Improvements in the analysis of food contaminations deriving from packaging materials

Dissertation zur Erlangung des Doktorgrades der Naturwissenschaften (Dr. rer. nat.)

Fakultät Naturwissenschaften
Universität Hohenheim

Institut für Lebensmittelchemie

vorgelegt von

Thorsten Rothenbacher

aus Konstanz

2009

Die vorliegende Arbeit wurde am 03.12.2009 von der Fakultät Naturwissenschaften der Universität Hohenheim als „Dissertation zur Erlangung des Grades eines Doktors der Naturwissenschaften“ angenommen.

Die mündlichen Prüfung fand am 28.01.2010 mit folgenden Prüfern statt:

Dekan:		Prof. Dr. H. Breer
Berichterstatter:	1. Prüfer:	Prof. Dr. W. Schwack
	2. Prüfer:	Prof. Dr. H.-D. Isengard
	3. Prüfer:	Prof. Dr. W. Vetter

Preliminary Remarks

Parts of the work presented in this thesis have already been published or submitted to international peer reviewed journals and/ or were presented on scientific conferences as oral or poster presentations:

Full papers:

1. T. Rothenbacher, M. Baumann and D. Fuegel. 2-Isopropylthioxanthone (2-ITX) in food and food packaging materials on the German market. *Food Additives and Contaminants* 2007; **24**: pp. 438-444
2. T. Rothenbacher, W. Schwack. Determination of epoxidized soybean oil by gas chromatography/single quadrupole and tandem mass spectrometry stable isotope dilution assay. *Rapid Communications in Mass Spectrometry* 2007; **21**: pp. 1937-1943
3. T. Rothenbacher, W. Schwack. Non-targeted multi-component analytical screening of plastic food contact materials using fast interpretation of deliverables via expert structure-activity relationship software *Journal of AOAC INTERNATIONAL* 2009; **92** (3): pp. 941-950*
4. T. Rothenbacher, W. Schwack. Rapid and nondestructive analysis of phthalic acid esters in toys of poly(vinyl chloride) by direct analysis in real time–single quadrupole mass spectrometry. *Rapid Communications in Mass Spectrometry* 2009; **23**: pp. 2829–2835
5. T. Rothenbacher, W. Schwack. Rapid identification of additives in poly(vinyl chloride) lid gaskets by direct analysis in real time ionisation and single-quadrupole mass spectrometry. *Rapid Communications in Mass Spectrometry* 2010; **24**: pp. 21-29

* Reprinted from The Journal of AOAC INTERNATIONAL, Rothenbacher, T., and Schwack, W. (2009) J. AOAC Int. 92, 941. Copyright 2009 by AOAC INTERNATIONAL.

Poster presentations:

1. “Schnelles Screening mit DART-MS am Beispiel von Weichmachern in PVC“. Poster at the Deutscher Lebensmittelchemikertag 2009 (14.-16. Sept., Berlin) “
2. “Nachweis von Phthalsäureestern in Spielzeug und Babyartikeln aus PVC mit DART™-MS in 4 min“. Poster at the Deutscher Lebensmittelchemikertag 2008 (08.-09. Sept., Kaiserslautern)
3. “Identifizierung von Additiven in Deckeldichtungen mit DART™-MS in 5 min?“. Poster at Deutscher Lebensmittelchemikertag 2008 (08.-09. Sept., Kaiserslautern)
4. “Bestimmung von epoxidiertem Sojaöl mittels GC-MS(/MS) SIVA”. Poster at Deutscher Lebensmittelchemikertag 2007 (10.–12. Sept., Nürnberg-Erlangen)

Oral presentations:

1. “Schnelle und zerstörungsfreie Analytik von Phthalsäureestern in Spielzeug und Babyartikeln mittels DART-MS”. Presentation at ANAKON 2009 (17.-20. Mar., Berlin))
2. “Schnelles Screening für Weichmacher in Spielzeug und Deckeldichtungen aus PVC mittels DART-MS“. Presentation at Tagung des Regionalverbandes Südwest der Lebensmittelchemischen Gesellschaft 2009 (02.-03. Mar., Koblenz)
3. “Bestimmung des Weichmachers ESBO mittels GC/MS-SIVA“. Presentation at Tagung des Regionalverbandes Südwest der Lebensmittelchemischen Gesellschaft 2007 (05.-06. Mar., Gießen)

Contributions to the dissertation

All experimental and analytical work including analyses and interpretations of all obtained data was performed by Thorsten Rothenbacher, except otherwise mentioned in this section. Moreover, conceptions and the preparations of the original versions of the manuscripts leading to publications originated from his initiative. Professor Dr. W. Schwack was the supervisor of this work and helped to find optimum formulations and forms for the English text manuscripts, except for paper 1. In case of paper 1, samples selections, analysis strategy and the sample preparation for food contact materials were developed by Thorsten Rothenbacher and sample preparation in foods by Rüdiger Weishaar. Practical work was done by lab assistants of the CVUA Stuttgart under supervision of Thorsten Rothenbacher, Dr. Markus Baumann, Diane Fügel, and Rüdiger Weishaar. The publication text was from Thorsten Rothenbacher with suggestions from Dr. Markus Baumann and Diane Fügel. Sample preparation of 2-isopropylthioxanthone in foods and the spiking procedure was from Diane Fügel, the instrumental analysis part was written by Dr. Markus Baumann. For paper 2 the practical works according the sample preparation and the solving of analytical standards in isooctane was carried out by lab assistants of the CVUA Stuttgart under the supervision of Thorsten Rothenbacher. In case of paper 3 practical work was made by lab assistants of the CVUA Stuttgart under the supervision of Werner Altkofer and Thorsten Rothenbacher according sample preparation, preparation of $^{13}C_{18}$-labelled ethyl 9,10,12,13-diepoxyoctadecanoate and $^{13}C_{18}$-labelled methyl 9,10,12,13-diepoxyoctadecanoate.

Table of Contents

I. General Introduction

I.1. Constituents of plastic materials

The packaging of food and beverages composes about 60% of the European packaging market and seems to be growing.[1,2] The principal intrinsic requirements for food packaging materials are a control over transfer of moisture and other gases/vapours, a wide temperature range in storage and use, low cost and the absence of toxic constitutents.[3] The packaging of food is needed to prevent the food of unintentional changes. It protects from mechanical damage, microbiological interference and chemical degradation such as oxidation, moisture transfer, and ultraviolet light. Furthermore it is used to transport information that concerns consumers, food industry and plastic recycling industry.

I.1.a. Polymers

Plastics primarily consist of polymers, in which each molecule represents a long chain or a network of repeating units, depending on the monomers. In terms of chemical composition, there are the homopolymers and the heteropolymers. The former exist of the same repeating block and the latter are polymers with different building-block units, regulary or irregulary distributed throughout their lengh. Copolymers, as an example for heteropolymers, possess exactly two different monomers which are polymerised together. Furthermore plastic polymers can be divided into two groups: i) polymers which are linear and extend in one dimension and ii) into cross-linked polymers that built more dimensional giant molecules. Different production parameters also influence the polymer molecule. Low density polyethylene, e.g., is processed between 1-3*105 kPa and 100-300 ºC and therefore contains chain branches of different length. Contrary in high density polyethylene chain branches are inexistent. All these parameters influence the physical properties of the polymer.[4]

I.1.b. Non polymeric constituents

Other constituents of plastics may derive from the production process like polymerisation residues including monomers, oligomers, catalysts, solvents,

emulsifiers and wetting agents, or can be raw material impurities, plant contaminants, inhibitors, decomposition and side reaction products. In order to change characteristics of polymers and to optimise production processes, additives are added. These additives can be subdivided into different groups which provide different efforts:

Nucleating agents are added to trigger a heterogeneous nucleation of the plastic melt and to give crystals of regular size. They can be organic compounds like salts of organophosphates or benzoic and phthalic acid, but also inorganic compounds like silica for example, used as finely ground filler. Utilisation is practiced in amounts 0.1-0.3% in polypropylene (PP), polyamide (PA) and polyethylene terephthalate (PET).[5]

Lubricants affect the melt rheology and facilitate processing due to decrease of internal and external friction. The former improves the polymeric chain movements and the latter the friction between the plastic surface and the processing equipment. Typical lubricants are fatty alcohols C_{12}-C_{22}, fatty acids C_{14}-C_{18} and their esters with fatty alcohols, glycerol or pentaerythritol, amides or diamides and other similar molecules. They may be used in all plastics, except PA and PET in concentrations up to 1.5%.[5]

By use of friction or rubbing of polymeric surfaces against each other, static electricity can be generated. *Antistatic agents* reduce the chargeability of plastic material because they form a conducting layer through the absorption of atmospheric moisture on the surface.[4] They can be applied on the plastic's surface or into the plastic mass during the processing.[5] If an "internal antistatic" is used and the plastic's surface is cleaned, the inner antistatic agent may migrate onto the plastic material's surface and build a film similar to the former one.[6] Typical external antistatic agents are cationic- and anionic active substances like quaterny ammonium or sulfonium salts of hydrocarbons C_{10} and typical internal antistatic agents are non-ionic agents like ethoxylated fatty alkylamines. Their content in mostly all plastics is about 0.1 to 2%.[5]

Blowing or foaming agents can mainly be used in polyvinyl chloride (PVC) and polystyrene (PS). They reduce consumption of raw materials, improve moisture transfer properties of the plastic material and provide cushioning effects. Depending on the processing physical foaming agents like carbon dioxide, nitrogen or hydrocarbons C_4-C_5 are common. Due to global warming, ozone depletion and the Montreal Protocol chlorinated and fluorinated hydrocarbons should no longer be used.[7,8] There exist also various chemical foaming agents that generate inert gases and are mixed into the plastic mass during the processing, like e.g. azodicarbonamide, sodium carbonate and diisopropylhydrazodicarboxylate.

Plasticisers are the probably most known additives. They improve processibility, flexability and stretchability of the polymer and are used in PA and PVC.[5] Plasticisers divide into two groups, the internal and the external plasticisers. Internal plasticisers are held in polymer systems by chemical bonds, while external plasticisers maintain their molecular identity in the polymer system and their comparability with it by hydrogen bonding and Van der Waals attraction. Well known plasticisers are phthalic acid esters, di-(ethylhexyl)adipate, and epoxidized soy bean oil.[9-11] As PVC may contain plasticisers up to 50%, low molecular weight plasticisers became its most problematic additives.

Plastics have to be stabilised to withstand chemical and physical stress during processing, storage and application. Therefore *stabilisers* defend from deteriorating agents like oxygen, high-energy radiations and heat. According their mechanisms, they can be subdivided into antioxidants, photoantioxidants, photostabilisers, heat stabilisers and antiacids. Other kinds of stabilisers like metal deactivators, antiozonants, fire retardants and biostabilisers are not used in food contact materials.[5] By use of thermal processing or triggered by UV-radiation, radicals are produced in the polymer. These macroradicals form alkylperoxyls which abstract an H-atom from another molecule, therefore forms another macroradical and turns into a hydroperoxide. The hydroperoxide also decomposes into radicals which may continue the typical radical chain reactions or release smaller molecules via ß-scission.[12] In order to prevent these reactions, there are so called *primary or chain breaking antioxidants* which are suitable to stabilise radicals, like steric hindered phenols and aromatic amines.[13] The concentration is up to 0.3% in plastic.[5]

Secondary or hydroperoxide decomposing antioxidants reduce hydroperoxides to alcohols. Usual secondary antioxidants possess a thioether or a phosphit group that will get oxidised during hydroperoxide decomposing.[13] Substances which provide similar functionality, but additionally are more photostable than common antioxidants, are the *photoantioxidants*. These are hindered amine stabilisers which often posses a 2,2,6,6-tetramethylpiperidine moiety. Photoantioxidants known to be very effective are oligomeric molecules, which are used in a concentration range from 0.2 to 0.5%.[14-16] Chromophores which are often impurities in plastic material, may trigger the photodegradation by the formation of singlet oxygen and acceleration of hydroperoxide decomposition. To prevent, *UV absorbers* which absorb from wavelenghs of 200 up 400 nm are used. In these molecules, like for example benzophenone, the energy of the absorbed light leads to mesomeric changing of the molecule and finally to radiationless transition of the molecule to the ground state. UV absorbers are used in a range of 0.25 to 0.5 %.[5,17] Antioxidants are used in almost all kinds of plastics. *Antiacids* neutralise acids arising from residues of catalysts or from thermodegradation of PVC and therefore are used especially in polyolefines or PVC. They can be for example zinc or calcium salts of weak organic acids or fatty acids, epoxidised oils and inorganic salts.[18]

If products with high water content are packed, small water droplets may condensate on the inside surface of the plastic film and becloud bright packagings. *Antifogging agents* are used to prevent a clear view of the packaging. They are non-ionic ethoxylates or hydrophilic fatty acid esters like sorbitol stearate and are applied internally or on the surface of the packaging material in ranges from 0.5 to 4%.[4,5]

Otherwise there are packaging materials that should not be clear, but possess other optical properties. Therefore *dyes and pigments* are applied. Pigments include a wide range of both organic and inorganic products, and are dispersed into a polymer in its liquid phase. After the polymer solidifies, the dispersed pigment particles are retained physically within the solid polymer matrix. In contrast, dyes are exclusively coloured organic compounds and are dissolved more or less completely in a polymeric mass and are usually retained as a result of intermolecular forces. Additionally there exists also fluorescent or so called optical brightening or whitening agents which are used for white coloured plastics. White plastics possess commonly

a strong absorption band in the UV region of the absorption spectrum which tails into the visible region, leading to a yellowish impression. Whitening agents absorb UV radiation and re-emit the energy by means of fluorescence in the blue to blue-violet region of the visible spectrum and therefore cause brilliant white plastics with a bluish impression.[4,5,19]

I.2. Regulation of plastic food contact materials by the European Union

I.2.a. General regulations

Generally, food contact materials (FCM) are regulated by the so called "Framework Regulation" of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food.[20] Its purpose is to ensure functioning of the internal market, whilst a high level of health protection and the interests of consumers are maintained. Therefore, article 3 forces the general requirements "that materials and articles, including active and intelligent 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; or bring about an unacceptable change in the composition of the food; or bring about a deterioration in the organoleptic characteristics thereof". Very important for plastic material also is article 5, which enables specific measures for a list of substances and their purity for use in the manufacturing of materials and articles. It authorizes the use of specific migration limits (SMLs) and overall migration limits (OMLs) and the promulgation of rules to check on compliance, for example for sample collection and the methods for analysis. In absence of these specific measures, article 6 allows national provisions in these terms, provided they comply with the rules of the treaty.

In order to define "good manufacturing practice" the regulation 2023/2006 forces an effective quality assurance and control system at all sectors and stages of the manufacturing, processing, and distribution of FCM, except for the production of starting substances.[21] The term "starting substance" is defined according the Commission as any substance, regardless of its chemical nature (e.g. compound, mixture, monomer, oligomer, prepolymer natural or synthetic macromolecules), that

is used in any type of polymerisation process including the modification of natural or synthetic substances.[22]

Several measures for plastic FCM have been released in the Commission directive 2002/72/EC in its actual amended version.[23-28] This directive covers mono- and multilayer materials and articles exclusively made of plastics, but does not cover those composed of two or more layers, of which one or more does not exclusively consist of plastics, even if the one intended to come into direct contact with food does consist of plastics. For this case, one exception is given for plastic layers that form gaskets in lids. Article 2 of 2002/72/EC declares an OML of 60 mg kg^{-1} or 10 mg dm^{-2} surface, depending on the product. Furthermore the use of starting substances and additives is limited by force of article 3 and 4 to only those substances listed in this directive with the requirement that given SMLs are maintained. This implements the idea of a positive list of substances, which exclusively have to be used in the manufacturing process. This idea was created 1972, but not implemented into legislative until 2008, but will get into force on 1st January 2010 and therefore can be regarded as a milestone.[29] The directive 2002/72/EC also applies in articles 3 and 8 rules for the verification of the compliance with the migration limits and therefore refers to its annex I and the directives 82/711/EEC and 85/572/EEC, in which rules for migration testing are given.[30-33] Regulations for layers which are not in direct contact with food and are separated from the food by a functional barrier are given additionally in article 7a.

I.2.b. Regulations covering specific substances

Beneath the already mentioned regulation and directives, there are some regulations covering specific substances. Directive 78/142/EEC limits the presence of vinyl chloride monomer to 1 mg kg^{-1} in materials and articles prepared with vinyl chloride polymers or copolymers and to a not detectable migration in food, defined as 0.01 mg kg^{-1}.[34] Methods for its analysis in plastics and in food are given in the directives 80/766/EEC and 81/432/EEC.[35,36]

The use of the epoxy derivatives bis(hydroxyphenyl)methane bis(2,3-epoxypropyl)ether (BFDGE) and other novolac glycidyl ethers (NOGE) is not allowed in food contact materials, and SMLs are given for 2,2-bis(4-hydroxyphenyl)

propane bis(2,3-epoxypropyl) ether (BADGE) and its water and hydrochlorine adducts in the Commission regulation (EC) no. 1895/2005.[37]

In order to provide time for the lid producing industry to solve migration problems of additives in fatty food, the regulation no. 372/2007 in its amended form lays down transitional SMLs until 30 April 2009 for plasticisers in gaskets of lids.[38,39]

The use of recycled plastic material in the manufacturing process of FCM and especially conditions and applications for the authorisation of such a process are treated in the regulation no. 282/2008.[40]

As a *future scope* the Commission directive 2002/72/EC should be changed in the terms of regulating also plastic layers in multi-material multi-layer materials and articles.[41] Authorised substances for the manufacturing process shall be presented including their functions and limitations in the food contact material. Also the guidelines on migration testing will be more concrete and adapted to the actual level of knowledge. For some food, e.g. dried products, the corresponding food simulant will be a blend of poly(p-phenylene oxide) with polystyrene, traded under the name Tenax®.[42]

In the case of Germany, the implementations of the directives mentioned above into national legislation are made by the Bedarfsgegenständeverordnung.[43]

I.3. Substance transfer into food and its impact on human health

I.3.a. Migration

To provide a cost-effective production and wide functionality, the packaging industry increased its portfolio over the last decades. As a consequence of an increasing number of substances used for food packaging and efforts in the analysis of food contact materials, concerns with regard to harmful substances in FCM have also arisen in the last decades.[44] They rely on the fact that there is a possibility for substances to leave the packaging material and diffuse into the foodstuff. This process of food contamination is called *migration* and may alter organoleptic and toxicological properties of the foodstuff.[5,22,45-47]

Migration occurs as foreseeable physical process and can be divided into two parts: i) diffusion inside the polymer, followed by ii) a partition of the migrant in the two phase system food and polymer. The diffusion follows in most cases the Fick's laws.[48] As a consequence the process of migration can be *mathematically modelled* in complex equitations. The migrated amount of a substance through the contact surface depends on the initial migrant concentration in the packaging material, the volume and density of packaging material and food, the *diffusion coefficient* of the migrant in the packaging material and the *partition coefficient* of the migrant between the FCM and the food.[49] The partition coefficient drastically may depend on temperature, chemical groups and the molecular size and structure of the migrant as well as the fat content of food and the degree of crystallinity of the food structure.[50] In case of multilayer materials, additionally the diffusion coefficients, density and thickness of each layer and partition coefficients of the migrant between adjacent layers have to be considered.[49] Comparisons between analysed and calculated data of migration proved in general a good correlation between the two methods, but sometimes also large deviations, like for example a predicted migration of 57 µg kg^{-1}, but measured to be 157 µg kg^{-1}.[51,52] Therefore, the European legislation allows in article 8 of the directive 2002/72/EC migration modelling to prove compliance, but not to demonstrate non-compliance.[53]

In order to prevent substances of the packaging from migrating into food, *functional barriers* can be incorporated into food contact materials, lowering diffusion of migrants.[54] But even in case of absolute powerful functional barriers, a contamination via the set-off procedure is possible.[55] The *set-off* can be defined as the unintentional transfer of substances, which derive from the external surface, to the inner food-contact surface. Possible mechanisms in this process are blocking, rubbing, peeling and migration by diffusion.[56] By use of these mechanism even substances that are separated via an effective functional barrier can contaminate food, like 2-isopropylthioxanthone deriving from the outside of multilayer cartons, for example.[57]

I.3.b. Risk exposure

The migration of toxic substances from packaging into food may harm human health. This *risk* can mathematically be described as following: Risk = hazard (toxicity) • exposure.[49]

The exposure of a migrant depends on the sum of the products that contain the migrant, of the concentration of the migrant in eaten foods and the amount of each eaten food. The *daily dose of an individual* can be expressed as following:

$$DD_{jk} = \frac{1}{Wj} \sum_{l=1}^{n(k)} w_{jkl} c_{jkl} ,$$

where DD_{jk} is the daily dose for an individual j on day k consuming up to n(k) items on that day. W_j is the weight of the individual j and c_{jkl} is the concentration of the migrant in the food item l, whilst w_{jkl} denotes the weight of item l on day k eaten by the individual j.[58]

To judge toxicity hazards, *acceptable daily intake* (ADI) or *tolerable daily intake* (TDI) values are used, which have been released by the Scientific Committee on Food and the European Food Save Authority (EFSA). ADI or TDI values generally derive from the dose at which adverse effects are not observed in toxicity tests (NOAEL) for residues and contaminats, respectively. The NOAEL is divided by a safety factor, usually 100, to get the ADI or TDI value.[59] In order to create *specific migration limits*, the ADI and TDI values are multiplied with a factor of 60. This factor is derived from the convention that a person of 60 kg daily could ingest up to 1 kg of the contaminated foodstuff.[22] In some cases of migrants in fatty food, fat reducing factors (FRF), which result in higher SML values, were released. They consider that the total daily fat consumption by European adults does not exceed 200 g of fat per person per day.[60]

I.4. Plastic food contact material analysis

First of all potential migrants and their toxicological potency have to be identified in food contact materials. In case of toxicological outcomes, the migrants have to be determined in foods which have been in contact with the packaging. The literature on migrants' analysis is scant. There is more emphasis concerning

migrations into food simulants than into food itself and analytical methods are still in the research and development stage.[61] The analytical procedures typically involve sample preparation, extraction, clean up and a final determination step. For the analysis in polymer materials solvents are used that extract the migrant or dissolve the polymer and migrant, followed by precipitation of the polymer.[62] In foodstuffs extraction is also done by solvents, but often solid phase extraction (SPE) or size exclusion or gel permeation chromatography (SEC or GPC) as cleaning step is necessary.[61] For determination most applied instruments nowadays are gas or liquid chromatography coupled with mass spectrometry (GC/MS or LC/MS).[47,63]

I.4.a. Targeted analysis

As food additives extremely vary in their physical and chemical properties, a “multi method” to determine all additives does not exist. An actual example for the efforts that have to be made to analyse regulated substances is the analysis of gaskets in lids of metal twist closures for glass jars. The gaskets usually are made of PVC that contains plasticisers, stabilisers, slipping and blowing agents and pigments. Only focussed on plasticisers that migrate into food and possess an SML, 1,2-cyclohexane-dicarboxylic acid,1,2-diisononyl ester (DINCH), bis(2-ethylhexyl) adipate (DEHA), dibutyl sebacate (DBS), tributyl O-acetylcitrate (ATBC), and the phthalic acid esters benzyl butyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), di-"isodecyl" phthalate (DIDP), di-"isononyl" phthalate (DINP) and dioctyl phthalate (DNOP), acetylated mono- and diglycerides of fatty acids (AcPG), epoxidized soybean oil (ESBO) and polyadipates (PAD) are specific regulated and have to be checked.[38,64] Phthalic acid esters, DEHA, ATBC and DBS mainly are extracted from food, cleaned via GPC or SPE and determined via GC/FID or GC/MS, if available by use of stable isotope dilution assay.[65-70] There also exist multi methods that determine phthalates, DBS, DEHA and ATBC together or more special methods that use injector-internal thermal desorption GC/MS or headspace solid-phase microextraction.[71-75] AcPG can be a mixture of different compounds or a single substance like octadecanoic acid-12-(acetyloxy)-2,3-bis(acetyloxy)propyl ester and can be determined in food by injector-internal thermal desorption GC/MS or by food extraction, subsequent GPC and GC/MS analysis.[76,77] Since soy bean oil mainly consists of triglycerides of linolenic, linoleic, oleic, stearic and palmitic acid, there is a vast variety of different triglycerides in ESBO and its analysis is more difficult.[78] Beneath one method that proposes extraction and direct analysis by use of liquid

chromatography- electrospray ionisation- tandem mass spectrometry (HPLC/ESI-MS/MS), GC methods seem to be preferred.[79] Thus for use of GC separation, a transesterification step is necessary, followed by different processing depending on the instrumental setup.[80-82] Additionally, it should be controlled via an analysis of the fatty acids composition, that ESBO was determined and not epoxidised line seed oil, because the latter is not covered by Commission directive 2002/72/EC. The exact analysis of PAD is a challenge. After isolation of the part of PAD below 1000 Da by SEC, the extract needs to be transesterified and determined by use of GC/MS. Afterwards the amount of migrated PAD has to be calculated by use of a conversion factor (CF) which depends on the used PAD and takes into account the relation of the different molecular weight of the transesterification product and the original PAD.[83] The CF therefore has to be analysed separately, which was not considered in previous released methods.[84,85] To put it in a nutshell, for the analysis of the regulated plasticisers of gaskets which migrated into food, strenuous efforts have to be made. In spite of these efforts, the results do not consider impurities of the additives nor decomposition and reaction products. Regarding ESBO, for example, structural and toxicological reaction products are formed in toxicological possibly relevant amounts, especially during heating of PVC, which were impossible to identify until now and actually are not regulated.[86,87] This leads to the more complicated kind of analysis, the identification of health hazards deriving from migrants.

I.4.b. Non targeted analysis

First of all the sensivity of an analysis method has to be considered. For this purpose, the US Food and Drug Administration established 1995 the *threshold of regulation*, which allows migrants whose dietary concentrations are up to 0.5 µg kg^{-1} with the reserve, that carcinogens or substances that may be carcinogens are excluded from this regulation.[88] On base of the works of Kroes et al., a task force of the International Life Sciences Institute released a *threshold of toxicological concern* that depends on the structure of the substance and is applicable to chemicals with low mass and known structure, but does not cover allergenicity, accumulation and endocrine disruption.[89,90] Therefore, a substance whose intake does not exceed 1.5 µg day^{-1} and whose structure does not raise concerns for potentional genotoxicity would not be expected to be a safety concern. In case of genotoxic related doubts, there is a negligible risk if the daily intake does not exceed 0.15 µg and the

substance is not an essential metal or metal containing compound, polyhalogenated dibenzodioxin, dibenzofuran or biphenyl, or an aflatoxin-like, azoxy or N-nitroso compound.[90] According the principles for establishing an SML, these values correspond to SMLs of 90 µg kg^{-1} and 9 µg kg^{-1} respectively. The European Commission regulates in directive 2002/72/EC in its actual form, article 7a a SML of 10 µg kg^{-1} for a migrant that is not covered by the positive list, but also excludes from this regulation substances that are classified as proved or suspect 'carcinogenic','mutagenic' or 'toxic to reproduction' according Annex I or VI of Council Directive 67/548/EEC.[53,91] The SML of 10 µg kg^{-1} also applies to a group of compounds, if they are structurally and toxicologically related, in particular isomers or compounds with the same relevant functional group, which drastically may lower the SML for a specific substance that belongs to such a group. To put it in a nutshell, the limit for unregulated substances is very low and therefore a very high sensivity is demanded for the identification of health hazards deriving from migrants.

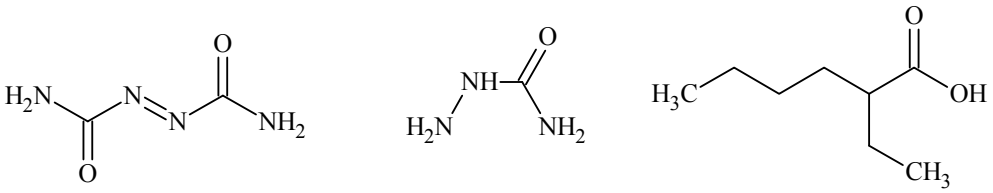
Analysis is complicated in addition by the huge amount of additives used for production of plastic FCM and the fact, that compositions of raw materials are treated by the suppliers as industrial secrets. The Synoptic Document lists all monomers and additives notified to the European Commission in view of their use for FCM.[92] It includes about 3000 substances, while aids to polymerisation, colorants, inks, adhesives and solvents are not mentioned in principle, and the list only presents the notified auxiliary material. Additionally there can also be impurities in additives or possible degradation products. So comprehensive analysis of migrates from food packaging materials is a real challenge.

In order to analyse the toxicological potential of migrants, some different approaches have been released. Short-term toxicity tests can be used, but generally they do not provide the necessary sensivity.[93,94] Another approach is to mark the specific structures of substances, which are responsible for the toxicological properties. However, until now this method does not apply owing to a general lack of known suitable markers and a barely specificity of existing derivatisation reagents.[95] The more classic analytical approach is to fractionate migrants with a molecular mass below 1000 D via GPC or SEC, in order to remove toxically insignificant substances whose absorptions by the human gastrointestinal tract are negligible and

also to clean the migrate from matrix.[22,96] Afterwards the extract has to be analysed by LC/MS or GC/MS. In this case, the operator is often challenged with the identification of a forest of unknown peaks followed by toxicological assessment. The substance identification only by use of mass spectra databases can be considered as critical.[63] Also the limits of detection reported so far, e.g. 20 µg kg^{-1} for compounds in the very unproblematic matrix water, determined after enrichment by solid-phase extraction.[97] Another critical part can be the toxicological assessment due to missing data information.

I.5. An actual example for alarming migrants deriving from one product

There have been a number of alarming findings that caused an increase of research. Lids of glass jars got into analysts' focus in the last years and will now be explained as an example for such processes. In 2003, semicarbazide (SEM, Figure 1, **1**), suspected to be genotoxic and carcinogenic, has been discovered in different kinds of food that were packed in glass jars closed with metal lids.[98]



1 **2** **3**

Figure 1: Chemical structures of SEM (**1**), ADC (**2**) and 2-EHA (**3**)

Further investigations identified SEM as a minor thermal decomposition product of the blowing agent azodicarbonamide (ADC, Figure 1, **2**), that was used in the plastic gasket of the lids for over 20 years.[99,100] Although extended research showed that FCM are not the only source for food contaminations with SEM, lids that were foamed with ADC were banned in the European Community.[101,102] One year after the discovering of SEM in food, 2-ethylhexanoic acid (2-EHA, Figure 1, **3**), a substance supposed to be teratogenic, was found in baby foods and fruit juices.[103] Salts of 2-EHA are used as a stabiliser in PVC.[104] A survey from 2007 of 63 samples from 15 different countries in Europe showed that the intake of 2-EHA deriving from contaminated baby food generally does not exceed the TDI for infants of 6–12 months, and in most cases, the levels of 2-EHA were at 13-fold below the TDI.[77]

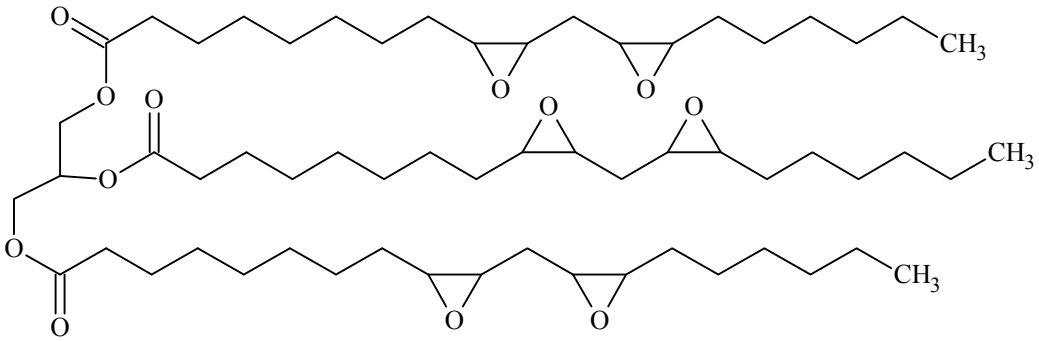


Figure 2: Chemical Structure of the major component in ESBO

In the meantime in 2005 recent publications showed that the analysed content of the plasticiser and stabiliser epoxidised soy bean oil (ESBO, Figure 2) in fatty food far exceeded the levels which had been found before and only two of 86 products complied with the limit of 60 mg kg^{-1} with amounts of ESBO in food up to 580 mg kg^{-1}.[105-107] As a consequence of the non compliance of lids with the SML of ESBO, further investigations on other plasticisers were made. They proved a high migration potential manifold exceeding legal limits for DEHA, phthalic acid esters and their substitution product DINCH. An analysis survey which was published 2006 showed, that DINP and DIDP, both with an SML of 9 mg kg^{-1}, migrated up to 270 mg kg^{-1} and 740 mg kg^{-1}, and DEHP up to 825 mg kg^{-1} (SML 1.5 mg kg^{-1}), respectively.[76] The migration of DINCH and DEHA also exceeded the limits of 60 mg kg^{-1} and 18 mg kg^{-1} with 710 mg kg^{-1} and 180 mg kg^{-1}, respectively. As the lid industry was alarmed and more research on lids showed ways to reduce the contamination, the analysed values decreased, but still lots of lids did not comply with the legal limits.[108-110] In order to give the lid industry a chance to investigate for solutions of these problems, the European Commission slackened the restrictions of the SMLs until 30 April 2009.[39] Newer approaches showed, that the migration of PAD is many times lower than that of other PVC plasticisers and that migration decreases with increasing molecular mass.[111] The use of high molecular weight PAD in gaskets of PVC or a change of the polymer type itself may be possible solutions for the future.[111]

As this actual example shows, there is a lot of research needed to safeguard FCM and respectively the consumers.

I.6. Phthalic acid esters - a transferable problem for toys made of plastic materials

Phthalic acid esters (PAEs) are plasticisers in food contact materials, but their applications also cover a wider scope. They are constituents of foils, floor coverings, tubes, cables, dyes, lacquers, cosmetics and even additives in pharmaceuticals.[112,113] As they can be considered an environmental contaminant they also occur in food not due to packaging but other processes or in mother's milk.[114] An important source for the PAE exposure of children can be the mouthing of soft plastic material.[115,116] For example mouthing actually is responsible for 90% of the exposure of European infants and toddlers with di-isononyl phthalate (DINP) whereas the daily exposure is about 1 μg kg^{-1} body-weight.[117] The Commission Directive 2005/84/EC prohibits to place toys and childcare on the market, that contain bis (2-ethylhexyl) phthalate, dibutyl phthalate or benzyl butyl phthalate at concentrations of more than 0.1% by mass of the material. It is equivalent for DINP, di-isodecyl phthalate or di-n-octyl phthalate, if the articles can be placed in the mouth by children.[118] This directive is implemented like the others directives mentioned above into the German legislation via the "Bedarfsgegenständeverordnung". According the European Union rapid alert system for all dangerous consumer products (RAPEX), 140 toys or childcare products were withdrawn from the market during the year 2008 due to their contents of PAEs, which did not comply with the legislation.[119] As analysis of PAEs in toys and childcare is also a time consuming process which includes extraction with organic solvents and evaporation steps followed by the analysis with GC, HPLC or HPTLC, a fast and reliable method to identify PAEs in toys and childcare may ease analysis and therefore guarantee a higher level of health security for children.[120-123]

I.7. Aims of the study

Experts rightfully consider the use of packaging materials as the largest and least controlled source of food contaminations with organic materials in Europe.[124] Since the analysis of contaminants deriving from packaging material is faced to multiple difficulties, as for example the generally unknown formulation of material in combination with the chemical variety and huge number of additives, methods to identify sensitively contaminants deriving from FCMs and to measure substances in

food have to be created. In order to exclude human health hazards the analysis should be focussed on the identification of harmful substances.

New methods should be as easy as possible and applicable for the majority of analysts with standard instruments in order to improve the situation. The health of consumers has also to be safeguarded in similar respects, like the exposure of children and toddlers with phthalic acid esters.

Beneath these general objectives, the specific aims were:

1. To develop an improved GC/MS method to identify substances in food contact materials that may migrate into food followed by an evaluation of these substances.
2. To develop a method that enables to get an overview of the use of 2-Isopropythioxanthone in food packaging materials, well suited for routine surveillance.
3. To evaluate, if a rapid identification of the complex additives in lids of glass jars is possible by Direct Analysis in Real Time- Mass Spectrometry.
4. To develop a facilitated gaschromatographic method that enables the analysis of epoxidised soy bean oil in fatty food and children's food.
5. To prove if Direct Analysis in Real Time- Mass spectrometry may be a tool for a rapid identification of phthalic acid ester plasticisers in toys and childcare products of polyvinylchloride.

