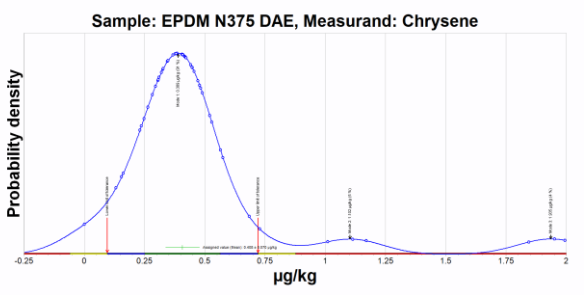
Table C-Sample 3-Standard deviations

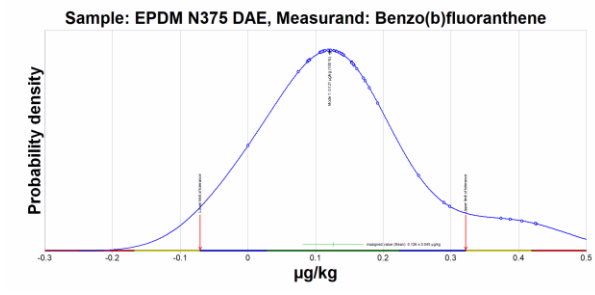
Standard Deviation - Sample 3 (PVC N772)								
ng/dm^2	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
LAB01	-	-	-	-	-	-	-	-
LAB02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB03	0.57	0.00	0.00	0.00	0.00	0.48	0.00	0.00
LAB04	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00
LAB05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB06	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00
LAB07	0.30	0.64	0.10	0.09	0.17	0.12	0.26	0.66
LAB08	0.07	0.13	0.13	0.00	0.17	0.16	0.17	0.00
LAB09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB10	0.49	0.43	0.54	0.52	0.57	0.54	0.40	0.59
LAB11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB12	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00
LAB14	0.02	0.00	0.11	0.03	0.03	0.15	0.05	0.01
LAB15	0.00	0.16	0.02	0.00	0.00	0.05	0.07	0.00
LAB17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

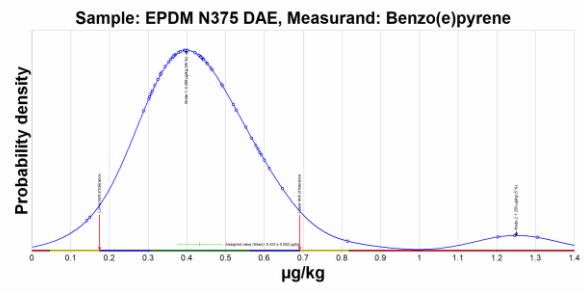
5.6 Statistics in terms of migration per weight (µg/kg)-ISO 5725-5

