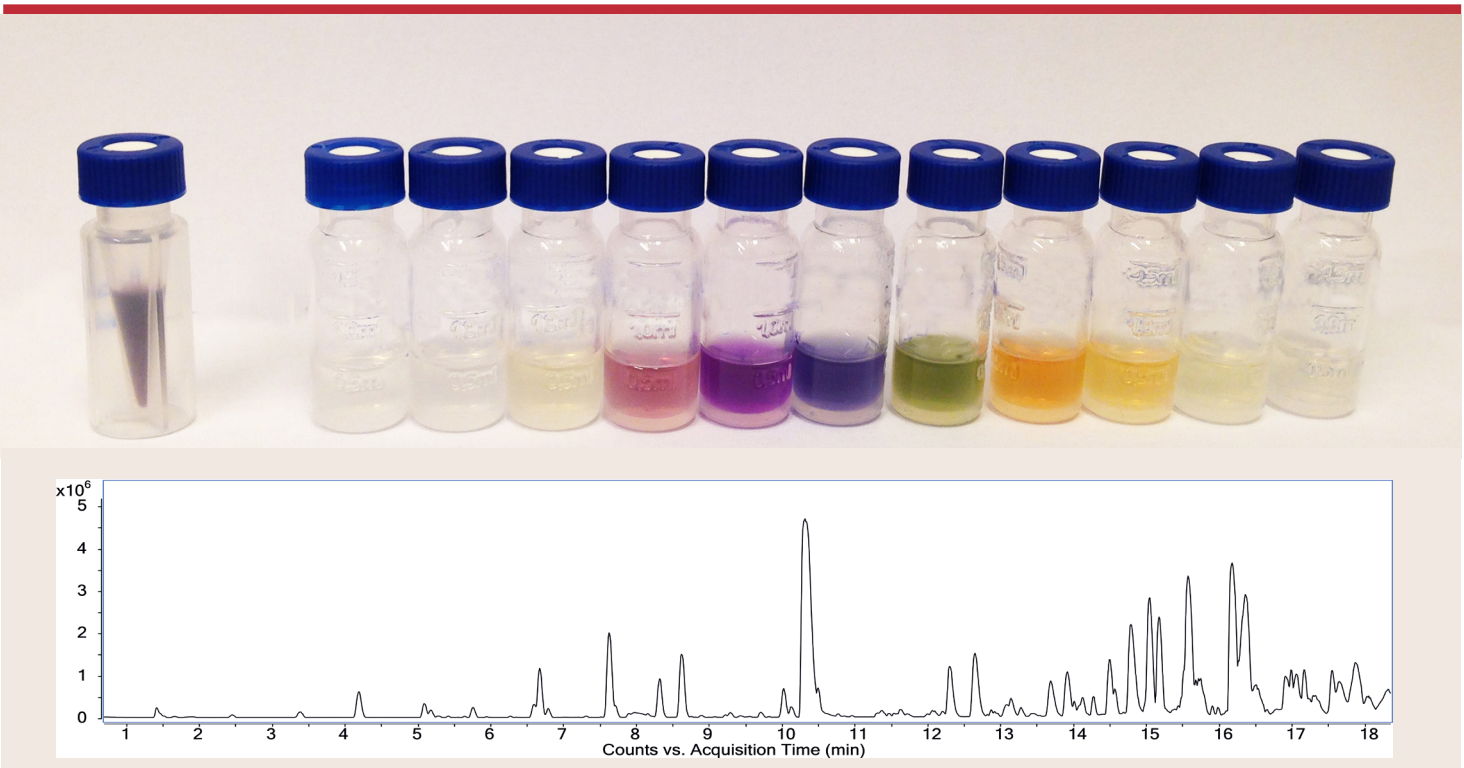
Chemical identification of contaminants in paper and board food contact materials



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Chemical identification of contaminants in paper and board food contact materials

PhD Thesis

Linda Bengtström

Division of Food Chemistry
National Food Institute
Technical University of Denmark

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Safety of food packaging materials - Development of a bioassay guided strategy for the identification of contaminants in paper and board

Author and place of employment

Linda Bengtström
Division of Division of Food Chemistry
National Food Institute
Technical University of Denmark

Supervisors

Senior researcher Jens Højslev Petersen
Division of Division of Food Chemistry
National Food Institute
Technical University of Denmark

Deputy Head of Division, Senior researcher Kit Granby
Division of Division of Food Chemistry
National Food Institute
Technical University of Denmark

Researcher Xenia Trier
Division of Division of Food Chemistry
National Food Institute
Technical University of Denmark

Evaluation committee

Team leader and senior scientist, Dr. Emma Bradley, The Food and Environmental Research Agency, UK
Ass. Professor Bo Svensmark, Department of Plant and Environmental Sciences, Copenhagen University, DK
Senior scientist, Dr. Julie Boberg, National Food Institute, Technical University of Denmark, DK

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Chemistry is like cooking,

just don't lick the spoon.

-Unknown

Preface

The practical work for this PhD has been conducted at The National Food Institute, Technical University of Denmark. In addition, practical work was also performed at the Food and Environmental Research Agency (FERA), UK under the supervision of Dr. Malcolm Driffield. The work presented in this thesis was funded by the Danish Government (Fødevareforlig II). The stay at FERA was generously funded by a grant from the NordFluor network.

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Thanks to all the co-workers at Dept. K (Food Chemistry) for giving me such a happy time during my years at the department as well as being helpful. Thank you to my office mates; Kasper, Anne-Mette and Line for all the good times and for sharing my belief that no question is too silly not to be googled. A shout out for all my PhD peers in the ULF network at the National Food Institute. Thank you for all your cromulent support and for all the laughs.

As part of my PhD project I got the opportunity to visit the Food and Environmental Research Agency (FERA) in York, UK. I had the pleasure of being supervised by Dr. Malcolm Driffield, whom together with his research group introduced me to new techniques, instrumentation and strategies for identification. Everyone was very welcoming and helpful throughout my stay in York. Thanks to Michael Dickinson and Antony Lloyd for helping me acquire and interpret data. Likewise, thanks to Lisa Parker Gomm for being such a sweet landlord in the lovely city of York.

Finally, I want to thank my friends and *la familia*. A special thank you goes to my mother Monica Bengtström, my father Hans Bengtström and my little sister Malin Bengtström. Lastly, I want to thank Jill, my Poison.

København/Malmö (København M)

December 2014

Linda Bengtström

PhD student

Summary

Paper and board are used for a variety of food contact materials, such as baking paper, microwave popcorn bags and packaging for cereals as well as fast foods. Despite this extensive use, there are currently large data gaps about the chemical composition of different paper and board food contact materials and the toxicological effects of these compounds.

The aim of this study was to develop a rationalised interdisciplinary strategy for the screening and identification of compounds with potential adverse health effects in paper and board materials. The first step in the proposed strategy was to develop a comprehensive extraction process that is compatible with both chemical and toxicological analyses. For this purpose, a purge-and-trap method was developed for the collection of small volatile organic compounds; in addition semi- and non-volatile compounds were extracted by a boiling ethanol reflux system.

After an initial *in vitro* screening of 20 different paper and board samples for endocrine disruptive effects, mutagenicity and effects on metabolism of foreign compounds, five samples with adverse effects were selected for fractionation. The fractions were tested in cell assays in a second screening. The fractionation was used to reduce the number of compounds to be identified as well as the matrix effect.

Next, the fractions were analysed by gas chromatography and liquid chromatography coupled to high resolution mass spectrometry. These two techniques were designed to be as complimentary as possible and by them in combination increased the possibility to identify compounds with potential adverse health effects. Several steps in the tentative identification by gas chromatography can be automated, due to the standardisation of this technique that enables searches in vast mass spectral libraries. Such libraries are missing for liquid chromatography, and a large part of the tentative identification must be performed manually. To facilitate the tentative identification by liquid chromatography, an accurate mass database containing approximately 2100 entries of compounds with reported use in paper and board was built. The results from this study indicate that both isotope ratio and hits in the accurate mass database greatly increases the possibility of a correct tentative identification.

After lists of tentatively identified compound had been produced for a certain toxicological assay, compound were selected for further testing based on previously reported effects, structural similarities to known ligands, and availability of analytical standards for identified compounds. Any positive annotation through databases should be regarded as tentative, and therefore analytical standards were used to confirm the identification.

After confirmation, equivalence factors for the initially observed toxicological effect and from all the confirmed compounds tested in the same toxicological assay were calculated. The initially observed effects on the metabolism of xenobiotics could to a minor extent, though not fully, be attributed to dyes used in printing inks. In addition, it was concluded that the endocrine disruptive effects could largely be explained by monomers and plasticisers present in a recycled fibre sample and by sizing agents in virgin fibres.

Resume' (summary in Danish)

Papir og pap anvendes til en række forskellige materialer i kontakt med fødevarer, såsom bagepapir, mikrobølge-popcorns poser og emballager til morgenmadsprodukter og fastfood. Trods den omfattende anvendelse, mangler der viden både om den kemiske sammensætning af forskellige fødevarekontaktmaterialer af pap og papir og de toksikologiske effekter af disse forbindelser.

Formålet med dette PhD-studie var at udvikle en rationel interdisciplinær strategi til screening og identifikation af kemiske stoffer i materialer af pap og papir med potentielt sundhedsskadelige effekter. Det første skridt i vores foreslåede strategi er at udvikle en omfattende ekstraktionsteknik, der er kompatibel med både analytisk kemiske og toksikologiske analyser. Til dette formål har vi udviklet en aktiv prøveudtagningsmetode til opsamling af små flygtige organiske forbindelser. Semi- og ikke-flygtige forbindelser blev ekstraheret i et reflux system med kogende ethanol.

Efter en indledende *in vitro* screenings-runde af 20 forskellige pap- og papirprøver blev fem prøver med positive virkninger udvalgt til fraktionering. Sure og basiske HPLC-fraktioner blev derefter testet i cellebaserede assays i en anden screeningsrunde. Fraktioneringen blev anvendt til at reducere antallet af forbindelser, der skal identificeres, samt at reducere eventuel matrix effekt i den massespektrometriske detektion.

Derefter blev fraktionerne analyseret ved gaskromatografi og væskekromatografi koblet til højtopløseligt massespektrometri. Disse to teknikker blev designet til at være så komplementære som muligt. Ved også at inkludere væskekromatografi i stedet for udelukkende at anvende gaskromatografi, er muligheden for at identificere forbindelser med potentielt sundhedsskadelige effekter styrket. Flere trin i den tentative gaskromatografiske identifikation kan automatiseres og på grund af standardiseringen muliggøres søgninger i store massespektrometriske biblioteker. Disse biblioteker mangler i væskekromatografi, og en stor del af den tentative identifikation skal derfor udføres manuelt. For at lette den tentative identifikation ved væskekromatografi opbyggede vi en database indeholdende præcise masser for ca. 2.100 kemiske forbindelser rapporteret at være forekommende i pap og papir. Resultater fra undersøgelsen viser, at både isotopforholdet og hits for de nøjagtige masser i databasen i høj grad forbedrer muligheden for en tentativ identifikation.

Ud fra en liste af tentativt identificerede forbindelser udarbejdet til de specifikke assays blev udvalgt stoffer til yderligere verifikation af identitet. Denne udvælgelse var baseret på tidligere rapporterede effekter, strukturelle ligheder med kendte ligander, og tilgængeligheden af standarder for identificerede forbindelser, Da enhver positiv identifikation fra databaser bør betragtes som tentativ, blev kromatografisk og massespektrometrisk sammenligning med analytiske standarder anvendt til at verificere identifikationen.

Ækvivalensfaktorer for de oprindeligt observerede toksikologiske effekter og fra summen af alle de verificerede forbindelser fra samme assay blev beregnet. Ud fra disse resultater, konkluderede vi at de oprindeligt observerede effekter på metabolismen af miljøfremmede stoffer kun i mindre grad kunne henføres til tre farvestoffer. De hormonforstyrrende effekter kunne i vid udstrækning kunne forklares ved tilstedeværelse af henholdsvis blødgørere i genbrugsfibre og ved lim anvendt i nye fibre.

List of abbreviations

AA	Abietic acid
AhR	Aryl hydrocarbon receptor
APCI	Atmospheric pressure chemical ionisation
APPI	Atmospheric pressure photoionisation
AR	Androgen receptor
BBP	Benzyl butyl phthalate
BP	Benzophenone
BPA	Bisphenol A
BPB	Bisphenol B
BPC	Base peak chromatogram
BPS	Bisphenol S
CAS	Chemical Abstracts Service
CID	Collision induced dissociation
DBP	Di-butylphthalate
DEHP	Di(2-ethylhexyl)phthalate
DHAA	Dehdyroabietic acid
DIBP	Diisobutyl phthalate
di-PAP	Dialkylated polyfluoroalkyl phosphate surfactant
DMSO	Dimethyl sulfoxide
EDC	Endocrine disruptive compound
EI	Electron ionisation
EQ	Equivalence factor
EQ_{calc}	Calculated equivalence factor
EQ_{meas}	Measured equivalence factor
ER	Estrogen receptor
ESI	Electrospray ionisation
eV	Electronvolt
FCM	Food contact material
GC	Gas chromatographic
GR	Glucocorticoid receptor
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometer
IAS	Intentionally added substance
IP	Identification points
IS	Internal standard
LC	Liquid chromatographic
m/z	mass-to-charge ratio
MFG	Molecular Formula Generator
MP	Methylparaben
MRM	Multiple reaction monitoring
MS	Mass spectrometer
MS/MS	Tandem mass spectrometry

NCI	Negative chemical ionisation
NIAS	Non-intentionally added substance
nrf2	Nuclear factor (erythroid-derived 2)-like 2
nVOC	Non-volatile organic compound
PAPS	Polyfluoroalkyl phosphate surfactants
PCI	Positive chemical ionisation
PFOA	Perfluorooctanoic acid
PFS	Poly- and perfluorinated surfactants
pK_a	Acid dissociation constant
PPARα/γ	Peroxisome proliferator-activated receptors
ppm	Parts per million
QqQ MS	Triple quadrupole mass spectrometer
QSAR	Quantitative structure–activity relationship
qTOF MS	Quadrupole time of flight mass spectrometer
RAR	Retinoic acid receptor
RP	Reverse phase
Rt	Retention time
S/N	Signal to noise ratio
sVOC	Semi-volatile organic compound
TOF MS	Time of flight mass spectrometer
TTC	Threshold of toxicological concern
UHPLC	Ultra high performance liquid chromatography
VOC	Volatile organic compound
vVOC	Very volatile organic compound

List of publications

Papers

Paper 1

Linda Bengtström, Xenia Trier, Kit Granby, Anna Kjerstine Rosenmai & Jens Højslev Petersen (2014) Fractionation of extracts from paper and board food contact materials for *in vitro* screening of toxicity, Food Additives & Contaminants: Part A, 31:7, 1291-1300

Paper 2

Linda Bengtström, Lisbeth Krüger Jensen, Kit Granby , Xenia Trier, Malcolm Driffield, Jens Højslev Petersen (2014) Identification of contaminants in paper and board food contact materials using bioassay guided screening and high resolution mass spectrometry . Manuscript in preparation to be submitted to Analytica Chimica Acta

Paper 3

Rosenmai, A.K., Bengtström, L., van Vugt-Lussenburg, B.M.A., Trier, X., Pedersen, J.H., Granby, K., Taxvig, C., and Vinggaard, A.M. (2014). A strategy to identify problematic chemicals in food contact materials of paper and board. Manuscript in preparation

Paper 4

Linda Bengtström, Jens Højslev Petersen, Kit Granby, Xenia Trier, Mona-Lise Binderup (2014). Identification of unknown mutagenic compounds in microwave popcorn bags. Manuscript in preparation to be submitted to Food and Chemical Toxicology as a Short Communication

Posters

Poster 1

Analysis of migration of polyfluorinated compounds from paper packaging into food matrices.
Linda Bengtström, Mette Regitze von Barner, Gitte Alsing-Pedersen, Kit Granby, Xenia Trier.
Presented at 4th International Nordfluor Workshop (NECC2012), Åbo/Turku, Finland, 2012.

Poster 2

Characterization and biodegradation of two technical mixtures of side-chain fluorinated acryl copolymers.
Christian Eschauzier, Xenia Trier, Linda Bengtström, Tobias Frömel, Pim de Voogt, Thomas P. Knepper
Presented at 4th International Workshop: Per- and Perfluorinated Alkyl Substances, Analysis - Fate - Human Exposure – Regulation, Idstein, Germany, 2012

Poster 3

Extraction method for the collection of volatile organic compounds in paper and cardboard food packaging materials.
Linda Bengtström, Xenia Trier, Kit Granby, Jens Højslev Petersen
Presented at ILSI, 5th International Symposium on Food Packaging, Berlin, Germany 2012

Oral presentations

Oral presentation 1

"Migration of polyfluorinated compounds from food packaging into food products"

Presented at 4th International Nordfluor Workshop (NECC2012), Åbo/Turku, Finland, 2012.

Oral presentation 2

"Bioassay guided identification of chemicals in paper and board food contact materials with toxicological effects"

Upcoming oral presentation, invited speaker at the 4th International Fresenius Conference "Residues of Food Contact Materials in Food", Cologne/Germany, March 2015

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1. Introduction and objectives

Food packaging is advantageous. It protects the food we eat from spoilage by external factors such as physical damage, microbes, light and oxidation. However, foods and beverages can be aggressive towards the packaging. They could for example be acidic, moist, fatty or salty. In addition, some of the foods and beverages are stored while hot in the packaging or before consumption, or stored for an extended time period. Certain properties of the food or the packaging, or the usage of the packaging, could mean that some substances in the packaging material leach into the food or beverage. This leaching, also called migration, of compounds could have a negative impact, both on food quality and on human health. In fact, food packaging has been shown to contribute significantly to human exposure of compounds with an adverse health effect (Grob et al. 2006a).

Paper and board are food packaging materials used for a variety of products, such as cereals and flour, frozen foods, fast foods and fresh produce. Second to plastics, paper and board are the most commonly used food contact materials (FCMs) and it is estimated that around one fifth of all packaging sold annually in the United States is fibre based FCMs (Rexam 2011). Consumers are therefore likely to eat food packed in paper and board FCMs in their everyday life and thus may potentially be exposed to migrating substances with adverse health effects from this source.

Paper and board are natural products with a variation in chemical composition, since the starting material consists of many different organic substances (Roberts 1996a). Furthermore, many types of paper and board are chemically treated with substances to improve particular qualities in the material, such as ability to repel grease or printability (Roberts 1996b). In addition, fibre-based food packaging could also have complex structures comprising several layers with different origins and properties (Roberts 1996a).

In order to assure that the packaging does not endanger human health, the potential for compounds within the materials to migrate, and thus exposure, in amounts high enough to have adverse health effects should be investigated. Due to the chemical complexity of paper and board, many of the substances present are unknown and are therefore also of unknown toxicity. Assessing each individual compound and every possible mixture of compounds from an almost infinite number of chemical mixtures to which humans can be exposed is an impossible task. Several interdisciplinary studies have therefore combined chemistry and toxicology to perform bioassay guided screening of extracts from paper and board FCMs (Bradley et al. 2008; Ozaki et al. 2005; Koster et al. 2014; Vinggaard et al. 2000;

Binderup et al. 2002; Honkalampi-Hämäläinen et al. 2010; Lopez-Espinosa et al. 2007). Chemical analysis cannot in itself give information on the potency of identified compounds to cause adverse health effects. In order to determine the toxicity of a certain compound, there is a need for toxicological data. Combining chemistry with toxicology allows for a screening that excludes samples with no relevance for the investigated toxicological effect early in the process from further investigations.

The hypothesis for this project was that a bioassay guided strategy; combining chemical and toxicological methodologies can be used to identify problematic compounds present in food packaging materials of paper and board. To answer the hypothesis; several milestones for this project were set;

- Development of a comprehensive extraction method compatible with both chemical and toxicological analyses
- Development of a fractionation method for extracts from samples with toxicological response
- Form a co-ordinating overall strategy for a rationalized interdisciplinary process for the detection and identification of compounds with potentially adverse health effects
- Determine the identity and concentration of compounds with toxicological effects

The thesis is structured as follows; a theoretical background for the thesis is presented in Chapter 2 and 3. An overview of the different paper and board materials as well as commonly used additives and the recycling process is presented in Chapter 2. Chapter 3 is a general introduction of the methods used in this study for separation and detection of analytes. A brief overview of the materials and methods used for extraction and identification of genotoxic, endocrine disruptive compounds (EDCs) and compounds with effects in the metabolism of xenobiotics in 20 different fibre-based FCMs is presented in Chapter 4. The results and discussion of the study is presented in Chapter 5. Overall conclusions and future perspectives are presented in Chapter 6 and 7 respectively.

2. Paper and board as food contact materials

This chapter provides a short description of paper and board as materials. The chemical structure of paper, types of pulp, additives in paper and migration will also be discussed.

Since paper making is an old process, there is a tendency not to think of the production of paper as a complicated process. However, this is far from the truth as modern paper mills and paper products might be highly sophisticated and specialised. Additionally, many types of paper and board are chemically treated, during as well as after production, with substances to improve certain material properties, such as water-impermeability or printability (Schaffrath & Tillmann 2013). Fibre-based food packaging could also have complex structures comprising of numerous layers with different origins and properties glued together by adhesives (Roberts 1996a).

Unlike plastic FCMs, fibre-based FCMs are not subject to any specific regulation by the European Union (EFSA 2012). However, like all FCMs in the European market, paper and board must meet the general demands described in the regulation of EC No 1935/2004 (2004), specifically:

"Materials and articles … shall be manufactured in compliance with good manufacturing practice so that, under normal or foreseeable conditions of use, they do not transfer their constituents to food in quantities which could endanger human health; …."

The demand cited above is stricter than for example food manufacturing processes in general or cooking, since the exposure of chemicals from packaging is considered avoidable (Grob 2014). It is the responsibility of the packaging industry to follow the good manufacturing practice described in the framework. However, there is currently a gap between legislation and reality, as many of the compounds in paper and board FCMs have not been toxicologically evaluated, meaning that a comprehensive safety assurance is not possible (Grob 2014).

The fibres from which paper are made can be derived from a variety of plant sources. Therefore the definition of paper is broad; it is a sheet material made up from a network of natural cellulosic fibres deposited from an aqueous suspension, see Figure 1 (Schaffrath & Tillmann 2013). The current primary source of cellulose fibre is wood (Roberts 1996b).

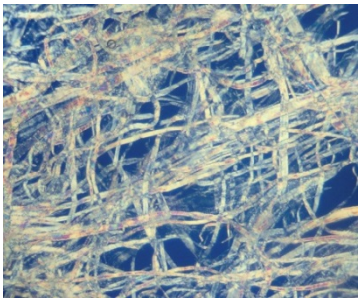


Figure 1. Structure of cellulose fibres in tissue paper at 200x magnification. Notable is the network of natural cellulose fibres.

Wood is a complex and non-homogenous material, with a natural chemical variability (Sjöström & Alen 1998). It is built-up by many different types of specialised cells, ensuring stability, metabolism and water supply (Hopkins & Hüner 2004; Nobel 2009). There are two types of wood mostly used for paper making; softwood and hardwood. Softwood, for example conifers such as spruce, has a long fibre-length, which contributes to the strength of the paper and is therefore used in greater quantities in paper making than hardwood (Heinemann 2013). However, the shorter fibre length of hardwood such as oaks and birches, is important for assisting in the formation of the paper sheet (Roberts 1996b).

2.1 Chemical structure of wood

The chemical composition of the finished paper product will vary greatly depending on the chemical treatment of the wood during the pulping process (Schaffrath & Tillmann 2013). However, there are four major constituents in common for all wood relevant in paper making; cellulose, hemi-cellulose, lignin and extractives such as rosin.

Cellulose is the primary structural component of the cell wall, and after the delignification process, it is also the primary component of paper (Roberts 1996b). Cellulose is a linear polymer made out of β-D linked glucose (Hopkins & Hüner 2004; Sjöström & Alen 1998), see Figure 2. In most wood species used for paper making, the cellulose content in the cell wall is around 40% to 45% (Sjöström & Alen 1998). A single cellulose chain can contain as many as 3000 or more glucose units (Hopkins & Hüner 2004).

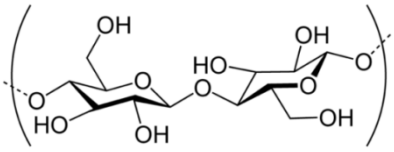


Figure 2. Molecular structure of a cellulose polymer. About 50 of these polymer chains are organized parallel into one microfibril, the basic unit of the cell walls.

Lignin, a highly complex polymer of relatively simple phenolic alcohols is the second most abundant class of organic molecules in the cell wall (Nobel 2009). The presence of lignin in the ready paper product is highly undesirable (Roberts 1996b). It causes paper to become frail and, through oxidation, causes yellowing and discolouration of the paper (Heinemann 2013). In high quality paper most of the lignin has been removed.

Hemi-cellulose is a heterogeneous and partly uncharacterised mixture of polysaccharides in the cell wall, their only shared trait is their extractability in strong alkaline solutions (Hopkins & Hüner 2004). The most common components of hemi-cellulose are; glucose, galactose, mannose, xylose, arabinose, gluconic acid. Some findings suggest that the hemi-cellulose assists in inter-fibre bonding or contributes in the swelling of the pulp and enhances the plasticity of the wet fibres during sheet formation (Roberts 1996b).

A small proportion of the wood, often between 2-5%, consists of so-called extractives (Roberts 1996b). This loose term includes lipophilic compounds extractable by organic solvents, such as ethanol or dichloromethane, and comprises varying compound classes such as alkanes, fatty acids, resin acids, terpenes and phenolic components (Sjöström & Alen 1998). Some of these substances are removed during the pulping process, although some may still be retained in the final paper product (Roberts 1996b). The resin acids found among the extractives are isomers of, or closely related, to abietic acid (AA), such as dehdyroabietic acid (DHAA), see Figure 3. The resin acids are also of importance in the paper making process, as they are used as anti-slip and sizing agents during the wet-end chemistry part of the pulping process or as varnishes to improve pigment wetting during printing (Roberts 1996a; Leach & Pierce 1993).

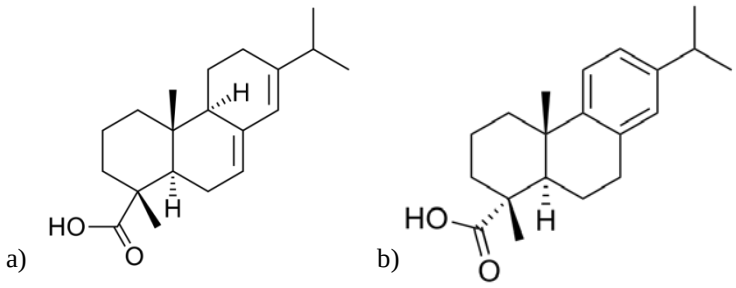


Figure 3. Structure of a) abietic acid (AA) and b) dehdyroabietic acid (DHAA), two of the most abundant substances of wood rosin.

2.2 Types of pulp

Pulp is defined as the mechanically or chemically separated fibrous material of wood, or isolated strands of fibres, dispersed in water (Biermann 1993; Heinemann 2013). The aim of the pulping process is to remove lignin, while the cellulose and hemicelluloses remain in the pulp (Sjöström & Alen 1998). The four types of pulps described in this section are the ones most relevant for manufacturing paper and board FCMs. The delignification improves the reactivity of the remaining polysaccharides, and the paper strength is thus improved due to the increase in crosslinking (Harmsen et al. 2010). The conditions for chemical pulping, by alkaline, neutral or acidic conditions, are so severe that carbohydrates are degraded (Roberts 1996a). This causes a reduction in both paper strength and yield (Heinemann 2013).

Mechanical pulp is produced by using mechanical grinding of the wood by using only water or water steam (Biermann 1993). Since there is no delignification process or other chemical treatment to the pulp, the finished paper product will contain the same chemical constituents as the raw wood material. Due to the relatively high lignin content, and thus a low paper quality, the use of mechanical pulp is limited to newspaper and paperboard (Biermann 1993).

Acidic sulphite pulping uses sulphur dioxide and/or alkali salts of sulphur oxide for delignification (Biermann 1993). One of the advantages of sulphite pulp is the relatively smaller pores formed in the finished paper product, causing the paper to hold more water than Kraft paper, a property which is useful for grease proofed paper (Biermann 1993).

Kraft pulping is the most commonly used method for pulp production in the world, including the production of paper and board food contact material (Roberts 1996b). The delignification process is done in a strong alkaline solution using sodium hydroxide and sodium sulphide (Harmsen et al. 2010). However, as this method also dissolves some of the hemi-cellulose, Kraft pulping results in a lower yield than for instance sulphite pulping (Heinemann 2013). Some of the extractives, such as AA and DHAA, are collected as by-products during the Kraft pulping process (Sjöström & Alen 1998).

Chemi-mechanical pulp can be viewed as a hybrid between the mechanical pulping and the chemical pulping processes. In short, a relatively mild chemical treatment is followed by an abrasive mechanical treatment, producing a pulp with improved printing abilities and strength when wet (Biermann 1993). Since almost no lignin is removed from the pulp, this method is most suitable for wood with low lignin content (Harmsen et al. 2010).

2.3 Additives and contaminants in paper

Additives in paper can be introduced in many of the steps during the papermaking process, such as during sheet formation or printing process. There are three types of paper products most relevant for paper and board FCMs; paper, paperboard and cardboard. Out of these three, paperboard is the most structurally complex, and could comprise of several layers of mixed origins. Some of the major sources of additives, or intentionally added substances (IAS), found in paper and board are constituents in printing inks, adhesives, sizing agents and coatings (Muncke 2011). Furthermore, non-intentionally added substances (NIAS), impurities from the manufacturing process and degradation products, also contribute to the overall content of substances in paper and board (Nerin et al. 2013).

Several other studies have previously reported the presence and migration of compounds, both IAS and NIAS, with mutagenic or ED effects in paper and board FCMs (Rosenmai et al. 2013; Begley et al. 2005; Castle et al. 1997; Koster et al. 2014; Ozaki et al. 2004; Honkalampi-Hämäläinen et al. 2010; Kirchnawy et al. 2014; Mertl et al. 2014). In addition, as recycled paper contains more contaminants than virgin paper this paper type poses a larger risk for migration of compounds with an adverse health effect (Binderup et al. 2002; Triantafyllou et al. 2005; Vinggaard et al. 2000; N. A. Suciu et al. 2013; Biedermann & Grob 2010).

The traditional toxicological methodology for safety assurance is to individually assess compound for effects. However, this strategy may risk an underestimation of human exposure as the real exposure is a multicomponent chemical mixture (Backhaus & Faust 2012). In addition, assessing each individual compound and every possible mixture of compounds from an almost infinite number of chemical mixtures to which humans can be exposed as well as the endless possibilities for modes of action of the compounds in the mixture is an impossible task (Hadrup 2014).

Recently, a more comprehensive approach of toxicological evaluation of different mixtures of compounds have demonstrated *in vitro* and *in vivo* effects (Charles & Darbre 2013; Smith et al. 2013; Silva et al. 2011; Axelstad et al. 2014; Krüger et al. 2008). In addition, when investigating certain EDCs in mixtures, such as pesticides and BPA in low doses, the observed effects of the mixture were considerably higher than when the compounds were tested individually (Silva et al. 2002; Hass et al. 2012).

Consumers are generally exposed to low levels of compounds migrating from FCM through their entire lives. In addition, there are also several other sources of the cumulative intake of compounds with

potentially adverse health effects, such as industrial chemicals, pesticides and environmental contaminants that humans could be exposed to simultaneously (Hass et al. 2012). However, food packaging have been shown to contribute significantly to human exposure of compounds with an adverse health effect (Grob et al. 2006a).

2.3.1 Sizing agents and surface coatings

The surface of dried paper after pulping is too rough and porous to be suitable for printing. Paper and board are therefore surface sized by the application of a water-soluble polymer, such as starch or a cellulose derivate, to enhance the printing properties (Sangl et al. 2013). Sizing agents in paper and board are added to change the absorption and/or frailness of the finished product (Thorn & Au 2009). Nowadays, the general trend towards neutral and alkaline papermaking means that the majority of sizing agents used for neutral conditions are cationic rosin sizes, such as AA and DHAA see Figure 3, and alkyl ketene dimer or alkyl succinic anhydride sizes for alkaline conditions (Thorn & Au 2009; Roberts 1996b). Derivatives from AA are also used in alcohol-based lacquers (see Section 2.3.3) for printing as well as in adhesives (see Section 2.3.4) in multi-layer paper and board packaging (Aznar et al. 2011; Ozaki et al. 2005; Leach & Pierce 1993). Some studies have identified the resin acids AA and DHAA in recycled fibre-based packaging as genotoxic (Ozaki et al. 2005; Ozaki et al. 2006).

Another group of compounds used for surface coatings are bisphenols, such as bisphenol A (BPA). In addition to surface coatings, BPA is used in epoxy resin based paints, adhesives, monomers in polycarbonate plastic, printing inks, carbonless and thermal paper and resin-based composites (EFSA 2006). Moreover, BPA, see Figure 4, has been found in recycled paper used for FCMs (Triantafyllou et al. 2002; Vinggaard et al. 2000; N. A. Suciu et al. 2013). BPA has been extensively investigated for toxicological effects and has for example showed an array of ED effects such as disturbed mammary gland development (Moral et al. 2008), changes to lipid metabolism (Seidlová-Wuttke et al. 2005) and changes in behaviour (Xu et al. 2011) in rodents.

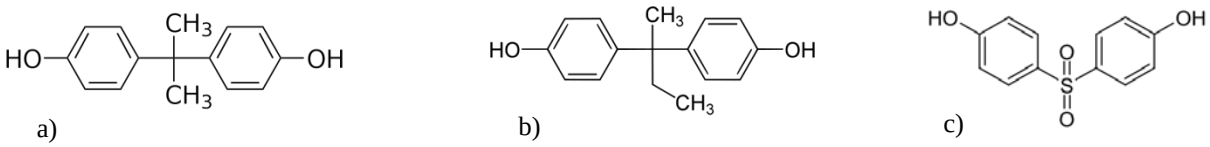


Figure 4. Molecular structure of a) bisphenol A (BPA), b) bisphenol B (BPB) and c) bisphenol S (BPS).

In addition, BPA is well known for its estrogenic activity by binding to the Estrogen receptor (ER) (Gould et al. 1998; Grignard et al. 2012) as well as its androgen receptor (AR) antagonism (Kitamura et al. 2005; Vinggaard et al. 2008). Human health effects linked to BPA have been examined, and although there are still large knowledge gaps, it appears that BPA influence multiple endocrine-related pathways in complex modes of action (Bondesson et al. 2009; Rubin 2011). Due to these recent findings, there have been efforts to phase out BPA from certain FCMs, mainly from plastic baby bottles (European Comission 2011). The industry has therefore started to substitute BPA with other bisphenol analogues such as bisphenol S (BPS) and bisphenol B (BPB) (Viñas et al. 2010; Grumetto et al. 2008), see Figure 4. However, BPS and BPB are also associated with endocrine disruptive effects (Rosenmai et al. 2014).

2.3.2 Greaseproof paper

The most important functional property of greaseproof papers is their resistance to grease, fat and oil (Kuusipalo 2003). Greaseproof paper is mainly used for baking paper and baking moulds. There are two types of greaseproof paper products, either through mechanical processing or surface coating. The distinct properties of mechanically processed greaseproof paper are mainly due to the high degree of beating which creates a large fibrous network, which in turn creates a paper of high density (Aulin 2007). Cellulose is impermeable to fat, therefore a surface layer of high density paper blocks the grease from penetrating further into the material (Kjellgren 2007).

The level of mechanical beating needed to achieve the high density necessary for greaseproof paper is associated with high costs (Aulin 2007). Adding fluorochemicals, such as fluorosurfactants, directly to the pulp or as a coating is a cheaper way of making the paper repellent towards oils and fats (Kissa 2002). The migration of covalently bound fluorochemicals in covalently bound coatings is significantly smaller than when unbound in the pulp (Dinglasan-Panlilio & Mabury 2006). Poly- and perfluorinated surfactants (PFS) have exceptional properties such as being both water and oil repellent and staying unaffected by high temperatures and other chemicals (Kissa 2002). They are used in the food packaging industry as cheaper options to mechanically greaseproof papers. However, PFS migrate from paper and board into foods (Lau et al. 2007; Begley et al. 2005; Begley et al. 2008; Trudel et al. 2008; Trier et al. 2011).

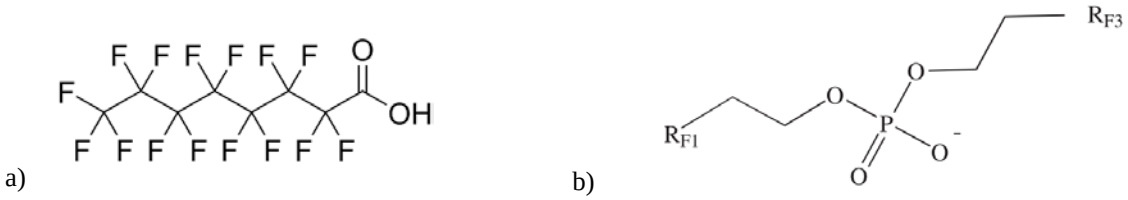


Figure 5. Molecular structure of a) perfluorooctanoic acid (PFOA) and b) dialkylated polyfluoroalkyl phosphate surfactants (diPAPS) (Rosenmai et al. 2013)

Some of the PFS, such as perfluorooctanoic acid (PFOA) and dialkylated polyfluoroalkyl phosphate surfactants (diPAPS), see Figure 5, are categorised as developmental toxicants and are suspected of for example ED effects in both rodents and humans (Rosenmai et al. 2013; Jensen & Leffers 2008; Joensen et al. 2009; Philo et al. 1994). The alternative chemicals that recently emerged on the market, mainly polyfluoroalkyl phosphate surfactants (PAPS), also show ED effects (Rosenmai et al. 2013)

2.3.3 Dyes, printing inks and lacquers

Most paper and board packaging materials are printed with a technique that requires low viscosity water or solvent based inks (Leach & Pierce 1993). The ink is dried by solvent evaporation, leaving a dry film of ink and resin on the surface (Leach & Pierce 1993). Dyes and pigments used for printing FCMs usually have complex compositions and structures, and are not easily classified (Barnes et al. 2007). However, inks for food packaging are based on substances with no odour or off-set, such as aromatic-free solvents and maleic resins (Barnes et al. 2007).

Some of the most widely used types of dyes for printing FCMs are basic dyes and solvent dyes (Leach & Pierce 1993). Basic dyes are soluble in water and ethanol, but have a poor solubility in other organic solvents. Examples of cationic basic dyes are Baso Red 546 and Basic Red 1 (Rhodamine B Base), see Figure 6. Solvent dyes can come from several different groups of compounds, with only the shared trait of being soluble in organic solvents (Leach & Pierce 1993). One example of a solvent dye is Solvent Violet 8, see Figure 6.

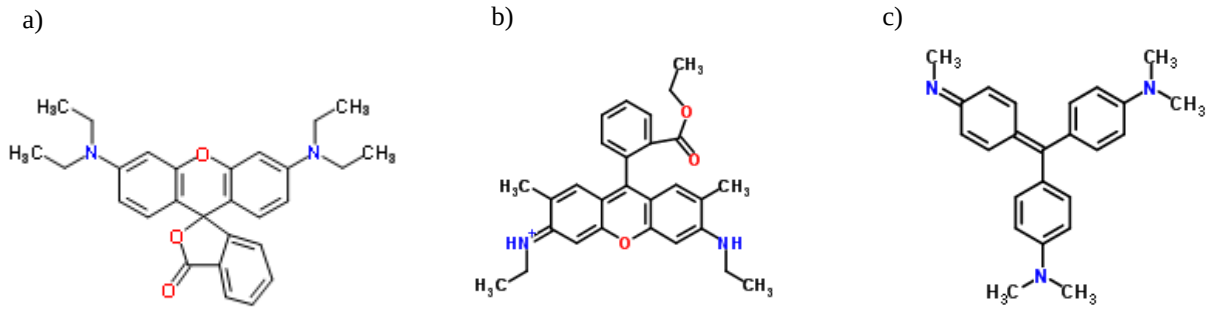


Figure 6. Molecular structures of a) Baso Red 546; b) Basic Red 1 and c) Solvent Violet 8. (From ChemSpider).

Phthalates, one of the most common groups of compounds present in food packaging, are used in printing inks, lacquers and adhesives and are regularly found in recycled paper and board (Poças et al. 2010; Fasano et al. 2012; N. a Suciu et al. 2013). The major source for human exposure for some of the phthalates, such as di-butylphthalate (DBP) and di(2-ethylhexyl)phthalate (DEHP), see Figure 7, is food (Fromme et al. 2007; Wormuth et al. 2006; Cirillo et al. 2011). These are also the most common phthalates in paper and board packaging (Vinggaard et al. 2000). Furthermore, phthalates migrates through paper and board packaging and contaminates both fatty and non-fatty foods (Gärtner et al. 2009).

DEHP and DBP have shown ED effects in rodents, with indications that females are more severely affected than males (Kavlock et al. 2002; Kavlock et al. 2002; Seidlová-Wuttke et al. 2005). Metabolites of DEHP and other phthalates have been found in urine of both adults and children (Frederiksen et al. 2010). In addition, there are evidence that phthalates reduce the activity of several lipid metabolism pathways (Johnson et al. 2011).

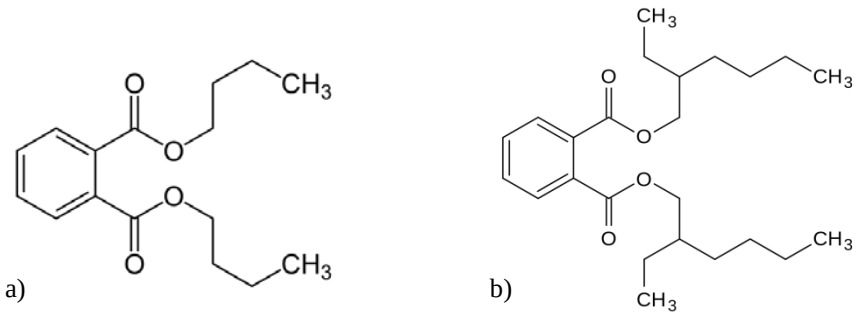


Figure 7. Molecular structure of a) di-butylphthalate (DBP) and b) di(2-ethylhexyl)phthalate (DEHP)

Some packaging is printed with photo-initiators added to the ink to facilitate a rapid drying process (Leach & Pierce 1993). For example, UV-cure inks and lacquers typically contain 5–10% photo-initiator (Anderson & Castle 2003). Although there are several photo-initiators available, the most commonly used is benzophenone (BP), see Figure 8, (Anderson & Castle 2003). BP is also present in recycled paper and has been found to migrate from the paper and board matrix into food (Anderson & Castle 2003; Jickells et al. 2005). However, studies on the ED effects of BP are inconsistent. For instance, no estrogenicity was observed in an uterotrophic assay and in an ER assay in one study (Yamasaki et al. 2002). Conversely, other studies on BP found a small activity in an ER assay and a significant anti-androgenic activity in another assay (Suzuki et al. 2005).

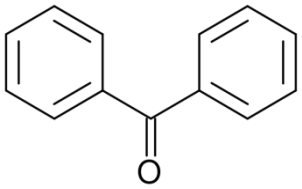


Figure 8. Molecular structure of benzophenone

2.3.4 Adhesives

Adhesives used for paper and board packaging are often complex formulations of adhesive components and modifying substances that have specialised functions (Canellas et al. 2010). The modifying substance could for instance function as base resin or binder, hardener, inhibitor, solvent, thickener, filler, carrier, plasticiser, flexibiliser, tackifier, film former, antioxidant, surfactant and wetting agent (Canellas et al. 2010). Some of the different groups of adhesives used in paper and board packaging are epoxy, isocyanate, epoxyhybrid, acrylic and cyanoacrylic adhesives (Petrie 2007).

2.3.5 Dispersants

Most paper and board materials undergo a de-inking process during recycling. An efficient de-inking process is expected to reduce ink and adhesive residue and other contaminant concentrations (Hubbe et al. 2007). However, some of the chemicals used for the de-inking process are likely to remain in the fibre and thus within the recycled paper product (Hubbe et al. 2007). The two most common methods for de-inking are ink washing and ink flotation. As the name implies, ink washing involves de-inking by simply washing the fibres (McKinney 1995). This technique uses sodium hydroxide, sodium silicate or hydrogen peroxide in combination with a dispersant, for example stearic acid, to remove ink from the pulp (McKinney 1995). Ink flotation separates the materials in the pulp based on their wetability, where

hydrophobic compounds adhere to air bubbles that rises to the water surface (Biermann 1993). Adhesive particles adhere to the fibres and are therefore particularly difficult to remove during the washing steps (Roberts 1996a).

2.4 Migration

Migration is a collection name for different processes where compounds transfer from the FCM into the food. The migration of compounds present in paper and board, such as sizing agents, dyes and lacquers, was not investigated in this study. Yet migration is important to mention as this process is directly linked to human exposure.

There are two types of migration described for paper and board FCMs; direct contact or mass transfer in air (Muncke 2011; Barnes et al. 2007; Johns et al. 2000a; Grob et al. 2006b). Porous materials such as paper and board offer little resistance towards the mass transfer of migrating compounds, see Figure 9, thus migration occurs regardless of direct contact with the foodstuff (Barnes et al. 2007; Bradley et al. 2005). The kinetics of migration from paper and board can be affected by properties in the packaging material, of the food as well as storage and usage conditions (Arvanitoyannis & Bosnea 2004; Triantafyllou et al. 2007a). In comparison to plastics, the migration from paper and board depends on additional mechanisms of diffusion controlled migration through a liquid (polymer) phase, including transport to and through the vapour phase and complex adsorption/desorption processes to fibres, coatings, printing inks and fillers at the material surface (Zülch & Piringer 2010). Migration through the vapour phase is decreased by a small pore size in the material, a compact material, thus the inter- and intermolecular bonds of cellulose, and a thick material (Roberts 1996b; Triantafyllou et al. 2007b). The application of coatings and polymers, that forms a dense layer, will also decrease the migration rate (Roberts 1996b).

Fatty and aqueous food also enhances migrations rates due to their absorption properties (Triantafyllou et al. 2007a; Binderup et al. 2002; Taylor et al. 2010; Vitrac et al. 2007; Begley et al. 2008). Interactions between the paper matrix and compounds increase with polarity, meaning that the migration rate decreases for polar compounds due to the hydroxyl-groups in the paper matrix (Zülch & Piringer 2010). In addition, migration through the vapour phase for volatiles is also affected by the pore size and the compactness of the material, including the inter- and intermolecular bonds of cellulose (Roberts 1996a).

Finally, storage conditions and modes of usage of the packaging also play an important role in migration rate (Barnkob & Petersen 2013; Johns et al. 2000; Anderson & Castle 2003). Even at low

temperatures, down to -20 °C, volatile organic compounds (VOCs) are able to migrate into food during extended storage times (Johns et al. 2000). However, migration of certain compounds, such as diisobutyl phthalate (DIBP) and BP, can also be decreased by using plastic or aluminium foil secondary packaging (Gärtner et al. 2009; Jickells et al. 2005).

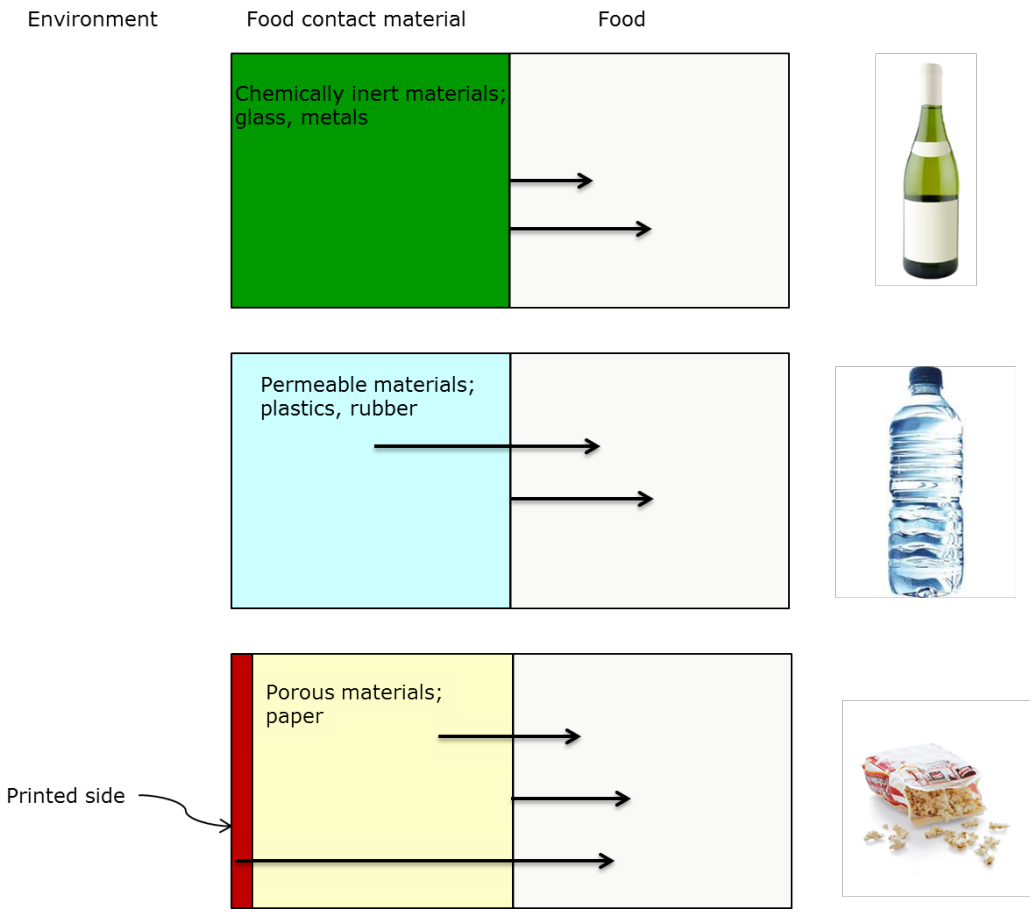


Figure 9. Migration processes for different packaging materials; glass or metal, plastic and paper. Porous materials such as paper and board offer almost no resistance towards mass transfer, allowing substances to migrate all the way through the material. Adapted from Barnes et al., 2007.

3. Principles of separation and detection

This chapter includes a brief introduction to mass spectrometry and a short description of the different methods used in this thesis for the qualitative and quantitative analyses of chemical contaminants. The fragmentation of compounds with different ionisation techniques will also be discussed.

3.1 Chromatographic separation and ionisation

3.1.1 Chromatographic separation

The aim of a chromatographic separation is to isolate analytes in order to avoid all compounds to elute all at once, leading to a cleaner spectrum and higher detector sensitivity (Croley et al. 2012; de Hoffman & Stroobant 2007). In addition, chromatographic separation reduces matrix effects, ion suppression or ion enhancement, thus when compounds interfere with detector response (Trufelli et al. 2011; Marchi et al. 2010). Matrix effects are dependent on both analyte and matrix properties (Marchi et al. 2010), as well as ionisation source design (Stahnke et al. 2012). Often, to compensate for matrix effects as well as other analytical variations, structural analogues with or without stable isotope labelling are used as internal standard (IS) (Stokvis et al. 2005).

There were two types of chromatographic separation techniques used in this project; gas chromatography (GC) and liquid chromatography (LC). Generally, GC is used for small and relatively non-polar volatile organic compounds (VOCs) and semi-volatile organic compounds (sVOCs) while LC is used for larger and polar or intermediate polar sVOCs and non-volatile organic compounds (nVOCs). GC have more favourable kinetic properties than LC, due to a larger number of theoretical plates in the column, meaning a more efficient separation (Poole 2003). However, LC offers more selectivity optimisation due to a larger variety of mobile phase compositions and solid phase alternatives in columns (Poole 2003).

3.1.2 Ionisation in mass spectrometry

Even though there are several different mass spectrometry (MS) techniques they all involve three steps; 1) analyte ionisation, 2) analyte isolation according to their mass-to-charge ratio (m/z) and 3) analyte detection.

Electron ionisation (EI) is mainly used in GC applications, as the analytes are already in gas phase when entering the MS. Due to the standardised ionisation conditions at 70 electronvolt (eV), EI produces highly reproducible fragmentation spectra that not only allows for valuable structural information of

the analytes to be obtained but also allows for the establishment of vast spectral libraries used for qualitative analyses (Portolés et al. 2011). However, due to the severe in-source fragmentation of EI at 70 eV, little or none of the quasi-molecular ion can be visualised (de Hoffman & Stroobant 2007). This severe fragmentation could to some level be avoided by using other softer ionisation techniques, such as positive and negative chemical ionisation (PCI and NCI respectively). However, these ionisation modes causes adduct formation, and can therefore not be used for examinations with the established mass spectral library.

The conversion of analytes to gas phase ions is essential for obtaining an efficient MS analysis. However, when using LC as the separation method, the analytes are dissolved in a liquid when entering the MS. Nowadays, electrospray ionisation (ESI) is one the most commonly used ionisation technique to convert the analytes into gas phase prior to the ionisation process. ESI ionisation efficiency is generally improved when analytes are already in ionic form in the solution as this facilitates the formation of droplets (Cech & Enke 2002). However, due to vendor specific modes of ionisation and adduct formation, there are no standardised ionisation conditions for ESI. There are several parameters affecting the adduct formation; the solvents and buffers used, pH of the mobile phase, as well as the analyte's proton donating or accepting properties and gas-phase acidities/alkalinities within the mass spectrometer (Schug & McNair 2002; Kind & Fiehn 2010). Overall, this means that there are no highly reproducible fragmentation spectra that could be used for vast and general mass spectral libraries.

In comparison to EI, the insource fragmentation of analytes in ESI are moderate at most, keeping the quasi-molecular ion intact (Portolés et al. 2011). The quasi-molecular ion is protonated in positive ESI mode [M+H] and deprotonated in negative ESI mode [M-H]. The ionisation polarity, ESI+ or ESI-, has significant impact on compound detection and fragmentation patterns, and thus on the identification. That is because compounds or product ions of compounds observed in one ionisation mode will not necessarily be observed in the opposite mode.

3.1.3 Mass spectrometry instruments

Triple quadrupole MS (QqQ MS) instruments operated in multiple reaction monitoring (MRM) are mainly used for tandem MS (MS/MS) applications for quantification purposes in a variety of applications such as detecting food packaging contaminants (Fasano et al. 2012; Petersen & Jensen 2010; Driffield et al. 2010), as well as pollutants (Herrmann et al. 2012) and pesticide residues (Núñez et al. 2012) in food. When a specific voltage and radio frequency is applied to a QqQ instrument, only

ions with a certain m/z will be able to pass through the quadrupole (Schreiber 2010) which enhances the instrument sensitivity.

MRM mode allows monitoring of transitions for each analyte, typically one precursor ion fragmented into a couple of product ions (Hird et al. 2014). These product ions are produced by either in-source fragmentation or by collision induced dissociation (CID) in a collision cell (Nielen et al. 2007). Additionally, the use of Rt windows enables an even more sensitive analysis for many more analytes, assuming previously established Rt's and that these remain stable during the entire analysis (Herrmann et al. 2012; Hird et al. 2014). However, when acquiring in MRM mode, there is no possibility for re-interrogation of data, except for the ion transitions already pre-programmed in the method.

Time of flight (TOF) MS instruments are mainly used for screening purposes due to their high mass accuracy in combination with high resolution and acquisition speed during full scan acquisitions (Hird et al. 2014). All these parameters are essential for a qualitative identification of unknown compounds. One of the advantages of full scan data acquisition is the possibility of re-interrogating the data, since all m/z are simultaneously acquired in contrast to UHPLC-ESI-QqQ MS. In addition to the high mass accuracy and acquisition speed a TOF MS hybrid, combining quadrupole with TOF (qTOF MS), enables an almost simultaneous acquisition of data at low collision and high collision energy. This feature provides valuable information of both the quasi-molecular ion (abundant in low collision energy spectra) and of the main product ions (abundant in high collision energy spectra) (Díaz et al. 2012).

3.2 Fragmentation

As compounds and functional groups fragment differently, fragmentation could also be used to reveal structural information of unknown compounds. General rules have been established for mass spectral fragmentation obtained by EI. For instance, the relative height of the quasi-molecular ion peak is largest for molecules with straight chains, and decreases with chain length (McLafferty & Tureček 1993). Fragmentation in EI is favoured at carbon atoms with alkyl-substitution (Bursey & McLafferty 1966), meaning that aromatic groups are considered the most stable functional groups. In addition, carbon bonds next to heteroatoms are more prone to cleavage, and cleavage is favoured when small stable molecules like water or ammonia can be expelled (McLafferty & Tureček 1993).

Fragmentation patterns in LC-MS are strongly dependent by experimental conditions such as collision energy and collision gas, as well as instrument design (Würtinger & Oberacher 2012; Webb et al. 1999). For the quasi-molecular ions, [M+H] or [M-H], produced in ESI there is a limited understanding of the

fragmentation rules. However, in their article from 2011, Weissberg & Dagan extensively describes fragmentation rules for some of the most commonly found functional groups. In addition to these rules, the neutral loss from certain functional groups can be used to identify structures and ultimately compounds (Levsen et al. 2007). Despite the apparent differences between the ionisation mechanisms of EI and ESI, when disassociation occurs at the same position in EI and ESI the fragments observed are the same (Levsen et al. 2007).

4. Methods

This section offers a general description of the methods used in this study such as sample preparation, extraction, fractionation and identification processes.

4.1 Sample preparation

Initially, 20 paper and board samples, see **paper 3**, were chosen for a primary screening. The samples selected was a wide-ranging collection of common and commercially available FCMs. The samples were chosen based on paper type, intended food product, material origin, surface modifications as well as intended storage and usage. Between 45 and 90 dm^2 , depending on the bulkiness of the sample, was cut into smaller pieces prior to the extraction. No IS was added during any step of the sample preparation, as these compounds could interfere with the *in vitro* testing of extracts and fractions.

4.2 Extraction methods

4.2.1 Purge-and trap extraction of volatile organic compounds (VOCs)

For the purpose of extracting VOCs from paper and board matrices for subsequent *in vitro* testing as well as chemical analysis, a purge-and-trap method, similar to a set-up used for air sampling (DS/EN 14662-2:2005) was developed. In order to collect the analytes from the paper matrix Tenax® (modified polyphenylene oxide) was used. Tenax® is used both as a food simulant for dry and fatty foods as well as for collection of air pollutants (DS/EN 14662-2:2005; 10/2011, 2011). A schematic representation for the set-up is presented in Figure 10. To investigate recovery, two paper samples; one with low grammage (45 g m^{-2}) and one with high grammage (550 g m^{-2}) were fortified with eleven volatile organic compounds (VOCs) and semi-volatile organic compounds (sVOC). These surrogates were chosen to represent different boiling points, vapour pressures and molecular weights.

Briefly, 6 dm^2 of shredded fortified paper samples were placed in a 2 L glass bottle in an oven set at 60 °C. The sampling time was set to 60 min. The inlet air was cleaned through a carbon filter outside the oven, and all connecting tubing was made of the chemically inert material Teflon. For the collection of VOCs from paper and board, single-bed thermal desorption glass tubes containing in total 300 mg Tenax® was used. The sorbent was kept in place by silanised glass wool. A pump, set at 350 mL min^{-1}, was used to drive the VOCs from the bottle through the desorption tube placed outside the oven. The desorption tubes were cooled with dry ice to approximately -15 °C. The surrogates were extracted from

the Tenax® by the addition of 1.2 mL ethanol into the collection tube (preferably by two portions of 0.8 mL and 0.4 mL respectively). Approximately 0.8 mL ethanol could be recovered from the Tenax®. The elution of ethanol was aided by a gentle stream of nitrogen through the collection tube.

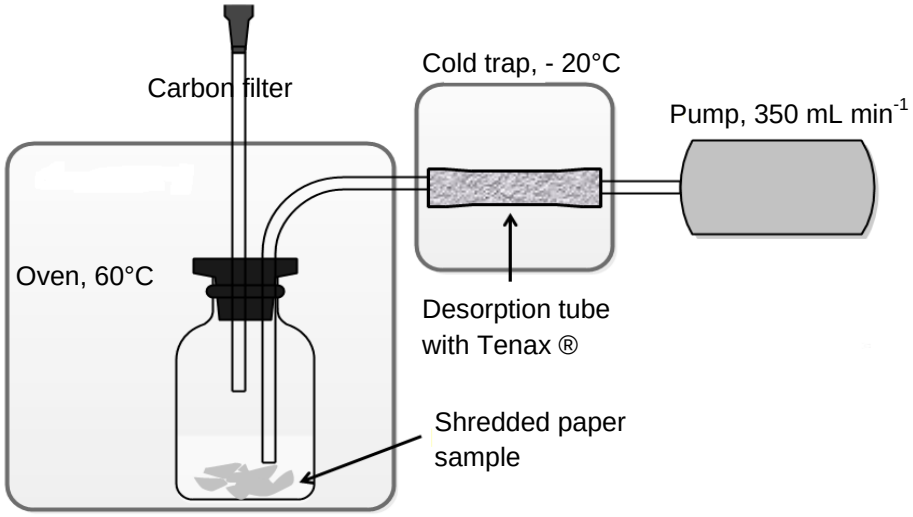


Figure 10. Set-up used for the purge-and-trap of VOCs from paper and board.

After extraction from the Tenax®, surrogates were analysed by a Agilent 6890A (Agilent Technologies, CA, US) plus gas chromatograph, equipped with a CTC Combi-PAL autosampler (Zwingen, Switzerland) and a tray cooler (kept at 10°C). The column used for the analysis was a CP WAX 52CB (30 m x 0.25 mm i.d. x 0.25 μm film thickness) (Agilent). High grade helium was used as carrier gas at a constant flow of 1.0 mL min^{-1}. Splitless injection mode, the transfer line was kept at 300°C, source at 250°C and quadrupole at 150°C. The total injection volume was 1μL. The column temperature program used was a 20 min linear gradient starting from temperature at 50°C to 250°C at 10°C min^{-1}. Samples were ionised by electron ionisation (EI) at 70eV and analysed in selective ion monitoring (SIM) mode. MS parameters, such as retention time (Rt) and precursor and product ions used for SIM, are presented in Table 1. The extracts from the fortified paper samples were diluted 1:10 *v/v* with ethanol prior to GC analysis. Quantification was performed in the Agilent ChemStation software. The extracts from the 20 samples used in the toxicological screening were produced in the same manner, except that 90 dm^2 were used.

Table 1.Surrogates used to fortify paper samples during method development for the purge-and-trap method. Qualifier ions are indicated in bold.

Compound	CAS number	Boiling point	Vapour pressure (mmHg)	Log $K_{O/W}$	Definition	Rt	Ions
Toluene	108-88-3	111	28.4	2.7	VOC	4.3	51, 65, **91**
Chlorobenzene	108-90-7	131	12.0	2.8	VOC	6.1	51, 77, **112**
Ethylbenzene	100-41-4	136	9.6	3.2	VOC	5.1	77, **91**, 106
Xylene (-p)	106-42-3	138	8.8	3.2	VOC	5.8	77, 91, **106**
Xylene (-m)	108-38-3	139	8.3	3.3	VOC	5.2	77, **91,** 106
Xylene (-o)	95-47-6	144	6.6	3.2	VOC	5.3	77, **91**, 106
Styrene	100-42-5	145	6.4	2.8	VOC	6.7	51, 78, **104**
1,2-Dichlorobenzene	95-50-1	147	1.36	3.4	VOC	9.5	75, 111, **146**
2,6-Diisopropylnaphthalene (DIPN)	24157-81-1	279	1.5E-3	6.2	sVOC	17.0	155, **197**, 212
Diisobutylphthalate (DIBP)	84-69-5	297	4.8E-5	4.4	sVOC	19.6	77, 105, **182**
Benzophenone (BP)	119-61-9	305	1.9E-3	3.2	sVOC	19.3	104, **149**, 223

Note: Boiling point is in °C at 760 Torr; vapour pressure is mm Hg at 25°C, log Ko/w is at 25°C

4.2.2 Extraction of semi- and non-volatile organic compounds (sVOCs and nVOCs)

In order to extract the sVOCs and nVOCs from the paper samples, a boiling ethanol reflux extraction, also called a Soxhlet extraction was used, as described in **paper 1**. Soxhlet extraction is a severe method intended to extract as much of the contaminants in the sample matrix as possible. The extracts were evaporated under nitrogen to concentrate them.

4.3 Toxicological screening of extracts

Combined extracts from the samples presented in **paper 3**, containing both VOCs, sVOCs and nVOCs, were screened for toxicological effects in the following *in vitro* assays; AR, ER, aryl hydrocarbon receptor (AhR), and peroxisome proliferator-activated receptors (PPARα/γ) reporter gene assays, glucocorticoid receptor (GR), retinoic acid receptor (RAR), nuclear factor (erythroid-derived 2)-like 2 (nrf2), and p53 CALUX reporter gene assays as well as mutagenicity tests; the Ames test and Comet assay.

4.4 Fractionation

The analysis of comprehensive extracts with toxicological effects by chromatographic methods will render very complex results that could be described as a forest-of-peaks analysis (Bradley et al. 2008; Bradley et al. 2010; Koster et al. 2014). Fractionation of the comprehensive extracts, and subsequent testing of the fractions in the same *in vitro* tests, is one strategy to narrow down the number of compounds to be identified. Five samples with toxicological response were chosen for fractionation and a subsequent second screening . The heterogenous sample, a microwave popcorn bag, was divided into three subsamples (susceptor part, adhesive part and bulk) before a second screening. In the high performance liquid chromatography (HPLC) based fractionation method described in **paper 1**; extracted compounds were separated on a reverse phase (RP) C_{18}-column with a linear gradient consisting of water and methanol. The gradient started at a low organic content in the mobile phase which was increased during the fractionation. Extracts were fractioned in two rounds; one round during acidic conditions and one round during alkaline conditions. The fractions, eleven in total per each round, were collected according to time.

4.5 Tentative identification

The tentative identification by GC-EI-qTOF MS and UHPLC-ESI-qTOF MS is described in detail in **paper 2**.

4.5.1 Tentative identification by GC-EI-qTOF MS

One of the advantages of identification of unknown compounds using GC-EI-qTOF MS is the standardised ionisation conditions, enabling searches in vast, commercially available spectral libraries such as the NIST library with over 270,000 available spectra. The emphasis of the tentative identification was aimed on detectability rather than meeting any identification criteria (see Section 4.6). The initial steps of the method, involving peak detection, deconvolution and library search were fully automated. Although, after these primary automated steps, several parameters were inspected manually (see Figure 11).

In order to make the laborious identification process more efficient, a cut-off based on the threshold of toxicological concern (TTC) was used, as previously described by Koster et al. (2014). However, in this study a cut-off based on a lower threshold of the TTC, at 25 ng dm^{-2}, for compounds with known genotoxic effect were used (EFSA Scientific Committee 2012). As no labelled IS was used, the exact

differences in detector response could not be determined. To compensate for these differences, one tenth of the peak area for d_4-DBP in the same concentration as the cut-off was used. Chromatographic peaks below this cut-off were not investigated further.

The mass spectral hits were scored within the analytical software according to mass match, abundance match, spacing match, fragment match and relative fragment intensity match. No mass spectral hits below 85 in the MassHunter software and below 800 in the Relative Match Factor in the NIST library were considered. After the initial automated steps, the obtained library hits were manually evaluated according to the flow chart presented in Figure 11. Initially, the Rt's for the suggested compounds from the library hits were compared to those of standards in the mixture. This comparison was made based on molecular weight and functionalities of the analyte and the standard. Next, the main fragments were inspected for the typical theoretical isotope patterns associated with the halogens chlorine and bromine, as well as for sulphur and silica. In addition, the isotope ratios for the main fragments were compared to those of the suggested formula within the MassHunter software (Agilent Technologies).

The following step for the tentative identification was an inspection of matching significant fragments, such as fragments that can be observed for the stable aromatic structures and characteristic fragments for phthalates. In addition, the list of tentatively identified compounds obtained by the UHPLC-ESI-qTOF MS method, see Section 4.5.2, was consulted for potential overlapping hits. Also, if the suggested compound from the library hit were in common for several fractions with the same toxicological response, the effect of the pH in the fractionation on the analyte-column interaction was compared between the fractions.

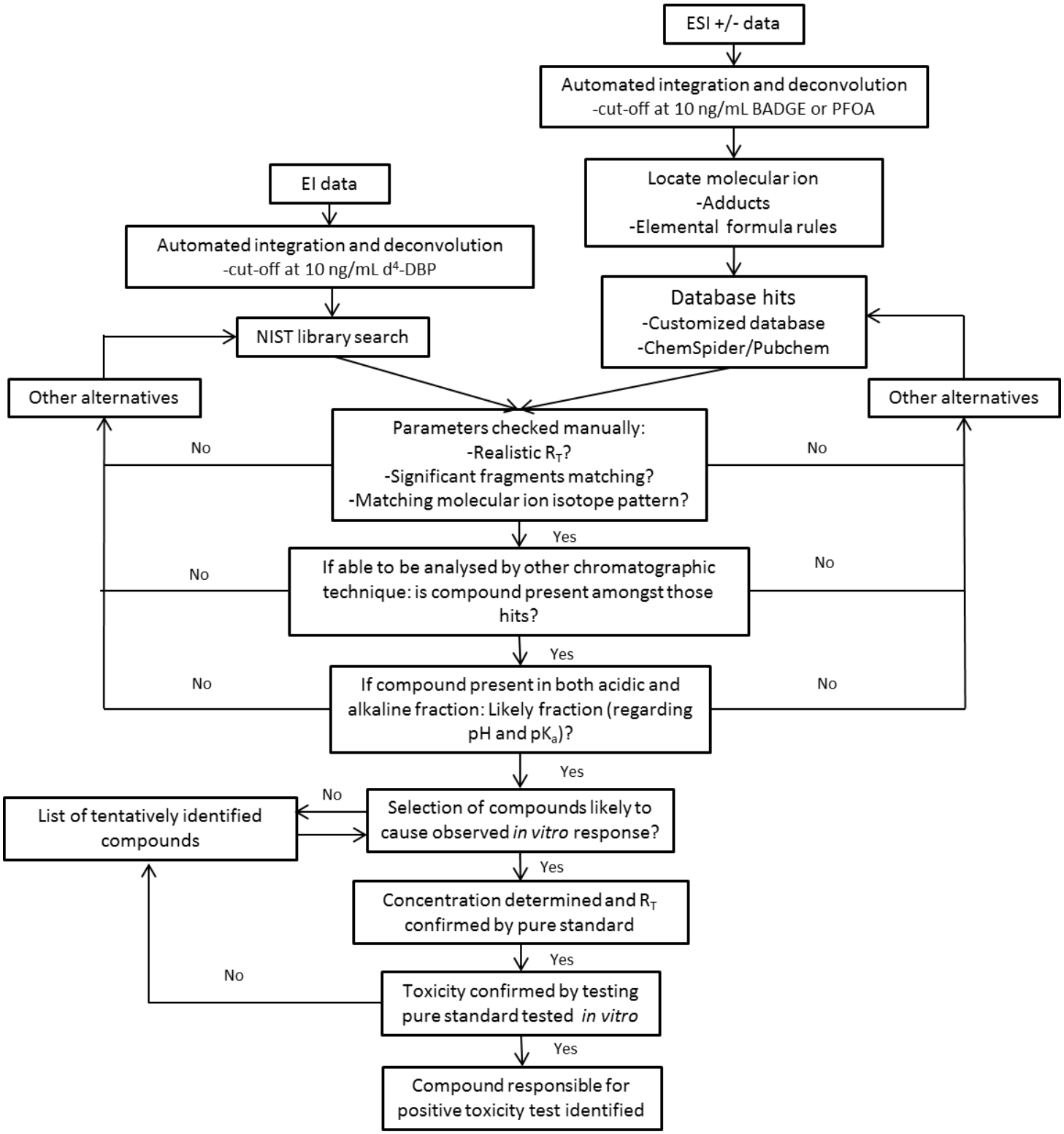


Figure 11. Flow chart for the tentative identification of unknown compounds in fractions analysed by either GC-EI-qTOF MS or UPLC-ESI-qTOF MS.

4.5.2 Tentative UHPLC-ESI-qTOF MS identification

The first step in tentatively identifying unknown compounds obtained by ultra-high performance liquid chromatography (UHPLC)-ESI-qTOF MS was a fully automated step of integration and deconvolution. The flowchart for the entire tentative identification process is presented in Figure 11. Next, the quasi-molecular ion, [M+H] or [M-H] in positive and negative mode respectively were located. When the quasi-molecular ion was found, the Molecular Formula Generator (MFG) feature within the MassHunter software generated possible molecular formulas for the most prominent spectral peaks. These molecular formula hits were ranked according to a weighted score within the MassHunter software according to mass match, abundance match and spacing match. No MFG hits with a score below 85 were considered. The MFG hits were then compared to the isotopic ratio to elucidate the most matching molecular formula(s).

The methods used for the tentative identification of compounds in the fractions were largely based on the "Seven Golden Rules" presented by Kind & Fiehn (2007) and further described by Godfrey & Brenton (2012), see Table 2 . These methods were used to reduce the number of suggested formulas for each spectral peak. Several of these rules, such as the nitrogen rule, the multiple element probability and the restriction of element numbers, are executed automatically by the MassHunter software. However, when measuring masses over 500 Da by accurate mass the nitrogen rule becomes defective (Kind & Fiehn 2007), and were therefore not used for analytes above this mass range.

Other rules described by Kind and Fiehn (2007), such as the isotope ratio, the hydrogen/carbon element ratio check as well as the heteroatom (N, O, P, S)/carbon element ratio check were inspected manually. In addition, the negative mass defect associated with fluorinated compounds was considered. Additionally, the same considerations as for tentative identification by GC-EI-qTOF MS was made, see Figure 11.