I.8. References

1. SPG Media Limited. European Packaging Sectors Breakdown. Available at http://www.packaging-gateway.com/market_statistics_europe.asp. Accessed 11 November 2008
2. SPG Media Limited. Food Packaging Market Forecast, Trends 2004-2008. Available at http://www.packaging-gateway.com/market_statistics_food.asp. Accessed 11 November 2008
3. Crosby N.T. Food Packaging Materials. Aspects of Analysis and Migration of Contaminats., Applied Science Publishers LTD, London. ISBN 0-85334-926-6

4. Robertson, G. L. *Food Packaging, Principles and Practice,* Taylor and Francis Group, LLC. ISBN 0-8493-3775-5

5. Piringer O.G.; Baner A.L. *Packaging Materials for Food,* Wiley-VCH, Weinheim, Germany. ISBN 3-527-28868-6

6. Woggon H, Uhde WJ. Antistatic finishing of plastics from the food-toxicological point of view. *Nahrung* 1977; **21**(3): pp.235-245

7. United Nations Environment Programme *The Montreal Protocol on Substances that Deplete the Ozone Layer,* Ozone Secretariat, United Nations Environment Programme, P. O. Box 30552, Nairobi, Kenya. ISBN 92-807-1888-6

8. Praller A. Foaming plastics with inert gases. Physical blowing agents. *Kunststoffe* 2005; **95**(6): pp.96-99

9. Petersen JH, Naamansen ET. DEHA-plasticized PVC for retail packaging of fresh meat. *Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung A: Food Research and Technology* 1998; **206**(3): pp.156-160

10. Freire MTD, Santana IA, Reyes FGR. Plasticisers in Brazilian food-packaging materials acquired on the retail market. *Food Additives & Contaminants* 2006; **23**(1): pp.93-99

11. Harrison N. Migration of plasticisers from cling-film. *Food Additives and Contaminants* 1988; **5**(Suppl. 1): pp.493-499

12. Society of Plastics Engineers ANTEC 2000: Plastics, the Magical Solution : Conference Proceedings, May 7-11 2000, Orlando, Florida, CRC Press. ISBN 978-1566768559

13. Pospisil J. Transformation products of antioxidants: the role in stabilization mechanisms. *Angewandte Makromolekulare Chemie* 1988; **158-159**: pp.209-219

14. Freitag W, Wurster R, Mady N. Determination of the polymeric light stabilizer Tinuvin 622 in polyolefins. *Journal of Chromatography* 1988; **450**(3): pp.426-429

15. Trones R, Andersen T, Hegna DR, Greibrokk T. Hindered amine stabilizers investigated by the use of packed capillary temperature programmed liquid chromatography. II: Poly-(N-b-hydroxyethyl-2,2,6,6-tetramethyl-4-hydroxypiperidyl succinate). *Journal of Chromatography, A* 2000; **902**(2): pp.421-426

16. Todesco RV, Ergenc N. Additives in plastics applications. *Chimia* 2002; **56**(5): pp.225-238

17. SpecialChem S.A. Ultraviolet Absorbers. Available at http://www.specialchem4adhesives.com/tc/uv-light-stabilizers/index.aspx?id=uvabsorbers. Accessed 31 October 2008

18. Geoffrey Pritchard *Plastics Additives: An A-Z Reference,* Springer, Berlin. ISBN 978-0-412-72720-7

19. Christie RM. Pigments, dyes and fluorescent brightening agents for plastics: an overview. *Polymer International* 1994; **34**(4): pp.351-361

20. European Communities. Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. *Official Journal of the European Union* 2004; **L 336**: pp.4-17

21. European Commission. Commission Regulation (EC) No 2023/2006 of 22 December 2006 on good manufacturing practice for materials and articles intended to come into contact with food. *Official Journal of the European Union* 2006; **L 384**: pp.75-78

22. Health & Consumer Protection Directorate of the European Commission. Food Contact Materials. A practical guide. Available at http://ec.europa.eu/food/food/chemicalsafety/ foodcontact/practical_guide_en.pdf. Accessed 13 November 2007

23. European Communities. Commission Directive 2002/72/EC of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs (consolidated version 2007-04-20). *Official Journal of the European Union* 2002; **L 220**: pp.18-58

24. European Communities. Commission Directive 2004/1/EC of 6 January 2004 amending Directive 2002/72/EC as regards the suspension of the use of azodicarbonamide as blowing agent. *Official Journal of the European Union* 2004; **L 7**: pp.45-46

25. European Communities. Commission Directive 2004/19/EC of 1 March 2004 amending Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with foodstuffs. *Official Journal of the European Union* 2004; **L 71**: pp.8-21

26. European Communities. Commission Directive 2005/79/EC of 18 November 2005 amending Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with food. *Official Journal of the European Union* 2005; **L 302**: pp.35-45

27. European Communities. Commission Directive 2007/19/EC of 30 March 2007 amending Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with food and Council Directive 85/572/EEC laying down the list of simulants to be used for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs. *Official Journal of the European Union* 2007; **L 91**: pp.17-36

28. European Communities. Commission Directive 2008/39/EC of 6 March 2008 amending Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with food. *Official Journal of the European Union* 2008; **L 63**: pp.6-13

29. Heckman JH. Food packaging regulation in the United States and the European Union. *Regul Toxicol Pharmacol* 2005; **42**(1): pp.96-122

30. European Communities. Council Directive 82/711/EEC of 18 October 1982 laying down the basic rules necessary for testing migration of the constituents of plastic materials and articles intended to come into contact with foodstuffs. *Official Journal of the European Union* 1982; **L 297**: pp.26-30

31. European Communities. Commission Directive 93/8/EEC of 15 March 1993 amending Council Directive 82/711/EEC laying down the basic rules necessary for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs. *Official Journal of the European Union* 1993; **L 90**: pp.22-25

32. European Communities. Commission Directive 97/48/EC of 29 July 1997 amending for the second time Council Directive 82/711/EEC laying down the basic rules necessary for testing migration of the constituents of plastic materials and articles intended to come into contact with foodstuffs. *Official Journal of the European Union* 1997; **L 222**: pp.10-15

33. European Communities. Council Directive 85/572/EEC of 19 December 1985 laying down the list of simulants to be used for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs. *Official Journal of the European Union* 1985; **L 372**: pp.14-21

34. European Communities. Council Directive 78/142/EEC of 30 January 1978 on the approximation of the laws of the Member States relating to materials and articles which contain vinyl chloride monomer and are intended to come into contact with foodstuffs. *Official Journal of the European Union* 1978; **L 44**: pp.15-17

35. European Communities. Commission Directive 80/766/EEC of 8 July 1980 laying down the Community method of analysis for the official control of the vinyl chloride monomer level in materials and articles which are intended to come into contact with foodstuffs. *Official Journal of the European Union* 1980; **L 213**: pp.42-46

36. European Communities. Commission Directive 81/432/EEC of 29 April 1981 laying down the Community method of analysis for the official control of vinyl chloride released by materials and articles into foodstuffs. *Official Journal of the European Union* 1981; **L 167**: pp.6-11

37. European Communities. Commission Regulation (EC) No 1895/2005 of 18 November 2005 on the restriction of use of certain epoxy derivatives in materials and articles intended to come into contact with food. *Official Journal of the European Union* 2005; **L 302**: pp.28-32

38. European Commission. Commission Regulation (EC) No 372/2007 of 2 April 2007 laying down transitional migration limits for plasticisers in gaskets in lids intended to come into contact with foods (corrected form). *Official Journal of the European Union* 2007; **L 92**: pp.9-12

39. European Commission. Commission Regulation (EC) No 597/2008 of 24 June 2008 amending Regulation (EC) No 372/2007 laying down transitional migration limits for plasticisers in gaskets in lids intended to come into contact with foods. *Official Journal of the European Union* 2008; **L 134**: pp.12-13

40. European Commission. Commission Regulation (EC) No 282/2008 of 27 March 2008 on recycled plastic materials and articles intended to come into contact with foods and amending Regulation (EC) No 2023/2006. *Official Journal of the European Union* 2008; **L 86**: pp.9-18

41. DG SANCO/E/3/AS. Comission services working document in preparation of a Comission relating to plastic materials and articles intended to come into contact with foodstuffs. Available at personal communication with professor W. Schwack, Universtity of Hohenheim, Germany. 5 November 2008

42. DG SANCO/E/3/AS. Draft on Community Guidelines on migration testing of plastic materials and articles. Available at personal communication with professor W. Schwack, Universtity of Hohenheim, Germany. 5 November 2008

43. Bundesministerium für Gesundheit. Bedarfsgegenständeverordnung in der Fassung der Bekanntmachung vom 23. Dezember 1997. *Bürgerliches Gesetzbuch* 1998; **1**: pp.5-

44. Jung H.Han *Innovations in Food Packaging,* Elsevier, Oxford, England. ISBN 978 0123 11632 1

45. Widen H, Leufven A, Nielsen T. Identification of chemicals, possibly originating from misuse of refillable PET bottles, responsible for consumer complaints about off-odours in water and soft drinks. *Food Additives & Contaminants* 2005; **22**(7): pp.681-692

46. Arvanitoyannis IS, Bosnea L. Migration of substances from food packaging materials to foods. *Critical Reviews in Food Science and Nutrition* 2004; **44**(2): pp.63-76

47. Lau OW, Wong SK. Contamination in food from packaging material. *Journal of Chromatography, A* 2000; **882**(1+2): pp.255-270

48. Franz R. Migration modelling from food-contact plastics into foodstuffs as a new tool for consumer exposure estimation. *Food Additives & Contaminants* 2005; **22**(10): pp.920-937

49. K Barnes; R Sinclair; D Watson *Chemical migration and food contact materials,* Woodhead Publishing Limited, Cambridge, England. ISBN 978 1 84569 029 8

50. Tehrany EA, Desobry S. Partition coefficients in food/packaging systems: a review. *Food Additives & Contaminants* 2004; **21**(12): pp.1186-1202

51. O'Brien A, Cooper I. Practical experience in the use of mathematical models to predict migration of additives from food-contact polymers. *Food Additives and Contaminants* 2002; **19**(Suppl.): pp.63-72

52. Merck, E. Anfärbereagenzien für Dünnschicht- und Papier-Chromatographie, Merck.

53. European Communities. Commission Directive 2002/72/EC of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs. *Official Journal of the European Union* 2002; **L 220**: pp.18-58

54. Feigenbaum A, Dole P, Aucejo S, Dainelli D, Cruz Garcia C, Hankemeier T, N'gono Y, Papaspyrides CD, Paseiro P, Pastorelli S, Pavlidou S, Pennarun PY, Saillard P, Vidal L, Vitrac O, Voulzatis Y. Functional barriers: Properties and evaluation. *Food Additives & Contaminants* 2005; **22**(10): pp.956-967

55. Rothenbacher T, Baumann M, Fuegel D. 2-isopropylthioxanthone (2-ITX) in food and food packaging materials on the German market. *Food Additives and Contaminants* 2007; **24**(4): pp.438-444

56. Bradley EL, Castle L, Dines TJ, Fitzgerald AG, Gonzalez Tunon P, Jickells SM, Johns SM, Layfield ES, Mountfort KA, Onoh H, Ramsay IA. Test method for measuring non-visible set-off from inks and lacquers on the food-contact surface of printed packaging materials. *Food Additives & Contaminants* 2005; **22**(5): pp.490-502

57. European Food Save Authority. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2-Isopropyl thioxanthone (ITX) and 2-ethylhexyl-4-dimethylaminobenzoate (EHDAB) in food contact materials . Available at http://www.efsa.europa.eu/EFSA/Scientific_Opinion/afc_op_ej293_itx_opinion_en1.pdf. Accessed 11 November 2008

58. Holmes MJ, Hart A, Northing P, Oldring PKT, Castle L, Stott D, Smith G, Wardman O. Dietary exposure to chemical migrants from food contact materials: A probabilistic approach. *Food Additives & Contaminants* 2005; **22**(10): pp.907-919

59. Benford, D. The Acceptable Daily Intake: A Tool for Ensuring Food Safety, ILSI Press, Washington, U.S.A. ISBN 1-57881-091-4

60. European Food Save Authority. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to the introduction of a Fat (consumption) Reduction Factor for infants and children. Available at http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620770209.htm. Accessed 11 November 2008

61. Silva AS, Garcia RS, Cooper I, Franz R, Losada PP. Compilation of analytical methods and guidelines for the determination of selected model migrants from plastic packaging. *Trends in Food Science & Technology* 2006; **17**(10): pp.535-546

62. EU DG XII research programme AIR 941025 (1994-1997). Safety and quality control of plastics materials for food, synthetic report. Available at http://crl-fcm.jrc.it. Accessed 13 November 2007

63. Veiga-Rial M, Sarria-Vidal M, de la Montana-Miguelez J, Simal-Gandara J. Identification of residual constituents in plastic packaging for dairy products. *Recent Research Developments in Agricultural & Food Chemistry* 1999; **3**(Pt. 1): pp.305-311

64. Biedermann-Brem S, Biedermann M, Fiselier K, Grob K. Compositional GC-FID analysis of the additives to PVC, focusing on the gaskets of lids for glass jars. *Food Additives & Contaminants* 2005; **22**(12): pp.1274-1284

65. Gruber L, Wolz G, Piringer O. Analysis of phthalates in baby food. *Deutsche Lebensmittel-Rundschau* 1998; **94**(6): pp.177-179

66. Castle L, Mercer AJ, Startin JR, Gilbert J. Migration from plasticized films into foods. 3. Migration of phthalate, sebacate, citrate and phosphate esters from films used for retail food packaging. *Food Additives & Contaminants* 1988; **5**(1): pp.9-20

67. Startin JR, Parker I, Sharman M, Gilbert J. Analysis of di-(2-ethylhexyl)adipate plasticiser in foods by stable isotope dilution gas chromatography-mass spectrometry. *Journal of Chromatography* 1987; **387**: pp.509-514

68. Castle L, Gilbert J, Jickells SM, Gramshaw JW. Analysis of the plasticiser acetyltributyl citrate in foods by stable isotope dilution gas chromatography-mass spectrometry. *Journal of Chromatography* 1988; **437**(1): pp.281-286

69. Petersen JH, Breindahl T. Plasticisers in total diet samples, baby food and infant formulae. *Food Addit Contam* 2000; **17**(2): pp.133-141

70. Kueseng P, Thavarungkul P, Kanatharana P. Trace phthalate and adipate esters contaminated in packaged food. *Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes* 2007; **42**(5): pp.569-576

71. Lau OW, Wong SK. Determination of plasticisers in food by gas chromatography-mass spectrometry with ion-trap mass detection. *J Chromatogr A* 1996; **737**(2): pp.338-342

72. Simoneau C, Ezerskis Z, Morkunas V. Determination of plasticisers and additives in PVC gasket seals and in fatty sauces and migration study. *Food Contact Polymers 2007, International Conference, 1st, Brussels, Belgium, Feb.21-22, 2007* 2007: pp.8/1-8/5

73. Fiselier K, Biedermann M, Grob K. Injector-internal thermal desorption from edible oils. Part 2: Chromatographic optimization for the analysis of migrants from food packaging material. *Journal of Separation Science* 2005; **28**(16): pp.2144-2152

74. Fankhauser-Noti A, Grob K. Injector-internal thermal desorption from edible oils performed by programmed temperature vaporizing (PTV) injection. *Journal of Separation Science* 2006; **29**(15): pp.2365-2374

75. Holadova K, Prokupkova G, Hajslova J, Poustka J. Headspace solid-phase microextraction of phthalic acid esters from vegetable oil employing solvent based matrix modification. *Analytica Chimica Acta* 2007; **582**(1): pp.24-33

76. Fankhauser-Noti A, Biedermann-Brem S, Grob K. PVC plasticisers/additives migrating from the gaskets of metal closures into oily food: Swiss market survey June 2005. *European Food Research and Technology* 2006; **223**(4): pp.447-453

77. Ezerskis Z, Pastorelli S, Contini C, Simoneau C. Survey of 2-ethylhexanoic acid in baby food. *Food Additives & Contaminants* 2007; **24**(7): pp.792-797

78. Belitz, H.-D.; Grosch, W. *Food Chemistry,* Springer-Verlag, Berlin - Heidelberg - New York. ISBN 3-540-64704-X

79. Suman M, La Tegola S, Catellani D, Bersellini U. Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry Method for the Determination of Epoxidized Soybean Oil in Food Products. *Journal of Agricultural and Food Chemistry* 2005; **53**(26): pp.9879-9884

80. Castle L, Sharman M, Gilbert J. Gas chromatographic-mass spectrometric determination of epoxidized soybean oil contamination of food by migration from plastic packaging. *Journal - Association of Official Analytical Chemists* 1988; **71**(6): pp.1183-1186

81. Biedermann-Brem S, Biedermann M, Fankhauser-Noti A, Grob K, Helling R. Determination of epoxidized soy bean oil (ESBO) in oily foods by GC-FID or GC-MS analysis of the methyl diepoxy linoleate. *European Food Research and Technology* 2007; **224**(3): pp.309-314

82. Weller P, Herrnreiter M, Donaubauer A. A miniaturized method to determine epoxidized soybean oil in baby food. *LC-GC Europe* 2007; **20**(5): pp.280-282, 285

83. Biedermann M, Grob K. GC method for determining polyadipate plasticisers in foods: transesterification to dibutyl adipate, conversion to migrating polyadipate. *Chromatographia* 2006; **64**(9-10): pp.543-552

84. Biedermann M, Grob K. GC-MS characterization of oligomers in polyadipates used as plasticisers for PVC in food contact. *Packaging Technology & Science* 2006; **19**(3): pp.159-178

85. Castle L, Mercer AJ, Gilbert J. Gas chromatographic-mass spectrometric determination of adipate-based polymeric plasticisers in foods. *Journal - Association of Official Analytical Chemists* 1988; **71**(2): pp.394-396

86. Biedermann-Brem S, Grob K, Biedermann M. Analysis of reaction products (e.g. chlorhydrins) of ESBO in poly(vinyl chloride) type polymers and coatings. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene* 2001; **92**(5): pp.515-534

87. Biedermann-Brem S, Biedermann M, Grob K. Reaction of epoxidized soya bean oil (ESBO) with hydrogen chloride formed in PVC: Investigation on model systems. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene* 2004; **95**(3): pp.261-286

88. Begley TH. Methods and approaches used by FDA to evaluate the safety of food packaging materials. *Food Addit Contam* 1997; **14**(6-7): pp.545-553

89. Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F, Vos JG, Wurtzen G. Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. *Food and Chemical Toxicology* 2004; **42**(1): pp.65-83

90. Barlow, S. *Threshold of Toxicological Concern (TTC),* ILSI Press, Washington, U.S.A. ISBN 1-57881-188-0

91. European Communities. Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. *Official Journal of the European Union* 1967; **L 196**: pp.1-98

92. European Communities. Synoptic Document. Available at http://europa.eu.int/comm/food/food/chemicalsafety/foodcontact/synoptic_doc_en.pdf. Accessed 13 November 2007

93. Severin I, Dahbi L, Lhuguenot JC, Andersson MA, Hoornstra D, Salkinoja-Salonen M, Turco L, Zucco F, Stammati A, Dahlman O, Castle L, Savolainen M, Weber A, Honkalampi-Haemaelaeinen U, Von Wright A. Safety assessment of food-contact paper and board using a battery of short-term toxicity tests: European union BIOSAFEPAPER project. *Food Additives and Contaminants* 2005; **22**(10): pp.1032-1041

94. Mittag N, Foerster S, Hoppe S, Simat TJ, Sehr A, Renz S, Vobach M, Kammann U. Bioassay guided analysis of migrants from can coatings. *Journal fuer Verbraucherschutz und Lebensmittelsicherheit* 2006; **1**(4): pp.345-353

95. Coulier L, Muilwijk B, van Stee L, Rijk R, Peters R, Zondervan-van den Beuken E, van Dongen WD. Analytical strategy to assess the safety of food contact materials. *Food Contact Polymers 2007* 2007: pp.7/1-7/10

96. Grob K. Comprehensive analysis of migrates from food-packaging materials: A challenge. *Food Additives and Contaminants* 2002; **19**(Suppl.): pp.185-191

97. Skjevrak I, Brede C, Steffensen IL, Mikalsen A, Alexander J, Fjeldal P, Herikstad H. Non-targeted multi-component analytical surveillance of plastic food contact materials: Identification of substances not included in EU positive lists and their risk assessment. *Food Additives and Contaminants* 2005; **22**(10): pp.1012-1022

98. European Food Safety Authority. EFSA Expert Group looks at semicarbazide: too early for any conclusions. Available at http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620779504.htm. Accessed 08 September 2008

99. Stadler RH, Mottier P, Guy P, Gremaud E, Varga N, Lalljie S, Whitaker R, Kintscher J, Dudler V, Read WA, Castle L. Semicarbazide is a minor thermal decomposition product of azodicarbonamide used in the gaskets of certain food jars. *Analyst (Cambridge, United Kingdom)* 2004; **129**(3): pp.276-281

100. European Food Safety Authority. FAQ on Semicarbazide. Available at http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_SemicarbazideFAQs.htm. Accessed 08 September 2008

101. de la Calle MB, Anklam E. Semicarbazide: occurrence in food products and state-of-the-art in analytical methods used for its determination. *Analytical and Bioanalytical Chemistry* 2005; **382**(4): pp.968-977

102. European Communities. Commission directive 2004/1/EC amending Directive 2002/72/EC as regards the suspension of the use of azodicarbonamide as blowing agent. *Official Journal of the European Union* 2004; **L 7**: pp.45-46

103. Elss S, Gruenewald L, Richling E, Schreier P. Occurrence of 2-ethylhexanoic acid in foods packed in glass jars. *Food Additives and Contaminants* 2004; **21**(8): pp.811-814

104. Falbe, J.; Regitz, M. *Römpp Chemie Lexikon,* Georg Thieme Verlag, Stuttgart, Germany. ISBN 3-13-102759-2

105. Hammarling L, Gustavsson H, Svensson K, Karlsson S, Oskarsson A. Migration of epoxidized soya bean oil from plasticized PVC gaskets into baby food. *Food Addit Contam* 1998; **15**(2): pp.203-208

106. Fantoni L, Simoneau C. European survey of contamination of homogenized baby food by epoxidized soybean oil migration from plasticized PVC gaskets. *Food Additives & Contaminants* 2003; **20**(11): pp.1087-1096

107. Fankhauser-Noti A, Fiselier K, Biedermann S, Biedermann M, Grob K, Armellini F, Rieger K, Skjevrak I. Epoxidized soy bean oil (ESBO) migrating from the gaskets of lids into food packed in glass jars. *European Food Research and Technology* 2005; **221**(3-4): pp.416-422

108. Fankhauser-Noti A, Grob K. Migration of plasticisers from PVC gaskets of lids for glass jars into oily foods: Amount of gasket material in food contact, proportion of plasticiser migrating into food and compliance testing by simulation. *Trends in Food Science & Technology* 2006; **17**(3): pp.105-112

109. Ezerskis Z, Morkunas V, Suman M, Simoneau C. Analytical screening of polyadipates and other plasticisers in poly(vinyl chloride) gasket seals and in fatty food by gas chromatography-mass spectrometry. *Analytica Chimica Acta* 2007; **604**(1): pp.29-38

110. Pedersen GA, Jensen LK, Fankhauser A, Biedermann S, Petersen JH, Fabech B. Migration of epoxidized soybean oil (ESBO) and phthalates from twist closures into food and enforcement of the overall migration limit. *Food Additives & Contaminants, Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment* 2008; **25**(4): pp.503-510

111. Biedermann M, Fiselier K, Marmiroli G, Avanzini G, Rutschmann E, Pfenninger S, Grob K. Migration from the gaskets of lids into oily foods: first results on polyadipates. *European Food Research and Technology* 2008; **226**(6): pp.1399-1407

112. Schweizerische Eidgenossenschaft, Bundesamt für Gesundheit Direktionsbereich Verbraucherschutz. Factsheet Phthalate. Available at http://www.bag.admin.ch/themen/chemikalien/ 00228/01378/index.html?lang=de. accessed 07 October 2008

113. Butte W, Hostrup O, Walker G. Phthalates in house dust and air: associations and potential sources in the indoors. *Gefahrstoffe - Reinhaltung der Luft* 2008; **68**(3): pp.79-81

114. Pford J. Levels of Di(2-ethylhexyl)phthalate(DEHP) and dibutyl phthalates in some foodstuffs with plastic packagings and in mother's milk. *Deutsche Lebensmittel-Rundschau* 2004; **100**(11): pp.431-436

115. Earls AO, Axford IP, Braybrook JH. Gas chromatography-mass spectrometry determination of the migration of phthalate plasticisers from polyvinyl chloride toys and childcare articles. *Journal of Chromatography, A* 2003; **983**(1-2): pp.237-246

116. Wilkinson CF, Lamb JC, IV. The Potential Health Effects of Phthalate Esters in Children's Toys: A Review and Risk Assessment. *Regulatory Toxicology and Pharmacology* 1999; **30**(2, Pt. 1): pp.140-155

117. Wormuth M, Scheringer M, Vollenweider M, Hungerbuhler K. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? *Risk Anal* 2006; **26**(3): pp.803-824

118. European Communities. Commission Directive 2005/84/EC of the European Parliament and of the Council of 14 December 2005 amending for the 22nd time Council Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (phthalates in toys and childcare articles). *Official Journal of the European Union* 2005; **L 344**: pp.40-43

119. European Commission. Rapid Alert System for Non-Food Products (RAPEX) - Latest notifications. Available at http://ec.europa.eu/ consumers/dyna/rapex/rapex_archives_en.cfm. Accessed 03 February 2009

120. Rastogi SC. Gas chromatography analysis of phthalate esters in plastic toys. *Chromatographia* 1998; **47**(11/12): pp.724-726

121. Vandenburg HJ, Clifford AA, Bartle KD, Carlson RE, Carroll J, Newton ID. A simple solvent selection method for accelerated solvent extraction of additives from polymers. *Analyst (Cambridge, United Kingdom)* 1999; **124**(11): pp.1707-1710

122. Stringer R, Labunska I, Santillo D, Johnston P, Siddorn J, Stephenson A. Concentrations of phthalate esters and identification of other additives in PVC children's toys. *Environmental Science and Pollution Research International* 2000; **7**(1): pp.27-36

123. Hauri U, Schlegel U, Wagmann M, Hohl C. Determination of phthalates in toys and childcare articles made of PVC with HPLC and HPTLC. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene* 2002; **93**(4): pp.179-185

124. Grob K, Biedermann M, Scherbaum E, Roth M, Rieger K. Food contamination with organic materials in perspective: Packaging materials as the largest and least controlled source? A view focusing on the European Situation. *Critical Reviews in Food Science and Nutrition* 2006; **46**(7): pp.529-535

II. Non-targeted multi-component analytical screening of plastic food contact materials using fast interpretation of deliverables via expert structure-activity relationship software.

THORSTEN ROTHENBACHER[1], WOLFGANG SCHWACK[2]

[1] Chemisches und Veterinäruntersuchungsamt Stuttgart, Schaflandstraße 3/ 2, 70736 Fellbach, Germany.[§]
[2] Institut für Lebensmittelchemie, Universität Hohenheim, Garbenstr. 28, 70599 Stuttgart, Germany, e-mail: wschwack@uni-hohenheim.de, corresponding author

II.1. Abstract

Plastic packaging materials may release compounds into packed foodstuffs. To identify potential migrants of toxicological concern, resins and multilayer foils (mainly polyethylene), intended for the production of food contact materials, were extracted and analysed by gas chromatography - mass spectrometry (GC/MS). To identify even compounds of low concentrations, the software AMDIS was used and data evaluation was safeguarded by the Kovats Retention Index (RI) system. By this way, 46 compounds were identified as possible migrants. The expert structure-activity relationship software DEREK for Windows™ (DfW) was utilized to evaluate all identified substances in terms of carcinogenicity, genotoxicity, thyroid toxicity and miscellaneous endpoints for humans. Additionally a literature search for these compounds was carried out with Sci-Finder®, but relevant data were missing for 28 substances. Summarized seven compounds with adverse toxicological effects were

[§] Present adress: Institut für Lebensmittelchemie, Universität Hohenheim, Germany

identified. In addition, the RIs of 24 commercial additive standards, measured with a GC capillary column of middle polarity, are given.

II.2. Introduction

Huge sectors of plastic materials are food packagings. During the contact of packaging with food, components deriving from the plastic material can be transferred into the food.

The transferred components, so called migrants, are part of the polymer and can be additives, catalysts, impurities of monomers and additives, oligomers and monomers of the core polymer or from polymeric additives.[1] Depending on the processing, there are additional possibilities for the carry-over of possible food contaminations. During storage of packaging, for example, substances like printing ink components can be transferred via offset from the non- food contact material side to the food contact material (FCM) side and subsequently migrate into the foodstuff.[2] Possible degradation products of any migrant have also to be respected.[3,4]

To overview the number of substances used for the production of FCM, the European Synoptic Document (SD) or the U.S.-American List of “Indirect” Additives Used in Food Contact Substances (LIA) can be used.[5,6] The SD lists all monomers and additives notified to the European Commission in view of their use for the manufacturing of plastics and coatings that will later be FCMs and includes about 3000 substances, while aids to polymerisation, colorants, inks, adhesives and solvents are not mentioned in principle. The list only presents the notified auxiliary materials. Quite the same number of substances used for food material production are listed by LIA, but this list is not limited to plastic and coating materials. It deals with substances used in food-contact articles, called “indirect food additives”, and includes adhesives and components of coatings, paper and paperboard components, polymers, adjuvants and production aids. In conclusion there is a vast variety of possible and unknown contaminants deriving from FCM.

To evaluate health risks of a migrating substance, its toxicological properties and the concentration in the food in conjunction with the average daily food intake have to be considered. According to the structure-based threshold of toxicological concern risk assessment tool for application to substances present at low levels in the diet by Kroes et al., the intake of a toxicological and structural unknown and therefore possible genotoxic substance should not exceed 0.15 µg per day, if it is excluded that the substance is not an non-essential metal or metal containing compound, polyhalogenated dibenzodioxin, dibenzofuran or biphenyl, or an aflatoxin-like, azoxy or N-nitroso compound.[7] In United States there is a substance-independent threshold of regulation tolerated by the U.S. Food and Drug Administration of 0.5 µg kg^{-1} foodstuff.[8] Obviously, the determination of the toxicological potential of a big variety of probably unknown substances at trace levels is the claim.

There are different general approaches of late to face this analytical claim, but a striking solution has not been found. From the toxicological point of view, FCM migrates can be characterized by short-term toxicity tests.[9] The application to migrants of can coatings proved this concept as principally possible, but due to lack of sensivity not generally usable.[10] Heading to the analytical viewpoint, specific compound classes known for toxic effects are determined by, e.g., gas chromatography (GC). However, this method does not apply owing to i) a general lack of known suitable reagents for derivatization and ii) a barely specificity, as evaluated, e.g., for the GC analysis of aromatic amines with pentafluorobenzyl chloride derivatization and mass spectrometric detection (GC/MS).[11] Fundamentally, the ranges of instrumental applications have to be considered, in case of GC analysis primarily the vapor pressure and thermal stability of analytes. In spite of this, GC/MS screening analysis actually is the most applied analytical method for unknown migrants. In this case, the operator is challenged with the identification of a forest of unknown peaks followed by toxicological assessment. Critical points are substance identification only by use of mass spectra databases[12] and insufficient limits of detection for compounds in food simulants or food, e.g 20 µg kg^{-1} as reported for compounds in water, determined after enrichment by solid-phase extraction.[13]

The toxicological assessment as a subsequent step of the screening analysis is a part of the whole evaluation, which is as important as the analysis. Salts of 2-ethylhexanoic acid (2-EHA) used as a stabilizer for polyvinylchloride (PVC), for example, were identified as a main migrant of PVC in 1997,[3] but toxicological concerns for 2-EHA in food were not published before 2004.[14] Due to the fact that generally not every compound identified as a potential migrant will be listed in national legislation, a time-consuming literature database search for toxic properties is required, often resulting in imperfect or negative results. A more effective way could be the use of structure-activity relationship software. To improve the overall accuracy and specificity of toxicological predictions, a multiple combination of software is proposed,[15] but for evaluation of the method presented here, the use of one software seems to be sufficient. DEREK (Deductive Estimation of Risk from Existing Knowledge) for Windows™ (DfW), for example, is one of the most widely used commercial toxicity prediction programs. It contains expert knowledge rules in toxicology and predicts toxic properties for substances on the base of their molecular structure.[16] DfW is a well suited toxicological screening tool ranging in the lower price region and best predicting mutagenicity and carcinogenicity as compared to other software systems.[17]

The aim of this study was to establish an improved GC/MS screening method including toxic interpretation of identified substances. To improve and safeguard the data interpretation in view of correct substance identification, the software AMDIS (Automated Mass Spectral Deconvolution and Identification System) from the National Institute of Standards and Technology (NIST, U.S.A.) should be implemented, because it provides efforts in the identification of substances at trace levels, as proved for pesticides.[18-20] AMDIS additionally offers an integrated Kovats Retention Index (RI) system, to support correct peak identification.[21] Subsequent the toxic evaluation should be done by DfW. Due to contaminations in the range of traces, the polymer instead of migrates should be investigated to achieve better sensitivity. In the case of positive findings, however, the migrations of harmful substances into food or food-simulants have to be checked by specific analysis, depending on the food intended for packaging. For this new approach, several resins, foils and multilayer foils, intended for the production of FCM, were screened.

The experiments were focused mainly on polyethylene, because it is the mostly used material for food packaging.

II.3. Materials and methods

Chemicals and reagents

1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP), 2-(2-butoxyethoxy) ethanol, benzophenone, octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate, 2-ethyl-hexanoic acid, oleamide, 2-phenoxyethanol, bis(2-ethylhexyl) phthalate, dioctyl phthalate, (Z)-docos-13-enamide, 2,6-di-tert-butyl-p-cresol, octabenzone and the alkanes for the alkane standard mixture C_{16}-C_{38} (n-hexadecane, n-octadecane, n-eicosane, n-docosane, n-tetracosane, n-hexacosane, n-octacosane, n-triacontane, n-dotriacontane, n-tetratriacontane, n-hexatriacontane and n-octatriacontane) were purchased from Sigma-Aldrich (Taufkirchen, Germany). Methanol, ethanol, toluene, 2-ethylhexyl 4-methoxycinnamate, ε-caprolactam, bis(2-ethylhexyl) adipate, triacetine, diisobutyl phthalate, dimethyl heptanedioate, phthalic anhydride, α-tocopherolacetate, isooctane, tributyl phosphate, dibutyl phthalate, tris(2-ethylhexyl) phosphate and 4-methyl-m-phenylenediamine were from Merck (Darmstadt, Germany). Methanol, ethanol, isooctane, toluene and HFIP were distilled before use.

Samples

Eighteen polymeric resins, two single-layer foils and four multi-layer foils, all intended for the production of food contact materials, were provided from different fabricators. The resins 1-12 were made of low-density polyethylene (LDPE), the resins 13-15 of linear low density polyethylene (LLDPE), resins 16 and 17 of polyamide (PA), and resin 18 of 1,2-polybutadiene (PB). Foil 1 was made of polyethylene terephthalate (PET), foil 2 of amorphous polyethylene terephthalate (APET), foil 3 was a multilayer foil made of APET, PE and polyethylene vinyl acetate (EVA), foil 4 a multilayer foil made of metalized PET, PE and PA, foil 5 a multilayer foil made of APET and PE, and foil 6 a multilayer foil made of PA and PE.

Sample preparation

The foils were cut into pieces smaller than 0.16 cm^2. Samples of foil or resin pieces (0.1 g) were refluxed in 4 mL HFIP (APET, PET and PA) or toluene (PE and PB) until the plastic material was dissolved. For multilayer foils HFIP as well as toluene was used. Afterwards, 15 mL of methanol (PET, PA) or ethanol (PE, PB) were added and the suspension was filtered. The extracts were evaporated by a gentle stream of nitrogen. The residue was taken up in 1 mL isooctane, 10 µL of dodecane in isooctane (c=2 g L^{-1}) were added and the solution was analysed by GC/MS. To identify contaminations of solvents and instruments, blanks were prepared and analysed the same way.

GC/MS

A Thermo Finnigan Trace Gas Chromatograph with a straight splitless liner containing a small packing of deactivated glass wool at its bottom, a Phenomenex ZB50 column (30 m length, 0.25 mm id., 0.25 µm film) and a 0.7 m uncoated and deactivated retention gap, coupled with a Thermo Finnigan Polaris QE 230 Ion Trap Mass Spectrometer was used. While helium was used with a flow of 1 mL/min, 1 µL was injected at 300°C for one minute splitless. Then the split was changed to 1/30. The oven temperature gradient was 50°C(5min)/100°C(10°C/min)/ 300°C(15°C/min)/300°C(10min). The transfer line was at 330°C and electron impact ionization at 70 eV and a scan range from *m/z* 33-600 in positive detection mode were used.

Determination of RI and target list compounds

The alkane standard mixture C_{16}-C_{38} (each hydrocarbon at a concentration of 100 mg L^{-1} in isooctane) was injected to obtain the RI calibration data and to assure the GC/MS instrument supports analysis of substances with low vapor pressure.