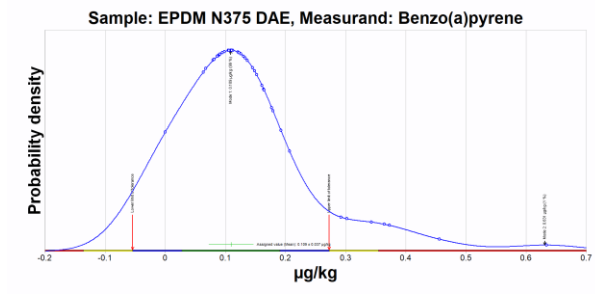
5.6.1 Kernel's density

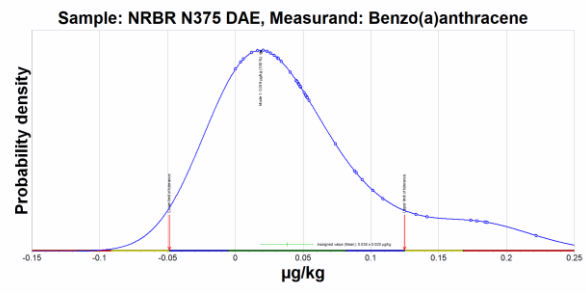


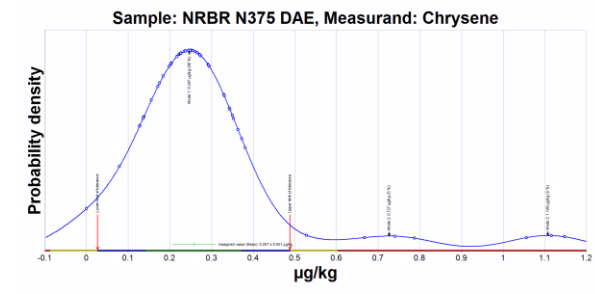


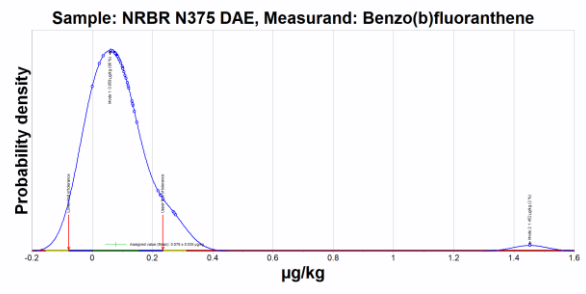


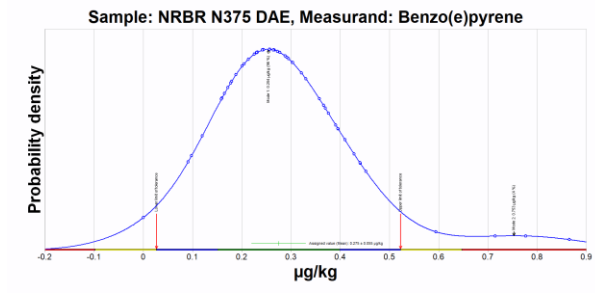


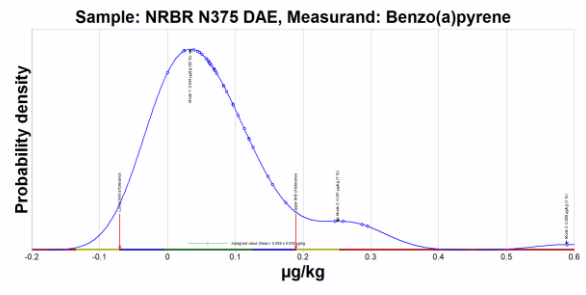




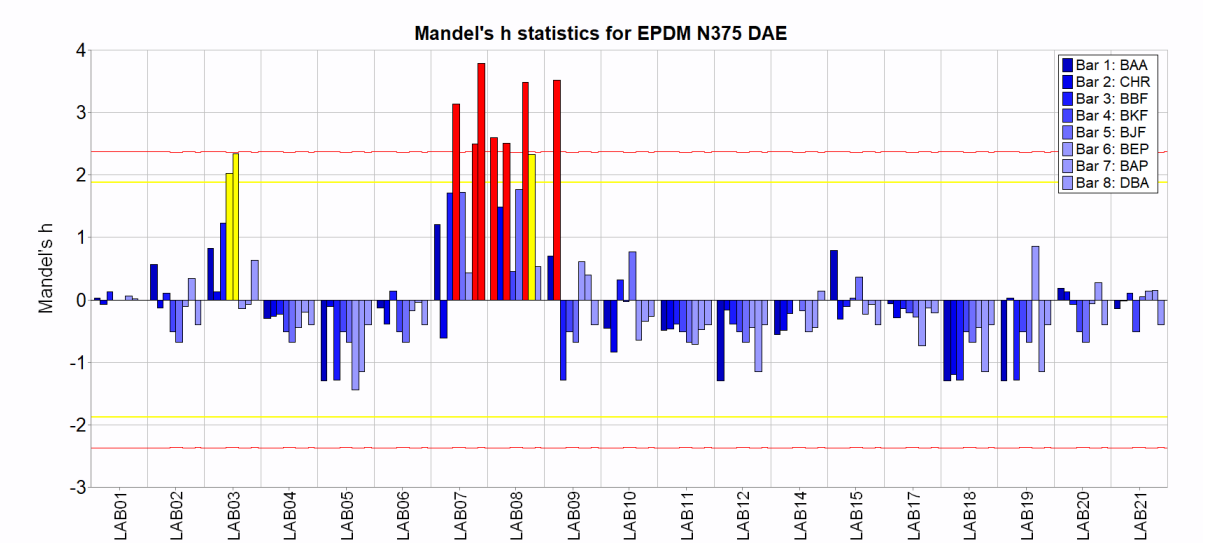


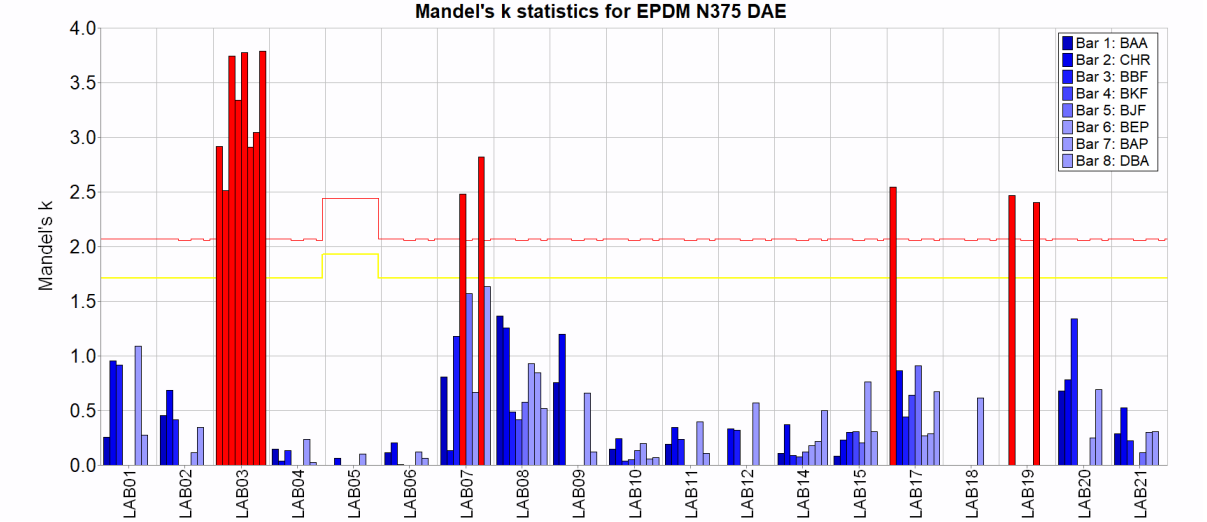


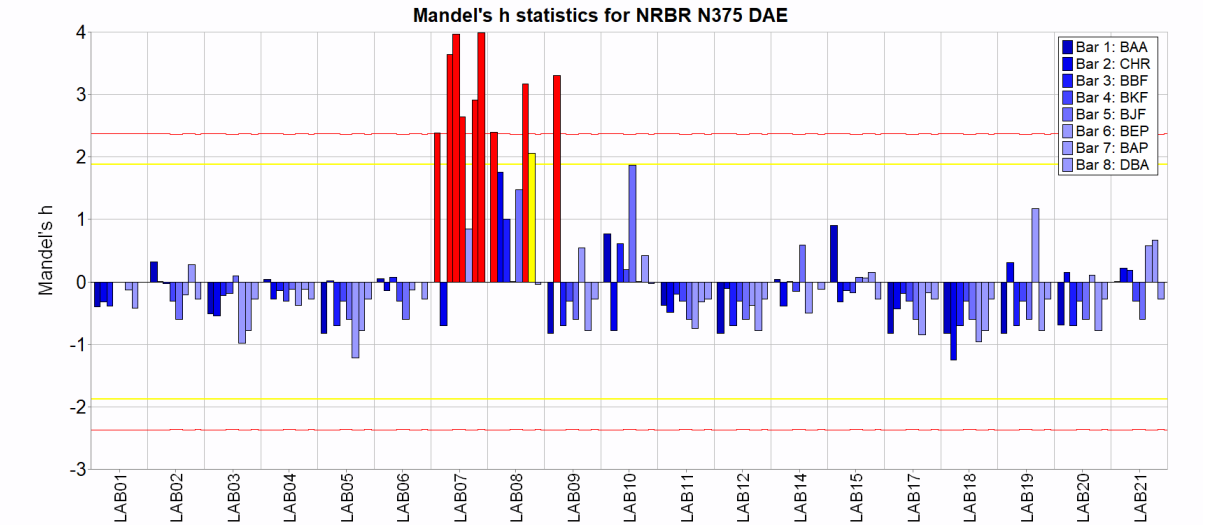


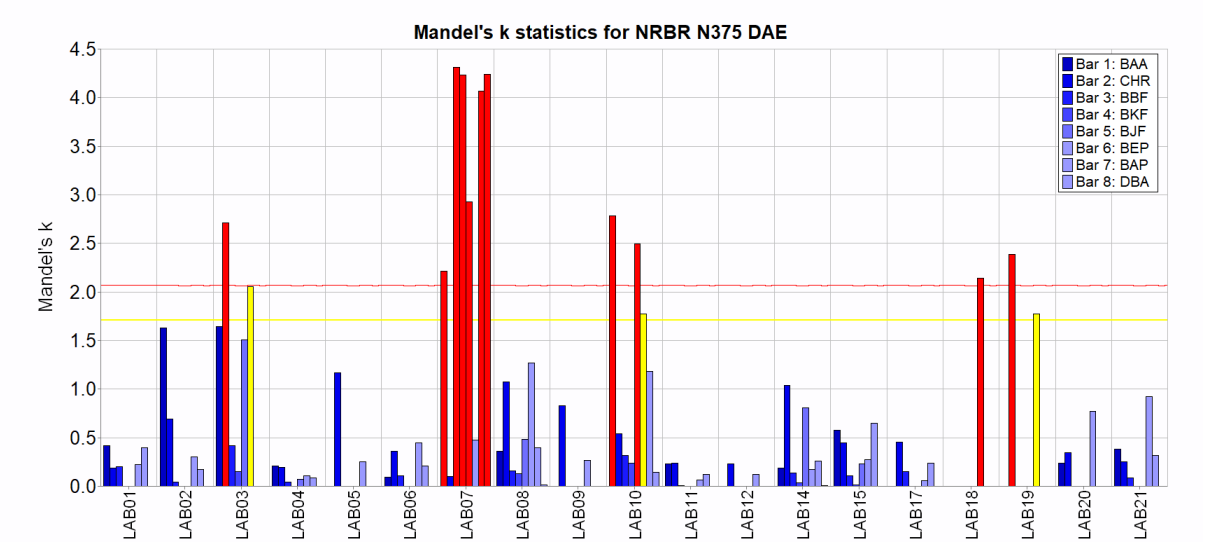


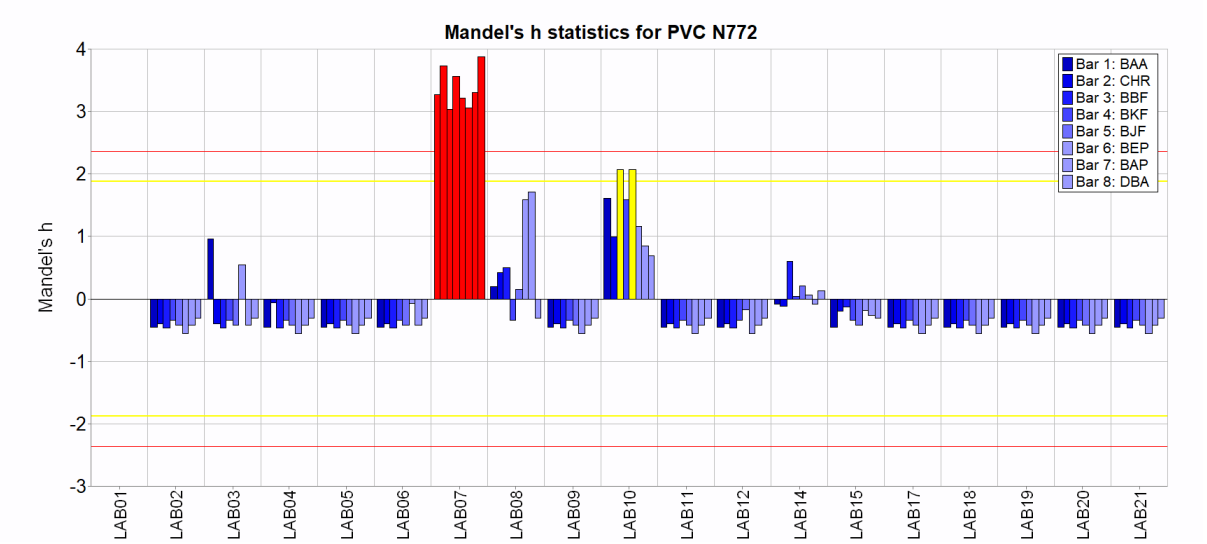
5.6.2 Mandels'h and k statistics

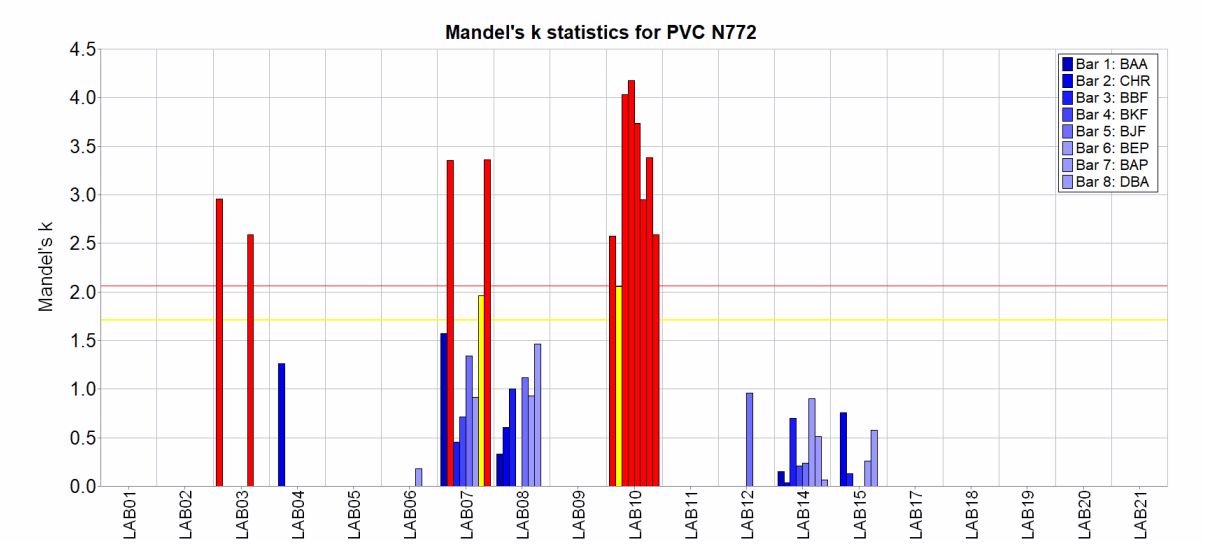




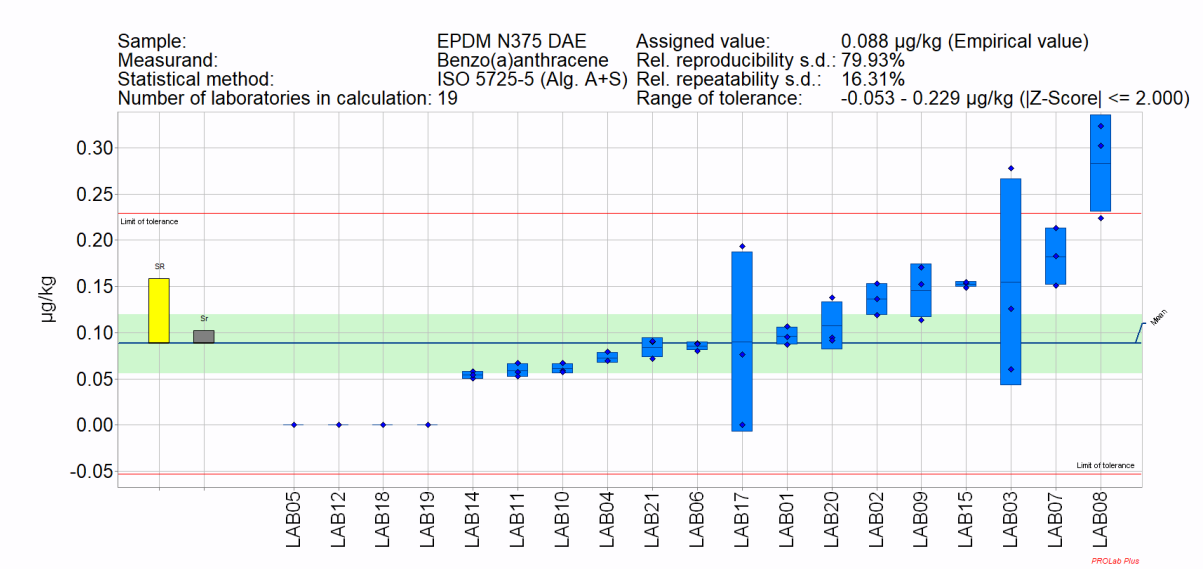


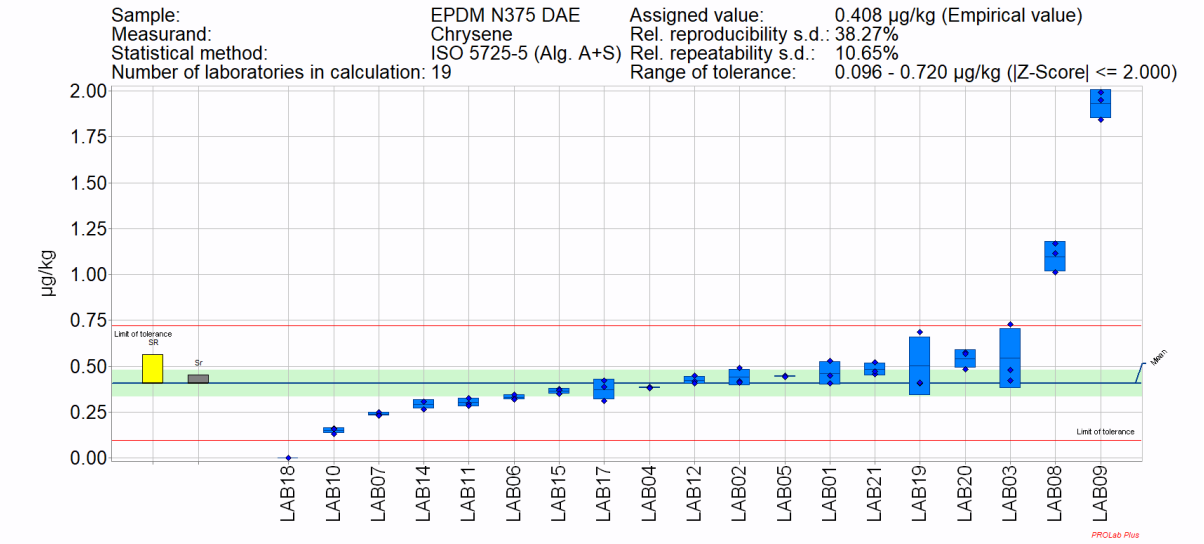

Mandel's k statistics for NRBR N375 DAE
Mandel's k
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Bar 1: BAA
Bar 2: CHR
Bar 3: BBF
Bar 4: BKF
Bar 5: BJF
Bar 6: BEP
Bar 7: BAP
Bar 8: DBA
LAB01
LAB02
LAB03
LAB04
LAB05
LAB06
LAB07
LAB08
LAB09
LAB10
LAB11
LAB12
LAB14
LAB15
LAB17
LAB18
LAB19
LAB20
LAB21