The molecular formulas were used to search a customised database. In the case of no hits obtained, the molecular formula was used to search large, generic databases such as ChemSpider or PubChem. The accurate mass window for all the queries was set to 10 parts per million (ppm). In the case of no generated molecular formula by the software, the mono-isotopic mass was used for queries in the generic databases. Reported usage of the suggested compounds in paper and board applications from patents listed in the ChemSpider and the PubChem databases was used to rank the hits.

Table 2. Summary of elemental formula rules used to reduce the number of suggested formulas from the molecular formula generator (MFG).

Name of rule	Description	Automated step /Manually inspected
Restrictions of element numbers	Exclusion of chemicals with unreasonable high element counts	Automated step
LEWIS and SENIOR	Only stable ionic compounds included	Automated step
Nitrogen	Odd monoisotopic molecular mass = even number of nitrogens*	Automated step
Isotope ratio	Average abundance of natural and stable isotope abundances for each element	Manually inspected
Hydrogen/Carbon element ratio check	Establishes likeliness for suggested formula(Usually $0.2 < H/C < 3.1$)	Manually inspected
Heteroatom (N, O, P, S)/carbon element ratio check	Restriction of unlikely high element ratios	Manually inspected
Element probability check	Restriction of unlikely combinations of a high number of heteroatoms	Manually inspected

* Only applicable for ESI ionisation, for EI ionisation the rule is; odd monoisotopic molecular mass = odd number of nitrogens

In order to elucidate the structure of unknown compounds and to compare it to candidates from the database search, the fractions were analysed by data-independent All ions mode in both polarities. In addition to a no collision mode, with only in-source fragmentation, spectra from two high collision modes (110V and 120V), causing analyte fragmentation, were acquired within the same analysis. All of the fragmentation rules for ESI presented in Section 3.2 were implemented in the tentative identification strategy by UHPLC-qTOF MS when applicable.

4.6 Identity confirmation and quantification

A total of 17 compounds, see **paper 2**, **3** and **4**, were selected from the complete lists of tentatively identified compounds for further chemical and toxicological testing. The selection was based on previously reported effects, structural similarities to known ligands, quantitative structure–activity relationship (QSAR) predictions and availability of analytical standards for identified compounds to be confirmed by GC-EI-qTOF MS or UHPLC-ESI-QqQ MS. The selected compounds were simultaneously confirmed and quantified, to be able to subsequently test the analytical standards in concentrations corresponding to the extracts in the *in vitro assays*.

For the confirmation of the tentative identification results obtained by UHPLC-ESI-QqQ MS and GC-EI-qTOF MS, both the relative Rt criteria (±0.5% for GC and ±2.5% for LC) and MS identification criteria are to be fulfilled (2002/657/EC, 2002). Identification points (IP) were used for MS criteria to be accepted: acquiring at least one MRM ion transition and one high resolution mass spectrometer (HRMS), such as the qTOF instruments used in this study, precursor ion renders more IP than the minimum requirement of four IP and allows the calculation of at least one ratio of the product ions (2002/657/EC, 2002). In addition to the IP points, a positive identification also requires at least one ion ratio to be measured (Commission 2002). The certainty of identity was increased even more in the case of matching high mass accuracy fragments acquired by HRMS

4.7 Toxicity confirmation

The response in the extract obtained from testing the selected and confirmed compounds individually in the respective cell assay was used to establish equivalence factors (EQ). The EQ for individual compounds were summarised to obtain the calculated EQ (EQ_{calc}). This EQ_{calc} was then compared to the measured EQ (EQ_{meas}) calculated from the original response from the extract when tested in the same *in vitro* assay. A detailed explanation of the calculation of EQs is described in **paper 2.**

5. Results and discussion - Strategy for a bioassay guided analysis and identification

In this chapter, the most important results from the individual chemical steps in the bioassay guided method developed in this study are presented and discussed. The overall strategy for the bioassay guided strategy presented in this study is presented in Figure 12. All Sections described in this chapter are represented by a step in this figure.

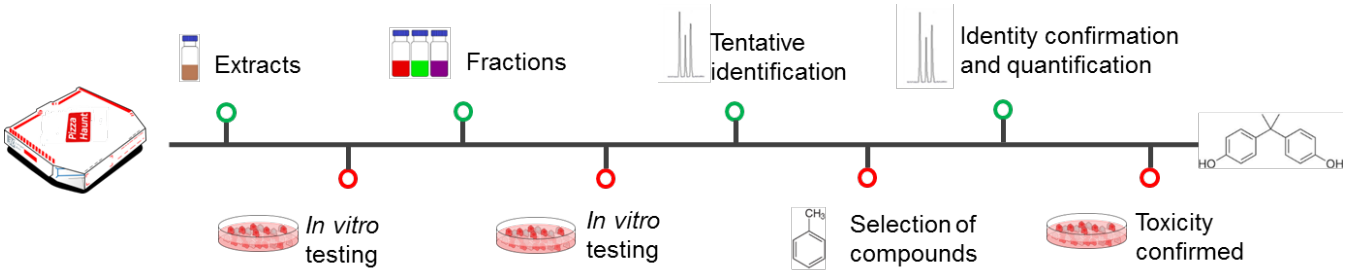


Figure 12. The overall strategy for the bioassay guided analysis proposed by this study.

5.1 Extraction methods

A bioassay guided screening for a comprehensive evaluation of paper and board FCMs sets high demands for an extraction method, as it should be compatible with both chemical analyses and *in vitro* tests. This includes choice of organic solvent to ensure extraction of a maximum number of compounds from the paper and board matrix, the concentration of the final extracts and the type of sample clean-up to avoid a loss of analytes. Moreover, most organic solvents available are not suitable for *in vitro* assays as they are highly cytotoxic. However, solvents such as ethanol and dimethyl sulfoxide (DMSO) are less cytotoxic than most other organic solvents. Ethanol and DMSO have both polar and non-polar properties and also similar polarities (log $K_{O/W}$ values), making them suitable for the extraction of the majority of the compounds found in paper and board. However, DMSO is a highly viscous liquid making pipetting an already viscous concentrated extract highly impractical. In addition, DMSO has a relatively high boiling point. Due to these circumstances, ethanol was chosen as the solvent used for the extraction methods. Furthermore, as cell assays require a low organic solvent content, around <0.5%-1%, to avoid cytotoxicity, extracts intended for *in vitro* testing must be highly concentrated. This concentration step was performed by evaporation, meaning that many of the analytes could evaporate before the solvent if DMSO was used. In order to further minimise the loss of analytes, there was no sample clean-up of the extracts prior to the *in vitro* testing.

5.1.1 Purge-and trap method for VOCs

Since migration rate in paper and board decreases with increasing molecular weight (Zülch & Piringer 2010), small VOCs (C_6-C_{15}) are relevant for evaluating migration from FCMs as they are easily transferred to the gas phase and migrate through the packaging and further into the foods (Barnes et al. 2007). In order to collect small VOCs (C_6-C_{15}), a purge-and-trap method, using Tenax®, based on methods adapted from air sampling (DS/EN 14662-2:2005), was developed. With the exception of DIBP, which is present in large amounts inherently in the recycled fibre, none of the sVOC surrogates were extracted from the fortified recycled cardboard by the purge-and-trap. In the fortified virgin paper sample, only very low amounts of the semi-volatiles DIPN, BP and DIBP could be detected after the purge-and-trap. These sVOCs could however be recovered after Soxhlet extraction.

The range of recovery for the VOCs in fortified virgin paper fibre with low grammage after the purge-and-trap varied from 58% for toluene to 101% for chlorobenzene, see Figure 13. The mean recoveries for all VOCs analysed were within an acceptable range (50%-120%) (2002/657/EC 2002), considering that no IS was used. Overall average recovery for all surrogate compounds classified as VOC in fortified virgin paper was 86%. Recoveries were calculated on the basis of the added ethanol, 1.2 mL, as this volume represented the true amount of ethanol. However, only two thirds of the added ethanol could be recovered for *in vitro* testing, the rest was bound in the Tenax®.

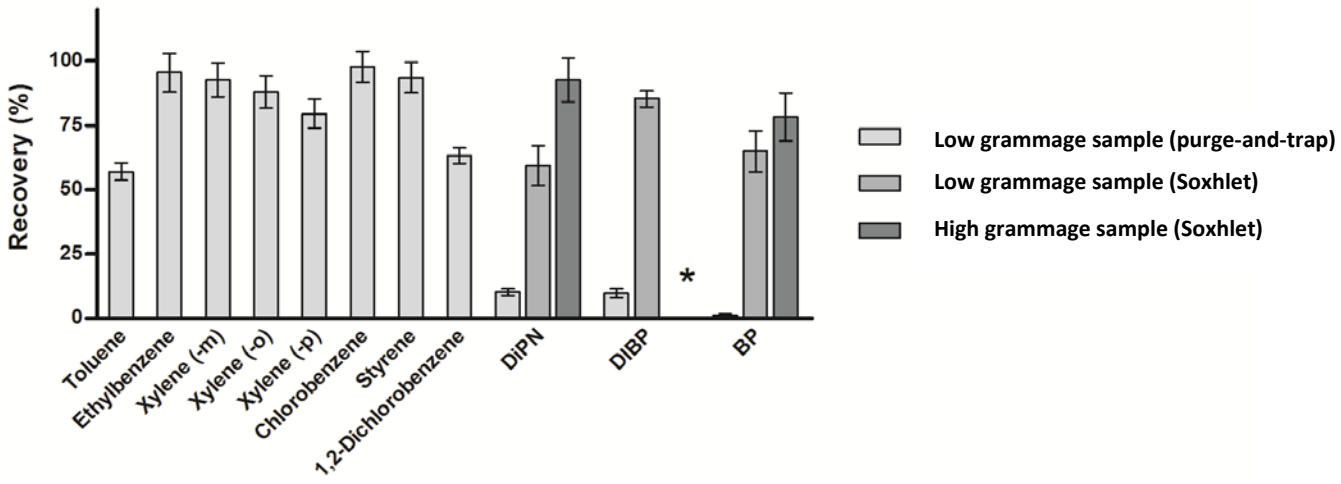


Figure 13. Recoveries of 167 ng surrogates dm^{-2} in fortified virgin fibre after purge-and-trap as well as after Soxhlet extraction and in fortified recycled fibre after Soxhlet extraction. Standard deviation is indicated as error bars. The surrogates are arranged from left to right according to their boiling point. *The recycled fibre contained too high concentrations of DIBP after the Soxhlet extraction to be quantified.

Toluene exhibits a lower recovery after purge-and-trap in virgin fibre than the other surrogates in this study, see Figure 13. This is also the compound with the lowest boiling point and vapour pressure. There was no toluene breakthrough observed when sampling tubes with two beds were tested during method optimisation. The low recovery is therefore likely due to an evaporation of toluene during the drying process after fortification with ethanol as solvent. Extracts from recycled fibre after Soxhlet extraction contained too high inherent concentrations, approximately 1000 times above the fortification level, of DIBP to be quantified. Studies have previously described significant concentrations in recycled paper (Poças et al. 2010).

Advantages of using Tenax® instead of other resins active charcoal or the polymer analogues, Carbosieve or Carbotrap™, are for instance a larger optimal molecular weight range for compounds relevant for FCMs and a lower affinity towards water (Ramírez et al. 2010). Another major advantage of polymer sorbents like Tenax® is the possibility to quantitatively elute collected polar and non-polar analytes with ethanol (Ramírez et al. 2010), in contrast to charcoal, which is only suitable for non-polar substances. All things considered, Tenax® was chosen for the purge-and-trap method, as it is able to collect both the non-polar compounds that migrate at a higher rate as well as the polar compounds, which is the majority of the substances found in paper and board materials.

However, the developed purge-and trap method has limitations, such as the range of compounds able to be collected by the Tenax® sorbent and paper types. This means very volatile organic compounds (vVOCs) will not be collected by the proposed method in larger quantities, even with the increased partitioning between the substance and polymer due to the low temperature of the trap at -20°C. However, these compounds are also likely too volatile to be retained within the paper matrix and would most likely have evaporated long before the packaging of food. Because of this, vVOCs were deemed outside of the scope for this study. It should also be noted that the purge-and-trap method described in this study represent a worst-case migration scenario as the experiments were performed in closed containers at elevated temperatures.

Although, several conditions, such as oven temperature (40°C, 60°C and 80°C), resin types (Tenax® and activated charcoal), resin amount (100 mg to 300 mg, one or two bed), cold trap temperature (ambient to -20°C) and pump rate (70 mL min^{-1} to 700 mL min^{-1}) were investigated there is need for a systematic testing as these tests were only performed once. In the future, it would be beneficial to perform a factorial design of experiments, including several factors tested at a high and a low level, in order to investigate which parameters are significant for recovery. These experiments would also indicate if the low recoveries for the sVOC analytes are due to a thermodynamic limitation, that the analytes are not transferred to gas phase, or a limitation in analyte-resin interaction.

5.1.2 Soxhlet extraction of sVOCs and nVOCs

To investigate the extraction efficiency of sVOCs and nVOCs, five surrogates with varying physic-chemical properties were used to fortify a paper sample made from virgin fibre and a cardboard sample made from recycled fibre, see **paper 1**. In terms of overall recovery, the boiling ethanol reflux resulted in acceptable recoveries of the five surrogates in the matrices, with a mean value of 71% in the virgin fibre paper sample and mean value 79% from the recycled fibre cardboard sample. However, the recycled paper contained too high endogenous concentration (approximately 1000 times higher) of one of the surrogates, AA, to be quantified.

Usually when validating a method, labelled IS are used to improve performance by compensating for matrix effects as well as other analytical variations of the extraction. However, as IS could interfere with the cell assays; these were consequently not used for any of the preparative extraction methods described in this study. Both methods developed for extraction of analytes was within acceptable ranges concerning recovery, repeatability and reproducibility (Commission 2002), even without the addition of IS.

5.2 Toxicological screening of extracts

In an initial screening, five combined extracts from the purge-and-trap method and the Soxhlet extraction had a toxicological effect in one or several of the toxicological assays were tested, see Table 3. The full list of the 20 samples initially screened is presented in **paper 3**. However, preliminary results showed that none of the extracts from the purge-and-trap method had a response when tested individually. Extracts from the Soxhlet extraction had a toxicological response, when tested individually, in the same assays as the initial combined extract and were thus selected for further analysis.

Table 3.The five samples selected for further analysis based on their toxicological response.

Sample no.	Usage	Material	Supplier	Pulp type	Printing	Grammage (g/m²)	Assay with positive response
S2	Plain paper	Paper	Paper industry	Virgin pulp	No	45	AR
S4	Sandwich wrapper	Paper	Retail	Virgin pulp	No	40	AR
S8	Pizza box	Corrugated fibreboard	Retail	Recycled	Yes	550	ER, AhR
S17	Microwave popcorn bag	Paper	Popcorn vendor	Recycled	Yes	90	Mutagenicity

5.3 Fractionation method

The aim for dividing extracts with a toxicological effect into fractions, see Figure 14, and subsequently testing these fractions by the same toxicological tests is to reduce the number of compounds to be identified and thus increase the efficiency of the entire workflow. Fractionation also acts as sample clean-up, removing potential interfering matrix components that could cause ion suppression during particularly LC-MS analysis (see Figure 15 and 16). There have been several attempts to describe fractionation of paper and board extracts for example by filtering (Bradley et al. 2010) or liquid-liquid extraction followed by gel permeation chromatography (Ozaki et al. 2005). In addition, solid-phase extraction (SPE) is useful for sample clean-up and concentration of analytes with known physico-chemical properties, such as bisphenol A (Dirtu et al. 2008; Grumetto et al. 2008) and various pesticides (Leandro et al. 2007). However, all of these fractionation methods are either too specific or not able to separate unknown compounds from different groups in a sufficient number of fractions to be feasible for the identification process. On the other hand, HPLC based fractionation have previously been successfully applied in order to remove paper and board bulk material that could interfere with the analysis (Biedermann & Grob 2013), as well as separating anabolic steroids in herbal preparations (Peters et al. 2010).

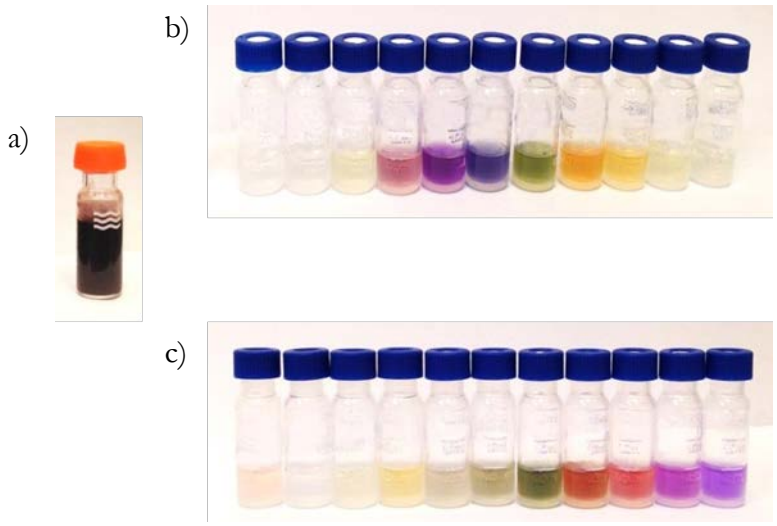


Figure 14. Pizza box a) extract and fractions obtained by b) acidic and c) alkaline fractionation conditions. The fractions are arranged from left to right according to their collection order, thus increasing organic content of the gradient.

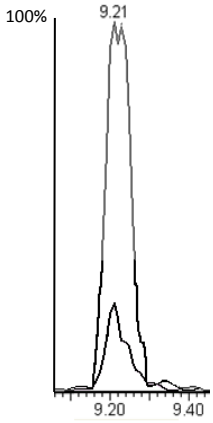


Figure 15. Overlay chromatogram of BPA from raw extract (low response) and fraction (high response) analysed by UHPLC-ESI-QqQ MS in negative mode. The matrix effect in the extracts causes a significant decrease in response.

In this study, an HPLC based fractionation was used (see **paper 1**) as this method offers the possibility to fine tune the fractionation process by changing several parameters such as mobile phases, columns and mobile phase gradient. In order to minimise loss of analytes, and in particular surfactants, the extracts were centrifuged instead of filtered. As the fractions, just like the extracts, have to be highly concentrated by evaporation prior to the *in vitro* assays, buffers with low boiling points for the mobile phases were selected. Both formic acid and ammonia, selected for the acidic and the alkaline fractionation respectively, readily evaporates before any potential analytes and will therefore have no effect on the *in vitro* analyses.

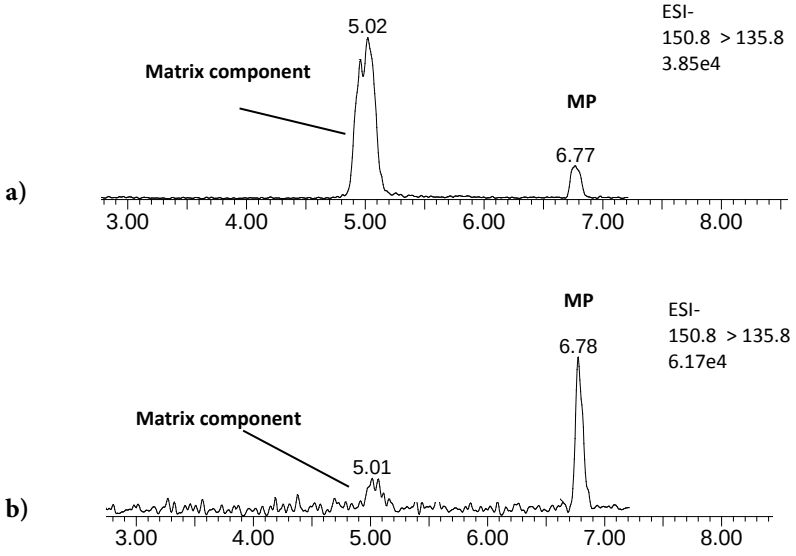


Figure 16. Chromatogram of methylparaben (MP) obtained by UHPLC-ESI-QqQ MS in positive mode from a) extract and b) fractionation. The matrix component found in the extract is visibly smaller in the fractionation.

With the intention to validate the fractionation method, extracts from paper samples fortified with five sVOCs and nVOCs, see Section 5.1.2, were fractioned according to the method described in **paper 1**. In terms of overall recovery, the fractionation only rendered a small loss of surrogate even after evaporating the fractions to dryness. In addition, both reproducibility and repeatability were within acceptable ranges according to the directives specified in 2002/657/EC 2002. However, the fractionation process is affecting method precision, causing a greater overall uncertainty.

5.4 Toxicological screening of fractions

All fractions produced were tested in the respective toxicological assays where the initial extracts had a toxicological response. Only a few of the fractions, see Table 4, had a positive response in the same cell assays as the original extracts. The extract from the susceptor part and the bulk part form S17, a microwave popcorn bag sample, had a toxicological response in the mutagenicity test (**paper 4**). The bulk subsample extract had a slightly higher effect than the susceptor, see Figure 17. However, neither of the fractions from any of the subsamples gave a positive response in the mutagenicity test. As the extract from the bulk subsample had a higher response than the susceptor, the bulk sample was selected for the tentative identification process.

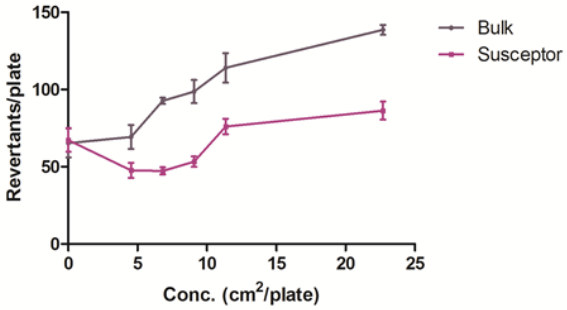


Figure 17. Results in the Ames test (TA98) with S9 mix of ethanol extracts of the two subsamples; bulk and susceptor, from sample S17, a microwave popcorn bag. Each point corresponds to the mean of three plates of one experiment. The standard deviation for each measure point is represented by horizontal bars.

*Table 4. Tentatively identified compounds, from fractions with toxicological effect, selected for further analysis. Solvent Violet 8 was selected for further testing in the Ah*R *assay and the Ames test.*

Compound	CAS number	Assay with toxicological response	Fractions	Tentatively identification method	Customised database hit (LC only)	ID confirmed?	Concentration in extract (µg dm^{-2})
Bisphenol A (BPA)	80-05-7	ER	S8 acidic 6, S8 alkaline 6	EI		Yes	21
Di-n-butyl phthalate (DBP)	84-74-2	ER	S8 acidic 6, S8 alkaline 6	EI		Yes	62
Benzyl Butyl Phthalate (BBP)	85-68-7	ER	S8 acidic 6	EI		Yes	22
Diisobutyl phthalate (DIBP)	84-69-5	ER	S8 acidic 6, S8 alkaline 6	EI		Yes	422
Dehydroabietic acid (DHAA)	1740-19-8	AR	S4 acidic 8, S4 alkaline 8	ESI+	x	Yes	7
Abietic acid (AA)	514-10-3	AR	S4 acidic 8, S4 alkaline 8	ESI+	x	Yes	752
4-oxoretinoic acid	38030-57-8	AR	S4 acidic 8	ESI+		No	
Isorhamnetin	480-19-3	AR	S4 acidic 8	ESI-		No	
Rhamnetin	90-19-7	AR	S4 acidic 8	ESI-		No	
Leucocrystal Violet	603-48-5	Ames test	S17	ESI+	x	Yes	2 E-1
Solvent Violet 8	52080-58-7	Ames test, AhR	S8 alkaline 9/ S17	ESI+	x	Yes	78 (S8)/ 13 (S17)
2-Mercaptobenzothiazole	149-30-4	AhR	S8 acidic 8, S8 alkaline 9	ESI-	x	No	
Basic red 1	989-38-8	AhR	S8 alkaline 9	ESI+	x	Yes	4
Baso Red 546 (Rhodamine B base)	509-34-2	AhR	S8 alkaline 9	ESI+		Yes	4
1-Isopropyl-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid	436811-11-9	AhR	S8 alkaline 9	ESI+		No	
Rhodamine 101	116450-56-7	AhR	S8 alkaline 9	ESI+		No	
2'-(Dibenzylamino)-6'-(diethylamino)-3H-spiro(2-benzofuran-1,9'-xanthen]-3-one	34372-72-0	AhR	S8 alkaline 9	ESI+		Yes	5

5.5 Tentative identification

The fractions selected for further analyses were subsequently analysed by GC-EI-qTOF MS and UHPLC-ESI-qTOF MS, see **paper 2**, **3** and **4**. In addition, using two separation methods rather than just one, enables an identification of compounds with a broad range of physico-chemical properties. Throughout the tentative identification process, there is a constant delicate balance between limiting the number of compounds to be identified and the risk of removing compounds with an actual toxicological effect.

Besides fractionation to limit the number of compounds, a cut-off based on the TTC for genotoxic effects was used (EFSA Scientific Committee 2012), to further reduce the number of compounds to be identified. The lowest TTC described by this report, for compounds with genotoxic effects or structural similarities to genotoxic compounds (EFSA Scientific Committee 2012), was used in this study, as the aim was identify compounds with a toxicological response in mutagenic assays as well as EDCs.

5.5.1 Tentative identification by GC-EI-qTOF MS

For an efficient identification process, it is important to have as many steps as possible automated (see **paper 2**). The advantage of using GC-EI-qTOF MS for identification purposes is that this analysis allows for many more automated steps, due to the availability of a vast and commercial spectral library. In addition, GC as a separation technique has more favourable kinetic properties than LC (Poole 2003). Consequently, GC-EI-qTOF MS is the first hand choice for a tentative identification process.

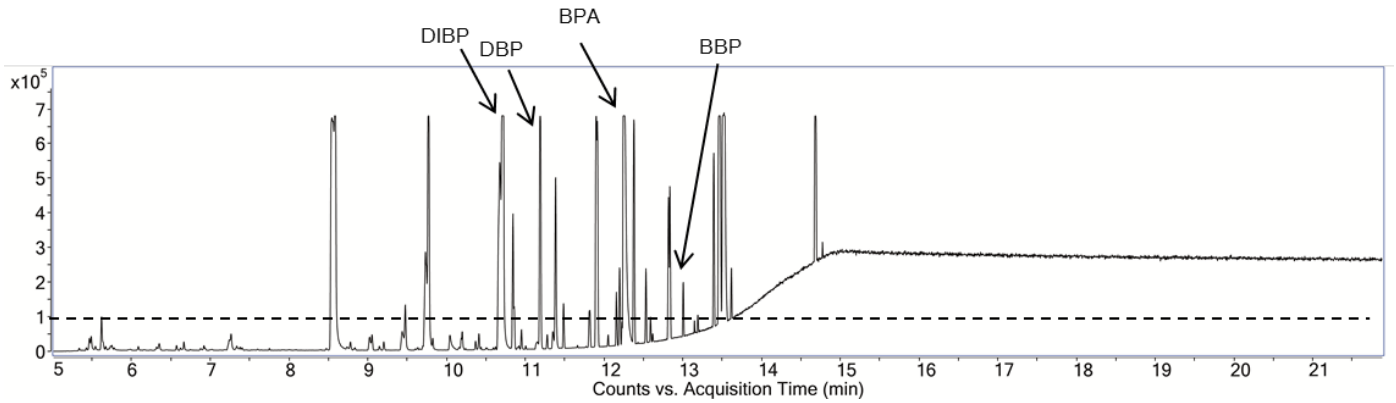


Figure 18. Base peak chromatogram (BPC) of acidic fraction 6 from sample S8 acquired by GC-qTOF. This fraction had a toxicological response in the ER assay. The four compounds; BPA, DBP, DIBP and BBP, selected for further testing are indicated by arrows in the chromatogram. The dotted line represents the cut-off.

However, due to the severe fragmentation caused by the EI, there are sometimes difficulties in identifying certain compounds because of the lack of quasi-molecular ions. There are other ionisation modes available for GC as well, such as positive and negative chemical ionisation (PCI and NCI respectively) that does not cause such severe fragmentation as EI. However, due to the adduct formation in PCI and NCI ionisation; the vast mass spectral library cannot automatically be used for the tentative identification. As this automated search is one of the major advantages with GC analysis, it was decided that PCI and NCI ionisation modes were not practical for a rationalised strategy.

In particular linear hydrocarbons, such as alkanes and fatty acids, were fragmented in too high extent for the quasi-molecular ion to be present in the mass spectra obtained by GC-EI-qTOF MS analysis. In these cases, the fragmentation patterns were found to be consistent with that of a linear hydrocarbon, but could not be assigned any specific compound. These linear hydrocarbons could originate from various sources within the paper production, such as contaminants from the de-inking process or from surface coatings and printing inks. As linear hydrocarbons are not associated with any toxicological response in the *in vitro* assays tested, the identification of these compounds was not confirmed.

All four compounds selected for further investigation in the ER assay, BPA, DIBP, DBP and benzyl butyl phthalate (BBP), were tentatively identified by GC-EI-qTOF MS, see Figure 18.

5.5.2 Tentative identification by UP LC-ESI-qTOF MS

The fractions with a toxicological response were also analysed by UHPLC-ESI-qTOF MS in order to develop an orthogonal and thus more comprehensive tentative identification process. Most compounds found in paper and board matrices are polar or semi-polar in order for them to be able to interact with the polar hydroxyl-groups in the cellulose. These compounds are better separated by UHPLC than by GC. In addition, the possibility to detect larger compounds (above m/z 550) with UHPLC-ESI-qTOF MS is favourable as the general threshold for the size of compounds that are able to pass the intestinal membrane of humans by passive diffusion is 1000 Da (Mitra et al. 2015). However, larger compounds, such as di-PAPs, could degrade into smaller constituents by the acidic environment and/or enzymes in the gut and thus be taken up. By using UHPLC-ESI-qTOF MS as a complimentary technique for the tentative identification, these larger as well as intermediately polar or polar compounds could also be analysed.

ESI was used as ionisation mode for the tentative identification process for the UHPLC-ESI-qTOF MS analysis. The advantages of ESI are that it is a soft ionisation technique, often leaving an abundant

quasi-molecular ion visible, and the adduct formation, facilitating the localisation of the quasi-molecular ion. Another advantage with ESI is the ability to perform a controlled CID fragmentation, by controlling the interface lens potentials. There are also other ionisation interfaces available for UHPLC, such as atmospheric pressure chemical ionisation (APCI) and atmospheric pressure photoionisation (APPI). In APCI, the evaporated mobile phase acts as the ionisation gas and forms the ions and much more energy is absorbed when the analyte ion is formed. Sometimes this absorbed energy is enough to fragment the quasi-molecular ion (Watson & Sparkman 2007). Therefore, there is no possibility of controlled CID fragmentation in APCI. In addition, APCI is only suitable for thermostable compounds below 1000 Da, and APPI is very selective towards compounds with aromatic structures. Nevertheless, ESI as ionisation technique appears to be more affected by matrix effects than for instance APCI and APPI (Trufelli et al. 2011). However, these effects are reduced by the fractionation as well as the dilution of the fractions. For the purpose of developing a comprehensive UHPLC analysis method, ESI was determined to be the most suitable and wide-ranging ionisation mode.

The first step when identifying unknown compounds acquired by UHPLC-ESI-qTOF MS after the automated integration and deconvolution was to locate the quasi-molecular ion (see **paper 1**). An example of the cut-off used to reduce the number of compounds to be identified is presented in Figure 19. Adducts can be helpful as they could facilitate this localisation when both species, i.e. the quasi-molecular ion and the adduct ion, are present. The comparison of co-eluting ions is enabled by recently released analytical software. If the mass difference of two of these ions matches the difference between two adduct masses specified in the search criteria of the software, it could be reasonably assumed that these two masses are in fact the same compound. By using this software, the uncertainty of localising the quasi-molecular ion could be minimised. The most observed adducts in the study was $[M+H+NH_3]$ and $[M+H-H_2O]$ in positive mode and $[M-H-H_2O]$ in negative mode. However, when adduct formation is favoured; there could be some difficulties to locate the actual quasi-molecular ion. After the localisation of the quasi-molecular ion, the MFG feature within the analytical software generated possible molecular formulas for the most prominent spectral peaks. These molecular formulas were then compared to the isotopic ratio to find the most matching formula(s). In agreement with earlier studies (Kind & Fiehn 2007; Kind & Fiehn 2006), matching isotope ratio appears to be more important than a high mass accuracy (<5 ppm) for the identification process. However, mass accuracy is a useful element for predicting a correct molecular formula during the preliminary steps.

Due to the non-standardised ionisation mode for UHPLC-ESI-qTOF MS as well as adduct formation, there are no vast mass spectral libraries available for this method, such as the NIST library for GC-EI-

qTOF MS. Even though some vendors have developed small mass spectral libraries, these are for specific purposes and are focused on only small subsets of analytes such as pesticides and illicit drugs (Hird et al. 2014). Accurate mass measurements obtained by HRMS instruments are specific and universal, theoretical accurate mass databases can be used for the identification of unknowns (Peters et al. 2010). To be able to perform a semi-targeted analysis for compounds suspected of being present in the fractions analysed by UHPLC-ESI-qTOF MS, an accurate mass customised database containing almost 2100 entries of compounds previously reported in paper and board, see Appendix B, was developed. The compounds in the database were both IAS and NIAS, and were collected from several different sources (Trier 2011; Ackerman et al. 2011; EuPIA 2011; European Comission 2000). The database consisted of compound names, Chemical Abstracts Service (CAS) numbers (if available), molecular formulas and mono-isotopic masses. The molecular formula obtained from the earlier steps in the tentative identification process was used to search the customised database, as well as the large, generic databases, if no hits were obtained in the customised database, for possible candidates.

A data-independent All Ions acquisition was used in order to elucidate structural information from the unknown compounds and to compare it to candidates from the databases. This included a no collision mode, with only in-source fragmentation, as well as spectra from two high collision modes acquired simultaneously. These fragment ions, if present, were used to strengthen a tentative identification by comparing the obtained high collision spectra with isotope matched product ions from the candidate compounds. However, as some of the compounds containing several aromatic structures did not fragment sufficiently, some higher collision energies would be necessary to fully take advantage of the data-independent All Ions acquisition.

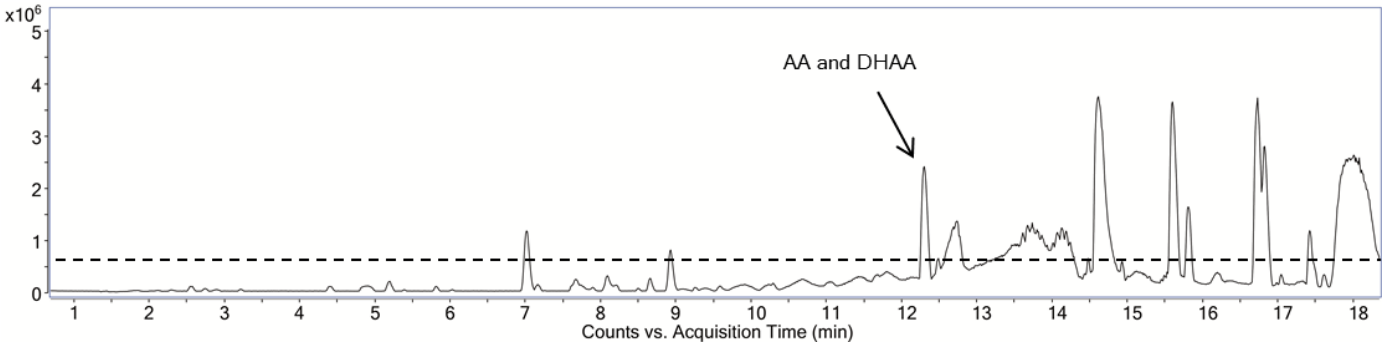


Figure 19. Base peak chromatogram (BPC) of alkaline fraction 8 from S4 acquired by UPLC-ESI+-qTOF MS. This fraction had a toxicological response in the AR assay. The co-eluting DHAA and AA, two of the compounds selected for further analysis are indicated in the BPC. The dotted line represents the cut-off.

Based on the findings of Berendsen et al. (2013), product ions considered nonspecific such as the loss of water or ammonia were not considered equally selective as compound-specific product ions . In total, 13 compounds tentatively identified by UHPLC-ESI-qTOF MS were selected for further analyses, see Table 4. Complete lists of tentatively identified compounds by both GC-EI-qTOF MS and UHPLC-ESI-qTOF MS operated in both positive and negative mode are presented as Appendix A, D and E**.** These lists also include potential sources of origin(s) from the different stages of paper production.

As predicted by Levsen et al. (2007), the same fragmentation pattern between EI and ESI for several compounds was observed. One example of these similarities is the fragmentation pattern of phthalates. In Figure 20, these similarities are represented by DBP, with a prominent spectral peak at *m*/z 149.0232 ($C_8H_5O_3^+$). This fragmentation, producing a protonated phthalic anhydride, have been described in detail by several research groups, recently by Jeilani et al. (2010). Another significant fragment, at *m*/z 223.0664 ($C_{12}H_{16}O_4^+$), are also matched in both acquisition methods. This shows that at least for some groups of compounds, the fragmentation pattern from EI could be helpful in identifying compounds ionised and fragmented by ESI. Also, the more severe fragmentation obtained from EI can be observed in Figure 20, as almost nothing remains of the quasi-molecular ion at *m*/z 279.1628 in spectra obtained by GC-EI-qTOF MS in comparison to the spectra from the UHPLC-ESI-qTOF MS acquisition.

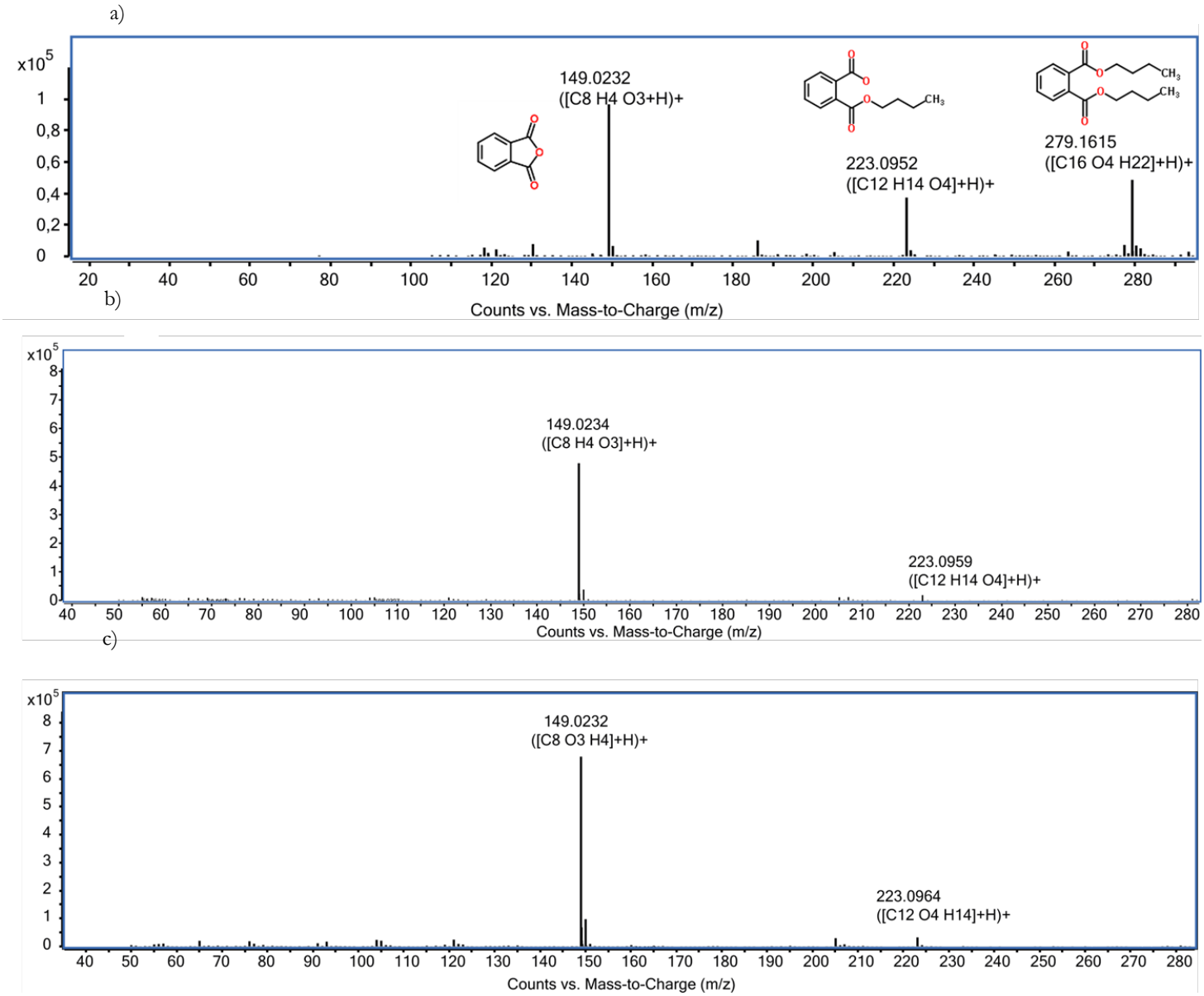


Figure 20. Fragmentation pattern of DBP in a) alkaline fraction 6 from sample S8 in obtained by UHPLC-ES+I-qTOF MS (at 100 V in collision energy, thus only in source fragmentation). b) Standard (5 μg/mL) with DBP and c) alkaline fraction 6 from sample S8 in obtained by GC-qTOF. In both cases, the fractions were diluted 1:1000 *v*/*v* with ethanol.

5.6 Toxicological assessment

After lists of tentatively identified compounds had been produced for a certain assay, compounds were selected for further testing based on previously reported effects, structural similarities to known ligands, i*n silico* predictions, such as QSAR modelling, and availability of analytical standards for identified compounds, see **paper 2, 3** and **4**. In the ER assay, literature studies revealed compounds with known effects in this assay on the list of tentatively identified compounds in the fractions. In the AR assay, the selection of compounds to be further assessed were based on an expert judgment including information on previously reported effects, read-across, and commercial availability of tentatively identified compounds. Therefore, QSAR modelling was not used to support the selection of compounds for further testing for the ER and AR assays.

However, in this study, QSAR modelling was used to predict the toxicity of tentatively identified compounds in the AhR assay as well as the mutagenicity test. The QSAR were found to be inadequate in the AhR assay, as there was a limited number of compounds in the dataset. This meant that a large number of compounds where outside of the domain of the model used to predict toxicity. Through read-across analysis, fifteen compounds were considered to share structural similarities with known AhR ligands. However, only seven of these selected compounds had commercially available standards and could be further investigated. However, for the mutagenicity tests, presented in **paper 4**, there were over 4100 compounds in the dataset, which makes the QSAR modelling in this case a powerful tool for selecting compounds for further testing.

5.7 Identity confirmation and quantification

Any positive annotation from either the customised database described in this thesis or any of the large, generic databases, should be regarded as tentative. The combinatory use of accurate mass and isotopic pattern is sufficient for screening purposes but not for a consistent identification (Ojanperä et al. 2012; Kind & Fiehn 2007). For a confirmed positive identification, the relative retention time as well as the fragmentation pattern of the tentative identified compounds should be correlated to those of analytical standards (Ojanperä et al. 2012).

A total of 17 compounds from the lists of tentatively identified compounds were selected for further analyses. All four compounds tentatively identified by GC-EI-qTOF MS were confirmed when relative Rt, fragmentation pattern and ion ratios were compared to analytical standards. Out of these four selected compound only DBP was present among the tentatively identified compounds by UHPLC-

ESI-qTOF MS. This is possibly due to the more severe matrix effect in the UHPLC acquisition due to the ESI interface, causing an ion suppression of these compounds. Of the 13 compounds tentatively identified by UHPLC-ESI-qTOF MS, six were confirmed when comparing to analytical standards in UHPLC-ESI-QqQ MS and five of these six compounds had entries in the customised database. The dye Baso Red 546 was the only one of the selected compounds that did not appear in the customised database. All confirmed compounds are soluble in both ethanol and methanol, and are therefore extracted from the matrix and eluted during fractionation.

One of the compounds tentatively identified with an entry in the customised database, 2-Mercaptobenzothiazole, were not confirmed when compared to an analytical standard. Other studies have found that a customised accurate mass database with matrix relevant entries greatly enhances the possibility of a correct identification of unknown compounds in UHPLC-ESI-qTOF MS analysis (Kind & Fiehn 2007; Mezcua et al. 2009). The results from this study clearly confirm these results; the use of a material matched accurate mass database is superior to using generic databases. These results also emphasise the importance of gathering as much information of chemical constituents in the matrix, in this case both natural components as well as IAS and NIAS from the paper production, as well as any production steps prior to the identification of unknowns in complex matrices.

BPA, identified and chemically confirmed in acidic and alkaline fractions nr 6 from sample S8, a pizza box, with ER effects, is a relatively non-polar compound found in for example surface coatings, printing ink and monomers. The fractions containing BPA were both collected when the organic mobile phase composition was increased from 50% to 60%. During acidic fractionation, BPA (acid disassociation constant (pK_a) 10.3) is neutral and during alkaline fractionation, the compound is in its ionised form. BPA therefore elutes faster in the alkaline fraction than in the acidic fraction due to a lower degree of analyte-stationary phase interaction on the RP column. However, as the fraction is collected for the relatively long period of five minutes, BPA elutes in the same fractions during both fractionation modes even though the differences in Rt's.

AA and DHAA, two relatively non-polar compounds, were identified and chemically confirmed in acidic and alkaline fractions nr 8 from sample S4, a sandwich wrapper, with effects in the AR assay. The fractions containing AA and DHAA were collected when the organic mobile phase composition was increased from 80% to 90%. During the acidic fractionation, AA and DHAA (pK_a 4.6) are neutral, which would explain the late elution time. However, during the alkaline fractionation, AA and DHAA are ionised and should therefore elute faster. However, due to the long collection period for individual

fractions, AA and DHAA elute in the same fractions during acidic and alkaline conditions even though actual differences in Rt's.

In the alkaline fraction nr 9 with effects in the AhR assay from sample S8, three dyes; Solvent Violet 8, Basic red 1 and Baso Red 546, were identified and chemically confirmed. The three dyes are either basic dyes or solvent dyes. The fractions containing the three dyes were collected when the organic content in the mobile phase was increased from 90% to 100%. During the alkaline fractionation, Basic Red 1 (pK_a 6.1) would be in its ionised form and elute faster than during acidic conditions. There are no compound specific pK_a values for Baso Red 546. In this case the diethylamine groups (pK_a 10.7) of the compound will be most affected by the variations in pH, as the ester structure (pK_a 25) will not be affected by the pH range used during fractionation. During acidic conditions, the diethylamine groups were neutral and during alkaline conditions, a majority (>90%) of the groups were ionised. The case of Solvent Violet 8 in fractions with AhR effects is discussed in detail in Section 5.8.

Solvent Violet 8, together with the dye Leucocrystal violet, was selected as the compound responsible for the mutagenic effect observed in the extract from the bulk sample from the microwave popcorn bag. When fractions from this sample were tested in the Ames test, no toxicological effects were observed. However, these fractions had been stored for a longer time period at 4-8°C prior to toxicological testing and could therefore have been degraded by for example oxidisation by other component present in the fractions. Earlier studies have shown that other solvent dyes are generally degraded by oxidation in wastewater (Ju et al. 2009). Another explanation of the absence of toxicological response in the fractions could be that the compound(s) responsible for the effect were precipitated, and were therefore no longer bioavailable. Additionally, the toxicological effect from the extract in the Ames test was reduced over time which supports the theory of an on-going degradation or precipitation process. It can therefore be concluded that the extracts and fractions should be tested *in vitro* as soon as possible after production. In addition, the extracts and fractions should also be stored under other conditions, such as at -20°C, to avoid a reduction in response.

As many of the compounds selected for further analysis elute in the same fraction during acidic and alkaline fractionation despite the differences in pH, it can be concluded that the time interval for the collection of fractions is too wide. A future improvement of the fractionation would involve collecting more than the eleven fractions in this study with narrower time intervals, to be able to fully take advantage of the acidic and alkaline fractionation.

5.8 Toxicity confirmation

The concentrations of the identified compounds in extracts were used to calculate equivalence factors as described in detail in **paper 3**. By using the equivalence factors, the initially observed toxicological effect in the extracts could be correlated with that of the concentration of confirmed compounds for the AR and ER assays, see Table 5. In the ER assay, the sum of the EQ_{calc} for the three compounds BPA, DBP and BBP were also higher than the EQ_{meas} for the extract of sample S8, which suggests that these compounds explain the response observed for the extract. The same was observed for AA and DHAA in the extract of sample S4 in the AR assay. This result suggests that the causative agents for the toxicological effect were identified. The slightly higher EQ_{calc} compared to the EQ_{meas} in both assays could be caused by other compounds being present in the extract that inhibits the confirmed compounds from activating the respective receptors.

Basic red 1 and Baso red 546 were only identified in the alkaline fraction with toxicological effect in the AhR assay, Solvent Violet 8 was confirmed in the alkaline as well as the acidic fraction. However, the concentration of Solvent Violet 8 was significantly higher in the alkaline fraction compared to the acidic fraction, 70 μg dm^{-2} and 0.7 μg dm^{-2} respectively. The structures of Basic Red 1 and Baso Red 546 were considered as similar to known AhR ligands during read-across assessment; these compounds were also included in the *in vitro* testing in order to elucidate potential cumulative effects. When analytical standards of Solvent Violet 8, Basic red 1 and Baso Red 546 were tested individually in the AhR assay, all dyes had a toxicological effect. However, when the EQ values from Solvent Violet 8, Basic red 1 and Baso Red 546 were added to calculate the EQ_{calc}, this value was smaller (<1%) than the EQ_{meas} calculated from the response in the extract. This indicates that the identified compounds tested *in vitro* cannot alone explain the observed response from the extract of sample S8.

The AhR is known to bind ligands such as polyaromatic hydrocarbons (PAHs), dioxins, polychlorinated biphenyls (PCBs) and other endocrine disruptors such as certain pesticides (Long 2003; Fujii-Kuriyama & Mimura 2005). These groups of compounds are highly potent and could cause a toxicological response in the AhR assay even at very low concentrations. In addition, all these compounds are readily soluble in organic solvents, especially water-miscible alcohols such as ethanol and methanol (Li & Andren 1994; Mackay et al. 1997), and should therefore in theory be extracted for the paper matrix and remain in solution during the fractionation process. The alkaline and acidic fractions number 8 positive in the AhR assay were therefore analysed in a targeted screening for a

selection of these compounds, see Appendix C. None of the compounds listed in Appendix C were found in the fractions after the re-interrogation of data.

Table 5. Calculated and measured equivalence factors (EQ_{calc} and EQ_{meas}) in μM of in the AR, ER and AhR reporter gene assay, respectively, for extract S8 and S4, as well as identified compounds causing changes in activity in extracts. [a]Concentrations (μM) for identified compounds in extract at maximum response.

ESTROGEN RECEPTOR ACTIVITY						
EXTRACT	BPA	DBP	BBP		TOTAL EEQ	
	0.08 μM[a]	0.19 μM[a]	μM[a]	EQ	EQ_{calc}	EQ_{meas}
S8	EQ: $1.11*10^{-5}$	EQ: $1.89*10^{-7}$	0.07	$1.99*10^{-7}$	$1.42*10^{-5}$	$2.23*10^{-6}$

ANDROGEN RECEPTOR ACTIVITY				
EXTRACT	DHAA	AA	TOTAL EEQ	
	3.91 μM[a]	485.2 μM[a]	EQ_{calc}	EQ_{meas}
S4	EQ: $2.14*10^{-4}$	EQ: $1.49*10^{-1}$	$1.49*10^{-1}$	$8.84*10^{-2}$

ARYL HYDROCARBON RECEPTOR ACTIVITY					
EXTRACT	Solvent violet 8	Basic Red 1	Baso Red 546	TOTAL EEQ	
	0.4 μM[a]	50 μM[a]	50 μM[a]	EQ_{calc}	EQ_{meas}
S8	EQ: $7.68'10^{-9}$	EQ: $6.34'10^{-9}$	EQ: $6.34'10^{-9}$	$2.0'10^{-8}$	$8.1*10^{-6}$

The testing of Solvent violet 8, found in sample S17, in the mutagenicity test is on-going, see **paper 4**. Due to the low concentrations of Leucocrystal violet (see Table 4) in the microwave popcorn bag extract, it was decided not to test this in the initial toxicity confirmation tests. Overall, the results from this study supports the findings reported by other studies, recycled paper contain more contaminants with potentially adverse health effects than virgin fibre (Binderup et al. 2002; Vinggaard et al. 2000; Biedermann & Grob 2010).

In those cases were the tentatively identification was regarded as inaccurate after being compared to analytical standards; the peaks are to be regarded as unidentified. Yet the compounds selected for further investigation with confirmed identity was found to be responsible for a majority of the observed toxicological effect in two of the three *in vitro* assays investigated where the full strategy was implemented. This means that even though there are some significant peaks in the fractions that could be viewed as unidentified, it is likely that a correctly identified compound tested *in vitro* would only contribute marginally to the overall observed effect in at least three of the four cases where the overall strategy was implemented.

5.9 Sources of compounds and human exposure

It can be concluded, based on the EQ value presented in Table 5, that BPA was the compound driving the toxicological effect in the ER assay. BPA was found in extracts from sample S8, a pizza box made from recycled fibreboard. This is in agreement with earlier studies (Triantafyllou et al. 2002; Vinggaard et al. 2000), where BPA was found in samples made from recycled paper. BPA has previously been established as an EDC due to its ability to bind to the ER (Gould et al. 1998; Grignard et al. 2012). Although diet is considered as the main contributor of BPA to human exposure (Beckman et al. 2014), there are no description of potential contaminant sources. It is therefore useful to gather more information about additional sources of contaminations before concluding that paper and board FCMs are significant contributors to the human exposure of BPA. Phthalates have a lower potency in the ER assay, in comparison to BPA. Even though some relatively high concentrations (see Table 4) of phthalates were found in the pizza box, these amounts were too low to contribute significantly the measured toxicity of the extract.

In the extract for sample S4 with effects in the AR assay, AA was found to be the main contributor to the measured toxicological effect, see Table 5. AA is naturally occurring as an extractive found in the wood (Roberts 1996b), and it can therefore be present in large amounts in the finished paper product. Although there are no data available on which pulping process was used to produce this paper sample, it can be assumed that it was acidic sulphite pulping. This pulping method produces paper with relatively small pores, which is useful for greaseproof paper (Biermann 1993). More importantly, this pulping method also maintains a large part of the extractives found in the original material through the pulping process and into the finished paper product.

A relatively high concentration of AA and a lower concentration of DHAA were found in sample S4 (see Table 4), a sandwich wrapper made from virgin fibre. Even though the Soxhlet extraction using 99.9% ethanol could be described as crude, both AA and DHAA are able to migrate under less severe extractions as well, such as 20% ethanol and water (Ozaki et al. 2006). These findings suggest that migration of AA and DHAA also occurs during more realistic conditions when assessing human exposure. AA has also been reported to migrate from FCMs into food, especially dry foods such as flour and sugar (Mitani et al. 2007). Since AA and DHAA is present in large amounts in paper products and are able to migrate into foods, it suggests that human exposure may occur. Studies have previously reported genotoxic effects of AA and DHAA in *in vitro* tests (Ozaki et al. 2004), however this is the first time antiandrogenic effects has been observed for DHAA.

The EQ values presented for the three dyes; Basic Red 1, Baso Red 546 and Solvent Violet 8, indicated that these compounds could not alone explain the response of the extract from sample S8 in the AhR assay. Further studies are thus needed to be able to fully explain the measured toxicological effects, preferably starting with the eight compounds selected through read-across selection, see **paper 2**, but without commercially available analytical standards. In addition, Solvent Violet 8 was also responsible for the mutagenic effect in the microwave popcorn bag. Solvent violet 8 is classified as a triamniophenylmethane solvent dye, and has previously been associated with genotoxic effects *in vivo* (Littlefield et al. 1989; Littlefield et al. 1985). The dark violet colour added by the dye appears black when printed on darker surfaces such as the unbleached paper of the microwave popcorn bag.

Overall, the identified compounds associated with the toxicological effects described in this study are all comparatively semi-polar. These compounds have the ability to migrate more readily from the paper and board FCMs than polar compounds, due to a lesser interaction between the semi-polar compounds and the hydroxyl-groups in the matrix. This is concerning, since it suggests that humans are exposed to these compounds through the food for which limited toxicological data is available. However, future studies are needed to further investigate the ability of identified compounds, especially the dyes, to migrate through the paper matrix, as they are usually applied on the non-food contact side, and into the food. In addition, there is need for studies to investigate the importance of pore size of some of the most common paper types for migration rate in order to determine which papers are most permeable for compounds originating from printing inks.

5.10 Example of the bioassay guided strategy

After extraction and *in vitro* testing of the initial 20 samples, an extract from sample S8, a pizza box made from recycled paper, had a positive response in the AhR assay, see Figure 21a. The extract was therefore fractionated and subsequently tested in the same cell assay. The results from the second screening in the AhR assay revealed a toxicological response in the acidic fraction number 9 and the alkaline fraction number 9, see Figure 21b. Both fractions were collected when the organic mobile phase composition was increased from 90% to 100%. The fractions were analysed by GC-EI-qTOF and UHPLC-ESI-qTOF MS in order to identify candidate compounds responsible for the measured effect in the AhR assay.

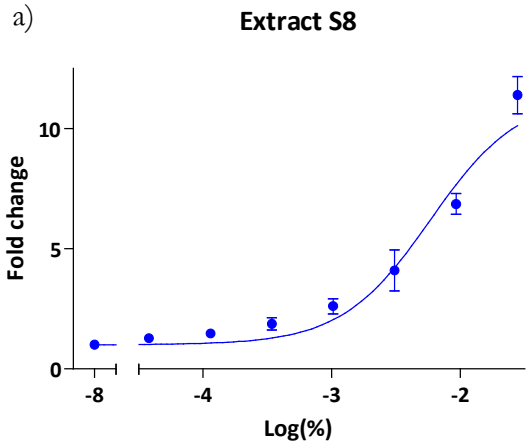
As an example of the entire workflow for tentative identification by UHPLC-ESI-qTOF MS and quantification, Figure 21c and d shows a base peak chromatogram (BPC) from alkaline fraction number 9 from sample S8 and the spectra obtained at R_T 7.6 minutes. The single ion in the spectra was

determined to be the quasi-molecular ion and fifteen possible molecular formulas were generated by the MFG. Next, the isotope ratio was used to select the most matching formula, as seen in Figure 21d. This formula, $C_{24}H_{27}N_3$, had the relatively high measured mass error of 7 ppm when compared to the theoretical monoisotopic mass of this compound, yet a perfect fit between the theoretical isotope ratio for the molecular formula and the measured isotope ratio. These results confirm those found by Kind & Fiehn (2007), that isotope ratio are more important for a correct tentative identification than mass accuracy.

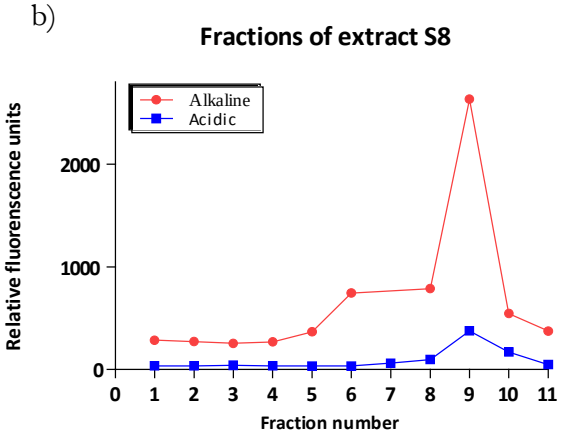
When the formula was run against the accurate mass database, the suggested formula was matched to that of Solvent Violet 8, see Figure 21e. Solvent Violet 8 is a dye used in printing inks, which is soluble in both ethanol and methanol. The compound would therefore be extracted by ethanol and eluted by methanol during the fractionation process. Although there is no specific pK_a value for Solvent Violet 8, the dimethylamine groups suggest a pK_a around 10.6. This means that during alkaline conditions, the compound is ionised, and during acidic conditions the compound is uncharged. When analysed in QSAR, this structure were outside the domain of the dataset for the AhR assay. However, earlier studies have suggested that printing inks could potentially be linked to toxicological effects in the AhR assay (Binderup et al. 2002).

Solvent Violet 8 was selected for further investigations due to structural similarities to known AhR ligands, see **Paper 2**. None of the compounds tentatively identified by GC-EI-qTOF MS was selected for further testing. When the fraction and standard was analysed by UHPLC-ESI-QqQ MS all parameters necessary for a positive identification; relative Rt, ion transitions and ion ratio matched, see Figure 21 f-h. A higher concentration of Solvent Violet 8 was found in the alkaline fraction than in the acidic fraction, suggesting that during acidic conditions the neutrally charged dye will elute in a later fraction with a higher organic composition in the mobile phase. When the analytical standard of Solvent violet 8 was analysed in the AhR assay, it was concluded that this compound alone could not explain the measured toxicological effect, see Section 5.8.

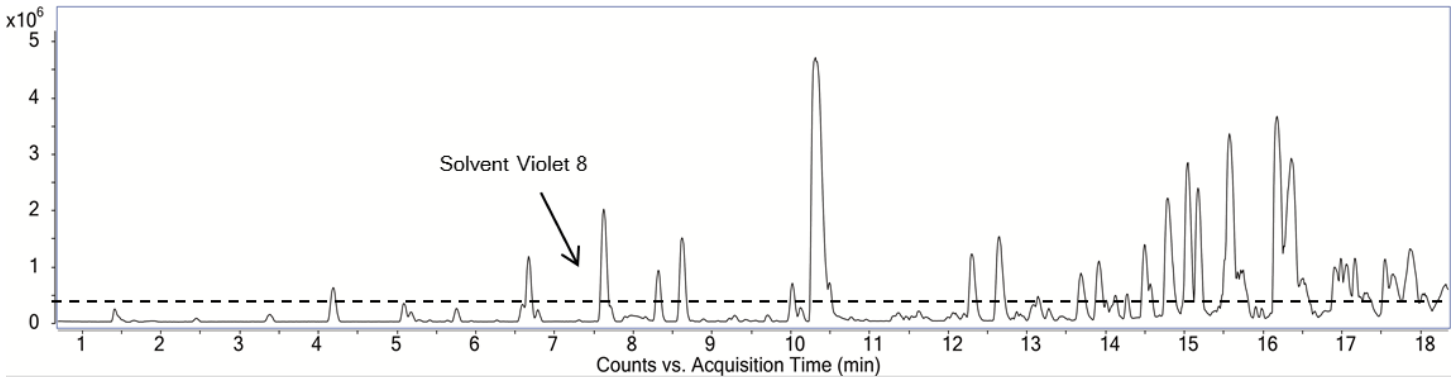
a)


Extract S8
Fold change
10
5
0
-8
-4
-3
-2
Log(%)

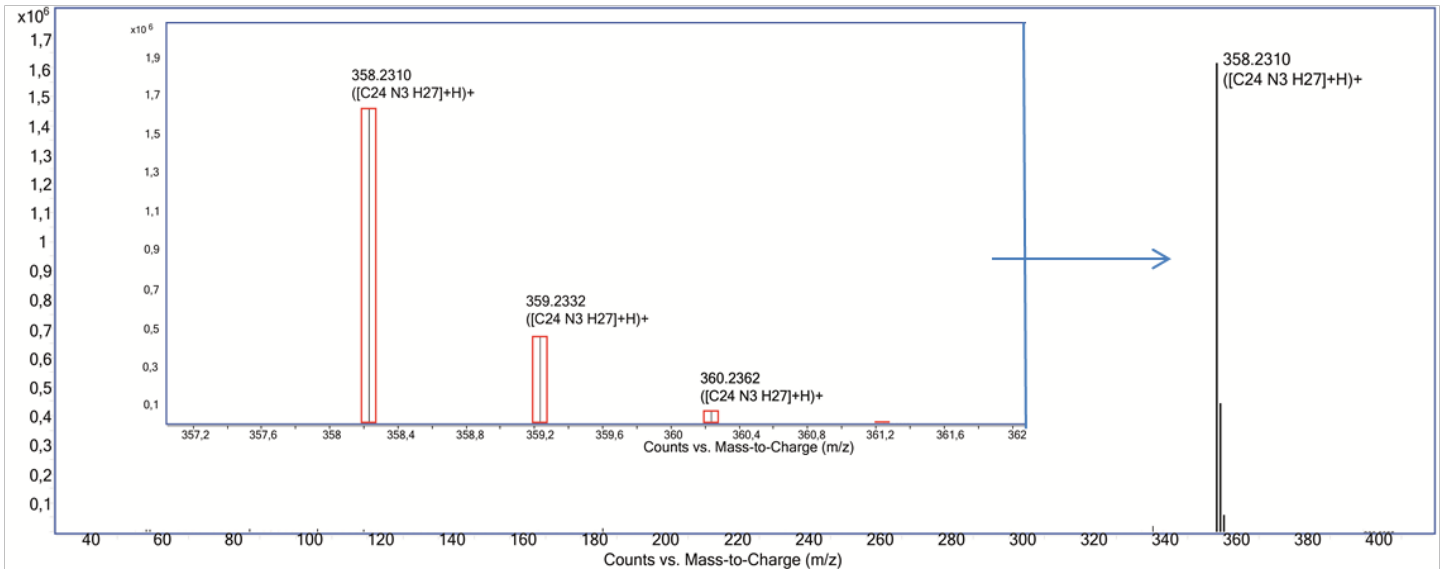
b)


Fractions of extract S8
Alkaline
Acidic
Relative fluorenscence units
2000
1000
0
0 1 2 3 4 5 6 7 8 9 10 11
Fraction number

c)


x10^6
5
4
3
2
1
0
Solvent Violet 8
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Counts vs. Acquisition Time (min)

d)


x10^6
1,7
1,6
1,5
1,4
1,3
1,2
1,1
1
0,9
0,8
0,7
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0,5
0,4
0,3
0,2
0,1
358.2310
([C24 N3 H27]+H)+
359.2332
([C24 N3 H27]+H)+
360.2362
([C24 N3 H27]+H)+
357,2 357,6 358 358,4 358,8 359,2 359,6 360 360,4 360,8 361,2 361,6 362
Counts vs. Mass-to-Charge (m/z)
358.2310
([C24 N3 H27]+H)+
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400
Counts vs. Mass-to-Charge (m/z)

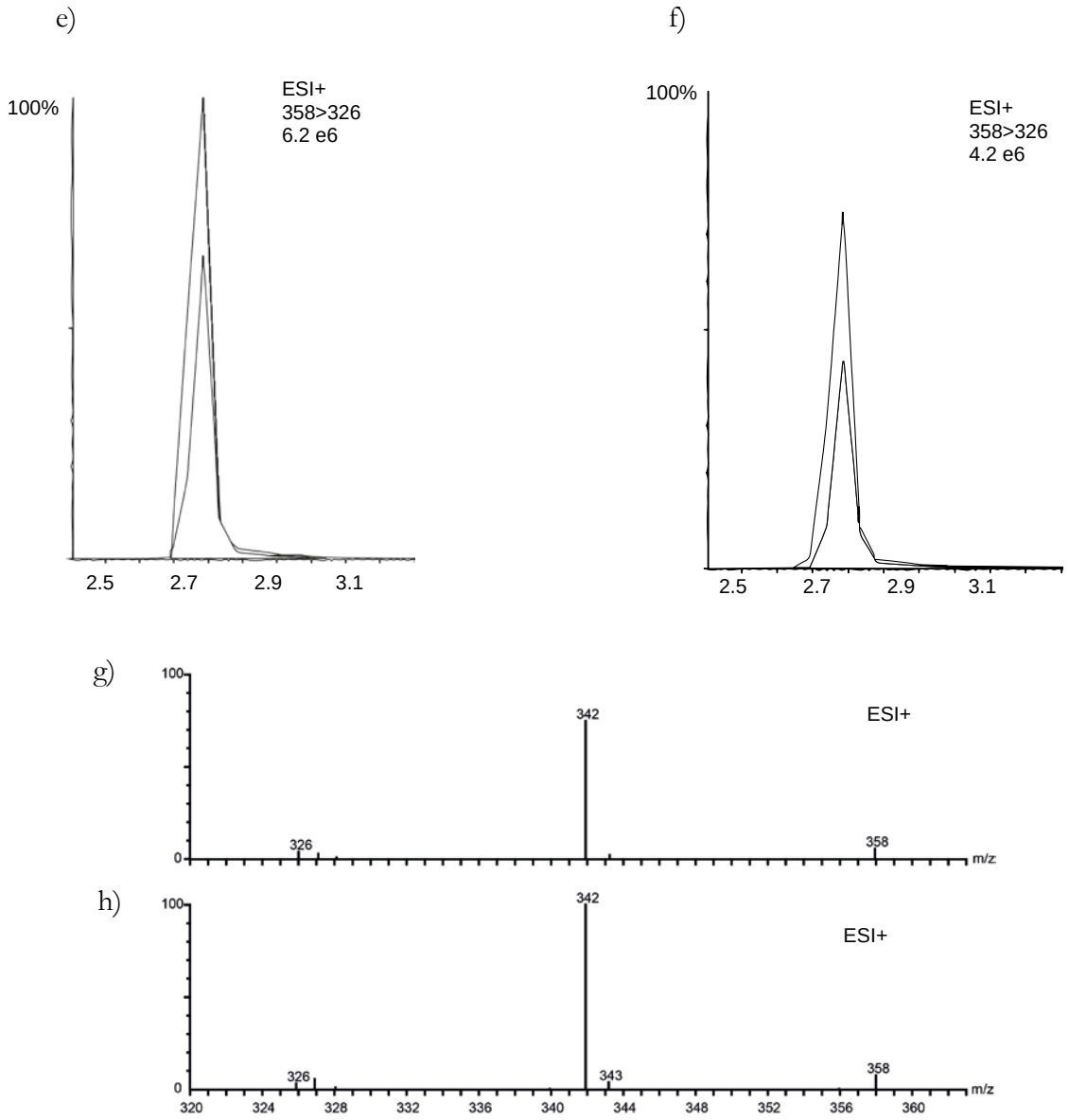

Figure 21. *Opposite side*: a) Toxicological response of extract from sample S8, a pizza box, in the AhR assay. Data from extract were normalized to controls and fitted to a sigmoidal dose-response model. b) Toxicological response (in relative fluorescence units) in the AhR assay of the fractions from S8. Graphs are based on one representative experiment in extract and fractions. Error bars represent standard deviations (SD) c) BPC of alkaline fraction number 9 from sample S8 in positive mode d) Spectra from the peak of Solvent Violet 8 obtained at 100 V with UHPLC-ESI-qTOF MS with suggested molecular formula ($C_{24}H_{27}N_3$) and the isotope pattern of suggested formula. *This side*: e) Retention time and ion transitions for the standard of Solvent Violet 8 obtained by UHPLC-QqQ MS/MS in MRM mode. f) Retention time and ion transitions for the fraction suspected of containing Solvent Violet 8 obtained the same method as the standard. g) Fragmentation pattern of Solvent violet 8 standard at 1 μg/mL h) Fragmentation pattern of alkaline fraction number 9 from sample S8 in positive mode (diluted 1:1000 *v/v* in ethanol).

6. Conclusions

The hypothesis for this project was that by combining chemical and toxicological methods, in a bioassay guided study, the identification of unknown problematic compounds in paper and board would be improved. To answer this hypothesis, a screening strategy enabling a rationalised workflow was developed, focusing the more time-consuming steps of, for example, identification on a subset of samples. Overall, this PhD study has been successful in showing that a bioassay guided analysis, combining both chemical and toxicological analyses, can be used to identify compounds present in paper and board FCMs with potentially adverse health effects. In addition, the use of a comprehensive extraction and identification strategy increases the possibility of analysing a wide range of analytes with different functional groups, molecular masses, vapour pressures and boiling points.

By using the bioassay guided strategy proposed in this study, compounds with ED effects, effects on the metabolism of xenobiotics or mutagenic effects were identified. The findings of several different toxicological effects in paper and board FCMs are of great concern and indicate the importance of using broad chemical and toxicological analyses in safety assessments of paper and board.

The concentration of compounds found in the extracts was successfully correlated in two of the three toxicological assays investigated with the originally measured toxicological effect. This proves that the suggested bioassay guided strategy is a powerful tool that can be used for future investigations and safety assessments of paper and board FCMs. The aim for the tentative identification process was to develop generic and complementary methods of analysis, covering as many different compounds as possible. Results from this study shows that by using two fundamentally different separation methods and by using two different detection modes enables the analysis and identification of a larger span of compounds than shown in earlier studies, which is important for the overall safety assessment of FCMs.

The tentative identification of compounds by GC-EI-qTOF MS were overall successful, as all compounds selected for further investigation by this method were confirmed by standards. Furthermore, the proposed method for the tentative identification of compounds in UHPLC-ESI-qTOF MS was especially successful for compounds with an entry in the material matched accurate mass database. The development of this customised database by gathering as much information about compounds being used in paper and board FCMs as possible was an important step towards the establishment of an effective tentative identification process for UHPLC-ESI-qTOF MS.

7. Future perspectives

In the future, the strategy proposed by this study can be used to screen new and existing paper and board products for potential adverse health effects using a toxicological test battery of several different end-points. Some of the strengths with this strategy are that it allows for fast turnover as well as having the potential for identification of unknown compounds and evaluating them for toxicological effects. Besides the fractions presented in this thesis, there are other fractions with a toxicological response in other of the tested assays that are yet to be analysed. In addition, further studies are required to be able to fully explain the observed effect in the AhR assay. Future studies would also involve investigations of the potential of the identified compounds ability to migrate into food in order to elucidate human exposure.