To obtain the RI of 2-(2-butoxyethoxy)ethanol, 2,6-di-tert-butyl-p-cresol, 2-ethyl-hexanoic acid, 2-ethylhexyl 4-methoxycinnamate, 2-phenoxyethanol, 4-methyl-m-phenylenediamine, benzophenone, bis(2-ethylhexyl) adipate, bis(2-ethylhexyl) phthalate, bis(2-methoxyethyl) phthalate, dibutyl phthalate, diisobutyl phthalate, dimethyl heptanedioate, dioctyl phthalate, ε-caprolactam, (Z)-docos-13-enamide, octabenzone, octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate, oleamide,

phthalic anhydride, triacetine, tributyl phosphate, tris(2-ethylhexyl) phosphate and α-tocopheryl acetate, each component was dissolved in isooctane in the concentration range of 20-50 mg L^{-1} and measured by GC/MS under conditions as described above for the samples, except a Varian VF-17ms column without precolumn was used. The obtained data were processed with AMDIS to set up a target list.

Software tools

AMDIS (version 2.62, NIST, U.S.A.)[22] was used to automatically detect peaks in chromatograms, deconvolute the centroided mass spectra and calculate the RIs. The adjacent peak subtraction was set to two, resolution, sensivity and shape requirements each to low.

Detected peaks were target-list searched and afterwards batch processed with NIST MS Search 2.0 (version 2.0 d) and the NIST/EPA/NIH Mass Spectral Library (Version NIST 05).[23]

Compound identification was on base of the mass spectra and its database search results in combination with the RI.

For the toxicological evaluation, the molecular structure of the identified substance was transferred from NIST MS Search 2.0 to DfW (software version 9.0.0)[24] via copy and paste. Then the structures were processed with DfW regarding carcinogenicity, genotoxicity, thyroid toxicity and miscellaneous endpoints for humans, the function “perceive tautomers” was enabled.

To evaluate identified substances by literature search, SciFinder® scholar (version 2006)[25] was used. First the substance was located via the substance identifier, followed by a search for references associated with adverse effects, including toxicity. If the title of the reference and the summary fit to the question of toxicological evaluation concerning carcinogenicity or genotoxicity, the literature was obtained and the toxicological data were extracted.

II.4. Results and discussion

The GC/MS screening analysis of the studied resins and foils provided chromatograms with a lot of peaks; an example is given in Figure 1.

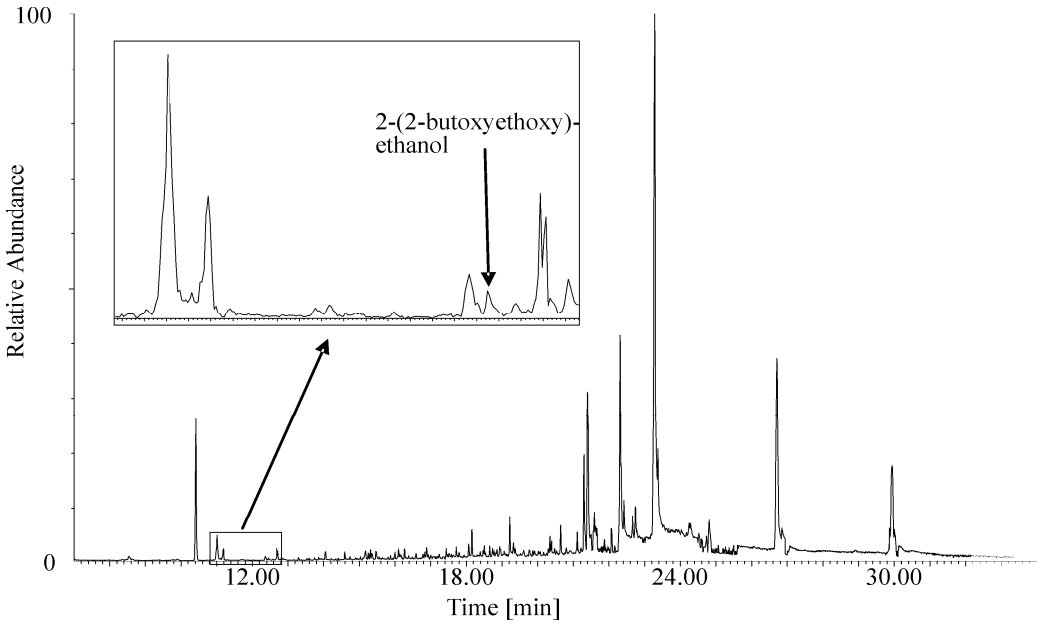


Figure 1. Total Ion Current of the GC/MS chromatogram of the extract of resin 5. The chromatogram is zoomed in on the range of 10.7-12.8 min to pick the peak identified as 2-(2-butoxyethoxy)-ethanol.

Comparable to the results of Veiga-Rial et al. these “peak forests” were mainly constitutional isomers of alkenes or saturated and unsaturated alcohols.[12] They could not exactly be characterized by GC/MS in combination with NIST due to missing database information. In spite of this, 46 different compounds were identified (Table 1).

Table 1. Substances identified in foils and resins including the measured RI (averaged if determined in more than one matrix), the RI predicted by NIST, the specific legislation according Commission Directive 2002/72/EC, additional toxicological information obtained by literature and the toxicology for humans according the prediction of the software DfW. Substances whose corresponding peak in the total ion current chromatogram possessed a low signal were marked with “traces”, see Figure 1.

substance	found in	CAS	measured RI [i.u.]	RI predicted [i.u.] (NIST)	specific legislation, additional toxicological information*	toxicology prediction by DfW#
1,8-diazacyclotetradecane-2,9-dione (addition product of two ε-caprolactam molecules)	resin 17	5776-79-4	2889	2005	nsl, nati	--
11-(Z)-eicosenamide	resin 11	10436-08-5	2917	2427	oml	--
2-(2-butoxyethoxy)ethanol, traces	resin 5, 9, 10, 14, 15	112-34-5	1393-1394	1211	SD-, nati	--
2,4,6-trimethylpyridine	foil 2	108-75-8	1110	1014	SD-, no cytotoxic activity found by in vitro tests[27]	--
2,4,6-tri-tert-butylphenol	resin 4	732-26-3	2135	1882	nsl, nati	--
2,4-di-tert-butylphenol in traces	resin 4, 15, 17, foil 2	96-76-4	1691	1555	SD-, nati	--
2,6-di-tert-butyl-p-cresol	resin 1, 7	128-37-0	1682	1501	SML 3 mg kg^{-1}	--
2-ethylhexyl 4-methoxycinnamate	resin 3, foil 2	5466-77-3	2680	2088	SD-, nati but estrogenic activity towards the estrogen receptor ERα[28]	--

substance	found in	CAS	measured RI [i.u.]	RI predicted [i.u.] (NIST)	specific legislation, additional toxicological information*	toxicology prediction by DfW#
2-ethylhexyltrans-4-methoxycinnamate	resin 8, 17, foil 1	83834-59-7	2681	2088	nsl, nati	peroxisome proliferation in human is improbable
2-methyl-m-phenylene diisocyanate	foil 6	91-08-7	1562	-	QM(T)=1mg kg^{-1} (calc. as NCO)	mutagenicity in vitro in human is open chromosome damage in vitro in human is plausible
2-phenoxyethanol	foil 2, 3, 5, 6	122-99-6	1495	1212	SD-, nati	peroxisome proliferation in human is improbable
3,5-di-tert-butyl-4-hydroxybenzaldehyde in traces	resin 4	1620-98-0	2052	1856	nsl, nati	--
4,4-dimethyl-2-cyclopentenone	foil 1, 2, 4	22748-16-9	1246	888	nsl, nati	chromosome damage in vitro in human is plausible
4-hydroxy-4-methylpentan-2-one	foil 2, 3	123-42-2	783	845	SD-, nati	--[4]

substance	found in	CAS	measured RI [i.u.]	RI predicted [i.u.] (NIST)	specific legislation, additional toxicological information*	toxicology prediction by DfW#
4-methyl-m-phenylene diisocyanate	foil 6	584-84-9	1555	-	QM(T)=1mg kg^{-1} (calc. as NCO)	mutagenicity in vitro in human is open chromosome damage in vitro in human is plausible
4-methyl-m-phenylenediamine	foil 6	95-80-7	1494	1417	nsl, genotoxic[29]	carcinogenicity in human is plausible mutagenicity in vitro in human is open
7,9-di-tert-butyl-1-oxaspiro-(4,5) deca-6,9-diene-2,8-dione	resin 4	82304-66-3	2230	2081	nsl, nati	chromosome damage in vitro in human is plausible
all-trans-Squalene (2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene)	resin 6, 12, 17	111-02-4	2997	2914	nsl, nati	--
bis(2-ethylhexyl) adipate	resin 1, 8, foil 3, 4	103-23-1	2586	2414	SML 18 mg kg^{-1}	peroxisome proliferation in human is improbable
bis(2-ethylhexyl) phthalate	resin 6, 16, 17	117-81-7	2864	2704	SML 1.5 mg kg^{-1}	peroxisome proliferation in human is improbable

substance	found in	CAS	measured RI [i.u.]	RI predicted [i.u.] (NIST)	specific legislation, additional toxicological information*	toxicology prediction by DfW#
diisobutyl phthalate	resin 2, 6-10, 13, 14, 18, foil 5	84-69-5	2169	1908	SD-, DNA-damaging impact of DiBP in human lymphocytes[30]	--
dimethyl terephthalate	resin 1	120-61-6	1790	1440	oml	--
docosane	resin 10, 13	629-97-0	2195	2208	nsl, nati	--
ε-caprolactam	resin 13, 16, 17, foil 4, 6	105-60-2	1590	1003	SML (T) 15mg kg^{-1}	--
(Z)-docos-13-enamide	resin 1, 5, 7, 11, 16, foil 6	112-84-5	3134	2625	oml	--
glycerol trioctanoate	foil 2, 3	538-23-8	3360	3143	-, mutagenic in vitro, cancerogenic in rats[31,32]	--
hexacosane	resin 10	630-01-3	2595	2606	nsl, nati	--
hexadecane	resin 10, 13	544-76-3	1598	1612	nsl, nati	--
hexadecyl 2-ethylhexanoate	resin 1, 3 13, 14	59130-69-7	2563	2510	nsl, nati	peroxisome proliferation in human is improbable teratogenicity in human is plausible
icosane	resin 10, 12, foil 6	112-95-8	1995	2009	nsl, nati	--
isopropyl laurate	resin 9, 15, foil 1, 2	10233-13-3	1716	1613	nsl, nati	--

substance	found in	CAS	measured RI [i.u.]	RI predicted [i.u.] (NIST)	specific legislation, additional toxicological information*	toxicology prediction by DfW#
isopropyl myristate	resin 10	110-27-0	1905	1814	nsl, nati	--
isopropyl palmitate	resin 1, 11	142-91-6	2131	2013	nsl, nati	--
methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate (irganox 1300)	resin 2, 4, 7, 8, 10, 11, 13, foil 3, 4	6386-38-5	2217	2134	nsl, nati	--
methyl palmitate	resin 18	112-39-0	2040	1878	SD-, nati	--
naphthalene	resin 13	91-20-3	1430	1231	nsl, known to cause cancer[33]	--
octacosane	resin 10, foil 6	630-02-4	2794	2804	nsl, nati	--
octadecane	resin 10, 12, 13	593-45-3	1795	1810	nsl, nati	--
octadecyl 2-ethylhexanoate	resin 1, 3, 17, foil 2, 3	59130-70-0	2765	2709	nsl, nati	peroxisome proliferation in human is improbable teratogenicity in human is plausible
octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate (Irganox 1076)	resin 2, 7-10, 13, 15, foil 4, 6	2082-79-3	3931	3823	SML 6 mg kg^{-1}	--
oleamide	resin 5, 8, 11	301-02-0	2697	2228	oml	--
palmitic acid	resin 3	57-10-3	2113	1967	oml	--

substance	found in	CAS	measured RI [i.u.]	RI predicted [i.u.] (NIST)	specific legislation, additional toxicological information*	toxicology prediction by DfW#
tetracosane	resin 10, 13, foil 3, 6	646-31-1	2394	2407	nsl, nati	--
tris(2,4-di-tert-butylphenyl) phosphate (suggested Irgafos 168 oxidation product)	resin 4, 5, 11, 15	95906-11-9	4040	Not in NIST	nsl, nati	--
tris(2,4-di-tert-butylphenyl) phosphite (Irgafos 168)	resin 4, 11, 15, foil 6	31570-04-4	3693	Not in NIST	oml	--
α-tocopheryl acetate	resin 3	58-95-7	3506	3308	nsl, nati	--

* The specific legislation is according the EC (Commission Directive 2002/72/EC). If a substance is mentioned in the Commission Directive 2002/72/EC, the specific migration limit (SML) is given. If there is no SML, the migration of the substance should fulfill the overall migration limit according article 2 of the directive and in this case it is marked with “oml”. If no specific legislation exists, the substance is marked with “nsl”. If the substance is not specially regulated, but cited in the Synoptic Document without specific toxicological data, it is marked with a “SD-“ instead of “nsl”. In case of no obtainable additional information about genotoxicity and mutagenicity literature search, the substance is marked with “nati”.

If the structure of the molecule does not possess a toxicophore regarding carcinogenicity, genotoxicity, thyroid toxicity and miscellaneous endpoints for humans, DfW does not suppose toxicological health effects and the substance is marked with “--“. The uncertainty terms used in DfW are as following: “Plausible” means that the weight of evidence supports the proposition, “improbable” that there is at least one strong argument that the proposition is false and there are no arguments that it is true and “open” that there is no evidence that supports or opposes the proposition [16].

The structural majority of them are hydrocarbons (7 compounds) and fatty acid esters (9 compounds). Some are known additives for plastic materials like the antioxidants α-tocopheryl acetate, methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate, tris(2,4-di-tert-butylphenyl) phosphite and octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate, the slipping agents (Z)-docos-13-enamide and oleamide and the derivates of the phthalates and bis(2-ethylhexyl) adipate, used as plasticisers. Some other identified compounds are not known additives, but have been found in earlier publications as contaminants of FCM, like 2-phenoxyethanol for example found in coating material of cookware products.[26] Caprolactam and its dimers were found as migrants from polyamide cooking utensils and 2,4-di-tert-butylphenol, a degradation product of irgafos antioxidants, as a migrant of polyolefin bottles.[13] Nevertheless, still 19 compounds are remaining, which are not expected to be in food contact materials. Concerning the toxic evaluation of the identified compounds, all 46 compounds were checked, because known additives or contaminants can also possess health risks. Of all these substances, only 13 compounds were regulated by the Commission Directive 2002/72/EC and eight are mentioned in the Synoptic Document without sufficient toxicological data. These eight substances should be reviewed by the European Food Safety Authority (EFSA) in the forthcoming years.

Therefore 33 identified compounds had to be evaluated, so the required level of own initiative was very high. Using SciFinder®, it took about five hours, but toxicological data comparable to an acceptable daily intake dose in the diet were not found for all substances. Only for five compounds, there was access to toxicologically relevant data like cytotoxic activity or genotoxicity. Therefore the SciFinder® search did not satisfy and left a great level of uncertainty for 28 substances. In contrast to this, the evaluation with DfW on base of the structure quickly gave results for each compound. For 26 of the 33 compounds to be evaluated negative effects were not predicted by DfW. For 2-ethylhexyl-(trans)-4-methoxycinnamate and 2-phenoxyethanol, a peroxisome proliferation in human is improbable, but not excluded. Thus, there could be the possibility that these compounds may lead to oxidative stress in the human body. DfW predicts for five compounds that they plausibly possess more negative health effects: 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione and 4,4-dimethyl-2-cyclopentenone damage human

chromosomes, 4-methyl-m-phenylenediamine is carcinogenic and hexadecyl 2-ethylhexanoate and octadecyl 2-ethylhexanoate are teratogenic for humans. In comparison to the evaluation via SciFinder® search, on the one hand, these adverse effects predicted by DfW have not been found in literature, except the carcinogenicity for 4-methyl-m-phenylenediamine.[29] On the other hand, naphthalene and surprisingly glycerol trioctanoate, too, are both reported as mutagenic[31-33] and diisobutyl phthalate possesses a DNA damaging impact in human lymphocytes according to the literature,[30] but DfW did not calculate a risk.

Regarding our data evaluation and the report of Dagan et al.,[20] the use of AMDIS saved time, because it automatically i) calculates the RIs for all detected peaks, ii) deconvolutes their mass spectra, iii) compares the data with the target list and iv) allows to search automatically for matches of the deconvoluted mass spectra in NIST. Although AMDIS featured fast processing, it sometimes excluded characteristic mass peaks of the spectrum of a compound. Therefore, to assure correct identification results, the deconvoluted mass spectrum of a compound was also manually compared with the native recorded mass spectrum as well as with the spectrum of the substances that were high-match proposed by the NIST library by an experienced GC/MS operator, who finally made the identification decision. Figure 1 illustrates a peak identified as 2-(2-butoxyethoxy)-ethanol to give an example for a substance which is found “in traces”. The mass spectrum of exactly this peak was treated with different kinds of data evaluation and compared to the mass spectrum of 2-(2-butoxyethoxy)-ethanol of the NIST database. Figure 2 demonstrates the reduction of the background by use of conventional background correction and deconvolution. The NIST database search, which can be considered as an important step of the method, gave no valueable results for the untreated spectrum. In case of the conventional background correction the database search delivered on the first position 2-(2-butoxyethoxy)ethyl thiocyanate, on the second position 17,20:20,21-bis[methylenebis(oxy)]-,cyclic 3-(1,2-ethanediyl acetal)pregn-5-ene-3,11-dione and on the third position with a probability of 2.2% the correct identification of 2-(2-butoxyethoxy)-ethanol. The search with the deconvoluted mass spectrum yielded a probability of 15.0% for the target compound in the first position. In this example, it is obvious that AMDIS improved correct substance identification significantly compared to the conventional data evaluation.

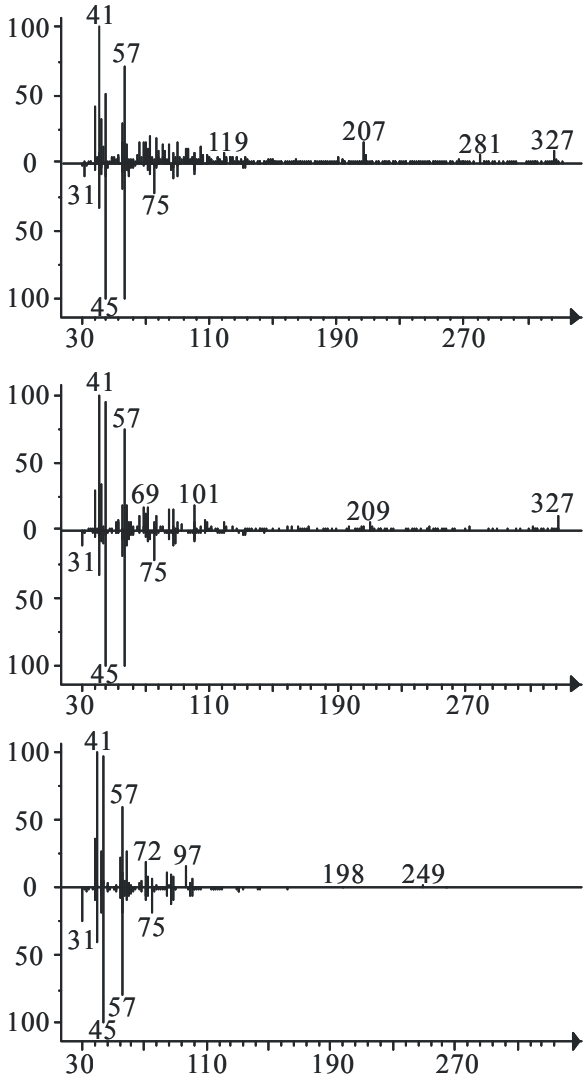


Figure 2. Three different processed mass spectra of the peak identified as 2-(2-butoxyethoxy) ethanol (see Figure 1) in a head to tail view. Each view allows comparing the obtained data in the head with the mass spectrum of the NIST database in the tail. Above is the unmodified scan, in the middle the scan with the subtracted background on both sides of the peak and below the scan extracted with AMDIS.

If the same substance was identified in different samples, the measured RIs of the substance varied up to ± 6, if the RI was over 3000 i.u., under there was less deviation. To carry out additional safeguarding, the RIs of substances, which were identified by their mass spectra, were compared with those provided by NIST (Table 1). It is significant that most of the measured RIs of the identified substances were higher than those listed by NIST. This is related to the use of a column of middle polarity for the analysis, which generally provides a better separation power than a column of low polarity, but NIST's structure-based RI estimates for a nonpolar column. The amount of the resulting ΔRIs, expressed in index units (i.u.), can be

judged according published group retention factors.[34] Table 2 shows the RI and ΔRI values for the standard compounds (target list compounds).

Table 2. RIs of measured standard compounds compared with the RIs predicted by NIST. The ΔRI is the difference of the predicted RI and the measured RI (the measured RI was set to 100%).

substance	CAS	RI [i.u.]	RI predicted [i.u.]	ΔRI [i.u.]	ΔRI [%]
2-(2-butoxyethoxy)ethanol	112-34-5	1313	1211	102	8
2,6-di-tert-butyl-p-cresol	128-37-0	1673	1668	5	11
2-ethyl-hexanoic acid	149-57-5	1210	1109	101	8
2-ethylhexyl 4-methoxycinnamate	5466-77-3	2655	2088	567	21
2-phenoxyethanol	122-99-6	1450	1212	238	16
4-methyl-m-phenylenediamine	95-80-7	1750	1417	333	19
benzophenone	119-61-9	1986	1603	383	19
bis(2-ethylhexyl) adipate	103-23-1	2567	2414	153	6
bis(2-ethylhexyl) phthalate	117-81-7	2858	2704	154	5
bis(2-methoxyethyl) phthalate	117-82-8	2450	1990	460	19
dibutyl phthalate	84-74-2	2292	2037	255	11
diisobutyl phthalate	84-69-5	2147	1908	239	11
dimethyl heptanedioate	1732-08-7	1550	1250	300	19
dioctyl phthalate	117-84-0	3064	2832	232	8
ε-caprolactam	105-60-2	1578	1003	575	36
(Z)-docos-13-enamide	112-84-5	3149	2629	520	17
octabenzone	1843-05-6	3273	2708	565	17
octadecyl 3-(3,5-di-tert-butyl-4-hydroxy-phenyl)propionate (Irganox 1076)	2082-79-3	3935	3823	112	3
oleamide	301-02-0	2708	2228	480	18
phthalic anhydride	85-44-9	1654	1443	211	13
triacetine	102-76-1	1494	1354	140	9
tributyl phosphate	126-73-8	1843	1620	223	12
tris(2-ethylhexyl) phosphate	78-42-2	2598	2463	135	5
α-tocopheryl acetate	7695-91-2	3479	3308	171	5

The data reveals that percental ΔRIs are low for compounds only containing few functional groups related to the number of non-polar groups, like for example Irganox 1070 (ΔRI 3%) and di-ethylhexyl-phthalate (ΔRI 5%). ΔRIs are increasing for substances with increasing functionality, like in the case of 2-ethylhexyl 4-methoxycinnamate (ΔRI 21%). The highest ΔRI value was found for ε-caprolactam (36%). This corresponds very well with the findings of Peng et al.[34], who showed that

the monosubstituted acid amido group possesses one of the largest group-retaining factor for polar columns.

Finally, there was left a question of the source of the identified compounds evaluated as toxicologically relevant. Di-tert-butyl-1-oxaspiro-(4,5) deca-6,9-diene-2,8-dione, that probably will cause a chromosome damage in humans, is a degradation product of Irganox 1010 (pentaerythritol tetrakis(3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate))[35] and has also been found in extracts of FCM before.[13] The same adverse effects possesses 4,4-dimethyl-2-cyclopentenone. It was found before in minor quantities in extrudates of food,[36] but is not known to be a FCM contaminant. The two isocyanates 2-methyl-m-phenylene diisocyanate and 4-methyl-m-phenylene diisocyanate were only detected in one multilayer foil and possibly derive from the glue between the layers. Another compound only found in the same multilayer foil is 4-methyl-m-phenylenediamine. It is also carcinogenic according DfW and supposably also derived from the glue, because it can be used as raw material for 4-methyl-m-phenylene diisocyanate and additionally is a degradation product of the hydrolyzed diisocyanate. Glycerol trioctanoate was found before as a lubricant in FCM[37] and should surprisingly be considered as mutagenic and genotoxic according to literature.[31,32] Naphthalene was found before in coating material of cookware products[26] and is described in literature to be carcinogenic.[32,33]

II.5. Conclusion

The method described allows a sensitive analysis of compounds in plastic materials accompanied by safeguarded identification via mass spectrometry and retention index system. Furthermore every resulting substance can be toxicologically evaluated in seconds and expeditiously data processing is implemented in the whole process. With regard to a forthcoming process of recycling in FCM production, the range of substances that possibly contaminate packed food may expand in the future. According to the opinion of the authors, the challenge of the identification of hazardous substances in the diet should be persecuted in the future on a larger scale. The use of the method described is an important aspect to resolve this hurdle.

II.6. Acknowledgment

The authors would like to thank CVUA Stuttgart for supply with equipment, materials and support and FABES Forschungs-GmbH for support.

II.7. References

1. Feigenbaum A, Scholler D, Bouquant J, Brigot G, Ferrier D, Franz R, Lillemark L, Riquet AM, Petersen JH, Van Lierop B, Yagoubi N. Safety and quality of food contact materials. Part 1: Evaluation of analytical strategies to introduce migration testing into good manufacturing practice. *Food Additives and Contaminants* 2002; **19**(2): pp.184-201
2. Rothenbacher T, Baumann M, Fuegel D. 2-isopropylthioxanthone (2-ITX) in food and food packaging materials on the German market. *Food Additives and Contaminants* 2007; **24**(4): pp.438-444
3. EU DG XII research programme AIR 941025. Safety and quality control of plastics materials for food, synthetic report. Available at http://crl-fcm.jrc.it. Accessed 13 November 2007
4. Piringer O.G.; Baner A.L. *Packaging Materials for Food,* Wiley-VCH, Weinheim, Germany. ISBN 3-527-28868-6
5. European Communities. Synoptic Document. Available at http://europa.eu.int/comm/food/food/chemicalsafety/foodcontact/synoptic_doc_en.pdf. Accessed 13 November 2007
6. U.S.Food and Drug Administration/ Center for Food Safety & Applied Nutrition. The List of "Indirect" Additives Used in Food Contact Substances. Available at http://www.cfsan.fda.gov/~dms/opa-indt.html.
7. Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F, Vos JG, Wurtzen G. Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. *Food and Chemical Toxicology* 2004; **42**(1): pp.65-83

8. Heckman JH. Food packaging regulation in the United States and the European Union. *Regul Toxicol Pharmacol* 2005; **42**(1): pp.96-122

9. Severin I, Dahbi L, Lhuguenot JC, Andersson MA, Hoornstra D, Salkinoja-Salonen M, Turco L, Zucco F, Stammati A, Dahlman O, Castle L, Savolainen M, Weber A, Honkalampi-Haemaelaeinen U, Von Wright A. Safety assessment of food-contact paper and board using a battery of short-term toxicity tests: European union BIOSAFEPAPER project. *Food Additives and Contaminants* 2005; **22**(10): pp.1032-1041

10. Mittag N, Foerster S, Hoppe S, Simat TJ, Sehr A, Renz S, Vobach M, Kammann U. Bioassay guided analysis of migrants from can coatings. *Journal fuer Verbraucherschutz und Lebensmittelsicherheit* 2006; **1**(4): pp.345-353

11. Coulier L, Muilwijk B, van Stee L, Rijk R, Peters R, Zondervan-van den Beuken E, van Dongen WD. Analytical strategy to assess the safety of food contact materials. *Food Contact Polymers 2007* 2007: pp.7/1-7/10

12. Veiga-Rial M, Sarria-Vidal M, de la Montana-Miguelez J, Simal-Gandara J. Identification of residual constituents in plastic packaging for dairy products. *Recent Research Developments in Agricultural & Food Chemistry* 1999; **3**(Pt. 1): pp.305-311

13. Skjevrak I, Brede C, Steffensen IL, Mikalsen A, Alexander J, Fjeldal P, Herikstad H. Non-targeted multi-component analytical surveillance of plastic food contact materials: Identification of substances not included in EU positive lists and their risk assessment. *Food Additives and Contaminants* 2005; **22**(10): pp.1012-1022

14. Elss S, Gruenewald L, Richling E, Schreier P. Occurrence of 2-ethylhexanoic acid in foods packed in glass jars. *Food Additives and Contaminants* 2004; **21**(8): pp.811-814

15. White AC, Mueller RA, Gallavan RH, Aaron S, Wilson AGE. A multiple in silico program approach for the prediction of mutagenicity from chemical structure. *Mutation Research, Genetic Toxicology and Environmental Mutagenesis* 2003; **539**(1-2): pp.77-89

16. Judson PN, Marchant CA, Vessey JD. Using Argumentation for Absolute Reasoning about the Potential Toxicity of Chemicals. *Journal of Chemical Information and Computer Sciences* 2003; **43**(5): pp.1364-1370

17. Hulzebos E, Sijm D, Traas T, Posthumus R, Maslankiewicz L. Validity and validation of expert (Q)SAR systems. *SAR QSAR Environ Res* 2005; **16**(4): pp.385-401

18. Stein SE. An integrated method for spectrum extraction and compound identification from gas chromatography/mass spectrometry data. *Journal of the American Society for Mass Spectrometry* 1999; **10**(8): pp.770-781

19. Zhang W, Wu P, Li C. Study of automated mass spectral deconvolution and identification system (AMDIS) in pesticide residue analysis. *Rapid Communications in Mass Spectrometry* 2006; **20**(10): pp.1563-1568

20. Dagan S. Comparison of gas chromatography-pulsed flame photometric detection-mass spectrometry, automated mass spectral deconvolution and identification system and gas chromatography-tandem mass spectrometry as tools for trace level detection and identification. *Journal of Chromatography, A* 2000; **868**(2): pp.229-247

21. Kovats E. Gas chromatographic characterization of organic compounds. I. Retention indexes of aliphatic halides, alcohols, aldehydes, and ketones. *Helvetica Chimica Acta* 1958; **41**: pp.1915-1932

22. AMDIS download page. Available at http://chemdata.nist.gov/mass-spc/amdis/. Accessed 03 May 2008

23. NIST/EPA/NIH Mass Spectral Library. Available at http://www.nist.gov/srd/nist1a.htm. Accessed 03 May 2008

24. DEREK for Windows™ . Available at http://www.lhasalimited.org/index.php?cat=2&sub_cat=64. Accessed 03 May 2008

25. SciFinder® scholar . Available at http://www.cas.org/SCIFINDER/SCHOLAR/index.html. Accessed 03 May 2008

26. Bradley EL, Read WA, Castle L. Investigation into the migration potential of coating materials from cookware products. *Food Additives and Contaminants* 2007; **24**(3): pp.326-335

27. Curvall M, Enzell CR, Pettersson B. An evaluation of the utility of four in vitro short term tests for predicting the cytotoxicity of individual compounds derived from tobacco smoke. *Cell Biology and Toxicology* 1984; **1**(1): pp.173-193

28. Gomez E, Pillon A, Fenet H, Rosain D, Duchesne MJ, Nicolas JC, Balaguer P, Casellas C. Estrogenic activity of cosmetic components in reporter cell lines: parabens, UV screens, and musks. *Journal of Toxicology and Environmental Health, Part A* 2005; **68**(4): pp.239-251

29. Severin I, Jondeau A, Dahbi L, Chagnon MC. 2,4-Diaminotoluene (2,4-DAT)-induced DNA damage, DNA repair and micronucleus formation in the human hepatoma cell line HepG2. *Toxicology* 2005; **213**(1-2): pp.138-146

30. Kleinsasser NH, Wallner BC, Kastenbauer ER, Weissacher H, Harreus UA. Genotoxicity of di-butyl-phthalate and di-iso-butyl-phthalate in human lymphocytes and mucosal cells. *Teratogenesis, Carcinogenesis, and Mutagenesis* 2001; **21**(3): pp.189-196

31. National Toxicology Program. Comparative toxicology studies of corn oil, safflower oil, and tricaprylin (CAS Nos.8001-30-7, 8001-23-8, and 538-23-8) in male F344/N rats as vehicles for gavage. Technical Report Series No. 426 (Research Triangle Park, NC). Available at http://ntp.niehs.nih.gov/?objectid=0709D0C9-A575-BA2F-3069EBD44EA674E2. Accessed 13 November 2007

32. Carcinogenic Potency Database. Carcinogenic Potency Database of the University of Berkley, California, USA. Available at http://potency.berkeley.edu/chempages/TRICAPRYLIN.html and http://potency.berkeley.edu/chempages/NAPHTHALENE.html. Accessed 13 November 2007

33. Office of Environmental Health Hazard Assessment of the State of California USA. Proposition 65 list. Available at http://www.oehha.ca.gov/prop65. Accessed 13 November 2007

34. Peng CT, Yang ZC, Ding SF. Prediction of retention indexes. II. Structure-retention index relationship on polar columns. *Journal of Chromatography* 1991; **586**(1): pp.85-112

35. Chen JT, Mossoba MM, Varner SL, Roach JAG, Sphon JA, Page SW. GC/matrix isolation/FTIR studies of decomposition products of Irganox 1010 in an aqueous ethanol system. *Proceedings of SPIE - The International Society for Optical Engineering* 1989; **1145**(Int. Conf. Fourier Transform Spectrosc., 7th): pp.600-601

36. Solina M, Johnson RL, Whitfield FB. Effects of glucose and acid-hydrolysed vegetable protein on the volatile components of extruded wheat starch. *Food Chemistry* 2006; **100**(2): pp.678-692

37. Tateo F, Bononi M. Identification of organic contaminants in disposable aluminium foil containers. *Food Additives and Contaminants* 2006; **23**(10): pp.1030-1037

III. 2-Isopropylthioxanthone (2-ITX) in food and food packaging materials on the German market

T. ROTHENBACHER, M. BAUMANN & D. FÜGEL‡

Chemisches und Veterinäruntersuchungsamt Stuttgart,
Schaflandstr. 3/2, D-70736 Fellbach, Germany

III.1. Abstract

To elucidate the occurrence of the photo-initiator 2-isopropylthioxanthone (2-ITX), more than 100 food products on the German market, packed in cartons, plastic cups and foils, were investigated. For this, a rapid method to detect 2-ITX in food packaging materials was established. In case of positive findings the accompanying foodstuffs were analysed in a subsequent step using different extraction methods, depending on the fat content of the food. Determination of the photo-initiator was done by high performance liquid chromatography with diode array and fluorescence detection (HPLC-DAD/FLD). The recoveries ranged between 94 and 106% for non-fatty (RSD $\leq$ 1.1) and between 80 and 105% for fatty foods (RSD $\leq$ 8.5). The limit of detection and the limit of quantification were determined to 2 and 5 µg L^{-1}. 2-ITX was detected in 36 out of 137 packages (26%) and significant migration occurred in 75% of the packaging materials tested positive. The amounts of 2-ITX ranged up to 357 µg kg^{-1} in orange juice.

III.2. Introduction

In September 2005 the Italian authorities informed the European Commission by a notification transmitted through the Rapid Alert System for Food and Feed

‡ To whom correspondence should be addressed. e-mail: Diane.Fuegel@cvuas.bwl.de

(RASFF) that they found baby milk contaminated with a substance called 2-isopropylthioxanthone (2-ITX, Figure 1).[1]

UV-curing inks are usually made of multifunctional acrylates, acrylated oligomers and pigments. Photo-initiators like 2-ITX are used to trigger the radical polymerization of the acrylic component of such inks and thus causing the liquid ink film to dry.[2] Compared to solvent based inks, UV-curing seemed a good alternative because the packaging material was no longer able to contaminate food with residues of organic solvents of the printing process. Nevertheless, there are new, possible contaminants in the packaging material, particularly with regard to acrylates and photo-initiators.[3] Generally, 2-ITX can migrate from the packaging into the foods. As intermediate layers of aluminium do not allow ink components to pass through packaging material, it was assumed that, in this particular case, 2-ITX got into the food by the so-called set-off effect. When the printed material is rolled on spools (e.g. carton-based packaging materials) or stacked (e.g. plastic cups), the external layer comes into contact with the internal layer. During this storage 2-ITX is transferred to the surface intended to come into contact with food and, consequently, can migrate into the foodstuff after packaging. At the beginning of this study 2-ITX was detected only in food packed in cartons. It is unknown if food packed in materials other than cartons can be affected. To assess the contamination of food by this photo-initiator, it is necessary to investigate food in cartons, as well as in other packaging materials like plastic cups or foils.