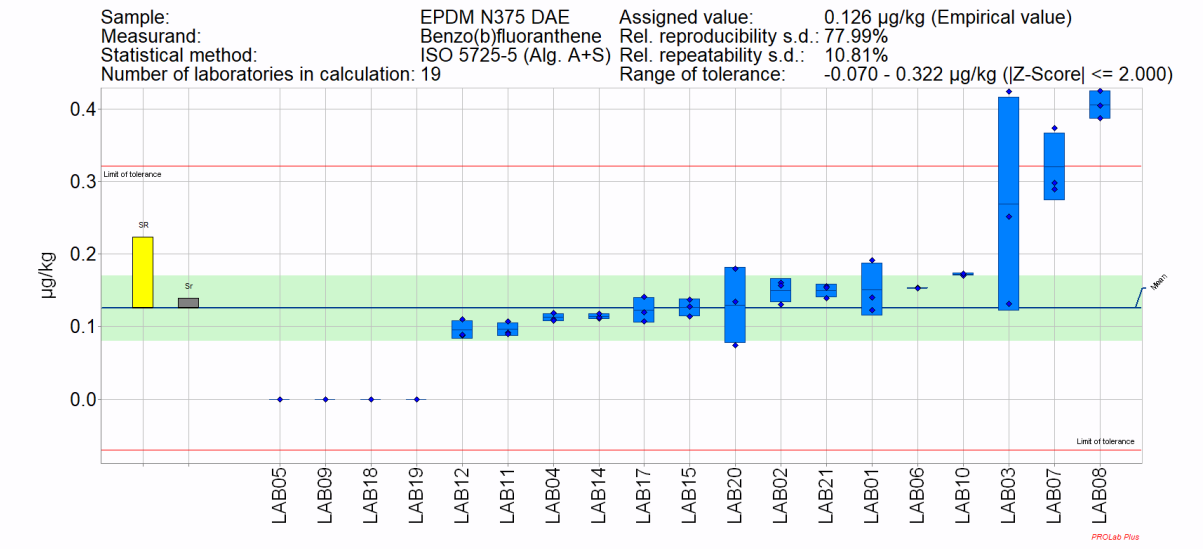

Mandel's h statistics for PVC N772
Mandel's h
4
3
2
1
0
-1
-2
-3
Bar 1: BAA
Bar 2: CHR
Bar 3: BBF
Bar 4: BKF
Bar 5: BJF
Bar 6: BEP
Bar 7: BAP
Bar 8: DBA
LAB01
LAB02
LAB03
LAB04
LAB05
LAB06
LAB07
LAB08
LAB09
LAB10
LAB11
LAB12
LAB14
LAB15
LAB17
LAB18
LAB19
LAB20
LAB21


Mandel's k statistics for PVC N772
Mandel's k
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Bar 1: BAA
Bar 2: CHR
Bar 3: BBF
Bar 4: BKF
Bar 5: BJF
Bar 6: BEP
Bar 7: BAP
Bar 8: DBA
LAB01
LAB02
LAB03
LAB04
LAB05
LAB06
LAB07
LAB08
LAB09
LAB10
LAB11
LAB12
LAB14
LAB15
LAB17
LAB18
LAB19
LAB20
LAB21

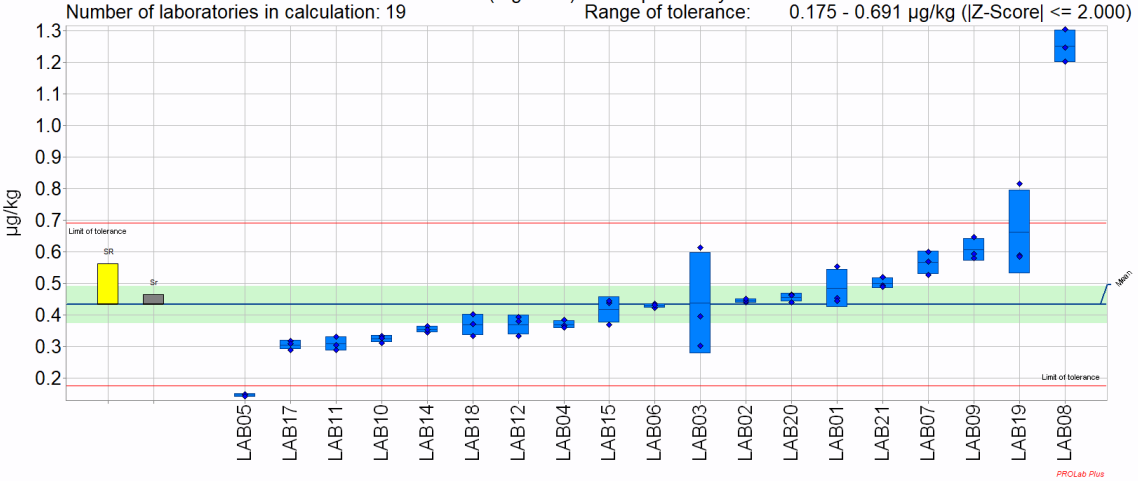
5.6.3 Inter-laboratory study results



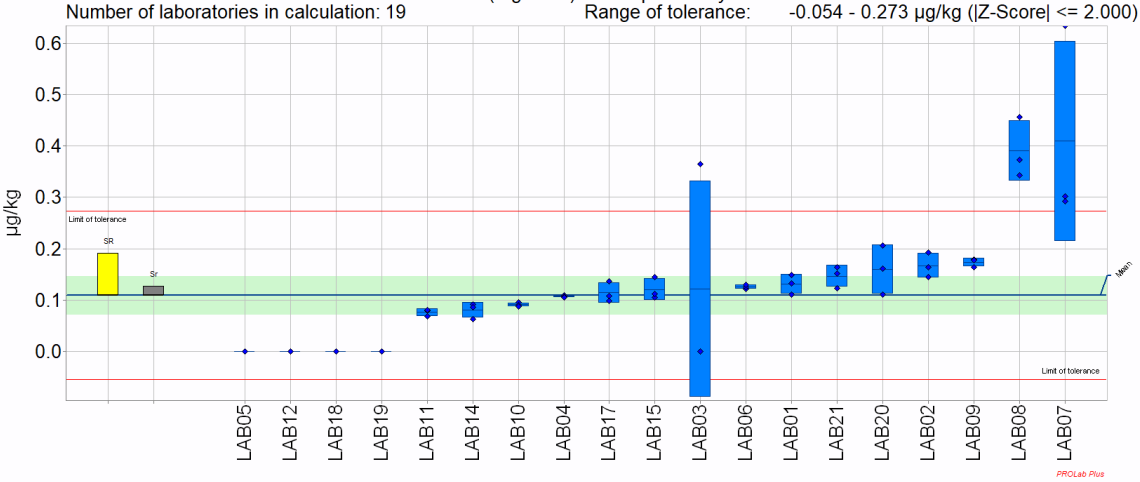




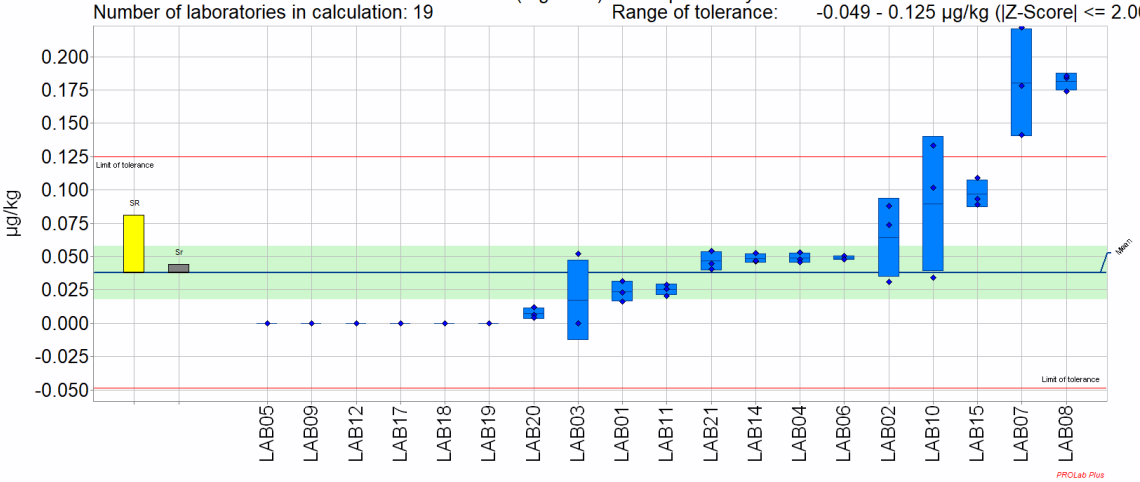
Sample: EPDM N375 DAE
Measurand: Benzo(e)pyrene
Statistical method: ISO 5725-5 (Alg. A+S)
Number of laboratories in calculation: 19
Assigned value: 0.433 µg/kg (Empirical value)
Rel. reproducibility s.d.: 29.79%
Rel. repeatability s.d.: 7.45%
Range of tolerance: 0.175 - 0.691 µg/kg (|Z-Score| <= 2.000)



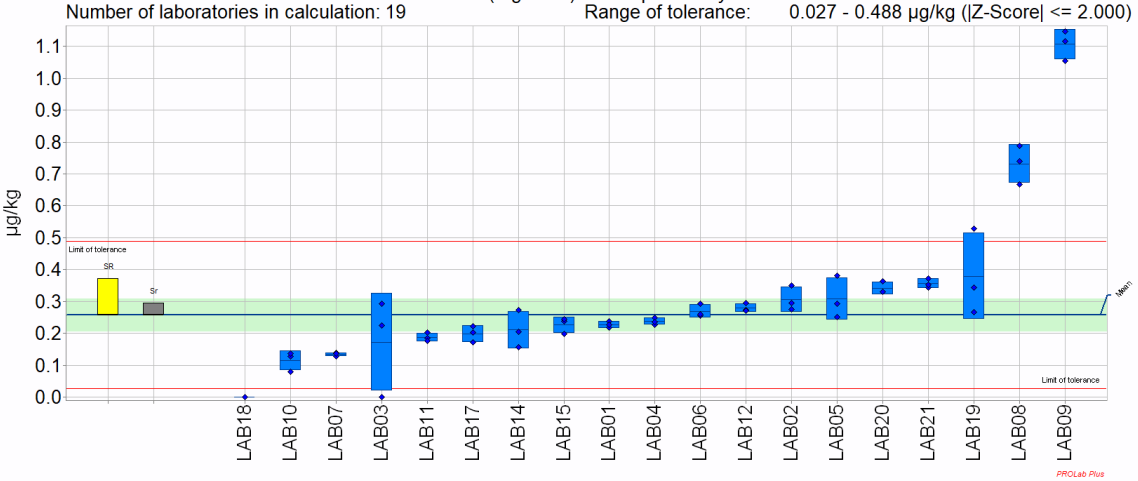
Sample: EPDM N375 DAE
Measurand: Benzo(a)pyrene
Statistical method: ISO 5725-5 (Alg. A+S)
Number of laboratories in calculation: 19
Assigned value: 0.109 µg/kg (Empirical value)
Rel. reproducibility s.d.: 74.86%
Rel. repeatability s.d.: 16.71%
Range of tolerance: -0.054 - 0.273 µg/kg (|Z-Score| <= 2.000)



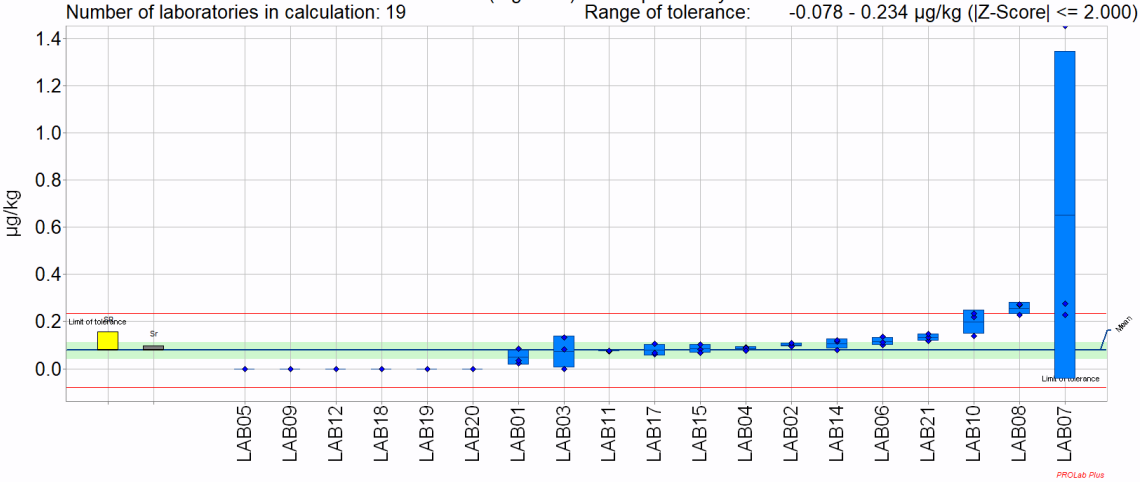
Sample: NRBR N375 DAE
Measurand: Benzo(a)anthracene
Statistical method: ISO 5725-5 (Alg. A+S)
Number of laboratories in calculation: 19
Assigned value: 0.038 µg/kg (Empirical value)
Rel. reproducibility s.d.: 113.76%
Rel. repeatability s.d.: 16.29%
Range of tolerance: -0.049 - 0.125 µg/kg (|Z-Score| <= 2.000)