Commercial availability of compounds was amongst the criteria for selection for further analysis, as analytical standards were required for chemical confirmation of identity as well as toxicological testing. An improvement could be to further fine-tune the fractionation in order to narrow down the number of compounds present in each fraction even more, and possibly even isolate a single compound in one fraction. This would allow for toxicological evaluation without an available commercial standard. Another improvement would be to further expand the customised database with relevant compounds, especially newly identified NIAS and emerging contaminants, as this enhances the possibility for a correct tentatively identification of unknowns. As the qTOF MS data was acquired in full scan mode, it is possible to re-interrogate the data to be able to tentatively identify unknowns with an updated version of the database and possibly be able to explain more of the observed toxicological effect by this new information. The ability to re-interrogate could also be used to detect trends in usage of certain compounds over time in paper and board FCMs.

Based on the results presented in this study along with other interdisciplinary studies, there is need to test a much broader spectrum of commercially available paper and board FCMs as well as a larger subset of products with toxicological effects to investigate whether this is a pervading issue.

There are many challenges ahead for both the industry as well as international and national agencies to ensure the safety of food contact materials. In addition to large knowledge gaps about the toxicological effect of individual compounds used in these products, there are also gaps between legislation and reality for the safety assurance of paper and board FCMs when referring to mixtures of several hundreds or thousands of compounds. European legislation states that migration of compounds from FCMs should not endanger human health. Yet, as many of the compounds in paper and board FCMs

never have been properly toxicologically evaluated, neither individually nor when present in mixtures, this comprehensive safety assurance is not possible. Up to date, investigations of toxicological effect of compounds or mixtures of compounds are generally regarded as both time-consuming and expensive. My hope is that this thesis could serve as a starting point towards a more efficient evaluation and identification of toxicological active compounds found in food contact materials.

8. References

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9. Appendices

9. 1 Paper 1

Linda Bengtström, Xenia Trier, Kit Granby, Anna Kjerstine Rosenmai & Jens Højslev Petersen (2014) Fractionation of extracts from paper and board food contact materials for in vitro screening of toxicity, Food Additives & Contaminants: Part A, 31:7, 1291-1300

9.2 Paper 2

Linda Bengtström, Lisbeth Krüger Jensen, Kit Granby , Xenia Trier, Malcolm Driffield, Jens Højslev Petersen (2014) Identification of contaminants in paper and board food contact materials using bioassay guided screening and high resolution mass spectrometry . Manuscript in preparation

9.3 Appendix A

Comprehensive lists of tentatively identified compounds in fractions with AhR effects

9.4 Appendix B

Accurate mass database used for the tentative identification process for data acquired by UHPLC-ESI-qTOF MS

9.5 Appendix C

List of selected compounds for targeted screening in fractions with AhR effects

9.6 Paper 3

Rosenmai, A.K., Bengtström, L., van Vugt-Lussenburg, B.M.A., Trier, X., Pedersen, J.H., Granby, K., Taxvig, C., and Vinggaard, A.M. (2014). A strategy to identify problematic chemicals in food contact materials of paper and board. Manuscript in preparation

9.7 Appendix D

Comprehensive lists of tentatively identified compounds in fractions with ER and AR effects

9.8 Paper 4

Linda Bengtström, Jens Højslev Petersen, Kit Granby, Xenia Trier, Mona-Lise Binderup (2014). Identification of unknown mutagenic compounds in microwave popcorn bags. Manuscript in preparation to be submitted to Food and Chemical Toxicology as a Short Communication

9.9 Appendix E

Comprehensive list of tentatively identified compounds in extract from microwave popcorn bag with mutagenic effects

9.1

Paper 1.

Linda Bengtström, Xenia Trier, Kit Granby, Anna Kjerstine Rosenmai & Jens Højslev Petersen (2014) Fractionation of extracts from paper and board food contact materials for in vitro screening of toxicity, Food Additives & Contaminants: Part A, 31:7, 1291-1300

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Fractionation of extracts from paper and board food contact materials for *in vitro* screening of toxicity

Linda Bengtström[a]*, Xenia Trier[a], Kit Granby[a], Anna Kjerstine Rosenmai[b] and Jens Højslev Petersen[a]

[a]*Division of Food Chemistry, National Food Institute, Technical University of Denmark, Copenhagen, Denmark;* [b]*Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark, Copenhagen, Denmark*

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Paper and board used as food contact materials (FCMs) are chemically complex matrices, partly due to the naturally occurring substances in paper and board, but also due to the chemical treatment of the paper used to make it suitable for food contact. In order to assure the safety of packaging materials, information on the exposure as well as on the toxicity of substances in the packaging must be obtained. This study describes a comprehensive method for the extraction and fractionation of substances present in paper and board FCMs for further investigation by *in vitro* testing and chemical analysis. The extraction efficiency and the fractionation process were validated by determining recoveries in extracts from paper and board fortified with five surrogates of known concentration. The recoveries for the five surrogates were between 20% and 104% in the raw extract and between 21% and 109% after extraction and fractionation. The fractionation both reduces the number of compounds to be identified and works as a sample clean-up by reducing matrix effects. Raw extracts and fractions from two paper and board FCMs were furthermore tested in the aryl hydrocarbon receptor (AhR) reporter gene assay. Both raw extracts and two of the fractions of the raw extracts gave a positive response in the AhR assay. The strategy of extraction followed by fractionation offers a powerful tool in order to make the workflow for screening FCMs for potentially adverse effects more efficient.

Keywords: food packaging; paper; cardboard; *in vitro* toxicological screening; extraction; fractionation

Introduction

Paper and board are the most common food packaging materials (FCMs) after plastics. Approximately 17% of all packaging annually sold in the United States is fibre-based food packages (Rexam 2011). Consumers are therefore likely to eat food packed in paper and board FCMs in their everyday life and thus may potentially be exposed to chemicals through this source. Porous materials such as paper and board offer little resistance towards the mass transfer of migrating compounds, thus migration occurs regardless of direct contact with the foodstuff (Barnes et al. 2007). Particularly recycled paper and board as FCM might pose a problem concerning migrating substances due to the varying origins of the starting materials (Biedermann & Grob 2013a). Some of these starting materials are not intended to end up in food packaging and could contain large amounts of substances with adverse health effects (Biedermann & Grob 2010; Biedermann & Grob 2013a).

A specific regulation for FCMs of paper and board does not exist in the European Union, as is the case for FCMs made of plastics (EFSA 2012). Though there are a number of national recommendations and legislations, they are not necessarily based on current risk assessment principles (EFSA 2012). Fibre-based food packaging is chemically complex, containing thousands of both naturally occurring and added substances, and can also comprise several layers with different origins and properties (Canellas et al. 2010; Honkalampi-Hämäläinen et al. 2010; EFSA 2012). Some of the major sources of potential migrating substances found in paper and board are constituents in printing inks, adhesives, sizing agents and coatings. Compounds known to be present in recycled FCMs of paper and board, such as PFOA and bisphenol A, have caused adverse effects in animal studies (Lau 2005; Moral et al. 2008; Xu et al. 2011). Since only a small fraction of the numerous chemicals in these types of materials have been identified, at present little is known with respect to the potential adversity of compounds used in FCM of paper and board.

In order to assure the safety of packaging materials, it must be investigated whether substances in the packaging materials could lead to migration, and thus exposure, in amounts that could have adverse health effects. This assessment includes the detection and identification of all potentially relevant compounds above a certain concentration level in a comprehensive analysis. There have been several attempts to describe a systematic comprehensive methodology for the analysis of migrating compounds in paper and board FCMs (Castle et al. 1997; Honkalampi-

*Corresponding author. Email: lben@food.dtu.dk

Hämäläinen et al. 2010; Biedermann & Grob 2013b). However, identification of unknown compounds in a complex mixture as paper matrix is both time consuming and painstaking and does not in itself give information on the potential to cause adverse health effects.

Therefore, some interdisciplinary studies have tried to screen paper and board FCMs by using both chemical analysis and *in vitro* tests (Vinggaard et al. 2000; Binderup et al. 2002; Lopez-Espinosa et al. 2007; Bradley et al. 2008; Koster et al. 2014). This process is used as a fast screening, excluding irrelevant samples and enabling further investigations on only toxicologically relevant samples. This approach leads to high demands on the extraction method, as it should be both comprehensive and compatible with *in vitro* assays. Even after an initial screening phase, the analysis of toxicologically relevant extracts by chromatographic methods will still give complex results, described as a forest-of-peaks analysis (Bradley et al. 2008, 2010; Koster et al. 2014). By fractioning the raw extracts into a number of fractions, and subsequently testing these in *in vitro* assays, the number of substances relevant for identification will be further reduced.

The aim of this study was to develop a generic method for the extraction and fractionation of chemicals present in paper and board FCMs with the purpose of testing these *in vitro*. We extracted semi-volatile and non-volatile organic compounds from two types of paper, both intended as being in direct contact with food, with a boiling ethanol reflux system, followed by a vaporisation step. These raw extracts were initially screened *in vitro* in the aryl hydrocarbon receptor (AhR) reporter gene assay. We then fractioned the samples by injecting the raw extracts in an HPLC system and collected the fractions according to time.

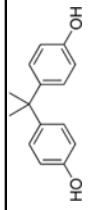
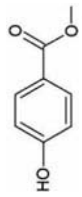
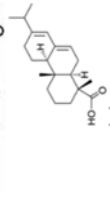
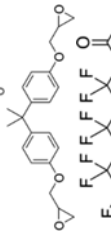
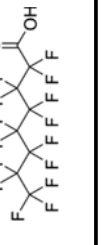
The extraction and fractionation method was validated by fortifying paper samples with five surrogates (Table 1), selected by reported use in fibre-based food contact materials and toxicological relevance. They were also chosen to represent different physico-chemical properties, such as molecular weight, partition coefficient (log*P*), vapour pressure, boiling point and acid disassociation constant (pK_a). A concentration of surrogates, 50 ng dm^{-2}, was chosen based on the threshold of toxicological concern (TTC) for compounds with genotoxic or endocrine disruptive effects (EFSA Scientific Committee 2012). This threshold of 0.15 µg/person/day, corresponds to 25 ng dm^{-2} assuming an intake of 1 kg of foods/person/day and that the food come into contact with 6 dm^2 of the particular FCM (EU 10/2011, 2011).

Materials and methods

Chemicals

Ethanol (99.9%), used for both the fortification of paper samples and extraction, was obtained from Merck (Darmstadt, Germany). The methanol (99.9%) used for

Table 1. Compounds used as surrogates for the fortification of paper samples.

Compound	Abbreviation	CAS number	Mw (g mol^{-1})	log $K_{O/W}$	Vapour pressure (Pa)	Boiling point (°C)	pK_a	Structure	Relevance
Bisphenol A	BPA	80-05-7	228.29	3.32	5.34E–07	401	10.29		Monomer and additive
Methylparaben	MP	99-76-3	152.15	1.882	5.55E–03	266	8.31		Possible occurrence in the raw material
Abietic acid	AA	514-10-3	302.45	6.1	5.96E–09	440	4.64		Occurrence in the raw material. Additive
Bisphenol A diglycidyl ether	BADGE	1675-54-3	340.41	3.71	3.66E–09	487	–		Used in epoxy resins
Perfluorooctanoic acid	PFOA	335-67-1	414.07	6.444	1.2E–03	188	3		Grease repellent

Note: Boiling point is in °C at 760 Torr; vapour pressure is Pa at 25°C; log $K_{O/W}$ and pK_a are at 25°C.

Table 2. Properties of paper packing materials used.

Sample	Type	Pulp	Recycled	Grammage (g m^{-2})
Virgin fibre	Paper	Spruce	No	40
Recycled fibre	Corrugated fibreboard	Recycled	Yes	550

mobile phases was purchased from Rathburn (Walkerburn, UK). The bisphenol A (BPA), methylparaben (MP), bisphenol A diglycidyl ether (BADGE), perfluorooctanoic acid (PFOA) and abietic acid (AA) standards were obtained from Sigma-Aldrich (Copenhagen, Denmark).

Fortification of paper samples

Paper and board samples, 6 dm^2 cut into 2.5 × 10 cm strips, were placed on a sheet of aluminium foil with folded edges. The characteristics of the paper and board samples used for the fortification are listed in Table 2. The paper samples were then soaked in the surrogate mix solution (25 ml, 12 ng ml^{-1}) (Table 1) and left to dry in a fume hood for approximately 60 min. The fortification of virgin paper was performed in two to three rounds, due to the large amount of surrogate mix solution. Three sample replicates were prepared for each paper or board sample. The paper samples in this study were fortified with a concentration of surrogates equivalent to 50 ng dm^{-2}.

Extraction of paper samples

Two different sizes of extraction systems were used. The fortified paper samples for chemical analysis only, 6 dm^2, were transferred to a 250 ml Soxhlet boiling reflux system chamber after the fortification. The chamber was connected to a 1 L round-bottomed flask containing 350 ml ethanol. Paper samples for both *in vitro* tests and chemical analyses (approximately 90 dm^2) were cut into strips and placed in a 500 ml Soxhlet boiling reflux system chamber. The chamber was connected to a 2 L round-bottomed flask containing 650 ml ethanol. The ethanol in the boiling reflux system was set to boil for 2 h under vacuum, after which the extract was transferred to a Büchi B-811 Extraction System (Flawil, Switzerland). The Büchi system was cleaned twice with ethanol between samples. The ethanol was evaporated without the application of vacuum until approximately 5 ml of the extract was left. The raw extract from the fortified paper samples was then further concentrated to approximately 0.5 ml under a gentle stream of nitrogen at 70°C, and diluted 1:10 v/v with ethanol. The raw extracts from paper samples for *in vitro* tests were concentrated in the same manner. As a control, the surrogate mix solution was also added directly to a round-bottomed flask with ethanol. There was no paper matrix in the chamber and the control was treated as the other samples.

HPLC fractionation

The extracts were fractioned using both acidic and factor eluents by a Waters 2695 chromatograph (Milford, MA, USA) coupled to a Gilson ASPEC XL (Middleton, WI, USA). The column used was a XTerra C18 column from Waters (5 µm, 250 × 4.6 mm i.d.) with a 0.2 µm in-line filter. Prior to the fractionation process the extracts were ultracentrifuged at approximately 9000*g* for 5 min (Ole Dich microcentrifuge 154, Hvidovre, Denmark). The supernatant were transferred to a vial, except for a portion of the extract which was removed for direct chemical analysis and *in vitro* testing. The pellet was resuspended in ethanol and also tested *in vitro*. The binary mobile phases consisted of water with 0.1% formic acid (mobile phase A) and methanol with 0.1% formic acid (mobile phase B), pH ~2, for the acidic fractionation and water with 5 mM ammonia (mobile phase A) and a methanol with 5 mM ammonia (mobile phase B), pH ~10, for the alkaline fractionation. The mobile phase composition was varied according to a linear gradient that increased from 10% to 100% B within 30 min, and was maintained at 100% B for 10 min and then returned to the initial conditions. Total run time was 55 min. The same gradient was applied for both the alkaline and the acidic separation. The injection volume was 100 µl and the flow rate was kept at 0.8 ml min^{-1}. The extracts from fortified paper were injected twice each for the alkaline and acidic fractionations, respectively, and fractions were collected in polypropylene tubes (50 ml; Sarstedt, Nümbrecht, Germany). The extracts from paper samples for the *in vitro* test were injected 10 times each, each injection corresponding to approximately 1.8 dm^2, for the alkaline and acidic fractionations, respectively, to obtain sufficient material for further analysis. The collection of fractions started at 3 min into the run and shifted every 5 min, except for the first fraction which was collected for only 2 min. First, 11 fractions were collected using acidic eluents, then another 11 were obtained with alkaline eluents. Next, the methanol in the fractions was exchanged for ethanol by a nitrogen vaporisation step as described above. A single injection of a surrogate mix (100 ng ml^{-1}) in both acidic and alkaline fractionations was also performed under the same conditions as described above and used as a control sample.

LC-MS/MS analysis of surrogates

Analytes were determined with a Waters Acquity UPLC™ chromatograph coupled to a Micromass mass spectrometer with an ESI. The column used was an XTerra CSH C18 column (2.5 µm, 150 × 2.1 mm) from Waters with a pre-column 0.2 µm filter (KrudKatcher Ultra, Phenomenex,

Værløse, Denmark). The data were acquired with MassLynx v.4.1 software. The mobile phases used for the separation were either water with 0.01% formic acid (mobile phase A1) and methanol with 0.01% formic acid (mobile phase B1); or water with 5 mM ammonium formate (mobile phase A2) and water with 5 mM ammonium formate (mobile phase B2). The chromatographic separation took place in 15 min. The mobile phase composition was varied according to a linear gradient that increased from 20% to 100% mobile phase B within 12 min, maintained at 100% mobile phase B for 3 min and then returned to the initial conditions. Total run time was 18 min. The flow rate was set at 0.2 ml min^{-1}; the injection volume was 3 µl. The capillary in negative mode voltage was –3 kV and +3 kV in positive mode. The desolvation gas flow was 700 l h^{-1} and cone gas flow 110 l h^{-1} of N_2. The source temperature was 120°C and desolvation temperature was 400°C. Argon was used as collision gas at 2.3×10^{-3} mbar. The chromatographic and mass spectrometric parameters for each surrogate are presented in Table 3.

Validation of chromatographic methods

Calibration curve and linearity

The calibration curves were plotted as peak area versus concentration of each surrogate. The calibration was performed using a seven-point calibration curve with concentrations of 0, 10, 20, 50, 100, 200 and 500 ng ml^{-1} for each standard. The calibration curve was weighted ($1/x$). Linearity was established by the coefficient of determination (R^2).

Precision

Precision was evaluated by determining repeatability and reproducibility. Repeatability was obtained by calculating the coefficient of variance (CV, %) for three injections of two samples for 1 day by using Equation (1):

$$\mathrm{CV}\% = \frac{\mathrm{SD}_r}{\text{Mean recovery of surrogate}} \times 100 \qquad (1)$$

where SD_r is the standard deviation within the analysis set.

Reproducibility was determined by calculating the CV % for three samples analysed for three days:

$$\mathrm{CV}\% = \frac{\mathrm{SD}_{iR}}{\text{Mean recovery of surrogate}} \times 100 \qquad (2)$$

where SD_{iR} is the total standard deviation for all samples and sets.

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

LOD was calculated as described in Equation (3); LOQ was defined as described in Equation (4):

$$\mathrm{LOD} = 3 \times \mathrm{SD}_{iR} + \text{blind} \qquad (3)$$

$$\mathrm{LOQ} = 5 \times \mathrm{SD}_{iR} + \text{blind} \qquad (4)$$

Specificity

The specificity of the method was obtained by injecting a matrix-matched sample and a fortified sample to determine that endogenous co-eluting components did not interfere with surrogate response.

Accuracy

The accuracy of the method was assessed by adding a known amount of surrogate standards (Table 1) to the sample matrices. The recovery (%) of each compound from fortified samples was calculated as follows:

$$\%\ \text{Recovery} = \frac{\text{Measured concentration of surrogate}}{\text{Theoretical concentration of surrogate}} \times 100 \qquad (5)$$

Aryl hydrocarbon receptor assay (AhR assay)

Stably transfected rat hepatoma (H4IIE-CALUX) cells provided by Dr Michael Denison (University of California, CA, USA) were used, and the assay was conducted as described in Rosenmai et al. (2014). The raw extracts were tested in threefold dilutions with the maximum concentration being a 400-fold dilution of raw extract in one experiment in triplicate. Fractions were tested in a 400-fold dilution as the only concentration in one to two experiments in duplicates.

As large amounts of 99.9% ethanol were used for the production of the extracts, there was a concern about benzene residues originating from the manufacturing process affecting the results of the *in vitro* tests. However, there was no positive response in blank samples, produced under the same conditions as the raw extracts and fractions although without paper matrix, indicating that any trace benzene was of no concern.

Results and discussion

Choice of extraction solvent

The produced extracts were tested in the *in vitro* AhR assay, and the method was therefore adjusted to fit this purpose through the choice of compatible organic solvent and a high concentration of analytes. Most organic solvents are not suitable for cell assays as they are highly cytotoxic and thus the number of candidate solvents is limited to such solvents as ethanol and dimethyl sulfoxide (DMSO). Ethanol and DMSO have a similar log*P* value,

Table 3. Chromatographic and mass spectrometric parameters for surrogate compounds used for the fortification of paper samples.

Compound	Ionisation	Mobile phase	Precursor ion (*m/z*)	Product ions (*m/z*)	Collision energy (V)	RT (min)	Fractions
BPA	ESI–	Water + 5 mM ammonium acetate:methanol + 5 mM ammonium acetate	227.2	212.1 133.1	20 25	8.27	Acidic: fraction 5, alkaline: fraction 5
MP	ESI–	Water + 5 mM ammonium acetate:methanol + 5 mM ammonium acetate	150.8	91.8 135.8	10 15	6.78	Acidic: fraction 4, alkaline: fractions 1 and 2
AA	ESI+	Water + 0.01% formic acid: methanol + 0.01% formic acid	303.5	257.2 200.8	15 20	13.56	Acidic: fractions 7 and 8, alkaline: fractions 5 and 6
BADGE	ESI+	Water + 0.01% formic acid: methanol + 0.01% formic acid	341.3	324.7 268.5	15 30	10.49	Acidic: fraction 6, alkaline: fraction 6
PFOA	ESI–	Water + 5 mM ammonium acetate:methanol + 5 mM ammonium acetate	413.1	168.9 369.2	15 12	10.12	Acidic: fraction 5, alkaline: fractions 4 and 5

making them capable of extracting similar classes of substances. To avoid cytotoxicity from the solvent, the maximum tested concentration in the AhR assay was a factor 400 dilution of the initial raw extract. For this reason the extracts initially have to be highly concentrated.

In this study we used ethanol extraction to simulate a worst-case migration scenario (Binderup et al. 2002). Ethanol also keeps substances extracted from paper and board in solution. Ethanol is considered to be a versatile solvent, and has both polar and non-polar properties. It is suitable as most substances present in paper and board are water soluble. However, ethanol as an extraction solvent has its limitations, such as a limited solubility for non-polar compounds such as for example alkanes, which are present in some non-water soluble lacquers and printing inks.

Initially we also considered DMSO as a candidate solvent due to its compatibility with *in vitro* assays. However, DMSO was ruled out due to high viscosity, making it difficult to pipette the already highly viscous extracts in a reproducible manner. Its boiling point of 189°C is also problematic as many of the semi-volatile substances would evaporate before the solvent during the vaporisation step.

Other studies have used modified polyphenylene oxide (MPPO) resin as a food simulant for dry and fatty foods in contact with the FCM (Triantafyllou et al. 2002; Bradley et al. 2008; Koster et al. 2014). The MPPO resin is suitable to simulate the transfer of volatile substances that can be transferred via the gas phase as well as direct transfer upon contact with hydrophobic substances. Therefore, the use of MPPO resin alone as a food simulant is not enough to give an overall depiction of migration from all types of FCMs into different foods (Bradley et al. 2008).

Recovery in raw extracts

When comprehensive extracts are analysed by *in vitro* tests, it is important to reduce the loss of substances to a minimum. Even if the recoveries of known analytes can be corrected for the concentrations, it is difficult to establish which recovery factor to correct unidentified substances with. Therefore, we designed the extraction method to minimise the losses of a variety of surrogates having a wide range of physico-chemical properties. Losses of substances are expected to be caused by, for example, vaporisation, adhesion to utensils or chemical degradation.

The paper samples in this study were fortified with a concentration of surrogates equivalent to 50 ng dm^{-2}, corresponding to 600 ng ml^{-1}. In both positive and negative mode the detector signal was significantly suppressed by the presence of sample matrix components in the raw extracts, which were especially apparent in the chromatogram obtained from MP in Figure 1. Therefore, dilution was necessary for the quantification of all surrogates except BADGE. Furthermore, BADGE exhibits a relatively low recovery in both samples compared with the

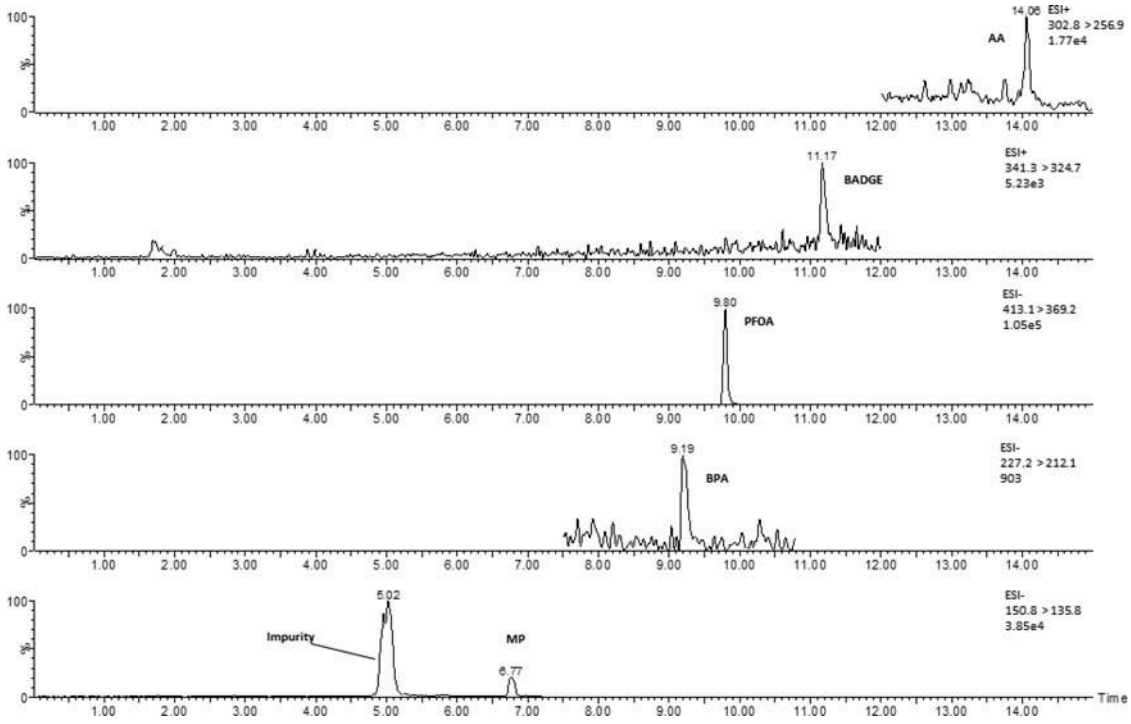


Figure 1. Illustrative chromatograms of the five surrogates in a raw extract from fortified virgin fibre. The extract was diluted 1:10 v/v. A large peak of a matrix component is present in the chromatogram from the MP.

other surrogates, as can be observed in Figure 2. It has been previously established that BADGE readily hydrolyses upon contact with water and/or acids (Philo et al. 1994), which explains the low recovery of BADGE.

The mean recovery for analytes from three replicates of the fortified paper samples is presented in Figure 2. In order to minimise the loss of surrogates, we evaporated the extract under a gentle stream of nitrogen. For the surrogates with the lowest boiling points – PFOA and MP – the mean recoveries for both samples were acceptable at 58% and 93% respectively (2002/657/EC 2002). It can therefore be assumed that other unknown substances with similar physico-chemical properties as PFOA and MP in paper and board matrices have acceptable recoveries.

The recycled fibre contained too high concentrations of the additive abietic acid (AA) to be quantified. AA is used as a sizing agent in paper to enhance the ability for printing inks to remain on the surface of the paper and not be soaked into the capillaries of the porous paper (Roberts 1996). The virgin fibre paper sample is not intended for printing, and thus lacks AA as an additive.

Choice of fractionation technique and buffers

After the initial *in vitro* test of the raw extract, both samples were further examined to identify the substance (s) causing the effect. Since the identification process is both painstaking and time consuming, the raw extracts

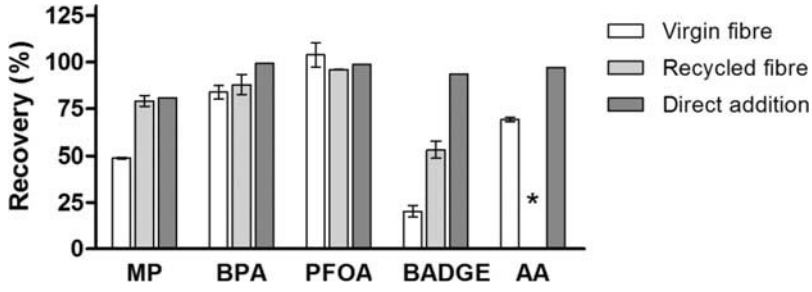


Figure 2. Recovery of 50 ng surrogates $(dm^{-2})^{-1}$ in fortified paper samples after extraction. The direct addition is used as a control sample. Recovery for BADGE is calculated from undiluted sample. Standard deviation is indicated as error bars. *The recycled fibre contained too high concentrations of the additive AA to be quantified.

were fractioned to limit the number of substances to be identified. Each fraction from each sample was then again tested *in vitro.*

Studies have reported different fractionation strategies for extracts from paper and board samples, such as filtering or by several liquid–liquid extractions followed by gel permeation chromatography (Ozaki et al. 2005; Bradley et al. 2008). In comparison with these methods, an advantage of using an HPLC-based fractionation method is that the separation process can be further optimised, e.g. by changing eluents and/or gradient. This makes it possible to separate substances that co-elute in one fractionation round into several sub-fractions in a second fractionation round. The use of several liquid–liquid extractions, as described by Ozaki et al. (2005), is not compatible with *in vitro* assays due to the cytotoxicity of the solvents used. Vaporising the non-compatible solvent and re-dissolving the extracted compounds with a compatible solvent would be futile as the extracted compounds would have a different solubility with this new solvent. The removal of bulk material from the matrix by HPLC fractionation has also been described by Biedermann and Grob (2013b). However, there are limitations to the developed HPLC fractionation method, such as the need for several injections in order to collect enough material for further analysis as well as the molecular size range of substances to be separated.

In order to minimise analyte loss, we centrifuged the raw extracts instead of filtering prior to fractionation. Centrifugation removed non-dissolved bulk material, and the pressure in the HPLC system was stable even after several injections of highly concentrated extract. Fractionation also acts as a sample clean-up, and some surrogates show a considerably higher recovery after fractionation than in the raw extracts because of a reduced matrix effect (Figures 1 and 3). The very large peak from a matrix component in the chromatogram from MP in the raw extract (Figure 1) is significantly smaller in the chromatogram from the same surrogate after the fractionation (Figure 3).

As buffers for the HPLC fractionation, we chose formic acid and ammonia due to their relatively low boiling points and vapour pressures. Theoretically, these two substances will evaporate before final analyses, and will therefore not in any way adversely affect the outcome of the cell assays. Moreover, only a small loss of surrogates could be observed after nitrogen vaporisation, even after vaporisation until dryness (Figure 4). A two-buffer system for fractionation, one acidic and one alkaline, allows substances with different properties to be affected differently by the pH. This in turn will affect the analyte retention time and also peak shape. Analytes with a pK_a value close to the pH of the buffer may have broad peaks and may elute in several fractions. These concentrations could be too low to cause an effect *in vitro* as the compounds of

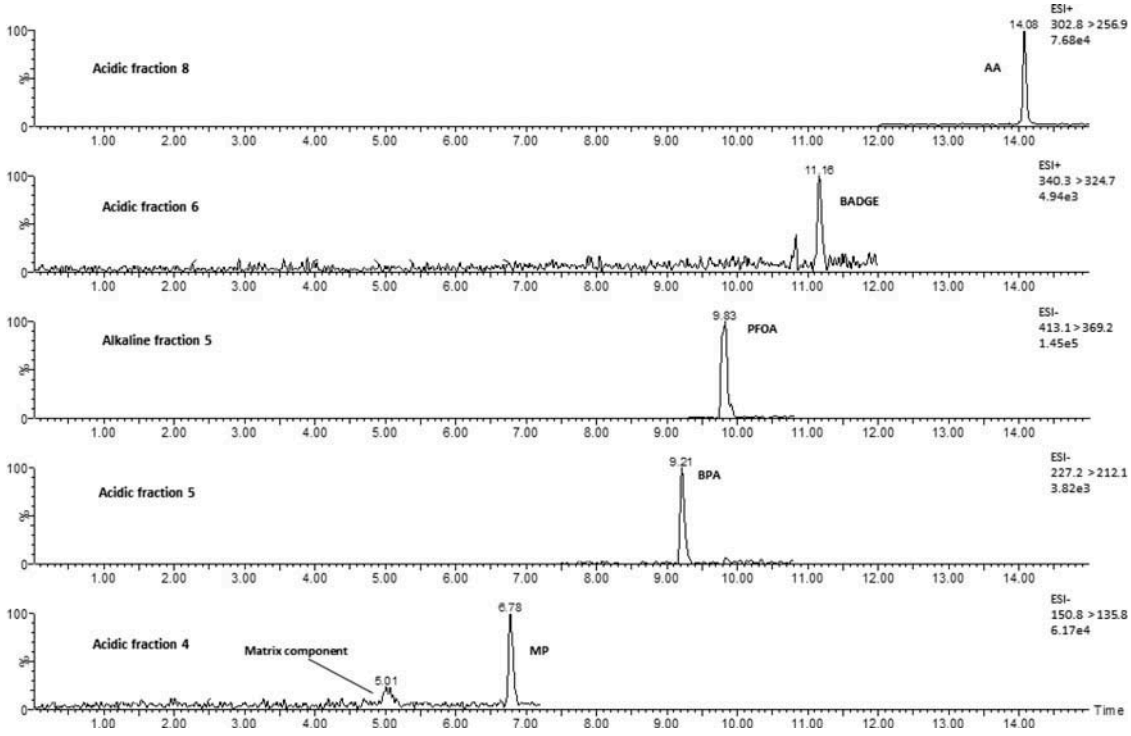


Figure 3. Illustrative chromatograms of the five surrogates in fractions from fortified virgin fibre. The fractions were diluted 1:10 v/v, except for acidic fraction number 6 with BADGE. The large peak of a matrix component in the MP chromatogram is clearly smaller in the fraction compared with the corresponding raw extract.

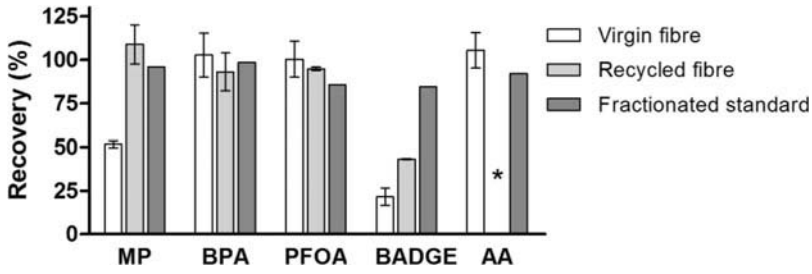


Figure 4. Recovery of 50 ng surrogates dm^{-2} in fortified paper samples after fractionation. All results are from the acidic fractionation, except for PFOA. Standard deviation is indicated as error bars. *The recycled fibre contained too high concentrations of) the additive AA to be quantified.

interest are separated into several fractions. The alkaline and acidic fractionation can also give hints about the functional groups of the substances, as these will be affected differently between the two fractionation processes. This may prove helpful in the identification of compounds giving response *in vitro*.

Recovery in fractionated extracts

The fractionation of the raw fortified extracts separated each of the surrogates into one or two fractions, out of 11, for the acidic and alkaline fractioning respectively according to their retention time (Table 3). It is not necessarily a negative outcome that some analytes elute in more than one fraction, as this can help with the identification process. If two adjacent fractions both show a toxicological response in the same *in vitro* assay, a first working hypothesis could be that the same compound is present in both fractions.

Recovery for PFOA was better in the alkaline fractions than in the acidic. The acidic mobile phase is close to the pK_a value for PFOA, rendering very broad peaks and a spread over several fractions. For PFOA, the retention time decreased as the pH of the mobile phase increased, and a sharper peak was obtained when using an alkaline mobile phase.

Method validation

Validation data after extraction and after fractionation are presented in Table 4. The linearity of the standard curve for each surrogate was R^2 = 0.96–0.99. Both LOD and LOQ for all surrogates were below the concentration required for quantitatively determining the value corresponding to a TTC of 25 ng $(dm^2)^{-1}$, or 300 ng ml^{-1} for 1 kg of foods (Table 4). When diluted 1:10 v/v, the TTC is equivalent to 30 ng ml^{-1} in the raw extract and fractions. Repeatability and reproducibility of the method were within acceptable ranges (2002/657/EC 2002). As there was too high concentration of AA in the recycled fibre, these replicates were not included in the calculations of repeatability and reproducibility (Table 4). The number of replicates of the virgin fibre alone was not enough to assure an adequate degree of freedom required for these calculations. Nevertheless, the fractionation process is affecting the method precision, leading to a greater overall uncertainty.

AhR reporter gene assay

A positive response of the raw extract from recycled fibre was observed in the AhR assay. After the fractionation process, each fraction, 11 acidic and 11 alkaline, was then tested in the same *in vitro* assay, where acidic fraction number 9 and alkaline fraction number 9 showed the

Table 4. Validation parameters for the method developed for the extraction and fractionation of contaminants in paper and board FCMs (n = 12).

Compound	Method	LOD (ng ml^{-1})	LOQ (ng ml^{-1})	Repeatability (CV%)	Reproducibility (CV%)
MP	Extraction	2	8	6	9
	Fractionation	2	7	16	17
BPA	Extraction	1	2	5	5
	Fractionation	2	8	14	17
PFOA	Extraction	2	6	14	17
	Fractionation	2	7	17	22
BADGE	Extraction	8	25	2	7
	Fractionation	5	17	24	24
AA	Extraction	1	3	–	–
	Fractionation	2	6	–	–

greatest change in response. Neighbouring fractions showed slight changes in response, suggesting that the compounds responsible for the effect was fractioned into only a subset of the fractions. The results also indicate that the substance responsible for the positive toxicological response is neutral, since it is the same fraction among both alkaline and acidic fractions that shows a positive toxicological response.

Future studies involve identifying the compound(s) accountable for this result. These investigations are in progress and will be published in upcoming papers.

Conclusions

This article presented a comprehensive extraction and fractionation method for paper and board used as FCMs. The severe extraction method with ethanol could be viewed as a worst-case scenario for migration, and is capable of extracting substances with different physico-chemical properties as well as compatible with *in vitro* assays. This strategy of extraction and following HPLC-based fractionation is a powerful tool to use when focusing on identifying only toxicologically relevant compounds from a complex mixture such as a paper matrix.

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9.2

Paper 2.

Linda Bengtström, Lisbeth Krüger Jensen, Kit Granby , Xenia Trier, Malcolm Driffield, Jens Højslev Petersen (2014) Identification of contaminants in paper and board food contact materials using bioassay guided screening and high resolution mass spectrometry . Manuscript in preparation to be submitted to Analytica Chimica Acta

Comments to the paper

In this paper, there is a reference to a manuscript in preparation; Rosenmai et al. 2014. This manuscript is listed in this thesis as Appendix 9.6.

Identification of unknown contaminants in paper and board food contact materials using bioassay guided screening and HRMS

Linda Bengtström[a], Xenia Trier[a], Lisbeth Krüger Jensen[a], Kit Granby[a], Malcolm Driffield[c], Jens Højslev Petersen[a]

[a] *Division of Food Chemistry, National Food Institute, Technical University of Denmark, Copenhagen, Denmark*

[b] *Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark, Copenhagen, Denmark*

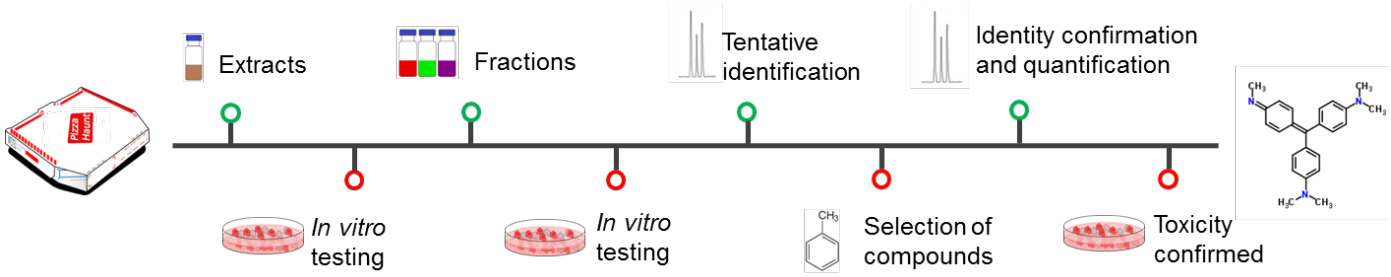
[c] *The Food and Environmental Research Agency, Sand Hutton, York, YO41 1LZ, UK*

Corresponding author:

Jens Højslev Petersen
Division of Food Chemistry, National Food Institute
Technical University of Denmark
Mørkhøj Bygade 19, 2860 Søborg, Denmark
+45 35 88 74 65
jhpe@food.dtu.dk

Identification of unknown contaminants in paper and board food contact materials using bioassay guided screening and HRMS

Graphical abstract



Abstract

This study describes the development and use of a bioassay guided screening strategy for identifying unknown contaminants in paper and board food packaging with potentially adverse health effects. Based on toxicological responses in an initial *in vitro* screening of extracts from several types of paper and board food packaging, samples were selected for further *in vitro* tests of subsamples generated by high performance liquid chromatography (HPLC) fractionation. A toxicological response in the aryl hydrocarbon receptor (AhR) assay, linked to metabolism of xenobiotics, was found in two fractions from a recycled paper sample. These two fractions were then analyzed by both gas chromatography (GC) and ultra high performance liquid chromatography (UHPLC) coupled to quadrupole time-of-flight mass spectrometers (qTOF MS) in order to tentatively identify unknown compound(s) causing the toxicological effect. To facilitate the tentative identification in UHPLC, an accurate mass database containing material relevant entries was built. Seven compounds, all found by UHPLC-qTOF MS, were suspected for the observed *in vitro* effect and subsequently quantitated. Out of these seven, three were confirmed by match of mass spectra and retention time of analytical standards in UHPLC tandem mass spectrometry. Of these three; two had entries in the database. The results from this study indicate that isotope ratio and material relevant accurate mass databases are useful for a tentative identification. When analytical standards were tested in the AhR assay in concentrations correlating to the extract, it was concluded that a small part of the effect could be attributed to Solvent Violet 8, Basic Red 1 and Baso Red 546, three pigments found in printing inks. This shows that a toxicological cocktail effect was present, but that other compounds not yet investigated contributed as well.

Key words: food packaging, identification, high resolution mass spectrometry, paper and board, bioassay guided screening, bio directed analysis

1. Introduction

The identification of unknown compounds in complex matrices by analytical chemistry is currently a challenge in several research fields such as forensic toxicology, environmental analysis as well as analysis of contaminants in foods. Food contact materials (FCMs) made from paper and board is one area where the identification of unknown compounds are of special concern, due to large knowledge gaps about which compounds are being used in these products and their toxicity [1], [2]. It has been estimated that up to 8000 compounds could be used in paper and board FCMs, and a large proportion of these compounds have not been sufficiently examined for toxicological effects [1], [2]. Moreover, food packaging has been shown to contribute significantly to human exposure of compounds with adverse health effect in humans [3].

Since paper and board are made from materials with a natural variation in chemical composition, the starting material consists of many different organic substances [4]. Additionally, many types of paper and board are chemically treated with substances to improve certain qualities in the material, such as grease-proofing or printability [4]. With such a chemically complex matrix as paper, identification and safety assessment of each individual substance would be both laborious and costly [5], and would not give any information on the potential of the identified substances to cause adverse health effects [6]. Several interdisciplinary studies have therefore combined chemical analysis and *in vitro* tests to screen paper and board FCMs [6]–[11]. This process excludes samples with no *in vitro* response and allows for further investigations of samples only with a toxicological response. However, the analysis of comprehensive extracts by chromatographic methods will give very complex results [5], [11], [12].

However, the complexity of the analysis and identification can be further reduced by fractioning the extracts, similar to the multiple heart-cutting 2D-LC technique, and subsequently testing these fractions *in vitro* [13]. In an earlier publication, we proposed a comprehensive extraction method of

semi- and non-volatile organic compounds followed by high-performance liquid chromatography (HPLC) based fractionation [14]. Special focus was put on the compatibility of the extracts with the chemical and toxicological analyses, thus that the extracts produced were able to keep the analytes in solution without being cytotoxic to the cells in the assays. During *in vitro* screenings of several FCMs, toxicological response in the aryl hydrocarbon receptor (AhR) assay for an extract as well as two fractions from a pizza box made from recycled fiber was measured [14]. The AhR assay is indicative for adverse health effects in the metabolism of xenobiotics.

The overall aim of this study was to develop a rationalized bioassay guided workflow for the identification of compounds with potential adverse health effects in paper and board FCMs by high resolution MS (HRMS). Accurate mass spectrometry with a high resolution is a powerful tool to investigate which compounds that contributed to the toxicological effect in the AhR assay. In this study, an Agilent quadrupole time-of-flight (qTOF) MS instrument which has a sufficiently high sensitivity and accuracy as well as resolution when scanning over wide *m/z* ranges was used for the analysis of the paper and board fractions.

A majority of the other interdisciplinary studies concerning screening and identification of contaminants in paper and board FCMs have identified substances based on gas chromatographic (GC) separation coupled MS analysis with electron ionization (EI) ionization [7], [8], [15]–[17]. However, in order for the analysis to be as orthogonal as possible, the fractions were analyzed by generically designed GC-EI and ultra-high performance liquid chromatography-electrospray ionization (UHPLC-ESI) separation methods coupled to qTOF MS instruments. By using two fundamentally different separation methods as well as three different ionization modes, EI as well as electrospray ionization in positive and negative mode (ESI+/-), the risk of not observing compounds due to lack of separation or ionization is reduced. Finally, analytical standards and

fractions were analyzed by UHPLC-triple quadrupole tandem MS (QqQ MS/MS) in order to confirm the identity of the tentatively identified compounds.

2. Materials and Methods

2.1 Test compounds and chemicals

Ethanol (99.9%), was purchased from Merck (Darmstadt, Germany) and the methanol (99.9%) was purchased from Rathburn (Walkerburn, UK). All aqueous solutions were prepared using ultrapure water obtained from a Millipore Milli-Q Gradient A10 system (Millipore, Bedford, MA, USA). HPLC MS grade 25 % ammonium hydroxide and formic acid were obtained from Sigma-Aldrich (St. Loiuse, MO, USA). UHPLC grade acetonitrile was obtained from Merck (Darmstadt, Germany). Di-n-butyl phthalate (DBP) (99%), deuterated di-n-butyl phthalate (d_4-DBP) (>98%), butyl-benzyl phthalate (BBP) (99%), di-isobutyl phthalate (DIBP) (99%), bisphenol A (99%), methylparaben, bisphenol A diglycidyl ether (BADGE) (95%), perfluorooctanoic acid (PFOA) (95%) and abietic acid (75%) were all obtained from Sigma-Aldrich. For UHPLC-QqQ MS/MS quantification and for AhR testing, the following standards were used; 2-mercaptobenzothiazole (98%), Rhodamine 101 (99%), Baso Red 546 (97%) and Solvent Violet 8 (85%) were from Sigma-Aldrich, 2'-(Dibenzylamino)-6'-(diethylamino)-3H-spiro[2-benzofuran-1,9'-xanthen]-3-one (98%) from TCI (Portland, OR, USA), 1-Isopropyl-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid (98%) were from Santa Cruz Biotechnology (Dallas, TX, USA) and Basic Red 1 (90%) were from Merck.

2.2 Production of extracts and fractions

Initially, 20 different paper and board samples intended for direct food contact were screened for *in vitro* effects (Rosenmai et al., in preparation). A printed pizza box made from recycled corrugated

fiber board (grammage 550 g m^{-2}), had a toxicological effect in the AhR assay, and was thus selected for fractionation and further analysis. Production of extracts and fractions are described in full detail in Bengtström et al. [14]. Briefly, samples (approximately 90 dm^2) were cut into shreds and placed in a Soxhlet boiling reflux system chamber (500 mL) connected to a 2 L round-bottomed flask containing 650 mL ethanol. The ethanol in the boiling reflux system was set to boil for 2 hours under vacuum, after which extracts were concentrated to approximately 10 mL in a Büchi B-811 Extraction System (Flawil, Switzerland). Extracts were then further concentrated to approximately 3 mL by a gentle stream of nitrogen at 70 °C. Extracts were fractioned using both acidic and alkaline eluents by a Waters 2695 chromatograph (Milford,MA,USA) coupled to a Gilson ASPEC XL (Middleton, WI). The column used was a Waters XTerra C18 column (5 µm, 250 mm x 4.6 mm i.d.) with a 0.2 µm in-line filter. For the acidic fractionation the binary mobile phases consisted of water with 0.1% formic acid (mobile phase A1) and methanol with 0.1% formic acid (mobile phase B1), pH ~2. For the alkaline fractionation the mobile phases were water with 5 mM ammonia (mobile phase A2) and methanol with 5 mM ammonia (mobile phase B2), pH ~10. The mobile phase composition was changed according to a linear gradient increasing from 10% to 100% B within 40 min, and maintained 100% B for 10 min and then returned to the initial conditions. Total run time was 55 min and the flow rate was kept at 0.8 mL min^{-1}. To obtain enough sample for toxicological testing, the extracts from paper samples for *in vitro* test were injected (100 µL) ten times each in acidic as well as alkaline conditions, with each injection corresponding to approximately 1.8 dm^2. Fractions were collected in polypropylene tubes (50 mL, Sarstedt, Nümbrecht, Germany). The collection of fractions started at 3 min into the run and shifted every 5 minutes except for the first fraction which was collected for 2 min. First, eleven fractions were collected using acidic eluents then another eleven was obtained with alkaline eluents. Lastly, the

cytotoxic methanol and water in the fractions was exchanged for the less cytotoxic ethanol by a nitrogen vaporization step as described earlier.

2.3 Aryl hydrocarbon receptor assay (AhR assay)

Test compounds were tested in stably transfected rat hepatoma (H4IIE-CALUX) cells provided by Dr. Michael Denison (University of California, USA), and the assay was conducted as previously described in Rosenmai et al. [18]. Extracts and fractions were tested as described in Bengtström et al. [14].

2.4 Tentative identification in fractions

Extracts and fractions with a toxicological response from the first respective second screening phase were analyzed by both GC-EI-qTOF MS and UHPLC-ESI-qTOF MS. Extracts and fractions were diluted 1:100 *v/v* and 1:10 *v/v* with ethanol (99.9%) respectively prior to analysis.

2.4.1 Tentative identification by GC-EI-qTOF MS

Separation was achieved by two coupled DB5 capillary columns (5% diphenyl – 95% dimethyl polysiloxane, 15m x 0.25mm , i.d., 25 µm film thickness) from Agilent Technologies (Santa Clara, USA). One mL splitless injections were made at 280°C. The separation gradient was; 0 min 40°C and kept at 40°C for 1 min, linearly increased for 16 min to 300°C, and kept at 300°C for 5 min. A 7200 GC-qTOF system (Agilent Technologies) mass spectrometer was operated with electron-ionization (EI) at 70eV in mass range *m/z* 50-550, scan range 5 spectra s^{-1}. Helium was used as carrier gas at 1.2 mL min^{-1}. A standard mix (10, 100 and 500 ng mL^{-1}) for each standard was analyzed before and after all the samples. Data analysis was performed by using MassHunter Qualitative software v. B06 (Agilent Technologies) using the NIST v. 11 mass spectral library.

2.4.2 Build-up of UHPLC-ESI-qTOF MS customized database

The customized accurate mass database contained the compound name, chemical formula and accurate monoisotopic mass of known suspect chemical groups such as bisphenol analogues, phthalates, bisphenol A diglycidyl ether (BADGE) and BADGE derivatives, dioxins, polychlorinated biphenyls (PCBs) and brominated flame retardants (BFRs), as well as naturally occurring substances in the paper material such as abietic acid and other resin components. Theoretical exact monoisotopic masses for compounds described in scientific research articles, from legislative lists and inventory lists were incorporated. This included Ackerman et al. [19] describing BADGE derivatives, as well as the European Printing Ink Association (EuPIA) inventory list [20] and EU reports such as the ESCO WG report on European nationally regulated substances in non-plastic FCMs [21].

2.4.3 Tentative identification by UHPLC-ESI-qTOF MS

The column XTerra CSH C18 column (2.6 µm, 2.1 x 100 mm) (Waters, Milford, MA, USA) was used at 40° C with the mobile phaes A; 5 mM ammonium hydroxide and 0.1 % formic acid in water and B; 0.1 % formic acid in acetonitrile. The separation gradient used was: 0 min 30% B, linear to 100% B at 15 min, then kept constant 100% B to 18 min, back to 30% B at 18.1 min and equilibration for 2 min. The flow rate was 0.25 mL min^{-1} and the injection volume was 3 µL. A standard mix (100 and 500 ng mL^{-1}) was prepared, and analyzed before and after all the samples. The QTOF-MS instrument was operated under the following conditions: Instrument used; 6550 iFunnel QTOF (Agilent Technologies) with an ESI + Agilent Jetstream Technology ion source in positive or negative ionization mode, operated in full scan in the data-independent All Ions MS/MS mode with a mass range of *m/z* 50-1700 in all acquisition modes. The collision cell was operated without CID in MS mode (transmission energy 7 eV) and with CID in MS/MS. The scan rate was 5

spectra s^{-1} in MS and MS/MS experiments. The source parameters were: drying gas temperature 225 °C, gas flow 13 L min^{-1}, nebulizer pressure 3 bar, sheath gas temperature 350 °C, sheath gas flow 7.5 L min^{-1}, VCap voltage +/-3 kV, desolvation gas flow 775 L h^{-1}, nozzle voltage 0 V and fragmentor voltage 110 V and 120 V. The Agilent fluorinated tune and calibration mixtures were used. Mass accuracy was typically <1 ppm for abundant peaks (ion counts >200). Data analysis was performed using MassHunter Qualitative software v. B06 (Agilent Technologies) and ProGenesis QI software (Nonlinear Dynamics Limited, UK). Injection orders for all the extracts and fractions were randomized and a quality control of pooled samples was used as a reference for the peak picking process.

2.4.4 Targeted screening

A targeted screening for dioxins, PCBs and BFRs, see Appendix C was performed by analyzing extracted ion chromatograms (EICs) of the quasi-molecular ions of these compounds from the total ion chromatogram (TIC) obtained by GC-EI-qTOF MS.

2.5 Quantitative identification by UHPLC-QqQ MS/MS

Selected tentatively identified compounds present in the fraction with toxicological response were quantified by LC-MS/MS using an eight-point external calibration curve (0, 10, 20, 50, 100, 200, 500 and 1000 ng mL^{-1} for all compounds in the mixture) of the seven selected compounds analyzed. Extracts and fractions were diluted 1:100 *v/v* and 1:1000 *v/v* with ethanol prior to analysis. Analysis was performed by a Waters Acquity UHPLC chromatograph coupled to a Micromass Quattro Ultima mass spectrometer with an ESI ionization interface. The column used was an XTerra CSH C18 column (2.5 μm, 150 × 2.1 mm) from Waters with a KrudKatcher Ultra pre-column 0.2 μm filter (Phenomenex, Torrance, CA, USA). Mobile phase A; 5mM ammonium formiate and 10 mM formic acid and mobile phase B; acetonitrile were used for separation. The gradient was: 0 min 25%

B, 1 min linear to 50% B, 4 min linear to 65% B, 4.5 min increased to 99% B, kept constant at 99% B to 5 min, back to 25% at 5.1 min and equilibrate for 1 min. The flow rate was set at 0.4 ml min^{-1}; the injection volume was 3 μL. The capillary voltage was +3 kV, desolvation gas flow 700 L h^{-1} and cone gas flow 110 L h^{-1} of N_2, source temperature 120°C, desolvation temperature 400°C. Argon was used as collision gas at 2.3×10^{-3} mbar. Data were acquired with MassLynx v.4.1 software and analyzed by the QuanLynx v 4.1 software. Chromatographic and MS parameters for each of the selected compounds are presented in Table 1. Linearity was established by the coefficient of correlation, R^2. Limit of detection (LOD) and limit of quantification (LOQ) was defined a three times and ten times the standard deviation of the lowest standard after the blank response was deducted.

3. Results and discussion

3.1 Sample preparation and initial in vitro screening

In recent years, several studies have reported an interdisciplinary approach combining analytical chemistry with *in vitro* tests in order to screen and assess cellulose-based FCMs [7], [8], [10]–[12], [22]. In this study we have further developed these methods into a strategy where a combination of analytical chemical and toxicological data has been applied to identify compounds with xenobiotic effects, see Fig. 1. An initial screening was carried out with 20 different paper and board samples in eleven different cell assays, covering genotoxicity, endocrine disruptive effects and metabolism of xenobiotics as described in Rosenmai et al. (in preparation). Results from this screening revealed a positive response in the AhR assay of the extract as well as the acidic fraction number 9 and the alkaline fraction number 9 from the pizza box sample, see Figure 2a-b, in the AhR assay as previously reported in Bengtström et al. [14]. These fractions were collected when the organic mobile phase composition was increased from 90% to 100%. A two-buffer system for fractionation,

one acidic and one alkaline, allows substances with different properties to be affected differently by the pH which in turn can give some indications of chemical properties when identifying substances.

In vitro testing is always a challenge when testing compounds with low water solubility, since the percentage organic solvent must be kept below 1% to prevent cell cytotoxicity of the solvent. This necessary dilution of extracts and fractions can result in non-homogeneously mixed solutions. Despite that long-chain and non-polar substances as well as surfactants are not normally soluble in solutions with such a low organic content, these compounds are expected to be dissolved, since the dilution is with cell culture media containing emulsifier and bovine serum albumin (BSA). The presence of BSA also has the advantage that it represents the likely delivery mode which can be expected in the human cells [24].

3.2 Tentative identification of compounds in fractions

The tentative identification process was in this study used to produce an accurate list of substances present in the fractions. The advantages of tentatively identifying compounds in the fractions rather than in the full extracts is the reduction of compounds to identify as well as the reduction of matrix effects [14]. The matrix effect typically suppresses the detector signal and interferes with the mass spectra. This is of particular importance for the UHPLC-ESI-qTOF MS analysis, as the ESI interface used appears to be more affected by matrix effects than other liquid chromatography ionization techniques, such as atmospheric pressure chemical ionization (APCI) [25]. Though, severe matrix effects are normally avoided by dilution, this approach is not effective for potent compounds present in low concentrations. Both methods, GC-EI-qTOF MS and UHPLC-ESI-qTOF MS, used for identification were designed to be as complimentary as possible, to be able to cover the broadest possible range of analytes. In addition, the methods used for separation were intended as generic as possible, including the choices of columns, DB-5 for GC and RP C_{18} for UHPLC, as

well as gradients and for UHPLC, the composition of the mobile phase. Generally, smaller (<550 Da) volatile and semi-volatile, as well as semi- to non-polar compounds can be identified by GC-EI-qTOF MS and larger (<1700 Da) semi- and non-volatile as well as semi-polar or polar compounds can be identified by UHPLC-ESI-qTOF MS. During the tentative identification process, it is important to keep a continuous balance between reducing the number of compounds to be analyzed and the risk of removing compounds with an actual toxicological effect.

3.2.1 Tentative identification by GC-EI-qTOF MS

A major advantage of using EI is the standardized ionization mode, enabling the establishment of a vast searchable mass spectral library. However, EI is considered a hard ionization method as the standardized ionization conditions leads to a characteristic, yet severe, fragmentation of the analyte [26]. This reproducible fragmentation allows searching for matching mass spectra in commercially available libraries, such as the NIST library. However, as only small (*m/z* 50-550) and volatile compounds are able to pass the GC, the mass range for analytes is limited. A representation of the workflow for the identification process for the data obtained by GC-EI-qTOF MS is presented in Figure 3, and a comprehensive list of tentatively identified compounds in the two fractions analyzed is presented in Appendix A.

To make the identification process more efficient, a cut-off similar to that used by Koster et al. [11] were used. Peaks with areas below the cut-off were not included in this study. The cut-off was chosen based on the lowest threshold of toxicological concern (TTC), that is used for compounds with genotoxic effects [27]. This threshold of 0.15 μg/person/day, corresponds to 25 ng dm^{-2} assuming an intake of 1 kg of foods/person/day and that the food consumed come into contact with 6 dm^2 of the particular FCM [28]. Because of uncertainties in measurements, in for example recovery during sample preparation and detector response, the analytical detection limit should be

below the TTC, corresponding to 125 ng mL^{-1} in the fraction. To compensate for these uncertainties, the standard with the lowest response at the TTC, d_4-DBP was used as a cut-off.

The initial steps in the identification process of GC-EI-qTOF MS data were fully automated, where peaks exceeding the cut-off were integrated and the mass spectra were compared to those in the NIST library. The resulting library hits were scored within the MassHunter software according to mass match, abundance match, spacing match, fragment match and relative fragment intensity match. No mass spectral hits below 85 in the MassHunter software and below 800 in the Relative Match Factor in the NIST library were considered. The library hits were then manually inspected as described in Figure 3. First, the retention time (Rt) for the suggested compound were established as realistic or not, based on the Rt for standards in the mixture with similar molecular weights and chemical functionalities. Secondly, the main fragments were inspected for bromine, chlorine, sulfur or silica, as these elements have typical isotopic patterns associated to ^{37}Cl (~32% relative abundance), ^{81}Br (~98%), ^{34}S (~4%) and ^{29}Si as well as ^{30}Si (~5 and ~3% respectively) [29]. The fragments for negative mass defect associated with fluorinated elements of the suggested compound were also examined. The isotope ratios for the main fragments were compared to those of the suggested formula. Next, suggested compounds were also inspected for matching significant and characteristic fragments. For example, fragments at *m/z* 65.0386 ($C_5H_5^+$), 77.0386 ($C_6H_5^+$), 91.0542 ($C_7H_7^+$) and 105.0335 ($C_7H_5O^+$) can often be observed for compounds containing aromatic substructures, and a fragment at *m/z* 149.0233 ($C_8H_4O_3^+$) can be characteristic for phthalates. In cases where these significant and isotope matched fragments did not match the suggested structure, the suggested compound was discarded.

As EI is a hard ionization technique, severe fragmentation for some compounds was observed. In particular linear hydrocarbons, such as alkanes and fatty acids, were fragmented to such an extent that a quasi-molecular ion was not present in the mass spectra. In these cases, the fragmentation

pattern could only be assigned as being consistent with that of a linear hydrocarbon, and not identified as any specific compound. Yet, as linear hydrocarbons are not associated with any toxicological response in the *in vitro* assays used, the identification of these compounds was not confirmed. These compounds are reported according to their class, rather than as tentatively identified, in Appendix A.

After that, the compounds in common for both fractions with toxicological response were compared. When compounds were present in both alkaline and acidic fractions, the effect of the pH on analyte-column interaction in the fractionation process must be considered and compared between the fractions. If the compound tentatively identified could be analyzed by UHPLC-ESI-qTOF MS in either positive or negative mode as well, the list of compounds from the UHPLC identification process was referred to for matching hits. In total, 29 compounds in the acidic fraction and 12 compounds in the alkaline fraction were identified by the process described in Figure 3.

3.2.2 Build-up of UHPLC-ESI-qTOF MS customized database

One disadvantage of UHPLC-ESI-qTOF MS analysis is that no generic mass spectral libraries are commercially available due to vendor specific differences in the parameters that govern ionization and collision induced disassociation. This includes adduct formation, ionization interfaces as well as the use of different mobile phases and cone voltages. Although, some vendors have developed small mass spectral libraries for specific purposes, these are focused on only small subsets of analytes such as pesticides and illicit drugs [30]. These libraries are not nearly as comprehensive as the vast mass spectral library used for GC-EI-MS. However, as the accurate *m/z* measured on qTOF MS instruments relate to the elemental composition of the molecules, and are specific and valid for all compounds. Futhermore, the use of a material matched accurate mass database greatly enhances the possibility of a correct tentative identification [13], [31], [32]. In order to perform a semi-targeted

311 analysis of the fractions analyzed by UHPLC-ESI-qTOF MS, a database with almost 2100 entries
312 of known intentionally added substances (IASs) as well as potential contaminants and known non-
313 intentionally added substances (NIASs) reported in paper and board was developed, see Appendix
314 B.

315 *3.2.3 Tentative UHPLC-ESI-qTOF MS identification*

316 The advantages of using UHPLC-ESI-qTOF MS analysis complementary to GC-EI-qTOF MS
317 analysis are the ability to analyze a wider *m/z* range as well as relatively polar compounds. The
318 mass range for acquisition by UHPLC-ESI-qTOF MS was *m/z* 50-1700 Da. When discussing
319 human exposure of oral uptake of compounds migrating from FCMs into the food, 1000 Da is
320 generally regarded as the highest molecular weight for compounds able to pass the intestinal
321 membrane by passive diffusion [33]. However, heavier and larger compounds could possibly
322 degrade into smaller in the acidic environment or enzyme activity in the gut and thus be taken up
323 [34].

324 Furthermore, ESI does not necessarily fragment the analytes as much as EI in GC, often leaving the
325 quasi-molecular ion visible in the mass spectra. The proposed workflow for identification of
326 unknown compounds in the fractions from paper and board samples is presented in Figure 3. As a
327 cut-off, one tenth of the peak areas for the standards BADGE and PFOA were used, as these had the
328 lowest response at 100 ng mL^{-1}, in positive and negative mode respectively. This was to
329 compensate for both variations in degree of ionization for different analytes and matrix effects such
330 as ion suppression.

331 When the quasi-molecular ion, $[M+H^+]$ or $[M-H^-]$, was identified, the Molecular Formula Generator
332 (MFG) feature within the MassHunter software was used to generate possible molecular formulas
333 for the most prominent spectral peaks. These molecular formula hits were ranked according to a

weighted score of mass match based on the mass accuracy of the quasi-molecular ion as well as the isotope distribution and isotope spacing of the quasi-molecular ion. No MFG hits with a score below 85 were considered. The software also automatically used elemental rules such as the nitrogen rule when generating molecular formulas. However, when measuring masses over 500 Da by accurate mass the nitrogen rule becomes unreliable [31], and were therefore not used for analytes above this mass range. Generated formulas with unlikely high element ratios were manually removed from further investigation.

The MFG hits were then compared to the isotopic ratio to elucidate the most matching molecular formula(s). The maximum mass error for the MFG was set to 10 ppm and the allowed elements were $C_{1\text{-}80}$, $H_{1\text{-}300}$, $O_{0\text{-}10}$, $N_{0\text{-}10}$, $P_{0\text{-}10}$, $S_{0\text{-}10}$, $Si_{0\text{-}10}$, $Cl_{0\text{-}10}$, $Br_{0\text{-}10}$ and $F_{0\text{-}25}$. Besides rationalizing the tentative identification process, using a cut-off also avoids analysis on low ion signal intensities. High signal intensities of both quasi-molecular ion and its isotope ratios enhance ion statistics, which in turn improves the mass accuracy and isotopic abundance measurements, leading to a better weighted score and higher assurance in determining correct elemental compositions.

Ionization polarity (ESI+/-) significantly influence on the ionization efficiency of the compounds and hence the detection of compounds. It also affects the fragmentation pattern as even at the same collision energy, bond dissociation may differ with positive and negative ionization, meaning the product ions observed in one ionization mode will not necessarily be observed in the opposite mode. The data-independent All Ions MS/MS acquisition included a no collision mode, with only in-source fragmentation except for the 7 eV used for acceleration of the ions through the quadrupole, as well as spectra from two higher collision energies acquired simultaneously. The product ions in the spectra were manually inspected for matching peak features in a co-elution plot (±0.2 min). These product ions, if existing, were used to support a tentative identification by

comparing the obtained MS/MS spectra of product ions, including their isotopes, with the spectra of analytical standards of the candidate compounds.

The initial steps for the tentative identification by UHPLC-ESI-qTOF MS were the same as described in Section 3.2.1, where peaks from the low collision energy chromatograms were automatically integrated and deconvoluted. Peaks were then manually inspected as described in Figure 3. The first manual step in the tentative identification process was to locate the molecular quasi-molecular ions. Adducts can be helpful in deducing the quasi-molecular ion when both species are present. On the other hand, when adduct formation is favored; there could be some difficulties to locate the actual quasi-molecular ion. However, recently released analytical software enables comparison of co-eluting ions. If the mass difference of two ions in the spectra matches the difference between two adduct masses specified in the search criteria in the software, it could be assumed that these two masses are in fact the same compound. To further enhance the certainty, the peak features for the suggested adducts and fragments were compared. By using these analytical software, the uncertainty of localizing the quasi-molecular ions could be reduced. The most observed adducts in the UHPLC-ESI-qTOF MS was $[M+NH_3+H]$ and $[M+H-H_2O]$ in positive mode and $[M-H-H_2O]$ in negative mode.

Second, the spectral peaks were inspected for Br, Cl, S or F elements by their specific isotope patterns as described above. The generated formulas were matched to the formulas in the customized database. For peaks with no database hit, the generic ChemSpider or PubChem databases to search for compounds with the suggested molecular formula or monoisotopic mass were used. For these queries, a mass defect below 10 ppm was used as search criteria. Reported use in paper and board FCMs, derived from patents, was also used to rate the suggested compounds in the general databases when no hit was found in the customized database.

A comparison of Rt's was also used to compare suggested compounds common for both fractions with toxicological response. If the compound identified could be analyzed by GC-EI-qTOF MS, the list of compounds from the GC identification process was consulted for matching hits. In total, 34 compounds were tentatively identified in positive and negative mode in the acidic fraction by the proposed method. The same number for the alkaline fractions was 51 tentative identified compounds. A complete list of tentatively identified compounds in the two fractions by both GC- and UHPLC-HRMS analysis is available in Appendix A. In total, 76 individual compounds were tentatively identified in the two fractions after they were analyzed by both separation methods.

3.3 Selection of compounds for further analysis

AhR is known to bind several exogenous ligands including polyaromatic hydrocarbons (PAHs), dioxins, PCBs and other endocrine disruptors such as certain pesticides and BFRs [35]–[37]. These compound groups are all highly potent, and could therefore cause a toxicological response in the AhR assay even at very low concentrations. When compounds were tentatively identified in the fractions by using the cut-off, no compounds from any of these groups were identified. The selection of compounds for further investigation was therefore based on reviewing both toxicological literature, structural similarities to compounds with previously shown effect in the AhR assay [38] as well as *in silico* modeling, quantitative structure–activity relationship (QSAR), of the listed compounds. This selection process is described further in Rosenmai et al. (in preparation).

There were only one compound overlapping found by both UHPLC-ESI-qTOF MS and GC-EI-qTOF MS, Bis(2-ethylhexyl) phthalate. All tentatively identified compounds selected for further analysis were obtained by UHPLC-ESI-qTOF MS, stressing the importance of using this technique as a complement to analysis by GC-EI-qTOF MS. From this list of tentatively identified compounds, see Appendix A, fifteen compounds were considered to share structural similarities

with known AhR ligands. However, only seven of these selected compounds had commercially available standards and were further investigated, see Table 1. The other eight were These eight compounds were Methyl 8,11,13-abietatrien-18-oate(CAS: 1235-74-1), Carbamodithioic acid, dimethyl-, 2-benzothiazolyl ester (9CI) (CAS: 3432-25-5) , Benzyl dimethylcarbamodithioate (CAS: 7250-18-2), Propane, 2,2-bis[4-(2-hydroxypropyloxy)-phenyl]- (CAS:116-37-0), 4-(1,5-diphenylpentan-3-yl)pyridine (CAS: 2057-47-8), 1,3-Dibenzyl-2-phenylimidazolidin (CAS: 4597-81-3), p-(Diethylamino)benzaldehyde diphenylhydrazone (CAS: 68189-23-1) and 2,4,6-Pyrimidinetriamine,5-[(2-methoxyphenyl)azo]-N,N',N''-tris(4-methylphenyl)- (CAS: 61038-65-1).

Five out of the compounds selected had a reported use in printing ink compositions. Earlier studies have suggested that printing inks could potentially be linked to toxicological effects in the AhR assay [7]. When analyzed in QSAR, none of the structures for any of the seven selected compounds were inside the domain of the dataset for the AhR assay. However, QSAR modeling is based on the available dataset of available toxicological data collected from literature. This means that for toxicological assays where the data is limited, such as for the AhR assay in this case, the QSAR prediction is insufficient.

A comprehensive analysis in its strictest sense is not achievable, as some compromises for the identification methods must be done, both in the sample preparation steps as well as in the identification process [39]. By using the GC-EI-qTOF MS and UHPLC-ESI-qTOF MS methods described in this study, very polar compounds will most likely not be detected since neither of the generic methods are suitable to separate these compounds.

3.4 Chemical identity confirmation and quantification

Extracts, fractions and analytical standards of the seven selected compounds were analyzed by UHPLC-QqQ MS/MS for confirmation of identity. Out of the seven compounds analyzed, the three dyes and one printing ink component; Solvent Violet 8, Basic red 1, Baso Red 546 and 2'-(Dibenzylamino)-6'-(diethylamino)-3H-spiro[2-benzofuran-1,9'-xanthen]-3-one, were confirmed in the extracts when relative Rt, product ions and ion ratios compared to those of the analytical standards. Two of these three compounds had an entry in the customized database, see Table 1.

The external calibration curves for the methods were established by plotting the peak area versus concentrations. All quantified compounds showed acceptable linearity (R^2>0.98, not weighted, not forced through 0) in the investigated range of 0-1000 ng mL^{-1}. The concentrations of the four confirmed compounds as well as the limit of detection (LOD) and limit of quantification (LOQ) in the tested extract are presented in Table 1.