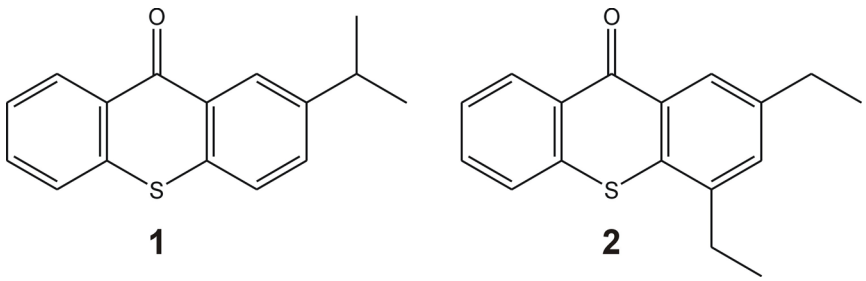


Figure 1. Chemical structures of the photo-initiators 2 ITX (1) and 2,4 DTX (2)

According to the opinion No 044/2005 of the German Federal Institute of Risk Assessment (BfR) of the 25th November 2005 and the press statement of the European Food Safety Authority (EFSA) on the 9th December 2005, toxicological data of 2-ITX is inadequate.[4,5] Existing *in vivo* genotoxicity studies do not indicate a

genotoxic potential for 2-ITX and, at a maximum migration level of 50 µg kg^{-1} food, 2-ITX appears unlikely to pose a health risk.[4,5]

Inks applied to the outer surface of food packaging materials are not covered by specific European legislation. One exception is Commission Directive 93/10/EEC According to this directive, the printed surfaces of regenerated cellulose film must not come into contact with the foodstuffs.[6] However, the only actual European legislation concerning 2-ITX in food and packaging materials, other than regenerated cellulose film, are the Framework Regulation (EC) No 1935/2004, Regulation (EEC) No 315/93 and the Regulation (EC) No 178/2002.[7-9] Pursuant to article 3 of Regulation (EC) No. 1935/2004, materials and articles intended to come into contact with food shall not transfer their constituents in food in quantities which could endanger human health or bring about unacceptable changes in composition or characteristics of foodstuffs. Following article 14 of Regulation (EC) No 178/2002, the food itself must not be placed on the market if it is injurious to health or unfit for human consumption, whether by extraneous matter or otherwise.

Another document concerning printing inks is the Resolution ResAP(2005)2 on packaging inks applied to the non-food contact surface of food-packaging materials and articles intended to come into contact with foodstuffs of the Council of Europe.[10] This resolution is not a legal norm, but it is assumed that the general requirements of article 3 of Regulation (EC) No. 1935/2004 for food contact materials are fulfilled if the packaging inks are in accordance with the requirements made.

Manufacturers of food packaging materials are responsible for ensuring that their products comply with the abovementioned regulations and that, from a technological viewpoint, they are suitable for the use for which they are intended. Therefore, the European Printing Ink Association (EuPIA) defined a guideline on printing inks.[11] According to this guideline, if only insufficient toxicological data are available, a substance is acceptable if its specific migration does not exceed 10 µg kg^{-1}. If three negative mutagenicity tests as requested by the EFSA-Guidelines are available, as in the case of 2-ITX, the specific migration limit is raised to 50 µg kg^{-1}.To assess the migration of 2-ITX into food, several methods have been developed. With respect to UV-curing inks and the determination of major acrylates

and widely used photo-initiators migrating into simulating solvents, gas chromatography coupled to a mass selective detector (GC-MS) was applied successfully with a recovery rate of 70-100%, depending on the simulant used.[3] In milk, yoghurt and fat 2-ITX was determined using accelerated solvent extraction and high performance thin layer chromatography (HPTLC)– fluorescence detection. Confirmation of the results was done by HPTLC-mass spectrometry.[12] The recoveries of this method ranged between 6-70%, corrected by internal standard up to 70-130%, with a limit of detection of 1 µg kg^{-1} in butter. A method to determine 2-ITX in fruit juices using pressurized liquid extraction and high performance liquid chromatography (HPLC) coupled to a single quadrupole, ion trap, or triple quadrupole MS detection systems gave recoveries of ~70% and detection limits up to 0.05 µg L^{-1}.[13]

Here, a fast and reliable method to determine 2-ITX in food and food contact materials, to enable an effective, routine surveillance of commodities on the German market, is described. Due to the UV-activity of photo-initiators it is possible to determine these compounds via their characteristic UV-spectra and fluorescence activity. Therefore, a method based on HPLC coupled to a diode array (DAD) and a fluorescence detector (FLD) for the quantitation of 2-ITX and other photo-initiators (Figure 1) was developed. Following a stepwise procedure, identification was done initially in food contact materials (multilayer cartons, plastic cups, and foil) and in case of positive findings, analysis was carried out on the wrapped foodstuffs.

III.3. Experimental

Chemicals

HPLC grade acetonitrile was purchased from Mallinckrodt Chemicals (Griesheim, Germany) and hexafluoro-2-propanol from Sigma-Aldrich (Taufkirchen, Germany). All further solvents were of gradient grade or distilled prior to use. Distilled water was produced by a Milli-Q water purification system (Millipore, Schwalbach, Germany). The sorbent used for the cleanup Bondesil-PSA (40 µm) came from Varian (Darmstadt, Germany). Analytical standard of 2-isopropylthioxanthone (2-ITX, purity 98%) was provided by IGM Resins (Krefeld, Germany) and

2,4-diethylthioxanthone (2,4-DTX, Figure 1) as internal standard (purity 98%) by Sigma-Aldrich (Taufkirchen, Germany). All further chemicals were at least analytical quality.

For preparation of 0.1 M citrate phosphate buffer (pH 6.0) 21 g citric acid monohydrate and 14.2 g disodium hydrogen phosphate are dissolved in approximately 900 mL water. After adjusting to pH 6.0 with sodium hydroxide, the solution is diluted up to 1000 ml with water. Solutions of 2-ITX and 2,4-DTX were prepared in acetonitrile and stored at 4°C in the dark.

Samples

A total of 137 samples of fatty and non-fatty food were collected randomly from October 2005 until April 2006 on the German retail market or partly direct from the food manufacturer. Following a stepwise procedure, the food contact materials were tested for 2-ITX initially while storing the homogenized fillings at -18 °C for further analysis.

Sample preparation

Food contact material. After separation of the food contact material from the filling approximately 4 cm^2 of the printed packaging material were cut into small pieces and extracted with 1 mL hexafluoro-2-propanol (EU DG XII Research Programm AIR 941025 (1994-1997) 1997) in an ultrasonic bath for 45 min. Then 4 mL ethanol were added and the mixture shaken intensively for 1 min. The precipitate is removed by filtration prior to HPLC analysis.

If 2,4-DTX was found originally in food packaging materials, no internal standard was added during the sample preparation (as described below) and both photo-initiators were determined by external standard calculation.

Non-fatty foods (e.g. juices, tomato puree). Sample preparation for non-fatty foods was based on the QuEChERS-method.[14] To 10 g of the homogenized sample material 10 mL acetonitrile are added. Extraction was done by shaking intensively for 1 min. Then 4 g magnesium sulphate and 1 g sodium chloride were added and the mixture was shaken intensively for 1 min. After addition of the internal standard and

gently shaking for 30 s, the mixture was centrifuged for 5 min at 3000 rpm. An 8 mL aliquot of the supernatant was mixed with 1.2 g anhydrous magnesium sulphate and 200 mg PSA. After shaking intensively for 30 s, the mixture was centrifuged for 1 min at 5300 rpm. The supernatant was directly subjected to HPLC analysis.

Fatty foods (e.g. yogurt, milk, sausage). To approximately 5 g of homogenized fatty food 5 mL 0.1 M buffer solution (pH 6.0) was added and the sample was extracted by shaking intensively for 1 min. After addition of the internal standard and 30 mL acetonitrile, the mixture was shaken for 10 min and quantitatively filtered through filter paper. Flask and filter paper were rinsed with 10 mL acetonitrile/water (3/1 v/v). After addition of 1.5 g sodium chloride and intensively shaking for 30 s, the filtrate was mixed with 20 mL *tert.*-butyl methyl ether/isohexane (80/20, v/v) before shaking gently again. The lower aqueous phase was discarded. The organic layer was washed two times with 20 mL water. After addition of 10 mL *tert.*-butyl methyl ether/isohexane (50/50 v/v) and, if necessary, separation of the aqueous phase, the organic layer was dried over anhydrous sodium sulphate. The solution was evaporated to dryness under vacuum. Finally, the residue was dissolved in 1 mL acetonitrile and directly subjected to HPLC analysis.

High performance liquid chromatography with diode array and fluorescence detection (HPLC-DAD/FLD)

Analysis was performed on a Agilent 1100 high performance liquid chromatograph (Agilent, Waldbronn, Germany) equipped with a LC-PAH Supelcosil (250 mm; 4.6 mm ID; 5 µm) column (Sigma-Aldrich, Taufkirchen, Germany) coupled to a diode array (260 nm; spectra recorded from 200 up to 500 nm) and a fluorescence detector (excitation 272 nm/emission 440 nm) connected in series. The system was run at 40 °C (stop time 10 min) in isocratic mode (dist. water/acetonitrile 15/85 v/v) with a flow rate of 1 mL min^{-1} and an injection volume set to 10 µL.

Limit of detection and quantitation (2-ITX), as well as the linearity range (2-ITX and 2,4-DTX), were determined by HPLC-FLD analysis of solutions of the photo-initiators in acetonitrile in the absence of matrix interferences according to DIN standard 32645 (DIN 1994).

Spiking procedure

Spiking of homogenized blank samples was performed by adding 2-ITX standard solution directly into the matrix. The spiked matrices were shaken briefly and left to stand 2 min before extraction to enable dispersion of the photo-initiator. For non fatty foods, recovery tests were conducted on blank homogenized orange and vegetable juice by spiking (five times each matrix and level) with 0.05 and 0.5 mg kg^{-1}. For fatty foods, blank homogenized milk and oil was spiked (six times each matrix and level) with 0.1 mg kg^{-1} of 2-ITX (Table 1).

III.4. Results and discussion

Validation of the method

Limits of detection and quantitation. The method for the determination of 2-ITX with HPLC-DAD/FLD (Figure 2) in acetonitrile was linear in the range 6-120 µg L^{-1} (Mandel test of linearity) for the FLD-signal, with a correlation coefficient of 0.9995. Due similarities in molecular structure and chemical properties 2,4-DTX was used as internal standard (linearity 5 up to 100 µg L^{-1}). According to DIN standard 32645, the limit of detection and quantification by fluorescence detection were determined to 2 and 5 µg L^{-1} 2-ITX in acetonitrile, respectively.[15] Confirmation of 2-ITX by its UV-spectrum could be achieved above 12 µg l^{-1}. The results of the samples analysed were only accepted, if the presence of the photo-initiator was approved by its characteristic UV spectrum (Figure 2).

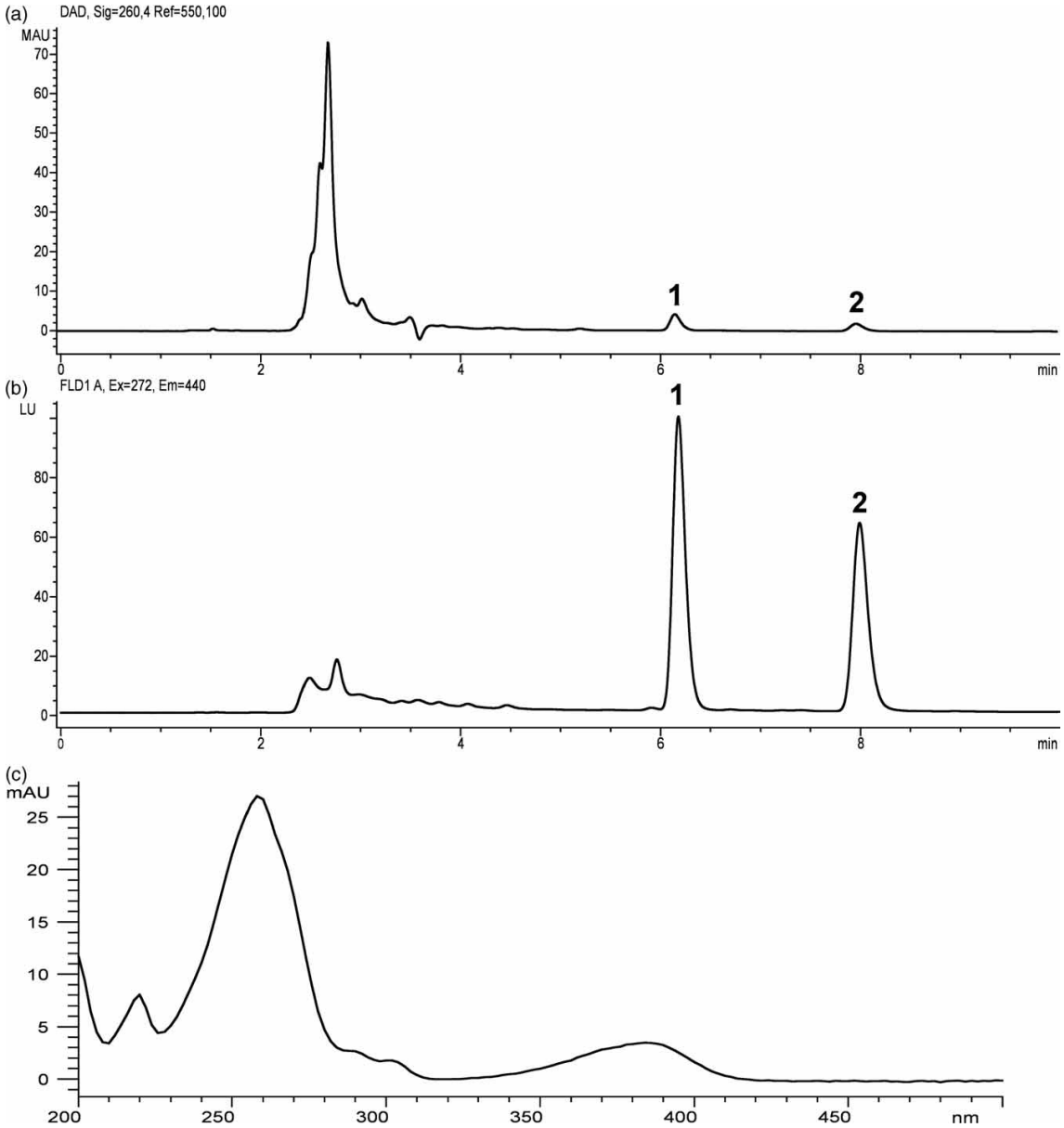


Figure 2. HPLC separation of an olive oil extract spiked with about 300 µg kg^{-1} 2 ITX (**1**) and 2,4 DTX (**2**). The DAD (**a**) and FLD Signal (**b**) are shown as well as the characteristic UV spectrum (**c**) of 2 ITX

Recoveries. For non fatty foods the recovery rates were >85% (data not shown). By application of the 2,4-DTX internal standard, the recovery rates were raised to 94-106% with a relative standard deviation (RSD) < 1.1% (Table I). For fatty foods, the signal of the internal standard showed partial interference. Therefore determination of 2-ITX in fatty foods was done by external calibration (Table 1).

Table 1. Recoveries rates and relative standard deviations (RSDs) from food samples spiked with 2-ITX.

food	fortification level [mg kg-1]	replicates	recovery rates [%]	mean [%]	RSD [%]
orange juice	0.05	5	95, 96, 96, 95, 95	95	0.4
	0.50	5	104, 104, 105, 103, 104	104	0.6
vegetable juice	0.05	5	94, 97, 95, 97, 97	96	1.1
	0.50	5	104, 105, 106, 105, 106	105	0.8
milk	0.10	6	80, 105, 93, 94, 100, 94	94	8.5
oil	0.10	6	95, 95, 90, 94, 95, 94	94	2.0

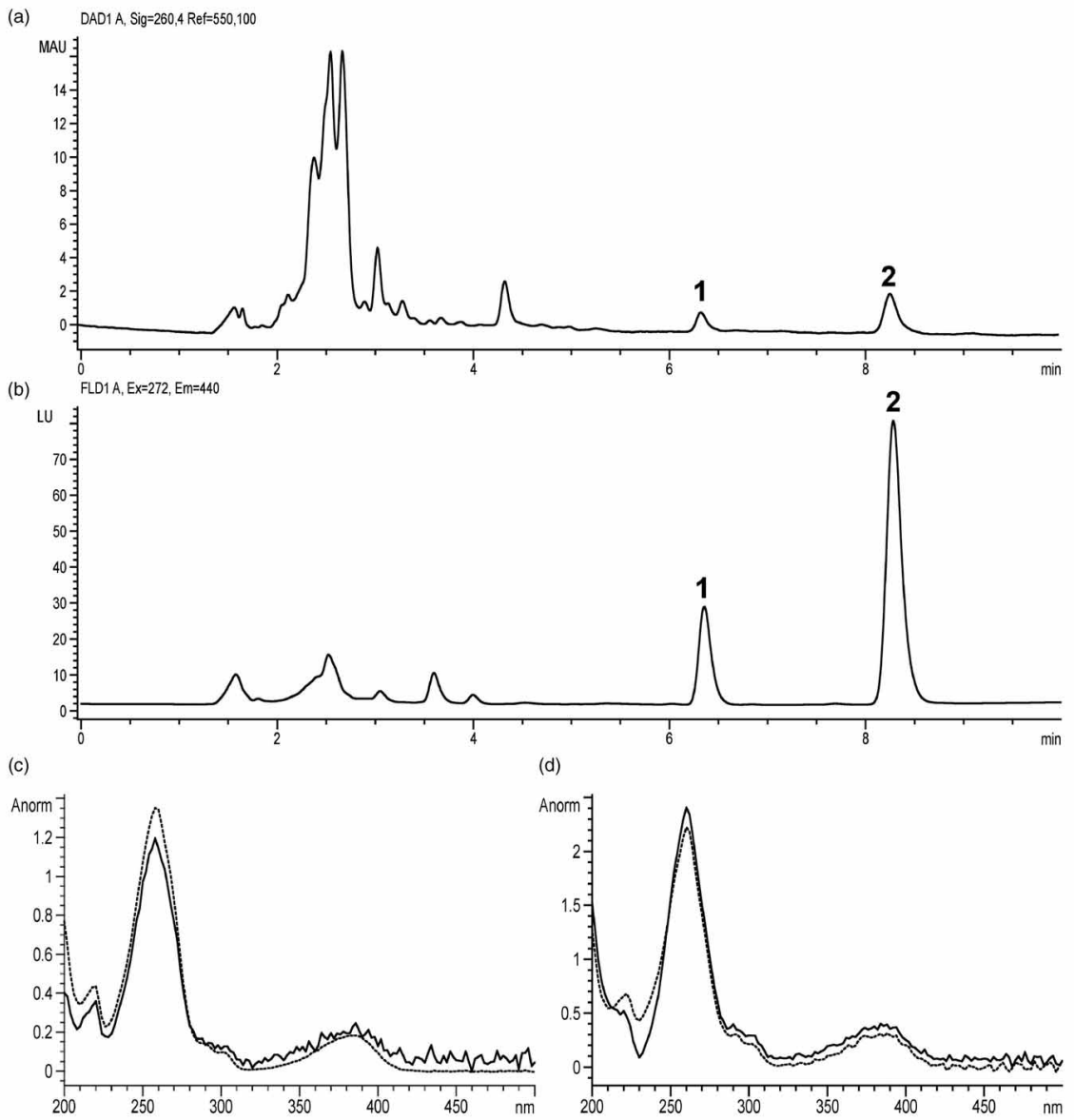


Figure 3. HPLC chromatograms of a real sample (yoghurt) showing 2 ITX (**1**) and 2,4 DTX (**2**). The DAD (**a**) and FLD Signal (**b**) are shown as well as the match of the UV spectra of 2 ITX (**c**) and 2,3 DTX (**d**) with the spectra deriving from standard solutions.

Analysis of real samples

Until now, 137 samples from the German market have been analysed for 2-ITX by the method developed (Figure 3). Most of the samples were packed in multilayer cartons and plastic cups, but also in plastic foil for butter and sausages. The results of the foodstuffs analysed are shown in Table 2. Of all the packages analysed, 2-ITX was detected in 36 samples (26%, Figure 4). In 27 of 36 positive tested food packaging materials (75%), significant migration of 2-ITX into the food could be observed with highest levels of 2-ITX found in orange juice (357 µg kg^{-1}) and baby food (208 µg kg^{-1}). In 13 samples (10%) the recommended migration level of 50 µg kg^{-1} was exceeded.

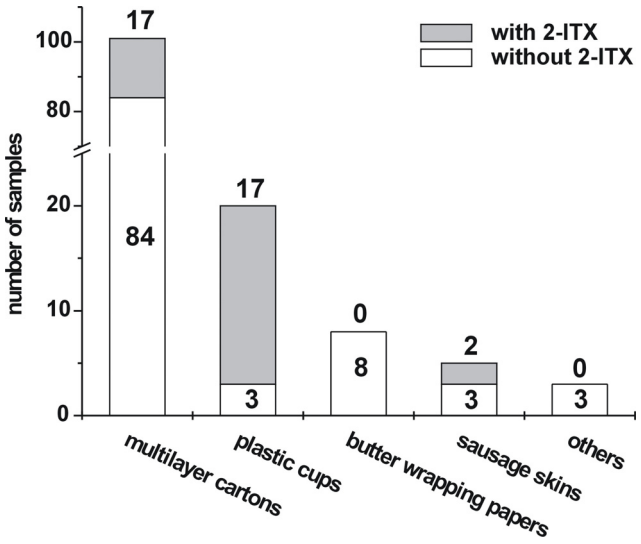


Figure 4. Overview of 2-ITX detected in food packaging materials.

Furthermore, the migration of 2-ITX into food was not only limited to printed multilayer cartons but also occured in food packed in printed plastic cups and foils (Figure 4). However, in these fillings analysed, the migration levels were always below 50 µg kg^{-1} food, at which level 2-ITX is not likely to pose a health risk.[4]

Latest results showed that 2,4-DTX the internal standard used in this study can be found in food packaging materials also, e.g. in plastic cups of yoghurt. The concentration of this contaminant in yoghurts was determined to be in the range of 15-48 µg kg^{-1} without addition of the internal standard, as described in the experimental section. Like 2-ITX, complete toxicological data about 2,4-DTX is not yet available.

Table 2. 2-ITX contents found in foods packed in multilayer cartons, plastic foils and cups.

food	packaging	number of				range of 2-ITX [µg kg^{-1}]
		samples	packaging positive	food positive	food >50 µg kg^{-1} 2-ITX	
fruit juice and nectar	multilayer cartons	31	6	5	5	48 – 357
vegetable juices	multilayer cartons	12	2	1	0	20
baby food	multilayer cartons	12	5	5	5	86 – 208
milk and cream	multilayer cartons	17	2	2	2	83 – 115
wine and other alcoholic beverages	multilayer cartons	15	0	0	0	-
tomato puree	multilayer cartons	5	0	0	0	-
sausages	plastic foil	5	2	1	0	9
yoghurt	plastic cup	19	17	11	0	7 – 40
butter	plastic foil	8	0	0	0	-
others	various	13	2	2	1	39 – 61
sum		137	36(26%)	27(20%)	13(10%)	

III.5. Conclusion

The method presented is fast and reliable for the determination of 2-ITX, as well as 2,4-DTX, in various food packaging materials and foods. With the strategy chosen to analyse the wrappings initially and, only in case of positive findings, the corresponding fillings, a rapid throughput could be achieved on a routine basis. The recovery rates of 2-ITX in food ranged between 94 and 105% with a relative standard deviation between 0.4 and 8.5%. In practice, the limit of quantitation for 2-ITX was below 50 µg kg^{-1} and, thus, allowed effective control of the maximum migration level of 50 µg kg^{-1} recommended by the German Federal Institute of Risk Assessment.[4]

Significant migration of 2-ITX from packaging materials into foodstuff was detected in 20% of the samples from the German market - up to 357 µg kg^{-1} in orange juice and 208µg kg^{-1} in baby food.

The occurrence of 2-ITX and 2,4-DTX in various food packaging materials, not limited to multilayer cartons, should direct the industry to utilize other, less-migrating photo-initiators. Moreover, the implementation of legislative standards for good manufacturing practice, with a positive list for printing inks and maximum migration limits, especially for substances with incomplete toxicological assessment, is essential.

III.6. Acknowledgement

We wish to thank Ms Pechstein, Ms Drewnik and Ms Moser for the skilful technical assistance, Mr. Dr. Weißhaar and Mr. Altkofer for valuable discussions and IGM resins for providing 2-ITX.

III.7. References

1. European Commission. Rapid Alert System for Food and Feed (RASFF): Isopropyl thioxanthone in milk for babies from Spain, Alert notification 2005.631, 2005 Sep t8. Available at http://ec.europa.eu/ food/food/rapidalert/ reports/week37-2005_en.pdf. Accessed 18 September 2006
2. European Food Safety Authority. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2-Isopropyl thioxanthone (ITX) and 2-ethylhexyl-4-dimethylaminobenzoate (EHDAB) in food contact materials . Available at http://www.efsa. europa.eu/EFSA/Scientific_Opinion/afc_op_ej293_itx_opinion_en1.pdf. Accessed 11 November 2008
3. Papilloud S, Baudraz D. Analysis of food packaging UV inks for chemicals with potential to migrate into food simulants. *Food Additives and Contaminants* 2002; **19**(2): pp.168-175

4. German Federal Institute of Risk Assessment. Constituents of printing inks in beverages from cartons. Opinion No 044/2005 of the German federal institute of risk assessment (BfR) on the 25th November 2005. Available at http://www.bfr.bund.de/cm/230/constituents_of_printing_inks_in_beverages_from_cartons.pdf. Accessed 18 November 2006

5. European Food Safety Authority. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food on a request from the commission related to 2Isopropyl thioxanthone (ITX) and 2ethylhexyl4dimethylaminobenzoate (EHDAB) in food contact materials. Available at http://www.efsa.europa.eu/etc/medialib/efsa/science/afc/afc_opinions/1256.Par.0002.File.dat/afc_op_ej293_itx_opinion_en1.pdf. Accessed 16 November 2006

6. European Communities. Commission Directive 93/10/EEC, relating to materials and articles made of regenerated cellulose film intended to come into contact with foodstuffs. *Official Journal of the European Union* 1993; **L 93**: pp.27-36

7. European Communities. Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. *Official Journal of the European Union* 2004; **L 336**: pp.4-17

8. European Communities. Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food. *Official Journal of the European Union* 1993; **L37**: pp.1-3

9. European Communities. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *Official Journal of the European Union* 2002; **31**: pp.1-24

10. Committee of Ministers of the Council of Europe. Resolution ResAP(2005)2 on packaging inks applied to the non-food contact surface of food packaging materials and articles intended to come into contact with foodstuffs. Available at https://wcd.coe.int/ViewDoc.jsp?id=908523&Site=COE&BackColorInternet=DBDCF2 &BackColorIntranet=FDC864&BackColorLogged=FDC864. Accessed 12 July 2009

11. European Printing Ink Assoc iation. Guideline on printing inks applied to the non-food contact surface of food packaging materials and articles. Available at http://www.eupia.org/doc/easnet.dll/GetDoc?APPL=2&DAT_IM=0205BB&TYPE=PDF. Accessed 18 September 2006

12. Morlock G, Schwack W. Determination of isopropylthioxanthone (ITX) in milk, yoghurt and fat by HPTLC-FLD, HPTLC-ESI/MS and HPTLC-DART/MS. *Anal.Bioanal.Chem.* 2006; **385**(3): pp.586-595

13. Sagratini G, Manes J, Giardina D, Pico Y. Determination of Isopropyl Thioxanthone (ITX) in Fruit Juices by Pressurized Liquid Extraction and Liquid Chromatography-Mass Spectrometry. *Journal of Agricultural and Food Chemistry* 2006; **54**(20): pp.7947-7952

14. Anastassiades M, Lehotay SJ, Stajnbaher D, Schenck FJ. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J.AOAC Int.* 2003; **86**(2): pp.412-431

15. Deutsches Institut für Normung e.V. DIN standard 32645, Chemical analysis, decision limit, detection limit and determination limit, estimation in case of repeatability, terms, methods, evaluation., Beuth Verlag GmbH, Berlin, Germany.

IV. Rapid identification of additives in gaskets for lids of polyvinyl chloride with direct analysis in real time ionisation and single quadrupole mass spectrometry (DART–MS)

Thorsten Rothenbacher, Wolfgang Schwack
Institut für Lebensmittelchemie, Universität Hohenheim,
Garbenstrasse 28, 70599 Stuttgart, Germany,
Correspondence to: W. Schwack, e-mail: wschwack@uni-hohenheim.de

IV.1. Abstract

Gaskets for lids of glass jars usually consist of polyvinyl chloride (PVC) containing plasticisers and additional additives, which may migrate into packed foodstuffs. Concerning legal regulations, migration has to be determined analytically, which is a big challenge due to the huge chemical variety of additives in use. Therefore, a rapid screening method by means of direct analysis in real time ionisation and single quadrupole mass spectrometry (DART™–MS) was developed. Introducing a plastisol sample into the DART™ interface, additives revealed protonated molecules and ammonium adducts as the typical ionisation products, and cleavages of ester bonds as typical fragmentation processes. Generally, additives present in the range of 1% could directly and easily be identified if ion suppressive effects deriving from specific molecules did not occur. These effects could be avoided by measuring toluene extracts of plastisol samples, which also improved the sensivity. Using this method, it was possible to identify phthalates, fatty acid amides, tributyl O-acetylcitrate, dibutyl sebacate, bis(2-ethylhexyl) adipate, 1,2-diisononyl 1,2-cyclohexanedicarboxylate, and even more complex additives like acetylated mono- and diacylglycerides, epoxidized soybean oil, and polyadipates with a limit of detection of ≤1% in PVC plastisols. Only in the case of epoxidized linseed oil were

levels ≥ 5% required for identification. The detection of azodicarbonamide as foaming agent within the manufacturing process was principally possible, but was not highly reproducible due to very low concentrations in plastisols.

IV.2. Introduction

Gaskets of metal twist closures for glass jars usually are manufactured from polyvinyl chloride (PVC) containing different additives, i.e., plasticisers, stabilisers, and slipping and blowing agents,[1] altogether also called a plastisol. The concentration of plasticisers is mainly 15% or more.[1,2] If packed foodstuff comes into direct contact with plastisols, additives or their reaction/degradation products can migrate into the foodstuff.[3] Hence, in the recent years food packed in glass jars were frequently found to be contaminated by additives, exceeding the limits of European food law.[2-11] Typically used additives are summarized in Table 1, divided into low and high molecular weight compounds. Due to different physicochemical and chemical properties, diverse analytical methods are required to analyse them. Commonly, the low molecular weight additives are extracted from foodstuffs followed by clean-up and concentration steps and analysed by gas chromatography with flame ionisation (GC/FID) or mass spectrometric detection (GC/MS), or by high performance liquid chromatography–mass spectrometry (LC/MS).[12-14] Acetylated mono- and diglycerides of fatty acids (AcPG) can be mixtures of different compounds or defined single substances, like 2,3-diacetoxy-propyl 12-acetoxystearate (APAS), which is the acetylated monoacylglyceride of hydrogenated ricinoleic acid, for example. According to the literature, mixtures of AcPGs can only be determined in food by injector-internal thermal desorption GC/MS, a method also suitable for other plasticisers, whereas APAS can be isolated from food extracts by gel permeation chromatography and determined by GC/MS.[3,15] For the epoxidized vegetable oils ESBO and ELO, extraction and direct determination by liquid chromatography/ electrospray ionisation–tandem mass spectrometry (LC/ESI-MS/MS) was reported,[16] whereas for GC analysis a transesterification step is mandatory and, depending on the method, an additional derivatisation reaction has to be used.[17-20] Polyadipates (PADs) are polyesters of 1,2-propanediol, 1,3- or 1,4-butanediol, or polypropyleneglycol with adipic acid, also end-capped with acetic acid, fatty acids (C12-C18), n-octanol or n-decanol.[21] As these units are also used in different mixtures, PADs represent a very

complex case of additives.[22] The analysis in food samples requires size exclusion chromatography, transesterification and the determination of dibutyl adipate as marker of PADs, followed by the analysis of the PAD in the gasket to calculate the amount of migrated PAD by use of a conversion factor, which is specific for different PADs.[23] To put it in a nutshell, for the analysis of the known plastisol additives in food samples, strenuous efforts have to be undertaken requiring different derivatization and clean-up steps as well as powerful and efficient instrumental setup. In order to optimize this workflow and especially to decide, if the distinct analysis of certain additives in food is necessary, a rapid and simple screening method for the identification of additives present in plastisols of gaskets of lids is desirable.

Until now only one method to determine additives in plastisols with focus on lids for glass jars was published.[1] The plastisol was dissolved in tetrahydrofuran, the PVC precipitated by ethanol, and the supernatant analysed by GC/FID or GC/MS. A second analysis including an additional transesterification step was necessary to detect the additives of higher molecular weight. Therefore, this method does not fulfill the requirements of being rapid and easy. Recently, it was mentioned that it might be possible to distinguish lids containing ESBO as the principal plasticiser from those containing phthalates as main plasticisers by means of Fourier transform infrared spectroscopy, but concrete results were not presented.[10]

Table 1. Known additives in PVC gaskets of lids.

compound	abbreviation	CAS	function
low molecular weight additives			
(Z)-docos-13-enamide (erucylamide)	EA	112-84-5	slipping agent
oleylamide	OA	301-02-0	slipping agent
azodicarbonamide	ADC	123-77-3	foaming agent
1,2-diisononyl 1,2-cyclohexanedicarboxylate	DINCH	166412-78-8	plasticiser
acetylated mono- and diglycerides of fatty acids	AcPG		plasticiser
benzyl butyl phthalate	BBP	85-68-7	plasticiser
bis(2-ethylhexyl) adipate	DEHA	103-23-1	plasticiser
bis(2-ethylhexyl) phthalate	DEHP	117-81-7	plasticiser
dibutyl phthalate	DBP	84-74-2	plasticiser
dibutyl sebacate	DBS	109-43-3	plasticiser

compound	abbreviation	CAS	function
diisodecyl phthalate	DIDP	26761-40-0	plasticiser
diisononyl phthalate	DINP	28553-12-0	plasticiser
dioctyl phthalate	DNOP	117-84-0	plasticiser
tributyl O-acetylcitrate	ATBC	77-90-7	plasticiser
high molecular weight additives			
epoxidized linseed oil	ELO	8016-11-3	plasticiser
epoxidized soybean oil	ESBO	8013-07-8	plasticiser
polyadipates	PAD		plasticiser

The direct analysis in real time (DART™) ion source provides surface desorption and soft ionisation of sample molecules on the basis of proton transfer reactions and, as an open atmospheric pressure interface, allows to directly introduce solid samples.[24,25] As recently shown, it is possible to identify phthalic acid esters in PVC toys down to 0.1% by DART™–MS as a rapid screening tool.[26] Therefore, it also should be possible to apply this technique to gaskets for lids of glass jars, but in contrast to toys, besides phthalates additional and more complex plasticisers have to be expected. Thus, the aim of the present study was to evaluate, if a rapid screening of typically used additives and especially plasticisers in plastisols can be performed by DART™–MS. Therefore, the formation of characteristic additive ions from standard solutions as compared directly from plastisols was studied, and limits of detection for the additives in plastisols were evaluated.

IV.3. Experimental

Chemicals and reagents

The following chemicals were of analytical grade unless otherwise specified. Toluene and dichloromethane were purchased from Roth (Karlsruhe, Germany), tetrahydrofuran, benzyl butyl phthalate (BBP) and sodium carbonate (anhydrous) from Merck (Darmstadt, Germany), and dibutyl phthalate (DBP), dioctyl phthalate (DNOP), dibutyl sebacate (DBS), diethyl phthalate (DEP), methyl stearate, methyl oleate, glycerol tristearate, glycerol trioleate, glycerol tripalmitate and glycerol trioctanoate from Sigma-Aldrich (Taufkirchen, Germany). Bis(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DINP), diisodecyl phthalate (DIDP), diisononyl 1,2-cyclohexanedicarboxylate (DINCH), tributyl O-acetylcitrate, bis(2-ethylhexyl) adipate (ATBC), polyadipates (PAD), acetylated mono- and diglycerides of fatty acids (AcPG), epoxidized soybean oil (ESBO), epoxidized linseed oil (ELO), oleamide

(OA), (Z)-docos-13-enamide (erucamide, EA), polydimethyldisiloxane, titanium dioxide and zinc dioctanoate were of industrial grade and provided by a lid producing company together with plastisol samples. Vinnolit P 70 was a gift of Vinnolit GmbH & Co. KG, Wacker Chemie AG (Burghausen, Germany).

Additive standard solutions and plastisol samples

To record the mass spectra of the additives, the respective standards were dissolved in toluene in a concentration of 0.1 g L^{-1}, in case of ESBO, ELO, the PADs and AcPGs 1 g L^{-1}.

Plastisols (numbered P1-P14, Table 2) were specially produced for this study by the pilot plant of a lid manufacturer. The formula of the plastisols generally contained the common additives at their typical concentrations, and the content of plasticisers in each plastisol was set to 40%. Additionally, P1 and P2 were foamed with ADC. In cases of AcPG and PAD, two different products deriving from different manufactures were used.