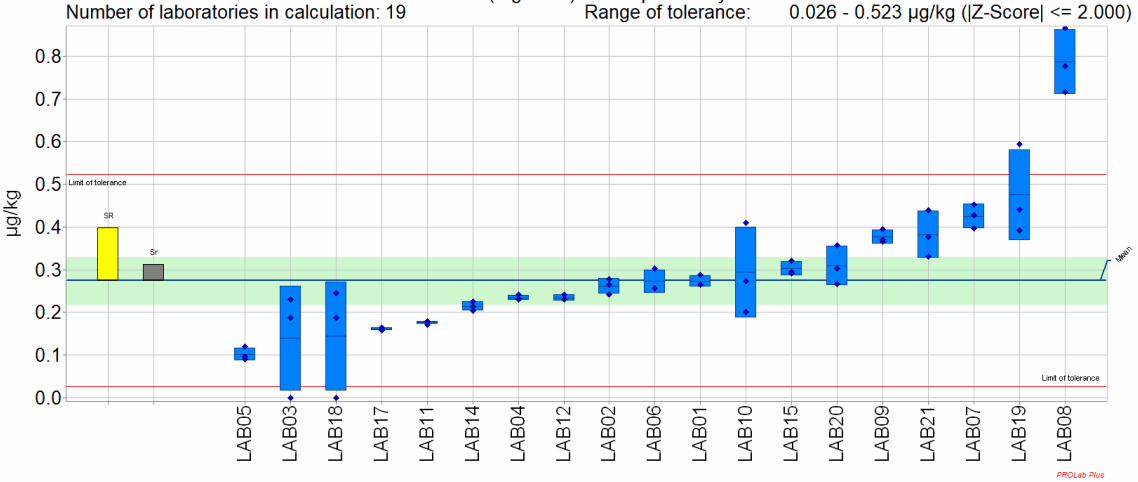
Sample: NRBR N375 DAE
Measurand: Chrysene
Statistical method: ISO 5725-5 (Alg. A+S)
Number of laboratories in calculation: 19
Assigned value: 0.257 µg/kg (Empirical value)
Rel. reproducibility s.d.: 44.82%
Rel. repeatability s.d.: 14.34%
Range of tolerance: 0.027 - 0.488 µg/kg (|Z-Score| <= 2.000)

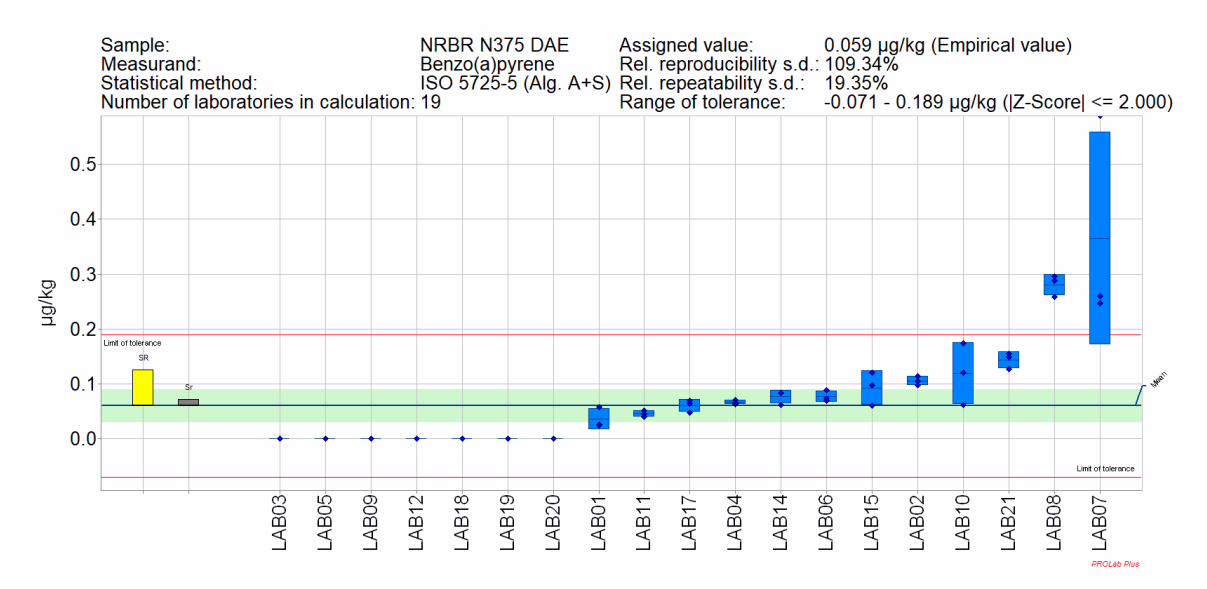


Sample: NRBR N375 DAE
Measurand: Benzo(b)fluoranthene
Statistical method: ISO 5725-5 (Alg. A+S)
Number of laboratories in calculation: 19
Assigned value: 0.078 µg/kg (Empirical value)
Rel. reproducibility s.d.: 100.01%
Rel. repeatability s.d.: 23.33%
Range of tolerance: -0.078 - 0.234 µg/kg (|Z-Score| <= 2.000)

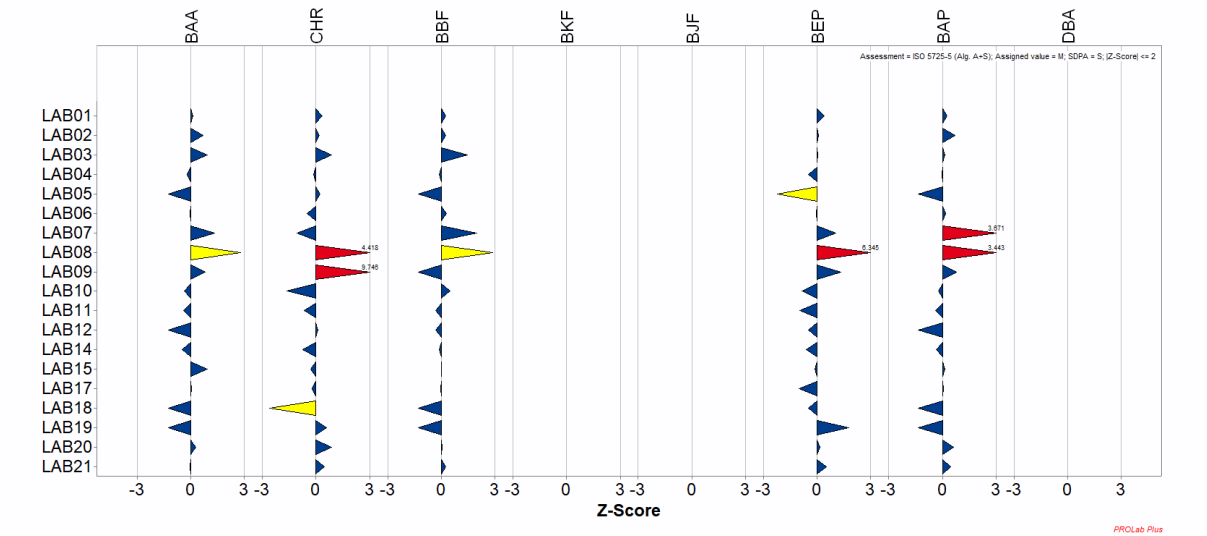


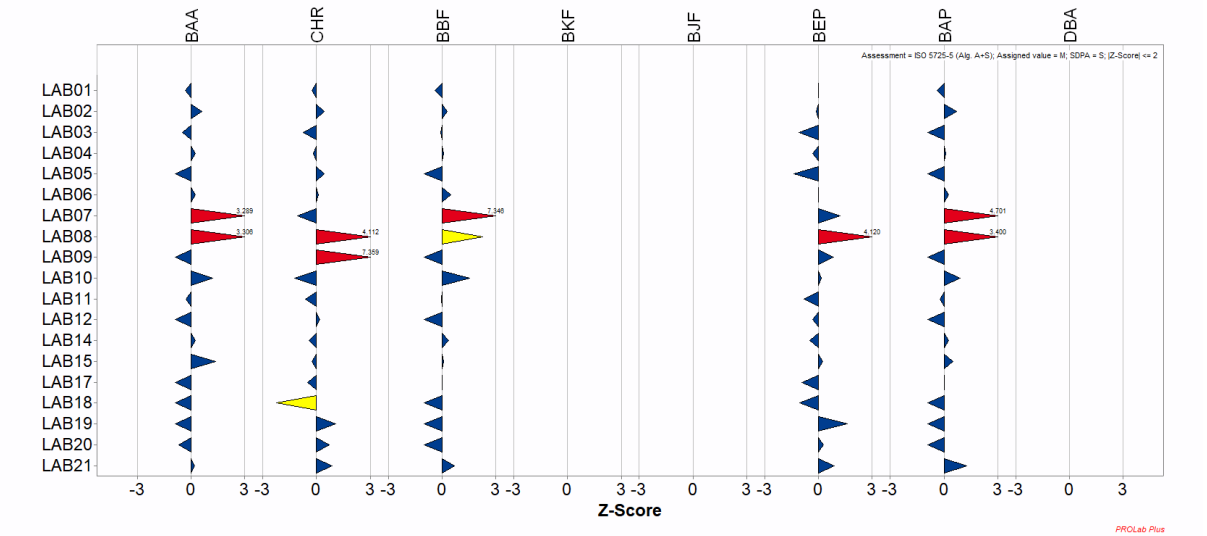
Sample: NRBR N375 DAE
Measurand: Benzo(e)pyrene
Statistical method: ISO 5725-5 (Alg. A+S)
Number of laboratories in calculation: 19
Assigned value: 0.275 µg/kg (Empirical value)
Rel. reproducibility s.d.: 45.18%
Rel. repeatability s.d.: 13.90%
Range of tolerance: 0.026 - 0.523 µg/kg (|Z-Score| <= 2.000)




Sample: NRBR N375 DAE
Measurand: Benzo(a)pyrene
Statistical method: ISO 5725-5 (Alg. A+S)
Number of laboratories in calculation: 19
Assigned value: 0.059 µg/kg (Empirical value)
Rel. reproducibility s.d.: 109.34%
Rel. repeatability s.d.: 19.35%
Range of tolerance: -0.071 - 0.189 µg/kg (|Z-Score| <= 2.000)
µg/kg
0.5
0.4
0.3
0.2
0.1
0.0
Limit of tolerance
SR
Sr
Limit of tolerance
LAB03 LAB05 LAB09 LAB12 LAB18 LAB19 LAB20 LAB01 LAB11 LAB17 LAB04 LAB14 LAB06 LAB15 LAB02 LAB10 LAB21 LAB08 LAB07
PROLab Plus

5.6.4 Z-scores


BAA CHR BBF BKF BJF BEP BAP DBA
Assessment = ISO 5725-5 (Alg. A+S); Assigned value = M; SDPA = S; |Z-Score| <= 2
LAB01 LAB02 LAB03 LAB04 LAB05 LAB06 LAB07 LAB08 LAB09 LAB10 LAB11 LAB12 LAB14 LAB15 LAB17 LAB18 LAB19 LAB20 LAB21
4.418
9.746
6.345
3.671
3.443
-3 0 3 -3 0 3 -3 0 3 -3 0 3 -3 0 3 -3 0 3 -3 0 3 -3 0 3
Z-Score
PROLab Plus


BAA CHR BBF BKF BJF BEP BAP DBA
Assessment = ISO 5725-5 (Alg. A+S); Assigned value = M; SDPA = S; |Z-Score| <= 2
LAB01 LAB02 LAB03 LAB04 LAB05 LAB06 LAB07 LAB08 LAB09 LAB10 LAB11 LAB12 LAB14 LAB15 LAB17 LAB18 LAB19 LAB20 LAB21
3.289
3.306
4.112
7.359
7.146
4.120
4.701
3.400
-3 0 3 -3 0 3 -3 0 3 -3 0 3 -3 0 3 -3 0 3 -3 0 3 -3 0 3
Z-Score
PROLab Plus