As an example of the entire workflow for tentative identification by UHPLC-ESI-qTOF MS and quantification, Figure 4a and 4b shows a base peak chromatogram (BPC) from alkaline fraction number 9 from the pizza box and the spectra obtained at Rt 7.6 minutes. Observe that the cut-off in Figure 4a is represented by a line based on peak height and not on peak area for practical reasons. In agreement with findings reported by Kind et al. [31], matching isotope patterns appears to be more important than a high mass accuracy (<5 ppm) for the tentative identification, see Figure 4b. In this case, the mass defect for the correct suggested formula, $C_{24}H_{27}N_3$, was 7 ppm. When the formula was compared to entries in the customized database, the suggested formula was matched to that of a dye used in printing inks; Solvent Violet 8. This compound is soluble in both ethanol and methanol, and it is therefore extracted from the matrix by ethanol as well as eluted by methanol during the fractionation process. Although there is no specific pK_a value for Solvent Violet 8, the methylamine and di-methylamine groups suggest a pK_a around 10.6. This means that during

alkaline conditions, the compound is ionized, and during acidic conditions the compound is neutral. When the extract, fractions and standard were analyzed by UHPLC-QqQ MS/MS all parameters necessary for a positive identification, see Figure 4 c-f. A higher concentration of Solvent Violet 8 was found in the alkaline fraction than in the acidic fraction during quantification, 70 µg dm^{-2} and 0.7 µg dm^{-2} respectively.

As many of the compounds selected for further analysis elute in the same fraction during acidic and alkaline fractionation despite the differences in pH, it can be concluded that the time interval for the collection of fractions is too wide and the gradient is too steep. A future improvement of the fractionation process would involve collecting many more fractions than the eleven produced for this study with narrower time intervals and with a slighter slope for the gradient, such as the gradients used in 2D-LC, in each of the conditions, to be able to fully take advantage of the acidic and alkaline fractionation.

3.5 Toxicity confirmation

The four standards of the confirmed compounds were subsequently tested in the AhR assay. Even if three of the compounds identified had a relative low concentration in comparison to Solvent Violet 8, see Table 1, these were included in the *in vitro* tests to investigate possible cocktail effects. Out of these four compounds, the three dyes; Solvent Violet 8, Basic red 1 and Baso Red 546 had a toxicological response. Details on the calculation of the toxicological equivalence factors are extensively described in Rosenmai et al. (in preparation). Solvent Violet 8 was found to be very cytotoxic, and only a very weak increase in AhR activity could be seen at the two lowest test concentrations (0.4 and 0.8 µM), which were the only non-cytotoxic concentrations. For Baso Red 546 and Basic Red 1, a weak increase in activity was seen at 50 µM, followed by relative marked increase at the highest tested concentration of 100 µM. Using the positive control of reference compound, the AhR equivalence factor were determined for the extract (EQ measured) and for the

three positive compounds (EQ calculated), see Table 2. The equivalence factor (EQ) calculated based on the response of the three positive compounds Solvent Violet 8, Baso Red 546 and Basic Red 1 (EQ calculated) was much lower than the equivalence factor determined for the extract (EQ measured), suggesting that the identified compounds cannot alone explain the response observed for the extract. Further studies are needed to be able to fully explain the measured toxicological effects, as a suggestion starting with the eight compounds selected with similar structures to known AhR ligands but without commercially available standards.

3.7 Targeted screening

One of the advantages of performing a full scan is the ability to perform a post-acquisition re-interrogation of data. As only a very small part of the initially observed toxicological effect could be explained by the printing inks, a targeted screening of compounds known to be highly potent in the AhR assay such as selected dioxins, PCBs and BFRs were performed, see Appendix C. This screening was performed without a lower limit of detection. None of the compounds presented in Appendix C were found when EICs of the quasi-molecular ions from the TIC obtained from the GC-EI-qTOF MS was analyzed.

5. Conclusions

Overall, the results from this study show that the procedure of bioassay guided fractionation in combination with hyphenated orthogonal HRMS analyses is useful for the detection and identification of unknown compounds with potentially adverse health effects in paper and board FCMs. The bioassay guided strategy presented here worked well in isolating first one sample with potentially adverse health effects out of a broad selection of paper and board FCMs, and secondly in isolating compounds in a fraction containing much less matrix interferences as well as fewer peaks than the original extract. Using HRMS based analysis, a substantial list of tentatively identified

compounds were produced. The compounds on this list could then be assessed for likely candidates for the measured toxicological response using *in silico* predictions such as QSAR as well as literature studies and read-across. However, the QSAR prediction was based on a limited dataset, and the information obtained from the *in silico* modeling was considered insufficient as many of the tentatively identified compounds were outside the domain.

Not all of the selected candidates were commercially available, and could therefore not be further investigated for either chemical confirmation and quantitation or toxicological confirmation. However, the presence of three compounds with AhR activity was discovered in the relevant extract. These compounds were correlated to a small part (<1%) of the measured toxicological effects to concentrations of confirmed compounds found in FCMs. When a targeted screening was performed in the fractions for known AhR ligands with high toxicological potency, such as PCBs, dioxins and BFR's, none of these compounds were found in the fractions. This means that the measured toxicological effect from the extracts is caused by unknown compound(s) not yet individually tested in *in vitro* tests. Future studies would involve testing analytical standards of the remaining selected compounds, to be one step closer to fully explain the measured effect in the AhR assay.

In addition, the results suggests that the use of an accurate mass database with material relevant entries are important for the tentative identification of unknown compounds when analyzed by UHPLC-ESI-qTOF MS. This study is a promising start, however, we recommend further studies to be conducted applying this strategy on a larger number of paper and board FCMs to further develop and refine the strategy. These further studies would also contribute to increase our understanding of the toxicity of compounds being used in paper and board FCMs.

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Appendix A: Comprehensive lists of tentatively identified compounds in fractions

Appendix B: Customized database

Appendix C: List of selected compounds for targeted screening

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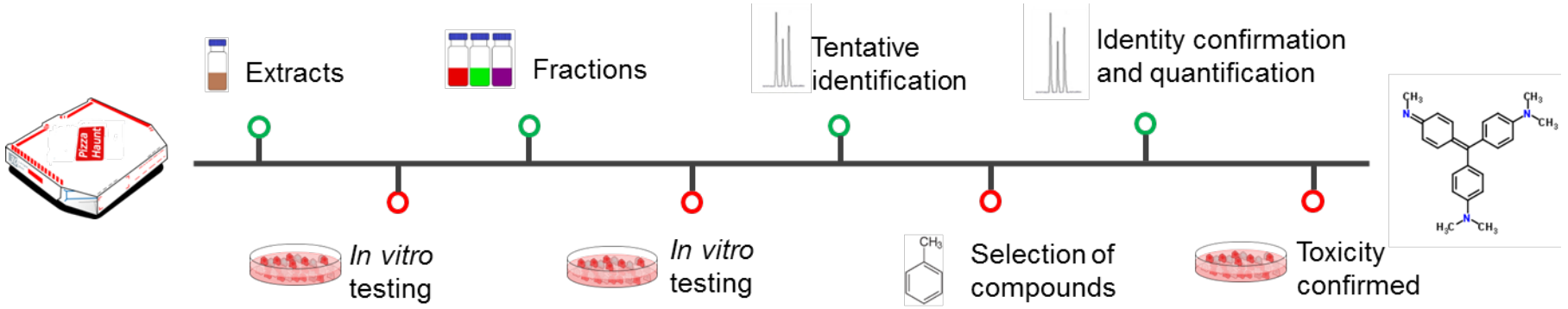


Fig 1. Overall strategy for the bio-directed analysis

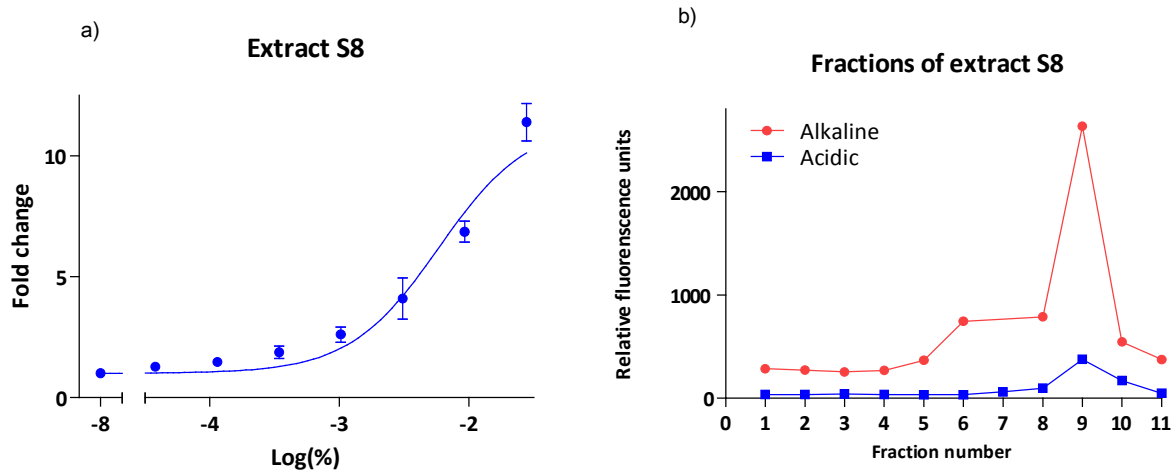


Figure 2. a) Arylhydrocarbon receptor (AhR) agonism in extract from the pizza box. Data from extract were normalized to controls and fitted to a sigmoidal dose-response model. b) Arylhydrocarbon receptor (AhR) agonism (presented in relative fluorescence units) in the fractions from the pizza box. Graphs are based on one representative experiment in extract and fractions. Error bars represent standard deviations (SD)

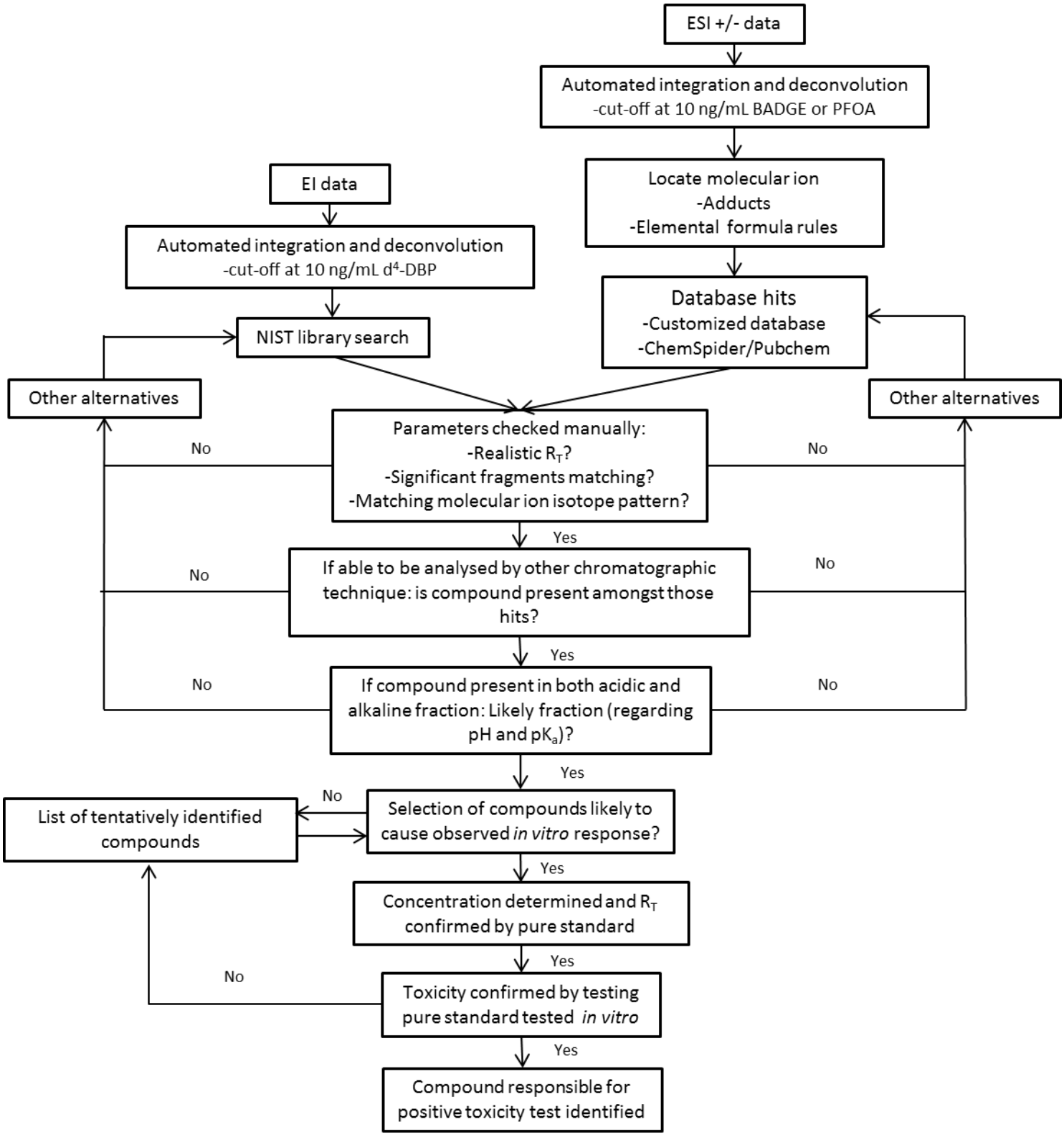


Fig 3.Workflow for the identification of compounds analyzed by GC-EI-qTOF MS and UHPLC-ESI-qTOF.MS.

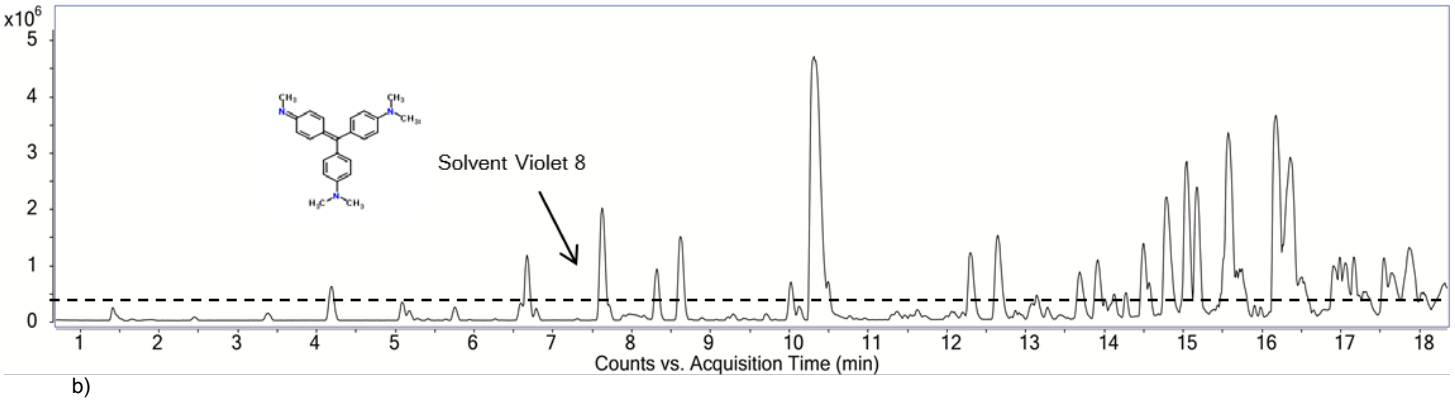
Table 1. Tentatively identified compounds selected for further investigation and the mass spectrometry parameters for each compound from the UHPLC-ESI-QqQ MS/MS analysis used for conformations.

Compound	CAS number	Tentatively identified in	Precursor ion	Product ions	Collision energy (eV)	Rt (min)	LOD/LOQ (ng mL^{-1})	Identity confirmed	Conc. in extract (µg dm^{-2})	Additional information
2-Mercaptobenzothiazole	149-30-4	Acidic fraction 9/ Alkaline fraction 9	168	188 135	20 10	2	2/20	No	-	Used in rubber and latex production as well as in paper manufacturing, and two-part cyanoacrylate adhesives
Solvent Violet 8	52080-58-7	Acidic fraction 9/ Alkaline fraction 9	358	342 326	50 30	2.6	<1/5	Yes	80	Entry in database. Used in ink
Basic red 1	989-38-8	Alkaline fraction 9	443	399 355	50 40	2.6	<1/5	Yes	1	Entry in database. Used in ink
Baso Red 546	509-34-2	Alkaline fraction 9	443	399 355	50 40	2.6	<1/5	Yes	1	Used in ink for ink-jet printers
1-Isopropyl-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid	436811-11-9	Alkaline fraction 9	259	186 242	20 10	1.2	<1/5	No	-	
Rhodamine 101	116450-56-7	Alkaline fraction 9	491	463 419	50 50	3	<1/5	No	-	Used in photoreceptor layers and optical filters
2'-(Dibenzylamino)-6'-(diethylamino)-3H-spiro[2-benzofuran-1,9'-xanthen]-3-one	34372-72-0	Alkaline fraction 9	567	475 399	50 50	5.4	<1/5	Yes	5	Entry in database. Used in printing inks

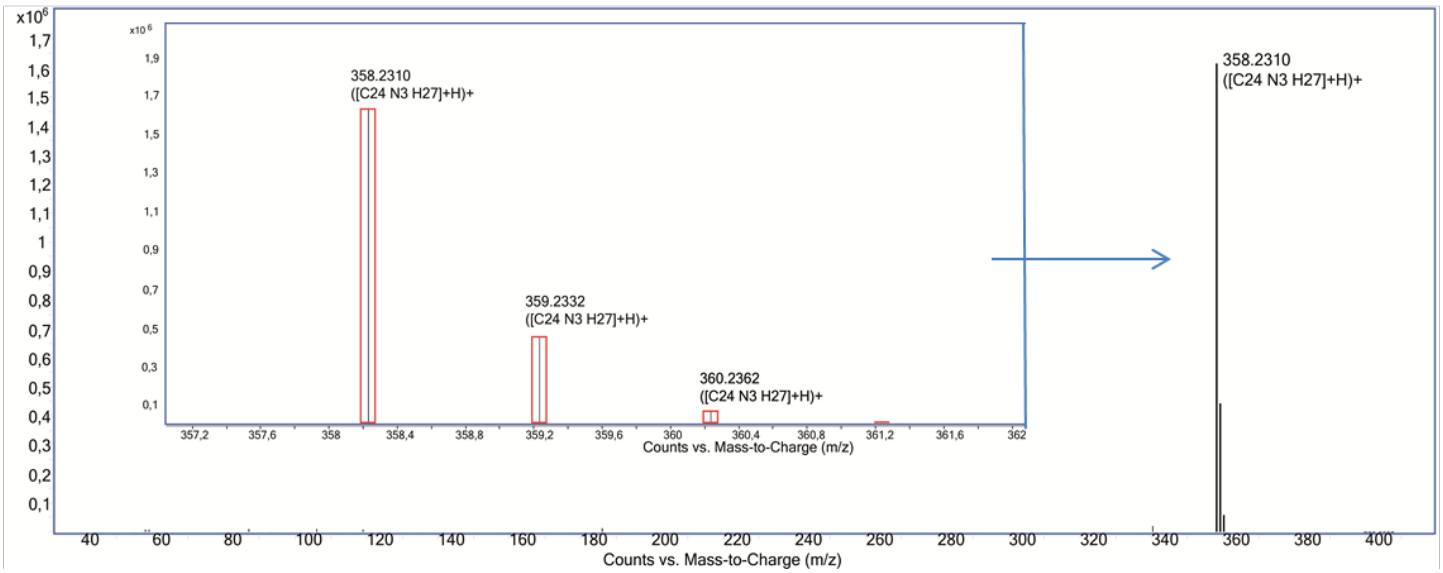
Table 2. Estimated and measured equivalence factors (EQ) for pizza box extract as well as identified compounds causing changes in activity in extracts including Solvent Violet 8, Baso Red 546 and Basic Red 1. Concentrations (µM) for identified compounds in extract at maximum response.

ARYL HYDROCARBON RECEPTOR ACTIVITY					
EXTRACT	Solvent violet 8	Baso Red 546	Basic Red 1	TOTAL EQ	
	0.4 µM[a]	50 µM[a]	50 µM[a]	EQ_{calc}	EQ_{meas}
S8	EQ: $7.68'10^{-9}$	EQ: $6.34'10^{-9}$	EQ: $6.34'10^{-9}$	$2.0'10^{-8}$	$8.1*10^{-6}$

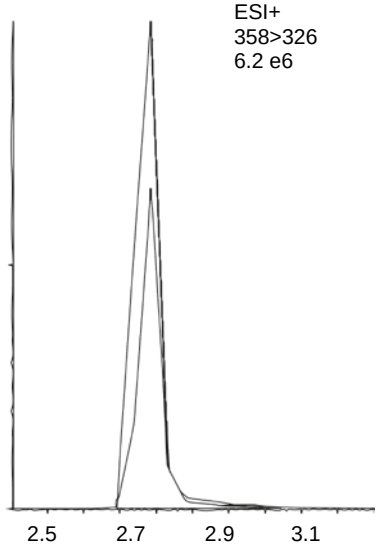
a)


x10^6
Solvent Violet 8
Counts vs. Acquisition Time (min)

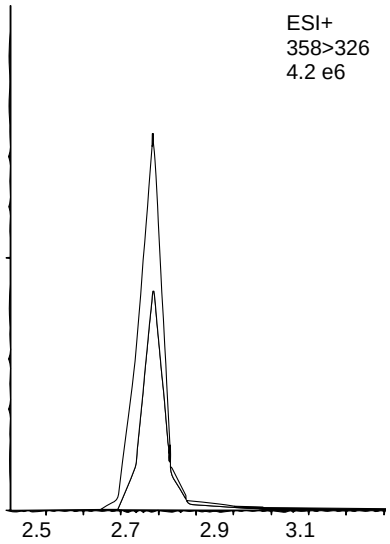
b)


358.2310
([C24 N3 H27]+H)+
359.2332
([C24 N3 H27]+H)+
360.2362
([C24 N3 H27]+H)+
Counts vs. Mass-to-Charge (m/z)
358.2310
([C24 N3 H27]+H)+
Counts vs. Mass-to-Charge (m/z)

c)


ESI+
358>326
6.2 e6

d)


ESI+
358>326
4.2 e6

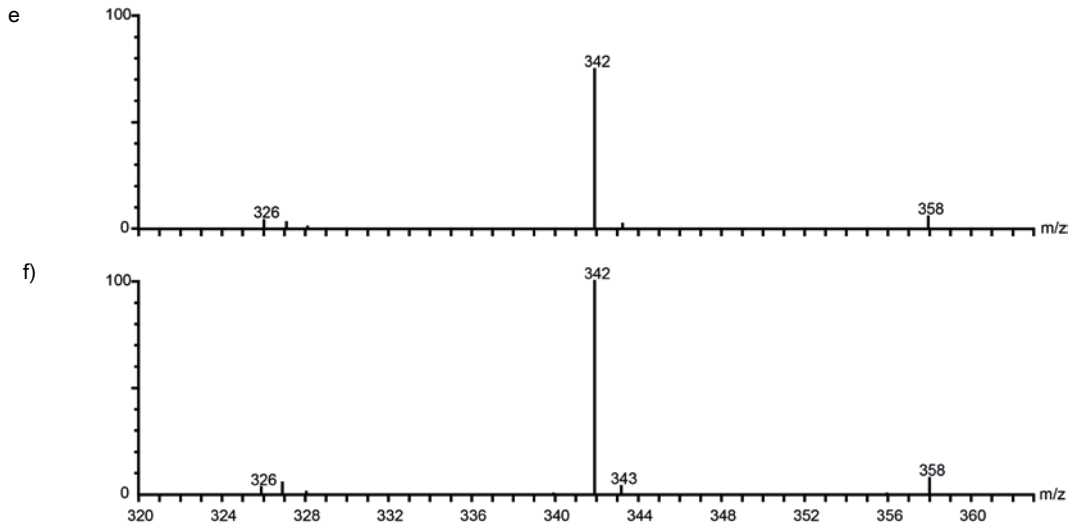


Figure 4. a) Base peak chromatogram of alkaline fraction number 9 from pizza box analyzed by UHPLC-ESI+-qTOF MS. The cut-off is represented by the dotted line. b) Spectra from the peak of Solvent Violet 8 obtained at 100 V with UHPLC-ESI-qTOF MS with suggested molecular formula ($C_{24}H_{27}N_3$) and the isotope pattern of suggested formula. c) Retention time and ion transitions for the standard of Solvent Violet 8 (200 ng mL^{-1}) obtained by UHPLC-ESI-QqQ MS in MRM mode. d) Retention time and ion transitions for the fraction suspected of containing Solvent Violet 8 obtained the same method as the standard. e) Fragmentation pattern of Solvent violet 8 standard at 1 µg/mL f) Fragmentation pattern of alkaline fraction number 9 from sample S8 in positive mode (diluted 1:1000 *v/v* in ethanol).

9.3

Appendix A

Comprehensive lists of compounds tentatively identified in fractions with positive toxicological effect in arylhydrocarbon receptor (AhR) assay

Appendix A: Tentatively identified compounds

Data presented in this Appendix are the results obtained from the tentative identification process of fractions with positive toxicological response in the AhR assay. Cut-offs are indicated as dotted-lines in the chromatograms.

Indicated in the columns are:

Compound name

CAS number: if available

Molecular formula

Retention time: In respective method

Ionization mode: GC-EI, LC-ESI+ and LC-ESI-

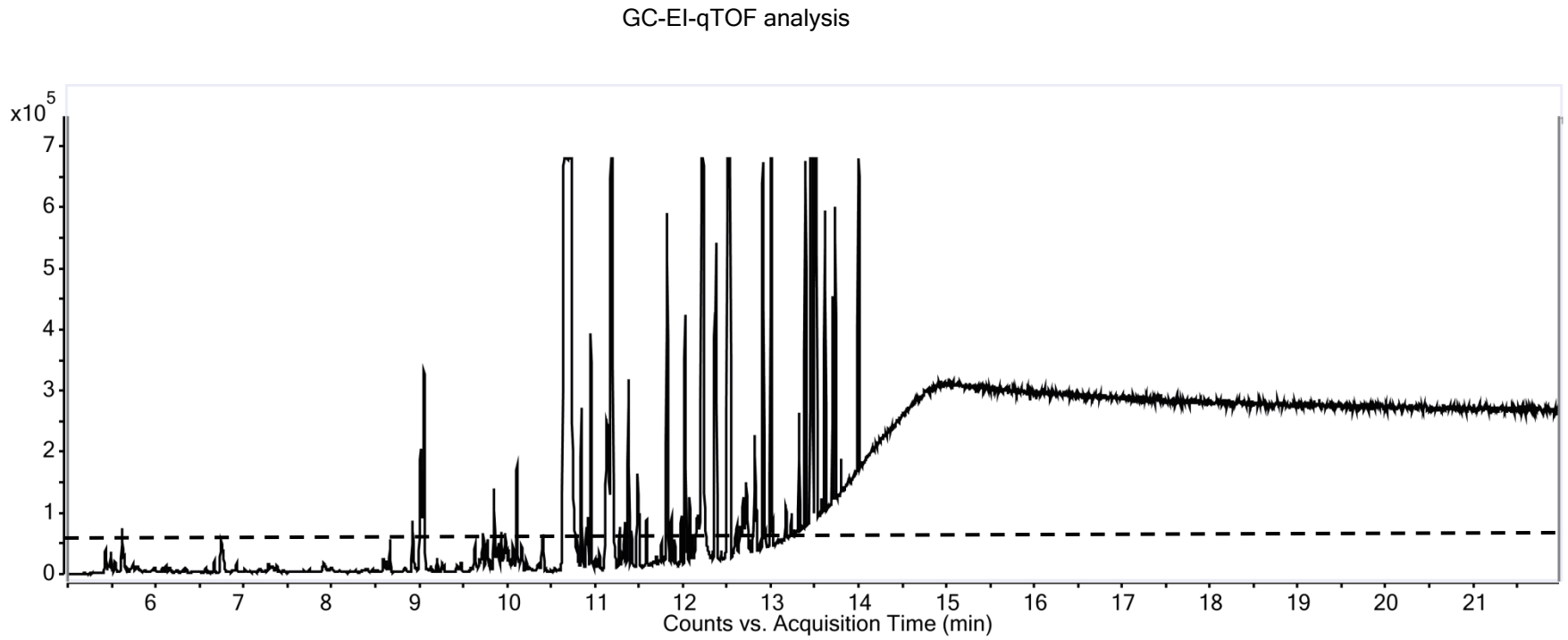
Customized database hit: The accurate mass database with matrix relevant entries were only used for compounds identified in LC-ESI+ or LC-ESI-

Number of ions: only used for compounds identified in LC-ESI+ or LC-ESI-. Adducts (if present) are also registered as these could facilitate localization of molecular ion.

Additional information: Significant isotope matched fragments, relevant usage in paper and board

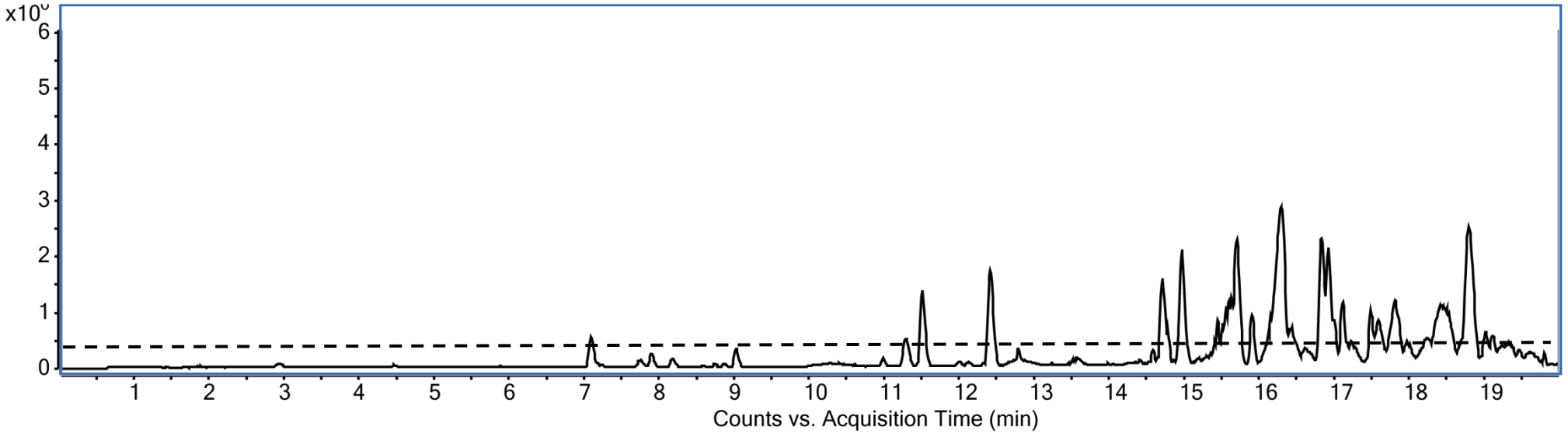
S8 acidic fraction 8

(positive response in AhR)



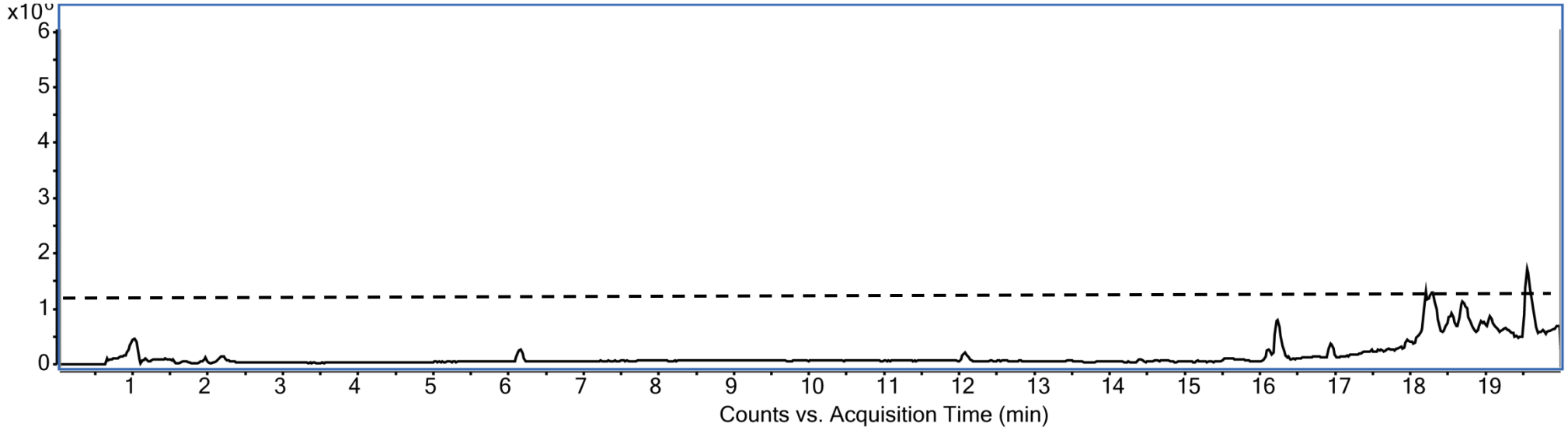
LC-ESI-qTOF analysis

(positive mode)



LC-ESI-qTOF analysis

(negative mode)



Compound	CAS number	Molecular formula	Retention time	EI	ESI +	ESI -	Customized database hit	Number of ions (for LC only)	Additional information
			5.62	x					Column impurity
Ethyl benzoate	93-89-0	C9H10O2	6.72	x					Used in liquid compositions for inkjet printing
2,4-Di-t-butylphenol	96-76-4	C14H22O	8.67	x					Used in ink set in inkjet printing
Lauric acid	143-07-7	C12H24O2	8.92	x					Used in alkyl resins, wetting agents and detergents
1-Isocyanatododecane	4202-38-4	C13H25NO	9.03	x					Used in coatings, adhesives and in printing
1-Isocyanatooctadecane	112-93-9	C19H37NO	9.05	x					Used in coatings, adhesives and in printing
4-(2,4,4-Trimethyl-2-pentanyl)phenol	140-66-9	C14H22O	9.85	x					Degradation product of alkylphenol surfactants
2,6-Di-iso-propylnaphthalene	24157-81-1	C16H20	10.11	x					Used in adhesives and in polymer production
1,2-diphenoxyethane	104-66-5	C14H14O2	10.41	x					Used in thermal paper (receipts)
Butyl cyclohexyl phthalate	84-64-0	C18H24O4	10.72	x					Fragments into C8H4O3 (phthalate specific fragment). Used in curable adhesives and in polymer production
			10.84	x					Aliphatic fragmentation pattern
Butyl 2-ethylhexyl phthalate	85-69-8	C20H30O4	10.92	x					Fragments into C8H4O3 (phthalate specific fragment). Used as a plasticizer
Hexadecanoic acid, methyl ester	112-39-0	C17H34O2	11.11	x					Used in thermal papers (receipts) in polymer films and as food flavouring
Dibutyl phthalate	84-74-2	C16H22O4	11.21	x					Used in in printing inks, resin solvent, paper coatings and in adhesives
			11.43	x					Fragments into CAS number 1746-11-8
1-hexadecanoic acid	57-10-3	C16H32O2	11.52	x					Common fatty acid that occurs in natural fats and in oils and non-drying oil for surface coatings
Ethyl Hexadecanoate	628-97-7	C18H36O2	11.55	x					Rheology control agent for coating compositions
			11.82	x					Aliphatic fragmentation pattern
1-eicosanol	629-96-9	C20H42O	11.99	x					Used in coatings
11-Octadecenoic acid, methyl ester	52380-33-3	C19H36O2	12.07	x					Rheology control agent for coating compositions
Methyl n-octadecanoate	112-61-8	C19H38O2	12.18	x					Occurs naturally as a flavour component of some foods as well as in lubricants and polymer production
1-Benzyloxy-naphthalene	607-58-9	C17H14O	12.21	x					Used in thermal paper (receipts)
Oleic acid	112-80-1	C18H34O2	12.25	x					Used as coatings for waterproof surfaces and food grade additives
Ethyl oleate	111-62-6	C20H38O2	12.39	x					Cationic surfactant
			12.41	x					Aliphatic fragmentation pattern, chlorinated

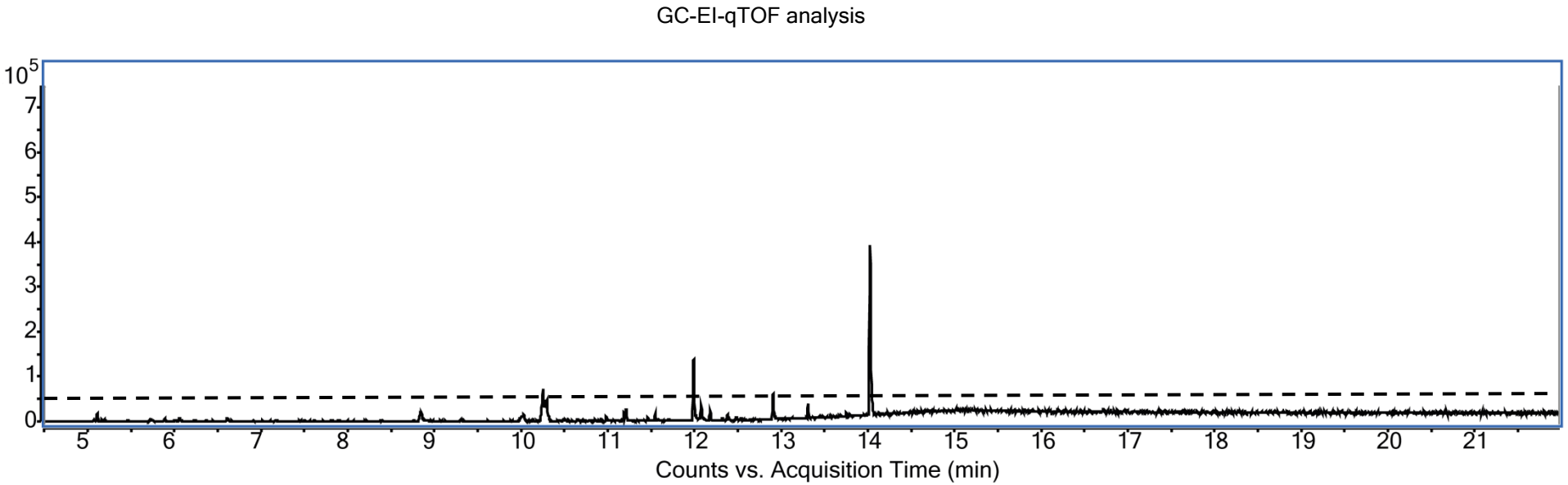
Compound	CAS number	Molecular formula	Retention time	EI	ESI +	ESI -	Customized database hit	Number of ions (for LC only)	Additional information
Methyl 17-methyloctadecanoate	55124-97-5	C20H40O2	12.48	x					Used in fibre washing process (ink flotation)
1,3-Dimethoxy-5-[(E)-2-phenylvinyl]benzene	21956-56-9	C16H16O2	12.73	x					
			12.91	x					Aliphatic fragmentation pattern
Benzyl Butyl Phthalate	85-68-7	C19H20O4	13.00	x					Used in in printing inks, resin solvent, paper coatings and in adhesives
			13.32	x					Aliphatic fragmentation pattern
2-(4-Fluoro-phenyl)-5-nitro-isoindole-1,3-dione		C14H7FN2O4	13.39	x					
2-(2-(Benzoyloxy)propoxy)propyl benzoate	20109-39-1	C20H22O5	13.49	x					Used in adhesive compositions
Methyl 4-methylbenzyl terephthalate	67801-55-2	C17H16O4	13.61	x					
1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro-9-hydroxy-1,4a-dimethyl-7-(1-methylethy	1802-09-1	C21H30O3	13.7	x					
Dicyclohexyl phthalate	84-61-7	C20H26O4	13.74	x					Fragments into C8H4O3 (phthalate specific fragment). Used in curable adhesives and in polymer production
Bis(2-ethylhexyl) phthalate	117-81-7	C24H38O4	14.03 (GC); 17.1 (LC)	x	x			2	Fragments into C8H4O3 (phthalate specific fragment). Used in curable adhesives and in polymer production
2-Ethyl-2-((4-hydroxybutoxy)methyl)propane-1,3-diol	81125-12-4	C10H22O4	2.932		x		x	2	Used as ink solvent
N-[4-(2-Thienyl)-1,3-thiazol-2-yl]propanamide		C10H10N2OS2	7.089		x			2	Fragments into C3H5NO
Carbamodithioic acid, dimethyl-, 2-benzothiazolyl ester (9CI)	3432-25-5	C10H10N2S3	7.76		x			2	Fragments into C3H5SN

Compound	CAS number	Molecular formula	Retention time	EI	ESI +	ESI -	Customized database hit	Number of ions (for LC only)	Additional information
4-(2-Ethoxyphenyl)-N-ethyl-1-piperazinecarbothioamide		C15H23N3OS	7.906		x			2	Fragments into C3H5SN
Benzyl dimethylcarbamodithioate	7250-18-2	C10H13NS2	9.027		x			2	Fragments into C3H5SN, used in printing inks
3-{[4-(2-Thienyl)-1,3-thiazol-2-yl]sulfanyl}propanoic acid		C10H9NO2S3	10.995		x			3	Fragments into C9H9NOS3 and C7H5NS3
		C19H21N3O	11.299		x			1	
Dehydroabietic acid	1740-19-8	C20H28O2	11.519		x		x	2	Very small fragment at C9H14, resin acid
(2E)-2-(1,3-Benzothiazol-2-ylsulfanyl)-3-(2-thienyl)acrylonitrile		C14H8N2S3	12.419		x			2	Fragments into C7H3NS2
		C19H37NOS	14.78		x			4	Fragments into C16H31NOS, C6H12S and C3H6S
		C27H29NO3S	14.974		x			1	
Oxazoline, 2- (8-heptadecenyl)-		C20H37NO	15.707		x			1	
N-(1'-Methyl-1,4'-bipiperidin-3-yl)-1-(1-piperidinyl)cyclohexanecarboxamide		C23H42N4O	15.707		x			2 (NH4+ adduct)	
		C20H49N7O4	15.707		x				
		C31H30N4O5	15.906		x			1	
		C28H57NO7	16.294		x			2	Fragments into C20H36NO2
		C32H53N9	16.294		x			1	
Stearamide	124-26-5	C18H37NO	16.838		x			1	Used in coatings and toners
		C18H35N3O	16.9		x			1	
		C25H49N5O	17.1		x			1	
		C22H32N6O2	17.1		x			1	
		C32H55NO7	17.487		x			1	
		C24H51N5O7	17.487		x			1	

Compound	CAS number	Molecular formula	Retention time	EI	ESI +	ESI -	Customized database hit	Number of ions (for LC only)	Additional information
		C41H55NO3	17.487		x			1	
3,6,9,12,15-Pentaoxadotriacontan-1-ol	35056-96-3	C27H56O6	17.592		x			1 (NH4+ adduct)	
3,6,9,12-Tetraoxanonacosan-1-ol	207385-29-3	C21H48N6O3	17.812		x			1 (NH4+ adduct)	
2-Mercaptobenzothiazole	149-30-4	C7H5NS2	6.617			x	x	2	C7H5NS. Used in rubber and latex production as well as in paper manufacturing, production of lithographic plates and two-part cyanoacrylate adhesives
Dodecyl hydrogen sulfate		C13H26O4S	12.082			x		2	Fragments into C13H24, used as surfactant
Propane, 2,2-bis[4-(2-hydroxypropyloxy)-phenyl]-	116-37-0	C21H28O4	16.234			x	x	2	Used in printing processes, epoxy resins and polymerization
Mesamoll mono SO3 C13	10157-76-3	C19H32O3S	16.234			x	x	1	Used in plastics
Benzyl octyl phthalate	68515-40-2	C23H28O4	16.234			x	x	1	C23H28O4, no phthalate specific fragments observed. Very low conc.
6-((1-Oxo-1,2,3,5,6,7-hexahydro-s-indacen-2-yl)methyl)-4-indanecarboxylic acid		C23H22O3	16.933			x		1 (-CO2)	
Methanol, tri-p-tolyl-	3247-00-5	C22H22O	16.933			x	x	1	Used in photosensitive resin composition

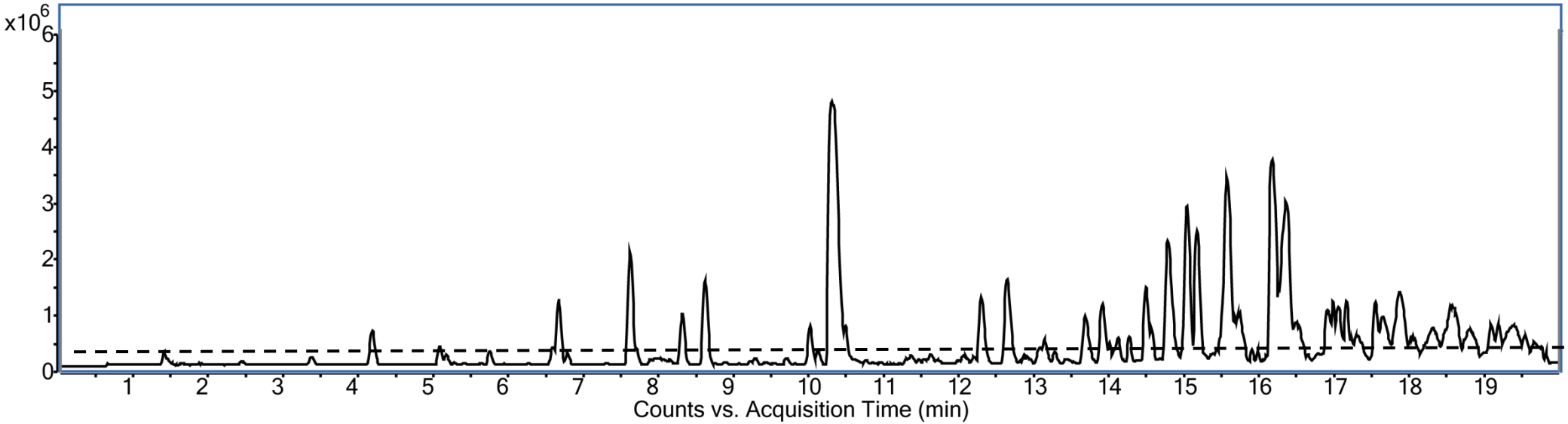
S8 alkaline fraction 8

(positive response in AhR)



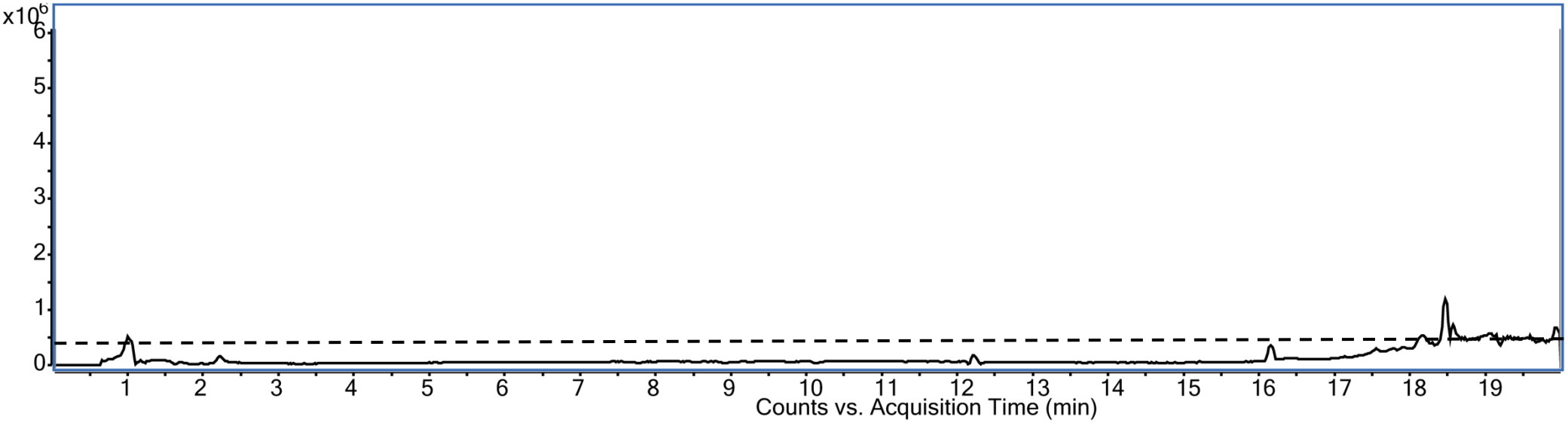
LC-ESI-qTOF analysis

(positive mode)



LC-ESI-qTOF analysis

(negative mode)



Compound	CAS number	Molecular formula	Retention time	EI	ESI+	ESI-	Customized database hit	Number of ions (for LC only)	Additional information
Dimethyl-n-decylamine	1120-24-7	C12H27N	8.85	x					Used in adhesives
2,6-Di-iso-propylnaphthalene	24157-81-1	C16H20	10.28	x					Used in adhesives and in polymer production
Dibutyl phthalate	84-74-2	C16H22O4	10.98	x					Used in platicizers and printing inks
2-Phenyldodecane	2719-61-1	C18H30	11.19	x					Dused in dyeing compositions
Hexadecanoic acid, methyl ester	112-39-0	C17H34O2	11.21	x					Used in thermal papers (receipts) in polymer films and as food flavouring
Ethyl Hexadecanoate	628-97-7	C18H36O2	11.55	x					Rheology control agent for coating compositions and pigment agent
11-Octadecenoic acid, methyl ester	52380-33-3	C19H36O2	12.07	x					Rheology control agent for coating compositions
Methyl n-octadecanoate	112-61-8	C19H38O2	12.18	x					Occurs naturally as a flavour component of some foods as well as in lubricants and polymers production
Ethyl oleate	111-62-6	C20H38O2	12.39	x					Cationic surfactant
1-Docosanol	661-19-8	C22H46O	12.91	x					Used in synthetic fibres and lubricants, thermal papers and toners
Methyl 8,11,13-abietatrien-18-oate	1235-74-1	C21H30O2	13.31	x					Resin acid
Bis(2-ethylhexyl) phthalate	117-81-7	C24H38O4	14.03 (GC)/ 17.01(LC)	x	x		x	2	Used in curable adhesives and in polymer production
Pentanamide	626-97-1	C5H11NO	1.414		x		x	4	Stabilizing agent for powder paints and dyes
		C13H23N3S	3.362		x			1	
		C14H25N3S	4.189		x			1	
Octadecyl 4-methylbenzenesulfonate	3386-32-1	C25H44O3S	5.089		x		x	1	Used in ink compositions
		C27H43NO	5.089		x			1	
4-(1,5-diphenylpentan-3-yl)pyridine	2057-47-8	C22H23N	5.759		x			1	
Benzeneethanamine, n,n-bis(phenylmethyl)-		C22H23N	5.759		x			1	

Compound	CAS number	Molecular formula	Retention time	EI	ESI+	ESI-	Customized database hit	Number of ions (for LC only)	Additional information
1,3-Dibenzyl-2-phenylimidazolidin	4597-81-3	C23H24N2	6.503		x			1	Used in the formation of olefin polymers (used in heat-sealable papers such as tea and coffee bags
p-(Diethylamino)benzaldehyde diphenylhydrazone	68189-23-1	C23H25N3	6.670		x			1	Used as photo initiator
2,2'-(Tridecylimino)diethanol		C17H37NO2	6.786		x			1	Used in olefin production
Solvent Violet 8	52080-58-7	C24H27N3	7.623		x		x	1	Used in ink as blue/violet dye
Basic red 1	989-38-8	C28H30N2O3	8.314		x		x	1	used in ink as red dye
Baso Red 546	509-34-2	C28H30N2O3	8.314		x		x	1	Used in ink (ink-jet printers)
		C25H29N3	8.618		x			1	
		C32H29N3	9.707		x			1	
1-Isopropyl-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid	436811-11-9	C15H18N2O2	10.021		x			4	
N-Benzyl-1-tetradecanamine		C21H37N	10.52		x			3	Fragments into C14H29N, C7H6. Used in organophilic clays for oil-repellent properties
		C30H33N3O3	12.293		x			1	
			12.649		x			2	m/z: 634.4576 and 474.3830
			12.649		x			2	m/z: 332.3353 and 240.2711
(9Z)-9-Icosenoic acid	29204-02-2	C20H38O2	12.995		x			2	Used in coatings
		C32H31N7O	13.11		x			1	
		C20H32N2O8/C20H28N8O6	13.11		x			1	
di-n-Undecylamine	16165-33-6	C22H47N	13.686		x			1	Used in cross-linking polymers for coating
Rhodamine 101	116450-56-7	C32H30N2O3	13.916		x			1	Used in photoreceptor layers and optical filters
Stearyldiethanolamine	10213-78-2	C22H47NO2	14.126		x			1	Used as organic filler and for sheet formation in paper making
2-(2-(4-Nonylphenoxy)ethoxy)ethanol (commercial name: Alfenol)	9062-77-5	C19H32O3	14.126		x			2 (+H2O adduct)	Used as detergent, emulsifier, wetting agent, defoaming agent

Compound	CAS number	Molecular formula	Retention time	EI	ESI+	ESI-	Customized database hit	Number of ions (for LC only)	Additional information
1,3,4-Tris(4-methylphenyl)-2,5-diphenyl-2,4-cyclopentadien-1-ol		C38H32O	14.492		x			1	Used for cellulose ester film
		C19H37NOS	14.786		x			4	Fragments into C16H31NOS, C6H12S, C3H6S
		C19H25N7O6	15.047		x			1	
4-[(4-Isopropoxyphenyl)sulfonyl] phenol	95235-30-6	C15H16O4S	15.047		x		x	1	Used in thermal papers (receipts)
		C46H34N6O4	15.047		x			1 (spectral peaks at 0.5)	Double charged from C23H17N3O2
2'-(Dibenzylamino)-6'-(diethylamino)-3H-spiro[2-benzofuran-1,9'-xanthen]-3-one	34372-72-0	C38H34N2O3	15.173		x		x	1	Used as dye
		C27H22N8O5	15.571		x			2	
		C26H26N8O/ C26H22N12O	15.571		x			2	
26-[4-(2,4,4-Trimethyl-2-pentanyl)phenoxy]-3,6,9,12,15,18,21,24-octaoxahexacosan-1-ol		C32H58O10	15.571		x		x	2	Used as a surfactant
		C60H76N8S4	16.178		x			1 (spectral peaks at 0.5)	Double charged from C30H38N4S2
Mandenol (Linoleic acid ethyl ester)		C20H36O2	16.45		x		x	2	
		C29H57N11O3	16.45		x			2 (+NH4+adduct)	
		C28H37NO	16.89		x			1	
		C20H39NO	16.91		x			1	
		C17H8Cl2N2O2	17.05		x			1	

Compound	CAS number	Molecular formula	Retention time	EI	ESI+	ESI-	Customized database hit	Number of ions (for LC only)	Additional information
9,12,15,18,21-Pentaoxanonacosane		C24H50O5	17.05		x			2 (+NH4+adduct)	
		C30H63NO8	17.5		x			2 (+H2O adduct)	
3,6,9,12,15-Pentaoxadotriacontan-1-ol	35056-96-3	C27H56O6	17.7		x			2 (NH4+ adduct)	
3,6,9,12-Tetraoxanonacosan-1-ol	207385-29-3	C21H48N6O3	17.8		x			2 (NH4+ adducts)	
		C4H4N2O6S	2.2			x		6	
2-[Formyl(2-hydroxyethyl)amino]ethyl (9E,12E)-9,12-octadecadienoate		C23H41NO4	12.2			x		1	
Dodecyl p-toluenesulfonate	10157-76-3	C19H32O3S	16.2			x	x	1	Used in plastics
		C16H10ClN3O3	16.2			x		1	

9.4

Appendix B

Accurate mass database used for the tentative identification process for data acquired by UPLC-ESI-qTOF MS

Monoisotopic mass	Formula	CAS number	Compound name
53,02655	C3H3N	107-13-1	Acrylonitrile
54,04695019	C4H6		butadiene
54,046951	C4H6	106-99-0	1,3-Butadiene
56,062599	C4H8	115-11-7	Isobutene
56,062599	C4H8	106-98-9	1-Butene
56,06260026	C4H8		Irganox 1076 thermal degradation products
57,05785	C3H7N	75-55-8	Aziridine, 2-methyl-
58,005478	C2H2O2	107-22-2	Glyoxal
58,04186481	C3H6O	123-38-6	propionaldehyde
58,041866	C3H6O	67-64-1	Acetone
58,041866	C3H6O	107-25-5	Methyl vinyl ether
58,041866	C3H6O	75-56-9	Propylene oxyde
58,078251	C4H10	106-97-8	Butane
58,078251	C4H10	75-28-5	Isobutane
59,03711379	C2H5NO		acetamide
59,07349929	C3H9N		isopropenylamine
60,02113	C2H4O2	64-19-7	Acetic acid
60,057514	C3H8O	67-63-0	2-Propanol
60,057514	C3H8O	71-23-8	1-Propanol
60,05751488	C3H8O		glycerol, glycerine
60,068748	C2H8N2	107-15-3	Ethylenediamine
60,068748	C2H8N2	57-14-7	Hydrazine, 1,1-dimethyl-
61,052765	C2H7NO	141-43-5	2-Aminoethanol
61,992329	C2H3Cl	75-01-4	Vinyl Chloride
62,036777	C2H6O2	107-21-1	Ethylene glycol
62,03677944	C2H6O2		ethylene glycol
68,062599	C5H8	78-79-5	2-Methyl-1,3 -butadiene
70,041862	C4H6O	9003-19-4	Polyvinyl ether
71,037117	C3H5NO	79-06-1	Acrylamide
71,037117	C3H5NO	9003-05-8	Polyacrylamide
72,021126	C3H4O2	79-10-7	Acrylic acid
72,057518	C4H8O	106-88-7	Butane, 1,2-epoxy-
72,057518	C4H8O	109-92-2	Ethyl vinyl ether
72,057518	C4H8O	109-99-9	Tetrahydrofuran
72,057518	C4H8O	78-93-3	2-Butanone
72,057999	C4H8O	123-72-8	Butyraldehyde
72,093903	C5H12	109-66-0	Pentane
72,093903	C5H12	78-78-4	Isopentane
73,052765	C3H7NO	68-12-2	Dimethylformamide
74,036781	C3H6O2	646-06-0	1,3-Dioxolane
74,036781	C3H6O2	556-52-5	2,3-Epoxypropanol
74,036781	C3H6O2	79-09-4	Propionic acid
74,036781	C3H6O2	79-20-9	Acetic acid, methyl ester

Monoisotopic mass	Formula	CAS number	Compound name
74,07316494	C4H10O		butanol
74,07316494	C4H10O		2-methylpropan-1-ol
74,073166	C4H10O	75-65-0	tert-Butanol
74,073166	C4H10O	78-83-1	Isobutanol
74,073166	C4H10O	71-36-3	1-Butanol
74,073166	C4H10O	78-92-2	2-Butanol
75,068413	C3H9NO	109-83-1	Ethanol, 2-(methylamino)-
76,016045	C2H4O3	79-14-1	Glycolic acid
76,016045	C2H4O3	79-21-0	Peroxyacetic acid
76,052429	C3H8O2	25322-69-4	Polypropyleneglycol
76,052429	C3H8O2	504-63-2	1,3-Propanediol
76,052429	C3H8O2	57-55-6	1,2-Propanediol
76,052429	C3H8O2	63625-56-9	Propylenglycol
76,052429	C3H8O2	109-86-4	Ethylene glycol monomethyl ether
78,013931	C2H6OS	67-68-5	Dimethyl sulphoxide
78,04695019	C6H6		benzene
78,04695019	C6H6		benzene
78,04695019	C6H6		benzene
78,046997	C6H6	71-43-2	Benzene
79,042198	C5H5N	110-86-1	Pyridine
80,06260026	C6H8		butadiene n=2
82,07825032	C6H10		2-methyl-2,4-pentadiene
84,043594	C2H4N4	461-58-5	Dicyanodiamide
84,05751488	C5H8O		cyclopentanone
84,093903	C6H12	110-82-7	Cyclohexane
84,093903	C6H12	592-41-6	1-Hexene
84,093903	C6H12	691-37-2	4-Methyl-1-pentene
85,052765	C4H7NO	110-67-8	Propionitrile, 3-methoxy-
85,052765	C4H7NO	1187-59-3	N-Methylacrylamide
85,052765	C4H7NO	79-39-0	Methacrylamide
86,036781	C4H6O2	108-05-4	Acetic acid, vinyl ester
86,036781	C4H6O2	3724-65-0	Crotonic acid
86,036781	C4H6O2	79-41-4	Methacrylic acid
86,036781	C4H6O2	9003-20-7	Polyvinyl acetate
86,036781	C4H6O2	96-33-3	Acrylic acid, methyl ester
86,036781	C4H6O2	96-48-0	γ-Butyrolactone
86,07316494	C5H10O		cyclopentanone
86,073166	C5H10O	107-88-0	1,3-Butanediol
86,084396	C4H10N2	110-85-0	Piperazine
87,06841392	C4H9NO		butanamide
88,052002	C4H8O2	107-92-6	Butyric acid
88,052429	C4H8O2	141-78-6	Acetic acid, ethyl ester
88,06366	C3H8N2O	96-31-1	Urea, 1,3-dimethyl-

Monoisotopic mass	Formula	CAS number	Compound name
88,088814	C5H12O	71-41-0	1-Pentanol
89,047676	C3H7NO2	4316-73-8	Sarcosine, monosodium salt
89,084061	C4H11NO	3710-84-7	N,N-diethylhydroxylamine
89,084061	C4H11NO	108-01-0	Dimethylaminoethanol
89,084061	C4H11NO	124-68-5	2-Amino-2-methylpropanol
89,995003	C2H2O4	144-62-7	Oxalic acid
90,031693	C3H6O3	625-45-6	Acetic acid, methoxy-
90,031693	C3H6O3	110-88-3	Trioxane
90,031998	C3H6O3	50-21-5	Lactic acid
90,042931	C2H6N2O2	9011-05-6	Urea-formaldehyde condensation products
90,068077	C4H10O2	107-98-2	1-Methoxy-2-propanol
90,068077	C4H10O2	110-63-4	1,4-Butanediol
90,068077	C4H10O2	110-80-5	Ethyleneglycol monoethyl ether
90,068077	C4H10O2	2163-42-0	2-Methyl-1,3-propanediol
90,068077	C4H10O2	75-91-2	tert-Butyl hydroperoxide
91,99320006	C2H4O2S		mercaptoacetic acid
92,002892	C3H5ClO	106-89-8	Epichlorohydrin
92,00289249	C3H5OCl		epichlorohydrin
92,04734	C3H8O3	56-81-5	Glycerol
92,04734412	C3H8O3		glycerol
92,04734412	C3H8O3		glycerol
92,062599	C7H8	108-88-3	Toluene
93,057846	C6H7N	62-53-3	Aniline
93,05784923	C6H7N		2-methyl-pyridine
93,05787426	C6H7N		Aniline
93,982155	C2H3ClO2	79-11-8	Monochloroacetic acid
94,04186481	C6H6O		phenol
94,04186481	C6H6O		phenol
94,04186481	C6H6O		phenol
94,042	C6H6O	108-95-2	Phenol
94,053101	C5H6N2	1072-63-5	1-Vinylimidazole
94,07670203	C7H8D		methylphenol
94,078247	C7H10	498-66-8	Bicyclo[2.2.1]hept-2-ene
95,953354	C2H2Cl2	75-35-4	Vinylidene chloride
96,021126	C5H4O2	98-01-1	2-Furaldehyde
97,969009	C2H4Cl2	107-06-2	1,2-Dichloroethane
97,969009	C2H4Cl2	1300-21-6	Dichloroethane
98,000397	C4H2O3	108-31-6	Maleic anhydride
99,068413	C5H9NO	2680-03-7	N,N-Dimethylacrylamide
99,068413	C5H9NO	872-50-4	N-Methylpyrrolidinone
99,068413	C5H9NO	3195-78-6	N-Vinyl-N-methylacetamide
99,104797	C6H13N	108-91-8	Cyclohexylamine
99,993614	C2F4	9002-84-0	Polytetrafluorethylene

Monoisotopic mass	Formula	CAS number	Compound name
99,993614	C2F4	116-14-3	Tetrafluorethylene
100,016044	C4H4O3		cis-butanedioic anhydride
100,016045	C4H4O3	108-30-5	Succinic anhydride
100,052429	C5H8O2	140-88-5	Acrylic acid, ethyl ester
100,052429	C5H8O2	111-30-8	Glutaraldehyde
100,052429	C5H8O2	25035-84-1	Polyvinyl propionate
100,052429	C5H8O2	80-62-6	Methacrylic acid, methyl ester
100,052429	C5H8O2	9003-33-2	Polyvinyl formal
100,088814	C6H12O	108-10-1	Methyl isobutyl ketone
100,088814	C6H12O	109-53-5	Isobutyl vinyl ether
101,047676	C4H7NO2	924-42-5	N-Methylolacrylamide
101,084064	C5H11NO		pentanamide
101,120445	C6H15N	121-44-8	Triethylamine
102,031693	C4H6O3	108-32-7	Carbonic acid, cyclic propylene ester
102,031693	C4H6O3	108-24-7	Acetic anhydride
102,0429274	C3H6N2O2		acetyl urea
102,068077	C5H10O2	108-21-4	Acetic acid, isopropyl ester
102,068077	C5H10O2	109-52-4	Valeric acid
102,068077	C5H10O2	75-98-9	Dimethylpropionic acid
102,068077	C5H10O2	109-60-4	Acetic acid, propyl ester
102,0680796	C5H10O2		pentanoic acid
102,104462	C6H14O	25917-35-5 111-27-3	Hexanol
103,0421992	C7H5N		benzonitrile
103,110947	C4H13N3	111-40-0	Diethylenetriamine
104,011002	C3H4O4	141-82-2	Malonic acid
104,062599	C8H8	100-42-5	Styrene
104,0626003	C8H8		styrene
104,083733	C5H12O2	2807-30-9	2-(Propyloxy)ethanol
104,083733	C5H12O2	1569-02-4	1-Ethoxy-2-propanol
104,083733	C5H12O2	126-30-7	2,2-Dimethyl-1,3-propanediol
104,094963	C4H12N2O	111-41-1	N-(2-Aminoethyl)ethanolamine
104,107002	C5H14NO	123-41-1	Choline and its salts
105,057846	C7H7N	100-43-6	4-Vinylpyridine
105,078979	C4H11NO2	111-42-2	Diethanolamine
106,018539	C4H7ClO	598-09-4	2-(Chloromethyl)-2-methyloxirane
106,0418648	C7H6O		benzaldehyde
106,0418648	C7H6O		benzaldehyde
106,042	C7H6O	100-52-7	Benzaldehyde
106,0629942	C4H10O3		diethylene glycol
106,062996	C4H10O3	111-46-6	Diethyleneglycol
106,078247	C8H10	100-41-4	Ethylbenzene
106,078247	C8H10	1330-20-7	Xylene
106,0782503	C8H10		2,6-dimethylbenzene

Monoisotopic mass	Formula	CAS number	Compound name
106,0782503	C8H10		butadiene n=3
106,0782503	C8H10		ethylbenzene
106,0782754	C8H10		xylene
106,986336	C2H5NS2	144-54-7	Methyldithiocarbamic acid
107,0734993	C7H9N		benzenmethanamine, aminotoluene
107,0734993	C7H9N		2,6-dimethylpyridine
107,073502	C7H9N	95-53-4	o-Toluidine
107,073502	C7H9N	106-49-0	p-Toluidine
107,0735243	C7H9N		o-Toluidine
107,988113	C2H4O3S	1184-84-5	Vinylsulphonic acid
107,988113	C2H4O3S	3039-83-6	Sodium vinylsulphonate
108,021126	C6H4O2	106-51-4	Benzoquinone
108,0211294	C6H4O2		benzoquinone
108,0211294	C6H4O2		p-benzoquinone
108,034195	C4H9ClO	36215-07-3	Propane, 1-chloro-3-methoxy-
108,0575149	C7H8O		HydroxyPh
108,057518	C7H8O	106-44-5	p-Cresol
108,057518	C7H8O	108-39-4	m-Cresol
108,057518	C7H8O	95-48-7	o-Cresol
108,057518	C7H8O	100-51-6	Benzyl alcohol
108,068748	C6H8N2	106-50-3	p-Phenylenediamine
108,0687483	C6H8N2		p-phenylenediamine
109,052765	C6H7NO	123-30-8	4-Aminophenol
110,036781	C6H6O2	108-46-3	1,3-Dihydroxybenzene
110,036781	C6H6O2	120-80-9	1,2-Dihydroxybenzene
110,037003	C6H6O2	123-31-9	1,4-Dihydroxybenzene
110,084396	C6H10N2	4078-19-7	1-Aziridinepropionitrile, .beta.-methyl-
111,068413	C6H9NO	9003-39-8	Polyvinylpyrrolidone
111,068413	C6H9NO	88-12-0	Vinylpyrrolidone
111,984657	C3H6Cl2	78-87-5	1,2-Dichloropropane
112,00798	C6H5Cl	108-90-7	Chlorobenzene
112,052429	C6H8O2	999-55-3	Acrylic acid, allyl ester
112,052429	C6H8O2	110-44-1	Sorbic acid
112,0524545	C6H8O2		m-Phenylenediamine
112,088814	C7H12O	1679-51-2	4-(Hydroxymethyl)-1- cyclohexene
112,125198	C8H16	111-66-0	1-Octene
112,125198	C8H16	25167-70-8	Diisobutene
112,1252005	C8H16		tert-octene
113,0476785	C5H7NO2		glutarimide
113,084061	C6H11NO	2210-25-5	N-Isopropylacrylamide
113,084061	C6H11NO	105-60-2	Caprolactam
113,084064	C6H11NO		caprolactam
114,068077	C6H10O2	106-92-3	Allyl-2,3-epoxypropyl ether

Monoisotopic mass	Formula	CAS number	Compound name
114,068077	C6H10O2	24937-78-8	Ethylene-vinyl acetate, copolymer
114,068077	C6H10O2	4454-05-1	2H-Pyran, 3,4-dihydro-2-methoxy-
114,068077	C6H10O2	689-12-3	Acrylic acid, isopropyl ester
114,068077	C6H10O2	925-60-0	Acrylic acid, propyl ester
114,068077	C6H10O2	97-63-2	Methacrylic acid, ethyl ester
114,1044651	C7H14O		2-ethyl-cyclopentanone
114,1408506	C8H18		octane
115,009186	C4H5NOS	55965-84-9	Mixture of 5-Chlor-2-methyl-2H-isothiazol-3-on and 2-Methyl-2H-isothiazol-3-on
115,009186	C4H5NOS	2682-20-4	2-Methyl-4-isothiazolin-3-one
115,063332	C5H9NO2	923-02-4	N-Methylolmethacrylamide
116,010956	C4H4O4	110-16-7	Maleic acid
116,010956	C4H4O4	110-17-8	Fumaric acid
116,0109586	C4H4O4		maleic acid
116,0334504	C2H4N4O2		Azodicarbonamide
116,04734	C5H8O3	818-61-1	Acrylic acid, monoester with ethyleneglycol
116,04734	C5H8O3	123-76-2	Levulinic acid
116,062599	C9H8	95-13-6	Indene
116,083733	C6H12O2	123-86-4	Acetic acid, butyl ester
116,083733	C6H12O2	142-62-1	Hexanoic acid
116,083733	C6H12O2	3126-95-2	Oxirane, (propoxymethyl)-
116,083733	C6H12O2	4016-14-2	Propane, 1,2-epoxy-3-isopropoxy-
116,083733	C6H12O2	106-36-5	propyl propanoate
116,083733	C6H12O2	110-19-0	Acetic acid, isobutyl ester
116,1201151	C7H16O		2,4-dimethylpentan-1-ol
116,120117	C7H16O	111-70-6	1-Heptanol
116,131348	C6H16N2	124-09-4	Hexamethylenediamine
116,131348	C6H16N2	280-57-9	Triethylenediamine
117,115364	C6H15NO	100-37-8	Diethylethanolamine
118,0266087	C4H6O4		butanedioic acid
118,026611	C4H6O4	110-15-6	Succinic acid
118,026611	C4H6O4	110-22-5	Diacetyl peroxide
118,053101	C7H6N2	1885-29-6	Anthranilonitrile
118,078247	C9H10	98-83-9	α -Methylstyrene
118,0782503	C9H10		alpha-methylstyrene
118,09938	C6H14O2	4457-71-0	3-Methylpentane-1,5-diol
118,09938	C6H14O2	107-41-5	2-Methyl-2,4-pentanediol
118,09938	C6H14O2	111-76-2	Ethyleneglycol monobutyl ether
118,09938	C6H14O2	629-11-8	1,6-Hexanediol
119,048347	C6H5N3	95-14-7	1H-Benzotriazole
119,0483472	C6H5N3		benzotriazole
119,0483472	C6H5N3		benzotriazole
120,05349	C3H8N2O3	140-95-4	N,N'-Bis(hydroxymethyl)urea
120,0575149	C8H8O		acetophenone