Additional plastisols were prepared with the standard composition of 2% polydimethyldisiloxane, 0.7% titanium dioxide, 0.05% zinc dioctanoate , 0.25% sodium carbonate, 55% PVC (Vinnolit P 70), 1% EA, 1% OA, and 40% plasticiser. In case of PAD containing plastisols, the PAD concentration was 0, 1, 2.5, and 5% with diethyl phthalate at 40, 39, 37.5, and 35%, respectively. For the preparation of plastisols, 200 mg of PVC and the additives were mixed in a 10-mL glass beaker with a spatula until a homogenous and colorless paste was obtained. The paste was coated on an aluminum foil, which was held at 200 ºC on a heating plate. After one minute, the aluminum foil was removed and the plastisol was allowed to cool off.

Extraction

About 20 mg plastisol were weighed into a 1.3-mL glass vial and 0.3 mL toluene was added. After 30 min extraction time at ambient temperature, the toluene extract was transferred into a second 1.3-mL vial by use of a disposable glass pipette.

Table 2. Composition of plasticisers in plastisols P1-P14 in mass percentages [%]. The general formula of the plastisols was 2% polydimethyldisiloxane, 0.7% titanum dioxide, 0.05% zinc octoate, 55% PVC, 1% EA and OA, and plasticisers and foaming agents according this table.

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14
DBP	0	0	0	1	2.5	0.5	0.1	0.05	0	0.1	0	0	0	0
BBP	0	0	0	1	2.5	0.5	0.1	0.05	0	0.01	0	0	0	0
DEHP	2.5	15	0	1	0	0.5	0	0.05	0	0.01	0	0.1	0	5
DNOP	0	0	0	0	1	0	0.5	0	0.05	0	0.01	0	0.1	5
DINP	2.5	0	0	1	0	0.5	0.1	0.05	0	0.01	0	0	0	0
DIDP	2.5	0	0	1	0	0.5	0.1	0.05	0	0.01	0	0	0	0
DEHA	2.5	0	22.5	1	13	0.5	30.9	0.05	36.85	39.65	39.69	38.4	38.4	10
ATBC	17.5	0	2.5	17	1	28.5	0.5	36.65	0.1	0	0	0	0	0
DBS	0	0	0	1	5	0.5	0.1	0	0	0	0	0	0	0
DINCH	2.5	0	0	1	0	0.5	0.1	0.05	0	0	0	0	0	0
ELO	10	0	0	5	0	2.5	0	1	0	0.1	0	0.5	0	10
ESBO	0	10	0	0	5	0	2.5	0	1	0	0.1	0	0.5	10
PAD1	0	10	0	0	5	0	2.5	0	1	0	0.1	0	0.5	0
PAD2	0	0	10	5	0	2.5	0	1	0	0.1	0	0.5	0	0
AcPG1	0	5	0	5	0	2.5	0	1	0	0.1	0	0.5	0	0
AcPG2	0	0	5	0	5	0	2.5	0	1	0	0.1	0	0.5	0
ADC	0.25	0.25	0	0	0	0	0	0	0	0	0	0	0	0
Na_2CO_3	0	0	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25

DART–MS

An Ion Sense DART™ 100 (KR Analytical, Sandbach, UK) with Vapur API-Interface and DART™-Control software (Version 2.19) coupled to a G1956B MSD single quadrupole mass spectrometer with ChemStation B.02.01 SR2 software (Agilent Technologies, Waldbronn, Germany) was used. The DART™'s needle voltage was set to 4000 V, discharge and grid electrode were operated at 280 V. For the DART™ helium 5.0 (purity > 99.999%) was set to a temperature of 250 ºC and used with a flow of 5-6 L min^{-1}, checked with a GFM 17 flowmeter (Analyt-MTC, Müllheim, Germany). The MSD was operated in positive fast-scan mode *m/z* 144-800 with a step size of 0.1 amu, a cycle time of 0.79 s $cycle^{-1}$, a capillary voltage of 6000 V, a fragmentor voltage of 50 V, a gain of 1.00 and a threshold of 0. Plastisol pieces of about 2 x 0.2 cm were held for about 45 s with the help of tweezers directly into the DART™ gas stream and solutions by means of DIP-it Liquid Samplers (KR Analytical, Sandbach, UK). Replication measurements of plastisols were generally taken from the same spot. The gap between the DART™ gas outlet and the Vapur API Interface inlet was 1.1 cm, the lengh of the ceramic tube 3.9 cm and the sampling point in the middle of the gap.

For measuring ESBO and ELO, the DART™ helium was set to 450 ºC and the mass scan range to *m/z* 800-1050. In case of ADC identification, the settings were 150 ºC for the helium temperature and *m/z* 70-300 for the scan range. Generally, each sample was measured six times, consecutively.

For high resolution mass spectrometry experiments, the DART™ interface was mounted onto an Orbitrap XL mass spectrometer (Thermo Scientific, Bremen, Germany) operated by XCalibur 2.0.7 software. The system was used in positive scan mode from *m/z* 140-1050 with a capillary temperature of 200 °C, a resolution of 60,000 and a scan time of one microscan with a maximum injection time of 0.5 s.

Concerning data evaluation, the set of obtained mass spectra over the whole time of a TIC current was averaged. To avoid false positive signals deriving from atmospheric conditions, the manual background correction of the MS software was used, and scans both ahead and behind the 'peak' were defined as background. This background correction securely prevented false positive identifications of additives.

Signal to noise ratios (S/N) for specific ions were calculated from the obtained mass spectra. The noise values of two different *m/z* ranges free of signals were averaged and related to the mass signal of interest. An ion was identified, if i) the S/N was >3 and ii) this occurred at least in four of six consecutive measurements.

IV.4. Results and discussion

Mass spectra of additive standards dissolved in toluene

The phthalic acid esters generally provided the protonated molecule, the corresponding $[M+18]^+$ signal and characteristic fragmentation products by DART™ ionisation as recently reported.[26] Similarly, the mass spectra of the slipping agents oleylamide (OA) and erucylamide (EA) were predominated by the protonated molecule at *m/z* 282.3 and 338.3, respectively, accompanied by an $[M+18]^+$ peak, but poor in fragments.

Since the other plasticisers under study more or less are fatty acid esters and partly rather complex in composition, methyl stearate, glycerol tristearate, glycerol trioleate, glycerol tripalmitate, and glycerol trioctanoate as model substances were firstly analysed by DART™–MS to experience ionisation principles. In all mass spectra, the $[M+18]^+$ was obtained as base peak, whereas the protonated molecule was below 50%, especially low for glycerol tristearate (5%). All triglycerides showed a fragment with an intensity of about 50%, to be explained by neutral loss of a fatty acid. In the case of, e.g., glycerol trioleate, it was recorded at *m/z* 603.5, and can be calculated as 885.8 amu ($[M+H]^+$ minus 282.3 amu for the loss of oleic acid). Therefore it can be concluded that the loss of an acid group from the protonated molecule under DART™ conditions is a typical fragmentation process for glycerides. Concerning the found $[M+18]^+$ signals, ammonium adducts are reported in literature and were especially observed, when a bottle of dilute ammonium hydroxide solution was opened nearby the DART™ source.[24] To prove the $[M+18]^+$ ions as products of environmental ammonia, solutions of EA and glycerol tristearate were analysed by DART™ coupled to an Orbitrap XL high resolution mass spectrometer. For EA, the protonated molecule at *m/z* 338.3407 (calculated 338.3417) and an $[M+18]^+$ at *m/z* 355.3672 (calculated 355.3683 amu) were obtained. In case of glycerol

tristearate, *m/z* 908.8618 for the $[M+18]^+$ (calculated 908.8641 amu) and *m/z* 607.5641 ($[M+H]^+$ minus stearic acid, calculated 607.5660 amu) were the dominant peaks in the mass spectrum. With deviations of about 3 ppm from the theoretical values, the accurate masses do support the important formation of ammonium adducts for esters and amides, even though an ammonium hydroxide solution was not used to trigger adduct formation.

Diisononyl 1,2-cyclohexanedicarboxylate (DINCH) and tributyl O-acetylcitrate (ATBC) offered the protonated molecule as base peak ion, but a more intense $[M+18]^+$ as compared to the phthalates, which was in the range of 20-40%. In the mass spectruma of dibutyl sebacate (DBS) and di(2-ethylhexyl) adipate (DEHA), the $[M+18]^+$ signal even reached 70-90% of the $[M+H]^+$ base peaks. As dimers, mainly $[2M+18]^+$ ions were found, whose intensities were about tenfold as compared to the $[2M+H]^+$ ions. This effect has also been shown before for DINP and DINCH.[26]

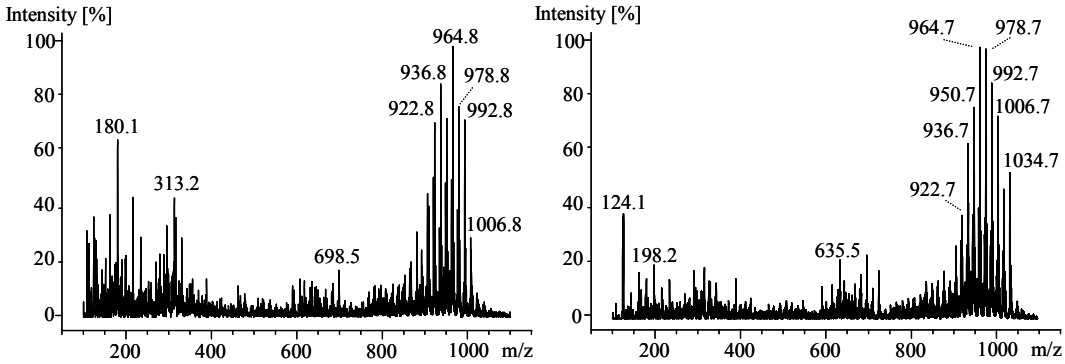


Figure 1. DART™–MS spectra of ESBO (left) and ELO (right).

Epoxidized soy bean oil (ESBO) and epoxidized linseed oil (ELO) mainly consist of epoxidized triglycerides (eTGs) of linolenic (Ln), linoleic (L), oleic (O), stearic (S) and palmitic (P) acid,[27] thus a couple of different eTGs have to be expected. Additionally, different eTGs have the same molecular formula or their masses only differ slightly as, for example, 77 ppm for the eTGs PLnLn and OLO. With intensities >80%, the mass spectrum of ESBO provided signals at *m/z* 936.7, 964.8, 978.7, and 992.7 (Figure 2), which were assigned to the $[M+NH_4]^+$ of eTGs PLL & POLn, OLO & SLL & SLnO, LLO & OLnO & LnLS, and LLL & LnOL & SLnLn, respectively. Due to the higher Ln content of linseed oil, ELO additionally showed two signals of a higher intensity at *m/z* 1020.7 and 1034.7 (Figure 1), which can be assigned to the ammonium adducts of epoxidized LnLnL and LnLnLn, respectively.

The ion at *m/z* 1034.7 did not appear in the spectrum of ESBO and is the only marker for ELO.

The mass spectrum of the acetylated mono-/diglyceride AcPG1 provided about 12 significant peaks (Figure 2). The base peak was found at *m/z* 376.2, and the second most intense peak was *m/z* 299.2 (50%). The first one was assigned to $[M+NH_4]^+$ of diacetoxypropyl dodecanoate, (APD), the second one to [M+H -acetic acid]$^+$. Measurements on the DART–OrbitrapXL system, resulting in *m/z* 376.2687 (calculated 376.2699) and *m/z* 299.2212 (calculated 299.2222), confirmed the assignments. The protonated APD and the ammonium adduct of dimeric APD yielded *m/z* 359.2 and 734.5, respectively. The signals of the main component APD are accompanied by the ammonium adducts of homologue C8, C10, C14, C16, and C18 fatty acid esters (Figure 2), including fragments formed by losses of a fatty acid or acetic acid. The analysis of AcPG2 resulted in a mass spectrum containing *m/z* 518.3 as base peak, *m/z* 501.3 with an intensity of 80%, *m/z* 441.3 (25%) and *m/z* 381.2, which were assigned to the $[M+NH_4]^+$, the $[M+H]^+$ and a fragmentation products formed by loss of one or two acetic acid moieties of 2,3-diacetoxypropyl 12-acetoxystearate as the main component of AcPG2. The by-product 2,3-diacetoxypropyl stearate is responsible for *m/z* 460.3 ($[M+NH_4]^+$, 10%).

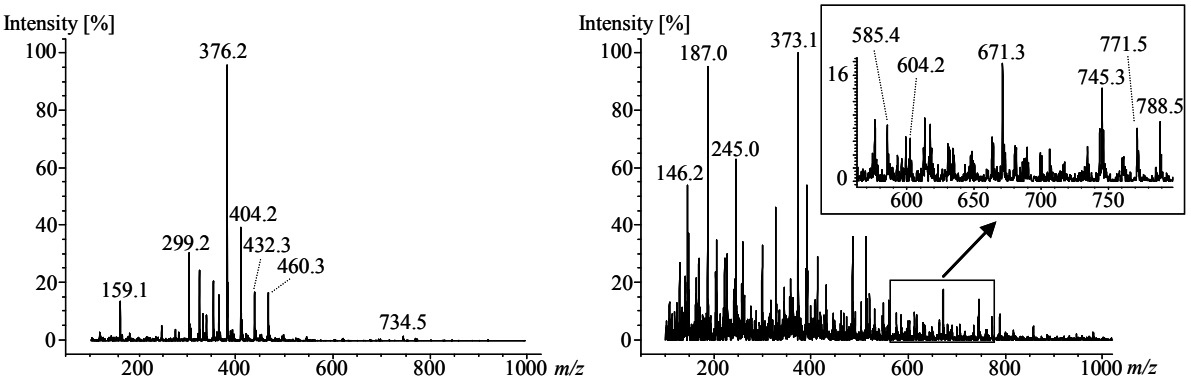


Figure 2. DART™–MS spectra of AcPG1 (left) and PAD1 (right)

As compared to the former plasticisers, the mass spectra of both polyadipates, PAD1 and PAD2, were highly complex and showed more than 40 peaks, exemplarily shown for PAD1 in Figure 2. In spite of this, the spectra of both PADs revealed *m/z* 373.1 (base peak), 390.2 and 745.3, which were assigned to the $[M+H]^+$, the $[M+NH_4]^+$ and the $[2M+H]^+$ of the cyclic adipate oligomer containing two adipate (A)

and two 1,2-propanediol (P) units. This PAD component was described as cy(A-P)$_2$ and identified in 7 of 14 commercially available PADs by Biedermann et al.[22]. DART™–MS signals at *m/z* 187.0 and *m/z* 204.1 were also significant for both PADs, which correspond to the $[M+H]^+$ and $[M+NH_4]^+$ of a cyclic 1,2-propanediol adipate (cy(A-P), which was identified by Biedermann et al. in the same PADs), but they also found *m/z* 187 as a very stable fragment of cy(A-P)$_2$ during GC/MS studies. The former assignments were confirmed by high resolution mass spectrometry resulting in signals at *m/z* 373.1847, 390.2113, 745.3624, 187.0959, and 204.1224, all of which with a deviation of about 2.5 ppm from the calculated exact masses. Besides the common signals, there also were differences in the mass spectra of PAD1 and PAD2. Signals, for example, at *m/z* 585.4, 602.4, 771.5, and 788.5 were only recorded in case of PAD1. The first two are assigned to the $[M+NH_4]^+$ and $[M+H]^+$, respectively, of an oligomer consisting of two A and one P, endcapped with decanol (D) and octanol (O), i.e., O-A-P-A-D, according to the nomenclature of Biedermann et al.,[22] whereas the latter two ions correspond to the respective adducts of O-A-P-A-P-A-D. High resolution mass spectrometry delivered *m/z* 585.4346, 602.4610, 771.5236, and 788.5519, which are in agreement with the calculated exact masses with a deviation of about 2.5 ppm. These two components were identified by Biedermann et al. in only one of the 14 investigated PADs. Therefore, the DART™–MS method should be suitable for rapid identification of a specific PAD deriving from a well known and characterized pool of PADs.

Data obtained from plastisol analysis

From the MS data obtained, the characteristic ions summarized in Table 3 were selected and used for the identification of additives in plastisols by DART™–MS.

Table 3. Selected ions and limits of detection for the identification of additives in plastisols by DART™–MS. If an ion was not detectable at a given percentage, the next higher detectable concentration present in a plastisol is added in parenthesis. For ions of ESBO, ELO, the PADs and AcPG 1, the LODs of the toluene extracts were additionally added.

additive	chemical formula of the characteristic molecule	*m/z*	adduct	LOD (%) in plastisols / LOD (%) in toluene extracts
DBP	$C_{16}H_{22}O_4$	279.2	H^+	1
		296.2	NH_4^+	2.5
BBP	$C_{19}H_{20}O_4$	313.1	H^+	1
		330.2	NH_4^+	2.5
DEHP	$C_{24}H_{38}O_4$	391.3	H^+	1
		408.3	NH_4^+	n.d. at 2.5% (15%)
DNOP	$C_{24}H_{38}O_4$	391.3	H^+	0.5
		408.3	NH_4^+	n.d. at 1%
DINP	$C_{26}H_{42}O_4$	419.3	H^+	0.5
		436.3	NH_4^+	n.d. at 2.5%
DIDP	$C_{28}H_{46}O_4$	447.3	H^+	0.5
		464.3	NH_4^+	n.d. at 2.5%
DEHA	$C_{22}H_{42}O_4$	371.3	H^+	1
		388.3	NH_4^+	n.d. at 2.5% (10%)
ATBC	$C_{20}H_{34}O_8$	403.2	H^+	0.5
		420.2	NH_4^+	n.d. at 2.5% (17%)
DBS	$C_{18}H_{34}O_4$	315.3	H^+	1
		332.3	NH_4^+	1
DINCH	$C_{26}H_{48}O_4$	425.4	H^+	0.5
		442.4	NH4+	n.d. at 2.5%
ESBO	C55H98O10	936.7	NH4+	n.d. at 40% / 1
	$C_{57}H_{102}O_{10}$	964.8	NH_4^+	n.d. at 40% / 1
	$C_{57}H_{100}O_{11}$	978.8	NH_4^+	n.d. at 40% / 1
	$C_{57}H_{98}O_{12}$	992.7	NH_4^+	n.d. at 40% / 1
ELO	$C_{55}H_{98}O_{10}$	936.7	NH_4^+	n.d. at 10% / 5
	$C_{57}H_{102}O_{10}$	964.8	NH_4^+	n.d. at 10% / 2.5
	$C_{57}H_{100}O_{11}$	978.8	NH_4^+	n.d. at 10% / 2.5
	$C_{57}H_{98}O_{12}$	992.7	NH_4^+	n.d. at 10% / 2.5
	$C_{57}H_{92}O_{15}$	1034.7	NH_4^+	n.d. at 10% / 5
PAD1	$C_{18}H_{28}O_8$	373.2	H^+	n.d. at 5% (10%) / 2.5
		390.2	NH_4^+	2.5 / 5
	$C_{33}H_{60}O_8$	585.4	H^+	5 / 1
	$C_{42}H_{74}O_{12}$	771.5	H^+	n.d. at 10% / 5
PAD2	$C_{18}H_{28}O_8$	373.2	H^+	n.d.at 5% / 1
		390.2	NH_4^+	2.5 / 5
AcPG1	$C_{19}H_{34}O_6$	359.2	H^+	n.d. at 5% / 5
		376.2	NH_4^+	2.5 / 1
AcPG2	$C_{27}H_{48}O_8$	501.3	H^+	0.5
		518.3	NH_4^+	0.1

Concerning data recording, the software provides curves representing the total or selected ion currents, respectively, according to the experiment time (Figure 3). Generally, the higher concentrated an additive is present in the plastisol, the higher the expected signal of the corresponding selected ion current is. However, the obtained 'peaks' did not repeatedly result in the same shape and intensity, although the plastisol samples were introduced into the DART™ interface in the same manner, as well as manually possible. Additionally, different additives obviously ionize with different latencies, for example EA and DIDP (Figure 3). This partly held true for different adducts of the same additive as shown for the $[M+H]^+$ and $[M+NH_4]^+$ of ATBC. If plastisol samples of highly concentrated and easily ionizable additives, e.g. 15% DEHA, are measured, possible memory effects may be suspected, resulting in a mass spectrometric background containing ions of the respective additives during the measurement of forthcoming samples. However, false positive signals were generally not observed, as exemplarily shown for the extracted ion counts of ATBC and DIDP during the analysis of the second plastisol (Figure 3).

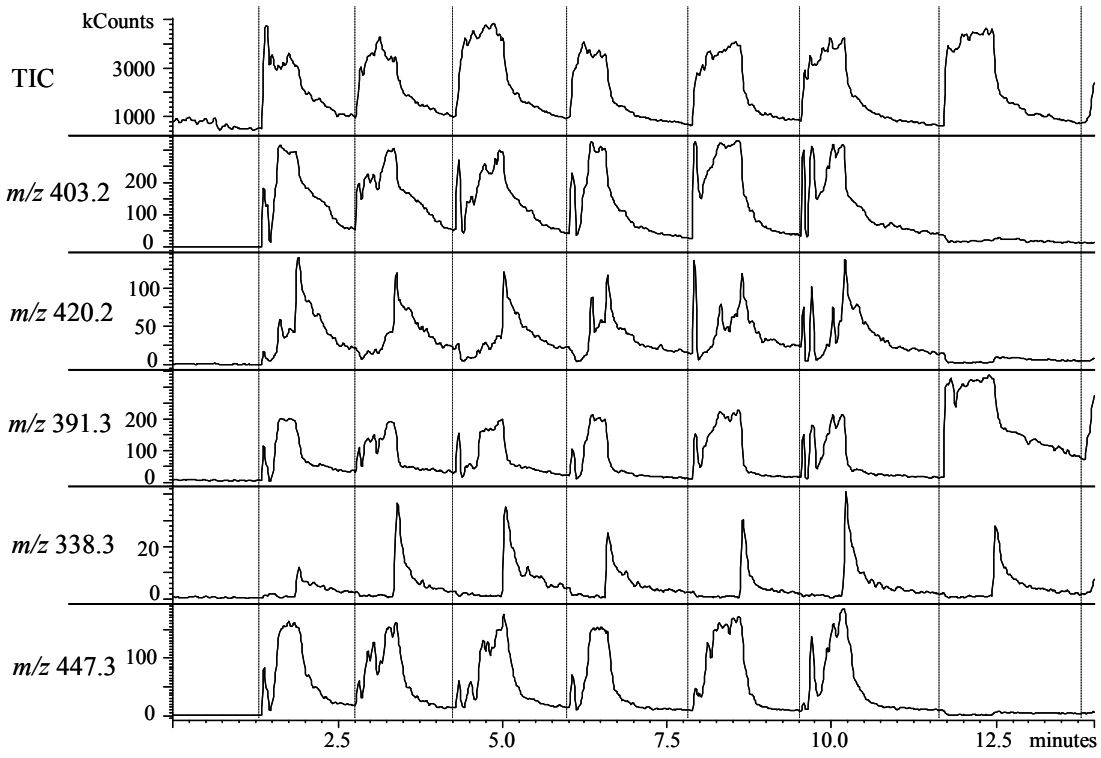


Figure 3. Total and selected ion currents (SICs) obtained during DART™–MS of plastisols: 0–1.25 min, blank (atmospheric situation); 1.25–11.5 min, P1 measured six times; 11.5– 14.0 min, P2 measured once; beginning of each measurement is indicated by a vertical line (SICs: *m/z* 403.2 and 420.2, $[M+H]^+$ and $[M+NH_4]^+$ of ATBC; *m/z* 391.3, $[M+H]^+$ of DEHP; *m/z* 338.3, $[M+H]^+$ of EA; *m/z* 447.3, $[M+H]^+$ of DIDP).

Identification of additives in the standardized plastisols P1-P14

The phthalic acid esters, DINCH, ATBC, AcPG, DBS and DEHA showed the same mass signals deriving from different plastisols as they were obtained from the toluene solutions. Identification of PAD1 and PAD2 through *m/z* 373.2 and *m/z* 390.2 was generally possible, but the signals may coincide with the M+2 satellites of DEHA, if it is present in high concentrations.

EA and OA, present at 1% in plastisols, were generally detectable through their protonated molecules at the defined LOD of S/N>3. The identification was significantly enhanced by measuring the toluene extracts, when both slipping agents provided clear signals with a S/N ratio of 20.

ESBO and ELO surprisingly were not detectable in the plastisols P1-P14. An additionally prepared plastisol with 40% ESBO showed the same result. Instead of the typical ESBO signals, only *m/z* 989.8, 1009.8, 1022.8 and 1042.7 (base peak) were detected within the relevant mass range in intensities that had been expected for ESBO signals. Further experiments showed that EA and OA, i) in combination and ii) both at a concentration of only 1%, strongly gave rise to ion suppressive effects according to ESBO ions. To avoid these effects, an extraction of ESBO with organic solvents was tested. Therefore, about 20 mg plastisol were extracted by 0.3 mL tetrahydrofuran, dichloromethane and toluene for 30 min and 12 h, respectively. Tetrahydrofuran dissolved the plastisol during both extraction times, but decrease of the ion suppressive effects did not occur. The same effects were observed for the 12-h extraction with dichloromethane. In case of the 12-h extraction with toluene and the 30-min extractions with both dichloromethane and toluene, the plastisol was not completely dissolved, and ESBO was well detectable. To avoid halogenated solvents, the 30 min extraction with toluene was preferred. Figure 4 exemplarily demonstrates the success of extraction as compared to direct measurement of plastisols; all used plasticisers were clearly identified. As the extraction procedure takes about half an hour and direct measuring of the plastisol including data evaluation can be performed during the extraction, the whole procedure of a plastisol analysis will only take approximately 45 min.

Additional efforts have been undertaken to differentiate ELO from ESBO in plastisols. On base of the different eTGs distribution of ESBO and ELO, peak height ratios should be indicators for the used additive. For example, the ratio of the TGs LLL & LnLO & SLnLn (epoxidized form in ESBO or ELO is identified by *m/z* 992.7) and PLL & POLn (epoxidized form in ESBO or ELO is identified by *m/z* 936.7) is reported as 1.7 and 8.0 for soy bean oil and linseed oil, respectively.[28]

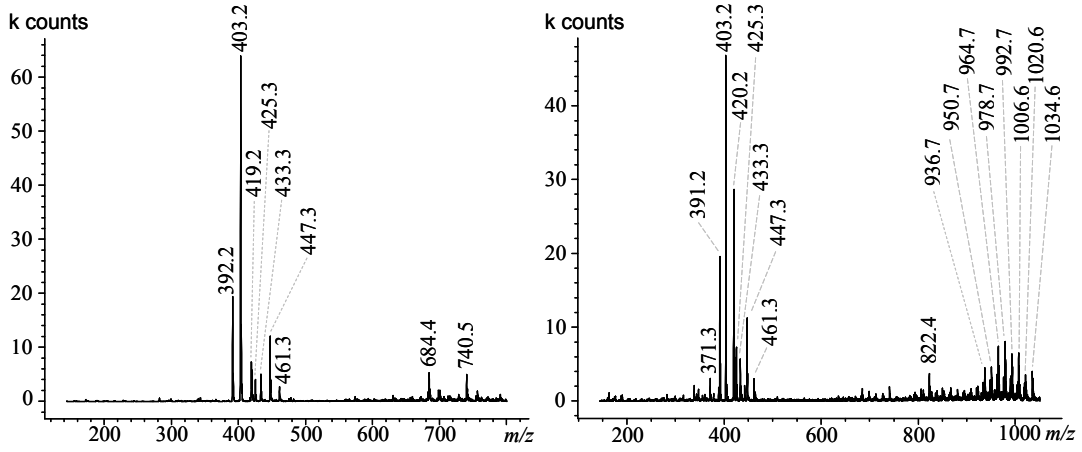


Figure 4. DART™–MS spectra of plastisol P1 directly measured (left) and after toluene extraction (right): DEHA (371.3), DEHP (391.2), ATBC (403.2, 420.2, 822.4 as $[2M+NH_4]^+$), DINP (419.2, on the right spectrum attaching to 420.2), DINCH (425.3), DIDP (433.2), ELO (936.7, 950.7, 964.7, 978.7, 992.7, 1006.6, 1020.6, 1034.6), impurities of DIDP or DINP (447.3, 461.3).

In six measurements of toluene extracts of the plastisols P1 (10% ESBO) and P2 (10% ELO), the averaged peak height ratio was 1.7 (± 0.5) for ESBO and 2.4 (± 1.4) for ELO, respectively. Thus, the obtained ratio was higher for ELO than for ESBO, but the expected strong difference was not found. If the cited ratios hold true for the respective plant oils, including natural variations, differences in response of eTGs under DART™ conditions may explain the discrepancy. Therefore, the best way to distinguish between ESBO and ELO is the identification of the eTG LnLnLn (*m/z* 1034.7), which was absent in any mass spectrum of ESBO, and, as compared to ESBO, the highly abundant *m/z* 1006.7.

Detection of azodicarbonamide

Due to the release of semicarbazide, a minor thermal decomposition product of the blowing agent azodicarbonamide (ADC), the use of ADC in the manufacturing process of gaskets for lids is forbidden in the European Community.[6,29] During heat

treatment of the plastisol's production process, ADC decomposes into gaseous products (34%) and the solid residues hydrazodicarbonamide (HDC, 34%) and urazole (27%).[30] As recorded DART™–MS spectra of plastisols showed intense signals in the mass range below 150 Da due to fragment and atmospheric ions, the former DART™ parameters used for additive identification could not be used. Therefore, the helium temperature was set to 150 ºC to i) decrease ionisation of high mass molecules and ii) to lower fragmentations. Additionally, the scan range was reduced to *m/z* 70-300. As a result, the total ion current surprisingly zeroed, when plastisol samples were introduced into the interface (Figure 5). Obviously, the ionisation of atmospheric molecules was suppressed by additives deriving from the plastisol sample. With respect to the scan range, however, the additive ions were not recorded resulting in a total ion current of nearly zero. Under these conditions, not ADC itself, but its main gaseous decomposition product hydroazodicarbonamide (HDC) was detectable through the protonated molecule at *m/z* 119 in the background corrected mass spectrum and also resulted in a small peak in case of the selected ion current (Figure 5). To avoid a decrease in concentration, the success of detectability was checked by introducing a new sample piece for repeated measurements. During a series of six replicates of plastisol P2, the HDC peak only was clearly detectable in three cases. However, in P3 foamed by Na_2CO_3, HDC could never be detected. Therefore, this method for the detection of an ADC usage principally worked, but was not sufficiently reproducible due to rather low concentrations.

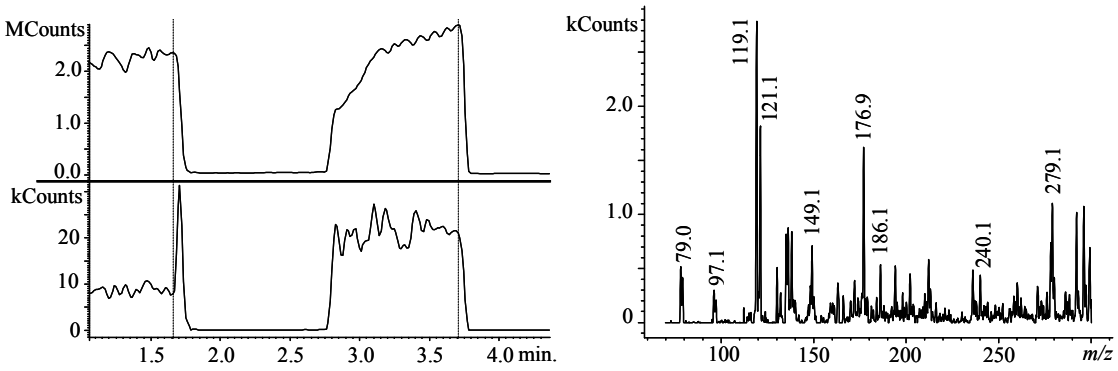


Figure 5. Left: Identification of HDC in plastisols; total ion (top) and selected ion (*m/z* 119) (bottom) current. Right: background corrected mass spectrum at 1.8 min.

Limits of detection

Considering the defined data evaluation method, the obtained results for the plastisols P1-P14 were interpreted to establish LODs for significant ions used for the identification of the additives. On basis of at least one characteristic ion, DNOP, DINP, DINCH, ATBC, and AcPG2 were detectable in concentrations of ≥0.5% in plastisols, whereas for DBP, BBP, DEHP, DBS, and DEHA, the LOD was 1% (Table 3). Detectability was also given at lower concentrations, but then only occurred in less than four of six consecutive measurements. Compared to the LODs of phthalic acid esters in plastisols that only contained DINCH as additional plasticizer,[26] the present results of more complex plastisols showed higher LODs. This may be due to ion suppression effects and the increased gas temperature, which enhanced fragmentation of molecular ions, as shown for DHEP and DNOP.[26] In the case of AcPG1, direct identification in the plastisol was only possible, if the concentration was above 2.5%; extraction with toluene resulted in an LOD of 1%. As the plastisols P1-P14 almost had high concentrations of interfering DEHA, additional plastisols were prepared at levels of 0–5% PAD1 or PAD2, respectively. DART™–MS measurements showed that *m/z* 373.3 ($[M+H]^+$ of cy(A-P)$_2$) was not detectable, but the ion at *m/z* 390.2 ($[M+NH_4]^+$ of cy(A-P)$_2$) of both PADs at a level of 2.5%, and *m/z* 585.4 of PAD1 at 5%. For some characteristic ions of the PADs, LODs were also lowered by toluene extraction (Table 3). The epoxidized vegetable oils were only detectable in toluene extracts; the resulting LODs were 1% for the characteristic ESBO ions, and 2.5% as well as 5% for typical ELO ions (Table 3). Significant identification of ELO is limited to 5% and does not cover mixtures of ESBO and ELO.

IV.5. Conclusion

The method presented shows great possibilities to identify additives in plastisols very easily and rapidly, compared to already published methods. As more clarity is brought into the results of ionisation and fragmentation processes, this method may be transferred easily to other applications dealing with similar subjects. DART™-MS may also be very useful to rapidly identify complex plasticisers as, for example, to distinguish between different kinds of PADs used in a plastisol.

IV.6. Acknowledgement

The authors would like to thank Wacker Chemie AG for providing the PVC and especially Ms Tatjana Resner for her commitment with DART™-MS measurements.

Thanks are also given to Dr. Jens Pfannstiel (Life Science Center, University of Hohenheim) for the chance to mount the DART™ interface onto the Orbitrap XL mass spectrometer.