5.6.5 Table (A, B, C): Original values, means and standard deviations

Table A-Sample 1-Original values

Sample 1 (EPDM N375 DAE)								
µg/kg	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
LAB01	0.11	0.53	0.19	<	<	0.55	0.15	<
	0.09	0.41	0.12	<	<	0.45	0.13	<
	0.10	0.45	0.14	<	<	0.44	0.11	<
LAB02	0.12	0.49	0.16	0.00	0.00	0.44	0.14	0.00
	0.15	0.42	0.16	0.00	0.00	0.44	0.16	0.00
	0.14	0.41	0.13	0.00	0.00	0.45	0.19	0.00
LAB03	0.13	0.42	0.25	0.10	0.18	0.40	0.00	0.00
	0.06	0.48	0.13	0.00	0.00	0.30	0.00	0.00
	0.28	0.73	0.42	0.26	0.32	0.61	0.36	0.30
LAB04	0.08	0.39	0.11	0.00	0.00	0.37	0.11	0.00
	0.07	0.39	0.11	0.00	0.00	0.38	0.11	0.00
	0.07	0.38	0.12	0.00	0.00	0.36	0.11	0.00
LAB05								
	0.00	0.45	0.00	0.00	0.00	0.15	0.00	0.00
	0.00	0.44	0.00	0.00	0.00	0.14	0.00	0.00
LAB06	0.08	0.33	0.15	0.00	0.00	0.42	0.12	0.00
	0.09	0.35	0.15	0.00	0.00	0.43	0.13	0.00
	0.09	0.32	0.15	0.00	0.00	0.43	0.13	0.00
LAB07	0.21	0.24	0.37	0.29	0.21	0.60	0.63	0.48
	0.18	0.25	0.29	0.12	0.08	0.57	0.30	0.34
	0.15	0.23	0.30	0.12	0.11	0.53	0.29	0.38
LAB08	0.22	1.01	0.39	0.06	0.11	1.25	0.37	0.07
	0.32	1.17	0.40	0.03	0.15	1.30	0.46	0.08
	0.30	1.11	0.43	0.04	0.14	1.20	0.34	0.12
LAB09	0.15	1.84	0.00	0.00	0.00	0.58	0.16	0.00
	0.17	1.95	0.00	0.00	0.00	0.65	0.18	0.00
	0.11	1.99	0.00	0.00	0.00	0.59	0.18	0.00
LAB10	0.07	0.16	0.17	0.02	0.08	0.33	0.10	0.02
	0.06	0.16	0.17	0.02	0.08	0.31	0.09	0.01
	0.06	0.13	0.17	0.02	0.07	0.33	0.09	0.01
LAB11	0.07	0.33	0.11	0.00	0.00	0.33	0.08	0.00
	0.06	0.30	0.09	0.00	0.00	0.29	0.07	0.00
	0.05	0.28	0.09	0.00	0.00	0.30	0.08	0.00
LAB12	0.00	0.45	0.11	0.00	0.00	0.39	0.00	0.00
	0.00	0.42	0.09	0.00	0.00	0.33	0.00	0.00
	0.00	0.41	0.09	0.00	0.00	0.38	0.00	0.00
LAB14	0.05	0.31	0.11	0.03	0.02	0.36	0.09	0.08
	0.06	0.26	0.11	0.02	0.03	0.34	0.09	0.05
	0.05	0.31	0.12	0.02	0.03	0.35	0.06	0.03
LAB15	0.15	0.38	0.14	0.04	0.06	0.44	0.15	0.00
	0.15	0.37	0.13	0.02	0.05	0.44	0.10	0.00
	0.15	0.35	0.11	0.01	0.07	0.37	0.11	0.00
LAB17	0.08	0.42	0.14	0.04	0.07	0.32	0.14	0.05
	0.19	0.39	0.11	0.00	0.00	0.31	0.11	0.00
	0.00	0.31	0.12	0.00	0.00	0.29	0.10	0.00
LAB18	0.00	0.00	0.00	0.00	0.00	0.37	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.00
LAB19	0.00	0.68	0.00	0.00	0.00	0.81	0.00	0.00
	0.00	0.41	0.00	0.00	0.00	0.59	0.00	0.00
	0.00	0.41	0.00	0.00	0.00	0.58	0.00	0.00
LAB20	0.09	0.48	0.07	0.00	0.00	0.44	0.11	0.00
	0.09	0.58	0.13	0.00	0.00	0.46	0.21	0.00
	0.14	0.57	0.18	0.00	0.00	0.46	0.16	0.00
LAB21	0.09	0.52	0.16	0.00	0.05	0.52	0.16	0.00
	0.09	0.46	0.14	0.00	0.04	0.49	0.12	0.00
	0.07	0.47	0.15	0.00	0.04	0.49	0.15	0.00

Table B-Sample 1-Mean values

Average - Sample 1 (EPDM N375 DAE)								
µg/kg	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
LAB01	0.10	0.46	0.15	-	-	0.48	0.13	-
LAB02	0.14	0.44	0.15	0.00	0.00	0.44	0.17	0.00
LAB03	0.15	0.54	0.27	0.12	0.17	0.44	0.12	0.10
LAB04	0.07	0.38	0.11	0.00	0.00	0.37	0.11	0.00
LAB05	0.00	0.44	0.00	0.00	0.00	0.15	0.00	0.00
LAB06	0.09	0.33	0.15	0.00	0.00	0.43	0.13	0.00
LAB07	0.18	0.24	0.32	0.17	0.13	0.57	0.41	0.40
LAB08	0.28	1.10	0.41	0.05	0.14	1.25	0.39	0.09
LAB09	0.15	1.93	0.00	0.00	0.00	0.61	0.17	0.00
LAB10	0.06	0.15	0.17	0.02	0.08	0.32	0.09	0.01
LAB11	0.06	0.30	0.10	0.00	0.00	0.31	0.08	0.00
LAB12	0.00	0.42	0.10	0.00	0.00	0.37	0.00	0.00
LAB14	0.05	0.29	0.11	0.02	0.03	0.35	0.08	0.05
LAB15	0.15	0.36	0.13	0.03	0.06	0.42	0.12	0.00
LAB17	0.09	0.37	0.12	0.01	0.02	0.30	0.11	0.02
LAB18	0.00	0.00	0.00	0.00	0.00	0.37	0.00	0.00
LAB19	0.00	0.50	0.00	0.00	0.00	0.66	0.00	0.00
LAB20	0.11	0.54	0.13	0.00	0.00	0.46	0.16	0.00
LAB21	0.08	0.48	0.15	0.00	0.04	0.50	0.15	0.00

Table C-Sample 1-Standard deviations

Standard Deviation - Sample 1 (EPDM N375 DAE)								
µg/kg	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
LAB01	0.01	0.06	0.04	-	-	0.06	0.02	-
LAB02	0.02	0.04	0.02	0.00	0.00	0.01	0.02	0.00
LAB03	0.11	0.16	0.15	0.13	0.16	0.16	0.21	0.17
LAB04	0.01	0.00	0.01	0.00	0.00	0.01	0.00	0.00
LAB05	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
LAB06	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00
LAB07	0.03	0.01	0.05	0.10	0.07	0.04	0.19	0.07
LAB08	0.05	0.08	0.02	0.02	0.02	0.05	0.06	0.02
LAB09	0.03	0.08	0.00	0.00	0.00	0.04	0.01	0.00
LAB10	0.01	0.02	0.00	0.00	0.01	0.01	0.00	0.00
LAB11	0.01	0.02	0.01	0.00	0.00	0.02	0.01	0.00
LAB12	0.00	0.02	0.01	0.00	0.00	0.03	0.00	0.00
LAB14	0.00	0.02	0.00	0.00	0.01	0.01	0.02	0.02
LAB15	0.00	0.02	0.01	0.01	0.01	0.04	0.02	0.00
LAB17	0.10	0.06	0.02	0.03	0.04	0.01	0.02	0.03
LAB18	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00
LAB19	0.00	0.16	0.00	0.00	0.00	0.13	0.00	0.00
LAB20	0.03	0.05	0.05	0.00	0.00	0.01	0.05	0.00
LAB21	0.01	0.03	0.01	0.00	0.01	0.02	0.02	0.00

Table A-Sample 2-Original values

Sample 2 (NRBR N375 DAE)								
µg/kg	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
LAB01	0.03	0.24	0.02	<	<	0.29	0.06	<
	0.02	0.22	0.04	<	<	0.27	0.03	<
	0.02	0.23	0.08	<	<	0.26	0.02	<
LAB02	0.03	0.35	0.10	0.00	0.00	0.28	0.10	0.00
	0.07	0.27	0.09	0.00	0.00	0.26	0.10	0.00
	0.09	0.29	0.11	0.00	0.00	0.24	0.11	0.00
LAB03	0.05	0.22	0.08	0.00	0.07	0.23	0.00	0.00
	0.00	0.29	0.13	0.05	0.03	0.19	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB04	0.05	0.25	0.09	0.00	0.03	0.24	0.07	0.00
	0.05	0.23	0.09	0.00	0.02	0.23	0.06	0.00
	0.05	0.23	0.08	0.00	0.02	0.23	0.06	0.00
LAB05	0.00	0.29	0.00	0.00	0.00	0.09	0.00	0.00
	0.00	0.38	0.00	0.00	0.00	0.12	0.00	0.00
	0.00	0.25	0.00	0.00	0.00	0.10	0.00	0.00
LAB06	0.05	0.29	0.14	0.00	0.00	0.30	0.09	0.00
	0.05	0.26	0.10	0.00	0.00	0.26	0.07	0.00
	0.05	0.26	0.11	0.00	0.00	0.26	0.07	0.00
LAB07	0.18	0.14	0.28	0.16	0.15	0.43	0.26	0.31
	0.14	0.13	0.23	0.14	0.10	0.40	0.25	0.21
	0.22	0.14	1.45	1.54	0.24	0.45	0.59	2.21
LAB08	0.18	0.74	0.27	0.02	0.10	0.78	0.29	0.05
	0.17	0.79	0.27	0.07	0.12	0.87	0.30	0.05
	0.19	0.67	0.23	0.04	0.10	0.71	0.26	0.05
LAB09	0.00	1.15	0.00	0.00	0.00	0.40	0.00	0.00
	0.00	1.06	0.00	0.00	0.00	0.37	0.00	0.00
	0.00	1.12	0.00	0.00	0.00	0.37	0.00	0.00
LAB10	0.03	0.08	0.14	0.02	0.06	0.20	0.06	0.01
	0.13	0.13	0.22	0.10	0.16	0.27	0.12	0.06
	0.10	0.14	0.24	0.09	0.17	0.41	0.17	0.08
LAB11	0.03	0.20	0.08	0.00	0.00	0.18	0.04	0.00
	0.03	0.18	0.07	0.00	0.00	0.17	0.04	0.00
	0.02	0.18	0.08	0.00	0.00	0.18	0.05	0.00
LAB12	0.00	0.27	0.00	0.00	0.00	0.23	0.00	0.00
	0.00	0.27	0.00	0.00	0.00	0.23	0.00	0.00
	0.00	0.29	0.00	0.00	0.00	0.24	0.00	0.00
LAB14	0.05	0.16	0.08	0.02	0.05	0.21	0.06	0.03
	0.05	0.20	0.12	0.03	0.05	0.20	0.08	0.03
	0.05	0.27	0.12	0.01	0.08	0.23	0.08	0.03
LAB15	0.09	0.24	0.08	0.02	0.04	0.29	0.12	0.00
	0.09	0.20	0.07	0.02	0.03	0.29	0.10	0.00
	0.11	0.24	0.10	0.02	0.03	0.32	0.06	0.00
LAB17	0.00	0.17	0.11	0.00	0.00	0.16	0.07	0.00
	0.00	0.20	0.06	0.00	0.00	0.16	0.05	0.00
	0.00	0.22	0.07	0.00	0.00	0.16	0.06	0.00
LAB18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.24	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00
LAB19	0.00	0.53	0.00	0.00	0.00	0.59	0.00	0.00
	0.00	0.27	0.00	0.00	0.00	0.44	0.00	0.00
	0.00	0.34	0.00	0.00	0.00	0.39	0.00	0.00
LAB20	0.01	0.36	0.00	0.00	0.00	0.36	0.00	0.00
	0.01	0.33	0.00	0.00	0.00	0.30	0.00	0.00
	0.00	0.33	0.00	0.00	0.00	0.27	0.00	0.00
LAB21	0.05	0.34	0.12	0.00	0.00	0.33	0.13	0.00
	0.04	0.37	0.13	0.00	0.00	0.44	0.15	0.00
	0.05	0.35	0.15	0.00	0.00	0.38	0.15	0.00