Monoisotopic mass	Formula	CAS number	Compound name
120,093903	C9H12	16219-75-3	5 -Ethylidenebicyclo [2.2.1]hept-2-ene
120,093903	C9H12	95-63-6	1,2,4-Trimethylbenzene
121,001991	C3H7NS2	79-45-8	Dimethyldithiocarbamic acid
121,089149	C8H11N	87-62-7	2,6-Xylidine
121,089149	C8H11N	103-69-5	Aniline, N-ethyl-
121,089149	C8H11N	618-36-0	Benzylamine, .alpha.-methyl-, (.+-.)-
121,0891494	C8H11N		2,4-dimethylaniline
121,0891494	C8H11N		2,6-dimethylaniline
121,0891494	C8H11N		2,4,6-trimethyl-pyridine
121,0891494	C8H11N		3-(1-methylethyl)-pyridine
121,0891744	C8H11N		2,6-Dimethylaniline
122,0367794	C7H6O2		benzoic acid
122,036781	C7H6O2	65-85-0	Benzoic acid
122,057907	C4H10O4	3586-55-8	Ethylene glycol bis(hydroxymethyl ether)
122,0731649	C8H10O		1-phenylethanol
122,073166	C8H10O	104-93-8	Anisole, p-methyl-
122,073166	C8H10O	123-07-9	Phenol, p-ethyl-
122,073166	C8H10O	576-26-1	2,6-Dimethylphenol
122,073166	C8H10O	95-65-8	3,4-xylenol
122,073166	C8H10O	95-87-4	2,5-Dimethylphenol
122,073166	C8H10O	105-67-9	2,4-Dimethylphenol
122,073166	C8H10O	108-68-9	3,5-dimethylphenol
122,0844234	C7H10N2		Toluene-2,6-diamine
122,0844234	C7H10N2		Toluene-2,4-diamine
122,1095504	C9H14		trans-2,6-dimethyl-2,4,6-heptatriene
123,0684139	C7H9NO		o-anisidine
124,052429	C7H8O2	150-76-5	4-Methoxyphenol
125,99868	C2H6O4S	77-78-1	Sulfuric acid, dimethyl ester
126,023628	C7H7Cl	100-44-7	Toluene, .alpha.-chloro-
126,065392	C3H6N6	108-78-1	2,4,6-Triamino-1,3,5-triazine
126,0653942	C3H6N6		melamine
126,068077	C7H10O2	96-05-9	Methacrylic acid, allyl ester
126,1408506	C9H18		nonene
127,0188769	C6H6NCl		4-chloroaniline
127,0494098	C3H5N5O		ammeline
127,0633285	C6H9NO2		azepane-2,7-dione
128,0334254	C3H4N4O2		ammelide
128,0374481	C8H4N2		phthalodinitrile
128,047348	C6H8O3	106-90-1	Acrylic acid, 2,3-epoxypropyl ester
128,058578	C5H8N2O2	77-71-4	5,5-Dimethylhydantoin
128,062607	C10H8	91-20-3	Naphthalene
128,0626253	C10H8		naphthalene
128,083725	C7H12O2	106-63-8	Acrylic acid, isobutyl ester

Monoisotopic mass	Formula	CAS number	Compound name
128,083725	C7H12O2	141-32-2	Acrylic acid, n-butyl ester
128,083725	C7H12O2	1663-39-4	Acrylic acid, tert-butyl ester
128,083725	C7H12O2	2210-28-8	Methacrylic acid, propyl ester
128,083725	C7H12O2	2998-08-5	Acrylic acid, sec-butyl ester
128,1565006	C9H20		nonane
128,968277	C2H4NNaS2	137-42-8	Methyldithiocarbamic acid, sodium salt
129,017441	C3H3N3O3		cyruanic acid
129,078979	C6H11NO2	1696-20-4	Morpholine, 4-acetyl-
129,078979	C6H11NO2	7659-36-1	Methacrylic acid, 2-aminoethyl ester
129,09021	C5H11N3O	6281-42-1	N-(2-Aminoethyl)ethyleneurea
129,1279402	C8H17O		2-ethylhexyl aldehyde
129,957901	C2H2CaO4	544-17-2	Calcium diformate
129,965942	C3H2N2S2	6317-18-6	Methylenebisthiocyanate
130,026611	C5H6O4	97-65-4	Itaconic acid
130,062988	C6H10O3	123-62-6	Propionic anhydride
130,062988	C6H10O3	25584-83-2	Acrylic acid, monoester with 1,2-propanediol
130,062988	C6H10O3	2238-07-5	Ether, bis(2,3-epoxypropyl)
130,062988	C6H10O3	2918-23-2	Acrylic acid, 2-hydroxyisopropyl ester (= acrylic acid, 2-hydroxy-1-methylethyl ester)
130,062988	C6H10O3	3121-61-7	Acrylic acid, 2-methoxyethyl ester
130,062988	C6H10O3	332-77-4	Furan, 2,5-dihydro-2,5-dimethoxy-
130,062988	C6H10O3	999-61-1	Acrylic acid, 2-hydroxypropyl ester
130,074234	C5H10N2O2	3699-54-5	2-Imidazolidinone, 1-(2-hydroxyethyl)-
130,078247	C10H10	1321-74-0	Divinylbenzene
130,078247	C10H10	29036-25-7	1H-Indene, methyl-
130,09938	C7H14O2	2426-08-6	Bis(2,3-epoxypropyl) butyl ether
130,09938	C7H14O2	590-01-2	butyl propanoate
130,09938	C7H14O2	111-14-8	Heptanoic acid
130,09938	C7H14O2	7665-72-7	Oxirane, [(1,1-dimethylethoxy)methyl]-
130,135757	C8H18O	104-76-7	2-Ethyl-1-hexanol
130,1357652	C8H18O		2-ethylhexanol
130,1357652	C8H18O		2-ethylhexanol
130,1357652	C8H18O		octanol
130,136002	C8H18O	111-87-5	1-Octanol
130,136002	C8H18O	123-96-6	Octan-2-ol
132,042007	C5H8O4	110-94-1	Glutaric acid
132,078644	C6H12O3	108-65-6	Acetic acid, 2-methoxyisopropyl ester
132,078644	C6H12O3	63697-00-7	Lactic acid, isopropyl ester
132,078644	C6H12O3	70657-70-4	1-Propanol, 2-methoxy-, acetate
132,078995	C6H12O3	107-71-1	tert-Butylperoxy acetate
132,0939004	C10H12		butadiene n=4
132,093903	C10H12	77-73-6	Dicyclopentadiene
132,115036	C7H16O2	5131-66-8	1-Butoxy-2-propanol
132,115036	C7H16O2	29387-86-8	Propyleneglycol monobutyl ether

Monoisotopic mass	Formula	CAS number	Compound name
133,063995	C7H7N3	29878-31-7	1H-Benzotriazole, 4-methyl-
134,02153	C4H6O5	617-48-1	Malic acid
134,036774	C8H6O2	553-86-6	2(3H)-Benzofuranone
134,057907	C5H10O4	4767-03-7	2,2-Bis(hydroxymethyl)propionic acid
134,0731649	C9H10O		3,4-dimethylbenzaldehyde
134,094299	C6H14O3	111-90-0	Diethyleneglycol monoethyl ether
134,094299	C6H14O3	25265-71-8	Dipropyleneglycol
134,094299	C6H14O3	110-98-5	1,1'-Oxydipropan-2-ol
134,094299	C6H14O3	77-99-6	1,1,1-Trimethylolpropane
134,1095504	C10H14		tert-butyl-benzene
135,06842	C8H9NO	99-92-3	Acetophenone, 4'-amino-
135,1047994	C9H13N		2-butyl-pyridine
135,1047994	C9H13N		2,4,5-trimethylaniline
136,052429	C8H8O2	93-58-3	Benzoic acid, methyl ester
136,052429	C8H8O2	123-11-5	p-Anisaldehyde
136,06366	C7H8N2O	88-68-6	2-Aminobenzamide
136,073563	C5H12O4	115-77-5	Pentaerythritol
136,088821	C9H12O	26998-80-1	2,3,4-Trimethylphenol
136,1000484	C8H12N2		1,3-benzenedimethanamine, m-xylylenediamine
136,100052	C8H12N2	1477-55-0	1,3-Benzenedimethanamine
136,100052	C8H12N2	539-48-0	1,4-Benzenedimethanamine
136,125198	C10H16	127-91-3	β -Pinene
136,125198	C10H16	138-86-3	Limonene
136,125198	C10H16	586-62-9	Terpinolene
136,125198	C10H16	80-56-8	α-Pinene
137,047684	C7H7NO2	88-72-2	Toluene, o-nitro-
137,047684	C7H7NO2	99-99-0	Toluene, p-nitro-
137,084064	C8H11NO		2-ethoxyaniline
137,084064	C8H11NO		2-methoxy-5-methylaniline
138,031693	C7H6O3	69-72-7	Salicylic acid
138,031693	C7H6O3	99-96-7	p-Hydroxybenzoic acid
138,068085	C8H10O2	14548-60-8	(Benzyloxy)methanol
138,079313	C7H10N2O		4-methoxy-m-phenylenediamine
138,079315	C7H10N2O	13811-50-2	N,N'-Divinyl-2-imidazolidinone
138,104462	C9H14O	78-59-1	Isophorone
139,030319	C3H9NO3S	107-68-6	Taurine, N-methyl-
139,1360996	C9H17N		aza-2,2,6,6-tetramethyl-3-cyclohexene
139,1360996	C9H17N		2,7,7-trimethyl-5-cycloheptene
139,1360996	C9H17N		aza-2,2,6,6-tetramethyl-3-cyclohexene
140,083725	C8H12O2	106-87-6	7-Oxabicyclo[4,1,0]heptane, 3-(2-oxiranyl)-
140,106201	C6H12N4	100-97-0	Hexamethylenetetramine
140,1201151	C9H16O		2-butyl-cyclopentanone
140,156494	C10H20	872-05-9	1-Decene

Monoisotopic mass	Formula	CAS number	Compound name
141,034527	C7H8NCl		4-chloro-o-toluidine
141,0789786	C7H11NO2		1-propyl-2,5-pyrrolidine-dione
141,078979	C7H11NO2	5117-12-4	4-Acryloylmorpholine
141,958832	C3H4Cl2O2	627-11-2	Formic acid, chloro-, 2-chloroethyl ester
141,995224	C4H8Cl2O	111-44-4	Ether, bis(2-chloroethyl)
142,0185676	C7H7OCl		Chloroanisole
142,018997	C7H7ClO	59-50-7	p-Chloro-m-cresol
142,062988	C7H10O3	106-91-2	Glycidyl methacrylate
142,09938	C8H14O2	585-07-9	Methacrylic acid, tert-butyl ester
142,09938	C8H14O2	97-88-1	Methacrylic acid, butyl ester
142,09938	C8H14O2	97-86-9	Methacrylic acid, isobutyl ester
142,135757	C9H18O	108-83-8	Diisobutyl ketone
142,1357652	C9H18O		nonanal
142,983932	C3H6NNaS2	128-04-1	Sodium dimethyldithiocarbamate
143,058243	C6H9NO3	9003-06-9	Acrylamide-acrylic acid, copolymer
143,0735243	C10H9N		2-Naphthylamine
143,094635	C7H13NO2	2439-35-2	Acrylic acid, 2-(dimethylamino)ethyl ester
144,042252	C6H8O4	24615-84-7	Hydracrylic acid, acrylate
144,0422587	C6H8O4		maleic acid ethyl ester
144,05751	C10H8O	135-19-3	2-Naphthol
144,078644	C7H12O3	106-74-1	Acrylic acid, 2-ethoxyethyl ester
144,078644	C7H12O3	2478-10-6	Acrylic acid, monoester with 1,4-butanediol
144,115005	C8H16O2	124-07-2	Caprylic acid
144,1150298	C8H16O2		2-ethylhexanoic acid
144,115036	C8H16O2	105-08-8	1,4-Bis(hydroxymethyl)cyclohexane
144,115036	C8H16O2	149-57-5	Ethyl hexanoic acid
144,115036	C8H16O2	624-54-4	pentyl propanoate
144,115036	C8H16O2	97-85-8	Isobutyric acid, isobutyl ester
144,151413	C9H20O	143-08-8	1-Nonanol
144,162643	C8H20N2	121-05-1	Ethylenediamine, N,N-diisopropyl-
146,036774	C9H6O2	91-64-5	Coumarin
146,057907	C6H10O4	3248-28-0	Dipropionyl peroxide
146,057907	C6H10O4	95-92-1	Diethyl oxalate
146,0579088	C6H10O4		hexanedioic acid, adipic acid
146,057999	C6H10O4	111-55-7	Acetic acid, diester with ethyleneglycol
146,057999	C6H10O4	124-04-9	Adipic acid
146,073166	C10H10O	61788-44-1	2,4-divinylphenol
146,094299	C7H14O3	138-22-7	Lactic acid, butyl ester
146,094299	C7H14O3	4435-53-4	3-Methoxybutyl acetate
146,094299	C7H14O3	54839-24-6	Acetic acid, ethoxyisopropyl ester
146,130676	C8H18O2	110-05-4	Di-tert-butyl peroxide
146,130676	C8H18O2	353260-22-7	2,4,4-Trimethylpentyl-2-hydroperoxide
146,153	C6H18N4	112-24-3	Triethylenetetramine

Monoisotopic mass	Formula	CAS number	Compound name
147,032028	C8H5NO2	85-41-6	Phthalimide
148,016037	C8H4O3	85-44-9	Phthalic anhydride
148,029114	C6H9ClO2	9003-22-9	Vinyl acetate - vinyl chloride, copolymer
148,0524295	C9H8O2		vinyl benzoate
148,055573	C5H12O3Si	2768-02-7	Vinyltrimethoxysilane
148,073563	C6H12O4	868-77-9	Methacrylic acid, monoester with ethyleneglycol
148,084793	C5H12N2O3	141-07-1	1,3-Bis(methoxymethyl)urea
148,101883	C8H17Cl	111-85-3	Octane, 1-chloro-
148,10994	C7H16O3	20324-32-7	1-(2-Methoxy-1-methylethoxy)-2-propanol
148,10994	C7H16O3	34590-94-8	Dipropyleneglycol monomethyl ether
148,970215	C4H4ClNOS	26172-55-4	5-Chloro-2-methyl-4-isothiazoline-3-one
148,970215	C4H4ClNOS	26172-55-4	3(2H)-Isothiazolone, 5-chloro-2-methyl-
149,009155	C4H5O6	96-49-1	Ethylene carbonate
149,033295	C5H11NS2	20624-25-3	Sodium ethylene bis dithiocarbamate
149,105194	C6H15NO3	102-71-6	Triethanolamine
150,016434	C4H6O6	87-69-4 133-37-9	Tartaric acid
150,016434	C4H6O6	87-69-4	L-Tartaric acid
150,068085	C9H10O2	122-60-1	2,3-Epoxypropyl phenyl ether
150,068085	C9H10O2	140-11-4	Acetic acid, benzyl ester
150,068085	C9H10O2	140-11-4	Benzyl acetate
150,068085	C9H10O2	937-41-7	Acrylic acid, phenyl ester
150,068085	C9H10O2	93-89-0	Benzoic acid, ethyl ester
150,089203	C6H14O4	109-16-0	Methacrylic acid, diester with triethylene glycol
150,089203	C6H14O4	112-27-6	Triethylene glycol
150,104462	C10H14O	585-34-2	3-Tert-butylphenol
150,104462	C10H14O	89-72-5	2-sec-Butylphenol
150,104462	C10H14O	98-54-4	4-tert-Butylphenol
150,104462	C10H14O	99-71-8	4-sec-Butylphenol
150,1044651	C10H14O		tert-butyl-phenol
151,009186	C7H5NOS	2634-33-5	1,2-Benzisothiazolin-3-one
151,063324	C8H9NO2	103-90-2	N-(4-Hydroxyphenyl) acetamide
151,076385	C6H14ClNO	3033-77-0	(2,3-Epoxypropyl)trimethylammonium chloride
152,047348	C8H8O3	100-09-4	p-Anisic acid
152,047348	C8H8O3	119-36-8	Salicylic acid, methyl ester
152,047348	C8H8O3	121-33-5	Vanillin
152,047348	C8H8O3	156-38-7	Acetic acid, (p-hydroxyphenyl)-
152,047348	C8H8O3	99-76-3	4-Hydroxybenzoic acid, methyl ester
152,083725	C9H12O2	37281-57-5	Poly(oxy-1,2-ethanediyl), .alpha.-(methylphenyl)-.omega.-hydroxy-
152,083725	C9H12O2	4169-04-4	1-Propanol, 2-phenoxy-
152,083725	C9H12O2	80-15-9	α , α -Dimethylbenzyl hydroperoxide
152,1201151	C10H16O		2-cyclopentyl-cyclo-pentanone
152,1201151	C10H16O		2-cyclopentylcyclopentanone
152,120117	C10H16O	76-22-2	Camphor

Monoisotopic mass	Formula	CAS number	Compound name
153,1517496	C10H19N		aza-1,2,2,6,6-pentamethyl-3-cyclohexene
153,995224	C5H8Cl2O	1575-61-7	Pentanoyl chloride, 5-chloro-
154,031601	C6H12Cl2	2163-00-0	Hexane, 1,6-dichloro-
154,062988	C8H10O3	760-93-0	Methacrylic anhydride
154,071823	C5H11N2NaO2	84434-12-8	N-(2-Aminoethyl)-beta-alanine, sodium salt
154,074234	C7H10N2O2	110-26-9	Methylenebisacrylamide
154,078247	C12H10	92-52-4	Biphenyl
154,0782503	C12H10		1,1-biphenyl
154,1357652	C10H18O		2-pentyl-cyclopentanone
155,045944	C7H6N3Na	64665-57-2	1H-Benzotriazole, 4(or 5)-methyl-, sodium salt
155,069473	C6H9N3O2	6642-31-5	6-Amino-1,3-dimethyluracil
155,0946287	C8H13NO2		1-butyl-2,5-pyrrolidine-dione
155,0946287	C8H13NO2		2-butyl-3,5-pyrrolidine-dione
155,1310142	C9H17NO		2,2,6,6-tetramethyl-4-piperidinone
156,034195	C8H9ClO	88-04-0	4-Chloro-3,5-dimethylphenol
156,078644	C8H12O3	2399-48-6	Acrylic acid, tetrahydrofurfuryl ester
156,115036	C9H16O2	2499-95-8	Acrylic acid, hexyl ester
156,1626487	C9H20N2		2,2,6,6-tetramethyl-4-aminopiperidine
157,110275	C8H15NO2	2867-47-2	Methacrylic acid, 2-(dimethylamino)ethyl ester
157,1466642	C9H19NO		2,2,6,6-tetramethyl-4-piperidinol
157,1466642	C9H19NO		4-hydroxy-2,2,6,6-tetramethylpiperidine
158,001358	C4H7NaO3S	1561-92-8	Methallylsulphonic acid, sodium salt
158,0843983	C10H10N2		1,5-diaminonaphthalene
158,094299	C8H14O3	106-31-0	Butyric anhydride
158,1095504	C12H14		butadiene n=5
158,130676	C9H18O2	112-05-0	Nonanoic acid
158,167068	C10H22O	112-30-1	1-Decanol
158,167068	C10H22O	78-69-3	Tetrahydro linalool
158,95787	C3H6KNS2	128-03-0	Potassium dimethylcarbamodithioate
160,073563	C7H12O4	13533-05-6	Acrylic acid, monoester with diethyleneglycol
160,0735839	C7H12O4		Monomethyl adipate
160,10994	C8H16O3	112-07-2	Acetic acid, 2-butoxyethyl ester
160,110001	C8H16O3	109-13-7	tert-Butylperoxy isobutyrate
161,012253	C3H8NNaO3S	4316-74-9	Ethanesulfonic acid, 2-(methylamino)-, monosodium salt
161,047684	C9H7NO2	550-44-7	Phthalimide, N-methyl-
161,097122	C8H16ClN	26062-79-3	Polydimethyldiallyl ammonium chloride
161,097122	C8H16ClN	7398-69-8	Diallyldimethyl ammonium chloride
161,105606	C6H19NSi2	999-97-3	Hexamethyldisilazane
161,929825	C2N2Na2S2	138-93-2	Cyanodithiocarbamic acid, disodium salt
161,974503	C3H3AlO6	7360-53-4	Aluminium triformate
161,974503	C3H3AlO6	7360-53-4	Aluminium triformate
162,011002	C4H7AlO5	139-12-8	Hydroxyaluminium di(acetate)
162,028534	C5H10N2S2	533-74-4	3,5-Dimethyl-1,3,5,2h-tetrahydrothiadiazine-2-thione

Monoisotopic mass	Formula	CAS number	Compound name
162,044754	C7H11ClO2	13248-54-9	Formic acid, chloro-, cyclohexyl ester
162,068085	C10H10O2	2177-70-0	Methacrylic acid, phenyl ester
162,068085	C10H10O2	2495-35-4	Acrylic acid, benzyl ester
162,089615	C6H18OSi2	107-46-0	Hexamethyldisiloxane
162,125595	C8H18O3	112-34-5	Diethyleneglycol butyl ether
162,1408506	C12H18		di-isopropyl-benzene
163,875412	C2Cl4	127-18-4	Tetrachloroethylene
164,000488	C7H6ClNaO	15733-22-9	p-Chloro-m-cresol, sodium salt
164,068466	C6H12O5	12441-09-7	Sorbitan
164,083725	C10H12O2	2210-79-9	2,3-Epoxypropyl-o-tolyl ether
164,083725	C10H12O2	97-54-1	Phenol, 2-methoxy-4-propenyl-
164,083725	C10H12O2	97-54-1	trans-isoeugenol
164,083725	C10H12O2	2315-68-6	Benzoic acid, propyl ester
164,083725	C10H12O2	97-53-0	Eugenol
164,094955	C9H12N2O	101-42-8	N,N-Dimethyl-N'-phenylurea
164,106201	C8H12N4	78-67-1	Azobis(isobutyronitrile)
164,1201151	C11H16O		2-tert-butyl-6-methyl-phenol
164,120117	C11H16O	2409-55-4	2-tert-butyl-4-methylphenol
164,120117	C11H16O	80-46-6	p-tert-Amylphenol
165,115356	C10H15NO	92-50-2	Ethanol, 2-(N-ethylanilino)-
166,0266087	C8H6O4		phthalic acid
166,0266087	C8H6O4		phthalic acid
166,0266087	C8H6O4		terephthalic acid
166,026611	C8H6O4	100-21-0	Terephthalic acid
166,026611	C8H6O4	121-91-5	Isophthalic acid
166,026611	C8H6O4	88-99-3	o-Phthalic acid
166,062988	C9H10O3	120-47-8	4-Hydroxybenzoic acid, ethyl ester
166,062988	C9H10O3	121-32-4	Ethyl vanillin
166,0629942	C9H10O3		octadecanol - corres acid 4
166,074234	C8H10N2O2	6342-56-9	Pyruvaldehyde, 1-(dimethyl acetal)
166,075882	C6H15O3P	122-52-1	Phosphorous acid, triethyl ester
166,09938	C10H14O2	98-29-3	4-tert-butylcatechol
166,09938	C10H14O2	1948-33-0	tert-butyl-Hydroquinone (TBHQ)
166,09938	C10H14O2	98-29-3	4-tert-Butylpyrocatechol
166,986343	C7H5NS2	149-30-4	2-Mercaptobenzothiazole
167,131012	C10H17NO	6837-24-7	N-Cyclohexyl-2-pyrrolidone
168,089874	C8H12N2O2	822-06-0	Hexamethylene diisocyanate
168,115036	C10H16O2	101-43-9	Methacrylic acid, cyclohexyl ester
168,187805	C12H24	112-41-4	1-Dodecene
169,0891744	C12H11N		4-Aminobiphenyl
169,110275	C9H15NO2	2873-97-4	Diacetone arylamide
169,1102787	C9H15NO2		1-pentyl-2,5-pyrrolidine-dione
170,013458	C8H7ClO2	501-53-1	Benzyl chloroformate

Monoisotopic mass	Formula	CAS number	Compound name
170,02153	C7H6O5	26677-99-6	Acrylic acid-maleic acid, copolymer
170,024689	C7H7ClN2O	140-39-6	Acetic acid, p-tolyl ester
170,057907	C8H10O4	2274-11-5	Acrylic acid, diester with ethyleneglycol
170,073166	C12H10O	1131-60-8	biphenyl-4-ol
170,073166	C12H10O	90-43-7	2-Phenylphenol
170,073166	C12H10O	92-69-3	4-phenylphenol
170,07319	C12H10O		diphenyl oxide
170,07319	C12H10O		2-hydroxybiphenyl
170,130676	C10H18O2	78-66-0	3,6-Dimethyl-4-Octyn-3,6-Diol
170,141907	C9H18N2O	5205-93-6	N-(Dimethylaminopropyl)methacrylamide
170,178299	C10H22N2	2855-13-2	1-Amino-3-Aminoethyl-3,5,5-trimethylcyclohexane
170,2034759	C12H26		dodecane
171,0354	C7H9NO2S	70-55-3	p-Toluenesulfonamide
171,0354	C7H9NO2S	1333-07-9	Toluenesulphonamide
171,0354	C7H9NO2S	88-19-7	o-Toluenesulphonamide
171,104797	C12H13N	10420-89-0	1-Naphthalenemethanamine, .alpha.-methyl-, (S)-
171,104797	C12H13N	3886-70-2	1-Naphthalenemethanamine, .alpha.-methyl-, (R)-
171,125931	C9H17NO2	2426-54-2	Acrylic acid, 2-(diethylamino)ethyl ester
171,1623143	C10H21NO		2,2,6,6-tetramethyl-piperidine methyl ether
171,1623143	C10H21NO		4-hydroxy-1,2,2,6,6-pentamethylpiperidine
172,019409	C7H8O3S	88-20-0	Toluene-2-sulphonic acid
172,019409	C7H8O3S	104-15-4	p-Toluenesulphonic acid
172,019409	C7H8O3S	70788-37-3	2(Or 4)-toluenesulphonic acid
172,073563	C8H12O4	1076-97-7	1,4-Cyclohexanedicarboxylic acid
172,133209	C9H18NO2	5039-78-1	Methacrylic acid, ester with trimethylethanolammonium chloride
172,146332	C10H20O2	334-48-5	n-Decanoic acid
172,146332	C10H20O2	26762-92-5	p-Menthane hydroperoxide
173,177963	C10H23NO	102-81-8	2-Dibutylaminoethanol
173,963913	C7H4Cl2O	457883-29-3	4,4'-Bis[(4-amino-6-morpholino-s-triazin-2-yl)amino]-2,2'-stilbenedisulphonic acid, disodium salt
174,029007	C8H7NaO3	5026-62-0	Benzoic acid, p-hydroxy-, methyl ester, sodium salt
174,029282	C8H7NaO3	5026-62-0	Methylparaben Sodium Salt
174,042923	C9H6N2O2	584-84-9	2,4-Toluene diisocyanate
174,042923	C9H6N2O2	91-08-7	2,6-Toluene diisocyanate
174,042923	C9H6N2O2	26471-62-5	Toluene diisocyanate
174,089203	C8H14O4	2224-15-9	Ethane, 1,2-bis(2,3-epoxypropoxy)-
174,089203	C8H14O4	502-44-3	Caprolactone
174,089203	C8H14O4	627-93-0	Adipic acid, dimethyl ester
174,089234	C8H14O4		Dimethyl adipate
174,125595	C9H18O3	927-07-1	tert-Butylperoxy pivalate
174,952789	C3H6KNOS2	51026-28-9	N-Hydroxymethyl-N-methyldithiocarbamic acid, potassium salt
175,979568	C7H6Cl2O	1777-82-8	Benzyl alcohol, 2,4-dichloro-
176,032089	C6H8O6	50-81-7	Ascorbic acid (vitamin C)

Monoisotopic mass	Formula	CAS number	Compound name
176,068466	C7H12O5	25395-31-7	Glycerol diacetate
176,083725	C11H12O2	18096-62-3	Indeno[1,2-d]-m-dioxin, 4,4a,5,9b-tetrahydro-
176,083725	C11H12O2	2495-37-6	Methacrylic acid, benzyl ester
176,104858	C8H16O4	2372-21-6	O,O-tert-Butyl isopropyl monoperoxycarbonate
176,120117	C12H16O	1130-60-8	2-Cyclohexylphenol
176,932007	C2H4KNO2S2	137-41-7	Methyldithiocarbamic acid, potassium salt
178,029984	C6H10O4S	111-17-1	Thiodipropionic acid
178,029984	C6H10O4S	693-36-7	Thiodipropionic acid, dioctadecyl ester
178,047745	C6H10O6	90-80-2	Gluconic acid lactone
178,058975	C5H10N2O5	1854-26-8	N,N'-Bis(hydroxymethyl)-4,5-dihydroxyethyleneurea
178,0782754	C14H10		Anthracene
178,0993797	C11H14O2		butyl benzoate
178,0993797	C11H14O2		2-tert-butyl-6-methyl-benzoquinone
178,09938	C11H14O2	136-60-7	Benzoic acid, butyl ester
180,042252	C9H8O4	120-61-6	Terephthalic acid, dimethyl ester
180,063385	C6H12O6	50-99-7	D-Glucose
180,063385	C6H12O6	57-48-7	Fructose, D-
180,068741	C12H8N2	66-71-7	phenanthroline
180,078644	C10H12O3	4191-73-5	4-Hydroxybenzoic acid, isopropyl ester
180,078644	C10H12O3	94-13-3	4-Hydroxybenzoic acid, propyl ester
180,0786693	C10H12O3		propyl paraben
180,115036	C11H16O2	88-32-4	2-tert-Butyl-4-hydroxyanisole
180,115036	C11H16O2	25013-16-5	2 -tert-Butylhydroxyanisole (BHA)
180,115036	C11H16O2	88-32-4	2-tert-Butyl-4-hydroxyanisole
181,110275	C10H15NO2	120-07-0	N-Phenyldiethanolamine
182,0215233	C8H6O5		isophthalic acid
182,070801	C6H15O4P	78-40-0	Phosphoric acid, triethyl ester
182,073166	C13H10O	119-61-9	Benzophenone
182,07319	C13H10O		Benzophenone
182,0790382	C6H14O6		sorbitol
182,079041	C6H14O6	69-65-8 87-78-5	Mannitol
182,079041	C6H14O6	50-70-4	Sorbitol
182,094299	C10H14O3	104-68-7	Ethanol, 2-(2-phenoxyethoxy)-
183,1623143	C11H21NO		1-aza-2,2,6,6-tetramethyl-3-cyclohexeneethanol
184,019409	C8H8O3S	26914-43-2 19922-72-6 4551-90-0 10525-12-9	Styrenesulphonic acid Benzenesulphonic acid, 4-ethenyl-, ammonium salt Potassium p-vinylbenzenesulphonate Benzenesulphonic acid, 2-ethenyl-, sodium salt
184,0524295	C12H8O2		diphenoquinonone
184,1000484	C12H12N2		4-aminodiphenylamine
184,1000734	C12H12N2		Benzidine
184,1252005	C14H16		butadiene n=6
184,146332	C11H20O2	103-11-7	Acrylic acid, 2-ethylhexyl ester

Monoisotopic mass	Formula	CAS number	Compound name
184,146332	C11H20O2	2499-59-4	Acrylic acid, n-octyl ester
184,146332	C11H20O2	29590-42-9	Acrylic acid, isooctyl ester
185,141998	C10H19NO2	105-16-8	Methacrylic acid, 2-(diethylamino)ethylester
185,876755	C3Cl2OS2	1192-52-5	4,5-Dichloro-1,2-dithiol-3-one
186,035065	C8H10O3S	25321-41-9	Xylenesulphonic acid
186,035065	C8H10O3S	28804-47-9	Toluenesulphonic acid, methyl ester
186,035065	C8H10O3S	80-48-8	p-Toluenesulphonic acid, methyl ester
186,161987	C11H22O2	2461-15-6	2-Ethylhexyl-2,3-epoxypropyl ether
186,198364	C12H26O	112-53-8	1-Dodecanol
187,0400313	C8H10NO2Cl		4-Chloro-2,5-dimethoxyaniline
187,0858	C9H9N5	91-76-9	2,4-Diamino-6-phenyl-1,3,5-triazine
188,044937	C9H9NaO3	35285-68-8	Aqueous solution of methyl p-hydroxybenzoate in hydrogen peroxide
188,079712	C7H12N2O4	6440-58-0	1,3-Bis(hydroxymethyl)-5,5-dimethylimidazolidine-2,4-dione
188,104858	C9H16O4	123-99-9	Azelaic acid
188,104858	C9H16O4	7328-17-8	Acrylic acid, 2-(2-ethoxyethoxy)ethyl ester
188,104858	C9H16O4	16096-30-3	Oxirane, 2,2'-[(1-methyl-1,2-ethanediyl)bis(oxymethylene)]bis-
188,968277	C7H4NNaS2	2492-26-4	2-Mercaptobenzothiazole, sodium salt
189,045959	C7H11NO3S	26447-09-6	Ammonium toluenesulphonate
189,045959	C7H11NO3S	4124-42-9	Ammonium toluene-4-sulphonate
189,047103	C12H10Cl		PCB 1
189,985519	C7H7ClO2S	26763-71-3	Toluenesulphonyl chloride
189,985519	C7H7ClO2S	98-59-9	Benzenesulfonyl chloride, 4-methyl-
190,102524	C8H18O3Si	78-08-0	Vinyltriethoxysilane
190,120514	C9H18O4	26402-23-3	Glycerol monohexanoate
190,120514	C9H18O4	88917-22-0	Dipropyleneglycol methyl ether acetate
190,1357652	C13H18O		3-methyl-5-tert-butyl-4-hydroxy styrene
190,156891	C10H22O3	29911-28-2	Dipropyleneglycol n-butyl ether
190,1721507	C14H22		1,3-di-tert-butyl-benzene
190,1721507	C14H22		1,3-di-tert-butyl-benzene
191,069473	C9H9N3O2	10605-21-7	Methyl benzimidazolecarbamate
191,094635	C11H13NO2	93-68-5	o-Acetoacetotoluidide
191,152145	C9H21NO3	122-20-3	Triisopropanolamine
192,005875	C9H4O5	552-30-7	Trimellitic anhydride
192,0270026	C6H8O7		citric acid
192,027008	C6H8O7	77-92-9	Citric acid
192,055115	C12H9NaO	132-27-4	2-Phenylphenol, sodium salt
192,078644	C11H12O3	56641-05-5	Poly(oxy-1,2-ethanediyl),.alpha.-(1-oxo-2-propenyl)-.omega.-phenoxy-
192,078644	C11H12O3	48145-04-6	Acrylic acid, 2-phenoxyethyl ester
192,1150298	C12H16O2		2,4-dimethylpropylbenzoate
192,1150298	C12H16O2		3-methyl-5-tert-butyl-4-hydroxy benzaldehyde
192,115036	C12H16O2	2208-05-1	Benzoic acid, 2-(dimethylamino)ethyl ester
192,136154	C9H20O4	24800-44-0	Tripropyleneglycol
193,08696	C8H16ClNO2	44992-01-0	Acrylic acid, ester with trimethylethanolammonium chloride

Monoisotopic mass	Formula	CAS number	Compound name
193,089142	C14H11N	948-65-2	2-Phenylindole
193,110275	C11H15NO2	10287-53-3	4-Dimethylaminobenzoic acid, ethyl ester
193,1103038	C11H15NO2		Ethyl-4-dimethylaminobenzoate
194,001358	C7H7NaO3S	657-84-1	Sodium toluene-4-sulphonate
194,001358	C7H7NaO3S	12068-03-0	Sodium toluenesulphonate
194,057907	C10H10O4	1459-93-4	Isophthalic acid, dimethyl ester
194,057907	C10H10O4	131-11-3	Phthalic acid, dimethyl ester
194,0579088	C10H10O4		dimethyl terephthalate
194,0579088	C10H10O4		dimethylphthalate
194,0579338	C10H10O4		Dimethyl phthalate
194,093994	C11H14O3	23676-09-7	4-Ethoxybenzoic acid, ethyl ester
194,094299	C11H14O3	94-26-8	butylparaben
194,094299	C11H14O3	614-45-9	p-tert-Butylperoxy benzoate
194,094299	C11H14O3	614-45-9	tert-Butylperoxy benzoate
194,094299	C11H14O3	93965-02-7	4,4'-Bis[[4-[bis(2-hydroxyethyl)amino]-6-(p-sulphoanilino)-s-triazin-2-yl]amino]-2,2'-stilbenedisulphonic acid, sodium salt, compound with diethanolamine
194,107178	C8H19O3P	4724-48-5	n-Octylphosphonic acid
194,115417	C8H18O5	112-60-7	Tetraethyleneglycol
194,115417	C8H18O5	25322-68-3	Polyethyleneglycol
194,130676	C12H18O2	26762-93-6	Diisopropylbenzene hydroperoxide
194,130997	C12H18O2	98-49-7	1-(4-Isopropylphenyl)-1-methylethyl hydroperoxide
194,141907	C11H18N2O	25646-77-9	Ethanol, 2-[(4-amino-3-methylphenyl)ethylamino]-, sulfate (1:1)(salt)
195,0354	C9H9NO2S	10154-75-3	3-(Phenylsulphonyl)propionitrile
195,125931	C11H17NO2	3490-06-0	Benzeneethanamine, 3,4-dimethoxy-N-methyl-
195,125931	C11H17NO2	3077-12-1	Ethanol, 2,2'-(p-tolylimino)di-
195,924942	C6H3Cl3O	25167-82-2	Trichlorophenol
195,924942	C6H3Cl3O	88-06-2	2,4,6-Trichlorophenol
196,055801	C10H12O2S	3454-29-3	Trimethylolpropane triglycidylether
196,058304	C6H12O7	133-42-6 526-95-4	Gluconic acid
196,058304	C6H12O7	526-95-4	Gluconic acid
196,088821	C14H12O	131-58-8	2-Methylbenzophenone
196,088821	C14H12O	134-84-9	4-Methylbenzophenone
196,088821	C14H12O	643-65-2	3-Methylbenzophenone
196,08884	C14H12O		2-Methylbenzophenone
196,08884	C14H12O		3-Methylbenzophenone
196,08884	C14H12O		4-Methylbenzophenone
196,145996	C12H20O2	26896-48-0	Tricyclodecanedimethanol
196,146332	C12H20O2	86178-38-3	3,3,5-Trimethylcyclohexyl acrylate
196,219101	C14H28	1120-36-1	1-Tetradecene
197,105194	C10H15NO3	25086-89-9	Vinyl acetate-vinylpyrrolidone, copolymer
197,116425	C9H15N3O2	3089-19-8	N-[2-(2-Oxo-1-imidazolidinyl)ethyl]methacrylamide
197,129486	C7H20ClN3O	42751-79-1	Dimethylamine-ethylenediamine-epichlorohydrin, copolymer

Monoisotopic mass	Formula	CAS number	Compound name
197,152817	C10H19N3O	29782-73-8	DL-Alanine, N-methyl-, monopotassium salt
197,177963	C12H23NO	947-04-6	Laurolactam
197,2128016	C13H25DN		13-oxo-tridecanoic acid amide
198,0588994	C9H12NO2S		N-Ethyl-p-toluene-sulphonamide
198,0681046	C13H10O2		4-Hydroxybenzophenone
198,0681046	C13H10O2		2-Hydroxybenzophenone
198,089203	C10H14O4	104-38-1	Ethanol, 2,2'-(p-phenylenedioxy)di-
198,089203	C10H14O4	1070-70-8	Acrylic acid, diester with 1,4-butanediol
198,089203	C10H14O4	19485-03-1	Acrylic acid, diester with 1,3-butanediol
198,089203	C10H14O4	97-90-5	Methacrylic acid, diester with ethyleneglycol
198,104462	C14H14O	1988-89-2	4-(1-phenylethyl)phenol
198,104462	C14H14O	4237-44-9	Phenol, o-(.alpha.-methylbenzyl)-
198,115692	C13H14N2	101-77-9	Bis(4-aminophenyl)methane
198,115692	C13H14N2	1208-52-2	2,4'-Diaminodiphenylmethane
198,115692	C13H14N2	6582-52-1	Aniline, 2,2'-methylenedi-
198,1157235	C13H14N2		4,4’-Methylenedianiline
198,1157235	C13H14N2		2,4’-Methylenedianiline
198,1157235	C13H14N2		2,2’-Methylenedianiline
198,948013	C3H6BrNO4	52-51-7	2-Bromo-2-nitro-1,3-propanediol
199,045563	C12H9NS	92-84-2	Phenothiazine
199,066696	C9H13NO2S	8047-99-2	N-Ethyl-toluenesulphonamide (NETSA)
199,2299999	C13H29N		Atmer 163 primary amine C13
200,05072	C9H12O3S	28631-63-2	Cumenesulfonic acid .
200,05072	C9H12O3S	37953-05-2	Cumenesulphonic acid
200,05072	C9H12O3S	80-40-0	p-Toluenesulfonic acid, ethyl ester
200,079712	C8H12N2O4	868-63-3	N,N'-(1,2-Dihydroxyethylene)bisacrylamide
200,083725	C13H12O2	620-92-8	Bis(4-hydroxyphenyl)methane
200,083725	C13H12O2	2467-02-9	2,2'-Methylenediphenol
200,083725	C13H12O2	2467-03-0	2-(4-hydroxybenzyl)phenol
200,0837296	C13H12O2		bisphenol F (BPF)
200,094955	C12H12N2O	101-80-4	Aniline, 4,4'-oxydi-
200,094988	C12H12N2O		4,4’-Diaminodiphenylether
200,104858	C10H16O4	66492-51-1	Acrylic acid, (5-ethyl-1,3-dioxan-5-yl)methyl ester
200,177628	C12H24O2	143-07-7	Lauric acid
200,177628	C12H24O2	68609-96-1	Oxirane, mono[(C8-10-alkyloxy)methyl] derivs
201,958832	C8H4Cl2O2	99-63-8	Isophthalic acid dichloride
202,039856	C8H15CoO2	13586-82-8	2-Ethylhexanoic acid, cobalt salt
202,060593	C10H11NaO3	35285-69-9	Benzoic acid, 4-hydroxy-, propyl ester, sodium salt
202,060593	C10H11NaO3	35285-69-9	Aqueous solution of propyl p-hydroxybenzoate in hydrogen peroxide
202,09938	C13H14O2	50976-02-8	Acrylic acid, dicyclopentadienyl ester

Monoisotopic mass	Formula	CAS number	Compound name
202,120514	C10H18O4	111-20-6	Sebacic acid
202,120514	C10H18O4	141-28-6	Adipic acid, diethyl ester
202,120514	C10H18O4	2425-79-8	1,4-Butanediol bis(2,3-epoxypropyl) ether
202,175522	C12H26S	112-55-0	Dodecylmercaptan
202,193283	C12H26O2	129228-21-3	3,3-Bis(methoxymethyl)-2,5-dimethylhexane
203,061615	C8H13NO3S	26447-10-9	Xylenesulphonic acid, ammonium salt
203,061615	C8H13NO3S	26447-10-9	Ammonium xylenesulphonate
204,115005	C13H16O2	12542-30-2	Acrylic acid, dicyclopentenyl ester
204,115036	C13H16O2	125-12-2	Cyclopentyl(phenyl)acetic acid
204,1150548	C13H16O2		1-Hydroxycyclohexyl-1-phenylketone
204,118393	C10H20O2S	25103-09-7	Isooctyl mercaptoacetate
204,1184006	C10H20O2S		2-ethylhexyl mercaptoacetate
205,110275	C12H15NO2	97-36-9	2',4'-Acetoacetoxylidide
205,1592403	C14H21O		2,4-di-t-butyl phenol
205,1592403	C14H21O		2,6-di-t-butyl phenol
205,974518	C2H8O7P2	68155-93-1	Dimethylacidpyrophosphate
206,001358	C8H7NaO3S	2695-37-6	Benzenesulfonic acid, 4-ethenyl-, sodium salt
206,0579088	C11H10O4		methyl vinyl terephthalate
206,079041	C8H14O6	16066-38-9	Dipropyl peroxydicarbonate
206,079041	C8H14O6	105-64-6	Bis(isopropyl) peroxydicarbonate
206,094299	C12H14O3	99880-64-5	Glycerol dibehenate
206,130676	C13H18O2	3101-60-8	Propane, 1-(p-tert-butylphenoxy)-2,3-epoxy-
206,130676	C13H18O2	7191-39-1	Acetophenone, 2'-(pentyloxy)-
206,1306798	C13H18O2		3-methyl-5-tert-butyl-4-hydroxy acetophenone
206,15181	C10H22O4	25498-49-1	Tripropyleneglycol monomethyl ether
206,1670653	C14H22O	18206-26-4	octylphenol
206,1670653	C14H22O		2,4-bis(1,1-dimethylethyl)phenol
206,1670653	C14H22O		2,4-di-tert-butylphenol
206,1670653	C14H22O		2,6-di-tert-butylphenol
206,167068	C14H22O	140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol
206,167068	C14H22O	96-76-4	2,4-Di-t-butylphenol
206,1670904	C14H22O	15214-89-8	4-tert-octylphenol
207,056534	C7H13NO4S	5165-97-9	2-Acrylamido-2-methylpropanesulphonic acid
207,056534	C7H13NO4S		Sodium 2-methyl-2-[(1-oxoallyl)amino]propanesulphonic acid
207,089539	C11H13NO3	92-15-9	o-Acetoacetanisidide
207,1026	C9H18ClNO2	5039-78-1	Methacrylic acid, ester with (N,N,N-trimethyl)ethanolammonium chloride
207,881857	C2O4Sn	814-94-8	Tin(II) oxalate
208,017014	C8H9NaO3S	1300-72-7	Sodium xylenesulphonate
208,019409	C10H8O3S	130-14-3	Sodium naphthalene-1-sulphonate
208,019409	C10H8O3S	68153-01-5	Naphthalenesulphonic acids
208,019409	C10H8O3S	120-18-3	2-naphthalenesulfonic acid

Monoisotopic mass	Formula	CAS number	Compound name
208,019409	C10H8O3S	85-47-2	1-Naphthalenesulphonic acid
208,029053	C12H9KO	13707-65-8	2-Phenylphenol, potassium salt
208,052429	C14H8O2	84-65-1	Anthraquinone
208,052429	C14H8O2	84-66-2	Phthalic acid, diethyl ester
208,0735589	C11H12O4		methylethyl terephthalate
208,10994	C12H16O3	6175-45-7	Acetophenone, 2,2-diethoxy-
208,146332	C13H20O2	3457-61-2	tert-Butyl cumyl peroxide
208,146332	C13H20O2	5888-33-5	exo-1,7,7-Trimethylbicyclo(2.2.1)hept-2-yl acrylate
209,1330255	C16H17		styrene dimer
209,9406229	C7H5OCl3		trichloroanisole
209,975296	C7H7KO3S	30526-22-8	Potassium toluenesulphonate
210,016434	C9H6O6	528-44-9	1,2,4-Benzenetricarboxylic acid
210,020493	C8H10CaO4	491589-22-1	cis-1,2-Cyclohexanedicarboxylic acid, calcium salt
210,042923	C12H6N2O2	3173-72-6	1,5-Naphthalene diisocyanate
210,089203	C11H14O4	20587-61-5	Ethanol, 2-[2-(benzoyloxy)ethoxy]-
210,102097	C8H19O4P	107-66-4	Phosphoric acid, dibutyl ester
210,136826	C11H18N2O2	28679-16-5	Mixture of (40% w/w) 2,2,4-trimethylhexane-1,6-diisocyanate and (60% w/w) 2,4,4-trimethylhexane-1,6-diisocyanate
210,136826	C11H18N2O2	15646-96-5	2,4,4-Trimethylhexane-1,6-diisocyanate
210,136826	C11H18N2O2	28679-16-5	trimethylhexamethylene diisocyanate
210,136826	C11H18N2O2	16938-22-0	2,2,4-Trimethylhexane-1,6-diisocyanate
210,1408506	C16H18		butadiene n=7
210,161987	C13H22O2	7779-31-9	3,3,5-Trimethylcyclohexyl methacrylate
210,161987	C13H22O2	84100-23-2	Acrylic acid, 4-(1,1-dimethylethyl)cyclohexyl ester
210,209595	C13H26N2	1761-71-3	Bis(4-Aminocyclohexyl)methane
212,058578	C12H8N2O2	1742-95-6	Naphthalimide, 4-amino-
212,068466	C10H12O5	121-79-9	Gallic acid, propyl ester
212,0837	C14H12O2		Bisphenol E
212,083725	C14H12O2	119-53-9	Benzoin
212,083725	C14H12O2	120-51-4	benzyl benzoate
212,104858	C11H16O4	2223-82-7	Acrylic acid, diester with 2,2-dimethyl-1,3-propanediol
212,1313736	C14H16N2		3,3’-Dimethylbenzidine
212,1565257	C16H20		Diisopropyl naphthalene
212,177628	C13H24O2	1330-61-6	Acrylic acid, isodecyl ester
212,177628	C13H24O2	2156-96-9	Acrylic acid, decyl ester
212,958939	C8H5BrFN	122-18-9	Benzylhexadecyldimethylammonium chloride
213,0823745	C10H15NO2S		N-butylbenzenesulfoneamide
213,118729	C11H19NOS	26530-20-1	2-Octyl-4-isothiazolin-3-one
213,2092645	C13H27NO		N,N-hexamethylenebisformamide
213,24565	C14H31N		Atmer 163 primary amine C14
213,962997	C8H7BrO2	2491-38-5	α-Bromo-4-hydroxyacetophenone
213,962997	C8H7BrO2	61791-99-9	2-Bromo-4'-hydroxyacetophenone
213,9865	C4HO2F7		PFBA
214,052002	C8H14CaO4	5743-36-2	Calcium butyrate

Monoisotopic mass	Formula	CAS number	Compound name
214,062988	C13H10O3	131-56-6	2,4-Dihydroxybenzophenone
214,062988	C13H10O3	611-99-4	4,4'-Dihydroxybenzophenone
214,062988	C13H10O3	102-09-0	Diphenyl carbonate
214,062988	C13H10O3	118-55-8	Salicylic acid, phenyl ester
214,084122	C10H14O5	4074-88-8	Acrylic acid, diester with diethyleneglycol
214,22966	C14H30O	112-72-1	Tetradecanol
215,115753	C10H17NO4	63225-53-6	Acrylic acid, 2-[[(butylamino)carbonyl]oxy]ethyl ester
216,006836	C6H9NaO5S	15717-25-6	Acrylic acid, ester with 3-hydroxy-1-propanesulfonic acid sodium salt
216,006836	C6H9NaO5S	1804-87-1	Sodium 2-sulphonatoethyl methacrylate
216,042252	C12H8O4	1141-38-4	2,6-Naphthalenedicarboxylic acid
216,063385	C9H12O6	50940-49-3	Butanedioic acid, mono[2-[(1-oxo-2-propenyl)oxy]ethyl] ester
216,0721191	C12H12N2S		4,4'-thiodianiline
216,115	C14H16O2		Bisphenol B
216,136154	C11H20O4	17557-23-2	Propane, 1,3-bis(2,3-epoxypropoxy)-2,2-dimethyl-
216,136154	C11H20O4	7328-16-7	acrylic acid, 2-(2-butoxyethoxy)ethyl ester
216,172546	C12H24O3	3006-82-4	tert-Butyl 2-ethylperoxyhexanoate
217,077271	C9H15NO3S	37475-88-0	Ammonium cumenesulphonate
217,906891	C6H2Cl3NaO	1320-79-2	Trichlorophenol, sodium salt
217,985138	C10H2O6	89-32-7	Pyromellitic anhydride
218,040146	C12H10O2S	127-63-9	Diphenyl sulphone
218,040253	C6H11NaO7	527-07-1	Gluconic acid, monosodium salt
218,079041	C9H14O6	102-76-1	Glycerol triacetate
218,107361	C11H19ClO2	42125-46-2	Carbonochloridic acid, 4-(1,1-dimethylethyl)cyclohexyl ester
218,15181	C11H22O4	26402-26-6	Glycerol monooctanoate
219,104797	C16H13N	90-30-2	N-Phenyl-1-naphthylamine
220,134247	C10H21ClN2O	51410-72-1	Methacrylamidopropyltrimethylammonium chloride
220,1463299	C14H20O2		2,6-di-tert-butylbenzoquinone
220,146332	C14H20O2	68400-54-4	1-Propanone, 1-[4-(1,1-dimethylethyl)phenyl]-2-hydroxy-2-methyl-
220,182709	C15H24O	128-37-0	2,6-Di-tert-butyl-p-cresol
220,182709	C15H24O	84852-15-3	4-(7-methyloctyl)phenol
220,1827154	C15H24O	104-40-5	nonylphenol
220,1827404	C15H24O		4-nonyl phenols
221,144714	C9H23NO3Si	919-30-2	3-Aminopropyltriethoxysilane
222,032654	C9H11NaO3S	32073-22-6	Benzene, (1-methylethyl)-, monosulfo deriv., sodium salt
222,032654	C9H11NaO3S	28348-53-0	Sodium cumenesulphonate
222,056198	C8H14O5S	3179-56-4	Acetyl cyclohexanesulphonyl peroxide
222,089203	C12H14O4	101-90-6	Resorcinol diglycidyl ether
222,089203	C12H14O4	16969-10-1	Acrylic acid, 2-hydroxy-3-phenoxypropyl ester
222,0892089	C12H14O4		diethyl terephthalate
222,089234	C12H14O4		Diethyl phthalate
222,089234	C12H14O4		Diethyl phthalate
222,110336	C9H18O6	80181-31-3	3-Hydroxybutanoic acid-3-hydroxypentanoic acid, copolymer
222,1255944	C13H18O3		octadecanol - corres acid 3

Monoisotopic mass	Formula	CAS number	Compound name
222,136826	C12H18N2O2	4098-71-9	1-Isocyanato-3 -isocyanatomethyl-3,5,5-trimethylcyclohexane
222,140854	C17H18	9011-11-4	α-Methylstyrene-styrene copolymers
222,161987	C14H22O2	7534-94-3	(1R,2R,4R)-1,7,7-trimethylbicyclo[2.2.1]hept-2-yl methacrylate
223,0081307	C12H9Cl2		PCB 2
223,1367903	C10H23O3S		Atmer 191 sulphonic acid C10
223,157227	C13H21NO2	38668-48-3	2-Propanol, 1,1'-[(4-methylphenyl)imino]bis-
223,990952	C8H9KO3S	30346-73-7	Potassium xylenesulphonate
224,104858	C12H16O4	106797-53-9	1-[4-(2-Hydroxyethoxy)phenyl]-2-hydroxy-2-methyl-1-propane-1-one
224,117752	C9H21O4P	513-02-0	Phosphoric acid, triisopropyl ester
225,09021	C13H11N3O	2440-22-4	2-(2'-Hydroxy-5'-methylphenyl)benzotriazole (UVA-P)
225,090212	C13H11N3O		Tinuvin P
225,1153891	C15H15NO		4-(dimethylamino)benzophenone
225,1265975	C14H15N3		4-amino-2,3'-dimethylazobenzidine
226,047745	C10H10O6	102-39-6	(1,3- Phenylenedioxy)diacetic acid
226,120514	C12H18O4	3159-98-6	1,4-Bis(-hydroperoxyisopropyl)benzene
226,120514	C12H18O4	13048-33-4	Acrylic acid, diester with 1,6-hexanediol
226,120514	C12H18O4	2082-81-7	Methacrylic acid, diester with 1,4-butanediol
226,120514	C12H18O4	721-26-6	1,3-Bis(α-hydroperoxyisopropyl)benzene
226,121002	C12H18O4	64194-22-5	Acrylic acid, 3-methyl-1,5-pentanediyl ester
226,1469986	C15H18N2		4,4'-methylenedi-o-toluidine
226,193283	C14H26O2	126-86-3	2,4,7,9-Tetramethyl-5-decyne-4,7-diol
226,957993	C8H6BrNO2	7166-19-0	2-Bromo-2-nitrostyrene
227,097992	C11H17NO2S	1907-65-9	N-Butyl-p-toluenesulphonamide
227,236145	C13H29N3	13590-97-1	Dodecylguanidine hydrochloride
227,2613001	C15H33N		Atmer 163 primary amine C15
228,072113	C13H12N2S	102-08-9	N,N'-Diphenylthiourea
228,078644	C14H12O3	118-58-1	Benzyl salicylate
228,078644	C14H12O3	131-57-7	2-Hydroxy-4-methoxy benzophenone
228,078644	C14H12O3	94-18-8	4-Hydroxybenzoic acid, benzyl ester
228,1150548	C15H16O2		bisphenol A
228,1361842	C12H20O4		Dibutyl maleate
228,172546	C13H24O3	26761-45-5	Neodecanoic acid, oxiranylmethyl ester
228,208923	C14H28O2	298695-60-0	3-Ethyl-3-[(2-ethylhexyloxy)methyl]oxetane
228,208923	C14H28O2	544-63-8	Myristic acid
228,2089301	C14H28O2		tetradecanoic acid, myristic acid
230,001358	C10H7NaO3S	1321-69-3	Naphthalenesulfonic acid, sodium salt
230,1518	C12H22O4		Di-n-propyl adipate
230,1518	C12H22O4		Di-iso-propyl adipate
230,15181	C12H22O4	106-79-6	Dimethyl sebacate
230,15181	C12H22O4	16096-31-4	Oxirane, 2,2'-[1,6-hexanediylbis(oxymethylene)]bis-
230,15181	C12H22O4	693-23-2	n-Dodecanedioic acid
231,125931	C14H17NO2	91-44-1	Coumarin, 7-(diethylamino)-4-methyl-
232,017014	C9H10Cl2N2O	330-54-1	Diuron

Monoisotopic mass	Formula	CAS number	Compound name
232,074524	C8H17NaO4S	126-92-1	2-Ethylhexylsulphuric acid, sodium salt
232,134247	C11H21ClN2O	26590-05-6	Acrylamide-diallyldimethylammonium chloride, copolymer
232,1827154	C16H24O		3,5-di-tert-butyl-4-hydroxystyrene
232,988998	C6H10KO5S	31098-20-1	Acrylic acid, 3-sulfopropyl ester, potassium salt
233,011353	C8H15O2Zr	18312-04-4	Caprylic acid, zirconium salt
234,037567	C8H10O8	123-23-9	4,4'-Dioxo-4,4'-dioxydibutyric acid
234,073959	C9H14O7	1321-57-9	(1-Methylethyl) dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate
234,110336	C10H18O6	16215-49-9	Dibutyl peroxydicarbonate
234,110336	C10H18O6	19910-65-7	Bis-sec-butyl peroxydicarbonate
234,1255944	C14H18O3		(2-methyl-6-tert-butyl-4-(propen-1-oic)-2,5-cyclohexadiene-1-one) acid
234,1526902	C15H10D6O2		BPA (methyl-d_6)
234,16198	C15H22O2		3,5-di-tert-butyl-4-hydroxybenzaldehyde
234,183105	C12H26O4	2167-23-9	di-tert-Butyl sec-butylidene diperoxide
234,198364	C16H26O	4130-42-1	2,6-Di-tert-butyl-4-ethylphenol
234,198364	C16H26O	121158-58-5	Phenol, dodecyl-, branched
235,0705392	C9H9N5O3		benzoguanamine
235,157227	C14H21NO2	14779-78-3	Benzoic acid, p-(dimethylamino)-, pentyl ester (padimate A)
236,083725	C16H12O2	84-51-5	Anthraquinone, 2-ethyl-
236,125992	C10H20O6	24937-93-7	Polyester of adipic acid with 1,3-butanediol
236,1412445	C14H20O3		3-methyl-5-tert-butyl-4-hydroxyphenyl propanoic acid
236,156494	C18H20	3910-35-8	Indan, 1,1,3-trimethyl-3-phenyl-
236,156494	C18H20	9017-27-0	α-Methylstyrene-vinyltoluene copolymers
236,156494	C18H20	6362-80-7	2,4-Diphenyl-4-methyl-1-pentene
236,1565006	C18H20		butadiene n=8
236,177628	C15H24O2	88-26-6	2,6-Di-tert-butyl-4-hydroxymethylphenol
236,21402	C16H28O	66068-84-6	4-(5,5,6-Trimethylbicyclo(2.2.1)hept-2-yl)cyclohexan-1-ol
237,078979	C15H11NO2	82-38-2	Solvent Red 111
237,1524404	C11H25O3S		Atmer 191 sulphonic acid C11
237,969315	C9H6N2S3	21564-17-0	2-(Thiocyanomethylthio) benzothiazole
238,006592	C9H11KO3S	28085-69-0	Potassium cumenesulphonate
238,084122	C12H14O5	211510-16-6 442536-99-4	Mixture of Oxy-phenyl-acetic acid 2-[2-oxo-2-phenyl-acetoxy-ethoxy]-ethyl ester and Oxy-phenyl-acetic 2-[2-hydroxy-ethoxy]-ethyl ester
238,09938	C16H14O2	103-41-3	Benzyl cinnamate
238,133392	C10H23O4P	3138-42-9	Phosphoric acid, dipentyl ester
238,240906	C15H30N2	6864-37-5	3,3'-Dimethyl-4,4'- diaminodicyclohexylmethane
239,853378	C3H2Br2N2O	10222-01-2	2,2-Dibromo-2-cyanoacetamide
239,930115	C5H6BrClN2O2	16079-88-2	1-Bromo-3-chloro-5,5-dimethyl-2,4-imidazolidinedione
239,988327	C6H12N2S4	137-26-8	Tetramethylthiuram disulphide
240,0786693	C15H12O3		Methyl-2-benzoyl benzoate
240,078995	C15H12O3	606-28-0	Benzoic acid, 2-benzoyl-, methyl ester
240,126266	C15H16N2O	621-00-1	Carbanilide, 4,4'-dimethyl-
240,208923	C15H28O2	2156-97-0	Acrylic acid, dodecyl ester
242,057907	C14H10O4	94-36-0	Dibenzoyl peroxide

Monoisotopic mass	Formula	CAS number	Compound name
242,115417	C12H18O5	37275-47-1	Acrylic acid, 2-ethyl-2-(hydroxymethyl)-1,3-propanediyl ester
242,260971	C16H34O	36653-82-4	1-Hexadecanol
243,2562147	C15H33NO		Atmer 163 secondary amine C13
244,073563	C14H12O4	131-53-3	2,2'-Dihydroxy-4-methoxybenzophenone
244,087952	C9H21O4V	5588-84-1	Oxotris(propan-2-olato)vanadium
244,1211778	C14H16N2O2		o-dianisidine
244,168793	C14H20N4	25551-14-8	Azobis(cyclohexanecarbonitrile)
244,2154577	C15D16O2		BPA-d_{16}
245,996429	C7H11KO5S	31098-21-2	Methacrylic acid, sulphopropyl ester
246,052826	C13H10O5	131-55-5	bis(2,4-dihydroxyphenyl)methanone
246,089203	C14H14O4	131-17-9	Phthalic acid, diallyl ester
246,089234	C14H14O4		Diallyl phthalate
246,146729	C12H22O5	12262-58-7	Cyclohexanone, peroxide
248,0896	C10H16O7	32074-56-9	Citric acid, diethyl ester
248,108002	C10H20O5Si	2530-85-0	Methacrylic acid, 3-(trimethoxysilyl)propyl ester
248,141251	C15H20O3	65983-31-5	2-(Tricyclo[5.2.1.02,6]dec-3-en-8-yloxy)ethyl acrylate
248,17763	C16H24O2		3,5-di-tert-butyl-4-hydroxyacetophenone
249,111343	C12H15N3O3	101-37-1	Cyanuric acid triallyl ester
249,111343	C12H15N3O3	1025-15-6	Triallyl isocyanurate
250,03	C12H10O4S		Bisphenol S
250,037964	C8H18OSn	818-08-6	Dibutyltin oxide
250,074234	C15H10N2O2	101-68-8	Diphenylmethane-4,4'-diisocyanate
250,074234	C15H10N2O2	5873-54-1	Diphenylmethane-2,4'-diisocyanate
250,074997	C9H16Cl2N4	4080-31-3	1-(3-Chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride
250,1205341	C14H18O4		Diisopropyl phthalate
250,142975	C12H18N4O2	80584-89-0	Ethanol, 2,2'-[[(4-methyl-1H-benzotriazol-1-yl)methyl]imino]bis-
250,142975	C12H18N4O2	88477-37-6	Ethanol, 2,2'-[[(methyl-1H-benzotriazol-1-yl)methyl]imino]bis-
250,156891	C15H22O3	5153-25-3	2-Ethylhexyl 4-Hydroxybenzoate
250,1568946	C15H22O3		3-methyl-5-tert-butyl-4-hydroxyphenyl methylpropanoate
250,1932801	C16H26O2		Triton X-45 n=1
250,193283	C16H26O2	79-74-3	2,5-Di-tert-pentylhydroquinone
251,059204	C10H14NNaO3S	70916-35-7	Benzenesulfonic acid, 4-(diethylamino)-, sodium salt
251,059204	C10H14NNaO3S	5123-63-7	Metanilic acid, N,N-diethyl-, sodium salt
251,061615	C12H12NO3S	24057-28-1	Pyridinium p-toluenesulfonate
251,1680904	C12H27O3S		Atmer 191 sulphonic acid C12
252,006866	C4H13O8PS	55566-30-8	Tetrakis(hydroxymethyl)phosponium sulfate
252,0221038	C12H10N2Cl2		3,3'-dichlorobenzidine
252,02211	C12H10Cl2N2	612-83-9	3,3'-Dichlorobenzidine
253,157898	C16H19N3	2481-94-9	Solvent Yellow 56
254,006271	C10H6O8	89-05-4	Pyromellitic acid
254,076538	C16H14OS	83846-86-0	4-Isopropyl thioxanthone
254,076538	C16H14OS	5495-84-1	2-Isopropyl thioxanthone

Monoisotopic mass	Formula	CAS number	Compound name
254,0765608	C16H14OS		2-Isopropylthioxanthone
254,0765608	C16H14OS		4-Isopropylthioxanthone
254,130676	C17H18O2	182121-12-6	9,9-Bis(methoxymethyl)fluorene
254,136551	C10H22O7	126-58-9	Dipentaerythritol
254,224579	C16H30O2	3076-04-8	Acrylic acid, tridecyl ester
254,224579	C16H30O2	373-49-9	Palmitoleic acid
255,920914	C4H6N2Na2S4	142-59-6	Nabam
256,1099694	C16H16O3		2,2-Dimethoxy-2-phenylacetophenone
256,16745	C14H24O4	14228-73-0	Cyclohexane, 1,4-bis[(2,3-epoxypropoxy)methyl]-
256,16745	C14H24O4	25134-51-4	Acrylic acid, acrylic acid 2-ethylhexyl ester, copolymer
256,2402303	C16H32O2		palmitic acid
256,2402303	C16H32O2		palmitic acid
256,2402303	C16H32O2		hexadecanoic acid, palmitic acid
256,240234	C16H32O2	57-10-3	Palmitic acid
256,9691584	C12H8Cl3		PCB 3
257,2718648	C16H35NO		Atmer 163 secondary amine C14
258,104462	C19H14O	2128-93-0	4-Benzoylbiphenyl
258,1044901	C19H14O		4-Phenylbenzophenone
258,110352	C12H18O6	1680-21-3	Acrylic acid, diester with triethyleneglycol
258,1831	C14H26O4		Di-n-butyl adipate
258,1831	C14H26O4		Di-iso-butyl adipate
258,183105	C14H26O4	105-99-7	Adipic acid, dibutyl ester
258,183105	C14H26O4	109-43-3	Sebacic acid, dibutyl ester
258,183105	C14H26O4	141-04-8	Adipic acid, diisobutyl ester
258,219482	C15H30O3	27194-74-7	1,2-Propyleneglycol monolaurate
260,024017	C14H9ClO3	85-56-3	2-(4-Chlorobenzoyl)benzoic acid
260,1058549	C10H21O4SNa		Atmer 191 C10
260,125977	C12H20O6	13236-02-7	Propane, 1,2,3-tris(2,3-epoxypropoxy)-
260,198761	C14H28O4	3006-86-8	1,1-Bis(tert-butylperoxy)cyclohexane
261,0961	C9H15N3O6	839-90-7	1,3,5-Tris(2-Hydroxyethyl)-1,3,5-triazine-2,4,6(1H,3H,5H)-trione
262,066467	C8H15NaO8	9004-32-4	Carboxymethyl cellulose, sodium salt
262,091339	C8H14N4O6	5395-50-6	Tetrahydro-1,3,4,6-tetrakis-(hydroxymethyl)-imidazo(4,5-d)imidazole-2,5(1H,3H)-dione as formaldehyde donator system with an average ratio of formaldehyde: acetylene diurea of 3.1:1 to 3.5:1
262,091339	C8H14N4O6	5395-50-6	Tetrakis(hydroxymethyl)glycoluril
262,168121	C15H22N2O2	5124-30-1	Dicyclohexylmethane-4,4′-diisocyanate
262,1721507	C20H22		butadiene n=9
262,229675	C18H30O	17540-75-9	4-sec-Butyl-2,6-di-tert-butylphenol
262,229675	C18H30O	27193-86-8	4-dodecylphenol
262,229675	C18H30O	28471-16-1	2,3,4-Tributylphenol
262,229675	C18H30O	5857-00-1	2,4,6-Tributylphenol
262,229675	C18H30O	5892-47-7	Phenol, 2,4,6-tris(1-methylpropyl)-
262,229675	C18H30O	732-26-3	Phenol, 2,4,6-tri-tert-butyl-
263,224915	C17H29NO	88-27-7	p-Cresol, 2,6-di-tert-butyl-.alpha.-(dimethylamino)-

Monoisotopic mass	Formula	CAS number	Compound name
263,8470282	C6HOCl5		Pentachlorophenol
263,889771	C6H6Br2N2	35691-65-7	1,2-Dibromo-2,4-dicyanobutane
263,9833	C5HO2F9		PFPA
264,0197	C6H5OF9		4:2 FTOH
264,063385	C13H12O6	30697-40-6	Phthalic acid, mono(2-hydroxyethyl) ester, acrylate
264,089874	C16H12N2O2	91-97-4	3,3'-Dimethyl-4,4'-diisocyanatobiphenyl
264,124084	C11H21ClN2O3	69418-26-4	Copolymer of acrylamide and 2-(N,N,N-Trimethylammonium)ethylacrylate, chloride
264,136169	C15H20O4	80-05-7	2,2-Bis(4-hydroxyphenyl)propane
265,1552176	C14H21N2O3		Nylon MXD6 n=1
265,167786	C15H23NO3	67362-76-9	Benzoic acid, 4-(dimethylamino)-, 2-butoxyethyl ester
265,1837405	C13H29O3S		Atmer 191 sulphonic acid C13
266,0377538	C13H12N2Cl2		4,4'-methylene-bis-(2-chloroaniline)
266,063782	C9H14O9	36291-32-4	Citric acid, monoester with glycerol
266,079041	C13H14O6	85-71-2	Phthalic acid, mixed esters with ethyl glycolate and methanol
266,094299	C17H14O3	70331-94-1	2,2'-Oxamidobis[ethyl-3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate]
266,130676	C18H18O2	68818-86-0	Anthracene, 9,10-diethoxy-
266,164703	C12H27O4P	126-71-6	Phosphoric acid, triisobutyl ester
266,164703	C12H27O4P	126-73-8	Phosphoric acid, tributyl ester
267,965363	C8H5NaO7S	6362-79-4	5-Sulphoisophthalic acid, salts
268,005798	C13H10Cl2O2	97-23-4	2,2′-Dihydroxy-5,5′-dichlorodiphenylmethane
268,0922109	C17H16OS		2,4-Diethyl-9H-thioxanthen-9-one
268,157562	C17H20N2O	85-98-3	Diethyldiphenylurea
268,1575883	C17H20N2O		4,4'-bis(dimethylamino)benzophenone
268,240234	C17H32O2	21643-42-5	Acrylic acid, tetradecyl ester
268,276611	C18H36O	143-28-2	Oleyl alcohol
269,093292	C9H19NO6S	13106-44-0	Choline, methyl sulfate, acrylate
269,115997	C15H15N3O2	2832-40-8	Disperse Yellow 3
269,118256	C14H20ClNO2	46830-22-2	Dimethyl(acryloyloxyethyl)benzylammonium chloride
269,308258	C18H39N	124-30-1	Octadecylamine
269,987671	C8H14O2S4	105-65-7	Bis(isopropyl) thioperoxydicarbonate
270,014069	C7H11O9P	37971-36-1	2-Phosphonobutane-1,2,4-tricaboxylic acid
270,089203	C16H14O4	3034-79-5	Bis(o-toluoyl) peroxide
270,0892089	C16H14O4		ethylene dibenzoate
270,110352	C13H18O6	57043-35-3	1,2-Cyclohexanedicarboxylic acid, mono[2-[(1-oxo-
270,12558	C17H18O3	87-18-3	Salicylic acid, 4-tert-butylphenyl ester
270,1384811	C14H23O3P		2,4-di-tert-butylphenylphosphate
270,161987	C18H22O2	80-43-3	Dicumyl peroxide
270,2922658	C18H38O		octadecanol, stearyl alcohol
270,292267	C18H38O	112-92-5	1-Octadecanol
271,2875148	C17H37NO		Atmer 163 secondary amine C15
272,142212	C12H25NaO3S	1510-16-3	Dodecane-1-sulphonic acid
273,062683	C8H12N5O4P	1071-93-8	Adipic acid, dihydrazide
273,078979	C18H11NO2	8003-22-3	Solvent Yellow 33

Monoisotopic mass	Formula	CAS number	Compound name
274,084137	C15H14O5	131-54-4	Benzophenone, 2,2'-dihydroxy-4,4'-dimethoxy-
274,1215049	C11H23O4SNa		Atmer 191 C11
274,214417	C15H30O4	27215-38-9	Glycerol monolaurate
275,1647196	C17H23O3		octadecanol - corres acid 2
276,006989	C8H13KO6S	93841-08-8	Acrylic acid, 2-(3-sulfopropoxy)ethyl ester, potassium salt
276,110992	C14H16N2O4	24731-73-5	Butanamide, N,N'-1,4-phenylenebis[3-oxo-
276,120911	C12H20O7	77-93-0	Citric acid, triethyl ester
276,1725446	C17H24O3		quinone methide,
277,110291	C18H15NO2	5232-99-5	2-Cyano-3,3-diphenylacrylic acid, ethyl ester
277,131409	C15H19NO4	5026-74-4	2-Oxiranemethanamine, N-[4-(oxiranylmethoxy)phenyl]-N-(oxiranylmethyl)-
277,131409	C15H19NO4	48145-04-6	Mixture of : Phenoxyethylacrylate Methyl-2-benzoylbenzoate 2-Benzyl-2-(dimethylamino)-4-morpholino butyrophenone Ethyl-4-Dimethylaminobenzoate
277,204193	C17H27NO2	21245-02-3	2-Ethylhexyl 4-(dimethylamino)benzoate
277,2042041	C17H27NO2		2-Ethylhexyl-4-(dimethylamino)benzoate
278,079041	C14H14O6	27697-00-3	1,2-Benzenedicarboxylic acid, mono[2-[(2-methyl-1-oxo-2-propenyl)oxy]ethyl] ester
278,139709	C12H23ClN2O3	35429-19-7	Acrylamide-N,N,N-trimethylaminoethyl methacrylate chloride, copolymer
278,151794	C16H22O4	84-74-2	Phthalic acid, dibutyl ester
278,151794	C16H22O4	1962-75-0	Terephthalic acid, dibutyl ester
278,151794	C16H22O4	84-69-5	Phthalic acid, diisobutyl ester
278,1518342	C16H22O4		Diisobutyl phthalate
278,1518342	C16H22O4		Di-n-butyl phthalate
278,1518342	C16H22O4		Diisobutyl phthalate
278,1518342	C16H22O4		Di-n-butyl phthalate
278,1881947	C17H26O3		octadecanol - corres acid 1
278,1881947	C17H26O3		3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionic acid
278,224579	C18H30O2	28290-79-1	Linolenic acid
279,1293246	C15H21NO2S		2-Methyl-4'-(methylthio)-2-morpholinopropiophenone
279,1596342	C16H23O4		MEHP
279,1993906	C14H31O3S		Atmer 191 sulphonic acid C14
280,094696	C14H16O6	84-72-0	Phthalic acid, mixed esters with ethyl glycolate and ethanol
280,240234	C18H32O2	60-33-3	Linoleic acid
280,991272	C8H12INO2	55406-53-6	3-Iodo-2-propynyl butyl carbamate
281,0408	C11H17Cl2NOS	64359-81-5	4,5-Dichloro-2-octyl-4-isothiazolin-3-one
281,040802	C11H17Cl2NOS	64359-81-5	3(2H)-Isothiazolone, 4,5-dichloro-2-octyl-
281,271851	C18H35NO	301-02-0	Oleamide
281,2718648	C18H35NO		Atmer SA 1758
281,2718898	C18H35NO		(9Z)-9-Octadecenamide
282,0210819	C12H12O4P2		Irgafos P-EPQ hydrolysis product
282,110352	C14H18O6	117-82-8	Phthalic acid, bis(2-methoxyethyl) ester
282,136841	C17H18N2O2	57834-33-0	Benzoic acid, 4-[[(methylphenylamino)methylene]amino]-, ethyl ester