IV.7. References

1. Biedermann-Brem S, Biedermann M, Fiselier K, Grob K. Compositional GC-FID analysis of the additives to PVC, focusing on the gaskets of lids for glass jars. *Food Additives & Contaminants* 2005; **22**(12): pp.1274-1284
2. Ezerskis Z, Morkunas V, Suman M, Simoneau C. Analytical screening of polyadipates and other plasticisers in poly(vinyl chloride) gasket seals and in fatty food by gas chromatography-mass spectrometry. *Analytica Chimica Acta* 2007; **604**(1): pp.29-38
3. Fankhauser-Noti A, Biedermann-Brem S, Grob K. PVC plasticisers/additives migrating from the gaskets of metal closures into oily food: Swiss market survey June 2005. *European Food Research and Technology* 2006; **223**(4): pp.447-453
4. Hammarling L, Gustavsson H, Svensson K, Karlsson S, Oskarsson A. Migration of epoxidized soya bean oil from plasticized PVC gaskets into baby food. *Food Addit Contam* 1998; **15**(2): pp.203-208
5. Fantoni L, Simoneau C. European survey of contamination of homogenized baby food by epoxidized soybean oil migration from plasticized PVC gaskets. *Food Additives & Contaminants* 2003; **20**(11): pp.1087-1096
6. Stadler RH, Mottier P, Guy P, Gremaud E, Varga N, Lalljie S, Whitaker R, Kintscher J, Dudler V, Read WA, Castle L. Semicarbazide is a minor thermal decomposition product of azodicarbonamide used in the gaskets of certain food jars. *Analyst (Cambridge, United Kingdom)* 2004; **129**(3): pp.276-281

7. Elss S, Gruenewald L, Richling E, Schreier P. Occurrence of 2-ethylhexanoic acid in foods packed in glass jars. *Food Additives and Contaminants* 2004; **21**(8): pp.811-814

8. Fankhauser-Noti A, Fiselier K, Biedermann S, Biedermann M, Grob K, Armellini F, Rieger K, Skjevrak I. Epoxidized soy bean oil (ESBO) migrating from the gaskets of lids into food packed in glass jars. *European Food Research and Technology* 2005; **221**(3-4): pp.416-422

9. Fankhauser-Noti A, Grob K. Migration of plasticisers from PVC gaskets of lids for glass jars into oily foods: Amount of gasket material in food contact, proportion of plasticiser migrating into food and compliance testing by simulation. *Trends in Food Science & Technology* 2006; **17**(3): pp.105-112

10. Pedersen GA, Jensen LK, Fankhauser A, Biedermann S, Petersen JH, Fabech B. Migration of epoxidized soybean oil (ESBO) and phthalates from twist closures into food and enforcement of the overall migration limit. *Food Additives & Contaminants, Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment* 2008; **25**(4): pp.503-510

11. European Communities. Commission Directive 2002/72/EC of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs. *Official Journal of the European Union* 2002; **L 220**: pp.18-58

12. Silva AS, Garcia RS, Cooper I, Franz R, Losada PP. Compilation of analytical methods and guidelines for the determination of selected model migrants from plastic packaging. *Trends in Food Science & Technology* 2006; **17**(10): pp.535-546

13. Lau OW, Wong SK. Determination of plasticisers in food by gas chromatography-mass spectrometry with ion-trap mass detection. *J Chromatogr A* 1996; **737**(2): pp.338-342

14. Lau OW, Wong SK. Contamination in food from packaging material. *Journal of Chromatography, A* 2000; **882**(1+2): pp.255-270

15. Simoneau C, Ezerskis Z, Morkunas V. Determination of plasticisers and additives in PVC gasket seals and in fatty sauces and migration study. *Food Contact Polymers 2007, International Conference, 1st, Brussels, Belgium, Feb.21-22, 2007* 2007: pp.8/1-8/5

16. Suman M, La Tegola S, Catellani D, Bersellini U. Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry Method for the Determination of Epoxidized Soybean Oil in Food Products. *Journal of Agricultural and Food Chemistry* 2005; **53**(26): pp.9879-9884

17. Castle L, Sharman M, Gilbert J. Gas chromatographic-mass spectrometric determination of epoxidized soybean oil contamination of food by migration from plastic packaging. *Journal - Association of Official Analytical Chemists* 1988; **71**(6): pp.1183-1186

18. Weller P, Herrnreiter M, Donaubauer A. A miniaturized method to determine epoxidized soybean oil in baby food. *LC-GC Europe* 2007; **20**(5): pp.280-282, 285

19. Biedermann-Brem S, Biedermann M, Fankhauser-Noti A, Grob K, Helling R. Determination of epoxidized soy bean oil (ESBO) in oily foods by GC-FID or GC-MS analysis of the methyl diepoxy linoleate. *European Food Research and Technology* 2007; **224**(3): pp.309-314

20. Rothenbacher T, Schwack W. Determination of epoxidized soybean oil by gas chromatography/single quadrupole and tandem mass spectrometry stable isotope dilution assay. *Rapid Communications in Mass Spectrometry* 2007; **21**(12): pp.1937-1943

21. European Commission. Commission Regulation (EC) No 372/2007 of 2 April 2007 laying down transitional migration limits for plasticisers in gaskets in lids intended to come into contact with foods (corrected form). *Official Journal of the European Union* 2007; **L 92**: pp.9-12

22. Biedermann M, Grob K. GC-MS characterization of oligomers in polyadipates used as plasticisers for PVC in food contact. *Packaging Technology & Science* 2006; **19**(3): pp.159-178

23. Biedermann M, Grob K. GC method for determining polyadipate plasticisers in foods: transesterification to dibutyl adipate, conversion to migrating polyadipate. *Chromatographia* 2006; **64**(9-10): pp.543-552

24. Cody RB, Laramee JA, Durst HD. Versatile new ion source for the analysis of materials in open air under ambient conditions. *Anal.Chem.* 2005; **77**(8): pp.2297-2302

25. Cody RB, Laramee JA, Nilles JM, Durst HD. Direct Analysis in Real Time (DART^{TM}) Mass Spectrometry; **78**. JEOL News 2005; 40(1): pp.8-12

26. Rothenbacher T, Schwack W. Rapid and nondestructive analysis of phthalic acid esters in PVC by direct analysis in real time-single quadrupole mass spectrometry (DART™-MS). Rapid Communications in Mass Spectrometry 2009; status: accepted

27. Belitz, H.-D.; Grosch, W. *Food Chemistry,* Springer-Verlag, Berlin - Heidelberg - New York. ISBN 3-540-64704-X

28. Rezanka T, Mares P. Determination of plant triacylglycerols using capillary gas chromatography, high-performance liquid chromatography and mass spectrometry. *Journal of Chromatography* 1991; **542**(1): pp.145-159

29. European Communities. Commission directive 2004/1/EC amending Directive 2002/72/EC as regards the suspension of the use of azodicarbonamide as blowing agent. *Official Journal of the European Union* 2004; **L 7**: pp.45-46

30. De la Calle MB, Anklam E. Semicarbazide: occurrence in food products and state-of-the-art in analytical methods used for its determination. *Analytical and Bioanalytical Chemistry*. 2005; **382**: 968-977

V. Determination of epoxidized soy bean oil by gas chromatography / single quadrupole and tandem mass spectrometry stable isotope dilution assay

THORSTEN ROTHENBACHER[*§], WOLFGANG SCHWACK[#]

[*] Chemisches und Veterinäruntersuchungsamt Stuttgart, Schaflandstraße 3/ 2, 70736 Fellbach, Germany

[#] Institut für Lebensmittelchemie, Universität Hohenheim, Garbenstr. 28, 70599 Stuttgart, Germany, e-mail: wschwack@uni-hohenheim.de, corresponding author

V.1. Abstract

PVC lids of glass jars often contain epoxidized soybean oil (ESBO), able to migrate and contaminate food. To establish a stable isotope dilution assay (SIDA), the $^{13}C_{18}$ labelled internal standard ethyl 9,10,12,13-diepoxyoctadecanoate (^{13}C(18:2E)Et) was synthesized, providing after sample preparation the same retention time as methyl 9,10,12,13-diepoxyoctadecanoate ((18:2E)Me), commonly used as marker for ESBO in GC analysis. For eleven different food matrices, the GC capillary columns VF-17ms, DB1701 and DB1 were tested with single quadrupole (GC-MS) as well as tandem mass spectrometric detection (GC-MS/MS). Overall, the VF-17ms column coupled with MS/MS detection showed the best results in terms of separation and sensitivity. The method validation for the matrix spiked olive oil resulted in a LOD of 5 mg kg^{-1}, a LOQ of 11 mg kg^{-1}, a mean recovery (n=5, c=106.5 mg kg^{-1}) of 99.7±5.5 % with a repeatability (within-run precision) of 6.0 %. By means of GC-MS a LOQ of 21 mg kg^{-1} and a mean recovery (n=5, c=106.5 mg kg^{-1}) of 103.3±0.8 % with a repeatability of 0.9 % were determined.

[§] Present adress: Institut für Lebensmittelchemie, Universität Hohenheim

V.2. Introduction

Epoxidized soybean oil (ESBO, CAS Registry Number 8013-07-8) is used as plasticiser and stabilizer for PVC, employed e.g. for closure gaskets of glas jars' metal lids. In 64 % of 158 lids collected at the Swiss market in June 2005, ESBO was the principal plasticiser in the gaskets with an average concentration of 35 to 40 % of the formulation weight.[1]

If food packed in glass jars comes into contact with the gasket, the plasticiser is able to migrate and contaminate the food. According to the Scientific Committee on Food (SCF), the tolerable daily intake (TDI) for ESBO is 1 mg kg^{-1} b.w.[2] The European specific migration limit (SML) was set to 60 mg kg^{-1} for food and 30 mg kg^{-1} for baby and infant food by the European Commission Directive 2005/79/EC.[3] The average weight of the gasket material of a lid coming into food contact is 228 mg, so with 35 % ESBO in the PVC of the lid for a 100 g jar, a maximum amount of 800 mg kg^{-1} plasticiser potentially migrating into the food can be estimated.[4] Former investigations showed ESBO contaminations of up to 580 mg kg^{-1} in food and 135 mg kg^{-1} in baby food.[4-7]

Using liquid chromatography tandem mass spectrometry, ESBO was determined in food sauces directly after extraction by dichloromethane. By selected reaction monitoring five signals, representing approximately 90 % of ESBO's triglycerides, were used for quantification.[8]

Concerning the analytical procedure of gas chromatography (GC), ESBO is determined as methyl 9,10,12,13-diepoxyoctadecanoate ((18:2E)Me) after transesterification. According to the Prileschajew reaction, complete epoxidation of linoleic acid, the main fatty acid of soy bean oil, results in four stereoisomers (Figure 1), due to the retained cis configuration of the reaction's transition state.[9] During GC analysis of the methyl esters, two peaks are obtained likely representing two pairs of enantiomers (structures **1**/**2** and **3**/**4**, Figure 1). Regarding the detection order in GC analysis the corresponding substance pairs are named (18:2E)Me(1) and (18:2E)Me(2). Because the content of (18:2E)Me(1) in ESBO is about twofold as compared to (18:2E)Me(2), the former is used for quantification.[10]

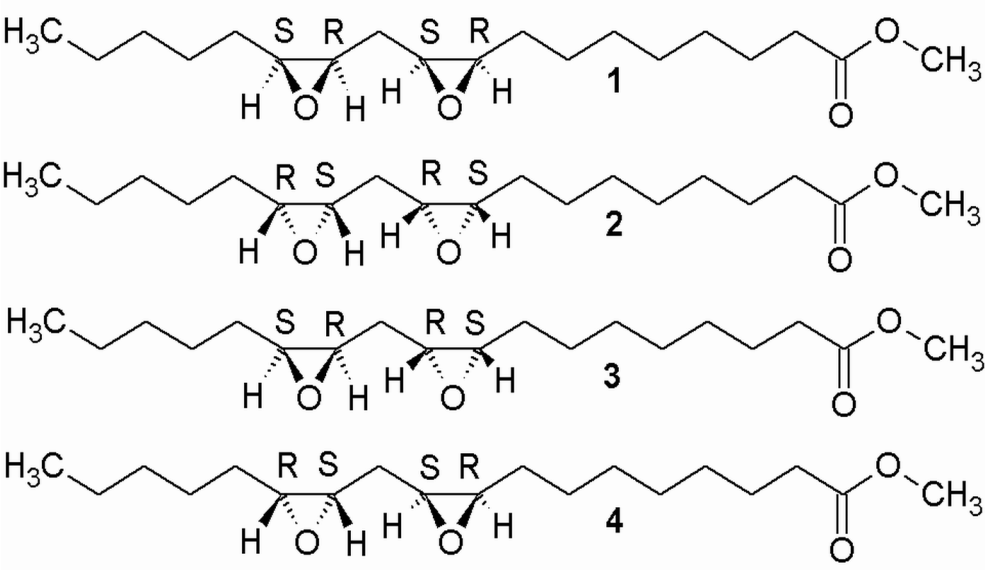


Figure 1. Structure formulae of the four possible stereoisomers (1 - 4) for (18:2E)Me, resulting from complete epoxidation of methyl linoleate. The capital letters (R, S) refer to the absolute configuration according to the Cahn-Ingold-Prelog rules.

If (18:2E)Me(1) is used as a marker, it must be guaranteed that its presence does not differ depending on both the origin of the soy bean oil used for the production of ESBO and the process. To prove the suitability of this marker for ESBO, the mean content of 77 lids was analysed to 33.0±1.9 % (18:2E)Me(1).[7]

The common GC analysis of ESBO in food was done by the use of ethyl 11,12,14,15-diepoxyeicosanoate as internal standard, trans-methylation of the lipids extracted from a sample, reaction of (18:2E)Me with cyclopentanone yielding 1,3-dioxolanes and GC-(EI)MS analysis.[11] The dioxolanes provide stability of the epoxidized compounds, optimize the gas chromatographic separation from fatty acid methyl esters, and shift the mass spectra to higher and more selective masses.

According to more time saving analysis, after addition of methyl 11,12,14,15-diepoxyeicosanoate as internal standard, ESBO was directly transmethylated in the food without prior fat extraction.[12] Additionally, (18:2E)Me(1) was directly determined without the transformation into dioxolanes by on-line liquid chromatography - gas chromatography with flame ionisation detection (LC-GC/FID).[7]

Using the same fast sample preparation, very recently a GC analysis was performed on polar stationary phases with FID detection for most food matrices, but in case of difficult matrices chemical ionisation with ammonia and mass

spectrometric detection was favoured.[13] However, the GC-FID method requires tenfold extract concentration before GC analysis, to achieve a detection limit of 20 mg kg^{-1}, and LC/GC-FID or GC-MS with chemical ionisation with ammonia, respectively, are not common equipments of food control laboratories. Therefore, regarding the advantages of fast sample preparation, it was the aim of the present study to use GC-MS and GC-MS/MS instruments with 70 eV electron impact ionisation for quantification of (18:2E)Me(1). For optimized analysis, $^{13}C_{18}$-labelled ethyl 9,10,12,13-diepoxyoctadecanoate (^{13}C(18:2E)Et) was introduced as internal standard, providing stable isotope dilution assay (SIDA) features after transesterification into the corresponding methyl ester. Furthermore, the chromatographic separation power of three different GC capillary columns was checked for eleven different food matrices by means of mass spectrometric detection in the selected ion monitoring (SIM) and the single reaction monitoring (SRM) mode.

V.3. Materials and Methods

Chemicals and reagents

All chemicals were of analytical grade unless otherwise specified. ESBO (Edenol D82) was a gift of the Official Food Control Authority of the Canton of Zürich (Switzerland) and originally came from Vernicolor (Switzerland). 3-Chloroperoxybenzoic acid and methyl linoleate were purchased from Sigma-Aldrich (Seelze, Germany), [1-18-^{13}C]-ethyl linoleate from Euriso-Top GmbH (Saarbrücken, Germany), sodium methoxide (30 % in methanol), all salts and solvents from Merck (Darmstadt, Germany).

Preparation of ^{13}C(18:2E)Et, ^{13}C(18:2E)Me and (18:2E)Me

According to the method described by Castle et al.,[11] solutions of 3-chloroperoxybenzoic acid (50.4 mg / 1 mL chloroform) and $^{13}C_{18}$-ethyl linoleate (25.5 mg / 1 mL chloroform) were mixed in an 15 mL test screw capped tube and kept at ambient temperature overnight. Thereafter extraction was performed three times by 5 mL sodium sulfite (10 % in water), 5 mL sodium hydrogen carbonate (10 % in water) and 5 mL water. The organic phase was diluted with 10 mL isooctane and filtered over 5 g of anhydrous sodium sulphate into a 50 mL volumetric flask. The

test tube was rinsed with 20 mL isooctane given over the filter into the flask, which was finally filled up to mark, to obtain the stock solution of the internal standard. By dilution (2 mL / 15 mL) the internal standard working solution was obtained and stored at -20 ºC.

To prepare ^{13}C(18:2E)Me and get solution S(^{13}C(18:2E)Me) for recording the mass spectrum, 1 mL of the above stock solution was diluted with 4 mL tetrahydrofuran (THF), and the transesterification was performed as described for the sample preparation procedure, but without adding any more THF.

Following the same protocol, (18:2E)Me was synthesized starting from unlabelled methyl linoleate. For GC-MS, 100 µL of the prepared (18:2E)Me stock solution were filled up with isooctane to 10 mL, to obtain solution S((18:2E)Me).

Samples

The following samples were purchased from local grocerys, all packed in glass jars: three pesto a la Genovese, two pesto rosso, one surrogate of salmon in oil, one garlic in oil and four baby food (vegetable with chicken, potatoes with carrots and meat, spaghetti with vegetables and meat, potatoes with beans and meat); olive oil for validation purposes (packed in a glass bottle with a closure made of metal and polyethylene).

Validation

For the on-matrix calibration, 21.3 mg ESBO were dissolved in 20 mL dioxane and suitably diluted with dioxane to spike olive oil at the following levels: 1.1, 5.3, 10.7, 21.3, 32.0, 42.6, 53.3, 106.5, 159.8, 213.0, 266.3, 319.5, 372.8, 426.0, 479.3 and 532.5 mg kg^{-1}. After addition of the ESBO solution the samples were homogenized by thorough agitation with a glass bar. All samples were used to create a calibration curve. To determine the limits of detection (LODs) and quantification (LOQs), the signal to noise ratios of (18:2E)Me(1) were determined with the MS Workstation software integrated tool (the noise was calculated as root mean square (rms)) and manually to obtain values qualified through expertise. For the LODs a manually determined signal to noise base of leastwise S/N = 3 and for the LOQs S/N = 9, respectively, was decisive. The repeatability (within-run precision) and

recovery were calculated on the base of five separately spiked olive oil samples of 106.5 mg kg^{-1}.

Sample preparation

The pestos and baby food samples, including the food adhering to the lid, were transferred into a beaker and homogenized with a ESGE Zauberstab (Unold AG, Hockenheim, Germany). In case of products in oil, only the oil was used for the analysis. Oil (100 mg), pesto (500 mg) or baby food (500 mg) samples were weighed into a 50 mL screw capped vial, 100 µL internal standard solution and 4 mL THF were added and mixed together. After the addition of 5 mL sodium methoxide solution (6 % in methanol) the vial was shaken vigorously for 90 s. Extraction was performed by the addition of 10 mL n-hexane and 10 mL disodium hydrogencitrate (15 % in water), and the organic phase was used for GC analysis.

Spiked olive oil samples (53, 107, 213, and 426 mg kg^{-1}) were prepared and used for a four-point calibration to determine ESBO in samples.

GC columns and oven temperatures for the analysis in food

(i) Varian (Darmstadt, Germany) VF-17ms (30 m lengh, 0.25 mm id., 0.25 µm film thickness), oven temperature gradient: 80°C (2 min) / 200°C (15°C min^{-1}) / 280°C (8°C min^{-1}) / 320°C (15°C min^{-1}) / 320°C (2 min).

(ii) J & W Scientific (Agilent, Waldbronn, Germany) DB 1701 (30 m lengh, 0.25 mm id., 0.25 µm film thickness), oven temperature gradient: 80°C (2 min) / 200°C (15 C min^{-1}) / 280°C (8°C min^{-1}) / 300°C (15°C min^{-1}) / 300°C (2 min).

(iii) J & W Scientific (Agilent, Waldbronn, Germany) DB 1 (30 m length, 0.25 mm id., 0.25 µm film thickness), oven temperature gradient: 80°C (2 min) / 320°C (15°C min^{-1}) / 320°C (2 min).

Gas chromatography- mass spectrometry (single and triple quadrupole conditions)

A Varian (Darmstadt, Germany) CP-3800 gas chromatograph with a GERSTEL (Mülheim an der Ruhr, Germany) KAS 3 split/ splitless injector coupled to a Varian 1200 triple quadrupole mass spectrometer was used with electron impact (EI) ionization at 70 eV in the positive-ion mode. Data acquisition and analysis were

performed using standard software supplied by the manufacturer (Varian, MS Workstation 6.2). The injection volume was 1 µL splitless. The injector started at a temperature of 80°C, increased by 12°C s^{-1} to 320°C and hold this temperature for 10 min. The ion source and transferline temperature were 300 and 350°C, respectively. Helium was used as carrier gas with a column head pressure of 117 kPa.

By using the MS in single quadrupole mode (GC-MS) the mass spectra of (18:2E)Me(1) (with solution S((18:2E)Me) and ^{13}C(18:2E)Me(1) (with solution S(^{13}C(18:2E)Me) were recorded from *m/z* 33-600 at a scan time of 0.5 scans s^{-1}. Product ion scans (PIS) were based on collision-induced dissociation (CID) occurring in the collision cell of the tandem quadrupole. A peak width of 1.0 atomic mass units (amu) was chosen for the precursor ions and the collision cell was operated with argon (pressure 0.27 Pa) and a collision energy of -10 V. The PIS for (18:2E)Me(1) scanned the precursor ions of *m/z* 155 in the range *m/z* 10-150 and for ^{13}C(18:2E)Me(1) the precursor ions of *m/z* 164 in the range *m/z* 8-170 with a scan time 0.2 scans s^{-1}, respectively.

For quantification by GC-MS, the SIM mode was used for *m/z* 155 [(18:2E)Me(1)] and 164 [^{13}C(18:2E)Me(1)], respectively, with a scan time of 0.2 scans s^{-1} and a peak width of 1.0 amu. The GC-MS/MS was run in SRM mode with the same scan time, a peak width of 1.5 amu for the transitions *m/z* 155 $\rightarrow$ 67 and *m/z* 164 $\rightarrow$ 72, respectively. For fragmentation the collision cell contained argon at a pressure of 0.20 Pa, and the collision energy was set to -10 V.

Gas chromatography- mass spectrometry (ion trap conditions)

Additional GC-MS^2 analyses were performed with a 3400 Saturn4D ion trap system (Varian, Darmstadt, Germany) in 70 eV electron impact ionization mode with a CID time of 20 ms, a CID amplitude of 1 V and a CID rf storage level of *m/z* 35. The carrier gas (helium) was used at a constant pressure of 22.5 PSI with a HP-5 (Agilent, Waldbronn, Germany) column (50 m length, 0.32 mm id., 0.17 µm film thickness), the oven temperature gradient of 80°C (2 min) / 300°C (10°C min^{-1}) / 300°C (2 min) and a transferline temperature of 300°C. For recording the mass spectra, 1 µL of S(^{13}C(18:2E)Me) and S((18:2E)Me) were injected in splitless mode.

The precursors *m/z* 109 for (18:2E)Me and *m/z* 117 for ^{13}C(18:2E)Me, respectively, were scanned from *m/z* 30-170.

V.4. Results and discussion

There are two great advantages of using $^{13}C_{18}$-labelled ethyl diepoxyoctadecanoate (^{13}C(18:2E)Et) instead of the common internal standard methyl diepoxyeicosanate (20:2E)Me.[7,13] During sample preparation, ^{13}C(18:2E)Et is also transesterified, together with the ESBO triglycerides, yielding the corresponding methyl ester ^{13}C(18:2E)Me as shown in the chromatograms of Figure 3. Therefore, controlling of the transesterification process as important and sensible part of sample preparation is enabled, which was also achieved by Castle et al.,[11] using ethyl diepoxyeicosanate as internal standard. However furthermore, the signals of the ^{13}C(18:2E)Me and the analyte are obtained under absolute identical instrumental conditions, regarding matrix effects. The chromatograms of the full scan GC-MS analysis, recorded with the VF-17ms column, of (18:2E)Me and ^{13}C(18:2E)Me provide peaks at the same retention time of 17.7 min representing (18:2)Me(1) and ^{13}C(18:2)Me(1), thus additionally providing the tools of SIDA.

The spectra of the full scan analysis show base peaks at *m/z* 155 and 164 for (18:2E)Me(1) and ^{13}C(18:2E)Me(1), respectively (Figure 2). Besides the base peak both mass spectra look rather similar in terms peak pattern as comparable fragmentation is to be expected. The significant fragment of (18:2E)Me(1) at *m/z* 155 was also found in the 70 eV spectra of both methyl 9,10-epoxyoctadecanoate and methyl 9,10-epoxyoctadec-12-enoate,[14] and in the spectra of dimeric fatty acids formed by heating of methyl 9,10-epoxyoctadecanoate, methyl 9,10-epoxyhexadecanoate, and methyl 9,10-epoxytetradecanoate.[15] Therefore, it can be concluded that *m/z* 155 is not only characteristic for (18:2E)Me, but generally typical for 9,10-epoxidized fatty acid esters or 9-ether bridged fatty acid ester dimers. By means of tandem mass spectrometry, the product ion scan (PIS) of *m/z* 155 ((18:2E)Me(1)) gave *m/z* 109 (base peak) and *m/z* 67 as most abundant product ions (Figure 2), whereas the PIS of *m/z* 164 (^{13}C(18:2E)Me(1)) provided *m/z* 117 (base peak) and *m/z* 72.

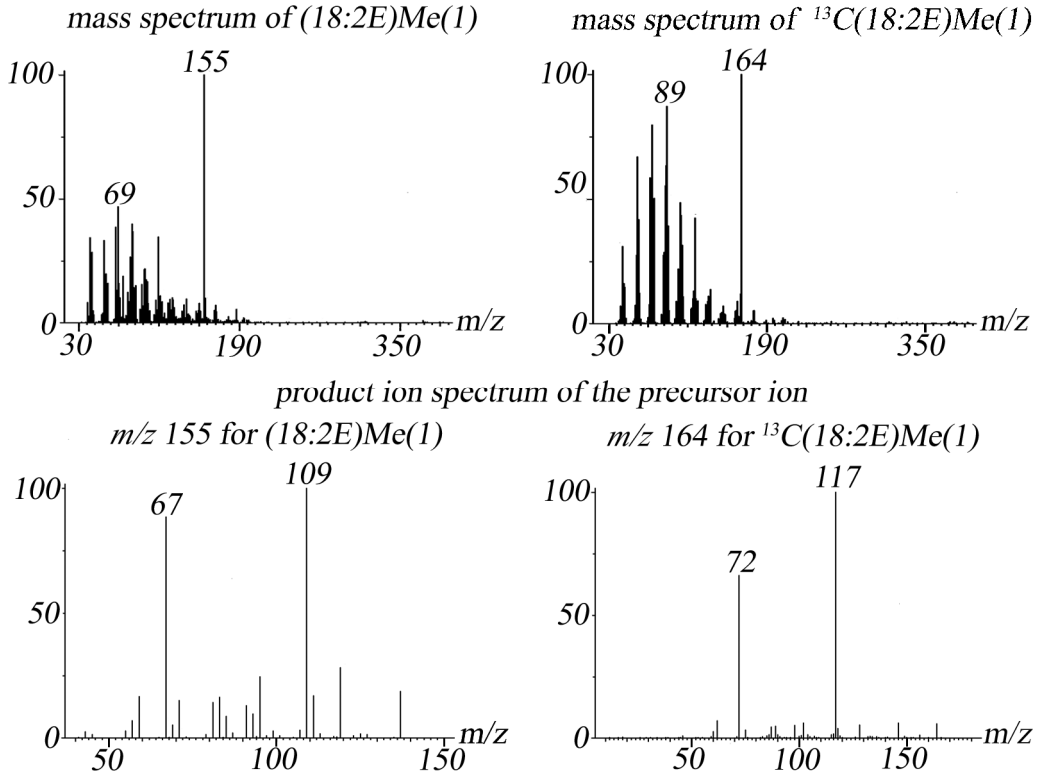


Figure 2. Mass spectra (EI, 70 eV) and product ion spectra (also 70 eV EI) of the precursor ions *m/z* 155 for (18:2E)Me(1) and *m/z* 164 for ^{13}C(18:2E)Me(1).

The spectra of the full scan analysis show base peaks at *m/z* 155 and 164 for (18:2E)Me(1) and ^{13}C(18:2E)Me(1), respectively (Figure 2). Besides the base peak both mass spectra look rather similar in terms peak pattern as comparable fragmentation is to be expected. The significant fragment of (18:2E)Me(1) at *m/z* 155 was also found in the 70 eV spectra of both methyl 9,10-epoxyoctadecanoate and methyl 9,10-epoxyoctadec-12-enoate,[14] and in the spectra of dimeric fatty acids formed by heating of methyl 9,10-epoxyoctadecanoate, methyl 9,10-epoxyhexadecanoate, and methyl 9,10-epoxytetradecanoate.[15] Therefore, it can be concluded that *m/z* 155 is not only characteristic for (18:2E)Me, but generally typical for 9,10-epoxidized fatty acid esters or 9-ether bridged fatty acid ester dimers. By means of tandem mass spectrometry, the product ion scan (PIS) of *m/z* 155 ((18:2E)Me(1)) gave *m/z* 109 (base peak) and *m/z* 67 as most abundant product ions (Figure 2), whereas the PIS of *m/z* 164 (^{13}C(18:2E)Me(1)) provided *m/z* 117 (base peak) and *m/z* 72.

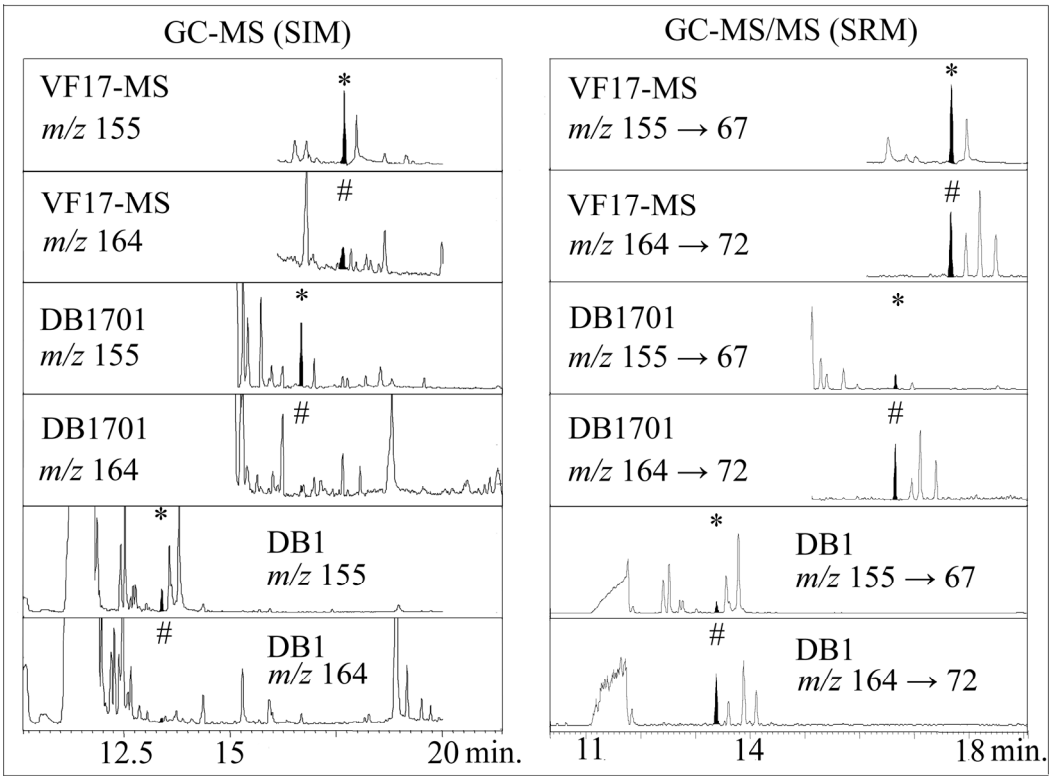


Figure 3. Chromatograms of the analysis of pesto rosso 1 obtained by GC-MS and GC-MS/MS with different capillary columns (VF-17ms, DB1701, DB1). The peaks of (18:2E)Me(1) and ^{13}C(18:2E)Me(1) are coloured black and signed by an asterisk or a number sign, respectively.

Additional GC-MS experiments, carried out with an ion trap system, proved the fragment at *m/z* 67 of (18:2E)Me(1) as product ion of *m/z* 109, and likewise identified *m/z* 72 as product ion of *m/z* 117 for ^{13}C(18:2E)Me(1). Further analyses showed that the mass spectra of ^{13}C(18:2E)Me and ^{13}C(18:2E)Et are identical (data not shown), proving that the alcohol rest is not included in the precursor ion *m/z* 164. Taking this observations and also the expected identical fragmentation of (18:2E)Me(1) and ^{13}C(18:2E)Me(1) into account, the number of carbons available in the fragments *m/z* 155, 109, and 67 for (18:2E)Me(1) and *m/z* 164, 117, and 72 for ^{13}C(18:2E)Me(1), respectively, can be calculated to nine, eight and five. Because the mass spectra of both methyl 6,7-epoxyoctadecanoate and methyl 11,12-epoxyoctadecanoate dimers do not provide a fragment of *m/z* 155[15] and under the assumption that the molecules providing *m/z* 155 resulting from both dimeric 9,10-epoxydized fatty acid esters and (18:2E)Me (molecular formula $C_{19}H_{34}O_4$) are of identical composition, *m/z* 155 should possess the molecular formula $C_9H_{15}O_2$ (loss of CH_3OH and $C_9H_{15}O$, Figure 4). Further neutral loss of H_2O and CO yields *m/z* 109 (C_8H_{13}) finally resulting in *m/z* 67 (C_5H_7) by loss of propene.

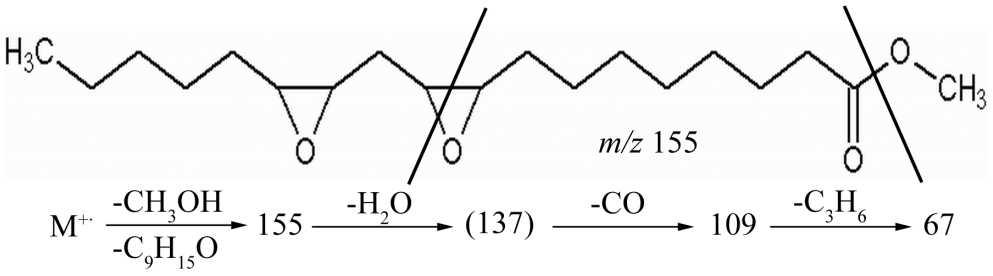


Figure 4. Proposed mass spectrometric fragmentation resulting in the most abundant fragments of *m/z* 155, 109 and 67 of (18:2E)Me(1).

By using the selected reaction monitoring (SRM) mode for detection in samples, selection of *m/z* 155 → 67 and 164 → 72 for (18:2E)Me(1) and ^{13}C(18:2E)Me(1), respectively, resulted in peak areas about two times larger than the transitions of *m/z* 155 → 109 and 164 → 117, respectively (data not shown). Therefore, monitoring of *m/z* 155 → 67 and 164 → 72 were preferentially used for the GC-MS/MS determination of ESBO in sample matrix.

According to recently released GC-MS methods, polar columns and on column injections of up to 5 µL were chosen.[13] Due to more stable column conditions for a period of analysis, only 1 µL was splitless injected into midpolar and unpolar columns providing temperature limits of 300 °C and higher. Gas chromatograms obtained for the determination of ESBO in a pesto rosso sample are exemplarily presented in Figure 3. It is visible, that chromatograms measured in SIM mode are almost noisier and show more interfering peaks than in the SRM mode. With regard to the number of detected peaks next to (18:2E)Me(1) and ^{13}C(18:2E)Me(1), a midpolar column like the VF-17ms (50% phenyl-, 50% dimethylpolysiloxane) or DB1701 ((14 %-cyanopropyl-phenyl)-methylpolysiloxane) gave the best results in terms of resolution. Concerning the MS detection mode, SRM is to be favoured over SIM, because peak interferences by matrix compounds are observable to a lesser extend for each tested column.

To evaluate the suitability of the columns for different food matrices, samples were prepared according the sample preparation and analysed by the use of the different GC columns. The results obtained should be identical, if there is no dependency on the used column or MS mode, respectively. Comparability between the different techniques is expressed by the relative standard deviation (RSD), which was only calculated in the case of similar and outliers free results (Table 1). Low rsds

minor 9 % were found in SRM mode for the samples pestos a la Genovese 1 and 2, garlic in oil and pesto rosso 1. High deviations of the results depending upon the used column were found in SRM mode with the DB 1 for pesto a la Genovese 3, pesto rosso 2 and baby food composed of vegetables with chicken, thus this column is not appropriate for these matrices. Respecting the results, the DB1701 was more usable, but in the chromatograms of pesto a la Genovese 2, pesto rosso 2 and garlic in oil co-elutions of (18:2E)Me(1) with matrix were observed. Concerning co-elution with matrix compounds, best results in SRM mode were obtained by means of the VF-17ms.

In SIM mode the determined sample values generally exhibit higher deviations than in SRM mode, and some samples could not be analysed because of high background noise interfering with ^{13}C(18:2E)Me (1). Therefore, a generally favoured column for pesto or oil matrices could not be proposed.

Concerning the limits of contamination, all oil and food sauce samples far exceeded the limit of 60 mg kg^{-1} ESBO, whereas one of the four baby food samples was above the limit of 30 mg kg^{-1}.

Table 1. Results of ESBO determination in different food samples by GC-MS (SIM mode) and GC-MS/MS (SRM mode) on different capillary columns (VF-17ms, DB1701, DB1).

	VF-17ms	DB1701	DB1	rsd [%]	VF-17ms	DB1701	DB1
	Contents [mg/kg] SRM mode				Contents [mg/kg] SIM mode		
pesto a la Genovese 1	214	199	214	4.1	111	n.d.	192
pesto a la Genovese 2	224	230	220	2.2	144	370	165
pesto a la Genovese 3	130	115	257	-	126	n.d.	107
pesto rosso 1	160	171	154	5.3	71	176	136
pesto rosso 2	332	298	247	14.6	107	162	n.d.
oil of garlic in oil	347	390	328	8.9	410	>532	379
vegetable with chicken*	122	132	89	19.7	104	152	141
oil of surrogate of salmon in oil	>532	>532	>532	-	>532	>532	>532

	VF-17ms	DB1701	DB1	rsd [%]	VF-17ms	DB1701	DB1
	Contents [mg/kg] SRM mode				Contents [mg/kg] SIM mode		
potatoes with carrots and meat*	<5	<5	<5	-	<5	<5	<5
spaghetti with vegetables and meat*	<5	<5	<5	-	<5	<5	<5
potatoes with beans and meat*	<5	<5	<5	-	<5	<5	<5

n.d.: not determinable because of high background noise interfering with the peak of ^{13}C(18:2E)Me(1).

* baby food

To evaluate the presented methods of ESBO determination, an in-house validation by using the VF-17ms column both in SRM and SIM mode was performed for spiked olive oil, a difficult matrix because of its high content of unsaturated fatty acids. Since high contents of ESBO were found in food, a wide calibration range from the limit of quantification (LOQ) up to 530 mg kg^{-1} was tested. An ESBO concentration in the range of 100 mg kg^{-1} was chosen to determine the repeatability for this matrix, because for oily food the limit of 60 mg kg^{-1} often is exceeded.