Table B-Sample 2-Mean values

Average - Sample 2 (NRBR N375 DAE)								
µg/kg	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
LAB01	0.02	0.23	0.05	-	-	0.27	0.04	-
LAB02	0.06	0.31	0.10	0.00	0.00	0.26	0.10	0.00
LAB03	0.02	0.17	0.07	0.02	0.04	0.14	0.00	0.00
LAB04	0.05	0.24	0.09	0.00	0.02	0.23	0.07	0.00
LAB05	0.00	0.31	0.00	0.00	0.00	0.10	0.00	0.00
LAB06	0.05	0.27	0.12	0.00	0.00	0.27	0.08	0.00
LAB07	0.18	0.13	0.65	0.61	0.17	0.43	0.37	0.91
LAB08	0.18	0.73	0.26	0.04	0.11	0.79	0.28	0.05
LAB09	0.00	1.11	0.00	0.00	0.00	0.38	0.00	0.00
LAB10	0.09	0.11	0.20	0.07	0.13	0.29	0.12	0.05
LAB11	0.03	0.19	0.08	0.00	0.00	0.18	0.05	0.00
LAB12	0.00	0.28	0.00	0.00	0.00	0.23	0.00	0.00
LAB14	0.05	0.21	0.11	0.02	0.06	0.21	0.08	0.03
LAB15	0.10	0.23	0.08	0.02	0.03	0.30	0.09	0.00
LAB17	0.00	0.20	0.08	0.00	0.00	0.16	0.06	0.00
LAB18	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00
LAB19	0.00	0.38	0.00	0.00	0.00	0.48	0.00	0.00
LAB20	0.01	0.34	0.00	0.00	0.00	0.31	0.00	0.00
LAB21	0.05	0.36	0.13	0.00	0.00	0.38	0.14	0.00

Table C-Sample 2-Standard deviations

Standard Deviation - Sample 2 (NRBR N375 DAE)								
µg/kg	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
LAB01	0.01	0.01	0.03	-	-	0.01	0.02	-
LAB02	0.03	0.04	0.01	0.00	0.00	0.02	0.01	0.00
LAB03	0.03	0.15	0.07	0.03	0.04	0.12	0.00	0.00
LAB04	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00
LAB05	0.00	0.07	0.00	0.00	0.00	0.01	0.00	0.00
LAB06	0.00	0.02	0.02	0.00	0.00	0.03	0.01	0.00
LAB07	0.04	0.01	0.69	0.80	0.07	0.03	0.19	1.13
LAB08	0.01	0.06	0.03	0.02	0.01	0.08	0.02	0.00
LAB09	0.00	0.05	0.00	0.00	0.00	0.02	0.00	0.00
LAB10	0.05	0.03	0.05	0.04	0.06	0.11	0.06	0.04
LAB11	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00
LAB12	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00
LAB14	0.00	0.06	0.02	0.01	0.02	0.01	0.01	0.00
LAB15	0.01	0.03	0.02	0.00	0.01	0.02	0.03	0.00
LAB17	0.00	0.03	0.02	0.00	0.00	0.00	0.01	0.00
LAB18	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00
LAB19	0.00	0.14	0.00	0.00	0.00	0.11	0.00	0.00
LAB20	0.00	0.02	0.00	0.00	0.00	0.05	0.00	0.00
LAB21	0.01	0.01	0.01	0.00	0.00	0.06	0.02	0.00

Table A-Sample 3-Original values

Sample 3 (PVC N772)								
µg/kg	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
LAB01	<	<	<	<	<	<	<	<
	<	<	<	<	<	<	<	<
	<	<	<	<	<	<	<	<
LAB02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB03	0.08	0.00	0.00	0.00	0.00	0.07	0.00	0.00
	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00
LAB04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00
LAB05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB06	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
LAB07	0.07	0.13	0.13	0.09	0.09	0.12	0.12	0.20
	0.11	0.09	0.14	0.10	0.08	0.13	0.14	0.19
	0.09	0.19	0.13	0.10	0.11	0.14	0.11	0.29
LAB08	0.02	0.04	0.04	0.00	0.02	0.08	0.08	0.00
	0.01	0.03	0.04	0.00	0.02	0.09	0.08	0.00
	0.01	0.02	0.03	0.00	0.00	0.06	0.06	0.00
LAB09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB10	0.09	0.08	0.14	0.09	0.11	0.10	0.07	0.10
	0.05	0.04	0.09	0.04	0.05	0.06	0.04	0.04
	0.02	0.02	0.06	0.01	0.03	0.03	0.02	0.02
LAB11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB12	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB14	0.01	0.01	0.04	0.01	0.02	0.03	0.01	0.02
	0.01	0.01	0.03	0.01	0.02	0.03	0.02	0.02
	0.01	0.01	0.05	0.01	0.01	0.01	0.01	0.02
LAB15	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.00
	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.00
	0.00	0.02	0.01	0.00	0.00	0.02	0.00	0.00
LAB17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table B-Sample 3-Mean values

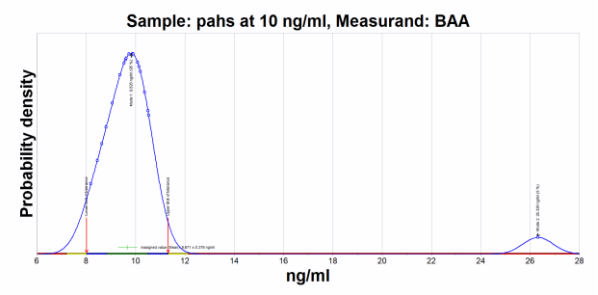
Average - Sample 3 (PVC N772)								
µg/kg	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
LAB01	-	-	-	-	-	-	-	-
LAB02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB03	0.03	0.00	0.00	0.00	0.00	0.04	0.00	0.00
LAB04	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
LAB05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB06	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
LAB07	0.09	0.14	0.13	0.10	0.09	0.13	0.12	0.22
LAB08	0.02	0.03	0.04	0.00	0.01	0.08	0.07	0.00
LAB09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB10	0.05	0.05	0.10	0.05	0.06	0.06	0.04	0.05
LAB11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB12	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
LAB14	0.01	0.01	0.04	0.01	0.02	0.02	0.01	0.02
LAB15	0.00	0.01	0.01	0.00	0.00	0.01	0.01	0.00
LAB17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

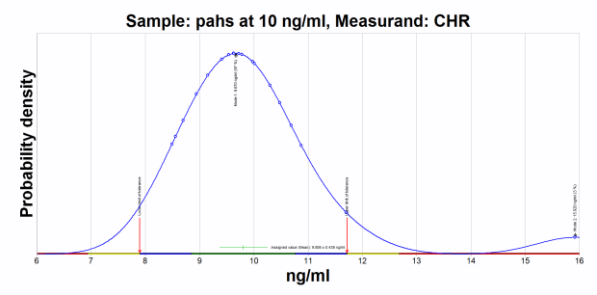
Table C-Sample 3-Standard deviations

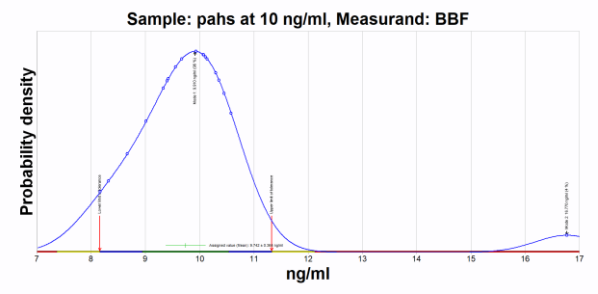
Standard Deviation - Sample 3 (PVC N772)								
µg/kg	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
LAB01	-	-	-	-	-	-	-	-
LAB02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB03	0.04	0.00	0.00	0.00	0.00	0.03	0.00	0.00
LAB04	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
LAB05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB07	0.02	0.05	0.00	0.01	0.02	0.01	0.02	0.06
LAB08	0.00	0.01	0.01	0.00	0.01	0.01	0.01	0.00
LAB09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB10	0.04	0.03	0.04	0.04	0.04	0.04	0.03	0.04
LAB11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB12	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
LAB14	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00
LAB15	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00
LAB17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LAB21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

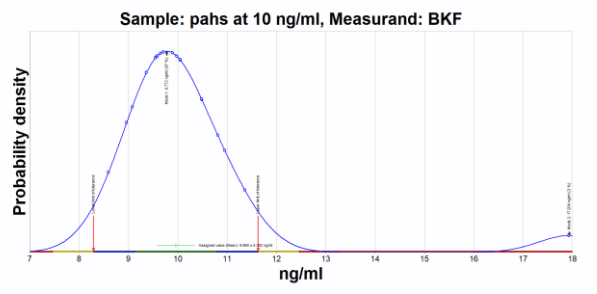
5.7 Statistics of 10 ng/ml control solution-ISO 5725-5