Monoisotopic mass	Formula	CAS number	Compound name
282,146729	C15H22O5	1034-01-1	Gallic acid, octyl ester
282,183105	C16H26O4	13048-34-5	Acrylic acid, decamethylene ester
282,2558803	C18H34O2		Oleic acid
282,25589	C18H34O2	26764-26-1	Octadecenoic acid
282,25589	C18H34O2	112-79-8	Elaidic acid
282,25589	C18H34O2	112-80-1	Oleic acid
282,997284	C11H9NO4S2	95154-01-1	Butanedioic acid, (2-benzothiazolylthio)-
283,011993	C8H15CeO2	7435-02-1	Caprylic acid, cerium salt
283,108948	C10H21NO6S	88992-91-0	1-Propanaminium, N,N-dimethyl-N-[2-[(1-oxo-2-propenyl)oxy]ethyl]-3-sulfo-, hydroxide, inner salt
283,264252	C18H35O2	26836-47-5	Sorbitol monostearate
283,287506	C18H37NO	124-26-5	Stearamide
283,2875148	C18H37NO		octadecanamide
283,953888	C6H12Cl3O4P	115-96-8	Phosphoric acid, trichloroethyl ester
284,060425	C6H12N4O9	1302-78-9	Bentonite
284,1541312	C15H25O3P		(nonylphenyl)phosphite
284,271515	C18H36O2	57-11-4	Stearic acid
284,2715304	C18H36O2		stearic acid, octadecanoic acid
284,282776	C17H36N2O	4559-86-8	Urea, tetrabutyl-
284,282776	C17H36N2O	4635-59-0	Butyryl chloride, 4-chloro-
285,1940084	C15H27NO4		Tinuvin 622 n=1
286,073517	C9H20O6P2	41203-81-0	Trimethylolpropane cyclic methylphosphonate (1:1) methyl methylphosphonate
286,120514	C17H18O4	126-00-1	Diphenolic acid
286,134888	C12H27O4V	1801-76-9	Tributoxyoxovanadium
286,141632	C14H22O6	1561-49-5	Dicyclohexyl peroxydicarbonate
286,214417	C16H30O4	6846-50-0	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate
286,2144345	C16H30O4		2,2,4-trimethyl-1,3-pentanediol diisobutyrate
287,246033	C16H33NO3	120-40-1	N,N-Bis(2-hydroxyethyl)lauramide
287,2824294	C17H37NO2		Atmer 163 C13
287,951172	C12H7Cl3O2	3380-34-5	2,4,4'-Trichloro-2'-hydroxydiphenyl ether
288,11438	C12H20N2O4S	13560-49-1	3-Aminocrotonic acid, diester with thiobis(2-hydroxyethyl) ether
288,137115	C12H25NaO4S	151-21-3	Dodecylsulphuric acid, sodium salt
288,137155	C12H25O4SNa		Atmer 191 C12
288,1878008	C22H24		butadiene n=10
290,06842	C8H20O7P2	7722-88-5	Tetrasodium pyrophosphate
290,06842	C8H20O7P2	112-57-2	Tetraethylenepentamine
290,1001676	C12H18O8		acetyl butyl citrate
290,1881947	C18H26O3		2-ethylhexyl-p-methoxycinnamate
290,1881947	C18H26O3		quinone methide methyl ester
290,188202	C18H26O3	5466-77-3	Acrylic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester
290,260956	C20H34O	26266-77-3	Hydroabietyl alcohol
290,9301861	C12H7Cl4		PCB 4
291,011963	C11H10BrN5	8002-09-3	Pine oil
291,158295	C15H21N3O3	6291-95-8	Trimethallyl isocyanurate

Monoisotopic mass	Formula	CAS number	Compound name
292,090668	C10H16N2O8	60-00-4	Ethylenediaminetetraacetic acid (EDTA)
292,2038448	C18H28O3		methyl-3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate
292,236206	C14H32N2O4	102-60-3	N,N,N′,N′,-Tetrakis(2-hydroxypropyl)ethylenediamine
293,2150406	C15H33O3S		Atmer 191 sulphonic acid C15
294,2194948	C18H30O3		Triton X-45 n=2
294,902832	C9H4Cl3NO2S	133-07-3	Folpet (Phthalimide, N-[(trichloromethyl)thio]-)
295,287506	C19H37NO	112-96-9	Octadecyl isocyanate
296,050934	C10H20N2S4	97-77-8	Tetraethylthiuram disulphide
296,125977	C15H20O6	15625-89-5	Trimethylolpropane triacrylate
296,1259884	C15H20O6		3,4-dimethylbenzylidene sorbitol
296,152466	C18H20N2O2	65816-20-8	Benzoic acid, 4-[[(ethylphenylamino)methylene]amino]-, ethyl ester
296,21402	C21H28O	68610-51-5	p-Cresol-dicyclopentadiene-isobutylene, copolymer
296,271515	C19H36O2	13402-02-3	Acrylic acid, hexadecyl ester
296,2715304	C19H36O2		methyl oleate
297,0961	C12H15N3O6	2451-62-9	Tris(2,3-epoxypropyl)isocyanurate (Teroxirone)
297,1940084	C16H27NO4		methyl-1-aza-2,2,6,6-tetramethyl-3-cyclohexeneethyl succinate
297,24353	C18H33O3	29894-35-7	Polyglycerol ricinoleate
298,018951	C10H18O2S4	105-77-1	Dibutylxanthogen disulphide
298,105255	C14H18O7	3524-68-3	Pentaerythritol triacrylate
298,156891	C19H22O3	3293-97-8	2-Hydroxy-4-n-hexyloxybenzophenone
298,1602904	C16H26O3S		Mesamoll mono SO3 C10
298,193268	C20H26O2	33145-10-7	2,4-Xylenol, 6,6'-isobutylidenedi-
298,2872055	C19H38O2		Methyl stearate
298,4608	C18H34O3	141-22-0	Ricinoleic acid
299,9503	C4HO3F9S		PFBS
300,1490458	C15H25O4P		(nonylphenyl)phosphate
300,157288	C15H24O6	42978-66-5	Acrylic acid,(1-methyl-1,2-ethanediyl)bis[oxy(methyl-2,1-ethanediyl)] ester
300,208923	C20H28O2	1740-19-8	Dehydroabietic acid
300,2089552	C20H28O2		Dehydroabietic acid
300,2219934	C18H33OCl		oleyol chloride
300,266449	C18H36O3	106-14-9	12-Hydroxystearic acid
301,2980795	C18H39NO2		Atmer 163 C14
302,136566	C14H22O7	17831-71-9	Acrylic acid, diester with tetraethyleneglycol
302,152805	C13H27O4SNa		Atmer 191 C13
302,167053	C22H22O	25640-70-4	Phenol, bis(1-phenylethyl)-
302,167053	C22H22O	2769-94-0	2,4-Bis(1-phenylethyl)phenol
302,172943	C15H26O6	60-01-5	Glycerol tributyrate
302,172943	C15H26O6	30499-70-8	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-, polymer with (chloromethyl)oxirane
302,224579	C20H30O2	8050-09-7	Rosin
302,224579	C20H30O2	514-10-3	Abietic acid
302,2246052	C20H30O2		Abietic acid
302,245697	C17H34O4	27214-38-6	Glycerol monomyristate
302,282104	C18H38O3	61725-89-1	Poly(ethylene propylene)glycol tridecyl ether

Monoisotopic mass	Formula	CAS number	Compound name
303,91748	C6H12N2S4Zn	137-30-4	Dimethyldithiocarbamic acid, zinc salt
304,034027	C14H12N2O2S2	2527-57-3	2,2'-Dithiobisbenzamide
304,092194	C20H16OS	83846-85-9	4-(p-Tolylthio)benzophenone
304,0922109	C20H16OS		4-(4-Methylphenylthio)benzophenone
304,166992	C18H24O4	42594-17-2	Dicyclopentadienedimethanol diacrylate
304,240234	C20H32O2	7771-44-0	Arachidonic acid
304,276611	C21H36O	501-24-6	Hydroginkgol
306,1831344	C18H26O4		Di-n-pentyl phthalate
306,232391	C16H35O3P	3658-48-8	Phosphonic acid, bis(2-ethylhexyl) ester
306,25589	C20H34O2	9014-92-0	Polyethyleneglycol dodecylphenyl ether
307,2306907	C16H35O3S		Atmer 191 sulphonic acid C16
308,035461	C14H12O6S	4065-45-6	2-Hydroxy-4-Methoxy-5-sulfonylbenzophenone(BP-4)
308,235138	C19H32O3	9016-45-9	Polyethyleneglycol nonylphenyl ether
308,235138	C19H32O3	26027-38-3	Polyethyleneglycol 4-nonylphenyl ether
309,303162	C20H39NO	10436-08-5	cis-11-Eicosenamide
310,156891	C20H22O3	70356-09-1	1,3-Propanedione, 1-[4-(1,1-dimethylethyl)phenyl]-3-(4-methoxyphenyl)-
310,162781	C13H26O8	13122-18-4	tert-Butyl 3,5,5-trimethylperoxyhexanoate
310,28717	C20H38O2	29204-02-2	Gadoleic acid
312,1361591	C19H20O4		BFDGE
312,136169	C19H20O4	39817-09-9	Bisphenol F diglycidyl ether
312,136169	C19H20O4	54208-63-8	Bis(2-hydroxyphenyl)methane bis(2,3-epoxypropyl) ether
312,136169	C19H20O4	2095-03-6	Oxirane, 2,2'-[methylenebis(4,1-phenyleneoxymethylene)]bis-
312,136169	C19H20O4	85-68-7	Phthalic acid, benzyl butyl ester
312,1361842	C19H20O4		Butyl benzyl phthalate
312,1361842	C19H20O4		Benzyl butyl phthalate
312,1474	C18H20N2O3	23949-66-8	2-Ethoxy-2'-ethyloxanilide
312,157288	C16H24O6	30145-51-8	Acrylic acid *3-[2,2-dimethyl-1-oxo-3-[(1-oxo-2-propenyl)oxy]propoxy]-2,2-dimethylpropyl ester
312,1759405	C17H28O3S		Mesamoll mono SO3 C11
312,302826	C20H40O2	506-30-9	Arachidic acid
312,302826	C20H40O2	822-23-1	Octadecyl acetate
312,3028305	C20H40O2		ethylstearate
313,1956258	C24H25		styrene trimer
313,225311	C17H31NO4	65447-77-0	1-(2-Hydroxyethyl)-4-hydroxy-2,2,6,6-tetramethyl piperidine-succinic acid, dimethylester, copolymer
313,9801	C6HO2F11		PFHxA
314,072601	C10H15N2NaO8	7379-28-4	Ethylenediaminetetraacetic acid, sodium salt
314,095245	C16H19NaO3S	1322-93-6	Naphthalenesulfonic acid, diisopropyl-, sodium salt
314,1154	C18H18O5		Diethylene glycol benzoate
314,2034508	C24H26		butadiene n=11
314,245697	C18H34O4	3851-87-4	Bis(3,5,5-trimethylhexanoyl) peroxide
314,2457346	C18H34O4		Di-n-butyl sebacate
314,282104	C19H38O3	29013-28-3	1,2-Propyleneglycol monopalmitate

Monoisotopic mass	Formula	CAS number	Compound name
315,113831	C17H18ClN3O	3896-11-5	2-(2'-Hydroxy-3'-tert-butyl-5'-methylphenyl)-5-chlorobenzotriazole (JC 30S)
315,3137296	C19H41NO2		Atmer 163 C15
316,055786	C20H12O2S	13354-35-3	9,10-Anthracenedione, 1-(phenylthio)-
316,121185	C20H16N2O2	2478-20-8	Solvent Yellow 44
316,16745	C19H24O4	901-44-0	2,2-Bis(4-hydroxyphenyl)propane-bis(2-hydroxyethyl) ether
316,1684551	C14H29O4SNa		Atmer 191 C14
316,1886	C16H28O6		Diacteyl lauroyl glycerol
317,2929941	C18H39NO3		dihydroxy oleamide
318,089203	C20H14O4	84-62-8	Phthalic acid, diphenyl ester
318,13147	C14H22O8	77-89-4	Triethyl acetylcitrate (Citroflex A2)
319,3239003	C22H41N		erucyl nitrile
320,11792	C18H21ClO3	25068-38-6	2,2-Bis(4-hydroxyphenyl)propane-epichlorohydrin copolymer
321,2463407	C17H37O3S		Atmer 191 sulphonic acid C17
321,3004848	C18H41O4		dihydroxy oleic acid
322,011353	C17H6O7	2421-28-5	Phthalic anhydride, 4,4-carbonyl-di
322,012329	C11H12Cl2N2O5	2832-19-1	Acetamide, 2-chloro-N-(hydroxymethyl)- (Chloramphenicol)
322,227295	C16H35O4P	298-07-7	Phosphoric acid, bis(2-ethylhexyl) ester
323,051849	C9H14N3O8P	26628-47-7	cmp-5 (Colour Former Red 3)
323,138214	C17H17N5O2	31482-56-1	Disperse Orange 25
323,236145	C21H29N3	6358-36-7	Basic Yellow 37
324,206573	C15H33O5P	9046-01-9	Polyethyleneglycol tridecyl ether phosphate
324,207031	C20H26N3O	73570-52-2	Phenoxazin-5-ium, 3,7-bis(diethylamino)-, nitrate
324,220154	C21H28N2O	90-93-7	4,4'-bis(Diethylamino)benzophenone
324,2201886	C21H28N2O		4,4'-bis(diethylamino)benzophenone
324,302826	C21H40O2	4813-57-4	Acrylic acid, octadecyl ester
324,8912137	C12H6Cl5		PCB 5
326,070801	C18H15O4P	115-86-6	Phosphoric acid, triphenyl ester
326,0708205	C18H15O4P		Triphenyl phosphate
326,101501	C16H14N4O4	4106-67-6	Pigment Yellow 5
326,10553	C21H14N2O2	10228-01-0	Quino[2,3-b]acridine-7,14-dione, 5,12-dihydro-2-methyl-
326,151794	C20H22O4	2451-84-5	Dibenzyl Adipate
326,1518	C20H22O4		Dibenzyladipate
326,172943	C17H26O6	87320-05-6	Acrylic acid,[2-[1,1-dimethyl-2-[(1-oxo-2-propenyl)oxy]ethyl]-5-ethyl-1,3-dioxan-5-yl]methyl ester
326,188202	C21H26O3	1843-05-6	2-Hydroxy-4-n-octyloxy benzophenone
326,1882197	C21H26O3		Chimassorb 81
326,191559	C18H30O3S	27176-87-0	Dodecylbenzenesulphonic acid
326,1915906	C18H30O3S		Mesamoll mono SO3 C12
327,207947	C14H33NO5S	4722-98-9	(2-Hydroxyethyl)ammonium dodecyl sulphate
328,142303	C18H20N2O4	52821-24-6	Basic Yellow 131
329,329376	C20H43NO2	2190-04-7	Octadecylammonium acetate
330,1467238	C19H22O5		BFDGE.H_2O
330,183105	C20H26O4	84-61-7	Phthalic acid, dicyclohexyl ester
330,1831344	C20H26O4		Dicyclohexyl phthalate

Monoisotopic mass	Formula	CAS number	Compound name
330,1841052	C15H31O4SNa		Atmer 191 C15
331,084473	C20H13NO4	17418-58-5	1-Amino-4-hydroxy-2-phenoxy-9,10-anthraquinone(Disperse Red 60)
332,068481	C20H12O5	2321-07-5	Fluorescein (Solvent Yellow 94)
333,147736	C20H19N3O2	27425-55-4	3-(1H-benzimidazol-2-yl)-7-(diethylamino)-2H-chromen-2-one (Disperse Yellow 82)
334,131744	C20H18N2O3	35773-42-3	3-(1,3-benzoxazol-2-yl)-7-(diethylamino)-2H-chromen-2-one (Disperse Yellow 232)
334,2144345	C20H30O4		Di-n-hexyl phthalate
334,235535	C17H34O6	995-33-5	4,4-Di-tert-butylperoxy n-butylvalerate
335,2619908	C18H39O3S		Atmer 191 sulphonic acid C18
336,060883	C23H12OS	16294-75-0	Solvent Orange 63 (14H-ANTHRA(2,1,9-MNA)THIOXANTHEN-14-ONE)
336,157288	C18H24O6	85-70-1	Phthalic acid, mixed esters with butyl glycolate and butanol
336,976257	C8H7NNa4O8	144538-83-0	Tetrasodium iminodisuccinate
337,134583	C20H20ClN3	632-99-5	Basic Violet 14
337,3106556	C22H41O2		erucic acid, docosenoic acid
337,334465	C22H43NO		Erucamide
337,334473	C22H43NO	112-84-5	Erucamide
337,33449	C22H43NO		(Z)-docos-13-enamide
338,172943	C18H26O6	3290-92-4	1,1,1-Trimethylolpropane trimethacrylate
338,20932	C19H30O5	1166-52-5	Gallic acid, dodecyl ester
338,245697	C20H34O4	2212-81-9	1,3-Bis(tertbutylperoxyisopropyl)benzene
338,2457096	C20H34O4		Triton X-45 n=3
338,318481	C22H42O2	112-86-7	Erucic acid
339,350128	C22H45NO	3061-75-4	Behenamide
340,0865	C19H17O4P		Cresyl diphenyl phosphate
340,152191	C17H24O7	68186-31-2	1,2,4-Benzenetricarboxylic acid, 2-ethylhexyl ester
340,16745	C21H24O4	1675-54-3	2,2-Bis(4-Hydroxyphenyl)propane bis(2,3-epoxypropyl) ether (=Badge)
340,16745	C21H24O4	474510-57-1	2-Hydroxy-1-(4-(4-(2-hydroxy-2-methylpropionyl)benzyl)phenyl-2-methyl-2-propanone
340,1674593	C21H24O4		BADGE
340,2072406	C19H32O3S		Mesamoll mono SO3 C13
340,2191009	C26H28		butadiene n=12
340,240234	C23H32O2	119-47-1	2,2'-Methylene bis(4-methyl-6-tert-butylphenol)
340,2613847	C20H36O4		Di-(2-ethylhexyl)fumarate
340,29776	C21H40O3	1330-80-9	1,2-Propyleneglycol monooleate
340,334137	C22H44O2	112-85-6	Behenic acid
340,334137	C22H44O2	123-95-5	Stearic acid, butyl ester
340,964783	C3H13N3O10Zr	32535-84-5	Zirconyl ammonium carbonate
341,069794	C15H17Cl2N3O2	60207-90-1	propiconazole
341,116425	C21H15N3O2	3271-22-5	2,4-Dimethoxy-6-(1-pyrenyl)-1,3,5-triazine
341,116425	C21H15N3O2	3271-22-5	2,4-Dimethoxy-6-pyren-1-yl-1,3,5-triazine
341,152466	C16H30MnO4	15956-58-8	2-Ethylhexanoic acid, manganese salt
342,116211	C12H22O11	57-50-1	Sucrose
342,1467	C20H22O5		Dipropylene glycol benzoate
342,173218	C23H22N2O	3008-87-5	7H-Dibenz[f,ij]isoquinolin-7-one, 4-(cyclohexylamino)-2-methyl-
342,313385	C21H42O3	1323-39-3	1,2-Propyleneglycol monostearate

Monoisotopic mass	Formula	CAS number	Compound name
343,986024	C6H6O4PF9		4:2 monoPAP
344,057251	C10H15N2Na3O7	139-89-9	N-(2-Hydroxyethyl)ethylenediaminetriacetic acid, trisodium salt
344,104218	C14H20N2O6S	55-55-0	Phenol, p-(methylamino)-, sulfate (2:1)
344,198761	C21H28O4	116-37-0	1,1'-Isopropylidenebis(p-phenyleneoxy)dipropan-2-ol
344,1997552	C16H33O4SNa		Atmer 191 C16
346,1627678	C16H26O8		acetyl dibutyl citrate
346,235535	C18H34O6	1338-39-2	Sorbitan monolaurate
346,235535	C18H34O6	16111-62-9	Bis(2-ethylhexyl) peroxydicarbonate
346,323578	C24H42O	134701-20-5	2,4-Dimethyl-6-(1-methylpentadecyl)-phenol
348,085846	C18H12N4O4	26747-90-0	2,4-Toluene diisocyanate dimeric
348,1128369	C19H21O4Cl		BFDGE.HCl
348,12793	C22H21O2P	75980-60-8	Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide
348,1572885	C19H24O6		BFDGE.2H_2O
348,230072	C21H32O4	3089-55-2	Benzyl octyl adipate
350,143555	C16H30O4Zn	136-53-8	Zinc bis(2-ethylhexanoate)
350,143555	C16H30O4Zn	136-53-8	2-Ethylhexanoic acid zinc salt
350,224579	C24H30O2	169198-26-9	5,7-Di-tert-butyl-3-(3,4- and 2,3-dimethylphenyl)-3H-benzofuran-2-one containing: a) 5,7-di-tert-butyl-3-(3,4-dimethylphenyl)-3H-benzofuran-2-one (80 to 100% w/w) and b) 5,7-di-tert-butyl-3-(2,3-dimethylphenyl)-3H-benzofuran-2-one (0 to 20% w/w)
350,258606	C18H39O4P	39471-52-8	Phosphoric acid, octadecyl esters
351,231049	C22H29N3O	25973-55-1	Tinuvin 328
352,115814	C17H20O8	4986-89-4	Pentaerythritol tetraacrylate
352,132416	C22H16N4O	85-86-9	Solvent Red 23
352,334137	C23H44O2	48076-38-6	Acrylic acid, eicosyl ester
353,068756	C15H21FeO6	14024-18-1	Tris(pentane-2,4-dionato)iron(III)
353,3293796	C22H43NO2		13-hydroxy-cis-14-docosenamide
354,189392	C14H34O6Si2	16068-37-4	1,2-Bis(triethoxysilyl)ethane
354,2228907	C20H34O3S		Mesamoll mono SO3 C14
354,234741	C27H30	41906-71-2	1H-Indene *2,3-dihydro-1,3-dimethyl-1-(2-methyl-2-phenylpropyl)-3-phenyl-
354,234985	C27H30	62604-62-0	4,6-Dimethyl-2,4,6-triphenyl-1-heptene
354,2553337	C21H10D14O4		d14 BADGE
354,277008	C21H38O4	140-03-4	Acetylricinoleic acid, methyl ester
355,057709	C20H12NaO5	518-47-8	Sodium fluorescein (Acid Yellow 73)
356,125977	C20H20O6	32647-67-9	Dibenzylidene sorbitol
356,2926598	C21H40O4		Glycerol monooleate
356,292664	C21H40O4	109-31-9	Azelaic acid, di-n-hexyl ester
356,292664	C21H40O4	68515-75-3	Adipic acid, dialkyl esters (C7-C9)
356,292999	C21H40O4	25496-72-4	Glycerol monooleate
357,160797	C20H24ClN3O	3864-99-1	2-(2'-Hydroxy-3,5'-di-tert-butylphenyl)-5-chlorobenzotriazole
358,1780239	C21H26O5		BADGE.H_2O
358,196655	C22H30O2S	96-69-5	4,4'-Thiobis(6-tert-butyl-3-methylphenol)

Monoisotopic mass	Formula	CAS number	Compound name
358,214417	C22H30O4	27987-25-3	Phthalic acid, bis(methylcyclohexyl) ester
358,2154053	C17H35O4SNa		Atmer 191 C17
358,235535	C19H34O6	30899-62-8	Glycerol monolaurate diacetate
358,2355639	C19H34O6		Acetylated glyceride (literature)
358,3083098	C21H42O4		Glycerol monostearate
358,308319	C21H42O4	31566-31-1	Stearic acid, monoester with glycerol
358,8522414	C12H5Cl6		PCB 6
359,980072	C10H20N2S4Zn	14324-55-1	Diethyldithiocarbamic acid, zinc salt
360,115356	C18H21AlO6	16899-72-2	2,4-Hexadienoic acid, aluminum salt, (E,E)-
360,127014	C12H24O12	8013-17-0	Invert sugar
360,214813	C18H32O7	77-94-1	Citric acid, tributyl ester
361,204193	C24H27NO2	6197-30-4	2-Cyano-3,3-diphenylacrylic acid, 2-ethylhexyl ester
361,276947	C26H35N	52047-59-3	2-(4-Dodecylphenyl)indole
361,347534	C22H48ClN	7173-51-5	Didecyldimethylammonium chloride
362,1647	C20H27O4P		Diphenyl 2-ethylhexyl phosphate
362,164703	C20H27O4P	1241-94-7	Phosphoric acid, diphenyl 2-ethylhexyl ester
362,245697	C22H34O4	3648-21-3	diheptyl phthalate
362,2457	C22H34O4		Diheptyl phthalate
362,272186	C25H34N2	2162-74-5	Bis(2,6-diisopropylphenyl) carbodiimide
363,0443	C11H9O2F10		Fluoroacrylate (5 CF entities)
363,250061	C19H42BrN	57-09-0	Hexadecyltrimethylammonium bromide
363,9769	C7HO2F13		PFHepA
364,013	C8H5OF13		6:2 FTOH
364,170624	C23H25ClN2	18015-76-4	Pigment Green 4 (Malachite Green)
366,1234016	C19H23O5Cl		BFDGE.H_2O.HCl
366,230713	C23H30N2O2	119313-12-1	1-Butanone, 2-(dimethylamino)-1-[4-(4-morpholinyl)phenyl]-2-(phenylmethyl)-
366,234751	C28H30		butadiene n=13
367,300568	C23H42ClN	68391-01-5	Dimethylalkyl(C12-C18)benzylammonium chloride
367,813507	C9H2Cl6O3	115-27-5	Hexachloroendomethylenetetrahydrophthalic anhydride
368,079712	C22H12N2O4	18600-59-4	2,2’-(1,4-Phenylene)bis[4H-3,1-benzoxazin-4-one]
368,198761	C23H28O4	68515-40-2	1,2-Benzenedicarboxylic acid, benzyl C7-9-branched and linear alkyl esters
368,2385407	C21H36O3S		Mesamoll mono SO3 C15
368,271515	C25H36O2	88-24-4	2,2'-Methylene bis(4-ethyl-6-tert-butylphenol)
368,339996	C22H44N2O2	93-81-2	N-(2-Aminoethyl)-N-(2-hydroxyethyl)oleamide
368,3654	C24H48O2		2-Ethylhexyl palmitate
368,365417	C24H48O2	557-59-5	Lignoceric acid
368,3654558	C24H48O2		2-Ethylhexyl palmitate
370,178009	C22H26O5	72004-73-0	Acrylic acid, 2-[4-[1-[4-(2-hydroxyethoxy)phenyl]-1-methylethyl]phenoxy]ethyl ester
370,3083	C22H42O4		Heptyl nonyl adipate
370,308319	C22H42O4	103-23-1	Adipic acid, bis(2-ethylhexyl) ester
370,308319	C22H42O4	123-79-5	Adipic acid, di-n-octyl ester
370,3083349	C22H42O4		Bis(2-ethylhexyl) adipate
370,3083349	C22H42O4		Dioctyl adipate

Monoisotopic mass	Formula	CAS number	Compound name
370,3083349	C22H42O4		Dioctyl adipate
370,3083349	C22H42O4		Bis(2-ethylhexyl) adipate
372,1784429	C18H28O8		Polyadipate (literature)
372,2310554	C18H37O4SNa		Atmer 191 C18
372,287567	C21H40O5	1323-38-2	Glycerol monoricinolate
373,251801	C25H31N3	603-48-5	Aniline, 4,4',4"-methylidynetris[N,N-dimethyl- (Leucomethyl green)
374,174011	C22H22N4O2	29190-28-1	Resorcinol, 2,4-bis(xylylazo)-
375,065369	C18H16N3NaO3S	587-98-4	Acid Yellow 36
375,252197	C21H33N3O3	745070-61-5	1,3,5-Tris (2,2-dimethylpropanamido)-benzene
376,144137	C21H25O4Cl		BADGE.HCl
376,1885886	C21H28O6		BADGE $2H_2O$
379,116821	C20H17N3O5	12217-80-0	4,11-Diamino-2-(3-methoxypropyl)-1H-naphth(2,3-f)isoindol-1,3,5,10(2H)-tetrone
380,011932	C20H10Cl2N2O2	3089-16-5	Quino[2,3-b]acridine-7,14-dione, 4,11-dichloro-5,12-dihydro-
380,163696	C24H20N4O	85-83-6	Solvent Red 24
380,246368	C24H32N2O2	119344-86-4	1-Butanone, 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(4-morpholinyl)phenyl]-
380,365417	C25H48O2	18299-85-9	Acrylic acid, docosyl ester
382,051788	C22H14CaO4	61789-36-4	Naphthenic acids, calcium salts
382,103821	C14H24Na2O7S	37294-49-8	Sulphosuccinic acid, isodecyl ester, disodium salt
382,2541908	C22H38O3S		Mesamoll mono SO3 C16
382,2719243	C22H38O5		Triton X-45 n=4
382,28717	C26H38O2	85-60-9	1,1-Bis(2-methyl-4-hydroxy-5-tertbutylphenyl)butane
383,220886	C22H29N3O3	79916-07-7	Phenoxazin-5-ium, 3,7-bis(diethylamino)-, acetate
384,0895146	C19H22O4Cl2		BFDGE.2HCl
385,824066	C9H4Cl6O4	115-28-6	Hexachloroendomethylenetetrahydrophthalic acid
386,1729386	C22H26O6		Irgaclear DM
386,2093241	C23H30O5		BADGE.EtOH
386,318481	C26H42O2	58446-52-9	Stearoylbenzoylmethane
386,341003	C24H42N4	80584-90-3	1H-Benzotriazole-1-methanamine, N,N-bis(2-ethylhexyl)-4-methyl-
388,212006	C21H24N8	4482-25-1	1,3-Benzenediamine-4,4'-[(4-methyl-1,3-phenylene)bis(azo)] bis[6-methyl-
389,116425	C25H15N3O2	3333-62-8	7-(2H-Naphtho-(1,2-D)triazol-2-yl)-3-phenylcoumarin
389,246704	C25H31N3O	467-63-0	Tris[4-(dimethylamino)phenyl]methanol (solvent Violet 9)
390,195984	C22H31O4P	29761-21-5	Phosphoric acid, diphenyl isodecyl ester
390,222687	C15H30N6O6	3089-11-0	N,N,N',N',N"",N""-Hexakis(methoxymethyl)-2,4,6-triamino-1,3,5-triazine
390,222687	C15H30N6O6	68002-20-0	1,3,5-Triazine-2,4,6-triamine, polymer with formaldehyde, methylated
390,277008	C24H38O4	117-81-7	Phthalic acid, bis(2-ethylhexyl) ester
390,277008	C24H38O4	117-84-0	Phthalic acid, di-n-octyl ester
390,277008	C24H38O4	27554-26-3	Phthalic acid, diisooctyl ester
390,277008	C24H38O4	6422-86-2	Terephthalic acid, bis(2-ethylhexyl)ester
390,277008	C24H38O4	68515-41-3	1,2-Benzenedicarboxylic acid, di-C7-9-branched and linear alkyl esters
390,277008	C24H38O4	68515-43-5	1,2-Benzenedicarboxylic acid, di-C9-11-branched and linear alkylesters
390,2770097	C24H38O4		DEHP
390,2770347	C24H38O4		Bis(2-ethylhexyl) phthalate
390,2770347	C24H38O4		Bis(2-ethylhexyl) terephthalate

Monoisotopic mass	Formula	CAS number	Compound name
390,2770347	C24H38O4		Dioctyl phthalate
390,2770347	C24H38O4		Bis(2-ethylhexyl) phthalate
390,2770347	C24H38O4		Di-n-octyl phthalate
392,250401	C30H32		butadiene n=14
392,271515	C27H36O2	4066-02-8	2,2'-Methylenebis(4-methyl-6-cyclohexylphenol)
392,8132691	C12H4Cl7		PCB 7
393,278015	C25H35N3O	125304-04-3	Phenol, 2-(2H-benzotriazol-2-yl)-6-dodecyl-4-methyl-, branched and linear
394,1547017	C21H27O5Cl		BADGE H_2O HCl
394,250793	C26H34O3	61167-58-6	Acrylic acid, 2-tert-butyl-6-(3-tert-butyl-2-hydroxy-5-methylbenzyl)-4-methylphenyl ester
394,4035476	C24H50N4		Chimassorb 944 part a
395,194397	C20H29NO7	64147-40-6	Castor oil, dehydrated
396,2698409	C23H10O3S		Mesamoll mono SO3 C17
396,323975	C24H44O4	140-04-5	Acetylricinoleic acid, butyl ester
396,3967	C26H52O2		2-Ethylhexyl stearate
396,396729	C26H52O2	22047-49-0	2-Ethylhexyl stearate
396,3967559	C26H52O2		2-Ethylhexyl stearate
397,178925	C20H34CoO4	61789-51-3	Naphthenic acid, cobalt salts
397,295898	C20H44ClNO4	6200-40-4	Bis(2-hydroxyethyl)-2-hydroxypropyl-3-(dodecyloxy)methylammonium chloride
397,3318	C24H46O4		Diisononyl adipate
398,023987	C22H14FeO4	1338-14-3	Naphthenic acids, iron salts
398,105988	C14H22O13	9005-32-7	Alginic acid
398,131653	C23H24Cl2N2	3521-06-0	Basic Blue 1
398,243347	C18H39O7P	78-51-3	Phosphoric acid, tris-(2-butoxyethyl)phosphate
398,266846	C22H38O6	15520-11-3	Percarbonic acid, bis (4-tert-butylcyclohexyl) ester
398,3396	C24H46O4	110-29-2	Adipic acid, n-decyl-, n-octyl ester
399,9439	C6HO3F13S		PFHxS
400,2249741	C24H32O5		BADGE PrOH
401,326725	C23H45O5		Acetylated glyceride (literature)
402,2253681	C20H34O8		ATBC
402,225372	C20H34O8	77-90-7	Tri-n-butyl acetyl citrate
402,2253931	C20H34O8		Acetyltributyl citrate
402,298126	C22H42O6	26266-57-9	Sorbitan monopalmitate
402,335388	C24H50S2	27458-90-8	Di-tert-dodecyl disulphide
405,923431	C18H6Cl4N2O	20749-68-2	8,9,10,11-tetrachloro-12-phthaloperinone (Solvent Red 135)
406,229675	C30H30O	18254-13-2	2,4,6-Tris(1-phenylethyl)phenol
406,229675	C30H30O	25640-71-5	Phenol, tris(1-phenylethyl)-
407,930634	C12H19Cl7	63449-39-8	1,2,3,4,6,7,10-Heptachlorododecane
410,2854909	C24H42O3S		Mesamoll mono SO3 C18
412,1208147	C21H26O4Cl2		BADGE 2HCL
412,355255	C25H48O4	103-24-2	Azelaic acid, bis(2-ethylhexyl)ester
412,3553	C25H48O4		Di(2-ethylhexyl)azelate
413,0411	C12H9O2F12		Fluoroacrylate (6 CF entities)
413,973694	C8HF15O2	3825-26-1	Perfluorooctanoic acid, ammonium salt

Monoisotopic mass	Formula	CAS number	Compound name
413,9737	C8HO2F15		PFOA
414,136841	C28H18N2O2	1533-45-5	4,4'-Bis(2-benzoxazolyl)stilbene
414,204254	C24H30O6	135861-56-2	Bis(3,4-dimethylbenzylidene)sorbitol
414,2406242	C25H34O5		BADGE BuOH
414,261749	C22H38O7	137-66-6	Ascorbyl palmitate
414,370911	C25H50O4	30233-64-8	Glycerol monobehenate
414,4914	C24H30O6	79072-96-1	Bis(4-ethylbenzylidene)sorbitol
415,1203	C14H22N3NaO10	7578-43-0	Diethylenetriaminepentaacetic acid, sodium salts
416,2198888	C24H32O6		BADGE MeEtOH
417,2582261	C32H33		styrene n=4
418,169769	C26H27O3P	162881-26-7	Phenyl bis(2,4,6-trimethylbenzoyl) phosphine oxide
418,192993	C30H26O2	40470-68-6	1,1'-Biphenyl, 4,4'-bis[2-(2-methoxyphenyl)ethenyl]-
418,2355388	C24H34O6		BADGE H_2O PrOH
418,236511	C19H39NaO6S	83721-45-3	2,3-Bis[(2-ethylhexyl)oxy]propane-1-sodium sulphate
418,237	C19H39NaO6S	62174-79-2	1, 3-Bis[(2-ethylhexyl)oxy]propane-2-sodium sulphate
418,2660511	C32H34		butadiene n=15
418,3083	C26H42O4		n-Octyl-n-decyl phthalate
418,308319	C26H42O4	68515-48-0	Phthalic acid, diesters with primary, saturated C8-C10 branched alcohols, more than 60 % C9
418,3083349	C26H42O4		Diisononyl phthalate
418,3083349	C26H42O4		Diisononyl phthalate
418,3446953	C27H46O3		Irganox 1076 thermal degradation products
420,215759	C18H37NaO7S	13150-00-0	Ethanol, 2-[2-[2-(dodecyloxy)ethoxy]ethoxy]-, hydrogen sulfate,sodium salt
420,302826	C29H40O2	77-62-3	2,2'-Methylenebis(4-methyl-6-(1-methyl-cyclohexyl) phenol)
421,7796483	C12HO2Cl7		1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin
423,127777	C18H21N3O9	40220-08-4	Tris(2-hydroxyethyl) isocyanurate triacrylate
424,153992	C25H20N4O3	16403-84-2	(4-((5-Carbamoyl-o-tolyl)azo)-3-hydroxynaphth-2-anilide) Pigment Red 268
424,283356	C25H44OS2	110553-27-0	2,4-Bis(octylthiomethyl)-6-methylphenol
424,301141	C25H44O3S		Mesamoll mono SO3 C19
424,355255	C26H48O4	166412-78-8	1,2-Cyclohexanedicarboxylic acid, diisononyl ester
424,3552851	C26H48O4		Diisononyl cyclohexane-1,2-dicarboxylate
425,210327	C27H27N3O2	147315-50-2	2-(4,6-Diphenyl-1,3,5-triazin-2-yl)-5-(hexyloxy)phenol
426,132996	C24H18N4O4	6448-95-9	Pigment Red 22
426,2981391	C24H42O6		Triton X-45 n=5
426,3709	C26H50O4		Di(2-ethylhexyl)sebacate
426,3709	C26H50O4		Diisodecyl adipate
426,370911	C26H50O4	2432-87-3	Sebacic acid, di-n-octyl ester
426,370911	C26H50O4	27178-16-1	Adipic acid, diisodecyl ester
426,370911	C26H50O4	122-62-3	Sebacic acid, bis(2-ethylhexyl) ester
426,7742968	C12H3Cl8		PCB 8
427,231842	C20H33N3O7	57116-45-7	1-Aziridinepropanoic acid *2-[[3-(1-aziridinyl)-1-oxopropoxy]methyl]-2-(hydroxymethyl)-1,3-propanediyl ester
428,083069	C24H16N2O4S	10128-55-9	N-(2-(4-Oxo-4H-3,1-benzoxazin-2-yl)phenyl)-2-naphthalenesulfonamide
428,20462	C21H32O9	28961-43-5	Acrylic acid, triester with polyethylene glycol triether with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol

Monoisotopic mass	Formula	CAS number	Compound name
428,313782	C24H44O6	620-67-7	Glycerol triheptanoate
428,313782	C24H44O6	1338-43-8	Sorbitan monooleate
429,358025	C25H49O5		Acetylated glyceride (literature)
430,1714988	C26H26N2O2S		Uvitex OB
430,171509	C26H26N2O2S	7128-64-5	2,5-Bis(5-tert-butyl-2-benzoxazolyl)thiophene
430,2355388	C25H34O6		BADGE EtEtOH
430,329437	C24H46O6	1338-41-6	Sorbitan monostearate
430,381073	C29H50O2	59-02-9	α-Tocopherol
431,043945	C21H14NNaO6S	4430-18-6	Acid Violet 43
432,2511889	C25H36O6		BADGE H_2O BuOH
432,2511889	C25H36O6		BADGE.2EtOH
434,2093241	C27H30O5		BADGE Ph
434,2304534	C24H34O7		BADGE H_2O MeEtOH
434,287964	C22H42O8	9005-67-8	Polyethyleneglycol sorbitan monostearate
434,352509	C24H51O4P	78-42-2	Phosphoric acid, tris(2-ethylhexyl) ester
436,145813	C18H36O2SSn	15535-79-2	Di-n-octyltin mercaptoacetate
438,313385	C29H42O3	4221-80-1	3,5-Di-tert-butyl-4-hydroxybenzoic acid, 2,4-di-tert-butylphenyl ester
438,3167911	C26H46O3S		Mesamoll mono SO3 C20
438,349792	C30H46O2	35958-30-6	1,1-Bis(2-hydroxy-3,5-di-tert-butylphenyl)ethane
440,14801	C25H20N4O4	6655-84-1	Pigment Red 17
440,148468	C25H20N4O4	36968-27-1	Pigment Red 266
440,386566	C27H52O4	22788-19-8	1,2-Propyleneglycol dilaurate
442,225647	C28H30N2O3	509-34-2	Baso Red 546
442,2719243	C27H38O5		BADGE.HexOH
442,29306	C24H42O7	25395-66-8	Ascorbyl stearate
442,381073	C30H50O2	8001-75-0	Ceresin
443,337325	C25H47O6		Acetylated glyceride (literature)
443,9796368	C8H6O4PF13		6:2 monoPAP
444,110992	C28H16N2O4	4051-63-2	4,4'-Diamino-1,1'-bianthracene-9,9',10,10'-tetrone (Pigment Red 177)
444,215759	C20H37NaO7S	1639-66-3	Succinic acid, sulfo-, 1,4-dioctyl ester, sodium salt
444,2817012	C34H36		butadiene n=16
444,308716	C24H44O7	1337-33-3	1,2,3-Propanetricarboxylic acid, 2-hydroxy-, octadecyl ester
446,3396	C28H46O4	53306-54-0	1,2-Benzenedicarboxylic acid, bis(2-propylheptyl) ester
446,3396	C28H46O4	84-77-5	Phthalic acid, di-n-decyl ester
446,3396	C28H46O4	68515-49-1	Phthalic acid, diesters with primary, saturated C9-C11 alcohols more than 90 % C10
446,339635	C28H46O4		Diisodecyl phthalate
446,339635	C28H46O4		Diisodecyl phthalate
446,339635	C28H46O4		Di-n-decyl phthalate
447,230988	C30H29N3O	70321-86-7	2-[2-Hydroxy-3,5-bis(1,1-dimethylbenzyl)phenyl]benzotriazole
447,2310626	C30H29N3O		Tinuvin 234
447,290405	C27H42ClNO2	121-54-0	Ammonium, benzyldimethyl[2-[2-[p-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethyl]-, chloride

Monoisotopic mass	Formula	CAS number	Compound name
448,2461035	C25H36O7		BADGE H_2O EtEtOH
448,276611	C33H36O	85305-20-0	Phenol, 2,4,6-tris[1-(methylphenyl)ethyl]-
448,276611	C33H36O	83804-01-7	Tris(1-(methylphenyl)ethyl)phenol
450,295746	C24H43NaO6	25383-99-7	Stearoyl-2-lactylic acid, sodium salt
450,326294	C27H47O3P	161717-32-4	2,4,6-Tris(tert-butyl)phenyl-2-butyl-2-ethyl-1,3-propanediol phosphite
450,393372	C26H50N4O2	124172-53-8	N,N'-Bis(2,2,6,6-tetramethyl-4-piperidyl)-N,N'-diformylhexamethylene diamine
452,211639	C27H33O4P	2502-15-0	Phenol, 4-(1-methylethyl)-, phosphate (3:1)
452,211639	C27H33O4P	68937-41-7	Phenol, isopropylated, phosphate (3:1)
452,2198888	C27H32O6		BADGE H_2O Ph
452,3324411	C27H48O3S		Mesamoll mono SO3 C21
452,365417	C31H48O2	19546-20-4	2,2-Bis(3,5-di-n-octyl-4-hydroxyphenyl)propane
454,1484051	C22H30O6S2		Mesamoll di SO3 C10
454,241425	C20H38O11	9004-67-5	Methylcellulose
455,740676	C12O2Cl8		octachlorodibenzo-p-dioxin
457,228485	C29H32ClN3	2185-86-6	Basic Blue 11
458,266839	C27H38O6		BADGE BuEtOH
458,2949818	C28H43O3P		di(2,4-di-tert-butylphenyl)phosphate
460,093994	C24H17ClN4O4	6471-50-7	Pigment Red 14
460,282489	C27H40O6		BADGE 2PrOH
460,7353245	C12H2Cl9		PCB 9
463,0379	C13H9O2F14		Fluoroacrylate (7 CF entities)
463,9705	C9HO2F17		PFNA
464,0069	C10H5OF17		8:2 FTOH
464,186676	C27H29ClN2O3	3068-39-1	Basic Red 1:1
466,220276	C24H34O9	94108-97-1	Acrylic acid,2-[[2,2-bis[[(1-oxo-2-propenyl)oxy]methyl]butoxy]methyl]-2-ethyl-1,3-propanediyl ester
467,28244	C32H37NO2	42887-26-3	9,10-Anthracenedione, 1-[(4-dodecylphenyl)amino]-
467,29953	C24H41N3O6	64265-57-2	1-Aziridinepropanoic acid, 2-methyl-,2-ethyl-2-[[3-(2-methyl-1-aziridinyl)-1-oxopropoxy]methyl]-1,3-propanediyl ester
468,1640552	C23H32O6S2		Mesamoll di SO3 C11
470,1775825	C26H32O4P2		Irgafos P-EPQ hydrolysis product
470,2973512	C36H38		butadiene n=17
470,3243538	C26H46O7		Triton X-45 n=6
471,368625	C27H51O6		Acetylated glyceride (literature)
472,105286	C18H36N2S4Zn	136-23-2	Dibutyldithiocarbamic acid, zinc salt
474,2981391	C28H42O6		BADGE.PrOH.BuOH
474,407288	C31H54O3	67845-93-6	3,5-Di-tert-butyl-4-hydroxybenzoic acid, hexadecyl ester
474,4072956	C31H54O3		Irganox 1076 thermal degradation products
476,2774036	C27H40O7		BADGE H_2O BuEtOH
478,202332	C28H31ClN2O3	989-38-8	Basic Red 1
481,139008	C26H19N5O5	6985-92-8	Pigment Red 175
482,1797052	C24H34O6S2		Mesamoll di SO3 C12
486,306305	C30H44FO2P	118337-09-0	2,2'-Ethylidenebis(4,6-di-tert-butyl phenyl) fluorophosphonite
486,3262819	C30H47O3P		di(nonylphenyl)phosphite

Monoisotopic mass	Formula	CAS number	Compound name
486,392029	C28H54O6	62568-11-0	Sorbitan monobehenate
487,112793	C24H17N5O7	6471-49-4	Pigment Red 23
487,26236	C33H33N3O	6786-83-0	α,α-Bis(4-(dimethylamino)phenyl)-4-(phenylamino)naphthalene-1-methanol (Solvent Blue 4)
488,3137891	C29H44O6		BADGE 2BuOH
490,2719243	C31H38O5		BADGE tBuPh
490,2930537	C28H42O7		BADGE.EtOEtOH.PrOH
492,226166	C22H44O4Sn	68928-76-7	Dimethylbis[(1-oxoneodecyl)oxy]stannane
492,2723183	C27H40O8		BADGE 2MeEtOH
494,2435167	C27H39O6Cl		BADGE.BuEtOH.HCl
494,6963521	C12HCl10		PCB 10
496,1953553	C25H36O6S2		Mesamoll di SO3 C13
496,3130013	C38H40		butadiene n=18
499,9375	C8HO3F17S		PFOS
500,3349435	C27H48O8		Acetylated glyceride (literature)
502,3211965	C30H47O4P		di(nonylphenyl)phosphate
502,451477	C30H63O3P	25448-25-3	Triisodecyl phosphite
503,048065	C14H18N3Na5O10	140-01-2	Diethylenetriaminepentaacetic acid, pentasodium salt
504,169037	C18H32O16	9004-53-9	Dextrin
504,3087038	C29H44O7		BADGE.EtEtOH.BuOH
504,345093	C30H48O6	1528-48-9	Triheptyl benzene-1,2,4-tricarboxylate
505,522278	C34H67NO	16260-09-6	Oleyl palmitamide
506,233612	C30H35ClN2O3	2390-63-8	Basic Violet 11
508,271851	C29H42NaO4P	85209-91-2	2,2'-Methylene bis(4,6-di-tert-butylphenyl)sodium phosphate
508,282489	C31H40O6		BADGE H_2O 2tBuPh
509,30423	C33H39N3O2	2725-22-6	2,4-Bis(2,4-dimethylphenyl)-6-(2-hydroxy-4-n-octyloxyphenyl)-1,3,5-triazine
510,2110054	C26H38O6S2		Mesamoll di SO3 C14
510,367004	C28H50N2O6	2516-92-9	1-Piperidinyloxy, 4,4'-[1,10-dioxo-1,10-decanediyl)bis(oxy)]bis[2,2,6,6-tetramethyl]
512,2998704	C28H40N4O5		Nylon MXD6 n=2
513,0347	C14H9O2F16		Fluoroacrylate (8 CF entities)
513,9673	C10HO2F19		PFDA
514,330994	C32H42N4O2	68310-04-3	1,3-Benzenediol, 4-[(2,4-dimethylphenyl)azo]-2-[(4-dodecylphenyl)azo]-
514,3505686	C28H50O8		Triton X-45 n=7
514,405579	C30H58O4S	123-28-4	Thiodipropionic acid, didodecyl ester
520,3036184	C29H44O8		BADGE 2EtEtOH
521,3208263	C40H41		styrene n=5
522,3286513	C40H42		butadiene n=19
524,1715375	C22H41O4SnCl		di-n-octyltin-(ethylmaleate) chloride
524,189392	C25H32O12	60506-81-2	Dipentaerythritol pentaacrylate
524,2266554	C27H40O6S2		Mesamoll di SO3 C15
527,4464208	C35H59O3		quinone methide
527,4464208	C35H59O3		cinnammate
528,043701	C20H24CaO10S2	8061-52-7	Lignin sulphonates, calcium salt

Monoisotopic mass	Formula	CAS number	Compound name
528,2511889	C33H36O6		BADGE 2Ph
528,402588	C30H56O7	7147-34-4	Citric acid, tris(2-ethylhexyl) ester
528,6573798	C12Cl11		PCB 11
530,433533	C34H58O4	119-06-2	Phthalic acid, di-n-tridecyl ester
530,4698959	C35H62O3		Irganox 1076
530,46991	C35H62O3	2082-79-3	Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate
531,274664	C33H39O6		3-ring-BADGE
532,3400039	C31H48O7		BADGE.BuOH.BuEtOH
532,521912	C36H68O2	3687-45-4	Oleic acid, oleyl ester
536,408997	C33H60OS2	110675-26-8	2,4-Bis(dodecylthiomethyl)-6-methylphenol
536,528076	C34H68N2O2	5518-18-3	N,N'-Ethylenebispalmitamide
537,584839	C36H75NO	143925-92-2	Amines, bis(hydrogenated tallow alkyl) oxidised
538,2423055	C28H42O6S2		Mesamoll di SO3 C16
539,75708	C15H12Br4O2	79-94-7	3,3',5,5'-Tetrabromobisphenol A
540,1181	C16H21O5F13		Polyethoxylate alcohol/Fluoroalkoxylate(6;5)
542,15741	C24H44Cl6	63449-39-8	Paraffins, chlorinated
543,9732496	C10H6O4PF17		8:2 monoPAP
544,391663	C37H52O3	1843-03-4	1,1,3-Tris(2-methyl-4-hydroxy-5-tert-butylphenyl) butane
544,391663	C37H52O3	1843-03-4	1,1,3-Tris(2-methyl-4-hydroxy-5-tert-butylphenyl) butane
546,392029	C33H54O6	272460-97-6	1-(4-[(4-Benzoylphenyl)thio]phenyl)-2-methyl-2-[(4-methylphenyl)sulfonyl]-1-propan-1-one
546,392029	C33H54O6	27251-75-8	Triisooctyl benzene-1,2,4-tricarboxylate
546,392029	C33H54O6	3319-31-1	Tris(2-ethylhexyl) benzene-1,2,4-tricarboxylate
546,392029	C33H54O6	89-04-3	Trioctyl trimellitate
546,3921	C33H54O6		Tri(2-ethylhexyl)trimellitate
548,3349185	C31H48O8		BADGE.EtEtOH.BuEtOH
548,3443014	C42H44		butadiene n=20
548,422974	C37H56O3	123968-25-2	Acrylic acid, 2,4-di-tert.-pentyl-6-[1(3,5-di-tert.pentyl-2-hydroxyphenyl)ethyl]phenyl ester
549,2788266	C26H53O2SSn		di-n-octyltin-(ethylhexylthioglycolate) chloride
552,2579556	C29H14O6S2		Mesamoll di SO3 C17
552,3927	C34H52N2O4	32687-78-8	N,N'-Bis(3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionyl)hydrazide
552,51178	C35H68O4	33587-20-1	1,2-Propyleneglycol dipalmitate
554,0729457	C20H31O8SnCl		n-octyltin-bis(ethylmaleate) chloride
554,155029	C22H42O4S2Sn	69226-44-4	Di-n-octyltin ethyleneglycol bis(mercaptoacetate)
556,170288	C22H44O4S2Sn	26636-01-1	Dimethyltin bis(isooctyl mercaptoacetate)
556,170288	C22H44O4S2Sn	57583-35-4	Dimethyltin bis(ethylhexyl mercaptoacetate)
558,3767833	C30H54O9		Triton X-45 n=8
562,049683	C28H20Na2O6S2	27344-41-8	4,4'-Bis(2-sulphostyryl)biphenyl, disodium salt
563,0315	C15H9O2F18		Fluoroacrylate (9 CF entities)
564,0005	C12H5OF21		10:2 FTOH
564,156006	C32H25ClN4O4	68227-78-1	Pigment Red 147
564,51178	C36H68O4	61788-89-4	Acids, fatty, unsaturated (C18), dimers, distilled
566,256958	C38H34N2O3	34372-72-0	2'-(Dibenzylamino)-6'-(diethylamino)-3H-spiro[2-benzofuran-1,9'-xanthen]-3-one Green DCF)

Monoisotopic mass	Formula	CAS number	Compound name
566,2736056	C30H46O6S2		Mesamoll di SO3 C18
568,224304	C30H36N2O7S	26694-69-9	Xanthylium, 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethyl-, ethyl sulfate (1:1)
568,282489	C36H40O6		BADGE.BPA
568,282489	C36H40O6		Cyclo-di-BADGE
568,3036184	C33H44O8		BADGE.BuOEtOH.hydroxyPh
568,3723667	C30H52N2O8		Tinuvin 622 n=2
569,227051	C24H45CeO6	24593-34-8	2-Ethylhexanoic acid, cerium salt
570,413147	C32H58O8	144-15-0	tris(2-ethylhexyl) 2-(acetyloxy)propane-1,2,3-tricarboxylate
570,468201	C34H66O4S	16545-54-3	Thiodipropionic acid, ditetradecyl ester
570,485962	C34H66O6	26322-14-5	Dihexadecyl peroxodicarbonate
570,523193	C36H74S2	2500-88-1	Dioctadecyl disulphide
573,020874	C9H28N3O15P5	15827-60-8	Diethylenetriaminepenta(methylenephosphonic acid)
574,3599515	C44H46		butadiene n=21
575,0793926	C32H16N8Cu		Copper phthalocyanine blue
576,3662186	C33H52O8		BADGE 2BuEtOH
578,190186	C24H42O8Sn	15571-60-5	Di-n-octyltin dimaleate
578,199951	C28H34O13	29570-58-9	Acrylic acid, hexaester with dipentaerythritol
580,270996	C26H54Br2N4	61269-61-2	N,N'-Bis(2,2,6,6-tetramethyl-4-piperidyl)hexamethylenediamine-1,2-dibromoethane, copolymer
580,2892557	C31H48O6S2		Mesamoll di SO3 C19
582,185974	C24H46O4S2Sn	69226-46-6	Di-n-octyltin 1,4-butanediol bis-mercaptoacetate
584,1444	C18H25O6F13		Fluoroalkoxylate(6;6)
584,372253	C30H57NaO7S	2673-22-5	Sulphosuccinic acid, ditridecyl ester, sodium salt
584,4288439	C33H60O8		Polyadipate (literature)
585,5979	C38H80ClN	107-64-2	Dimethyldioctadecylammonium chloride
586,2930537	C36H42O7		BADGE H_2O BPA
586,3505686	C34H50O8		Irganox 245
586,350586	C34H50O8	36443-68-2	Triethyleneglycol bis[3-(3-tert-butyl-4-hydroxy -5-methylphenyl) propionate]
588,389526	C33H56N4OS2	991-84-4	2,4-Bis(octylmercapto)-6-(4-hydroxy-3,5-di-tert-butylanilino)-1,3,5-triazine
588,438965	C36H60O6	53894-23-8	tris(7-methyloctyl) benzene-1,2,4-tricarboxylate
588,559021	C38H72N2O2	110-31-6	N,N'-Ethylenebisoleamide
589,61615	C40H79NO	10094-45-8	Octadecylerucamide
589,9951279	C12H9O4PF18		4:2 diPAP
592,035034	C21H17ClN8O7S2	6539-67-9	Reactive Yellow 3
592,5906797	C38H76N2O2		Bis(stearoyl)ethylenediamide
592,590698	C38H76N2O2	110-30-5	N,N'-Ethylenebisstearamide
593,574707	C38H75NO3	14351-40-7	Stearic acid, 2-stearamidoethyl ester
594,3049057	C32H50O6S2		Mesamoll di SO3 C20
594,3192685	C35H46O8		BADGE.H2O.diisoPrPh
594,440002	C37H58N2O4	69851-61-2	Benzenepropanamide,N,N'-1,3-propanediylbis[3,5-bis(1,1-dimethylethyl)-4-hydroxy-
599,9311	C10HO3F21S		PFDS
600,3756015	C46H48		butadiene n=22
600,5566942	C35H68N8		Chimassorb 944 part b

Monoisotopic mass	Formula	CAS number	Compound name
602,4029981	C32H58O10		Triton X-45 n=9
602,454651	C37H62O6	84864-66-4	1,2-Didecyl 4-octyl benzene-1,2,4-tricarboxylate
602,454651	C37H62O6	67989-23-5	1,4-didecyl 2-octyl benzene-1,2,4-tricarboxylate
604,308289	C33H50O6P2	26741-53-7	Bis(2,4-di-tert-butylphenyl)pentaerythritol diphosphite
604,41864	C32H60O10	9005-64-5	Polyethyleneglycol sorbitan monolaurate
604,41864	C32H60O10	9005-65-6	Polyethyleneglycol sorbitan monooleate
604,543091	C39H72O4	105-62-4	1,2-Propyleneglycol dioleate
605,317017	C39H44ClN3O	73309-46-3	Basic Blue 81
608,3205558	C33H52O6S2		Mesamoll di SO3 C21
608,371304	C37H52O7		BADGE BuEtOH *t*BuPh
608,574341	C39H76O4	6182-11-2	1,2-Propyleneglycol distearate
610,1365198	C28H34O9S3		Mesamoll tri SO3 C10
613,0283	C16H9O2F20		Fluoroacrylate (10 CF entities)
618,717896	C45H30O3	227099-60-7	1,3,5-Tris(4-benzoylphenyl) benzene
620,537964	C39H72O5	25637-84-7	Glycerol dioleate
622,256653	C38H38O8	7328-97-4	Ethane, 1,1,2,2-tetrakis[p-(2,3-epoxypropoxy)phenyl]-
624,036011	C26H18N4Na2O8S2	3051-11-4	Brilliant yellow
624,1521699	C29H36O9S3		Mesamoll tri SO3 C11
624,3087038	C39H44O7		BADGE (n=1) (di-BADGE)
624,569275	C39H76O5	1323-83-7	Glycerol distearate
624,5692756	C39H76O5		glycerol distearate
625,3834266	C48H49		styrene n=6
626,3912516	C48H50		butadiene n=23
628,1706	C20H29O7F13		Fluoroalkoxylate(6;7)
629,9512124	C11H4O4PF21		10:2 monoPAP
630,179077	C40H26N2O6	83524-75-8	Pigment Black 32
630,485962	C39H66O6	4130-35-2	1,2,4-Benzenetricarboxylic acid, tris(decyl) ester
632,2371185	C28H48O8Sn		Di-n-octyltin-bis(ethylmaleate)
632,339539	C35H54O6P2	80693-00-1	Bis(2,6-di-tert-butyl-4-methylphenyl)pentaerythritol diphosphite
632,38269	C32H64O4Sn	77-58-7	Dibutyltin dilaurate
636,486633	C40H64N2O4	23128-74-7	1,6-Hexamethylene-bis(3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionamide)
637,23999	C40H35N3O3S	6417-46-5	2-Methyl-4-{[4-({4-[(3-methylphenyl)amino]phenyl}{4-[(3-methylphenyl)imino]-2,5-cyclohexadien-1-ylidene}methyl)phenyl]amino}benzenesulfonic acid (Pigment Blue 56)
637,7999222	C22H6N4O2Cl8		Yellow 110
638,1678199	C30H38O9S3		Mesamoll tri SO3 C12
638,454651	C40H62O6	35074-77-2	1,6-Hexamethylene-bis(3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate)
638,548523	C39H74O6	538-24-9	Glycerol trilaurate
640,1118	C18H21O5F17		Fluoroalkoxylate(8;5)
640,3763894	C41H52O6		BADGE 2tBuPh
642,395386	C38H58O6S	41484-35-9	Thiodiethanol bis(3-(3,5-di-tert-butyl-4-hydroxy phenyl) propionate)
643,9668624	C12H6O4PF21		10:2 monoPAP
646,4292128	C34H62O11		Triton X-45 n=10
646,451477	C42H63O3P	31570-04-4	Phosphorous acid, tris(2,4-di-tert-butylphenyl) ester

Monoisotopic mass	Formula	CAS number	Compound name
646,4514824	C42H63O3P		Irgafos 168
652,18347	C31H40O9S3		Mesamoll tri SO3 C13
652,4069017	C50H52		butadiene n=24
654,487305	C42H62N4O2	65087-00-5	1,3-Benzenediol, 2,4-bis[(4-dodecylphenyl)azo]-
658,3340832	C40H52O4P2		Irgafos P-EPQ hydrolysis product
658,399536	C41H50N6O2	103597-45-1	2,2'-Methylenebis[6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol]
660,2853815	C39H45O7Cl		Di-BADGE HCl
662,1385267	C26H38O12Sn		n-octyltin-tris(ethylmaleate)
662,446397	C42H63O4P		oxidized Irgafos 168
663,0251	C17H9O2F22		Fluoroacrylate (11 CF entities)
664,564209	C41H76O6	25151-96-6	Pentaerythritol dioleate
664,658997	C41H84N4O2	35674-65-8	1,3-Bis(3-octadecylureido)propane
665,304993	C33H47NO13	7681-93-8	Natamycin
666,19912	C32H42O9S3		Mesamoll tri SO3 C14
668,408997	C44H52N4O2	6706-82-7	(3E,3'''E)-3,3'-{1,1-Cyclohexanediylbis[(2-methyl-4,1-phenylene)(1E)-2-hydrazinyl-1-ylidene]}bis[1,1'-bi(cyclohexane)-1,5-dien-4-one] (Solvent Yellow 29)
672,1968	C22H33O8F13		Fluoroalkoxylate(6;8)
672,583862	C39H80N2O4S	72749-55-4	Imidazolium compounds, 2-(C17- and C17-unsaturated alkyl)-1-[2-(C18- and C18- unsaturated amido)ethyl]-4,5-dihydro-1-methyl-, methylsulfates
674,2252293	C28H55O4S2SnCl		n-octyltin-bis(ethylhexylthioglycolate) chloride
677,499573	C36H72NO8P	8002-43-5	Lecithin
678,200745	C28H38O19	126-14-7	Sucrose octaacetate
678,4225517	C52H54		butadiene n=25
680,2147701	C33H44O9S3		Mesamoll tri SO3 C15
684,138	C20H25O6F17		Fluoroalkoxylate(8;6)
684,3662186	C42H52O8		BADGE (n=1) PrOH
684,414978	C36H68O4Sn	1912-84-1	Oleic acid, tin(Il) salt
686,3818687	C42H54O8		BADGE.BPA.BuEtOH
688,445984	C36H72O4Sn	6994-59-8	Tin distearate
688,498413	C45H69O3P	26523-78-4 and 1333-21-7	Tris(mono and dinonylphenyl) phosphite
688,4984326	C45H69O3P		TNPP
689,572327	C39H80NO6P	63438-80-2	(2-Carbobutoxyethyl)tin-tris(isooctyl mercaptoacetate)
689,9887407	C14H9O4PF22		4:2/6:2 diPAP
692,559021	C42H76O7	29116-98-1	Sorbitan dioleate
694,2304202	C34H46O9S3		Mesamoll tri SO3 C16
694,307617	C34H56CaO8P2	65140-91-2	3,5-Di-tert-butyl-4-hydroxybenzylphosphonic acid, monoethyl ester, calcium salt
694,969116	C26H16N3Na3O10S3	3861-73-2	Solvent Blue 37
696,2620593	C39H46O7Cl2		Di-BADGE 2HCl
696,590393	C42H80O7	29589-99-9	Distearyl citrate
698,3818687	C43H54O8		BADGE (n=1) BuOH
699,424744	C42H57N3O6	40601-76-1	1,3,5-Tris(4-tert-butyl-3-hydroxy-2,6-dimethylbenzyl)-1,3,5-triazine-2,4,6(1H,3H,5H)-trione

Monoisotopic mass	Formula	CAS number	Compound name
700,3611333	C42H52O9		BADGE (n=1) MeEtOH
701,608887	C42H84ClNO4	67846-68-8	Di(hydrogenated tallow fatty acids-2-hydroxyethyl ester)dimethyl ammonium chloride
702,3767833	C42H54O9		BADGE (n=1) H_2O PrOH
704,4382018	C54H56		butadiene n=26
704,4933472	C45H69O4P		tris(nonylphenyl)phosphate
706,471069	C35H69Cl3N8	71878-19-8	Poly[6-[(1,1,3,3-tetramethylbutyl)amino] -1,3,5-triazine-2,4-diyl]-[(2,2,6,6-tetramethyl-4-piperidyl)-imino]hexamethylene[(2,2,6,6-tetramethyl-4-piperidyl)imino]
708,2460702	C35H18O9S3		Mesamoll tri SO3 C17
713,0219	C18H9O2F24		Fluoroacrylate (12 CF entities)
714,3767833	C43H54O9		BADGE (n=1) EtEtOH
714,558716	C48H74O4	36388-36-0	1,2-Benzenedicarboxylic acid, 1,2-bis[[tetradecahydro-1,4a-dimethyl-7-(1-methylethyl)-1-phenanthrenyl]methyl] ester
716,223	C24H37O9F13		Fluoroalkoxylate(6;9)
716,3924334	C43H56O9		BADGE (n=1) H_2O BuOH
718,3505686	C45H50O8		BADGE (n=1) Ph
718,371698	C42H54O10		BADGE (n=1) H_2O MeEtOH
722,2617203	C36H50O9S3		Mesamoll tri SO3 C18
728,1642	C22H29O7F17		Fluoroalkoxylate(8;7)
729,4460268	C56H57		styrene n=7
730,4538519	C56H58		butadiene n=27
732,387348	C43H56O10		BADGE (n=1) H_2O EtEtOH
736,2773704	C37H52O9S3		Mesamoll tri SO3 C19
736,3611333	C45H52O9		BADGE (n=1) H_2O Ph
742,4080835	C45H58O9		BADGE (n=1) BuEtOH
744,257385	C31H60O6S3Sn	57583-34-3	Monomethyltin tris(ethylhexyl mercaptoacetate)
744,257385	C31H60O6S3Sn	54849-38-6	Monomethyltin tris(isooctyl mercaptoacetate)
744,4237335	C45H60O9		BADGE (n=1) 2PrOH
744,507996	C40H80O4Sn	3648-18-8	Di-n-octyltin dilaurate
750,202026	C48H26N6O4	3049-71-6	Pigment Red 178
750,2930204	C38H54O9S3		Mesamoll tri SO3 C20
752,3894022	C36H72O4S2Sn		Di-n-octyltin-bis(ethylhexylthioglycolate)
752,389404	C36H72O4S2Sn	26401-97-8	Di-n-octyltin bis(isooctyl mercaptoacetate)
752,389404	C36H72O4S2Sn	15571-58-1	Di-n-octyltin bis(2-ethylhexyl mercaptoacetate)
756,4695019	C58H60		butadiene n=28
759,4445233	C42H59N6O7		Nylon MXD6 n=3
760,1316	C20H25O6F21		Fluoroalkoxylate(10;5)
760,2492	C26H41O10F13		Fluoroalkoxylate(6;10)
760,4186481	C45H60O10		BADGE (n=1) H_2O BuEtOH
764,18103	C37H38N2Na2O9S2	72243-90-4	Acid Violet 48
764,3086705	C39H56O9S3		Mesamoll tri SO3 C21
770,168945	C34H30N10O8S2	7342-13-4	4,4’-bis[[4-Methoxy-6-anilino-s-triazine-2-yl]amino]-2,2’-stilbenedisulphonic acid
770,168945	C34H30N10O8S2	3426-43-5	4,4'-Bis[(4-anilino-6-methoxy-s-triazin-2-yl)amino]-2,2'-stilbenedisulphonic acid, disodium salt

Monoisotopic mass	Formula	CAS number	Compound name
770,5180529	C42H74O12		Polyadipate (literature)
772,1904	C24H33O8F17		Fluoroalkoxylate(8;8)
772,4550337	C47H64O9		BADGE (n=1) 2BuOH
774,4131688	C49H58O8		BADGE (n=1) tBuPh
774,595093	C54H78O3	1709-70-2	1,3,5-Trimethyl-2,4,6-tris(3,5-di-tert-butyl-4-hydroxybenzyl)benzene
776,4135628	C45H60O11		BADGE (n=1) 2MeEtOH
778,1246345	C35H38O12S4		Mesamoll tetra SO3 C10
782,485152	C60H62		butadiene n=29
783,518616	C48H69N3O6	27676-62-6	1,3,5-Tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,5H)-trione
784,306458	C34H64O8S2Sn	63397-60-4	Bis(2-carbobutoxyethyl)tin-bis(isooctyl mercaptoacetate)
789,9823535	C16H9O4PF26		6:2 diPAP
792,1402845	C36H40O12S4		Mesamoll tetra SO3 C11
792,4237335	C49H60O9		BADGE (n=1) H_2O 2tBuPh
794,475769	C50H66O8	32509-66-3	Ethyleneglycol bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate]
796,3975188	C51H56O8		BADGE.2BPA
800,407715	C54H56O6	68937-90-6	Acids, fatty (C18 unsaturated), trimers
800,424988	C40H72O8Sn	10039-33-5	Di-n-octyltin bis(2-ethylhexyl maleate)
802,440552	C40H74O8Sn	1185-73-5	Di-n-octyltin bis(ethyl maleate)
802,440552	C40H74O8Sn	33568-99-9	Di-n-octyltin bis(isooctyl maleate)
804,1578	C22H29O7F21		Fluoroalkoxylate(10;6)
804,2754	C28H45O11F13		Fluoroalkoxylate(6;11)
804,4448629	C47H64O11		BADGE (n=1) 2EtEtOH
804,712463	C51H97O4P	3135-18-0	3,5-Di-tert-butyl-4-hydroxybenzylphosphonic acid, dioctadecyl ester
806,1559346	C37H42O12S4		Mesamoll tetra SO3 C12
806,451111	C36H70O19	9004-64-2	Hydroxypropyl cellulose
808,5008021	C62H64		butadiene n=30
810,485962	C54H66O6	57569-40-1	Terephthalic acid, diester with 2,2'-methylenebis(4-methyl-6-tert-butylphenol)
812,3924334	C51H56O9		BADGE (n=1) 2Ph
815,4159085	C51H59O9		5-ring-BADGE
816,4812484	C49H68O10		BADGE (n=1).BuEtOH.BuOH
820,028015	C31H23N6Na3O11S3	67969-87-3	Trisodium 7-[(E)-{4-[({2-methoxy-4-[(E)-(3-sulfonatophenyl)diazenyl]phenyl}carbamoyl)amino]-2-methylphenyl}diazenyl]-1,3-naphthalenedisulfonate (Direct Yellow 118)
820,1715847	C38H44O12S4		Mesamoll tetra SO3 C13
822,504395	C48H66N6O6	88122-99-0	Tris(2-ethylhexyl)-4,4',4"-(1,3,5-triazine-2,4,6-triyltriimino)tribenzoate
833,5086271	C64H65		styrene n=8
834,1872347	C39H46O12S4		Mesamoll tetra SO3 C14
834,5164521	C64H66		butadiene n=31
836,2428	C26H41O10F17		Fluoroalkoxylate(8;9)
842,366943	C38H74O6S3Sn	27107-89-7	Mono-n-octyltin tris(2-ethylhexyl mercaptoacetate)
842,366943	C38H74O6S3Sn	26401-86-5	Mono-n-octyltin tris(isooctyl mercaptoacetate)
842,3669522	C38H74O6S3Sn		n-octyltin-tris(ethylhexylthioglycolate)
846,38855	C40H62O19	126-13-6	Sucrose acetate isobutyrate