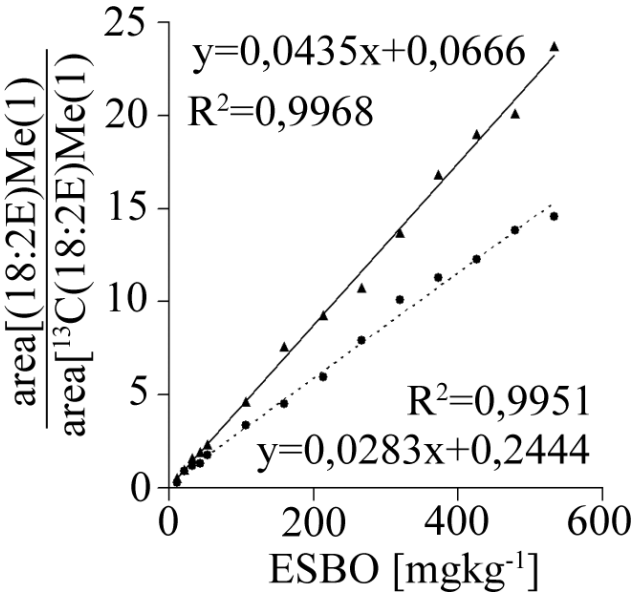


Figure 5. Calibration curves of ESBO in spiked olive oil by means of the SIM(●) and SRM(▲) detection mode.

The linearity test of the calibration curves (Figure 5) according Mandel[16] gave 0.27 for the SRM and 8.55 for the SIM mode, respectively. The critical value for the Mandel linearity test with a level of confidence of 99 % is 9.64. Therefore the linear

calibration for both modes is proved within a range of up to 530 mg kg^{-1}. The coefficients of correlation (R^2) were absolutely satisfying.

Table 2. Mean recovery and repeatability (within-run precision) for spiked olive oil and signal to noise ratios of (18:2E)Me(1) in the chromatograms of spiked olive oil (c=5, 11 and 21 mg kg^{-1}) obtained manually and from the MS software tool (all results obtained by means of the column VF-17ms).

	mean recovery (n=5, c=106.5 mg ESBO kg-1)	repeatability (n=5, c=106.5 mg ESBO kg-1)	S/N ratio determined manually for c=5/11/21 mg kg-1	S/N ratio (rms#) determined by software for c=5/11/21 mg kg-1
SRM mode	99.7 ± 5.5 %	6.0 %	4.2/11.6/31.5	31/37/100
SIM-mode	103.3 ± 0.8 %	0.9 %	5.6/7.2/14.2	23/48/65

[#] Noise was calculated as root mean square (rms)

Based on the more severe and manually determined signal to noise ratios (Table 2), the limits of detection (LODs) and quantification (LOQs) resulted in LODs of about 5 mg kg^{-1} for both detection modes. However, the LOQs were quite different with 11 and 21 mg kg^{-1} in the SRM and SIM mode, respectively. Regarding the SML of 60 mg kg^{-1} recommended by the Commission Directive 2005/79/EC, both MS detection modes fulfil the analytical requirements for the determination of ESBO.[3] The mean recovery and repeatability (within-run precision) were also excellent and sufficient in every respect (Table 2).

V.5. Conclusion

Based on the latest experiences of ESBO's fast sample preparation for GC analysis without both prior fat extraction and derivation of ESBO's fatty acid methyl esters into dioxolanes, ^{13}C(18:2E)Et as internal standard successfully provides standardization of the transmethylation process and additionally allows SIDA. However, to reap these benefits, a matrix interference free chromatography is required, which was optimally achieved by means of a VF-17ms GC column coupled with tandem mass spectrometric detection in the SRM mode. The validation for the difficult matrix olive oil offered very well scored results.

V.6. Acknowledgement

The authors would like to thank CVUA Stuttgart and especially Mr. Altkofer and Ms. Morandini for supply with equipment, materials and support.

V.7. References:

1. Fankhauser-Noti A, Biedermann-Brem S, Grob K. PVC plasticisers/additives migrating from the gaskets of metal closures into oily food: Swiss market survey June 2005. *European Food Research and Technology* 2006; **223**(4): pp.447-453
2. European Communities. Synoptic Document. Available at http://europa.eu.int/comm/food/food/chemicalsafety/foodcontact/synoptic_doc_en.pdf. Accessed 13 November 2007
3. European Communities. Commission Directive 2005/79/EC of 18 November 2005 amending Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with food. *Official Journal of the European Union* 2005; **L 302**: pp.35-45
4. Biedermann-Brem S, Biedermann M, Fiselier K, Grob K. Compositional GC-FID analysis of the additives to PVC, focusing on the gaskets of lids for glass jars. *Food Additives & Contaminants* 2005; **22**(12): pp.1274-1284
5. Hammarling L, Gustavsson H, Svensson K, Karlsson S, Oskarsson A. Migration of epoxidized soya bean oil from plasticized PVC gaskets into baby food. *Food Addit Contam* 1998; **15**(2): pp.203-208
6. Fantoni L, Simoneau C. European survey of contamination of homogenized baby food by epoxidized soybean oil migration from plasticized PVC gaskets. *Food Additives & Contaminants* 2003; **20**(11): pp.1087-1096
7. Fankhauser-Noti A, Fiselier K, Biedermann-Brem S, Grob K. Epoxidized soy bean oil migrating from the gaskets of lids into food packed in glass jars. Analysis by on-line liquid chromatography-gas chromatography. *J Chromatogr A* 2005; **1082**(2): pp.214-219

8. Suman M, La Tegola S, Catellani D, Bersellini U. Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry Method for the Determination of Epoxidized Soybean Oil in Food Products. *Journal of Agricultural and Food Chemistry* 2005; **53**(26): pp.9879-9884

9. Rebek J, Jr., Marshall L, McManis J, Wolak R. Convergent functional groups. 2. Structure and selectivity in olefin epoxidation with peracids. *Journal of Organic Chemistry* 1986; **51**(10): pp.1649-1653

10. Fankhauser-Noti A, Fiselier K, Biedermann-Brem S, Grob K. Assessment of epoxidized soy bean oil (ESBO) migrating into foods: Comparison with ESBO-like epoxy fatty acids in our normal diet. *Food and Chemical Toxicology* 2006; **44**(8): pp.1279-1286

11. Castle L, Sharman M, Gilbert J. Gas chromatographic-mass spectrometric determination of epoxidized soybean oil contamination of food by migration from plastic packaging. *Journal - Association of Official Analytical Chemists* 1988; **71**(6): pp.1183-1186

12. Suter B, Grob K, Pacciarelli B. Determination of fat content and fatty acid composition through 1-min transesterification in the food sample. Principles. *Z.Lebensm.-Unters.Forsch.A* 1997; **204**(4): pp.252-258

13. Biedermann-Brem S, Biedermann M, Fankhauser-Noti A, Grob K, Helling R. Determination of epoxidized soy bean oil (ESBO) in oily foods by GC-FID or GC-MS analysis of the methyl diepoxy linoleate. *European Food Research and Technology* 2007; **224**(3): pp.309-314

14. Dr William W Christie. The Lipid Library. Available at http://www.lipidlibrary.co.uk/. Accessed 22 November 2006

15. Gilbert J, Shepherd MJ, Startin JR, Eagles J. Dimeric epoxy fatty acid methyl esters: formation, chromatography and mass spectrometry. *Chemistry and Physics of Lipids* 1981; **28**(1): pp.61-68

16. IUPAC. Guidelines for Calibration in Analytical Chemistry Part 1: Fundamentals and Single Component Calibration. *Pure and Applied Chemistry* 1998; **70**(4): pp.993-1014

VI. Rapid and nondestructive analysis of phthalic acid esters in toys of polyvinyl chloride by direct analysis in real time–single quadrupole mass spectrometry (DART–MS).

THORSTEN ROTHENBACHER, WOLFGANG SCHWACK

Institut für Lebensmittelchemie, Universität Hohenheim, Garbenstr. 28, 70599 Stuttgart, Germany, Correspondence to:W. Schwack, e-mail: wschwack@uni-hohenheim.de

VI.1. Abstract

In the European Community, selected phthalic acid esters (PAE) are restricted in their use for the manufacturing of toys and childcare articles to a content of 0.1% by weight. As they are mainly used as plasticisers for polyvinyl chloride (PVC), a rapid screening method for PVC samples with direct analysis in real time ionisation and single quadrupole mass spectrometry (DART™–MS) was developed. Using the most intensive protonated molecules, a limit of detection (LOD) of 0.05% was obtained for benzyl butyl phthalate, bis(2-ethylhexyl) phthalate and di-isononyl phthalate, while for dibutyl phthalate, di-n-octyl phthalate and di-isodecyl phthalate the LOD was 0.1%. Validation of identification by ammonium adducts and characteristic fragments was possible to a content of ≥1% for all PAEs, except for benzyl butyl phthalate (≥5%). Based on fragmentation products, bis(2-ethylhexyl) phthalate clearly could be distinguished from di-n-octyl phthalate, if the concentrations were ≥5% and ≥1% at measured DART™ helium temperatures of 130 and 310 ºC, respectively. The complete analysis of one sample only took about 8 minutes. At the generally used gas temperature of 130 ºC, most toy and childcare samples did not sustain damage if their shape fitted into the DART™ source.

VI.2. Introduction

Phthalic acid esters (PAE) represent an important group of plasticisers for polyvinyl chloride (PVC), which also were used since many years for the fabrication of toys and childcare articles made from PVC. Since in 1999 several European Member States expressed concerns about the risk of adverse effects of phthalates on the health of children,[1] six PAE in toys and childcare articles were temporarily banned from the European market. The facts that i) the toxicology of all phthalates in humans was not totally clarified, ii) especially bis(2-ethylhexyl) phthalate (DEHP) clearly showed adverse effects on the development and reproduction of laboratory animals,[2,3] and iii) a high level of health protection, especially for children, was demanded, led to a permanent regulation for phthalates.[4] Actual results show that different PAE act on the same target organ, but they individually possess complex modes of action.[5] Therefore, the council directive 76/769/EEC of the European Commission in its actual form prohibits to place toys and childcare articles on the market, which contain bis(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP) or benzyl butyl phthalate (BBP) at concentrations of more than 0.1% by mass of the material. This is equivalent for di-isononyl phthalate (DINP), diisodecyl phthalate (DIDP) or di-n-octyl phthalate (DNOP), if the articles can be taken into the mouth by children.[6]

According to a survey of 72 toys made from PVC and purchased in 17 different countries, nearly all toys or at least their PVC subsections contained PAE, mainly in the range 10-40% by weight.[7] More actual data were obtained from the European Union rapid alert system for all dangerous consumer products (RAPEX),[8] in which toys or childcare products are named together with the reason of hazardousness. A search for the phrase ‘phthalate’ in the notifications of 2008 resulted in 143 hits, but cosmetics, clothing and stationery products were also included herein, and the search result only represented those entries, in which a nomenclature was used that contained the term ‘phthalate’. Entries that used a nomenclature like e.g. ‘* phthalic acid ester’ did not result in a hit. A closer look at each alert of 2008 identified 140 toys or childcare products having been withdrawn from the market due to PAE violations. In seven samples, the PAE content was below 3%, and in nine samples just an analysis result of >0.1% was given. Vice versa, 95% of the notified samples revealed PAE at 3% or more.

For the determination of PAE in plastic toys according published methods, about 1 g of the sample is directly soxhlet extracted with dichloromethane for 16 h[9] or is finely grounded and extracted twice by sonication in hexane for 30 min.[7] The extracted phthalates are determined by gas chromatography with flame ionisation or mass spectrometric detection. Extraction of PVC by accelerated solvent extraction also led to good results.[10] Another approach for the analysis of PAE in PVC consists in completely dissolving the plastic in tetrahydrofuran and precipitating PVC by the addition of methanol. Determination was performed by high performance liquid chromatography or high performance thin-layer chromatography (HPTLC).[11] HPTLC was especially used to facilitate quantification of the isoalkyl PAE DINP and DIDP, which both are mixtures of several compounds, resulting in a sharply separated HPTLC zone instead of a hump during HPLC for both DINP and DIDP.

As toys or childcare products contain alternative plasticisers substituting PAE, the analysis of PAE is possibly done to no avail. To avoid wasting of solvents, instrumental measuring and human working time, rapid identification methods are worthwhile to check the presence of phthalates in the specimens prior to exact determination. As PAEs are regulated differentially by authorities, the method should be at least able to distinguish DEHP, DBP and BBP from DIDP, DINP and DNOP. Following this objective, different pure phthalates were measured by fourier transform Raman spectroscopy (FTRS), but an identification in PVC was successfully demonstrated only for DEHP.[12] However, due to similarities in the spectra of the common dialkyl phthalates it is doubtful, whether it would be possible to qualitatively determine the exact kind of phthalate ester present in a sample containing a mix of phthalates.

Therefore, the aim of the present study was to evaluate, if a rapid screening test for PAE in PVC materials can be developed by using direct analysis in real time–mass spectrometry (DART™–MS), with an open interface allowing to directly insert solid specimens.[13,14] DART™-MS was successfully employed for the analysis of different compounds in almost solid samples, like strobilurin fungicides in wheat,[15] flavors in perfumery raw materials,[16] and isopropylthioxanthone[17] or lubricant additives[18] from HPTLC plates.

As DART™ is a rather new ionisation technique, characteristic ions of phthalates as standards and in PVC plastics containing additives (also called plastisols) had to be identified, the sensivity of identification to be evaluated, and the applicability to toy samples of known PAE concentrations to be shown.

VI.3. Experimental

Chemicals and reagents

The following chemicals were of analytical grade unless otherwise specified. Toluene was from Roth (Karlsruhe, Germany) benzyl butyl phthalate (BBP) from Merck (Darmstadt, Germany), dibutyl phthalate (DBP) and dioctyl phthalate (DNOP) from Sigma-Aldrich (Taufkirchen, Germany). Bis(2-ethylhexyl) phthalate (DEHP), diisodecyl phthalate (DIDP), diisononyl phthalate (DINP), and 1,2-cyclohexanedicarboxylic acid 1,2-diisononyl ester (DINCH) were of technical grade and were provided by a plastisol producing company. Vinnolit P 70 was used as PVC material and was provided by Vinnolit GmbH & Co. KG, Wacker Chemie AG (Burghausen, Germany).

Plastisol samples

Twenty five different plastisols of three different types containing a) DBP & DINP, b) DEHP & DIDP and c) BBP & DNOP at mass percentages of 0%, 0.05%, 0.01%, 0.5%, 1%, 5%, 10%, 15%, and 25% were prepared. Generally, plastisols contained 60% PVC and the respective PAE, which were filled up to 40% with DINCH. In case of the plastisols with 25% PAE, PVC was only present at 50%, and DINCH was not used. To prepare the plastisols, about 0.5-1 g of the PVC and the platicisers were mixed with a metal spatula in a 10 mL glass beaker until the mixture was a homogenous and colourless paste. Then, it was coated with about 1 mm thickness on a heating plate covered by aluminum foil at 200ºC. After one minute, the aluminum foil was removed from the plate to cool off the plastisol.

Toy samples

Five toys made from PVC and containing PAE or DINCH were provided by Chemisches und Veterinäruntersuchungsamt Stuttgart (Germany) and were already analysed for plasticisers: a horror mask (42.6% DEHP), childrens' swimming aids

(16.2% DEHP and 23.0% DINP), 2 heads of puppets (containing 22.0% DINP or 35.4% DINCH, respectively), and a fish (30.8% DINCH). Additionally, three about 25 year old toys with unknown content of PAEs were used for analysis: one lizard, one bicycle driver and one squeaky toy.

DART™–MS

An Ion Sense DART™ 100 with Vapur API-Interface, DART™ control software version 2.19 (KR Analytical, Sandbach, UK) and a G1956B MSD single quadrupole mass spectrometer with ChemStation B.02.01 SR2 software (Agilent Technologies, Waldbronn, Germany) were used. The DART™'s needle voltage was 4000 V, discharge and grid electrode were each at 280 V. DART™ was operated at 200 °C with helium 5.0 (purity > 99.999%) at a flow rate of 5–6 L min^{-1}, controlled by a GFM 17 flowmeter (Analyt-MTC, Müllheim, Germany). The MSD was operated in positive fast-scan mode *m/z* 140-920 with a step size of 0.1 amu, a cycle time of 0.79 s $cycle^{-1}$, a capillary voltage of 6000 V, a fragmentor voltage of 200 V, a gain of 1.00 and a threshold of 0. The data were recorded in profile mode.

Mass spectra of the target PAE were obtained by dissolving the substances in toluene (0.1–1 g L^{-1}) and introducing the samples by means of DIP-it liquid samplers (KR Analytical) under the same MS conditions as described above. Plastisol specimens of about 2 x 0.2 cm were manually introduced into the DART™ gas stream by use of tweezers. Replicate measurements of plastisols were performed from the same spot, when a depletion of the signals was not observed. The gap between the DART™ gas outlet and the Vapur API Interface inlet was 1.1 cm, the lengh of the ceramic tube 3.9 cm and the sampling point in the middle of the gap (Figure 1). Each sample was measured six times, consecutively, while keeping it in the same position in the helium stream for about 30 seconds. When the sample was inserted, the signal increased in a split second, remained at a constant level and, after sample removal, generally decreased within several seconds, depending on the PAE content. In replicate measurements, the signal heights showed deviations concerning the intensity.

For data evaluation, the set of mass spectra recorded during the measuring time (30 s) and resulting in a total ion current 'peak' was averaged. Background

correction was performed manually by subtracting scans both ahead and behind the peak.

Concerning limits of detection for a specific ion of the known plastisol samples, a signal to noise (S/N) ratio of 3, referring to the height of the mass signal, was taken as a basis, having been fulfilled for at least four of the six measurements, whereat an averaged noise value of two different regions in the background corrected mass spectrum was used.

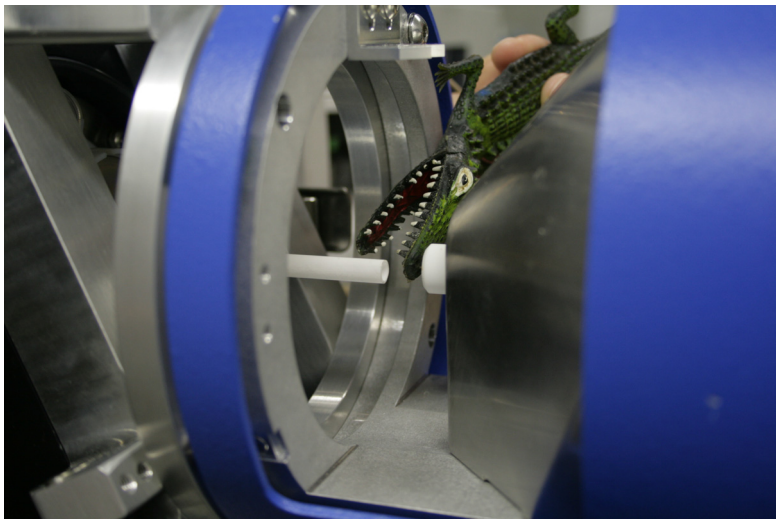


Figure 1. Positioning of a sample in the DART™ source

Gas chromatography- mass spectrometry (GC/MS)

A Fisons (Manchester, UK) GC-8000 gas chromatograph with a split/ splitless injector coupled to a Fisons MD800 quadrupole mass spectrometer was used with electron ionization (EI) at 70 eV in the positive-ion mode. Data acquisition and analysis were performed using standard software supplied by the manufacturer (Mass Lab software version. 1.3). The injection volume was 1 µL splitless. The GC oven started at a temperature of 80°C, increased by 12°C s^{-1} to 300°C and hold this temperature for 10 min. The ion source and transferline temperature were 200 and 300°C, respectively. Helium was used as carrier gas with a column head pressure of 70 kPa with a Phenomenex (Aschaffenburg, Germany) ZB50 column (30 m length, 0.25 mm id., 0.25 µm film) and a 0.7 m uncoated and deactivated retention gap. The MS was operated with a scan range from *m/z* 50-420 with a scan time of 0.6 scans s^{-1}.

VI.4. Results and discussion

DART–MS spectra of phthalic acid esters

The phthalic acid esters generally provided the protonated molecule as base peak and an $[M+18]^+$ peak in different intensities of about 10-40%, which was assigned to the respective ammonium adduct (Figure 2 and 3). Additionally, proton and ammonium adducts of dimer PAE could be detected, especially if solutions of >100 mg L^{-1} were measured. Fragmentation products of *m/z* 149 and 167 were obtained in low intensities for all PAE, to be interpreted by consecutive neutral losses of alcohol and alkene moieties (Figure 4).

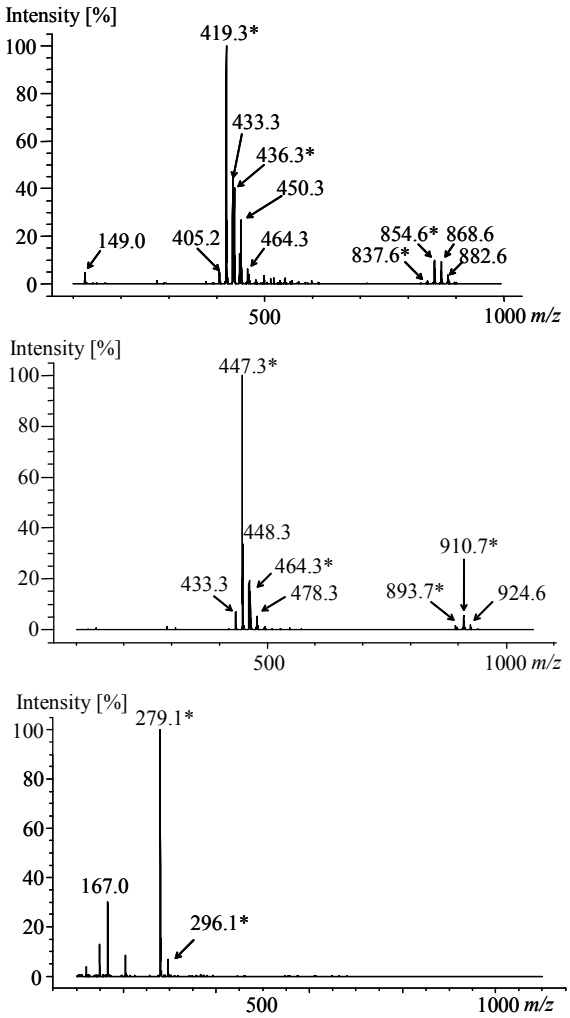


Figure 2. Typical DART™–MS spectra obtained from PAE disolved in toluene, exemplarily shown for DINP (top), DIDP (middle) and DBP (bottom). The proton and ammonium adducts are marked with an asterisk.

Since technical grade PAE are used in fabrication processes, impurities have also to be respected, which easily can be made visible by DART™–MS as shown for

DINP and DIDP (Figure 2). Additional ions ($[M+H]^+$) with intensities of 5–20% surround the main protonated molecules at distances of 14 and 28 mass units accompanied by the respective ammonium adducts. Obviously, the technical alcohols used for PAE preparation significantly had impurities of homologues.

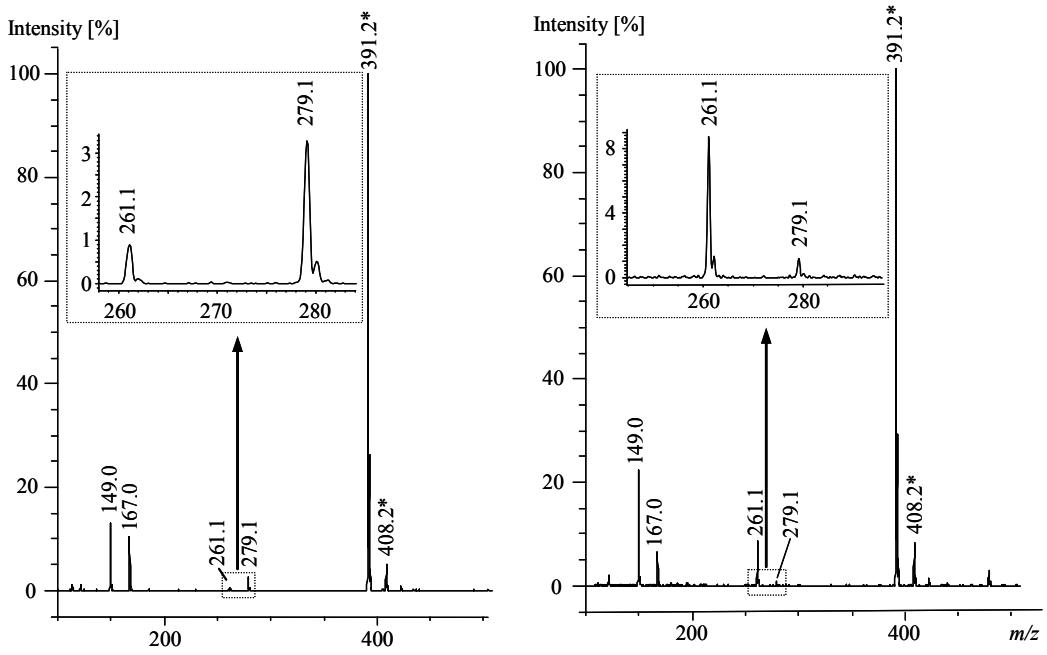


Figure 3. DART™-MS spectra of DEHP (left) and DNOP (right), both at a concentration of 1 g L^{-1} in toluene. The proton and ammonium adducts are marked with an asterisk.

From the couple of characteristic PAE ions obtained by DART™–MS (Table 1), the protonated molecules of highest intensities were selected for identification of the respective PAE in PVC samples, and the ammonium adducts were used as additional qualifiers.

Differentiation of DEHP and DNOP

Interestingly, there is a great chance to differentiate between the isomers DEHP and DNOP, which may be important due to different treatment by the European legislation. Both DEHP and DNOP offer fragments at *m/z* 261 and 279, but in clearly reversed ratios of intensity (Figure 3). As the loss of octanol from the protonated molecule is favored by DNOP, DEHP preferably eliminates 2-ethyl-1-hexene (Figure 4). By (ESI)MS/MS experiments, it could be shown that a loss of water from *m/z* 279, also resulting in *m/z* 261, does not occur (data not shown). Similar experiences were made during GC/(EI)MS analyses, when the fragment at *m/z* 261 was more stable deriving from DNOP than from DEHP (Figure 5). Thus, the

results strongly support the proposed fragmentation processes (Figure 4) and the possibility to certainly differentiate DEHP from DNOP.

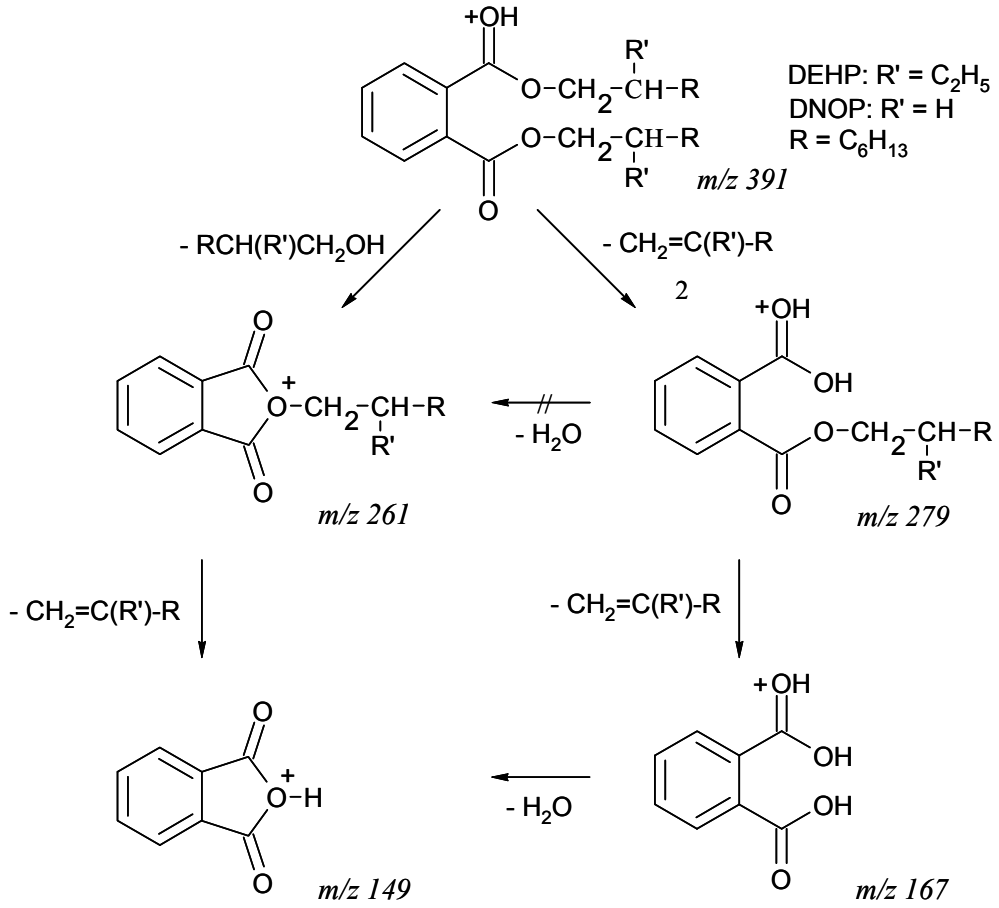


Figure 4. Proposed fragmentation processes of phthalic acid esters, especially shown for the differentiation of DEHP and DNOP.

In DNOP containing plastisols, *m/z* 261 could be detected in a concentration range of 5–25%, but *m/z* 279 only at 25%. In the case of DEHP, both fragments were detectable at concentrations ≥5% in plastisol samples. It was possible to increase the sensitivity by increasing the DART™ helium setting to 450°C, which resulted in detectability of the characteristic fragments at concentrations of 1% in plastisols for both DEHP and DNOP.

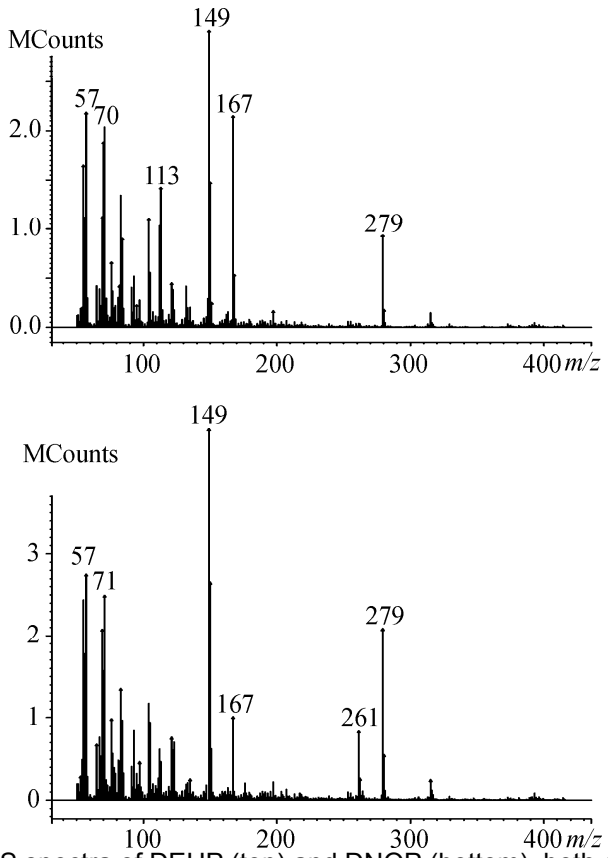


Figure 5. GC/70eV-EI-MS spectra of DEHP (top) and DNOP (bottom), both at a concentration of 0.01 g L^{-1} in toluene.

Since there also is the possibility of a DBP impurity with the protonated molecule at *m/z* 279 in DNOP and DEHP, it had to be checked that *m/z* 279 just is a fragment of DNOP and DEHP. A GC/MS analysis proved a DBP content below 0.5% for both PAE (data not shown).

Limits of detection and blank values

Since toy samples have to be manually introduced into the DART™ interface expectedly associated with low repeatability and since there is no chance to apply internal standards, calibration and quantification is complicated. Therefore, PVC plastisols with different concentrations of PAE (0–25%) were prepared to derive limits of detection (LOD) from the obtained MS signals. Diisononyl 1,2-cyclohexanedicarboxylate (DINCH) was additionally used to provide a plasticiser level of at least 40% in all samples. To largely overcome the variations by manual operation, samples were generally measured six times, consecutively. The LOD was defined, if the signal to noise ratio (s/n) for a specific ion of the background corrected mass spectrum (Figure 6) was ≥3 for at least four of the six recordings. As six

measurements took 3 minutes, the analysis time mainly depends on the data analysis. Calculating this with 5 min, screening a sample takes about 8 min.

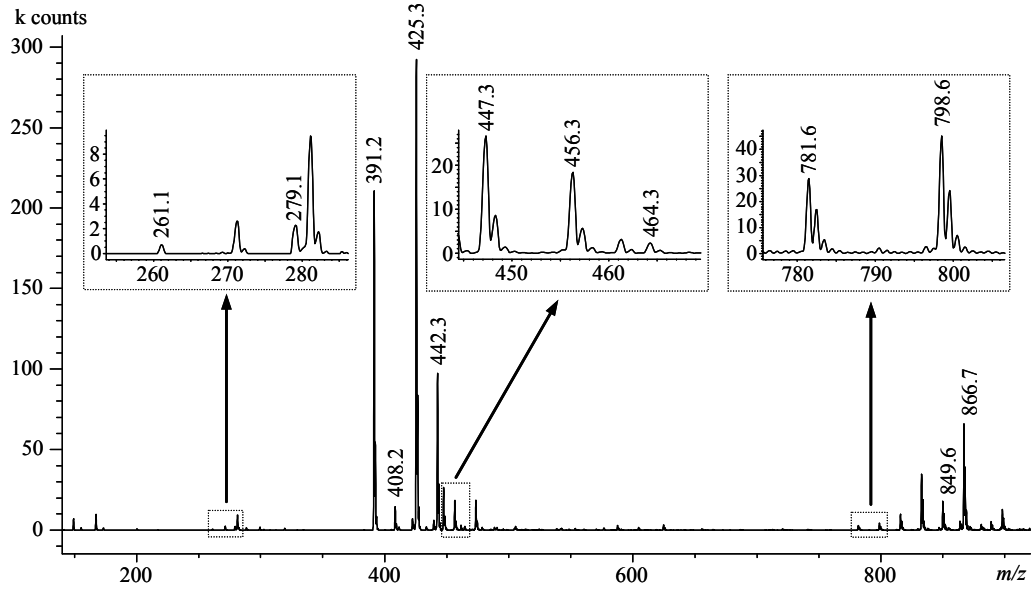


Figure 6. DART™-MS spectrum of a PVC sample containing 5% DEHP, 5% DIDP and 30% DINCH. DEHP: *m/z* 261.1, 279.1, 391.2 $[M+H]^+$, 408.2 $[M+NH_4]^+$, 781.6 $[2M+H]^+$, 798.6 $[2M+NH_4]^+$; DIDP: *m/z* 447.3 $[M+H]^+$, 464.3 $[M+NH_4]^+$; DINCH: 425.3 $[M+H]^+$, 442.3 $[M+NH_4]^+$, 849.6 $[2M+H]^+$, 866.7 $[2M+NH_4]^+$.

The LODs determined by this procedure were ≤0.1%, if the most intensive signal of the protonated molecules were used (Table 1). Therefore, the DART™-MS method is able to identify PAE in PVC down to the actual limit of the council directive 76/769/EEC.[6] A validation of identification by using the ammonium adducts was possible for four of the tested PAE, if the concentration was ≥1% (Table 1). In the case of BBP and DEHP, validation was only possible with the next higher concentration tested. The protonated dimers almost were only detectable at higher concentrations of 5–10%.

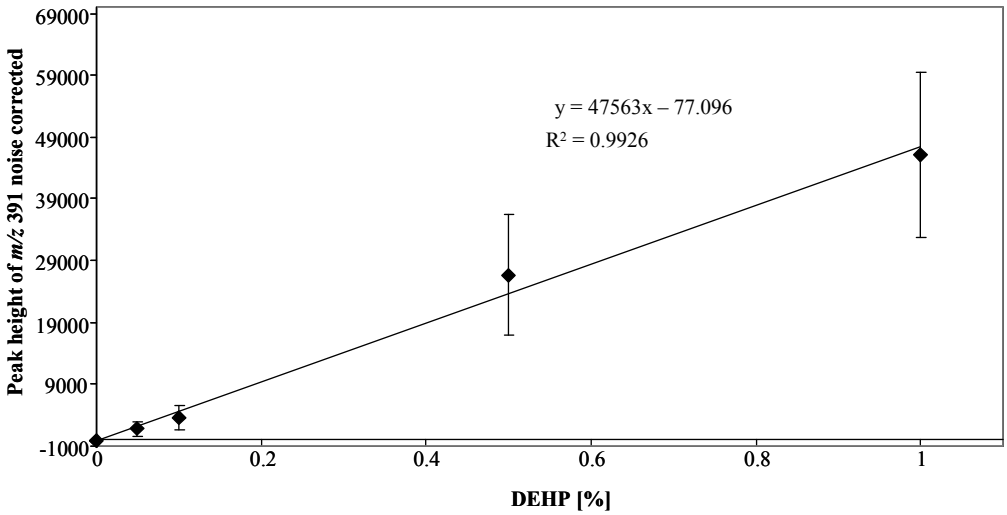


Figure 7. Calibration curve exemplarily obtained from plastisol samples containing DEHP in the range 0–1%.