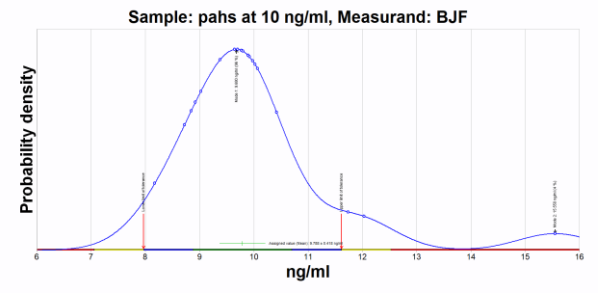
5.7.1 Kernel's density

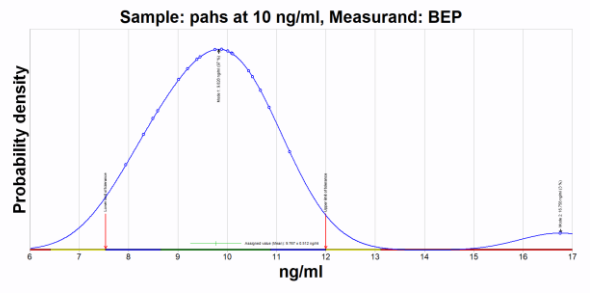


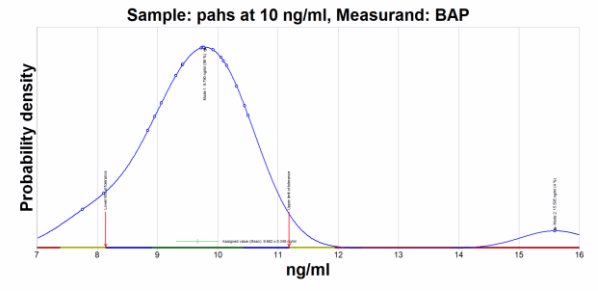


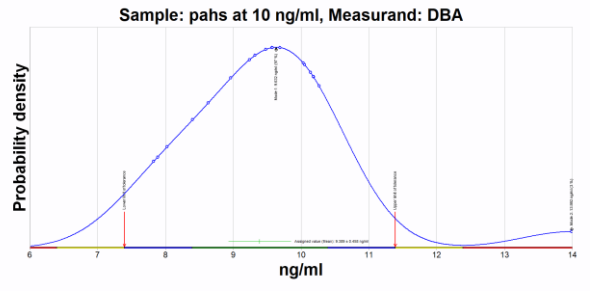




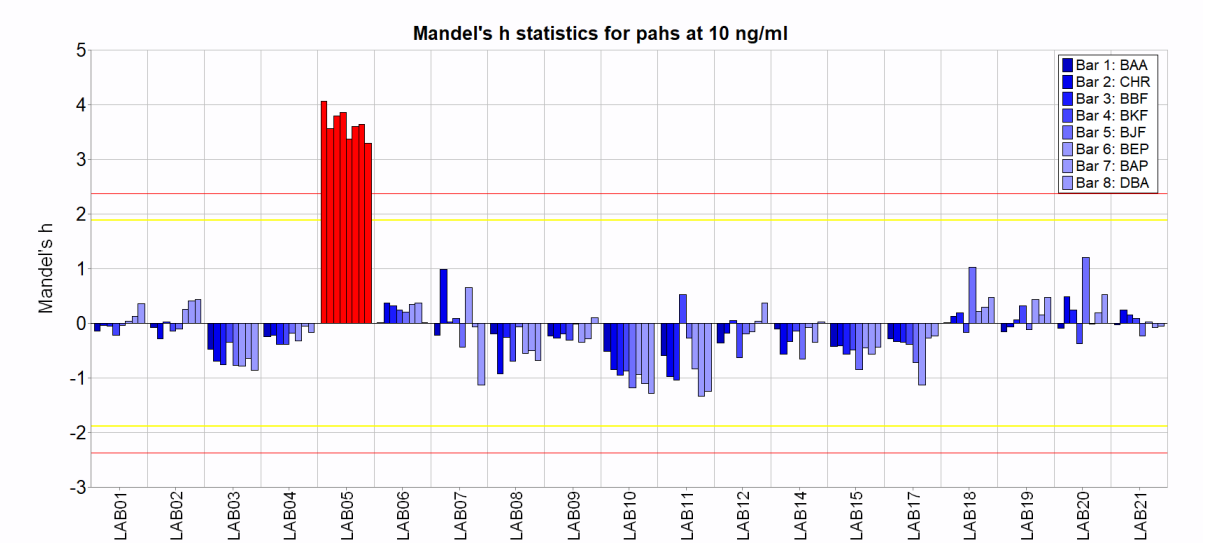




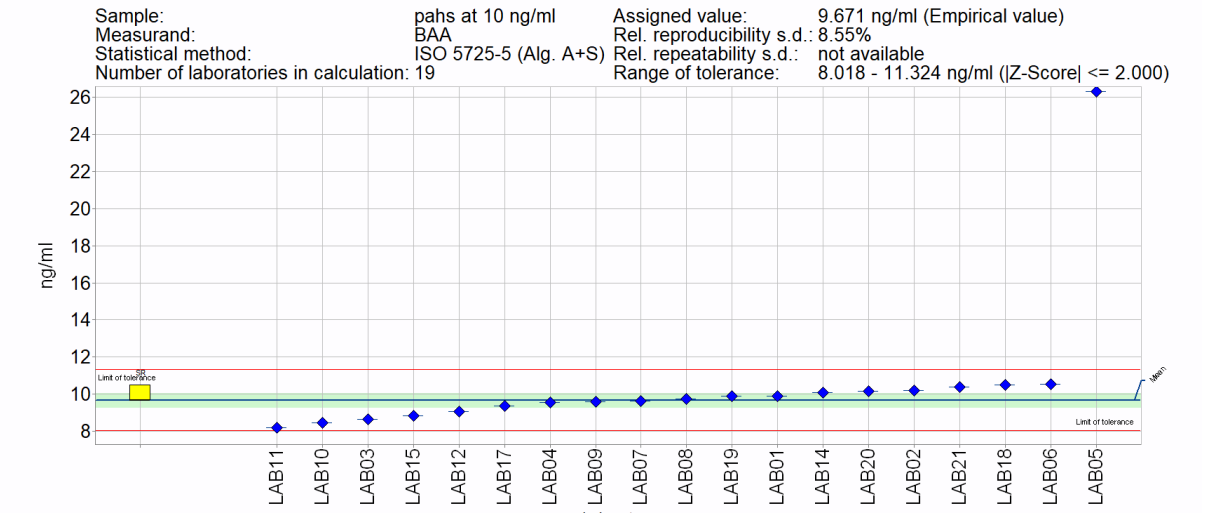


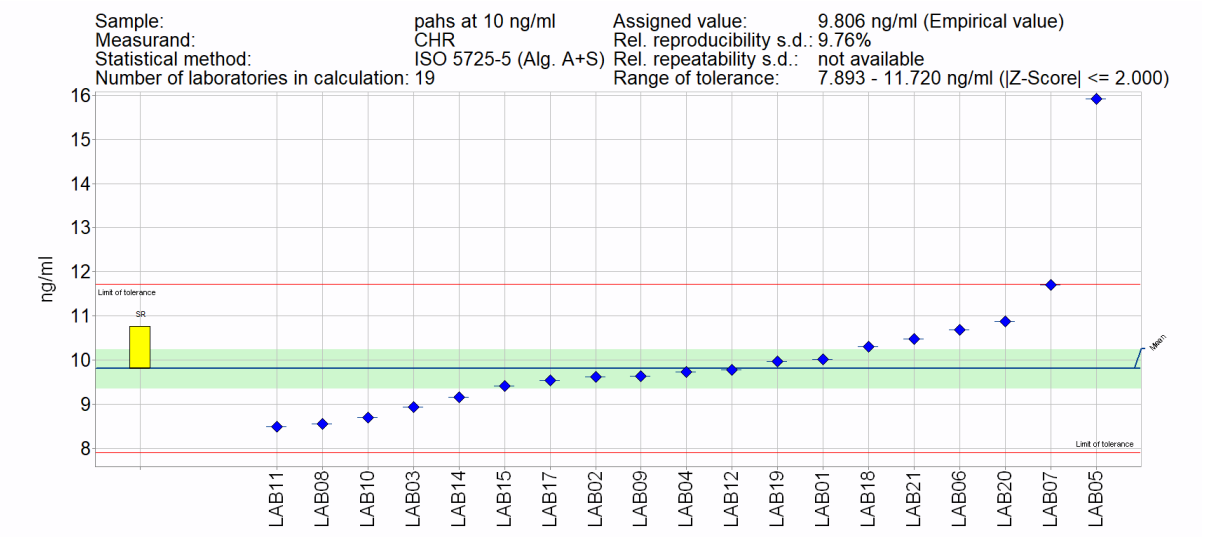


5.7.2 Mandel's h statistics

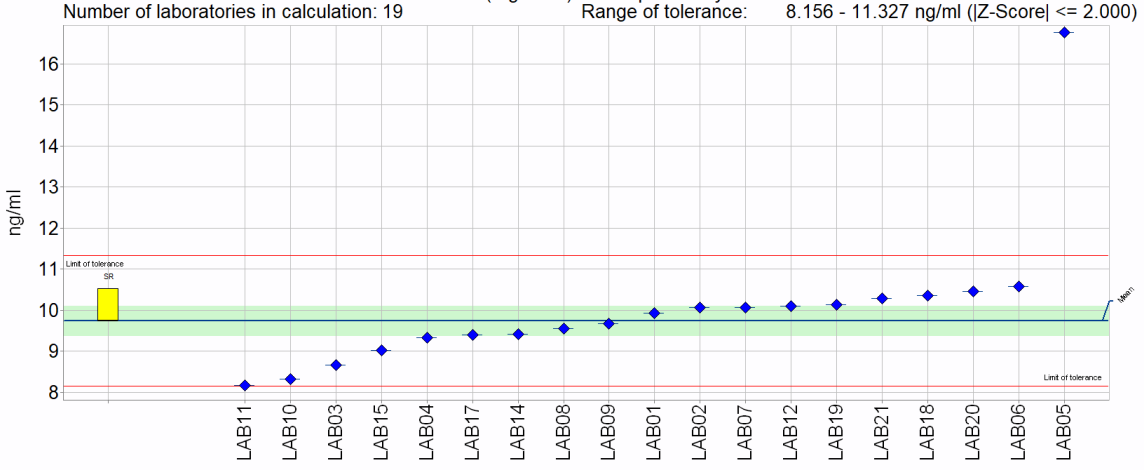


5.7.3 Inter-laboratory study results

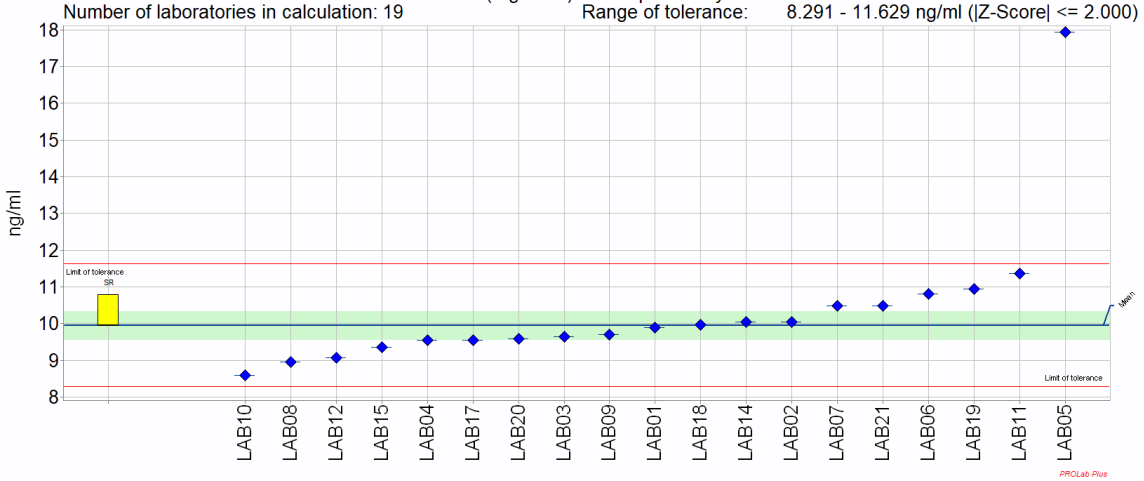




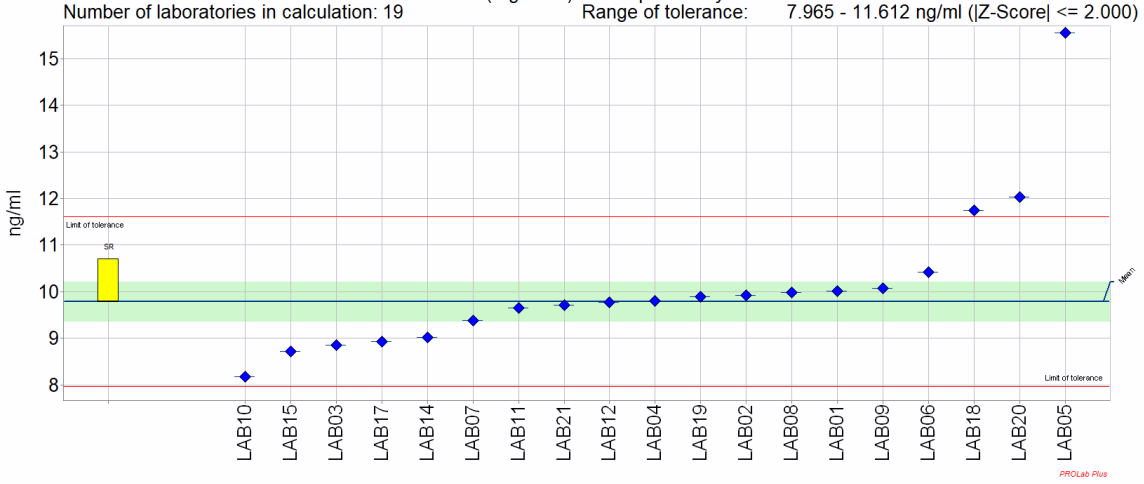
Sample:	pahs at 10 ng/ml	Assigned value:	9.742 ng/ml (Empirical value)
Measurand:	BBF	Rel. reproducibility s.d.:	8.14%
Statistical method:	ISO 5725-5 (Alg. A+S)	Rel. repeatability s.d.:	not available
Number of laboratories in calculation:	19	Range of tolerance:	8.156 - 11.327 ng/ml (|Z-Score| <= 2.000)



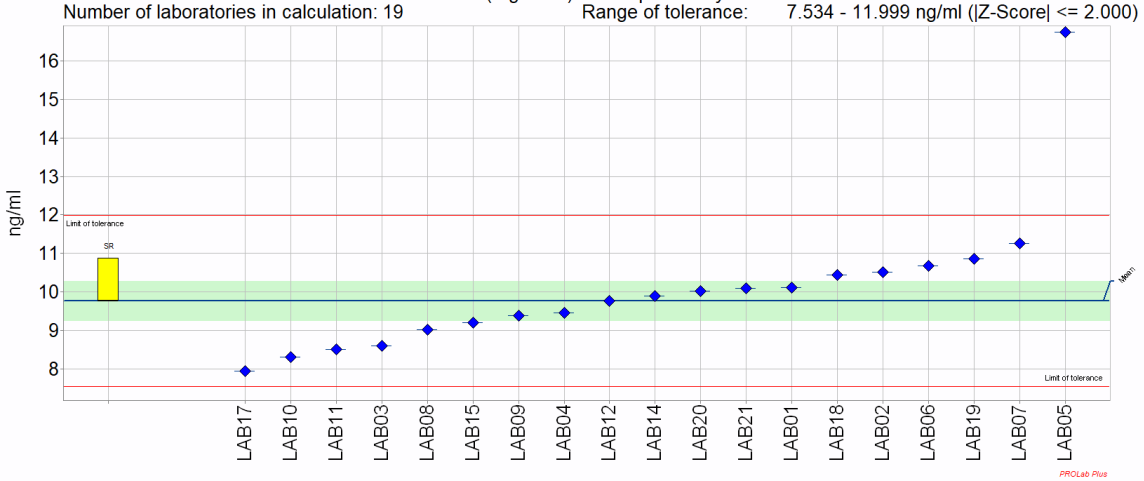
Sample:	pahs at 10 ng/ml	Assigned value:	9.960 ng/ml (Empirical value)
Measurand:	BKF	Rel. reproducibility s.d.:	8.38%
Statistical method:	ISO 5725-5 (Alg. A+S)	Rel. repeatability s.d.:	not available
Number of laboratories in calculation:	19	Range of tolerance:	8.291 - 11.629 ng/ml (|Z-Score| <= 2.000)