Monoisotopic mass	Formula	CAS number	Compound name
846,4905838	C54H72O4P2		Irgafos P-EPQ hydrolysis product
848,184	C24H33O8F21		Fluoroalkoxylate(10;7)
848,2028848	C40H48O12S4		Mesamoll tetra SO3 C15
848,3016	C30H49O12F13		Fluoroalkoxylate(6;12)
851,3925862	C51H60O9Cl		5-ring-BADGE HCl
851,550725	C45H77N3O12		Tinuvin 622 n=3
852,37085	C53H58O6P2	154862-43-8	Bis(2,4-dicumylphenyl)pentaerythritol-diphosphite
852,4237335	C54H60O9		BADGE(n=1).BPA
860,5074632	C51H72O11		BADGE (n=1) 2BuEtOH
860,5321022	C66H68		butadiene n=32
862,2185349	C41H50O12S4		Mesamoll tetra SO3 C16
864,514587	C44H88O4S2Sn	84030-61-5	Di-n-dodecyltin bis(isooctyl mercaptoacetate)
872,2967852	C41H60O12S4		Mesamoll tetra SO3 C21
876,2341849	C42H22O12S4		Mesamoll tetra SO3 C17
878,757446	C54H102O8	54140-20-4	Sorbitan tripalmitate
879,929993	C23H47Cl5Na2O2S2Sn2	68442-12-6	Reaction products of oleic acid, 2-mercaptoethyl ester, with dichlorodimethyltin, sodium sulphide and trichloromethyltin
880,1514	C22H29O7F25		Fluoroalkoxylate(12;5)
880,269	C28H45O11F17		Fluoroalkoxylate(8;10)
884,78302	C57H104O6	122-32-7	Glycerol trioleate
886,5477522	C68H70		butadiene n=33
887,369264	C51H61O9Cl2		5-ring-BADGE 2HCl
889,9759663	C18H9O4PF30		6:2/8:2 diPAP
890,249835	C43H54O12S4		Mesamoll tetra SO3 C18
890,8302413	C57H110O6		glycerol tristearate
892,3279	C32H53O13F13		Fluoroalkoxylate(6;13)
894,574524	C48H86CaO12	5793-94-2	Stearoyl-2-lactylic acid, calcium salt
898,429565	C42H82O6S3Sn	67649-65-4	Mono-n-dodecyltin tris(isooctyl mercaptoacetate)
904,2654851	C44H56O12S4		Mesamoll tetra SO3 C19
908,4499483	C57H64O10		BADGE (n=2) (tri-BADGE)
912,2364	C26H41O10F21		Fluoroalkoxylate(10;8)
912,5634023	C70H72		butadiene n=34
916,274475	C40H44N12O10S2	4404-43-7	4,4'-Bis[[4-anilino-6-[bis(2-hydroxyethyl)amino]-s-triazin-2-yl]amino]-2,2'-stilbene-disulphonic acid
918,2811351	C45H58O12S4		Mesamoll tetra SO3 C20
918,7160244	C55H98O10		Epoxidised linseed oil
918,7887954	C57H106O8		Epoxidised soy bean oil
924,1776	C24H33O8F25		Fluoroalkoxylate(12;6)
924,21698	C40H38N12Na2O8S2	16090-02-1	4,4'-Bis[(4-anilino-6-morpholino-s-triazin-2-yl)amino]-2,2'-stilbenedisulphonic acid, disodium salt
924,2953	C30H49O12F17		Fluoroalkoxylate(8;11)
924,5176339	C59H72O9		BADGE (n=1) 2tBuPh
925,4526879	C57H65O11		Tri-BADGE H_20
936,3541	C34H57O14F13		Fluoroalkoxylate(6;14)

Monoisotopic mass	Formula	CAS number	Compound name
937,5712273	C72H73		styrene n=9
938,5790524	C72H74		butadiene n=35
938,815002	C57H110O9	139-44-6	Glycerol tris(12-hydroxystearate)
941,207825	C32H44N11NaO15S3	85154-06-9	4-[[4-[Bis(2-hydroxyethyl)amino]-6-methoxy-s-triazin-2-yl]amino]-4'-[[4-methoxy-6-[(2-sulphoethyl)amino]-s-triazin-2-yl]amino]-2,2'-stilbenedisulphonic acid, sodium salt, compound with diethanolamine
943,418801	C57H64O10Cl		Tri-BADGE HCl
946,7473245	C57H102O10		Epoxidised soy bean oil
946,7473245	C57H102O10		Epoxidised linseed oil
948,872131	C60H116O7	7775-50-0	Trioctadecyl 2-hydroxypropane-1,2,3-tricarboxylate
949,178223	C12Br10O	1163-19-5	Ether, bis(pentabromophenyl)
955,987	C35H24N6Na4O13S4	3214-47-9	Tetrasodium 3,3'-{carbonylbis[imino(2-methyl-4,1-phenylene)-2,1-diazenediyl]}di(1,5-naphthalenedisulfonate) (Direct Yellow 50)
956,2627	C28H45O11F21		Fluoroalkoxylate(10;9)
956,804443	C60H108O8	26266-58-0	Sorbitan trioleate
960,237976	C40H42N12Na2O10S2	4193-55-9	4,4'-Bis[[4-anilino-6-[bis(2-hydroxyethyl)amino]-s-triazin-2-yl]amino]-2,2'-stilbene-disulphonic acid, disodium salt
960,7265891	C57H100O11		Epoxidised soy bean oil
960,7265891	C57H100O11		Epoxidised linseed oil
961,215027	C14H4Br10	84852-53-9	1,2-Bis(2,3,4,5,6-pentabromophenyl)ethane
964,5947024	C74H76		butadiene n=36
968,3215	C32H53O13F17		Fluoroalkoxylate(8;12)
968,5074632	C60H72O11		BADGE(n=2).PrOH
970,5231132	C60H74O11		BADGE(n=1).BPA.BuEtOH
972,316956	C36H60O30	10016-20-3	α-Dextrin
974,7058536	C57H98O12		Epoxidised soy bean oil
974,7058536	C57H98O12		Epoxidised linseed oil
976,21228	C40H42KN12NaO10S2	70942-01-7	4,4'-Bis[[4-anilino-6-[bis(2-hydroxyethyl)amino]-s-triazin-2-yl]amino]-2,2'-stilbene-disulphonic acid, potassium sodium salt
979,3954787	C57H65O10Cl2		Tri-BADGE 2HCl
980,3803	C36H61O15F13		Fluoroalkoxylate(6;15)
980,862	C60H116O9	26658-19-5	Sorbitan tristearate
988,135986	C36H36N12O14S4	47910-88-3	4,4’-bis[[4-[bis(2-Hydroxyethyl)amino]-6-(m-sulphoanilino)-s-triazine-2-yl]amino]-2,2’-stilbenedisulphonic acid
988,2301	C26H41O10F25		Fluoroalkoxylate(12;7)
988,6851182	C57H96O13		Epoxidised soy bean oil
989,696533	C54H105CeO6	10119-53-6	Stearic acid, cerium salt
989,9695791	C20H9O4PF34		8:2 diPAP
990,6103525	C76H78		butadiene n=37
992,185974	C40H42K2N12O10S2	71230-67-6	Dipotassium 4,4'-bis[6-anilino-4-[bis(2-hydroxyethyl)amino-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate
992,9445918	C59H116N12		Chimassorb 944 n=1
1000,2889	C30H49O12F21		Fluoroalkoxylate(10;10)
1006,589176	C56H78N8O9		Nylon MXD6 n=4

Monoisotopic mass	Formula	CAS number	Compound name
1012,3477	C34H57O14F17		Fluoroalkoxylate(8;13)
1014,26001	C42H44N14Na2O10S2	27344-06-5	4,4'-Bis[[4-anilino-6-[(2-carbamoylethyl)-(2-hydroxyethyl)amino]-s-triazin-2-yl]amino]- 2,2'-stilbenedisulphonic acid, disodium salt
1014,548419	C58H85AlO9P2	151841-65-5	Aluminium hydroxybis [2,2'-methylenebis (4,6- di-tert.butylphenyl)] phosphate
1016,626003	C78H80		butadiene n=38
1016,643647	C57H92O15		Epoxidised linseed oil
1024,4065	C38H65O16F13		Fluoroalkoxylate(6;16)
1025,911011	C32H20Cl2N10Na4O12S4	37138-26-4	4,4'-Bis[[4-chloro-6-(4-sulphoanilino)-s-triazin-2-yl]amino]-2,2'-stilbenedisulphonic acid, tetrasodium salt
1032,2563	C28H45O11F25		Fluoroalkoxylate(12;8)
1034,647084	C68H92O4P2		Irgafos P-EPQ
1034,647095	C68H92O4P2	119345-01-6	Reaction product of di-tert-butylphosphonite with biphenyl, obtained by condensation of 2,4-di-tert-butylphenol with Friedel Craft reaction product of phosphorus trichloride and biphenyl
1034,647095	C68H92O4P2	38613-77-3	Tetrakis(2,4-di-tert-butyl-phenyl)-4,4'-biphenylylene diphosphonite
1041,633828	C80H81		styrene n=10
1044,3151	C32H53O13F21		Fluoroalkoxylate(10;11)
1050,641999	C68H92O5P2		Irgafos P-EPQ oxidation product
1056,3739	C36H61O15F17		Fluoroalkoxylate(8;14)
1059,018066	C69H134O6	18641-57-1	Glycerol tribehenate
1060,347168	C69H48N4O8	178671-58-4	Pentaerythritol tetrakis (2-cyano-3,3-diphenylacrylate)
1066,046753	C39H30N10Na4O13S4	50925-42-3	Direct Yellow 86
1066,636914	C68H92O6P2		Irgafos P-EPQ oxidation product
1068,4327	C40H69O17F13		Fluoroalkoxylate(6;17)
1076,18811	C40H44N12O16S4	5131-70-4	4,4'-Bis[[4-[bis(2-hydroxyethyl)amino]-6-(m-sulphoanilino)-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonic acid
1076,2825	C30H49O12F25		Fluoroalkoxylate(12;9)
1088,3413	C34H57O14F21		Fluoroalkoxylate(10;12)
1089,963192	C22H9O4PF38		8:2/10:2 diPAP
1100,4001	C38H65O16F17		Fluoroalkoxylate(8;15)
1112,4589	C42H73O18F13		Fluoroalkoxylate(6;18)
1120,3087	C32H53O13F25		Fluoroalkoxylate(12;10)
1132,3675	C36H61O15F21		Fluoroalkoxylate(10;13)
1134,369751	C42H70O35	7585-39-9	β-Dextrin
1134,729083	C60H102N4O16		Tinuvin 622 n=4
1144,4263	C40H69O17F17		Fluoroalkoxylate(8;16)
1145,696428	C88H89		styrene n=11
1156,4851	C44H77O19F13		Fluoroalkoxylate(6;19)
1161,925505	C22H5O4PF42		10:1 diPAP
1164,115967	C40H40N12Na4O16S4	16470-24-9	4,4'-Bis[[4-[bis(2-hydroxyethyl)amino]-6-(p-sulphoanilino)-s-triazin-2-yl]amino]-2,2'- stilbenedisulphonic acid, tetrasodium salt
1164,3349	C34H57O14F25		Fluoroalkoxylate(12;11)
1176,3937	C38H65O16F21		Fluoroalkoxylate(10;14)
1176,784058	C73H108O12	6683-19-8	Pentaerytritol tetrakis(3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate)

Monoisotopic mass	Formula	CAS number	Compound name
1176,784079	C73H108O12		Irganox 1010
1182,116943	C42H38N14Na4O14S4	37515-76-7	Tetrasodium 4,4'-bis[[4-[(2-cyanoethyl)(2-hydroxyethyl)amino]-6-[(4-sulphonatophenyl)amino]-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate
1185,925505	C24H5O4PF42		10:2 diPAP
1188,4525	C42H73O18F17		Fluoroalkoxylate(8;17)
1200,5114	C46H81O20F13		Fluoroalkoxylate(6;20)
1201,116943	C77H148O8	115-83-3	Pentaerythritol tetrastearate
1208,3611	C36H61O15F25		Fluoroalkoxylate(10;12)
1220,178467	C44H48N12Na4O16S4	67786-25-8	Benzenesulfonic acid *2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxypropyl)amino]-6-[(4-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-, tetrasodium salt
1220,4199	C40H69O17F21		Fluoroalkoxylate(10;15)
1221,004272	C24H22F34N3O8PS2	30381-98-7	Bis[2-[N-ethyl(perfluorooctane)sulphonamido]ethyl] phosphate, ammonium salt
1229,112061	C78H148O9	61752-68-9	Sorbitan tetrastearate
1232,4788	C44H77O19F17		Fluoroalkoxylate(8;18)
1239,043823	C78H144O6P2	13003-12-8	4,4'-Butylidene-bis(6-tert-butyl-3-methylphenyl-ditridecyl phosphite)
1252,3873	C38H65O16F25		Fluoroalkoxylate(12;13)
1253,733829	C70H97N10O11		Nylon MXD6 n=5
1258,251343	C64H50CaN8O14S2	43035-18-3	Pigment Red 247
1260,692505	C56H108O30	9004-65-3	Methylhydroxypropylcellulose
1264,4462	C42H73O18F21		Fluoroalkoxylate(10;16)
1276,505	C46H81O20F17		Fluoroalkoxylate(8;19)
1296,4136	C40H69O17F25		Fluoroalkoxylate(12;14)
1304,013794	C40H38N12Na6O18S6	41098-56-0	4,4'-Bis[[4-diethylamino-6-(2,5-disulphoanilino)-s-triazin-2-yl]amino]-2,2'-stilbene-disulphonic acid, hexasodium salt
1307,960693	C40H28N12Na8O20S4	174305-36-3	N,N'-Ethylidenebis[(3-sulpho-4,1-phenylene)imino(6-[(4-sulphophenyl)amino]-s-triazin-4,2-diyl]]bis[N-(carboxymethyl)glycine], octasodium salt
1308,4724	C44H77O19F21		Fluoroalkoxylate(10;17)
1320,5312	C48H85O21F17		Fluoroalkoxylate(8;20)
1331,97229	C40H34N12Na6O20S6	52301-70-9	4,4'-Bis[[4-morpholino-6-(2,5-disulphoanilino)-s-triazin--2-yl]amino]-2,2'-stilbene-disulphonic acid, hexasodium salt
1340,4398	C42H73O18F25		Fluoroalkoxylate(12;15)
1352,4986	C46H81O20F21		Fluoroalkoxylate(10;18)
1367,993042	C40H38N12Na6O22S6	68971-49-3	4,4'-Bis[[4-[bis(2-hydroxyethyl)amino]-6-(2,5-disulphoanilino)-s-triazin-2-yl]amino]-2,2'-stilbenedisulphonic acid, hexasodium salt
1384,466	C44H77O19F25		Fluoroalkoxylate(12;16)
1396,5248	C48H85O21F21		Fluoroalkoxylate(10;19)
1417,907442	C75H127N5O20		Tinuvin 622 n=5
1424,5561	C50H89O21F21		Fluoroalkoxylate(10;20)
1428,4922	C46H81O20F25		Fluoroalkoxylate(12;17)
1463,911499	C90H132NO9P3	80410-33-9	2,2',2""-Nitrilo[triethyl tris(3,3',5,5'-tetra-tert-butyl-1,1'-bi-phenyl-2,2'-diyl)phosphite]
1472,5184	C48H85O21F25		Fluoroalkoxylate(10;18)
1500,878482	C84H116N12O13		Nylon MXD6 n=6

Monoisotopic mass	Formula	CAS number	Compound name
1514,056641	C52H44N14Na6O20S6	68134-04-3	Hexasodium 2,2'-[vinylenebis[(3-sulphonato-4,1-phenylene)imino[6-[(3-amino-3-oxopropyl)(phenylmethyl)amino]-1,3,5-triazine-4,2-diyl]imino]]bis(benzene-1,4-disulphonate)
1516,5446	C50H89O22F25		Fluoroalkoxylate(12;19)
1576,5658	C52H93O24F25		Fluoroalkoxylate(12;20)
1591,485636	C94H182N20		Chimassorb 944 n=2

9.5

Appendix C

List of compounds in the targeted analysis for selected dioxins, polychlorinated biphenyls (PCBs) and brominated flame retardants (BFRs) in fractions with positive toxicological effect in aryl hydrocarbon receptor (AhR) assay

Appendix C.

Compound	Formula	Monoisotopic mass	Compound type
Bis(pentabromophenyl) ether	C12Br10O	949,1782	BFR
3,3',5,5'-Tetrabromobisphenol A	C15H12Br4O2	539,7571	BFR
4,5,6,7-Tetrabromo-1,3-isobenzofurandione	C8Br4O3	459,6581	BFR
Hexabromobenzene	C6Br6	545,5100	BFR
Pentabromotoluene	C7H3Br5	481,6151	BFR
Decabromobiphenyl	C12Br10	933,1833	BFR
2,2',4,4',5,5'-Hexabromobiphenyl	C12H4Br6	621,5413	BFR
3,3',4,4'-Tetrabromobiphenyl	C12H6Br4	465,7203	BFR
3,3',4,4',5,5'-Hexabromobiphenyl	C12H4Br6	621,5413	BFR
2,3,7,8-Tetrachlorodibenzo-p-dioxin	C12H4Cl4O2	319,8965	Dioxin
3,3',4,4'-Tetrachlorobiphenyl	C12H6Cl4	289,9224	PCB
3,3',4,4',5,5'-Hexachlorobiphenyl	C12H4Cl6	360,8780	PCB
2,3,7,8-Tetrabromooxanthrene	C12H4Br4O2	495,0694	PCB
2,3,7,8-Tetrabromodibenzo(b,d]furan	C12H4Br4O	479,6995	Dioxin
1,2,3,7,8-pentabromodibenzofuran	C12H3Br5O	557,6100	Dioxin
PCB 77 og 81	C12H6Cl4	289,9224	PCB
PCB 77 og 81	C12H6Cl4	289,9224	PCB
TCDF	C12H4Cl4O2	319,8965	Dioxin
TCDF	C12H4Cl4O2	319,8965	Dioxin
TCDD	C12H4Cl4O2	319,8965	Dioxin
TCDD	C12H4Cl4O2	319,8965	Dioxin
PCB 126	C12H5Cl5	323,8834	PCB
PCB 126	C12H5Cl5	323,8834	PCB
PeCDF	C12H3Cl5O	337,8627	Dioxin
PeCDF	C12H3Cl5O	337,8627	Dioxin
PeCDD	C12H3Cl5O2	353,8576	Dioxin
PCB 169	C12H4Cl6	357,8444	PCB
PeCDD	C12H3Cl5O2	353,8576	Dioxin
PCB 169	C12H4Cl6	357,8444	PCB
HxCDF	C12H2Cl6O	371,8237	Dioxin
HxCDF	C12H2Cl6O	371,8237	Dioxin
HxCDD	C12H2Cl6O2	387,8186	Dioxin
HxCDD	C12H2Cl6O2	387,8186	Dioxin
HpCDF	C12HCl7O	405,7847	Dioxin
HpCDF	C12HCl7O	405,7847	Dioxin
HpCDD	C12HCl7O2	421,7796	Dioxin
HpCDD	C12HCl7O2	421,7796	Dioxin
OCDF	C12Cl8O	439,7457	Dioxin
OCDF	C12Cl8O	439,7457	Dioxin
OCDD	C12Cl8O2	455,7407	Dioxin

Compound	Formula	Monoisotopic mass	Compound type
OCDD	C12Cl8O2	455,7407	Dioxin
PCB 28	C12H7Cl3	255,9613	PCB
PCB 28	C12H7Cl3	255,9613	PCB
PCB 52	C12H6Cl4	289,9224	PCB
PCB 52	C12H6Cl4	289,9224	PCB
PCB 101	C12H5Cl5	323,8834	PCB
PCB 105	C12H5Cl5	323,8834	PCB
PCB 114	C12H5Cl5	323,8834	PCB
PCB 118	C12H5Cl5	323,8834	PCB
PCB 123	C12H5Cl5	323,8834	PCB
PCB 138	C12H4Cl6	357,8444	PCB
PCB 153	C12H4Cl6	357,8444	PCB
PCB 156	C12H4Cl6	357,8444	PCB
PCB 157	C12H4Cl6	357,8444	PCB
PCB 167	C12H4Cl6	357,8444	PCB
PCB 170	C12H3Cl7	391,8054	PCB
PCB 180	C12H3Cl8	391,8054	PCB
PCB 189	C12H3Cl9	391,8054	PCB

9.6

Paper 3

Rosenmai, A.K., Bengtström, L., van Vugt-Lussenburg, B.M.A., Trier, X., Pedersen, J.H., Granby, K., Taxvig, C., and Vinggaard, A.M. (2014). A strategy to identify problematic chemicals in food contact materials of paper and board. Manuscript in preparation

Comments to Paper 3

At present only data from AR, ER, and AhR reporter gene assays are included in the paper. Six other assays, namely nrf2, p53, RAR, GR CALUX reporter gene assays as well as PPARa/y reporter gene assays and data on cytotoxicity will be part of the final paper. There are still additional experiments conducted to verify some of the findings described in the paper. These include,

- **Cell viability tests**: In some cases we observed increased responses in the cell viability plates in the ER reporter gene assay. We are in the process of conducting additional experiments to shed light on this issue, which will be included in the final paper.

In this paper there are references to Supplementary Materials. This is Appendix D in this thesis.

A strategy to identify problematic chemicals in food contact materials of paper and board

Anna Kjerstine Rosenmai[a], Linda Bengtström[b], Camilla Taxvig[a], Xenia Trier[b], Jens Højslev Petersen[b], Kit Granby[b] & Anne Marie Vinggaard[a]

[a]*Division of Toxicology & Risk Assessment, National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark*

[b]*Division of Food Chemistry, Toxicology & Risk Assessment, National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark*

Corresponding Author

Anna Kjerstine Rosenmai

akjro@food.dtu.dk

Division of Toxicology & Risk Assessment
National Food Institute
Technical University of Denmark
Mørkhøj Bygade 19
DK-2860 Søborg
Denmark

Phone + 45 35 88 76 00

Running Title

Bioassay guided analysis of extracts from food contact materials

Abstract

Food contact materials (FCMs) constitute a source of human exposure to chemicals. Lists of compounds intended for use in these materials exist, but limited knowledge is available on the potential toxicological effects of many of the compounds allowing for appropriate risk assessment. In this study, we aimed at developing a strategy which allows for screening of FCMs and identification of potentially problematic compounds in these materials by means of applying analytical chemistry tools as well as bioassay guided analysis in combination.

A step-by-step approach was developed in which extracts from FCMs were tested *in vitro*, active extracts underwent fractionation, fractions were tested *in vitro*, and tentative identification of compounds was conducted in active fractions. Selected tentatively identified compounds were tested individually *in vitro* and quantified in the extracts. The battery of *in vitro* assays covered endpoints related to endocrine disruption, oxidative stress, cytotoxiticy and genotoxicity.

All 20 extracts led to effects on aryl hydrocarbon receptor activity, whereas only a subset of extracts led to effects on estrogen receptor (ER) and androgen receptor (AR) activity. Two extracts were selected for further investigation by use of the step-by-step strategy, one extract from a pizza box and one from a sandwich wrapper. By doing so, we successfully identified di-butyl phthalate, butyl benzyl phthalate, and bisphenol A (BPA) as causing ER activity in the pizza box of which BPA mainly caused the effect. In the sandwich wrapper extract, which caused AR antagonism, we identified two causative agents, dehydroabietic acid (DHAA) and abietic acid, of which the latter was present at high concentrations and thus mainly caused the effect in the extract. This is to our knowledge the first reporting of the antiandrogenicity of DHAA.

Collectively these data suggest that applying the strategy is a useful tool to assess potential hazards posed by chemicals in FCMs of paper and board, as well as identifying causative agents.

Keywords

Biodirected analysis, bioassay guided analysis, food contact material, paper, board, food

Introduction

Assuring food safety is a challenge in modern society as the potential sources of food contamination are many, one of which is compounds migrating from food contact materials (FCM) (Borchers et al. 2010; Grob et al. 2006). We already know of several compounds, which are used in FCMs and which have the potential to cause adverse effects, such as bisphenol A (BPA) and phthalates. BPA is used in applications such as polycarbonate plastic bottles, as a monomer used for epoxy resin coatings in cans for foods and drink (EFSA 2006), and have been found in recycled paper (Geens et al. 2011; Vinggaard et al. 2000), whereas phthalates are used in applications such as PVC tubing, food-packaging films, and have been measured in paper and board (Cao 2010). BPA and some phthalates are known endocrine disruptors, and have shown adverse effects *in vivo* (Christiansen et al. 2014; Foster 2006; Miyawaki et al. 2007).

Besides these known compounds there are multiple other compounds used in FCMs. Compiled lists of food contact substances include up to 8000 compounds (Neltner et al., 2013, Geueke et al., 2014), some of which are intended for FCMs. These lists do not differentiate between compounds actually being used and compounds which are not (Geueke et al., 2014), and the majority are not sufficiently examined for toxicological effects (Neltner et al., 2013). Though no specific EU regulation exists on the use of chemicals in FCMs of paper and board, there is a frame work directive, which states that compounds used in FCMs may not migrate into foods in amounts which can adversely affect human health (Regulation No 1935/2004). Based on the aforementioned data, it becomes apparent that a strategy is needed to obtain information on usage and potential effects of compounds in FCMs.

The vast numbers of compounds, for which little is known on usage and adverse effects, pose challenges as testing all the compounds would be extremely time consuming. Thus, several

previous studies have investigated effects of mixtures of compounds originating from FCM of paper and board by applying a bioassay guided analysis approach. In these studies extracts from FCMs of paper and board were tested *in vitro* assessing endpoint such as genotoxicity, cell toxicity, and endocrine disruption (Binderup et al. 2002; Lopez-Espinosa et al. 2007; Ozaki et al. 2004; Ozaki et al. 2005; Vinggaard et al. 2000; Weber et al. 2006). On several occasions compounds were identified by use of these strategies, which could explain the observed effects either fully or partly. These strategies can be applied to enable a future prioritization of studies to be conducted on the identified compounds.

In this study we wanted to develop a test strategy to obtain information on both the potential use and effects of compounds present in FCM of paper and board by applying both analytical chemistry tools and bioassay guided analysis. A broad battery of *in vitro* assays was used to examine effects of compounds present in the materials including endpoints associated with endocrine disruption, oxidative stress, and genotoxicity. Extracts from 20 FCMs were tested in these assays and two samples underwent further investigation by testing fractions of the extract. Finally the compounds that most likely were responsible for the effect in question were identified. Fractions were produced by LC based fractionation in two separate rounds, one with acidic and one with alkaline conditions. Tentative identification of compounds in fractions exhibiting *in vitro* activity was conducted by using hyphenated techniques (time-of-flight mass spectrometers (qTOF) coupled to either gas or liquid chromatography (GC or LC)). Finally identified compounds which led to activity *in vitro* were quantified by use of GC-qTOF and LC-MS/MS.

Materials and Methods

The step-by-step procedure in the FCM strategy is shown in Figure 1. Raw extracts from 20 FCMs were tested in vitro and two FCMs were selected for further investigation. Fractions were produced

by liquid chromatography (LC) based fractionation in two separate rounds, one with acidic and one with alkaline conditions and these were tested in vitro. Identification of compounds in fractions exhibiting *in vitro* activity was conducted by using hyphenated techniques (time-of-flight mass spectrometers (qTOF) coupled to either gas or liquid chromatography (GC or LC)). Finally identified compounds which led to activity *in vitro* were quantified by use of GC-qTOF and LC-MS/MS.

Test compounds and chemicals

Ethanol (99.9%) used for the extraction and re-dissolving of evaporated extracts was purchased from Merck (Darmstadt, Germany). Methanol (99.9%) used for mobile phases for HPLC fractionation was purchased from Rathburn (Walkerburn, Scotland). All aqueous solutions were prepared using ultrapure water obtained from a Millipore Milli-Q Gradient A10 system (Millipore, Bedford, MA, USA). HPLC MS grade formic acid and a water solution of 25 % ammonium hydroxide were obtained from Sigma-Aldrich (St. Loiuse, MO, USA). HPLC grade acetonitrile was obtained from Merck (Darmstadt, Germany). Standards for the UPLC-qTOF method; bisphenol A (BPA), methylparaben, perfluorooctanoic acid (PFOA), bisphenol A diglycidyl ether (BADGE) as well as deuterated BPA (d_{16}-BPA) used for the quantitative determination of bisphenol were obtained from Sigma-Aldrich. Di-butyl phthalate (DBP), deuterated di-butyl phthalate (d_4-DBP), butyl-benzyl phthalate (BBP), di-isobutyl phthalate (DiBP) were used for quantitative determination in fractions and extracts. Abietic acid (AA), dehydroabietic acid (DHAA), isorhamnetin and rhamnetin (Sigma-Aldrich) as well as 4-oxoretinoic acid (Santa Cruz Biotechnology, TX, USA) were used for quantification in extracts and fractions thereof.

Paper and board samples

In total 20 different paper and board samples were applied in the test strategy. See Table 1 for paper and board characteristics.

Production of extracts and fractions

The paper and board sample extracts and fractions were produced as described in Bengtström et al (2014a). Selected samples with a positive toxicological response in the initial screening phase were fractionated (indicated in Table 1 and 3 with the marking *). New extracts were produced before fractionation. The fractionation was performed in two separate rounds, one during alkaline conditions and one during acidic conditions.

Identification of compounds in fractions of extracts by HRMS

The extracts and corresponding fractions exhibiting *in vitro* activity were analysed by high resolution mass spectrometry (HRMS) techniques, GC-qTOF and LC-qTOF (Agilent Technologies, Santa Clara, CA, USA). The methods for the identification process are presented in full detail in Bengtström et al. (in preparation). In brief, the highly concentrated extracts and fractions were diluted 1:100 *v/v* prior to identification. Cut-offs were based on the threshold of toxicological concern for substances with suspected genotoxic (Cramer Class III) or endocrine disruptive effects (EFSA Scientific committee, 2012) for both identification methods. Peaks with areas below this threshold were not further investigated.

In GC-qTOF, the extracts and fractions were ionized by electron ionization (EI) at 70 eV. Only peaks above the area corresponding to one tenth d_4-DBP, to compensate for differences in detector response, of the response of the internal standard, were analysed by the GC-qTOF. Analysis was performed in the Agilent MassHunter Qualitative software with the NIST library v.1.1.

Data obtained by the UPLC-qTOF analysis was analysed by using MassHunter Qualitative software as well as ProGenesis QI software (Nonlinear Dynamics Limited, UK). Only peaks with area above one per cent of that corresponding to BADGE for positive mode and PFOA for negative mode were considered. Differences in detector response and ion suppression were compensated for by this cut-off. Compounds were identified by using a customized library containing approximately 2300 matrix specific entries (Bengtström et al., in preparation) as well as the ChemSpider and PubMed database.

Quantitative determination of phthalates by GC-qTOF

Phthalates were quantified by the accredited method described in Petersen & Jensen (2010). All quantified phthalates showed good linearity (R^2>0.98, not weighted, not forced through 0). The phthalate standards used for an external calibration curve were run using the same parameters and settings as described in Bengtström et al. (in preparation). Extracts were diluted 1:1000 *v/v* with ethanol prior to analysis. The data was analysed by the Agilent MassHunter Quantitative software.

Quantitative determination by LC-MS/MS

All extracts were diluted 1:1000 *v/v* with ethanol before analysis. Quantitation of bisphenol A (BPA) was based on an accredited method, described in Table 2. The eight-point calibration curve (0, 7.5, 15, 30, 75, 150, 225 and 300 ng ml^{-1}) consisted of BPA standard in methanol and water (75:25, *v/v*). Internal standard, d_{16}-BPA at 150 ng mL^{-1}, was added to both calibration curve and samples. The mass transition reactions used for BPA quantification were *m/z* 227.2>212.1 as quantifier, *m/z* 227.2>133.1 as qualifier and *m/z* 241.2>223.1 for d_{16}-BPA.

A seven point calibration curve (0, 10, 20, 50, 100, 200 and 500 ng mL^{-1}) with a standard mixture of the five compounds recognized as the most likely to cause the positive response in AR assay in

ethanol was analysed according to Table 2. Masses used for quantification of AA was *m/z* 301.5>167.7 as quantifier and *m/z* 301.5>138.8 as qualifier; and for DHAA *m/z* 299.5>157 as quantifier and *m/z* 299.5>137 as qualifier. The mass transition reactions used for 4-oxoretinoic acid quantification were *m/z* 313.2>254.2 as quantifier, *m/z* 313.2>163.1 as qualifier. For isorhamnetin *m/*z 315>300 and *m/*z 315>151 was used as quantifier and qualifier respectively; and for rhamnetin *m/*z 315>300 and *m/*z 315>165 was used as quantifier and qualifier.

The calibration curves for all methods were obtained by plotting the peak area versus concentrations. All compounds quantified showed good linearity (R^2>0.98, not weighted, not forced through 0) in their respective method and investigated range. Data from both quantifications were analysed by the Waters QuanLynx (v 4.1) software.

Reporter gene assays for testing of extracts, fractions, and identified compounds

Nine reporter gene assays were applied to test the extracts from FCMs. These assays included the androgen receptor (AR), estrogen receptor (ER), aryl hydrocarbon receptor (AhR), and peroxisome proliferator-activated receptors (PPARα/γ) reporter gene assays. Furthermore,the glucocorticoid receptor (GR), retinoic acid receptor (RAR), nuclear factor (erythroid-derived 2)-like 2 (nrf2), and p53 CALUX reporter gene assays were applied. The experimental procedures and materials used for these assays have been described previously (Piersma et al. 2013; Rosenmai et al. 2014; Taxvig et al. 2012; Vinggaard et al. 2002). The AR reporter gene assay was conducted in both agonist and antagonist mode (0.1 nM R1881 added) on extracts. Extracts were tested in a maximum concentration of 0.25-1 % of extract provided in 2-3.3 fold dilutions. The vehicle concentrations were constant except in the nrf2, p53, RAR and GR CALUX reporter gene assays, in which the vehicle was diluted accordingly. The experiments were repeated 1-3 times. Data from the nrf2, p53, RAR, and GR CALUX as well as PPARα/γ reporter gene assays are currently not included in this

paper, but will be included before submission of the manuscript. Data from the Comet assay and Ames test obtained from testing the extracts will be published elsewhere.

All fractions of extract S4 and S8 were tested in the AR and ER reporter gene assay, respectively, in one concentration of 0.25 % of fraction provided. 1-2 experiments were conducted in 2-4 replicates.

Tentatively identified compounds were tested in the ER and AR reporter gene assays. Compounds included DHAA, AA, rhamnetin, isorhamnetin, and 4-oxo-retinoic acid in the AR reporter gene assay and BPA, DiBP, BBP, and DBP in the ER reporter gene assay.

All *in vitro* experiments were conducted with a positive control, which was 17β-estradiol in the ER reporter gene assay and R1881 or hydroxyflutamide (OHF) in the AR reporter gene assay in the agonism or antagonism mode, respectively.

In vitro data processing and calculation of equivalence factors

For extracts and tentatively identified compounds, statistical analysis was conducted on individual experiments in which data was normalized to vehicle controls. Residuals to means within each exposure group was tested for whether means of these were statistically different (ANOVA) and successively pooled and tested for normal distribution (D'agostino Pearson's Omnibus test). Data for which pooled residuals were normally distributed were analyzed by a one-way ANOVA (post-test Dunnett) and all other data were analyzed by the Kruskal Wallis test (post-test Dunn). Exposure groups, in which cell toxicity was observed was removed before statistical analysis was conducted. Data obtained from testing fractions underwent no further data processing.

The overall criteria for reporting *in vitro* effects for extracts and selected identified compounds were that means between exposure groups should exhibit statistically significant differences, the effects should be dose-dependent, and should be reflected in the majority of conducted experiments.

In antagonist mode the lowest observable effect concentrations (LOECs) were reported as the concentration in which a ≥ 25% decrease was observed, which was reflected in higher concentrations, and in agonist mode LOECs were reported if ≥ 50% increase was observed which was reflected in higher exposure concentrations. The greatest change in response in non-cytotoxic concentrations in the ER, AR, and AhR reporter gene assays are reported as the tentative maximum efficacy (E_{max}).

Data from extracts selected for further testing, identified compounds in extracts and fractions, and positive controls, in the respective assay were fitted to a four parameter sigmoidal curve fit in which the upper and lower limits were fixed at 1 and E_{max}. Based on these curve fits calculated hill slope and EC_{50} values were obtained, which was used for determination of estrogen equivalence factors (EEQs) and androgen equivalence factors (AEQs) for both extracts (EQ_{meas}) and for identified compounds in extracts (EQ_{calc}). The following equations were used to calculate EQ factors,

$$response = bottom * \frac{top-bottom}{1+10^{(\log(EC_{50})-concentration)*hillslope}} \quad (1)$$

$$concentration = log(EC_{50}) - \frac{log\left(\frac{top-response}{response-bottom}\right)}{hillslope} \quad (2)$$

Here top and bottom represent the highest and lowest y-values, respectively, and Hill slope and EC_{50} values are estimated from the curve fit.

The identified compounds were quantified in the extract and based on the parameters obtained from testing these individually; the estimated response in the extract was calculated by using equation (1). The concentration of the identified compound inserted were those leading to E_{max} in the extract. Successively this calculated response was inserted in equation 2, in which all the parameters inserted were based on the positive control. Now the identified compounds are converted into EQ

factors of the positive control. The EQs for individual compounds were summarized to obtain the EQ_{calc}. For the extract the EQ_{meas} was calculated by inserting the E_{max} for the extract into equation (2) with all the parameters in the equation being based on the positive control.

Results

In vitro effects of extracts

An overview of data obtained for the extracts in the AR, ER, and AhR reporter gene assay are given in Table 3 with the determined LOECs (dm^2/mL) and tentative efficacies (E_{max}) (%). Concentration-response relationships for extracts S4 and S8 that were further investigated for determination of causative agents are shown in Figure 2.

Several of the extracts led to AR agonism (S2, S3, S4, S11, S16, and S18) and antagonism (S2, S3, S4, S5, S13, and S18). Notably, extract S4, which was further examined by fractionation and identification of causative agents both led to agonism and antagonism in the AR reporter gene assay. This dual modality on AR activity was also observed for extract S2, S3, and S18. S4 was selected for further examination as it showed dual modality, with a marked effect on agonism and a 50% decrease in response in the antagonist mode with a LOEC of $2.3*10^{-2}$ (dm^2/mL). Twelve extracts did not affect AR activity.

Nine of the extracts led to increased ER activity with tentative E_{max} values ranging from 63-245% increase compared to the control and tentative LOECs ranging from $1.3*10^{-3}$-$5.9*10^{-2}$ (dm^2/mL). Extract S8 was chosen for further investigation as it showed high potency and materials were available for fractionation. 11 extracts did not increase ER activity. Indications of cytotoxicity were observed in some cases at higher extract concentrations.

All extracts caused increased AhR activity in the reporter gene assay of which S8 and S14 exhibited marked potency and efficacy in the range of 2.3-4.8 dm^2_{paper}/mL and more than 1000% increase in response. Extract S2-S4 were among the less active samples leading to approximately 100% increase in response and LOECs of around $2.1*10^{-1}$-$6.3*10^{-2}$ dm^2/mL.

In vitro effects of fractions

Data from the ER and AR reporter gene assays for the fractions, both alkaline and acidic, are shown in Figure 2. The fractions selected to undergo further identifications were those giving the greatest change in response in the respective assay. Fraction 8 of extract S4 showed a marked decrease in response in both the acidic and alkaline mode and was chosen for further identification. Acidic fraction 9 and 10 also led to a decreasing trend compared to the remaining fractions, but were not further tested. Fractions of extract S8 led to a marked increase in acidic fraction 6 and alkaline fraction 7, and these were chosen for further investigation. Several other acidic fractions led to increased responses, but were not further tested.

Identification of compounds in selected fractions and extracts

A comprehensive list of all peaks and compounds tentatively identified for fractions with positive response in the ER or AR reporter gene assays is submitted as Supplementary Materials xxx. The selection of compounds for further investigation *in vitro* was based on an expert judgement including information on previously reported effects, read-across, and commercial availability of tentatively identified compounds.

In sample S8, three compounds, bisphenol A (BPA), di-butyl phthalate (DBP), di-isobutyl phthalate (DIBP), present in both the acidic and alkaline fraction with positive ER response, were chosen for further investigation. Moreover, butyl-benzyl phthalate (BBP), found in the acidic fraction with ER response, was also selected for additional testing. All of these compounds were also present in the

extract. For the fractions of sample S4 with positive AR response, five compounds, dehydroabietic acid (DHAA), abietic acid (AA), isorhamnetin, rhamnetin and 4-oxoretinoic acid, were selected for further toxicological analysis.

In vitro testing of identified compounds

Of the four selected compounds tested for ER activity, BPA, BBP, and DBP led to increased activity, whereas DiBP did not lead to any effect in this assay. Among the five selected compounds tested for AR antagonism, four caused marked effects, namely isorhamnetin, 4-oxo-retinoic acid, abietid acid and dehydroabietic acid. Marked cytotoxicity accompanied the decreased response for rhamnetin and thus we could not establish whether this compound had antagonistic effects on AR activity. Results for identified compounds affecting the ER and AR activity are shown in Figure 2.

Quantification of identified compounds

In order to confirm the identity and to quantify the compounds present in the extracts, standards of the selected tentatively identified compounds were analysed by either GC-qTOF or LC-MS/MS. Relative retention times and fragmentation patterns for both BPA and the phthalate standards, analysed by GC-qTOF, confirmed the initial identification as correct, according to criteria set by 2002/657/EC (2000), whereas only two of five identified compounds in the AR active extract, DHAA and AA, had matching relative retention times between standards and fraction (>0.2 min) when analysed by LC-MS/MS. Moreover, DHAA and AA were the only compounds out of the five tested that had an entry in the customized database. Concentrations for quantified compounds causing effects are given in Table 4.

Calculated and measured equivalence factors

The EEQ calculated based on identified compounds were higher than that measured, EEQ_{calc} = $1.42*10^{-5}$µM versus $2.23*10^{-6}$ µM. The same was evident for AEQ values based on identified compounds in the AR reporter gene assay, AEQ_{calc} = $1.49*10^{-1}$ µM versus $8.84*10^{-2}$µM. The calculated and measured EQ are shown in Table 4.

Discussion

In this study we aimed at developing a strategy to identify potentially problematic compounds in FCMs of paper and board by applying a step-by-step approach, as illustrated in Figure 1. 20 FCMs were investigated of which several caused effects in the *in vitro* assays, Table 3. The full strategy (Figure 1) was applied on two extracts, S4 and S8, which were active in the AR and ER reporter gene assays, respectively. Applying the work-flow to these extracts illustrated that the strategy could be used to identify causative agents present in the extracts, as we successively identified DHAA, AA, BPA, DBP, and BBP, which could more than explain the responses of the extracts.

The food contact material strategy

The strategy involved several steps including, 1) preparation of extracts from FCMs of paper and board, 2) testing of extracts *in vitro*, 3) fractionation of selected active extract, 4) testing of fractions in the assay in which the extract led to effect, 5) tentative identification of compounds in active fractions, 6) evaluation to narrow down the list of identified compounds, 7) *in vitro* testing of final list of identified compounds, 8) verification of presence of substances in the extract as well as quantification of active identified compounds, and 9) evaluation of contribution to effect in extract of identified compounds by calculating EQ factors.

In vitro effects of extracts

315 The extracts tested in the three *in vitro* assays showed activity to a varying degree depending on the
316 assay. All extracts led to effects in the AhR reporter gene assay, whereas in the ER and AR reporter
317 gene assays effects were only observed for some extracts.

318 Several of the paper and board extracts induced ER activity, which is in accordance with findings of
319 estrogenicity of kitchen rolls reported previously (Vinggaard et al., 2000). Kitchen rolls made from
320 recycled materials in particular led to effects, which was suggested primarily to be caused by the
321 occurrence of BPA in these materials (Vinggaard et al., 2000). In the present study eight of 14
322 extracts fully or partly based on recycled materials caused effects and one extract out of six made of
323 virgin paper caused effect. The results suggest that estrogenic potential is prevalent in recycled
324 compared to virgin paper, though recycled paper in itself is not a clear marker of estrogenicity, as
325 six recycled FCMs did not lead to effects.

326 The AhR activity was induced by all the extracts tested suggesting that common compounds are
327 present in the extracts leading to effects, or that many different compounds present in FCMs have
328 the ability to activate the receptor. Four extracts were previously examined for ability to activate the
329 AhR, which all led to effects to varying degrees (Binderup et al., 2002), supporting the findings of
330 the current study. Binderup et al. (2002) suggested mono-ortho PCBs as contributing to the effects
331 to a minor degree. However, other well-known AhR activators were not examined, such as dioxins,
332 non ortho-PCBs and PAHs (Binderup et al., 2002). We are in the process of investigating the
333 extracts further to shed light on potential reasons for the activities observed (Bengtström et al.,
334 Manuscript in preparation).

335 *Identification of causative agents*

336 The list of tentatively identified compounds in active fractions underwent an expert evaluation. This
337 evaluation was based on previously reported effects, known biophores for the effect in question,

read-across, and commercial availability of compounds, leading to a final candidate list. This process was successful as most of the final candidate compounds led to effects in the assays and were present in the extract. Of the nine preliminary identified compounds on the final list, seven caused effects in the respective assay, namely DBP, BBP, BPA, DHAA, AA, isorhamnetin, and 4-oxo-retinoic acid, of which five were also identified and quantified in the extracts.

The tentative identification was conducted by use of high resolution mass spectrometry (HRMS), qTOF, which is necessary when examining a complex matrix as paper and board. HRMS has high sensitivity when scanning broad mass ranges as well as high mass resolution. The advantages of this is two-fold: 1) broad ranges of compounds can be identified, which is essential, as we wanted as many compounds as possible to be identified and 2) high mass resolution allows for accurate identification, causing a higher degree of certainty in the tentative identification, ultimately leading to a reduced list of candidate compounds.

We further applied two chromatographic separation methods, GC and LC. This enabled analysis of a broader spectrum of compounds, compared to using only one of the methods, as GC-MS separates small, non-polar and thermostable volatiles, whereas LC-MS separates larger, more polar, thermo-labile compounds. The four compounds selected for further investigation from fractions positive in ER were identified by GC-qTOF, whereas the five compounds selected for further investigation from AR positive fractions were tentatively identified by LC-qTOF. These data illustrate that by using these two methods in combination, we obtain more knowledge on compounds present in the extracts, as none of the AR active compounds were identified by GC-qTOF.

Utilizing GC-qTOF in combination with EI ionization allowed for identification by use of a vast commercially available mass spectral library. In contrast, no such library is available for identification using LC-qTOF, which is a major disadvantage, and thus we build a customized

361 database for this purpose (Bengtström et al., in preparation). Only DHAA and AA had matches in
362 the customized database, whereas the other AR active compounds did not, and further these were
363 not confirmed in the extract when compared to standards. This illustrates that using a customized
364 database enhances the likelihood for correct identification, as DHAA and AA was found in both the
365 database and in the extracts, which is in-line with that previously reported (Kind and Fiehn 2007).

366 *Equivalence factors*

367 In the AR reporter gene assay four of the five preliminary identified compounds in extract S4,
368 namely DHAA, AA, isorhamnetin, and 4-oxo-retinoic acid, all inhibited AR activity, whereas no
369 AR antagonism at non-cytotoxic concentrations of rhamnetin could be detected. Only the identity
370 of DHAA and AA were confirmed in the extract. The sum of the calculated AEQ values for these
371 two compounds was higher than that measured in the extract, suggesting that these compounds can
372 explain the response observed for the extract.

373 In the ER reporter gene assays the sum of the calculated EEQ factors for identified compounds were
374 higher than the EEQ value measured for the extract, suggesting that we have identified the causative
375 agents. The somewhat higher EEQ_{calc} compared to the EEQ_{meas} could be caused by other
376 compounds being present in the extract, which inhibit the ability of BPA, DBP, and BBP to activate
377 the ER. Furthermore, an assumption for the calculated EEQ is that dose-addition occurs in the
378 extract, however if this is not the case, this could add to the observed differences in EEQ factors.
379 Finally, the lesser EEQ measured in the extract could also be caused by cytotoxicity. The
380 experiments were conducted for all extracts, fractions, and identified compounds before evaluation
381 of results and only during data processing did we observe a consistent cytotoxicity at concentration
382 in which effects were observed. It was decided to evaluate the data nevertheless, to see if it was

possible to identify causative agents irrespectively. Overall, we were successful in identifying well-known EDs in the extract of the pizza box made from recycled fibres.

Identified causative agents

BPA, DBP, and BBP were identified as ER active compounds in the pizza box (S8). The origin of BPA could be due to the presence of recycled materials in FCMs as suggested by Vinggaard et al. (2000). As BPA is used in thermal paper (Liao and Kannan 2011; Mendum et al. 2011), this may be a source of contamination of recycled paper. Phthalates are used in inks, lacquers, and adhesives, and are considered general environmental contaminants and therefore occur in recycled paper and board (Fasano et al. 2012; Pocas et al. 2010; Suciu et al. 2013). Human sources of exposure to these three compounds are many and diet is believed to contribute significantly (EFSA 2013). All three compounds are routinely detected in most – if not all - in human urine samples (Calafat et al. 2008).

The ability of BPA, DBP, and BBP to exhibit estrogenicity *in vitro* has been reported previously (Ghisari and Bonefeld-Jorgensen 2009; Gould et al. 1998; Grignard et al. 2012; Kitamura et al. 2005; Krishnan et al. 1993; Mankidy et al. 2013; Paris et al. 2002; Shen et al. 2009; Zhang et al. 2011) and thus it was not surprising that these compounds exhibited activity in the ER reporter gene assay. In our study the potency of BPA is greater than BBP and DBP and thus despite the higher concentrations of DBP in the extract, it is BPA that drives the effect, which is illustrated by the higher EEQ value for BPA, than that of the phthalates.

Besides the *in vitro* estrogenicity of BPA, this compound has led to low-dose effects *in vivo* such as disturbed mammary gland development (Moral et al. 2008), behavioural changes (Xu et al. 2010), as well as decreased anogenital distance (Christiansen et al. 2014). Whereas phthalates have led to effects such as malformations of external genitalia, cryptorchidism, and changes in nipple retention

and anogenital distance (Foster 2006). Based on these findings it is of concern that these compounds are present in FCMs of paper and board.

DHAA and AA are resin acids present in different types of resins, and thus the compounds may very well be present in paper and board materials, as they are naturally occurring constituents of wood (Roberts 1996; Sjöström and Alen 1998). During the pulping process, DHAA and AA can be removed, but they can also be present in the final paper product (Roberts 1996). Besides the natural occurrence in wood, these compounds are also used in the paper making process (Leach and Pierce 1993; Roberts 1996). In the BIOSAFEPAPER project and a study by Ozaki et al. (2006), DHAA and AA were identified in several of the FCMs investigated (Ozaki et al. 2006; Weber et al. 2006). In the latter study the compounds were detected in 15 out of 20 FCMs of paper and board and were shown capable of migrating into food simulants (Ozaki et al. 2006). Furthermore, DHAA and AA were detected in foods packed in FCM of paper (Mitani et al. 2007), suggesting that human exposure may occur through food consumption.

In the AR reporter gene assay DHAA and AA have similar dose-response relationships, Figure 2, however as AA is present in higher amounts, this compound drives the effect in the extract. No data is available on the ability of DHAA to exert AR antagonism. However AA has been found to exhibit antiandrogenic potential on AR activity in two *in vitro* assays (Rostkowski et al. 2011) in accordance with our findings. Furthermore, AA has shown the ability to inhibit 5α-reductase activity (Roh et al. 2010), which is responsible for converting testosterone into its more potent form, dihydrotestosterone. This mechanism-of-action is different from that examined in the present study, AR activity, however these two mechanisms might lead to a greater overall antiandrogenic potential of AA.

Besides these specific antiandrogenic effects, studies in fish have reported antiestrogenic effects based on decreased plasma vitellogenin levels with DHAA exposure (Christianson-Heiska et al. 2008; Orrego et al. 2010). Further, masculinization in female mosquitofish was observed, when exposed to effluents from a pulp and paper mill, which contained DHAA and AA amongst many other identified compounds (Ellis et al. 2003). These data suggest androgenic potential of these compounds, however since other compounds was present in the effluent, it cannot be excluded that these might overrule a potential antiandrogenic effect of DHAA and AA. Overall, these studies imply that DHAA and AA have the potential to interfere with hormone systems, which could be a concern if human exposure occurs.

In the present study, we have illustrated that biodirected analysis is a valuable tool for examining FCMs of paper and board for presence of potentially problematic compounds. In the strategy several *in vitro* assays were included in which several of the extracts caused effects. This highlights that the 'contamination level' of FCMs may be high and that we need to focus more on this potential source of human exposure to chemicals. However, it is noteworthy to mention that rejecting FCMs as potentially problematic based on these three assays solely is unadvised, as they only represent a subset of endpoints that could be investigated. By applying the strategy, we successfully identified three compounds with estrogenic potential as well as two compounds with antiandrogenic potential, which are present in the selected FCMs of paper and board. We recommend further studies to be conducted applying this strategy on FCMs of paper and board in order to improve an refine the strategy further. Large numbers of FCMs still remain to be tested in this set-up and many more could contain potentially problematic compounds.

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Supplementary materials

Supplementary data analyzable

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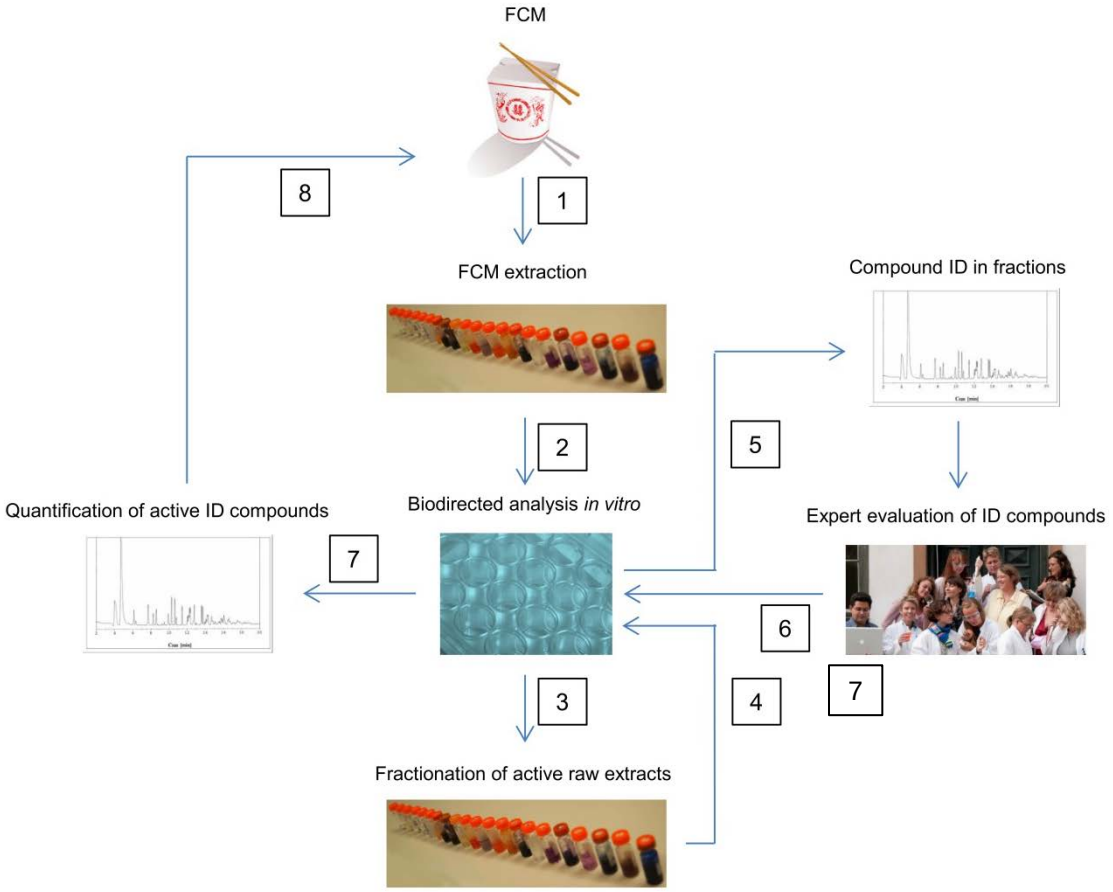


Figure 1: Work-flow for the top-down approach applied to food contact materials of unknown composition. Numbers indicate the step-by-step progression from extraction from the food contact material to evaluation of active components of this. Abbreviations: ID = identification, FCM = food contact material.

Table 1: Sample overview of the tested FCMs of paper and board with indication of usage or intended use, material type, suppliers, pulp type, whether the materials were printed and the mass volumes.

Sample no.	Usage	Material	Supplier	Pulp type	Printing	Grammage (g/m^2)
S2	Plain paper	Paper	Paper industry	Virgin pulp	No	45
S3	Baking paper	Paper	Retail	Virgin pulp	No	45
S4	Sandwich wrapper*	Paper	Retail	Virgin pulp	No	40
S5	Baking paper	Paper	Retail	Virgin pulp	No	40
S6	Baking mold	Paper	Retail	Virgin pulp	Yes	40
S7	Fluor bag[a]	Paper	Retail	Virgin pulp	Yes	80
S8	Pizza box*	Corrugated fibreboard	Retail	Recycled	Yes	550
S9	Susceptor for microwave popcorn	Paperboard	Printing industry	Virgin and recycled	Yes	190
S10	Sausage tray	Paperboard	Printing industry	Virgin and recycled	Yes	270
S11	Microwave pizza tray	Paperboard	Printing industry	Virgin and recycled	Yes	475
S12	Frozen fish box	Paperboard	Printing industry	Virgin and recycled	Yes	400
S13	Cake tray	Paperboard	Printing industry	Virgin and recycled	Yes	420
S14	Tomato punnet	Paperboard	Printing industry	Virgin and recycled	Yes	400
S16	TEPP Chinese Zineth	Paperboard	Printing industry	Recycled	Yes	310
S17	Microwave popcorn bag	Paper	Popcorn vendor	Recycled	Yes	90
S18	TEPP Chinese Spark	Paperboard	Printing industry	Recycled	Yes	330
S19	Paperboard with UV print	Paperboard	Printing industry	Recycled	Yes	280
S20	Paperboard with water soluble print	Paperboard	Printing industry	Recycled	Yes	230
S21	Paperboard with offset print	Paperboard	Printing industry	Recycled	Yes	280
S22	Cereal box[a]	Paperboard	Retail	Recycled	Yes	380

[a]Contained food at purchase. *Samples for further fractionation.

Table xx. Methods used for quantification of a) bisphenol A and b) for compounds found in AR positive fractions.

a)

LC conditions			
Instrument	Waters Acquity UPLC System (Waters, Milford, USA)		
Column	Kinetex C18 (2.6 µm, 2.1x100 mm) (Waters)		
Column temperature	40°C		
Injection volume	3 µL		
Mobile phase	A: water		
	B: methanol		
Flow rate	0.3 mL min^{-1}		
Gradient	Time (min)	%A	%B
	0	80	20
	1	55	45
	4.5	20	80
	5.5	2	98
	13	2	98
Post time	2 min		
Total run time	15		

MS conditions	
Instrument	Micromass Quattro Ultima (Waters, Milford, USA) in MRM mode
Ionization mode	ESI -
Voltage	- 3 kV
Source temperature	120°C
Cone voltage	55 V
Cone gas flow	100 L h^{-1}
Desolvation temperature	500°C
Desolvation gas flow	775 L h^{-1}

b)

LC conditions			
Instrument	Agilent 1200 Series HPLC (Agilent Technologies, CA, US)		
Column	Gemini C18 (3µm 2 x 200 mm) (Phenomenex, CA, USA)		
Column temperature	40°C		
Injection volume	3 µL		
Mobile phase	A: water + 0.01 formic acid		
	B: methanol		
Flow rate	0.2 mL min^{-1}		
Gradient	Time (min)	%A	%B
	0	10	90
Total run time	10 min		

MS conditions	
Instrument	Micromass Quattro Ultima (Waters, Milford, USA) in MRM mode
Ionization mode	ESI-
Voltage	-2.5 kV
Source temperature	120°C
Cone voltage	20 V
Cone gas flow	135 L h^{-1}
Desolvation temperature	380°C
Desolvation gas flow	575 L h^{-1}

Table 3: Tentative lowest observable effect concentrations (LOECs) (dm^2/mL) and tentative efficacies (E_{max}) (%) for the 20 paper and board extracts examined in the AR reporter gene assay in agonist and antagonist mode, the ER reporter gene assay and the AhR reporter gene assay.

Sample no.	Usage	AR Agonism		AR Antagonism		AhR agonism		ER agonism	
		LOEC	E_{max}	LOEC	E_{max}	LOEC	E_{max}	LOEC	E_{max}
S2	Plain paper	$8.0*10^{-3}$	290	$2.2*10^{-1}$	45	$2.2*10^{-1}$	115	-	-
S3	Baking paper	$6.3*10^{-2}$	73	$2.3*10^{-3}$	65	$6.3*10^{-2}$	73	-	-
S4	Sandwich wrapper*	$7.8*10^{-3}$	532	$2.3*10^{-2}$	52	$2.1*10^{-1}$	81	-	-
S5	Baking paper	-	-	$2.5*10^{-2}$	28	$7.4*10^{-2}$	224	-	-
S6	Baking mold	-	-	-	-	$2.0*10^{-2}$	174	-	-
S7	Fluor bag[a]	-	-	-	-	$6.5*10^{-3}$	568	$5.9*10^{-2}$	82
S8	Pizza box*	-	-	-	-	$4.8*10^{-5}$	1040	$2.6*10^{-3}$	103
S9	Susceptor for microwave popcorn	-	-	-	-	$1.1*10^{-2}$	505	-	-
S10	Sausage tray	-	-	-	-	$3.9*10^{-3}$	992	$2.3*10^{-2}$	69
S11	Microwave pizza tray	$3.7*10^{-3}$	327	-	-	$3.7*10^{-3}$	260	-	-
S12	Frozen fish box	-	-	-	-	$3.6*10^{-3}$	223	$2.1*10^{-2}$	63
S13	Cake tray	-	-	$5.1*10^{-3}$	42	$1.7*10^{-3}$	274	-	-
S14	Tomato punnet	-	-	-	-	$2.3*10^{-5}$	1069	$5.7*10^{-3}$	105
S16	Imported Chinese 1	$1.3*10^{-2}$	104	-	-	$1.4*10^{-3}$	670	$1.3*10^{-2}$	160
S17	Microwave popcorn bag	-	-	-	-	$4.4*10^{-4}$	229	-	-
S18	Imported Chinese 2	$1.1*10^{-1}$	141	$1.1*10^{-1}$	36	$3.9*10^{-3}$	701	$3.5*10^{-2}$	132
S19	Paperboard with UV print	-	-	-	-	$6.5*10^{-4}$	634	$1.3*10^{-3}$	245
S20	Paperboard with water soluble print	-	-	-	-	$8.9*10^{-4}$	430	$1.8*10^{-3}$	226
S21	Paper board with offset print	-	-	-	-	$1.1*10^{-3}$	869	-	-
S22	Cereal box[a]	-	-	-	-	$4.3*10^{-4}$	329	-	-

Values are based on one representative experiment in cases where more than one experiment was conducted.

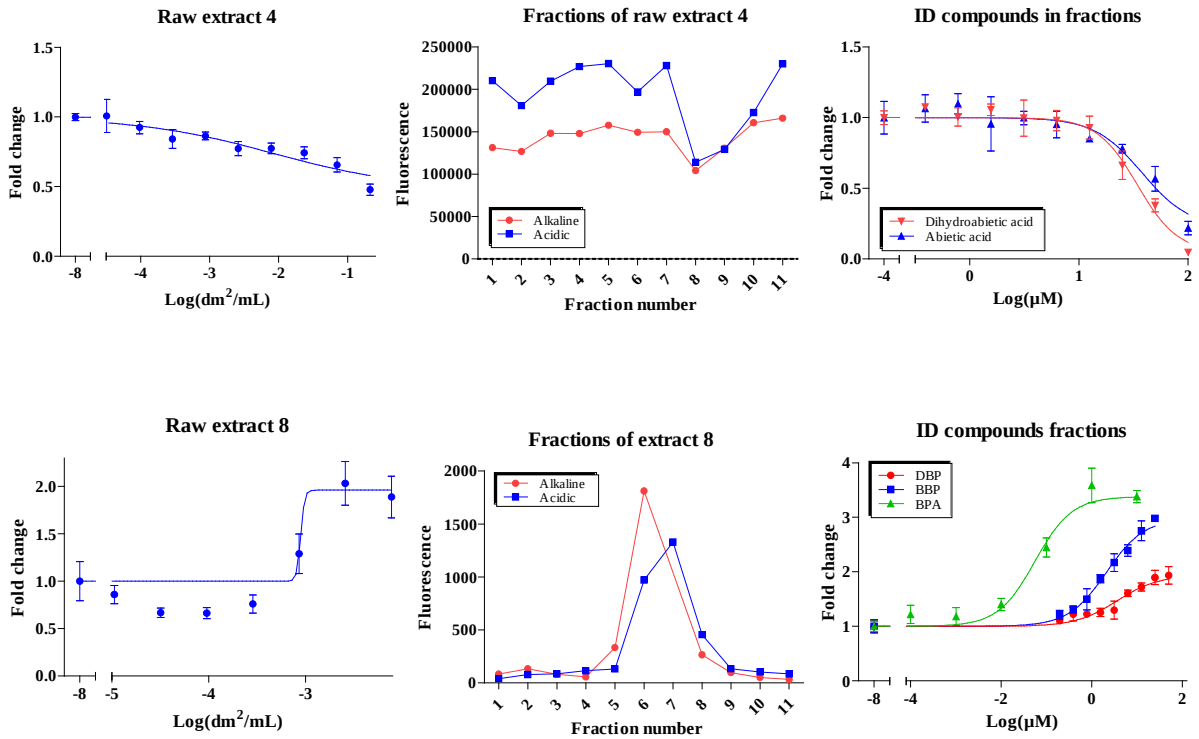


Figure 2: Androgen receptor (AR) antagonism of extract 4 (top, left), fractions of extract 4 (top, middle), and identified (ID) compounds in active fractions of extract 4 (top, right) and estrogen receptor (ER) agonism of extract 8 (bottom, left), fraction of extract 8 (bottom, middle), and identified (ID) compounds in fraction 6 alkaline and 7 acidic of extract 8 (bottom, right). Graphs are based on one representative experiment in extract (n=3), fractions (n=2), and ID compounds (n=3). Data from extract and identified compounds were normalized to controls and fitted to a sigmoidal dose-response model. Error bars represent standard deviations (SD).

Table 4: Estimated and measured equivalence factors (EQ) in µM of OHF and E2 in the AR and ER reporter gene assay, respectively, for extract S8 and S4, as well as identified compounds causing changes in activity in extracts including bisphenol A (BPA), di-butylphthalate (DBP), butyl-benzylphthalate (BBP), dehydroabietic acid (DHAA), and abietic acid (AA). [a]Concentrations (µM) for identified compounds in extract at maximum response.

ESTROGEN RECEPTOR ACTIVITY								
EXTRACT	BPA		DBP		BBP		TOTAL EEQ	
	µM[a]	EEQ	µM[a]	EEQ	µM[a]	EEQ	EEQ_{calc}	EEQ_{meas}
S8	0.08	$1.11*10^{-5}$	0.19	$1.89*10^{-7}$	0.07	$1.99*10^{-7}$	$1.42*10^{-5}$	$2.23*10^{-6}$

ANDROGEN RECEPTOR ACTIVITY						
EXTRACT	DHAA		AA		TOTAL EEQ	
	µM[a]	AEQ	µM[a]	AEQ	AEQ_{calc}	AEQ_{meas}
S4	3.91	$2.14*10^{-4}$	485.2	$1.49*10^{-1}$	$1.49*10^{-1}$	$8.84*10^{-2}$

9.7

Appendix D

Comprehensive lists of compounds tentatively identified in fractions with positive toxicological effect in Estrogen receptor (ER) assay and androgen receptor (AR) assay

Supplementary materials: Tentatively identified compounds

Data presented in this Supplementary material are the results obtained from the tentative identification process of fractions with positive toxicological response. Cut-offs are indicated as dotted-lines in the chromatograms.

Indicated in the columns are:

Compound name

CAS number: if available

Molecular formula

Retention time: In respective method

Ionization mode: GC-EI, LC-ESI+ and LC-ESI-

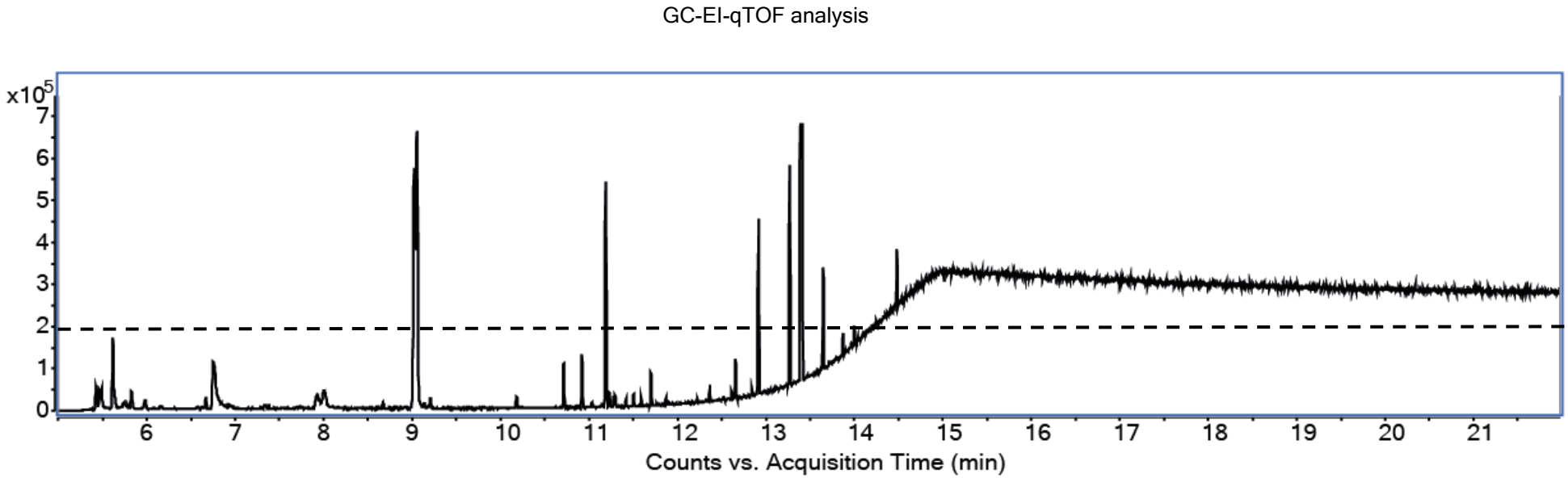
Customized database hit: only used for compounds identified in LC-ESI+ or LC-ESI-

Number of ions: only used for compounds identified in LC-ESI+ or LC-ESI-. Adducts (if present) are also registered as these could facilitate localization of molecular ion.