Despite the complications to be expected, a surprising linear correlation was obtained in the low concentration range of most interests, if the mean of six replicates was plotted versus the noise corrected height of the ion signal (Figure 7).

Concerning blank values for PAE, the method of direct sample measurement excludes contaminations from solvents or vessels. In spite of this, there exist atmospheric sources for PAE detectable by DART™-MS, if, for example, the flooring consists of PVC (Figure 8). Working with an open interface as DART, such background signals have generally to be kept in mind, demanding for a careful background correction to avoid false positive identifications. To check this concept, a sample only containing DINCH as plasticiser was analysed, at which no PAE signals were detectable.

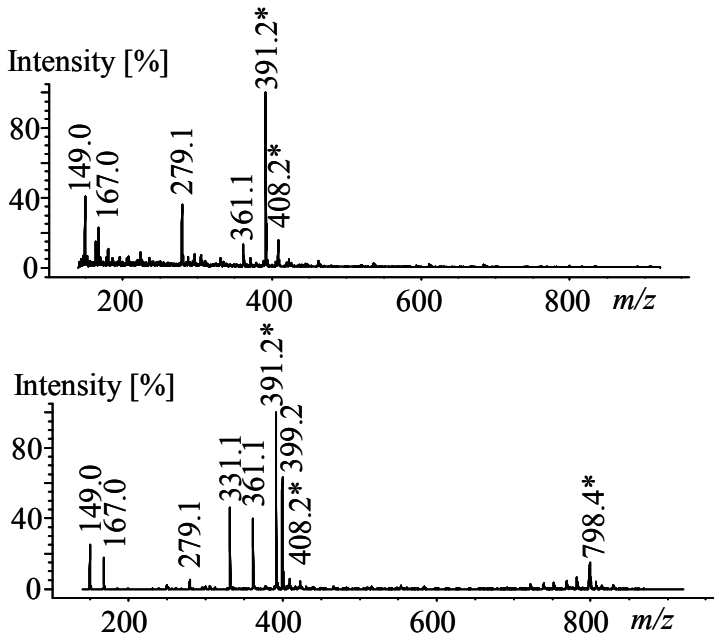


Figure 8. DART™-MS spectra of the laboratory atmosphere (top) and a piece of flooring (bottom), both showing ions of DEHP. The proton and ammonium adducts are marked with an asterisk.

Table 1. Characteristic DART™-MS ions (calculated) of phthalic acid esters with corresponding LODs given in mass percentages.

	DBP	BBP	DEHP	DNOP	DINP	DIDP
molecular formula	$C_{16}H_{22}O_4$	$C_{19}H_{20}O_4$	$C_{24}H_{38}O_4$	$C_{24}H_{38}O_4$	$C_{26}H_{42}O_4$	$C_{28}H_{46}O_4$
m/z $[M+H]^+$	279.2	313.1	391.3	391.3	419.3	447.3
LOD of $[M+H]^+$	0.1%	0.05%	0.05%	0.1%	0.05%	0.1%
m/z $[M+NH_4]^+$	296.2	330.2	408.3	408.3	436.3	464.4
LOD of $[M+NH_4]^+$	1%	5%	5%	1%	1%	1%
m/z $[2M+H]^+$	557.3	625.3	781.6	781.6	837.6	893.7
LOD of $[2M+H]^+$	5%	10%	1%	5%	10%	10%

Analysis of real samples and destructiveness of the method

Five toy samples pre-analysed by GC/MS were applied to the DART™-MS method. The mass spectra of a horror mask provided *m/z* 391 as base peak, the corresponding ammonium adduct and the dimeric ions, including the typical PAE fragments at *m/z* 149 and 167 (Figure 9). The signal ratio of *m/z* 261 and 279 proved the presence of DEHP instead of DNOP. A childrens' swimming aids provided the same mass signals characteristic for DEHP, but additionally showed the protonated molecule (*m/z* 419) and the ammonium adduct (*m/z* 436) of DINP (Figure 9). The mass spectra of a puppet head containing DINP only exhibited the corresponding signals of the protonated molecule and the ammonium adduct. Additionally, the $[2M+NH_4]^+$ was present with 10% intensity, but the $[2M+H]^+$ was missing. Similar results were obtained for another puppet head (Figure 9) and a fish, both only containing DINCH. Besides the protonated molecule and the ammonium adduct, the $[2M+NH_4]^+$ abundance at *m/z* 867 strongly predominated over the protonated dimer at *m/z* 850. Three further 25 years old toy samples (lizard, bike driver, squeaky toy) not analysed by GC/MS all provided ions clearly proving just the presence of DEHP.

Generally, the DART™ helium was set to 200 ºC, but the temperature measured at the point of sampling was measured to 130 ºC. Under these conditions, all studied samples did not sustain visible damages, except the 25 years old squeaky toy, which started to melt. To increase the sensitivity for PAE, the temperature was set to 450 ºC (measured 310 ºC), but most samples decomposed. Consequently, the operation conditions are limited, if the DART™-MS screening shall be used for nondestructive controls. As to be expected, however, there is another limit for a nondestructive analysis. Concerning the sample size, the shape of a toy had to be at least a cone of 5 cm in height and below 3 cm in diameter to easily be introduced into the DART™ source. If these shape requirements are not fulfilled, cutting into proper subsamples is essential. However, new designs of the DART™ ion source like the DART™-ET may allow wider sample shapes.

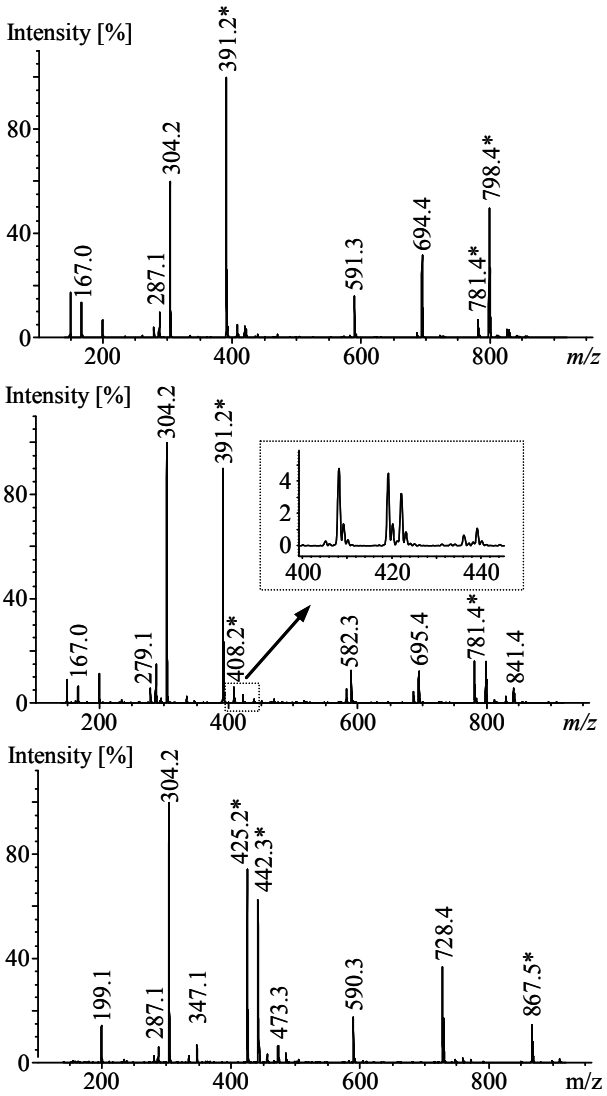


Figure 9. DART™-MS spectra of a horror mask containing 42.6% DEHP (top), childrens' swimming aids with 16.2% DEHP and 23.0% DINP (middle) and a puppet head containing 35.4% DINCH (bottom). The proton and ammonium adducts are marked with an asterisk.

VI.5. Conclusions

The presented DART™–MS method enables rapid identification of PAEs in PVC samples as toys and childcare articles at the limits actually fixed by European regulations. Validation of a positive finding based on the protonated molecule does work for a concentration of 1% for all PAE, except for BBP. As 95% of toys and childcare articles submitted in 2008 to RAPEX had more than 3% PAE, the obtained sensivity for validation is sufficient for most of the samples. Since for the present study just a single-quadrupole mass spectrometer was available, there expectedly will be the chance to increase the sensivity with a triple-quadrupole system.

Concludingly, DART™–MS provides a powerful up-to-date screening tool, well suited for surveillance purposes to screen with a high sample throughput.

VI.6. Acknowledgement

The authors would like to thank the Chemisches und Veterinäruntersuchungsamt Stuttgart for providing the samples, the Analytisches Institut Bostel for the chance to measure PAEs with ESI-MS/MS and especially Ms Tatjana Resner for her commitment with DART™-MS measurements.

VI.7. References

1. European Commission. Consumer affairs. Available at http://ec.europa.eu/consumers/safety/prod_legis/index_en.htm#Phthalates. Accessed 01 December 2008
2. Fay M, Donohue JM, De Rosa C. ATSDR evaluation of health effects of chemicals. VI. Di(2-ethylhexyl)phthalate. *Toxicology and Industrial Health* 1999; **15**(8): pp.651-746
3. Center for the Evaluation of Risks to Human Reproduction of the National Toxicology Program of the United States of America. Di-(2-ethylhexyl) Phthalate (DEHP) Evaluation. Available at http://ntp.niehs. nih.gov:8080/cs.html?charset=iso-8859-1&url=http%3A//cerhr.niehs. nih.gov/chemicals/dehp/dehp-eval.html&qt=DEHP&col=005main&n= 1&la=en. Accessed 01 December 2008
4. European Communities. Commission Directive 2005/84/EC of the European Parliament and of the Council of 14 December 2005 amending for the 22nd time Council Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (phthalates in toys and childcare articles). *Official Journal of the European Union* 2005; **L 344**: pp.40-43

5. European Food Safety Authority. Statement of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission on the possibility of allocating a group-TDI for Butylbenzylphthalate (BBP), di-Butylphthalate (DBP), Bis(2-ethylhexyl) phthalate (DEHP), di-Isononylphthalate (DINP) and di-Isodecylphthalate (DIDP). Available at http://www.efsa.europa.eu:80/cs/BlobServer/Statement/phthalategroup_minutes_statement1.pdf. Accessed 01 December 2008

6. European Communities. Council Directive 76/769/EEC of 27 July 1976 on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations. *Official Journal of the European Union* 1976; **L 262**: pp.201-203

7. Stringer R, Labunska I, Santillo D, Johnston P, Siddorn J, Stephenson A. Concentrations of phthalate esters and identification of other additives in PVC children's toys. *Environmental Science and Pollution Research International* 2000; **7**(1): pp.27-36

8. European Commission. Rapid Alert System for Non-Food Products (RAPEX) - Latest notifications. Available at http://ec.europa.eu/consumers/dyna/rapex/rapex_archives_en.cfm. Accessed 03 February 2009

9. Rastogi SC. Gas chromatography analysis of phthalate esters in plastic toys. *Chromatographia* 1998; **47**(11/12): pp.724-726

10. Vandenburg HJ, Clifford AA, Bartle KD, Carlson RE, Carroll J, Newton ID. A simple solvent selection method for accelerated solvent extraction of additives from polymers. *Analyst (Cambridge, United Kingdom)* 1999; **124**(11): pp.1707-1710

11. Hauri U, Schlegel U, Wagmann M, Hohl C. Determination of phthalates in toys and childcare articles made of PVC with HPLC and HPTLC. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene* 2002; **93**(4): pp.179-185

12. Norbygaard T, Berg RW. Analysis of phthalate ester content in poly(vinyl chloride) plastics by means of Fourier transform Raman spectroscopy. *Applied Spectroscopy* 2004; **58**(4): pp.410-413

13. Cody RB, Laramee JA, Durst HD. Versatile new ion source for the analysis of materials in open air under ambient conditions. *Anal.Chem.* 2005; **77**(8): pp.2297-2302

14. Cody RB, Laramee JA, Nilles JM, Durst HD. Direct Analysis in Real Time (DART^{TM}) Mass Spectrometry. *JEOL News* 2005; **40**(1): pp.8-12

15. Schurek J, Vaclavik L, Hooijerink H, Lacina O, Poustka J, Sharman M, Caldow M, Nielen MWF, Hajslova J. Control of strobilurin fungicides in wheat using direct analysis in real time accurate time-of-flight and desorption electrospray ionization linear ion trap mass spectrometry. *Anal.Chem.(Washington, DC, U.S.)* 2008; **80**(24): pp.9567-9575

16. Haefliger OP, Jeckelmann N. Direct mass spectrometric analysis of flavors and fragrances in real applications using DART. *Rapid Communications in Mass Spectrometry* 2007; **21**(8): pp.1361-1366

17. Morlock G, Ueda Y. New coupling of planar chromatography with direct analysis in real time mass spectrometry. *J.Chromatogr., A* 2007; **1143**(1-2): pp.243-251

18. Dytkiewitz E, Morlock GE. Analytical strategy for rapid identification and quantification of lubricant additives in mineral oil by high-performance thin-layer chromatography with UV absorption and fluorescence detection combined with mass spectrometry and infrared spectroscopy. *J.AOAC Int.* 2008; **91**(5): pp.1237-1243

VII. Summary

Food packaging is an important part of today's lifestyle. Its applications cover a wide different range which still is growing and plastic packaging plays a very important role at this. In order to enable diverse functions a widespread range of substances is used, covering most diverse chemical and physical characteristics. Therefore additives are used in the manufacturing process of polymers, fulfilling a lot of different functions. Most of the additives are not covalently bounded to the polymer and therefore are able to move inside the plastic material and also to break through the contact surface between polymer and food. These processes are called migration and can be ascribed to different and foreseeable physical processes. As migration of toxic substances from packaging into food may harm human health, the European legislation lays down rules to ensure consumers' safety. Beneath general regulations there are also specific regulations for plastic food contact material covering for example migration of substances into food and methods of analysis. Specific migration limits are established mainly on base of toxicological evaluations and consumers' consumption habits.

The analyst's work is to ensure the compliance of packaging material with the European legislation, especially in terms of health safety. The huge variety of used additives and their reaction and degradation products in combination with their different physical and chemical properties and in particular the fact that the used additives are only known to specific links of the packaging material producing chain set severe requirements to the analysis. Even today only a part of used additives is known to the European Community. Therefore an important part of the analysis primarily is the identification of hazardous substances, a non targeted analysis which can be considered as an analytical challenge and should be followed by toxicological evaluations. Chapter II deals with the non-targeted multi-component analytical screening of plastic food contact materials using fast interpretation of deliverables via expert structure-activity relationship software. To identify potential migrants of toxicological concern, resins and multilayer foils which were intended for the production of food contact materials were extracted and analysed by gas chromatography - mass spectrometry (GC-MS). In order to identify even compounds of low concentrations, the software AMDIS was used and data evaluation was

safeguarded by the Kovats Retention Index system. By this way, 46 compounds were identified as possible migrants. The expert structure-activity relationship software DEREK for Windows™ was utilized to evaluate all identified substances in terms of carcinogenicity, genotoxicity, thyroid toxicity and miscellaneous endpoints for humans. Additionally a literature search for these compounds was carried out with Sci-Finder®, but relevant data were missing for 28 substances. Summarized seven compounds with adverse toxicological effects were identified. In addition, the RIs of 24 commercial additive standards, measured with a GC capillary column of middle polarity, were given. Chapter II provides a valuable method to practically and efficiently identify substances of toxicological concern in food contact materials.

The analysis of specific substances can often be considered as challenging, too, due to lack of existing methods and the complexity of target substances and matrices. Actual examples like semicarbazide, a decomposition product of the foaming agent azodicarbonamide used for the gaskets of lids for glass jars and suspected to be genotoxic and carcinogenic, or 2-isopropylthioxanthone (ITX), a photo initiator for UV-curing inks which occurred in baby food, show that food can be seriously contaminated by packaging materials. Chapter III describes a survey that was made after the first findings of ITX in food have become public. In order to elucidate the occurrence of ITX in products on the German market more than 100 foods packed in cartons as well as in plastic cups and foils were investigated. For this, a fast method to detect ITX in food packaging materials was established. In case of positive findings the accompanying foodstuffs were analysed in a subsequent step using different extraction methods, depending on the fat content of the food. Determination of the photo-initiator was done by high performance liquid chromatography with diode array and fluorescence detection with recoveries between 94 and 106% for non-fatty foods with a relative standard deviation value (RSD) smaller than 1.2. For fatty foods the recoveries were between 80 and 105% with a rsd ≤ 8.5, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) were determined to 2 and 5 µg L^{-1}. ITX was detected in 36 out of 137 packages (26%) and significant migration occurred in 75% of the packaging materials tested positive with amounts ranging up to 357 µg kg^{-1} in orange juice and 208 mg kg^{-1} in baby food. In this survey of chapter III, 2,4 diethylthioxanthone, mainly used as a substitution product for ITX, was also found.

Other examples for food which is seriously contaminated by packaging materials are plasticisers in oily food migrating from the gaskets of lids for glass jars and exceeding manifold legislative migration limits. Generally the first step for the analysis of plasticisers in food, whose source might be the gasket of glass jars, is the identification of additives in gaskets, which also requires considerable efforts. Chapter IV shows how up to date instrumental equipment can be very helpful in the rapid identification of substances in plastics with direct analysis in real time ionisation and single quadrupole mass spectrometry (DART™-MS), using the example of additives in gaskets for lids made of polyvinylchloride (PVC). In chapter IV it is shown that solutions of substances measured with DART™-MS showed the $[M+H]^+$ and $[M+NH_4]^+$ as typical ionisation and the cleavage of ester bonds as typical fragmentation products of the molecules. The additives were identified rapidly and easily via DART™-MS generally in the range of 1% (wt), if ion suppressive effects did not occur. It was shown that in order to avoid these effects deriving from specific molecules, extracts of a plastic with appropriate organic solvents can improve sensivity. By use of this method it was possible to identify fatty acid amides, phthalates, tributyl O-acetylcitrate, dibutyl sebacate, bis(2-ethylhexyl) adipate, 1,2-cyclohexanedicarboxylic acid 1,2-diisononyl ester and even more complex additives like acetylated mono- and diglycerides of fatty acids, epoxidised soybean oil and polyadipates in ranges up to a content of 1% or better in plastic material. Epoxidised linseed oil could also be identified if contained at 5% or higher and false positive identifications only occurred for di-isononyl phthalate. The detection of the use of azodicarbonamide as foaming agent in the manufacturing process showed possibilities, but failed due to missing robustness. A great value of the method presented in chapter IV is the complete analysis time of about 45 minutes for very complex plastic samples. As more clarity was brought into the results of ionisation and fragmentation processes of DART™-MS, this method may be transferred easily to other applications dealing with similar subjects.

Experts consider the use of packaging materials as the largest and least controlled source of food contaminations with organic materials in Europe and improvements in the analysis of food contaminations deriving from packaging materials are an imperative exigence. The plasticiser epoxidised soy bean oil (ESBO) was also a substance which contaminated especially oily food and baby food, in

amounts several fold exceeding European specific migration limits. In chapter V the determination of ESBO in food by GC-MS and gas chromatography- tandem mass spectrometry (GC-MS/MS) stable isotope dilution assay (SIDA) is evaluated. To establish a SIDA, the $^{13}C_{18}$ labelled internal standard ethyl 9,10,12,13-diepoxyoctadecanoate was synthesized, providing after sample preparation the same retention time as methyl 9,10,12,13-diepoxyoctadecanoate, commonly used as marker for ESBO in GC analysis. For eleven different food matrices, the GC capillary columns VF-17ms, DB1701 and DB1 were tested with GC-MS as well as GC-MS/MS detection. Overall, the VF-17ms column coupled with MS/MS detection showed the best results in terms of separation and sensitivity. The method validation for the matrix spiked olive oil resulted in a LOD of 5 mg kg^{-1}, a LOQ of 11 mg kg^{-1}, a mean recovery (n=5, c=106.5 mg kg^{-1}) of 99.7±5.5 % with a repeatability of 6.0 %. By means of GC-MS a LOQ of 21 mg kg^{-1} and a mean recovery (n=5, c=106.5 mg kg^{-1}) of 103.3±0.8 % with a repeatability of 0.9 % were determined. The method provided in chapter V shows that a very fast sample preparation for GC analysis is even possible, if on-line liquid chromatography - gas chromatography with flame ionisation detection or GC-MS with chemical ionisation with ammonia is absent in the laboratory equipment.

There exist also other sources of the exposure of hazardous substances than food consumption. In case of babies, toddlers and children mouthing is responsible for the intake of phthalic acid esters (PAEs) in amounts higher than generally presumed. For toys and childcare it actually is responsible for 90% of the exposure of European infants and toddlers with di-isononyl phthalate (DINP), for example. For these products analysis is german to one of packaging material, fast and easy methods are lacking and improvements in this area have to be made. Chapter VI describes a rapid and nondestructive analysis for phthalic acid esters in PVC by use of DART™-MS. LODs for the $[M+H]^+$ ion for benzyl butyl phthalate (BBP), bis (2-ethylhexyl) phthalate (DEHP) and DINP was 0.05% (wt) and 0.1% for dibutyl phthalate, di-n-octyl phthalate (DNOP) and di-isodecyl phthalate, respectively. Safeguarding of the identification with other characteristic ions was possible to a contentent of 1% (wt) or higher for all PAEs, except for benzyl butyl phthalate (5%). Distinction of bis (2-ethylhexyl) phthalate and di-n-octyl phthalate on base of fragmentation products was successful at concentrations of 5% or higher, by use of a

gas temperature of 310 ºC even at 1%. As the generally used gas temperature was 131ºC, most toy and childcare samples do not get destroyed. The analysis of one sample generally took between 10 and 15 minutes. Summarised the method described in chapter VI is a powerful and up to date screening tool allowing a fast identification of PAEs in PVC up to the legislative European limits. It is very well suited for surveillance purposes in order to screen with a high sample throughput.

VIII. Zusammenfassung

Lebensmittelverpackungen sind ein wichtiger Teil des momentanen Lebensstils. Sie werden in einem weiten, sich stets vergrößernden Bereich angewendet und Verpackungen aus Kunststoff spielen hierbei eine sehr wichtige Rolle. Um unterschiedlichste Funktionen zu gewährleisten wird eine breitgefächerte Auswahl an Stoffen, welche mannigfachste chemische und physikalische Eigenschaften aufweisen, verwendet. Aus diesem Grund werden Additive, die diverse Funktionen erfüllen in der Produktion von Kunststoffen eingesetzt. Da die meisten Additive nicht kovalent an das Kunststoffpolymer gebunden sind, können sie im Kunststoff diffundieren und sogar die Grenzschicht zwischen Kunststoff und Lebensmittel überwinden. Diese Vorgänge können unterschiedlichen und vorhersehbaren physikalischen Prozessen zugeordnet werden und werden fachlich Migration genannt. Da die Migration von toxischen Stoffen aus Kunststoffverpackungen in Lebensmittel gesundheitsgefährdend sein kann, hat die europäische Gesetzgebung Normen erlassen um die Sicherheit der Verbraucher zu gewährleisten. Neben allgemeinen Normen gibt es auch sehr spezielle Regelungen, die zum Beispiel von der Migration von Stoffen in Lebensmittel und von Analysenmethoden handeln. Überwiegend auf der Basis von toxikologischen Untersuchungen und Verzehrsgewohnheiten von Verbrauchern wurden spezifische Migrationsgrenzwerte erlassen.

Analytiker überprüfen, ob Verpackungsmaterial den europäischen gesetzlichen Vorschriften und insbesondere der gesundheitlichen Unbedenklichkeit entspricht. Die große Anzahl an verwendeten Additiven sowie deren Reaktions- und Abbauprodukte zusammen mit ihren unterschiedlichen physikalischen und chemischen Eigenschaften, und insbesondere die Tatsache, dass die benutzten Additive nur einzelnen Gliedern der Kette des Herstellungsprozesses bekannt sind, wirken sich als sehr hohe Anforderungen für die Analytik aus. Momentan ist nur ein Teil der benutzten Additive der Europäischen Gemeinschaft bekannt. Deshalb spielt die Identifizierung von gesundheitsgefährdenden Substanzen als erster Schritt eine wichtige Rolle. Die sogenannte nicht zielgerichtete Analyse, welche als analytische Herausforderung betrachtet werden kann, sollte eine toxikologische Bewertung nach sich ziehen. Kapitel II handelt von der nicht zielgerichteten Analyse von

Lebensmittelverpackungen aus Kunststoff, indem die Ergebnisse rasch mittels einer Software gesundheitlich aufgrund von Struktur- Aktivitätsbeziehungen bewertet werden. Um toxikologisch bedenkliche, mögliche Migranten zu identifizieren, wurden Kunststoffgranulate und Mehrschichtfolien, die zur Lebensmittelverpackungsherstellung benutzt werden, extrahiert und mittels Gaschromatographie gekoppelt mit Massenspektrometrie (GC-MS) untersucht. Damit auch Komponenten geringer Konzentration identifiziert werden können wurde die Software AMDIS benutzt, und zusätzlich wurde die Auswertung durch das Kovats- Retentionsindexsystem abgesichert. Auf diese Art und Weise wurden 46 Stoffe als mögliche Migranten identifiziert. Die Struktur- Aktivitätsbeziehungs- Software DEREK for Windows™ wurde eingesetzt um alle identifizierten Stoffe bezüglich Kanzerogenität, Genotoxizität, Schilddrüsentoxizität und sonstigen Endpunkten, die für Menschen relevant sind, zu beurteilen. Zusätzlich wurde eine Literaturrecherche mit SciFinder® durchgeführt, die jedoch in den zu untersuchenden Bereichen für 28 Substanzen erfolglos blieb. Zusammengefasst wurden sieben gesundheitsgefährdende Stoffe identifiziert. Zusätzlich wurde von 24 kommerziell verwendeten Additiven der Kovats-Retentionsindex, der mit einer mittelpolaren GC-Säule bestimmt wurde, gegeben. Kapitel II übermittelt eine wertvolle Methode um praktisch und effizient toxikologisch bedenkliche Stoffe in Lebensmittelbedarfsgegenständen zu identifizieren.

Da weder analytische Methoden existieren und Zielanalyten, sowie deren Matrix sehr komplex sind, kann auch die Analyse von speziellen Analyten häufig als Herausforderung betrachtet werden. Aktuelle Beispiele wie Semicarbazid, ein Abbauprodukt von dem Treibmittel Azodicarbonamid, welches für die Dichtungsmasse von Gläschendeckel verwendet und im Verdacht steht genotoxisch und karzinogen zu sein, oder 2-Isopropylthioxanton (ITX), ein Fotoinitiator für UV-härtende Druckfarben, welcher in Babynahrung auftrat, zeigen, dass Lebensmittel von Verpackungen schwerwiegend kontaminiert werden können. Kapitel III beinhaltet eine Erhebung, die gemacht wurde nachdem erste ITX Funde in Lebensmitteln veröffentlicht wurden. Um das Vorhandensein von ITX in deutschen Produkten aufzuklären wurden über 100 Lebensmittel, die in sowohl in Kartonverpackungen, als auch in Kunststoffbechern und Folien verpackt waren, untersucht. Um dies durchzuführen wurde eine Schnellmethode entwickelt, die ITX in Lebensmittelverpackungen detektiert, Wurde ITX identifiziert, so wurde auch nachfolgend das entsprechende Lebensmittel untersucht, wobei entsprechend

dessen Fettgehalt unterschiedliche Extraktionsmethoden angewendet wurden. Die Bestimmung des Fotoinitiators wurde mittels Hochleistungsflüssigkeits-chromatographie mit Diodenarray- und Fluoreszenzdetektion und Wiederfindungsraten für nicht fetthaltige Lebensmittel zwischen 94 und 106% mit einer relativen Standardabweichung (RSD) kleiner als 1,2 durchgeführt. Für fetthaltige Lebensmittel waren entsprechend die Wiederfindungsraten zwischen 80 und 105% mit einer RSD kleiner oder gleich 8,5. Die Erfassungsgrenze wurde zu 2 µg/L und die Bestimmungsgrenze zu 5 µg/L bestimmt. ITX wurde in 36 von 137 Lebensmitteln identifiziert, dies entspricht einer Trefferquote von 26%. Eine signifikante Migration fand in 75% der positiv getesteten Verpackungen statt, mit Gehalten bis zu 357 µg/kg in Orangensaft und 208 µg/kg in Babynahrung. In dieser Erhebung von Kapitel III wurde auch 2,4-Diethylthioxanthon gefunden, welches überwiegend als Ersatzprodukt für ITX eingesetzt wurde.

Andere Beispiele, bei denen Lebensmittel schwerwiegend durch Lebensmittelverpackungen kontaminiert wurden, sind Weichmacher, die aus der Dichtungsmasse von Gläschendeckel migrierten und gesetzliche Grenzwerte mehrfach überschritten. Im Allgemeinen ist der erste Schritt der Analyse von verpackungsbedingeten Weichmachern in Lebensmitteln die Analyse der Weichmacher in der Dichtungsmasse, welche auch beträchtlichen Aufwand erfordert. Kapitel IV zeigt, wie modernes Equipment sehr hilfreich bei der schnellen Identifizierung von Substanzen in Kunststoffen mittels der direkten Echtzeitionisation gekoppelt mit der Einfachquadrupolmassenspektrometrie (DART™-MS), am Beispiel von Additiven in Dichtmassen für Gläschendeckel aus Polyvinylchlorid (PVC), sein kann. Kapitel IV führt aus, dass Lösungen von Stoffen, die mit DART™-MS gemessen werden, die Ionen $[M+H]^+$ und $[M+NH_4]^+$ als typische Ionisationsprodukte und eine Spaltung der Esterbindung als typische Zerfallsprodukte der Moleküle zeigen. Die Additive wurden schnell und einfach mit DART™-MS identifiziert, im Allgemeinen im Bereich von einem Massenprozent falls keine ionensuppresiven Effekte auftraten. Da diese Effekte von bestimmten Molekülen verursacht werden, konnte gezeigt werden, dass Extrakte von einem Kunststoff mit dem entsprechend geeigneten organischen Lösungsmittel die Nachweisempfindlichkeit erhöhen können. Mit dieser Methodik war es möglich Fettsäureamide, Phthalate, Tributyl-O-acetylcitrat, Dibutylsebacat, Bis(2-ethylhexyl)adipat, 1,2-Cyclohexanedicarboxyl-säure, 1,2-diisononyl ester und sogar komplexere Additive wie acetylierte Mono- und

Diglyceride von Fettsäureestern, Epoxidieres Sojaöl und Polyadipate bis zu einem Prozent oder geringer im Kunststoff nachzuweisen. Epoxidiertes Leinsamenöl konnte auch als solches ab einem Gehalt von 5% identifiziert werden und falsch positive Identifizierungen traten nur für Diisononylphthalat auf. Der Nachweis, ob Azodicarbonamid im Herstellungsprozess benutzt wurde zeigte Möglichkeiten auf, war aber nicht sehr robust. Ein sehr großer Nutzen der Methode, die in Kapitel IV vorgestellt wird, ist die Analysenzeit von ungefähr 45 Minuten für sehr komplexe Kunststoffproben. Nachdem mehr Gewissheit in die Ionisierungs- und Zerfallsprodukte der DART™-MS gebracht wurde, kann diese Methode auch auf andere Anwendungen, die von ähnlichen Fragestellungen handeln, übertragen werden.

Experten betrachten die Verwendung von Verpackungsmaterial als größte und am wenigsten kontrollierte Quelle für die Lebensmittel-kontamination mit organischen Stoffen in Europa, deshalb ist die Verbesserung von Analysenmethoden für Lebensmittelkontaminationen, die durch Verpackungen verursacht werden, eine zwingend erforderliche Notwendigkeit. Der Weichmacher Epoxidiertes Sojaöl (ESBO) war auch ein Stoff, welche insbesondere ölhaltige Lebensmittel und Babynahrungsmittel kontaminierte, und zwar in einem Umfang welcher europäische spezifische Migrationsgrenzwerte um ein mehrfaches überschritt. Kapitel V erforscht die Bestimmung von Epoxidiertem Sojaöl in Lebensmitteln mit der GC-MS und der Gaschromatographie gekoppelt mit der Dreifachquadrupolmassen-spektrometrie (GC-MS/MS)- Stabilisotopverdünnungsanalyse (SIDA). Um eine SIDA zu entwickeln wurde der $^{13}C_{18}$- markierte interne Standard Ethyl 9,10,12,13-diepoxyoctadecanoat synthetisiert, welcher nach der Probenaufarbeitung dieselbe Retentionszeit wie Methyl 9,10,12,13-diepoxyoctadecanoat, das allgemein in der Gaschromatographie als Marker für ESBO genutzt wird, besitzt. Die Gaschromatographiekapillarsäulen VF-17ms, DB1701 und DB1 wurden für elf unterschiedliche Lebensmittelmatrixe mittels GC-MS und GC-MS/MS getestet. Bezüglich Trennleistung und Empfindlichkeit zeigte vor allem die VF-17ms-Säule mit MS/MS Detektion die besten Ergebnisse. Eine Methodenvalidierung für die Matrix Olivenöl ergab eine LOD von 5 mg kg^{-1}, eine LOQ von 11 mg kg^{-1}, eine mittlere Wiederfindung (n=5, c=106,6 mg/kg) von 99,7±5,5% mit einer Wiederholbarkeit von 6,0%. Unter Verwendung von GC-MS wurde das LOQ zu 21 mg/kg und eine mittlere Wiederfindung (n=5, c=106,6 mg/kg) von 103,3±0,8% mit einer Wiederholbarkeit von

0,9% bestimmt. Die Methode welche in Kapitel V vorgestellt wird zeigt, dass selbst wenn im Analysenlabor keine onlinegekoppelte Hochleistungsflüssigkeits-chromatographie online gekoppelt mit Gaschromatographie mit Flammenionisations-detektion, oder GC-MS mit chemischer Ionisierung mit Ammoniak, zu Verfügung stehen, eine sehr schnelle Probenaufarbeitung für die gaschromatographische Analyse möglich ist.

Neben der Nahrung gibt es auch andere Aufnahmewege für gesundheitsgefährdende Stoffe. Bei Babys, Kleinkindern und Kindern ist das in den Mundnehmen von Dingen für die Aufnahme von Phathalaten verantwortlicher als es allgemein angenommen wird. Im Fall von Spielzeug und Babyartikel verursacht es zum Beispiel momentan bei Babys und Kleinkindern 90 % der Belastung mit Di-isononylphthalat (DINP). Für solche Produkte sind Analysenmethoden eng verwandt mit denen von Packungsmaterialien, schnelle und einfache Methoden fehlen jedoch und Verbesserungen in diesem Bereich sind notwendig. Kapitel VI beschreibt eine schnelle und zerstörungsfreie Analysenmethode für Phthalsäureester in PVC mittels der DART™-MS. Die Nachweisgrenzen für das $[M+H]^+$-Ion für Benzylbutylphthalat (BBP), Di-(2-ethylhexyl)phthalat (DEHP) und DINP waren 0,05 Gew-% und entsprechend 0,1 Gew-% für Dibutylphthalat, Di-n-octylphthalat (DNOP) und Di-isodecylphthalat. Eine Absicherung der Identifizierung mittels anderer charakteristischen Ionen war für alle Phthalate mit einem Gehalt von einem Gew.-% oder höher möglich, außer für BBP (5 Gew.-%). Die Unterscheidung von DEHP und DNOP mittels ihrer ionisierten Zerfallsprodukte war ab einer Konzentration von 5 Gew.-% erfolgreich. Wurde die Gastemperatur auf 310ºC erhöht, dann sogar ab einem Gew.-%. Da üblicherweise aber mit 131ºC gearbeitet wurde, wurden die meisten Spielzeug und Babyartikel nicht beschädigt. Die Analyse einer Probe nahm generell etwa zehn bis fünfzehn Minuten in Anspruch. Kurz und bündig ist die in Kapitel VI vorgestellte Methode ein mächtiges und zeitgemäßes Mittel für eine Übersichtsanalyse, welche eine schnelle Identifizierung der Phthalsäureester in PVC bis zu den Grenzwerten der europäischen Gesetzgebung erlaubt. Es ist für Überwachungszwecke sehr gut geeignet, um mit einem hohen Probendurchsatz Übersichtsanalysen durchzuführen.

Acknowledgements

Additionally to acknowledgements previously mentioned in this work, I particulary thank:

-My parents, my wife and my children for supporting me during this work,

-Prof. Dr. W. Schwack for his help in all kind of matters and the people of the Institut für Lebensmittelchemie of the Universität Hohenheim who assisted me, especially Ms. Tatjana Resner and Mr. Axel Lang,

-The Chemisches und Veterinäruntersuchungsamt Stuttgart for supporting me in the beginning of this work.