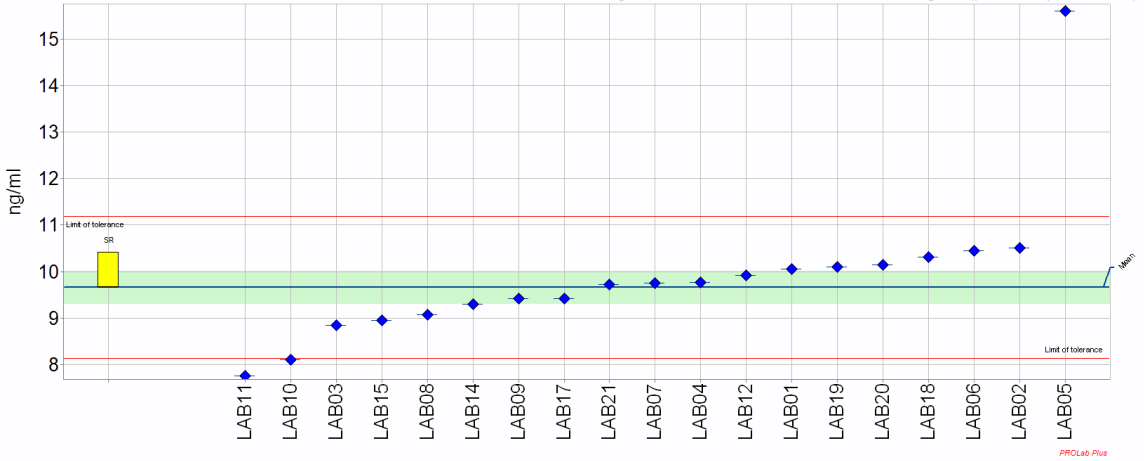
Sample:	pahs at 10 ng/ml	Assigned value:	9.788 ng/ml (Empirical value)
Measurand:	BJF	Rel. reproducibility s.d.:	9.31%
Statistical method:	ISO 5725-5 (Alg. A+S)	Rel. repeatability s.d.:	not available
Number of laboratories in calculation:	19	Range of tolerance:	7.965 - 11.612 ng/ml (|Z-Score| <= 2.000)



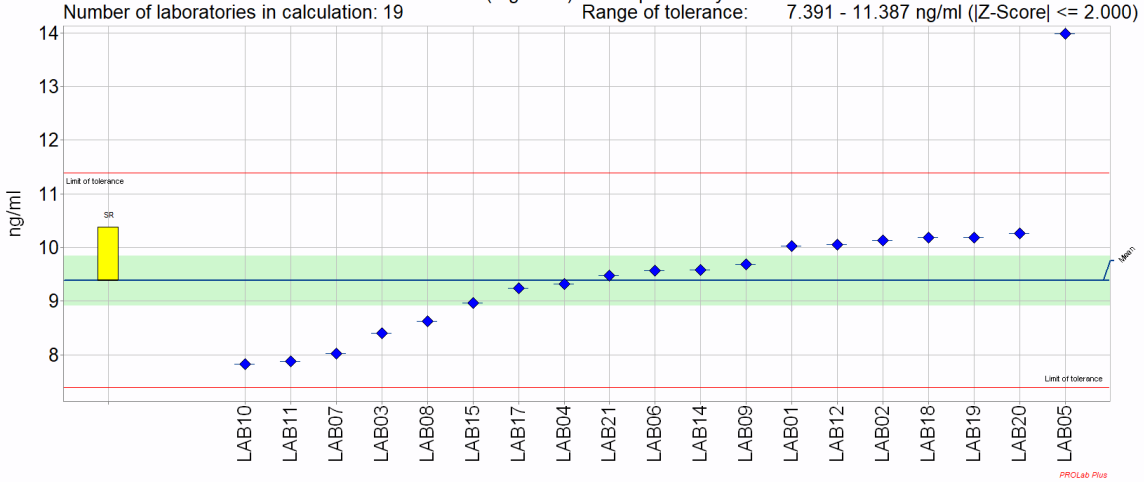
Sample:	pahs at 10 ng/ml	Assigned value:	9.767 ng/ml (Empirical value)
Measurand:	BEP	Rel. reproducibility s.d.:	11.43%
Statistical method:	ISO 5725-5 (Alg. A+S)	Rel. repeatability s.d.:	not available
Number of laboratories in calculation:	19	Range of tolerance:	7.534 - 11.999 ng/ml (|Z-Score| <= 2.000)



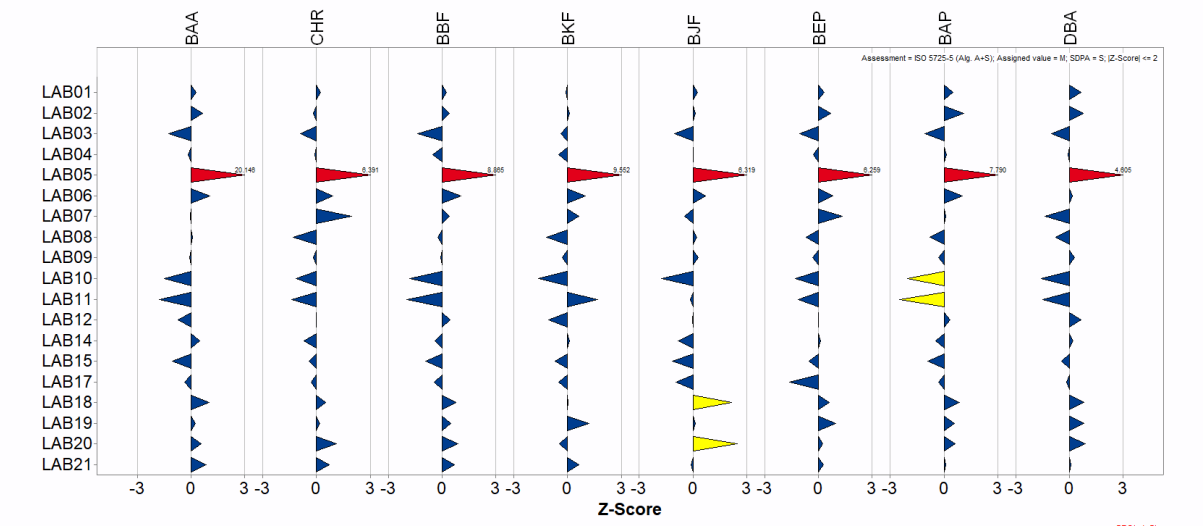
Sample:	pahs at 10 ng/ml	Assigned value:	9.662 ng/ml (Empirical value)
Measurand:	BAP	Rel. reproducibility s.d.:	7.88%
Statistical method:	ISO 5725-5 (Alg. A+S)	Rel. repeatability s.d.:	not available
Number of laboratories in calculation:	19	Range of tolerance:	8.139 - 11.184 ng/ml (|Z-Score| <= 2.000)



Sample:	pahs at 10 ng/ml	Assigned value:	9.389 ng/ml (Empirical value)
Measurand:	DBA	Rel. reproducibility s.d.:	10.64%
Statistical method:	ISO 5725-5 (Alg. A+S)	Rel. repeatability s.d.:	not available
Number of laboratories in calculation:	19	Range of tolerance:	7.391 - 11.387 ng/ml (|Z-Score| <= 2.000)



5.7.4 Z-scores



5.7.5 Table (A): Original values

Table A 10 ng/ml control solution: Original values.

Control solution at 10 ng/ml								
ng/ml	**BAA**	**CHR**	**BBF**	**BKF**	**BJF**	**BEP**	**BAP**	**DBA**
LAB01	9.90	10.01	9.93	9.88	10.02	10.11	10.06	10.03
LAB02	10.19	9.62	10.07	10.05	9.92	10.52	10.51	10.14
LAB03	8.63	8.94	8.66	9.65	8.85	8.59	8.84	8.40
LAB04	9.53	9.72	9.33	9.55	9.80	9.45	9.77	9.32
LAB05	26.32	15.92	16.77	17.93	15.55	16.75	15.59	13.99
LAB06	10.53	10.69	10.58	10.81	10.42	10.67	10.44	9.57
LAB07	9.62	11.70	10.07	10.48	9.38	11.27	9.75	8.02
LAB08	9.74	8.56	9.56	8.96	9.98	9.02	9.06	8.63
LAB09	9.59	9.63	9.67	9.70	10.07	9.39	9.42	9.69
LAB10	8.45	8.69	8.32	8.59	8.17	8.30	8.10	7.82
LAB11	8.19	8.49	8.17	11.36	9.64	8.49	7.76	7.88
LAB12	9.06	9.78	10.11	9.08	9.77	9.75	9.92	10.05
LAB14	10.08	9.15	9.42	10.04	9.02	9.88	9.30	9.58
LAB15	8.81	9.41	9.01	9.36	8.72	9.19	8.95	8.96
LAB17	9.37	9.54	9.40	9.56	8.92	7.95	9.42	9.24
LAB18	10.50	10.30	10.36	9.98	11.74	10.43	10.32	10.18
LAB19	9.89	9.97	10.14	10.95	9.89	10.85	10.10	10.18
LAB20	10.14	10.88	10.45	9.58	12.03	10.01	10.14	10.26
LAB21	10.37	10.47	10.29	10.49	9.70	10.09	9.72	9.48

5.8 Participants' comments

LAB	Comments/Deviation from SOP
LAB 1	None
LAB 2	None
LAB 3	Result for Chrysene is may be not as accurate as expected due to an interference - Recovery from the control sample is a little bit too low for the dibenzo[a,h]anthracene - One sample is missing (2C) due to the SPE purification. It happens a lot that the SPE was clogged during the loading step. - For bbf, bkf and bjf, it seems that if the LOQ of this analytical method was 5 times lower, it should be possible to get more quantified "results"
LAB 4	None
LAB 5	None
LAB 6	Measurements on a routine instrument with a prior cleanup of the source. Data below LOQ was set to zero.The Benzo[e]pyrene value in Material 3 is nearly at the LOQ and was therefore reported.Additional data for LOD/LOQ would be available.Additional Data on 60 m column also available
LAB 7	None
LAB 8	None
LAB 9	None
LAB 10	Most of our results are below the LOQ. Different problems occured during our analysis: 1 -The range of calibration concentrations from 0,5 to25 ng/ml is too big, because of heteroscedasticity. The quantitation in the range of sample concentrations is inaccurately. We would propose the use of standard concentration between 0,5 and 5 ng/ml. 2 -many interferences; the method suffers of insufficient selectivity. Integration of qualifier ions was not possible due to low sensitivity. 3- It might be better to look for another extraction solution with lower polarity. 4-Because of lack of sensitivity, we applied large volume injection (10 µl) instead of 1µl splitless injection.
LAB 11	Raw data also available
LAB 12	Samples/calibrants run also on a different instrument using our usual column and the routine method we normally use for PAHs analysis. The instrument is more sensitive that the one we have used using the column provided and your conditions. With the more sensitive instrument we can detect responses for more PAHs but they were all below the response for the lowest calibrant (and therefore concentrations would only be indicative). Average areas from two replicate injections bracketing samples are provided for calibrants
LAB 13	Very high LOD/LOQ. For some analytes >25 ng/mL
LAB 14	Reported values under LOQ might refer to noise
LAB 15	None
LAB 16	
LAB 17	None
LAB 18	LOD/LOQ given in the SOP were very challenging. In the SOP the lowest calibration level is set to 0,5 ng/ml and on the other side LOQ < 0,5 ng/ml for most of the PAH are requested. According to SOP we had to re-dissolve the dried extract in 1 ml of toluene. We were wondering about this relatively huge amount of solvent. Because, in 2016 we participated in another interlaboratory study (Migration of PAH from rubber samples) organized from our NRL and in their SOP we were asked to re-dissolve the dried extract in only 65 µl of solvent. Using 1 mL of solvent (and therefore having a high dilution factor) is probably the reason why we could hardly detect PAH.
LAB 19	Lab did not perform injection in pulsed splitless mode but in simple splitless mode.
LAB 20	None
LAB 21	None

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