Additional information: Fragmentation, relevant usage in paper and board

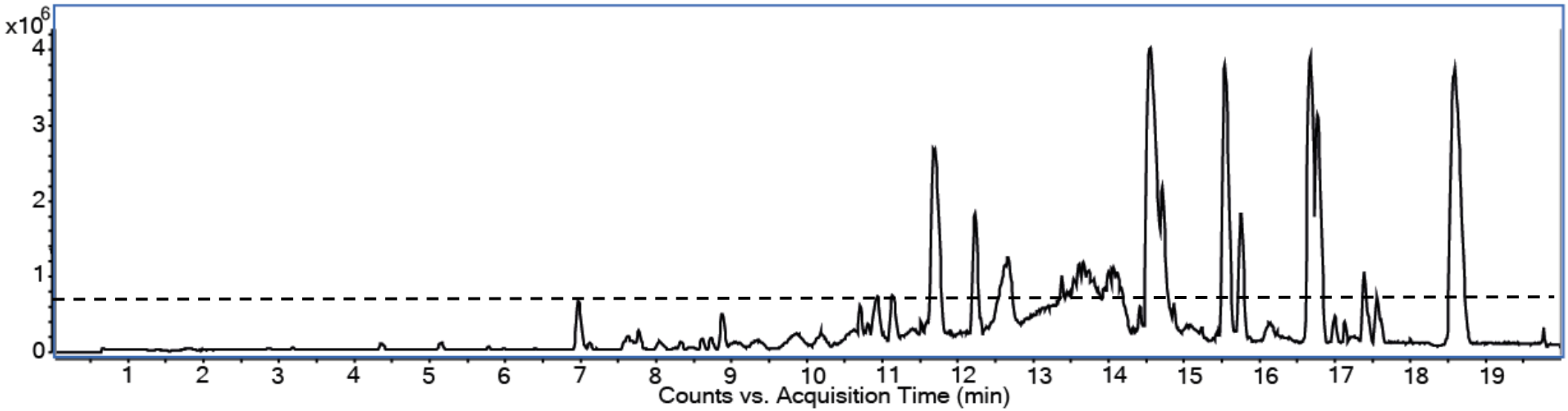
S4 acidic fraction 8

(positive in AR)



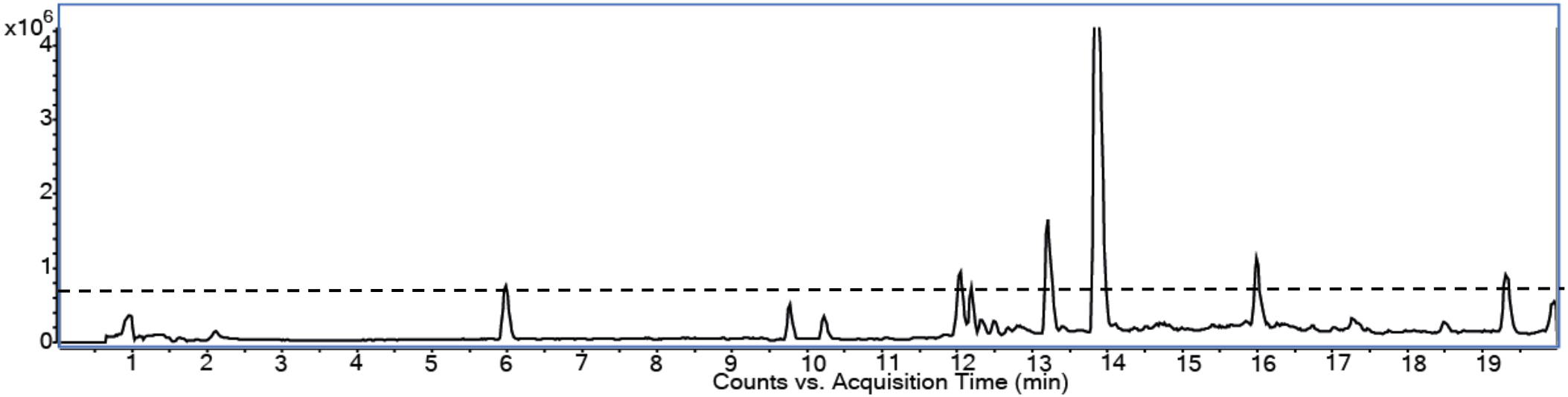
LC-ESI-qTOF analysis

(positive mode)



LC-ESI-qTOF analysis

(negative mode)

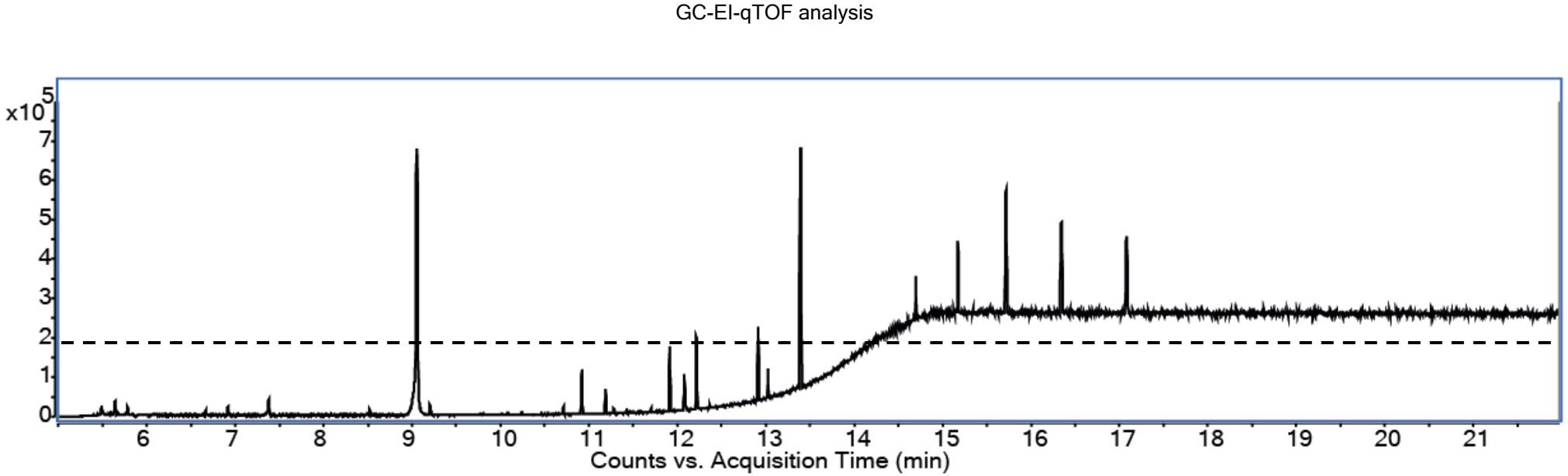


Compound	CAS number	Molecular formula	Retention time	GC-EI	LC-ESI+	LC-ESI-	Customized database hit	Number of ions (for LC only)	Additional information
1-Isocyanatooctadecane	112-96-9	C19H37NO	9.32	x					Used in coatings, adhesives and in printing
Phthalic acid, butyl cyclohexyl ester	84-64-0	C18H24O4	11.12	x					Used as plasticizer
			12.98	x					Aliphatic fragmentation pattern
			13.27	x					Aliphatic fragmentation pattern
2,2-diethoxyacetophenone	C12H16O3	6175-45-7	13.38	x					Used in resin compositions for ink jet printing
Methyl 6-methoxy-2,3-dihydro-1-benzofuran-2-carboxylate	C11H12O4	20166-65-8	13.64	x					
			14.48	x					Aliphatic fragmentation pattern
Propanamide, 3-(2-benzothiazolylthio)-	132605-19-7	C10H10N2OS2	6.95		x			2	Fragment into C3H5NO
			11.67		x			1	*m/z* 300.9967
		C18H30N4S	12.15		x			1	
Dehydroabietic acid	1740-19-8	C20H28O2	12.3		x		x	1	Resin acid
Abietic acid	514-10-3	C20H30O2	12.3		x		x	1 (-H2O loss)	Resin acid
Isorhamnetin	480-19-3	C16H12O7	12.66		x			1	Naturally occuring flavonol
Rhamnetin	90-19-7	C16H12O7	12.66		x			1	Naturally occuring flavonol
		C23H40O5	12.66		x			2 (NH4+ adduct)	Fragments into C12H18O. No matching structures available
3,6,9,12-Tetraazaoctadeca-14,16-dien-18-oic acid, 1-amino-, ethylester	61347-03-3	C16H33N5O2	14.50		x			4	
4-oxoretinoic acid	38030-57-8	C20H26O3	14.77		x			1 (NH4+ adduct)	
2-(8-Heptadecenyl)-2-oxazoline		C20H37NO	15.51 +15.81		x			2	Fragments into C3H5NO
			16.60		x			3	Molecular ion at *m/z* 1120.8380, fragments at *m/z* 569.4367 and *m/z* 284.2982 (C18H37NO)
		C26H55NO4S	17.40		x			7	Fragemnts into C24H48O2S, C13H28S, C11H16, C8H12, C7H10, C6H8

Compound	CAS number	Molecular formula	Retention time	GC-EI	LC-ESI+	LC-ESI-	Customized database hit	Number of ions (for LC only)	Additional information
2-Mercaptobenzothiazole	149-30-4	C7H5NS2	5.98			x	x	3	Fragments into C7H5NS , used in rubber and latex production as well as in paper manufacturing, production of litographic plates and two-part cyanoacrylate adhesives
		C21H26N4	9.77			x		1	
		C16H32N4	12.05			x		1	
		C18H34N4S	12.05			x		1	
		C17H30N14O	13.21			x		2	
		C1736O10	13.82			x		2	Fragments into C13H28O8
(2E)-3-(Tetradecylsulfanyl)acrylic acid		C17H32O2S	15.98			x		1	

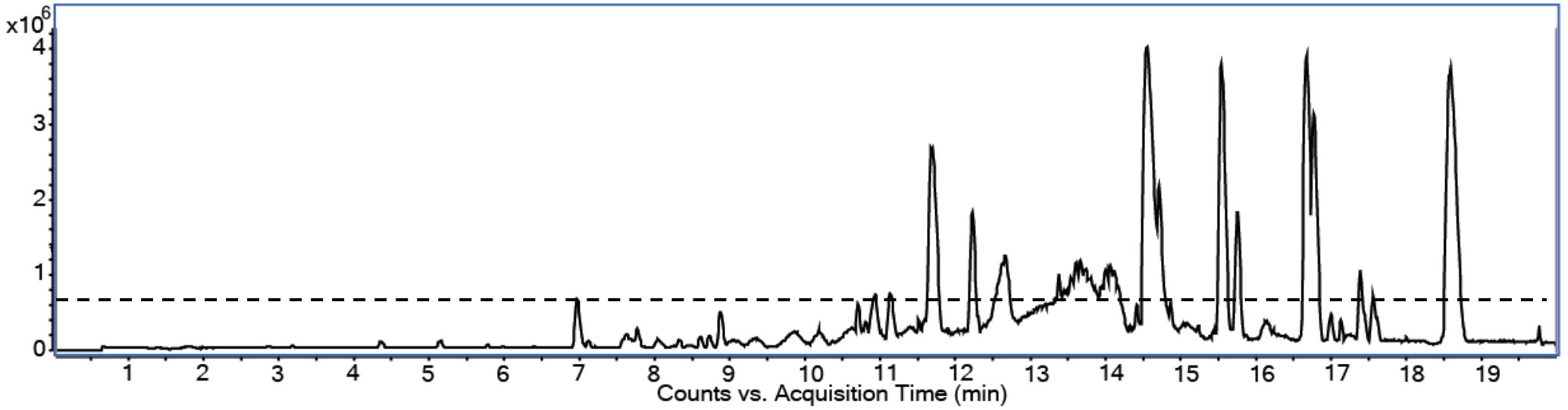
S4 alkaline fraction 8

(positive in AR)



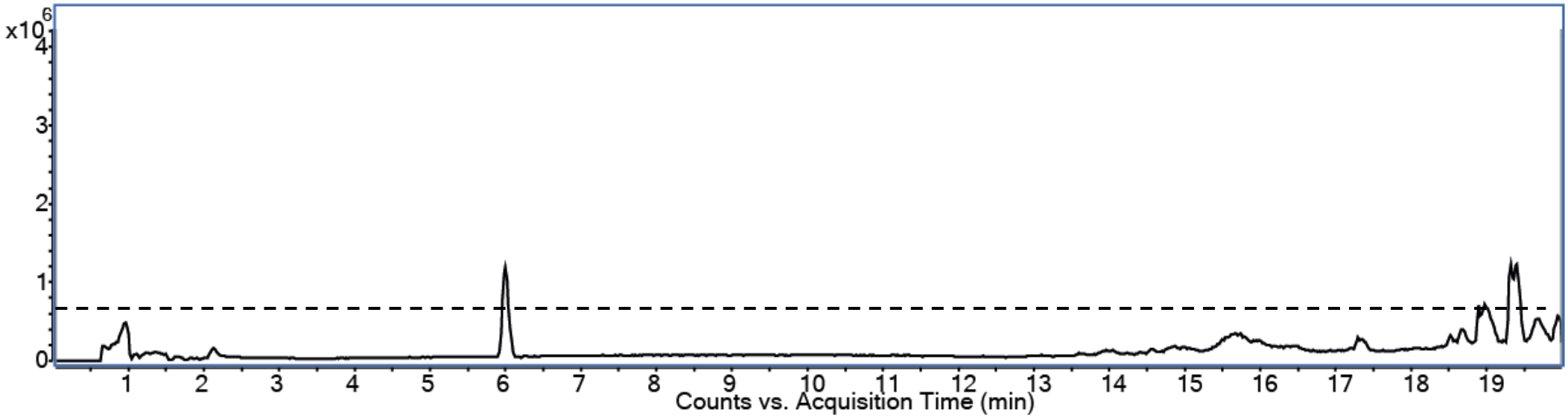
LC-ESI-qTOF analysis

(positive mode)



LC-ESI-qTOF analysis

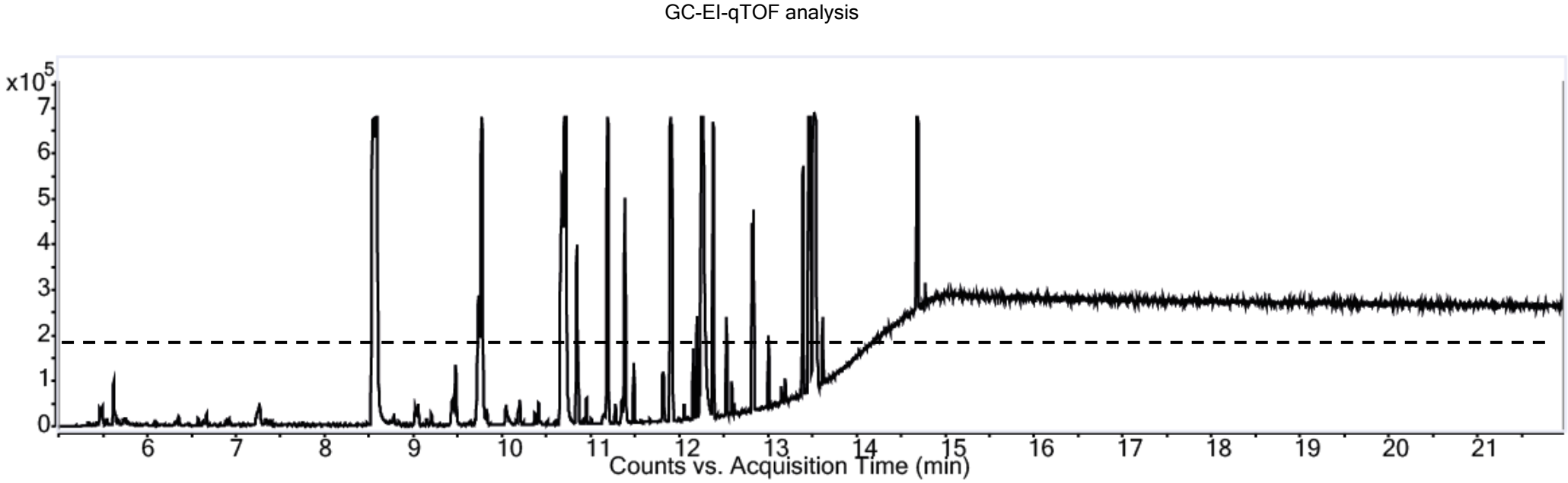
(negative mode)



Compound	CAS number	Molecular formula	Retention time	GC-EI	LC-ESI+	LC-ESI-	Customized database hit	Number of ions (for LC only)	Additional information
1-Isocyanatooctadecane	112-96-9	C19H37NO	9.32	x					Used in coatings, adhesives and in printing
4-hydroxy-3a,7a-dimethyl-4,5-dihydro-3H-2-benzofuran-1-one	54346-06-4	C10H14O3	12.91	x					
2,2-diethoxyacetophenone	C12H16O3	6175-45-7	13.39	x					Used in resin compositions for ink jet printing
			14.7	x					Aliphatic fragmentation pattern
			15.17	x					Aliphatic fragmentation pattern
			15.71	x					Aliphatic fragmentation pattern
			16.34	x					Aliphatic fragmentation pattern
			17.07	x					Aliphatic fragmentation pattern
		C10H10N2OS2	7.02		x			2	Fragments into C3H5NO
Benzyl dimethylcarbamodithioate	7250-18-2	C10H13NS2	8.93		x			2	Fragments into C3H5NS,used in ink compositions
Dehydroabietic acid	1740-19-8	C20H28O2	12.3		x		x	1 (M+H)	Resin acid
Abietic acid	514-10-3	C20H30O2	12.3		x		x	1 (M+H-H2O)	Resin acid
			14.55		x			4	328.2714, 286.2235, 117.0741, 75.0262
2-(8-Heptadecenyl)-2-oxazoline		C20H37NO	15.51, 15.8		x			2	Fragments into C3H5NO
			16.6		x			3	*m/z* 1120.8380, fragmentsinto *m/z* 569.4367 and *m/z* 284.2982 (C18H37NO)
		C26H55NO4S	17.40		x			7	Fragemnts into C24H48O2S, C13H28S, C11H16, C8H12, C7H10, C6H8
2-Mercaptobenzothiazole	149-30-4	C7H5NS2	5.98			x	x	2	Fragments into C7H5NS, used in rubber and latex production as well as in paper manufacturing, production of litographic plates and two-part cyanoacrylate adhesives

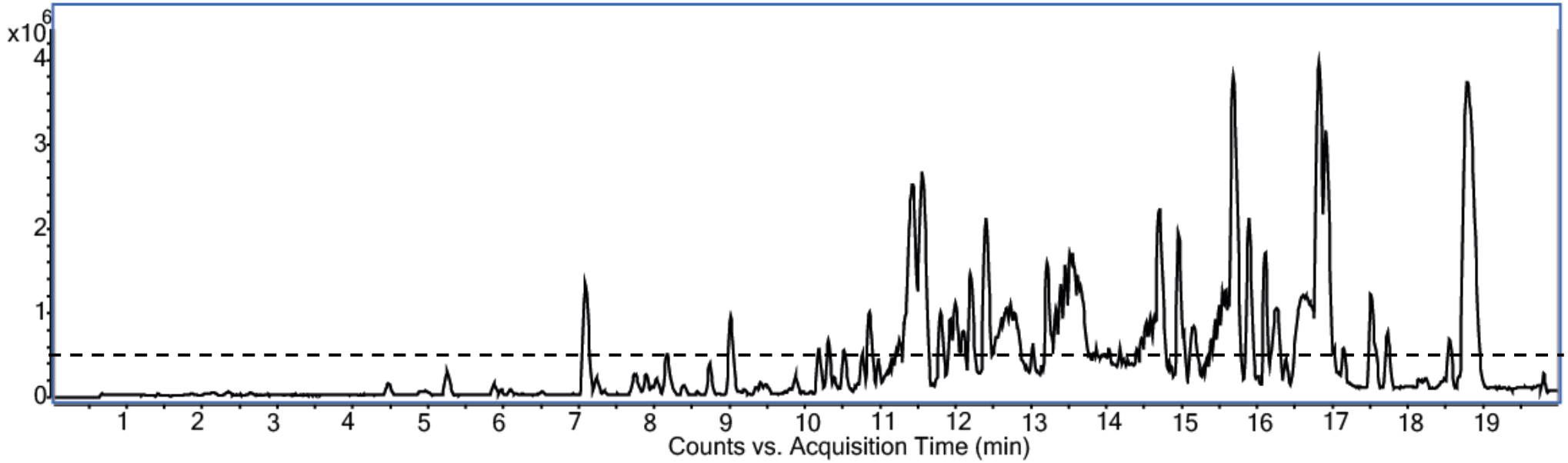
S8 acidic fraction 6

(positive in ER)



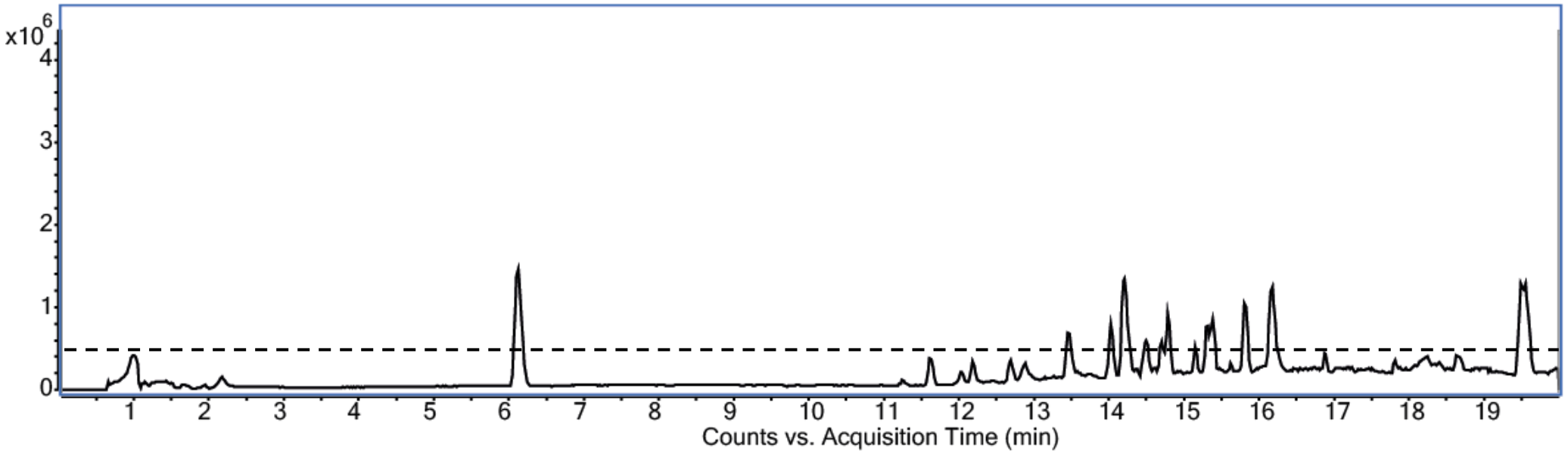
LC-ESI-qTOF analysis

(positive mode)



LC-ESI-qTOF analysis

(negative mode)

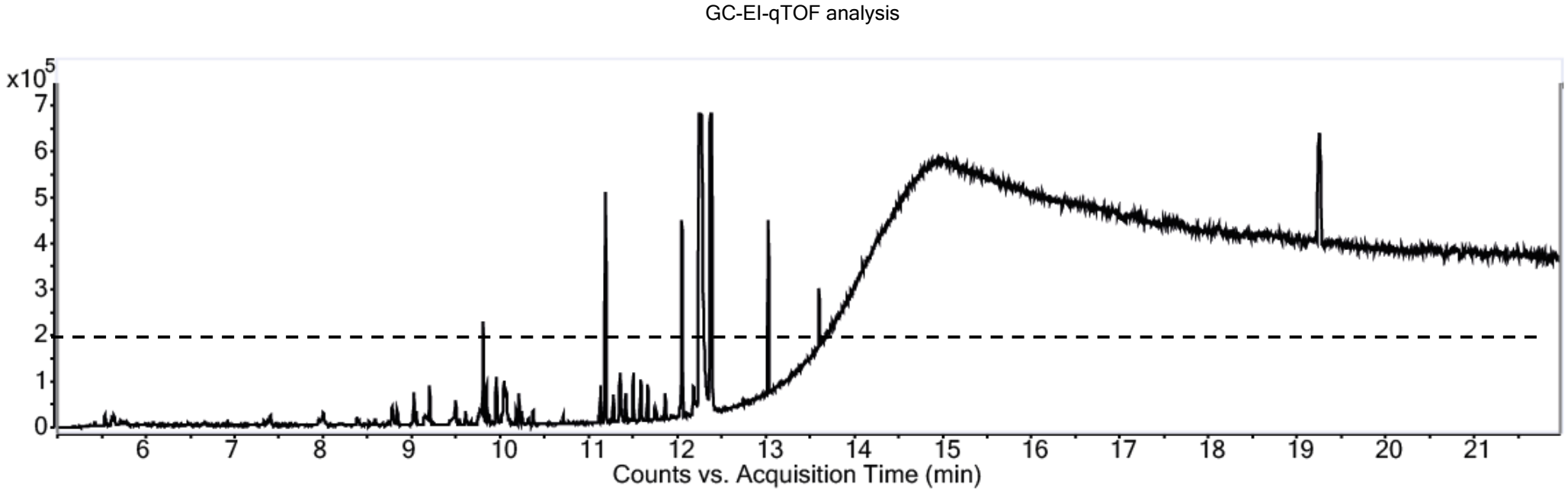


Compound	CAS number	Molecular formula	Retention time	GC-EI	LC-ESI+	LC-ESI-	Customized database hit	Number of ions (for LC only)	Additional information
N,N-Dimethyldodecylamine	112-18-5	C14H31N	8.58	x					
Vinyl benzoate	769-78-8	C9H8O2	9.48	x					Monomer for polyvinyl
Di-isobutyl phthalate	84-69-5	C16H22O4	10.68	x					Used in in printing inks, resin solvent, paper coatings and in adhesives
Dibutyl phthalate	84-74-2	C16H22O4	11.17 (GC); 12.25 (LC)	x	x		x	3	Used in in printing inks, resin solvent, paper coatings and in adhesives
2,2-Dimethoxy-1,2-diphenylethanone	24650-42-8	C16H16O3	10.85	x					Photo initiator. Associated with printing inks.
4a,9a-Dihydro-9,10-anthracenedione	84-65-1	C14H8O2	11.387	x					Digester additive in paper making process. Protecting cellulose (and hemicellulose) from alkaline degradation.
Methyl 4-(6-methyl-4-oxo-2-heptanyl)-1-cyclohexene-1-carboxylate	17904-27-7	C16H26O3	11.49	x					
Benzidine	92-87-5	C12H12N2	11.9	x					Used in production of dyes.
Bisphenol A	80-05-7	C15H16O2	12.25	x					Used for manufacturing epoxy, polycarbonate and other resins
Tributyl acetylcitrate	77-90-7	C20H34O8	12.53	x					Biodegradable plasticizer used in cellulose resin.
4'-Methoxy-2-hydroxystilbene	-	C15H14O2	12.84	x					Naturally occurring substance formed in hardwood.
Bis(2-ethylhexyl) (2E)-but-2-enedioate	141-02-6	C20H36O4	12.38	x					Fumarate. Possibly used to facilitate dye setting.
Benzyl Butyl Phthalate	85-68-7	C19H20O4	13.00	x					Used in in printing inks, resin solvent, paper coatings and in adhesives
1,3-Diphenyl isothianaphthene	16587-39-6	C20H14S	13.40	x					Used in production of vinyl polymer
2-(2-(Benzoyloxy)propoxy)propyl benzoate	20109-39-1	C20H22O5	13.46	x					Naturally occurring substance formed in hardwood.
Oxydi-2,1-ethanediyl dibenzoate	120-55-8	C18H18O5	13.52 (GC); 9.1 (LC)	x	x			2	Fragments into C10H11O2 in LC. Naturally occurring substance formed in hardwood.
Dipropyleneglycol dibenzoate	20109-39-1	C20H22O5	13.59	x					Used in photo resistant layers as well as adhesives
Diglycol dibenzoate	120-55-8	C18H18O5	14.69	x					Used as a plasticizer, in adhesives and in ink jet inks
Michler's ketone	90-94-8	C17H20N2O	7.1		x		x	2	Fragments into C8H11N, intermediate in the synthesis of dyes and pigments for paper
4,4-Dimethylandrost-5-ene		C21H34	10.85		x			1 (NH4+ adduct)	
2,4-Xylenol, 6,6'-isobutylidenedi-	33145-10-7	C20H26O2	11.4		x			1	Used in printing inks (for black)
			11.6		x			3	317.2086, 295.2264, 277.2186
Dipropylene glycol benzoate	27138-31-4	C20H22O5	11.8-12.0		x			2	Fragments into C10H11O2, used as plasticizer

Compound	CAS number	Molecular formula	Retention time	GC-EI	LC-ESI+	LC-ESI-	Customized database hit	Number of ions (for LC only)	Additional information
		C26H26N2O2	12.5		x			1	
		C31H38N2	13.2		x			1	
		C19H37NOS	14.75		x			3 (-H2O loss)	Fragments into C6H12S and C3H6S
			14.9		x			1	*m/z* 298.3151
			15.7		x			1	*m/z* 308.2898
			15.9		x			1	*m/z* 308.3001
		C40H36O	16.1		x			1	
			16.8		x			1	*m/z* 284.2992
Stearamide	124-26-5	C18H37NO	16.9		x		x	1	Used in toner pigments, as defoamer and in flexographic printing forms
		C27H44O2	17.55		x			4	Fragments into C25H40, C16H24, C11H16
N-Isopropylhexadecanamide	189939-61-5	C19H39NO	17.75		x			1	Used in amide containing copolymers
2-Mercaptobenzothiazole		C7H5NS2	6.15			x		2	Fragments into C7H5NS, used used in rubber and latex production as well as in paper manufacturing, production of lithographic plates and two-part cyanoacrylate adhesives
Polyglycerol ricinoleate	29894-35-7	C18H33O3	13.45			x		2	Fragments into C12H20O2, printing ink
Nonidet P-40	11130-43-1	C18H30O3	14.03			x		2	Approved use as a component of articles intended for use in packaging, transporting, or holding food (US FDA)
Ricinoleic acid	141-22-0	C18H34O3	14.21			x	x	1 (-H2O loss)	Used in printing ink
Methyl 9,10-Dihydroxystearate	1115-01-1	C19H38O4	14.78			x		2	Fragments into C18H34O3, used in ink jet printing
Polyglycerol ricinoleate	29894-35-7	C18H33O3	15.31			x	x	1	Used in printing ink
12-Hydroxystearic acid	106-14-9	C18H36O3	15.82			x		1	Used as printing ink, resin composition and laminates
Dehydroabietic acid	1740-19-8	C20H28O2	16.21			x	x	1	Resin acid

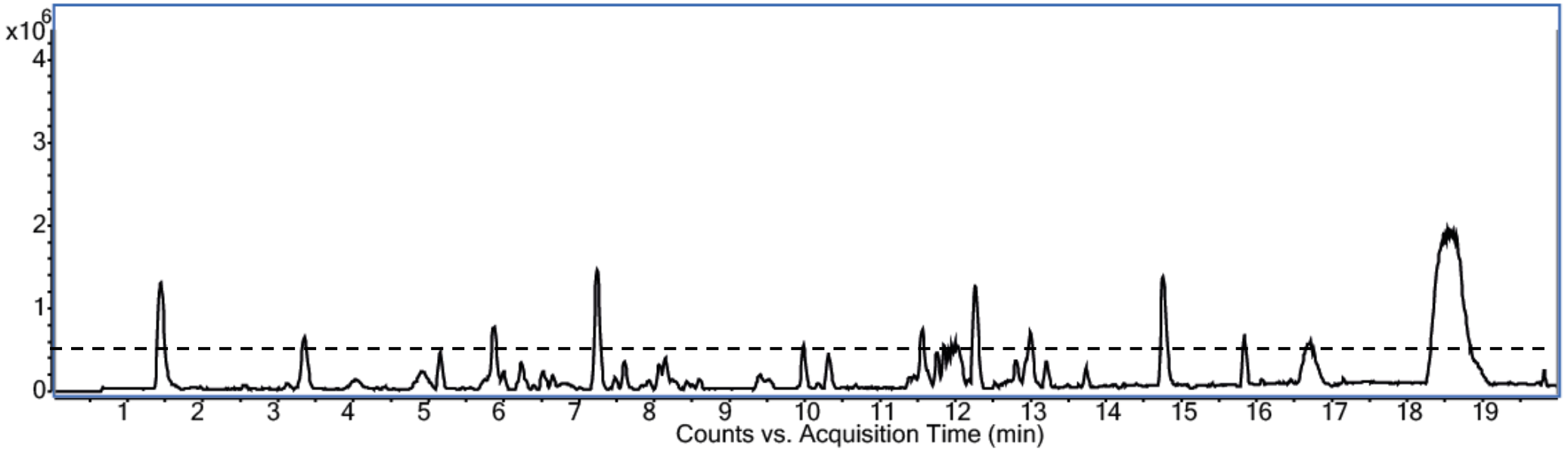
S8 alkaline fraction 6

(positive in ER)



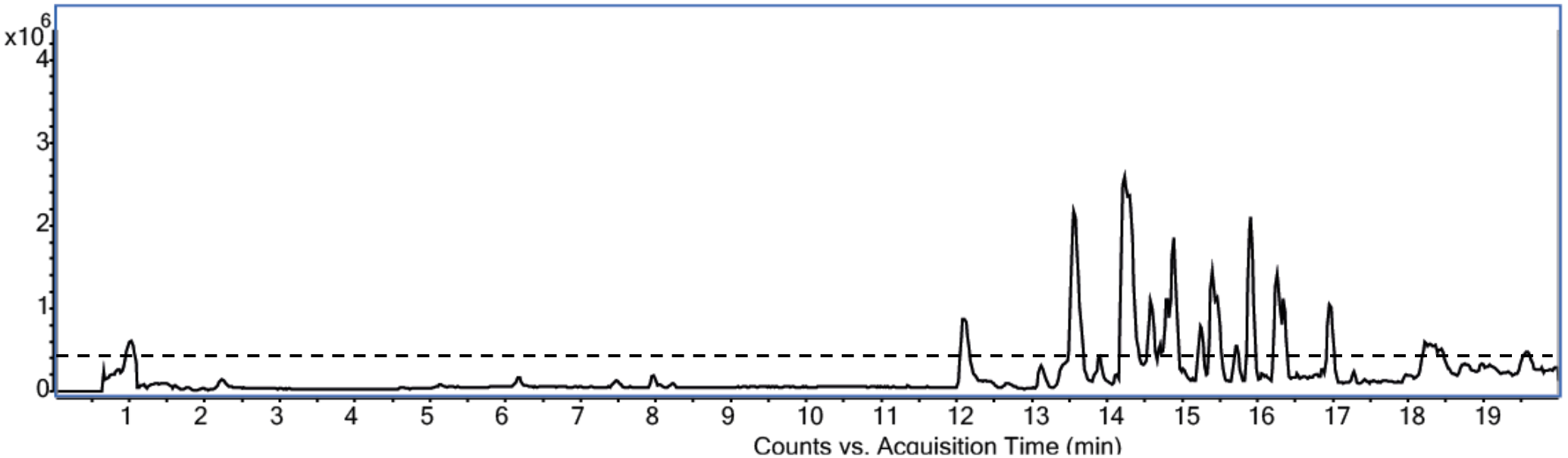
LC-ESI-qTOF analysis

(positive mode)



LC-ESI-qTOF analysis

(negative mode)



Compound	CAS number	Molecular formula	Retention time	GC-EI	LC-ESI+	LC-ESI-	Customized database hit	Number of ions (for LC only)	Additional information
4-Diethylaminobenzaldehyde	120-21-8	C11H15NO	9.81	x					Used in colorants
Isobutyl Benzoate	120-50-3	C12H16O2	9.95	x					Used in olefin production, and in defoaming processes
N-Propylbenzamide	10546-70-0	C10H13NO	10.0	x					Found in curable compositions in printing
Dibutyl phthalate	84-74-2	C16H22O4	11.12 (GC); 12.25 (LC)	x	x		x	3	Phthalate fragment at 149.023480. Also fragment at 205.086153 (C12H13O3). Used in in printing inks, resin solvent, paper coatings and in adhesives
Bis(2-ethylhexyl) (2E)-but-2-enedioate	142-02-6	C20H36O4	12.05	x					Used to facilitate dye setting
Bisphenol A	80-05-7	C15H16O2	12.27	x					Used in resins, paper coatings and in epoxy adhesives
Fumaric acid, 3-methylbut-2-yl, undecyl ester	-		12.37	x					
Methyl 8,11,13-abietatrien-18-oate	1235-74-1	C21H30O2	13.03	x					
2,4-Bis(1-phenylethyl)phenol	2769-94-0	C22H22O	13.9	x					Used in thermoplastics
Bisomer Amine D 700		C5H13NO	1.05		x			2	Fragments into C3H9N. Used in dye-containing curable compositions.
		C20H13N5	3.35		x			1	
		C22H40N4O4	4.93		x			4 (+NH4+ adduct)	Fragments at C9H18O3, C6H12O2,C3H6O and C22H43N5O4
		C20H23NO	5.16		x			3	Fragment: C11H13N and C8H9N
1,3-dibenzylurea	1466-67-7	C15H16N2O	5.88		x			2	Fragments into C8H7NO
4-Nonylphenyl dihydrogen phosphate		C15H25O4P	6.23		x		x	2 (-H2O loss)	Used as surfactant and thermal transfer dye sheets
N-Benzylbenzamide	1485-70-7	C14H13NO	7.2		x			2	Fragments into C7H4O
		C11H15NO	7.2		x			2	Fragments into C10H15N
Solvent Violet 8	52080-58-7	C24H27N3	7.61		x		x	1	Used as a dye
		C33H29N3O8	8.0		x			1	
1-benzyl-3-tert-butyl-1H-pyrazole-5-carboxylic acid	100957-85-5	C15H18N2O2	9.98		x			3	Fragments into C12H10N2O2, and C12H10N2O
N-Benzyl-1-tetradecanamine		C21H37N	10.34		x			3	Fragments into C14H29N and C7H6, used in thermal dye transfer

Compound	CAS number	Molecular formula	Retention time	GC-EI	LC-ESI+	LC-ESI-	Customized database hit	Number of ions (for LC only)	Additional information
			11.56		x			3	317.2086, 295.2264, 277.2186
Linolenic acid		C18H30O2	12.99		x			1 (-H2O loss)	
		C19H37NOS	14.75		x			3 (-H2O loss)	Fragments into C6H12S and C3H6S
			14.9		x				m/z 298.3151
			15.83		x				m/z 298.2812
		C13H22N4S	12.14			x		1	
Polyglycerol ricinoleate	29894-35-7	C18H33O3	13.51			x		2	Fragments into C12H20O2, used in printing ink
		C21H34N4O3S	14.23			x		1	
2-Dodecylbenzenesulfonic acid	27176-87-0	C18H30O3S	14.9			x		1 (-H2O loss)	Used as binder resin in toners
3-tetradecyldihydrofuran-2,5-dione	47165-57-1	C18H32O3	15.2-15.5			x	x	1	Used in paper size compositions
12-Hydroxystearic acid	106-14-9	C18H36O3	15.88			x	x	1	Used as printing ink, resin composition and in laminates
Ricinoleic acid	141-22-0	C18H34O3	14.2; 16.25			x	x	1 (-H2O loss)	Used in printing ink
Dehydroabietic acid	1740-19-8	C20H28O2	16.35			x	x	1	Resin acid
		C23H22O3	16.95			x		2	Fragments into C22H22O

9.8

Paper 4

Linda Bengtström, Xenia Trier, Kit Granby, Mona-Lise Binderup, Jens Højslev Petersen (2014). Identification of unknown mutagenic compounds in microwave popcorn bags. Manuscript in preparation to be submitted to Food and Chemical Toxicology as a Short Communication

Comments to paper 4

The results presented in this manuscript are preliminary. Additional data concerning the testing of Solvent Violet 8 in mutagenicity test is currently conducted and will be included in the final manuscript prior to submission.

In this paper, there are references to Appendix A. This is the Appendix E in this thesis.

Identification of unknown mutagenic compounds in microwave popcorn bags

Linda Bengtström[a], Xenia Trier[a], Kit Granby[a,], Mona-Lise Binderup[b], Jens Højslev Petersen[a]

[a] *Division of Food Chemistry, National Food Institute, Technical University of Denmark, Copenhagen, Denmark*

[b] *Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark, Copenhagen, Denmark*

Corresponding author:

Mona-Lise Binderup

Division on toxicology and risk assessment

Technical University of Denmark

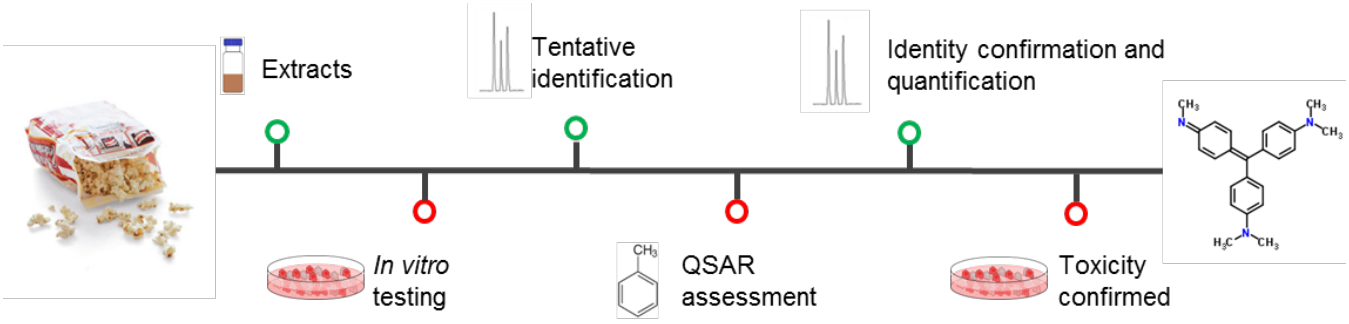
Mørkhøj Bygade 19, 2860 Søborg, Denmark

+45 35 88 75 91

mobi@food.dtu.dk

Identification of unknown mutagenic compounds in microwave popcorn bags

Graphical abstract



Abstract

In this interdisciplinary study we describe a strategy for the identification of unknowns with mutagenic effects in paper and board food contact materials. Extracts from a microwave popcorn bag tested positive in an initial screening round of a broad selection of packaging for mutagenic effects. The heterogeneous popcorn bag was divided into three subsamples; adhesive, susceptor and bulk, which were tested *in vitro* for mutagenicity. Both the bulk and the susceptor subsamples had a concentration related mutagenic effect, but the bulk was more potent. No effect was observed of the adhesive part. In order to tentatively identify the compound(s) responsible for this effect, the bulk sample was analysed by orthogonal GC-EI-qTOF and UPLC-ESI-qTOF methods. The mutagenicity of tentatively identified compounds was predicted by a QSAR model based on Ames in vitro test data. One compound; Solvent violet 8 were selected for testing of analytical standards *in vitro* as well as chromatographic verification. The presence of this compound in the sample was confirmed when compared to analytical standards. The result of the mutagenicity test will be available shortly.

Key words: food packaging, identification, high resolution mass spectrometry, mutagenicity assay, *Salmonella*/mictosome assay, popcorn bags

1. Introduction

Paper and board food contact materials (FCMs) are used for a variety of applications, such as packaging for dry foods and fast food as well as microwave popcorn bags. As the starting materials of paper and board are of plant origins, it consists of a complex mixture of different organic compounds with varying concentrations. Furthermore, many types of paper and board are chemically treated with compounds to improve material properties, such as grease-proofing or printability (Roberts 1996). Recently, paper FCMs in general and microwave popcorn bags in particular have been recognized as problematic due to large knowledge gaps of which chemicals are being used for the treatment of the products and the toxicological effects of these compounds (Trier et al. 2011; Sinclair et al. 2007; Begley et al. 2008; Neltner et al. 2013; Grob et al. 2010). In addition, some paper FCMs have previously been associated with mutagenic effects (Ozaki et al. 2004).

The identification of each individual substance would be both time-consuming and costly with such a chemically complex matrix as paper and board (Bradley et al. 2010). Moreover, a chemical analysis, although comprehensive, will not give any information on the potential of the identified compounds to cause adverse health effects (Honkalampi-Hämäläinen et al. 2010). To gain as much information as possible some studies have therefore combined chemical analysis and *in vitro* tests to screen paper and board FCMs (Binderup et al. 2002; Vinggaard et al. 2000; Lopez-Espinosa et al. 2007; Ozaki et al. 2004; Honkalampi-Hämäläinen et al. 2010; Koster et al. 2014). This process excludes samples with no *in vitro* response in the investigated assays and allows for further investigations of only samples with a positive toxicological response.

The aim of this study was to develop further a strategy for isolation and identification of samples and compound(s) with mutagenic effect in different paper and board FCMs by using an

interdisciplinary approach. The aim was also to correlate the concentration of identified compound(s) in the sample with the observed mutagenic effect.

2. Materials and methods

2.1 Chemicals and reagents

Ethanol (99.9%), used for the extraction and re-dissolving was purchased from Merck (Darmstadt, Germany). The methanol (99.9%) used for mobile phases for the HPLC fractionation was purchased from Rathburn (Walkerburn, UK). All aqueous solutions were prepared using ultrapure water obtained from a Millipore Milli-Q Gradient A10 system (Millipore, Bedford, MA, USA). HPLC MS grade 25 % ammonium hydroxide and formic acid were obtained from Sigma-Aldrich (St. Loiuse, MO, USA). UPLC grade acetonitrile was obtained from Merck (Darmstadt, Germany). Standards for the GC-EI-qTOF method; di-butyl phthalate (DBP), deuterated di-butyl phthalate (d_4-DBP), butyl-benzyl phthalate (BBP), and di-isobutyl phthalate (DIBP) and standards for the UPLC-ESI-qTOF method; bisphenol A, methylparaben, bisphenol A diglycidyl ether (BADGE), perfluorooctanoic acid (PFOA) and abietic acid were all obtained from Sigma-Aldrich. For UHPLC-MS/MS quantification and for mutagenicity testing, Solvent Violet 8 (85%, Sigma Aldrich) and Leucocrystal violet (Sigma Aldrich) standards were used.

2.2 Sample preparation and production of extracts

For the initial screening round approximately 90 dm^2 of each of 20 different samples were used. One sample showing mutagenic activity was obtained from a popcorn vendor. The characteristics of the sample were; grammage 90g m^2 and printed on the non-food contact side. Production of extracts by boiling ethanol reflux is described in full detail in Bengtström et al (2014). During a second screening round, the non-homogenous popcorn bag was divided into three subsamples; top of the bag with a sealing containing adhesives part, susceptor part and bulk part. The susceptor is the material on the bottom of the microwave popcorn bag absorbing the electromagnetic energy from

the microwave oven and converts it into heat, in order to facilitate the popping of the popcorn. Each subsample consisted of 90 dm^2 for extraction.

2.3 Mutagenicity tests

The ethanol extracts were tested for induction of point mutations in the Salmonella/microsome assay. Two tester strains were used: TA98 (frameshift mutations) and TA 100 (base pair substitution). The test was performed without and with Aroclor 1254 induced rat liver S9 mix with a protein content of 4 mg S9/ml. The assay was performed as a microsuspension assay as described in Binderup et al. 2002. The highest tested concentration was based on preliminary toxicity tests. Initially, extracts were tested in three concentrations in duplicate. Extracts with a positive response were then tested in five concentrations in triplicate. Negative (ethanol) and positive controls were included in all assays. The positive controls were: 2-aminoanthrazene (both strains with S9-mix), natriumazide (TA100 without S9-mix) and 2-nitrofluorene (TA98 without S9-mix).

2.4 Tentative identification

The tentative identification process as well as the GC-EI-qTOF and LC-ESI-qTOF instrumentation is described in detail in Bengtström et al (in preparation). The extracts were diluted 1:100 *v/v* prior to analysis. Cut-offs for peaks were based on the threshold of toxicological concern for compounds with suspected mutagenic effects, corresponding to 150 ng kg^{-1} packed food or 150 ng per 6 dm^2 of packaging, for both tentative identification methods. Peaks with areas below this threshold were not further investigated. In GC-EI-qTOF, the extracts and fractions were ionized by electron ionization (EI) at 70 eV. Analysis was performed in the Agilent MassHunter Qualitative software with the NIST library v.11. Data-independent All Ions (100 V, 110V and 120 V) were acquired on the UPLC-ESI-qTOF analysis was analysed by using MassHunter Qualitative software (Agilent Technologies) as well as ProGenesis QI software (Nonlinear Dynamics Limited, UK). Compounds

were identified by using a customized library containing approximately 2300 matrix specific entries (Bengtström et al., in preparation) as well as the public ChemSpider and PubChem databases.

2.5 Quantitative analysis by UPLC-MS/MS

Solvent violet 8 and Leucocrystal violet were quantitated by UPLC-MS/MS using an seven-point external calibration curve (0, 10, 20, 50, 100, 500 and 1000 ng mL^{-1} for both standards in the mixture). Extracts were diluted 1:1000 *v/v* and 1:10000 v/*v* with ethanol prior to analysis. Analysis was performed by a Waters Acquity UPLC chromatograph coupled to a Micromass Quattro Ultima mass spectrometer with an ESI ionization interface in postitive mode. The column used was an XTerra CSH C18 column (2.5 μm, 150 × 2.1 mm) from Waters with a KrudKatcher Ultra pre-column 0.2 μm filter (Phenomenex, Torrance, CA, USA). Mobile phase A; 5mM ammonium formiate and 10 mM formic acid and mobile phase B; acetonitrile were used for separation. The gradient was: 0 min 25% B, 1 min linear to 50% B, 4 min linear to 65% B, 7 min increased to 98% B, increased to 99% B to 8 min, back to 25% at 8.1 min and equilibrate for 1.9 min. The flow rate was set at 0.4 ml min^{-1}; the injection volume was 3 μL. The capillary in negative mode voltage; +3 kV, desolvation gas flow 700 L h^{-1} and cone gas flow 110 L h^{-1} of N_2, source temperature 120°C, desolvation temperature 400°C. Argon was used as collision gas at 2.3×10^{-3} mbar. Data were acquired with MassLynx v.4.1 software and analyzed by the QuanLynx v 4.1 software. Masses used for quantification of Solvent Violet 8 was *m/z* 358>326 (collision energy 50 eV) as quantifier and *m/z* 358>342 (collision energy 30 eV) as qualifier; and for Leucocrystal Violet *m/z* 374.2>239.1 (collision energy 50 eV) as quantifier and *m/z* 374.2>358.1 as qualifier (collision energy 30 eV). Retention time (Rt) was 2.5 min for Solvent Violet 8 and 5.5 for Leucocrystal violet. Limit of detection (LOD) and limit of quantification (LOQ) was defined a three times and ten times the standard deviation of the lowest standard after the blank response was deducted.

3. Results and discussion

3.1 Initial screening

An initial screening of an extract from the microwave popcorn bag revealed an effect in the mutagenicity test. The extract for the initial screening contained both volatile organic compounds and non-volatile organic compounds. In order to elucidate which part of the heterogeneous sample responsible for the observed effect, the microwave popcorn bag was divided into three subsamples; an adhesive part, a susceptor part and a bulk part. These subsamples were then subsequently tested in the same assays. Whereas a mutagenic effect was observed in the bulk and the susceptor subsamples. However, as the response obtained from the bulk subsample was significantly higher, see Figure 1, this sample was chosen for further investigation. These results indicate that mutagenic compound(s) are present in the susceptor part, but higher concentrations of the compound(s) are present in the bulk, which also include the susceptor. Additionally, a decrease in mutagenic effects over time was observed. This decrease could be explained by either an oxidation or a precipitation of the compound(s) causing the effect which affects the bioavailability of the compound(s).

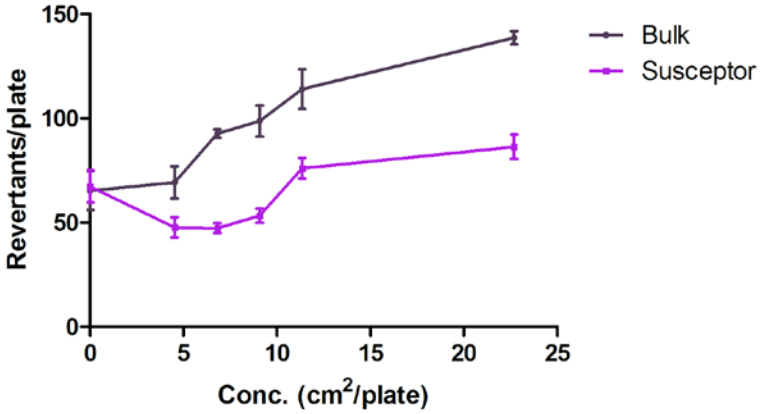


Figure 1. Results in TA98 with S9 mix of ethanol extracts of the two microwave popcorn bag subsamples with mutagenicl effects; susceptor and bulk; as well as the compound Solvent Violet 8. Each point corresponds to the mean of three plates SD (horizontal bars) of one experiment.

3.2 Tentative identification

The tentative identification of compounds in the extracts from the microwave popcorn bag bulk sample was performed as described in Bengtström et al. (in preparation). In short, extracts were analysed by both GC-EI-qTOF and UPLC-ESI-qTOF in order to make the identification method as generic and orthogonal as possible. Potential matrix effects, especially relevant for the UPLC-ESI-qTOF analysis as this ionization mode appears to be more affected than other techniques (Trufelli et al. 2011), were reduced by dilution. To reduce the number of compounds to be identified in the extracts, we used a cut-off similar to that used by Koster et al. (2014). However, the cut-off used for this study was based on the threshold of toxicological concern (TTC) for compounds with known genotoxic effects (Cramer Class III) (EFSA Scientific Committee 2012).

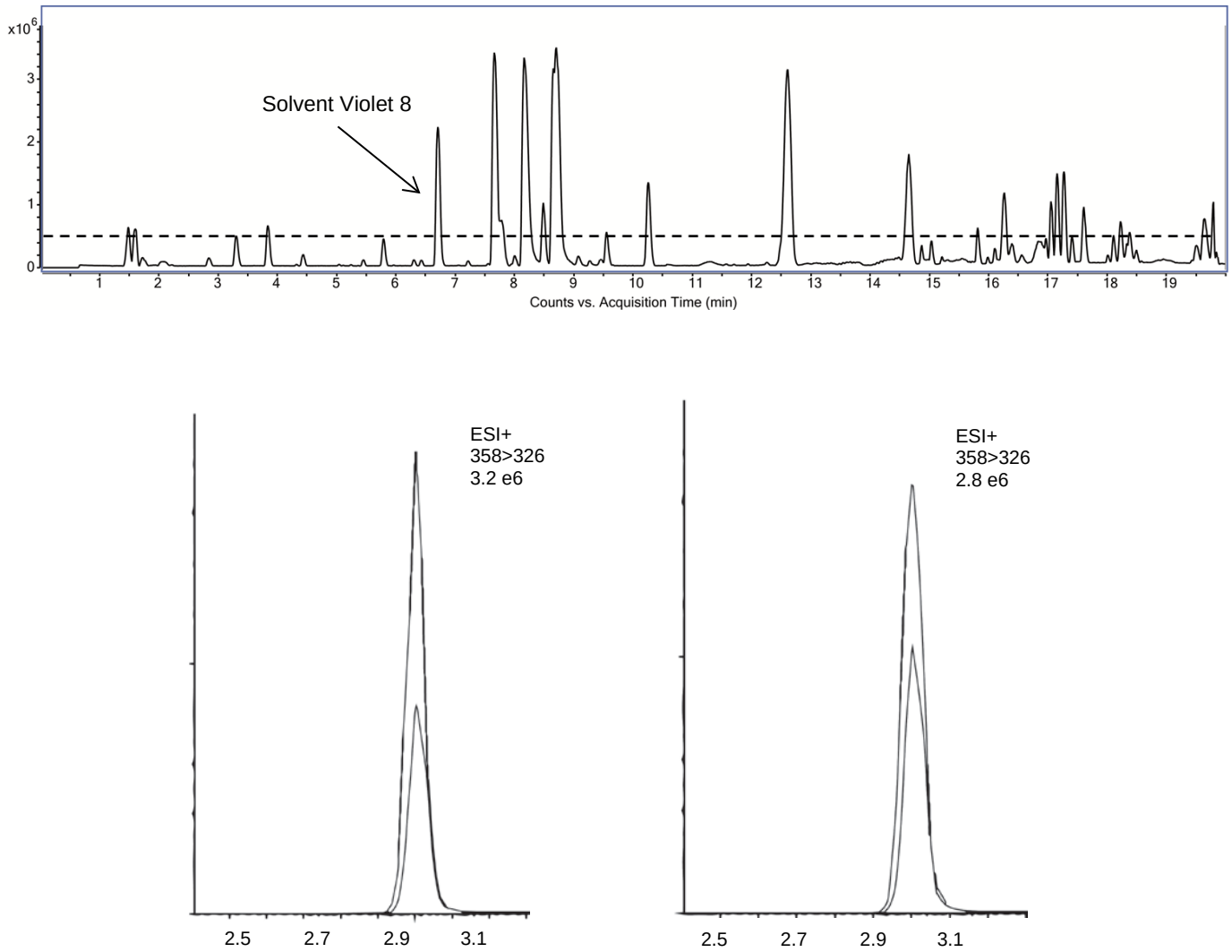
One of the major advantages with the identification of unknowns by GC-EI-qTOF is the standardized ionization mode, which enables searches in vast mass spectral libraries and a largely automated process. However, due the severe fragmentation that follows this ionization, the molecular ion is often small or even not visible in the spectra. In comparison, in spectra acquired by UPLC-ESI-qTOF, the molecular ion is often clearly visible. Though, as this ionization technique is not standardized and therefore there are no vast and generic libraries available, we developed a customized database containing approximately 2300 matrix relevant entries. Data from extracts were acquired in both ionization polarities, to increase the number of compounds to be detected, as well as at three levels of collision energies, see Bengtström et al. (in preparation).

3.3 Selection of compounds

In total, 35 compounds were tentatively identified by the two separation methods. A comprehensive list of tentatively identified compounds in extract from the microwave popcorn bag is presented in Appendix A. In order to select compounds with unknown toxicity for further investigations, we used an in-house quantitative structure–activity relationship (QSAR) database to search for correlations between tentatively identified compounds (see Appendix A) and predicted Ames positives for specific classes of known procarcinogens. The database includes model predictions based on Ames in vitro mutagenicity data, as described in detail in Jónsdóttir et al. (2012). Out of the list of tentatively identified compounds; two compounds were selected for further testing by QSAR modelling as well as reviewing toxicological literature. These two compounds, Leucocrystal violet and Solvent violet 8 are triaminophenylmethanes previously associated with genotoxic effects (Littlefield et al. 1989; Littlefield et al. 1985).

3.4 Quantitative analysis

The use of accurate mass and isotopic pattern is sufficient for screening purposes but not for a confirmed identification (Kind & Fiehn 2007; Ojanperä et al. 2012). Extracts and analytical standards of the two selected compounds were therefore analysed by UPLC-MS/MS for verification of identity. Both of the selected compounds; Leucocrystal violet and Solvent Violet 8 were confirmed in the extracts when relative Rt, product ions and ion ratios were compared to those of the standards. In addition, both compounds had an entry in the customized database and are reported as being used to print unbleached paper. The dark violet colour added by the dyes appears black when printed on darker surfaces such as unbleached paper.



177

178 Figure 2. a) Base peak chromatogram of extract from the bulk part of the microwave popcorn bag
179 analysed by ??-qTOF in ?? mode. The cut-off is represented by the dotted line. Solvent Violet 8 is
180 indicated by an arrow. b) Retention time and ion transitions for the standard of Solvent Violet 8
181 (200 ng mL^{-1}) obtained by UP LC-MS/MS in MRM mode. c) Retention time and ion transitions for
182 the extract of the bulk containing Solvent Violet 8 obtained the same method as the standard

183

Concentrations of the confirmed compounds as well as the limit of detection (LOD) and limit of quantification (LOQ) in the tested extract are presented in Table 1.The calibration curves for the methods were established by plotting the peak area versus concentration. All quantified compounds showed acceptable linearity (R^2>0.98, not weighted, not forced through 0) in the examined range. However, since only a low concentration of Leucocrystal Violet was found in the extract, it was decided not to test this compound in the mutagenicity tests.

Table 1.Tentatively identified compounds selected for further investigation.

Compound	CAS number	LOD/LOQ (ng mL^{-1})	Conc. in extract (µg mL^{-1})	Confirmed	Additional information
Solvent Violet 8	52080-58-7	0.5/5	300	Yes	Dye used in printing inks. Customized database entry.
Leucocrystal Violet	603-48-5	1/10	0.19	Yes	Dye used in printing inks. Customized database entry.

3.5 Verification of toxicity

The analytical standard of Solvent violet 8 was tested in concentrations correlating to those quantified in the extract, see Table 1.

4 Conclusions

In this study mutagenic effects of ethanol extracts from a microwave popcorn bag were observed using a microsuspension version of the *Salmonella*/microsome assay.

When the testing an analytical standard of Solvent Violet 8 is completed, it will be possible to evaluate if mutagenic effects could be fully or partially explained by the dye. The results from our study show that the procedure of bioassay guided screening in combination with orthogonal hyphenated HRMS analyses is a can be used for the detection and identification of unknown compounds with potential mutagenic effects in paper and board FCMs. Future studies would

involve testing this strategy on a larger number of microwave popcorn bags to elucidate whether this is a pervading issue for this type of paper products. In addition, testing more types of products will also increase the knowledge on whether paper and board contain mutagenic compounds, for the identification of such compounds and possibly also to avoid mutagenic compounds in FCM. In the future more realistic migration studies on the identified compounds should be performed in order to be able to make a risk assessment.

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Appendix A: Supplementary data

Supplementary data associated with this article can be found in Appendix A

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9.9

Appendix E

Comprehensive lists of compounds tentatively identified in fractions with positive toxicological effect in mutagenicity test (Ames test).

Supplementary materials: Tentatively identified compounds

Data presented in this Supplementary material are the results obtained from the tentative identification process of fractions with positive toxicological response. Cut-offs are indicated as dotted-lines in the chromatograms.

Indicated in the columns are:

Compound name

CAS number: if available

Molecular formula

Retention time: In respective method

Ionization mode: GC-EI, LC-ESI+ and LC-ESI-

Customized database hit: only used for compounds identified in LC-ESI+ or LC-ESI-

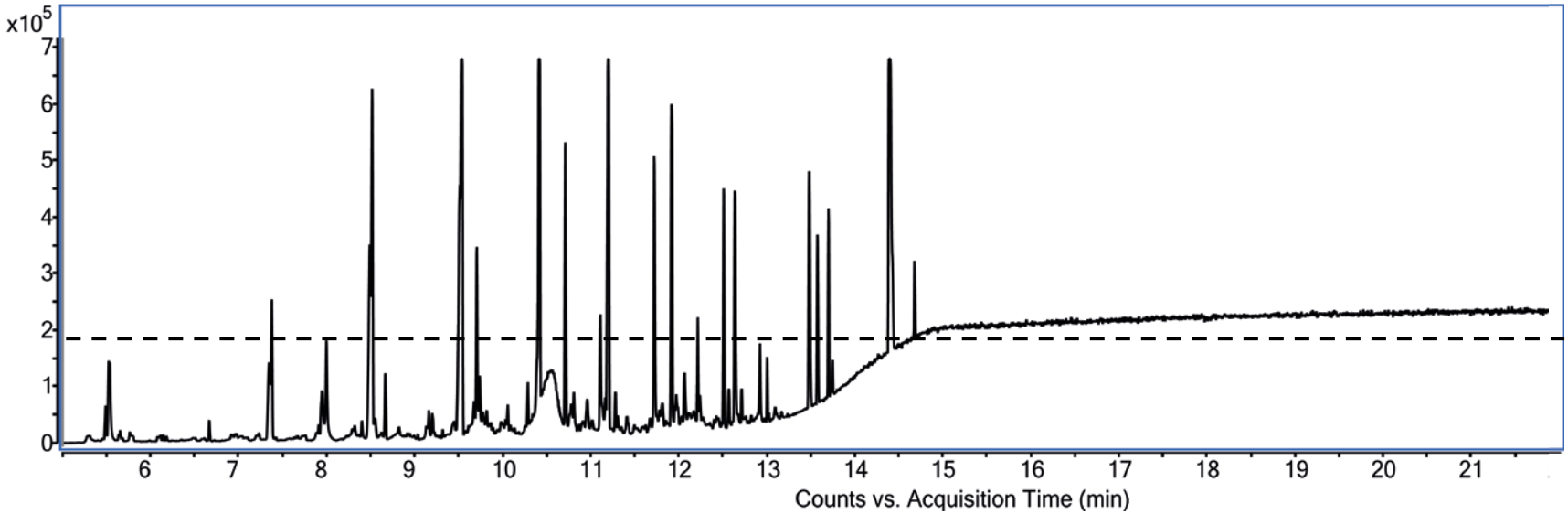
Number of ions: only used for compounds identified in LC-ESI+ or LC-ESI-. Adducts (if present) are also registered as these could facilitate localization of molecular ion.

Additional information: Fragmentation, relevant usage in paper and board

S17 bulk extract

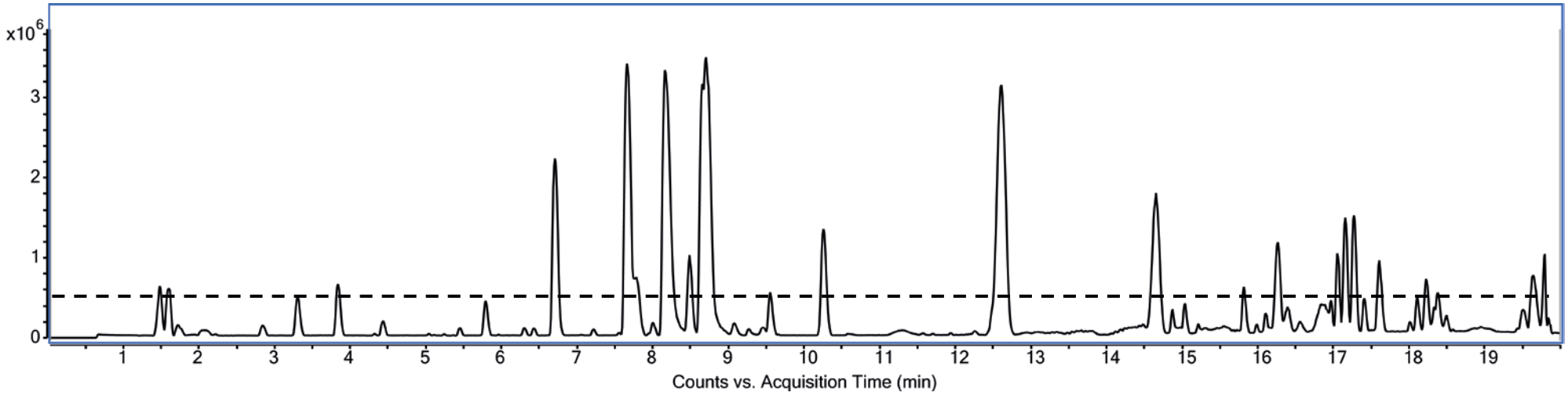
(Positive in mutagenicity tests)

GC-EI-qTOF analysis



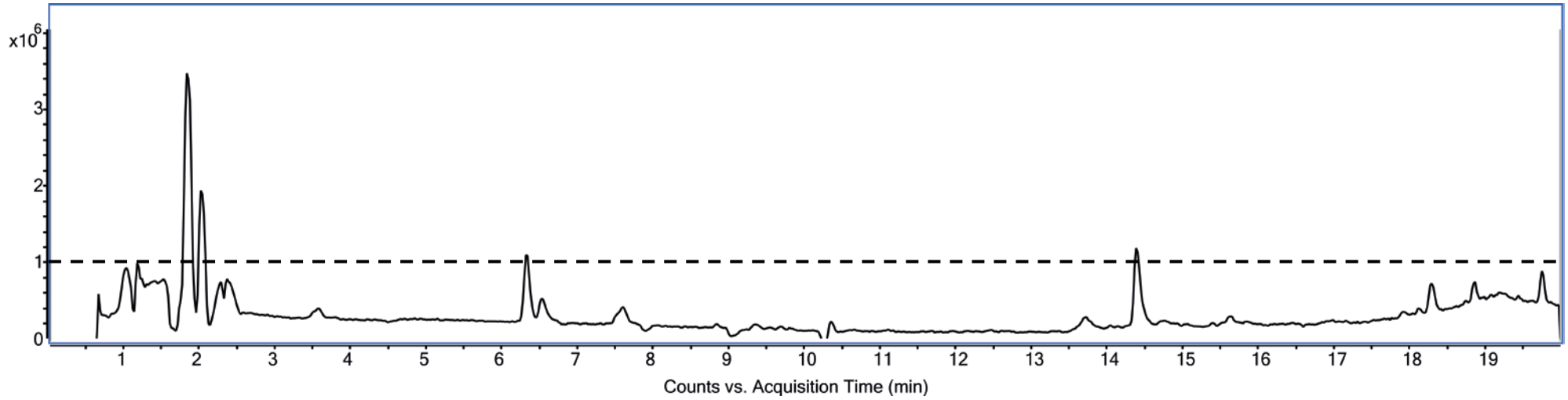
LC-ESI-qTOF analysis

(positive mode)



LC-ESI-qTOF analysis

(negative mode)



Compound	CAS number	Molecular formula	Retention time	EI	ESI+	ESI-	Customized database hit	Number of ions (for LC only)	Additional information
Benzaldehyde, 2,4-dimethyl-	15764-16-6	C9H10O	7.51	x					
Vanillin	121-33-5	C8H8O3	8.1	x					Naturally occurring substance formed wood
Phenol, 2,4-bis(1,1-demethylethyl)-	96-76-4	C14H22O	8.51	x					Used as an antifading agent in printing inks
			9.54	x					Aliphatic fragmentation pattern
			9.77	x					Aliphatic fragmentation pattern
Dodecanoic acid			10.34	x					Aliphatic fragmentation pattern
Phthalic acid, butyl oct-3yl ester			11.11	x					
			11.85	x					Aliphatic fragmentation pattern
Hexadecanoic acid, ethyl ester	628-97-7	C18H36O2	11.93	x					Used in in printing inks
			12.50	x					Aliphatic fragmentation pattern
			12.66	x					Aliphatic fragmentation pattern
			13.52	x					Aliphatic fragmentation pattern
Benzene, 1,3-dimethoxy-5-[(1E)-2-phenylethenyl]-	21956-56-9	C16H16O2	13.59	x					Naturally occurring substance formed in hardwood (stilbene)
			13.64	x					Aliphatic fragmentation pattern
Benzyl butyl phthalate	85-68-7	C19H20O4	14.50	x					Used in in printing inks, resin solvent, paper coatings and in adhesive
Diethylene glycol dobenzoate	120-55-8	C18H18O5	14.71	x					Used as dispersion agent for dyes and plastics
Valeric acid	109-52-4	C5H10O2	1.48		x		x	1 (+NH3)	Used in printing ink compositions
Diethylene glycol	111-46-6	C4H10O3	1.48		x		x	1 (+NH3)	Used in printing ink compositions as solvent
Isovaleraldehyde	590-86-3	C5H10O	1.48		x		x	1 (+NH3)	Used in printing ink compositions
Cyclopentanol	96-41-3	C5H10O	1.48		x		x	1 (+NH3)	Used in printing ink compositions
1,3-Butanediol	107-88-0	C5H10O	1.48		x		x	1 (+NH3)	Used in printing ink compositions
4,4'-Dicyanobiphenyl	1591-30-6	C14H8N2	1.60		x			1	Used in thermal paper resins and adhesives
			1.60		x			1	m/z 387.1526
8-Methyl-2,4-diphenyl-5,6,7,8-tetrahydrochromenium			3.30		x			1	
2,4-Bis(1-phenylethyl)phenol	2769-94-0	C22H22O	3.30		x		x	1	Used in the production of thermoplastic polycarbonate.

Compound	CAS number	Molecular formula	Retention time	EI	ESI+	ESI-	Customized database hit	Number of ions (for LC only)	Additional information
1,3,5-Triphenyl-1,3,5-triazinane	91-78-1	C21H21N3	3.84		x			1	Used as pigment in printing ink (photoreceptor)
3-(1,3-Dibenzyl-imidazolidin-2-yl)-pyridine	693243-72-0	C22H23N3	5.79		x			1	
4-[4-(2-Methyl-2-propanyl)phenyl]-2-phenyl-5,6,7,8-tetrahydrochromenium			6.67		x			1	
Solvent Violet 8	52080-58-7	C24H27N3	7.61		x		x	1	Used as dye
Benzenamine, 4,4',4"-methylidynetris[N,N-dimethyl-	603-48-5	C25H31N3	7.74		x		x		Used as dye
Decyl D-glucopyranoside (Triton X 190)	58846-77-8	C16H32O6	7.74		x			2	Fragments into C10H19O. Used as detergent (cleaning recycled paper and board)
15-Hydroxypentadecanoic acid	4617-33-8	C15H30O3	8.12		x			1	Used in polymerization processes
Leucocrystal Violet	603-48-5	C25H31N3	8.12		x			1	Dye used in printing inks
		C24H37N2O3	8.48		x			2	Fragments into C18H22N3O
		C20H29N5O2	8.64		x			2	Fragments into C9H10.
5-{2-Hydroxy-3-[(4-isopropylphenyl)amino]propyl}-3-methyl-1-oxo-1,5-dihydropyrido[1,2-a]benzimidazole-4-carbonitrile		C25H26N4O2	8.69		x			2 (+NH3)	Fragments into C9H10.
2-Butoxy-2-oxoethyl butyl phthalate	85-70-1	C18H24O6	9.54		x			4	Fragment into C8H4O3, C7H4O, C6H5
			10.24		x			5	m/z: 454.3387, 410.3131, 366.2872, 322.2607, 199.1708
2,4,7,9-Tetramethyl-5-decyne-4,7-diol	126-86-3	C14H26O2	12.55		x			1	Used in printing ink
			12.55		x			2	C27H49N2O5, fragments into C25H42NO4

Compound	CAS number	Molecular formula	Retention time	EI	ESI+	ESI-	Customized database hit	Number of ions (for LC only)	Additional information
Palmitoleate	2091-29-4/ 373-49-9	C16H30O2	14.60		x		x	1	Used in printing ink compositions
			14.60		x			1	C27H51N2O4
			14.60		x			1	C25H47N2O4
(2E,4E)-N-Isobutyl-2,4-hexadecadienamide	54794-69-3	C20H37NO	15.80		x			1	
Oleic acid	112-80-1		16.25		x			1	Used in printing ink compositions
Decyl methacrylate	3179-47-3	C14H26O2	17.06, 17.16, 17.27		x			1	
			17.06		x			1	C30H63N4O8
Gadoleic acid	29204-02-2	C20H38O2	17.60		x			1	Used in printing ink compositions
			1.85			x		1	C20H22NO6
D-(-)-Mannitol	69-65-8		1.85			x			

National Food Institute
Technical University of Denmark
Mørkhøj Bygade 19
DK - 2860 Søborg

Tel. 35 88 70 00
Fax 35 88 70 01

www.food.dtu.dk

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