Diese Dissertation haben begutachtet :

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DISSERTATION

Migration of benzophenone and diphenyl phthalate into selected foodstuffs

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CONTENT:

1 Abstract

As a part of the Foodmigrosure project - Modelling migration from plastics into foodstuffs as a novel and cost efficient tool for estimation of consumer exposure from food contact material (Project reference: QLK1-CT2002-2390) - migration tests at various time/temperature conditions were conducted with 17 different foodstuffs using a polyethylene plastic film containing two model substances of interest, benzophenone and diphenyl phthalate. Straightforward analytical methods for determination of benzophenone and diphenyl phthalate in the selected foodstuffs were established and the extent of the migration was measured as a function of time. Furthermore the influence of the storage temperature on the migration was studied and the extent of the migration between different foodstuff groups was compared. Influence of food parameters like fat content, alcohol content or water content on the migration were discussed.

2 Introduction

Packaging in general is primarily designed for product protection. In case of food packaging this protective function is undoubtedly the most important one besides other functions like selling a product and maintaining an efficient and cost-effective process cycle. Nowadays various materials are being employed in contact with foodstuffs in form of packaging ranging from glass and metals to paper and board, wood, waxes and plastics. Each material has its unique properties, many are tailored to a specific purpose, but they all have one thing in common – they all interact with the packed foodstuff. This interaction might be based on a chemical reaction between the packaging material and the foodstuff or on mass transfer from the packaging material into the foodstuff or vice versa. Components of the contact material, even though incorporated in the material, might move to the surface through the process of diffusion and subsequently transfer to the foodstuff. Part of the substances may have their origin on the surface of the contact material (adsorbed substances e.g. antistatics used in the production). The consequences of interactions between the packaging material and the packed foodstuff may range from slight aroma deterioration to serious health risk for the consumer. This way packaging, whose primary function is to protect the packed good, may just as well be the cause of dramatic quality loss of a product if not chosen reasonably.

To address the risk of mass transfer of packaging constituents into foodstuffs overall and specific migration limits were established for substances which are used in the packaging production process and might pose a risk to human health or cause an unacceptable change in the composition of the foodstuff. At Community level legislative framework covered by Regulation (EC) No. 1935/2004 on materials and articles intended to come into contact with food contains Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with food, where the migration limits applicable to plastic materials can be found. From all the food contact materials being used today plastic materials are those with the most

comprehensive regulations. The variety of substances used during production of plastic packaging materials is vast resulting in a variety of different materials. In combination with the different kinds of foodstuffs on the market there are countless foodstuff/packaging variations. Analytical methods therefore have to be adopted for migrant determination in varying matrices. This of course makes the analytical control and enforcement of the migration limits rather difficult and costly.

Compliance testing of food contact materials requires standardized procedures to be agreed upon in order to achieve comparability of analytical data. When a certain material is tested, the use of real foodstuffs is problematic because their properties vary depending on many factors like place of origin, production techniques, additives etc. rendering them unsuitable for compliance testing.

In order to achieve comparability between the migration test results obtained in different laboratories during compliance testing of packaging materials food simulants were introduced in the past and are still being used instead of real foodstuffs to measure migration. These simulants are a compromise, a simplification, and can not reflect the true nature of the various foodstuffs available. In some cases the migration measured into a food simulant might be an overestimation of the migration into a real foodstuff it represents thus adding an extra safety margin and might be seen as a positive step towards consumer protection. On the other hand the opposite situation has to be considered where the migration into the food simulant might be lower than it would be in the case of real foodstuff. For example in the past water was used as a food simulant for milk. However, measurements of styrene migration from polystyrene cups into milks with various fat contents and ethanol/water mixtures showed that pure water gave migration values considerably lower than all of the milks tested. Fifty percent ethanol was shown to correlate approximately with the milk containing 3,5% fat. (1) This case demonstrates the necessity of migration measurements to be conducted on real foodstuffs in order to obtain solid data upon which a mathematical model for estimation of consumer exposure can be based.

Migration modeling has the potential to identify situations where high mass transfer is likely, thus reducing time and effort analyzing huge amount of samples and enabling to focus on the crucial cases. Using sophisticated models critical situations can be detected where a violation of the migration limits might be expected. In these specific cases analytical determination in a laboratory can then be employed for confirmation.

However, mathematical models for migration modeling first need data upon which their calculations are to be based. Quality of the data the model is based upon determines the quality of its output. This puts a lot of stress on the quality of the input data.

This work is part of the project Foodmigrosure – Modelling migration from plastics into foodstuffs as a novel and cost efficient tool for estimation of consumer exposure from food contact material (Project reference: QLK1-CT2002-2390). The aim of this particular work was to conduct migration tests with real foodstuffs selected in such a way as to cover in the best possible way the broad spectrum of packed foodstuffs available on the market with respect to the expected migration behavior and develop an analytical method for determination of two selected analytes – benzophenone and diphenyl phthalate. A polyethylene film

containing specified amounts of the analytes (see Table 2) was used in this study. From the 21 foodstuffs selected in the Foodmigrosure project (see chapter 6) 17 were included in this particular study (see Table 8).

3 Food safety in the European Union – European food law and Regulation (EC) No 178/2002

The European Union adopted an integrated approach to food safety. Through so called farm-to-table measures and monitoring high level of food safety, animal health, animal welfare and plant health shall be assured.

The principles of the European food law are defined in the Regulation (EC) No 178/2002 „laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in means of food safety" which states:

(1) „...free movement of **safe and wholesome food** is an essential aspect of the internal market and contributes significantly to the health and well-being of citizens, and to their social and economic interests". Furthermore

(2) „...a **high level of protection of human life and health** should be assured in the pursuit of Community policies".

As this regulation should provide for an extensive concept of food safety:

(11) „In order to take a sufficiently comprehensive and integrated approach to food safety, there should be a broad definition of food law covering a wide range of provisions with a direct or indirect effect on the safety of food and feed, including **provisions on materials and articles in contact with food**, animal feed and other agricultural inputs at the level of primary production.

As materials and articles in contact with food represent an essential aspect of food safety it is necessary to investigate the possible interactions between these materials and the food.

3.1 Legislation on food contact materials – Regulation (EC) No 1935/2004

The first Directive concerning food contact materials was adopted by the Council on 23rd of November 1976. It was the Directive 76/893/EEC on the approximation of the laws of the Member States relating to materials and articles intended to come into contact with foodstuffs. Later on it was the Directive 89/109/EEC of 21st of December 1988 until it was repealed by the Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27th of October 2004 on materials and articles intended to come into contact with food (further also referred to as materials and articles), which is currently in power. The declared purpose of the regulation is to ensure the effective functioning of the internal market in the

Community whilst providing the basis for securing a high level of protection of human health and the interests of consumers (Article 1). The regulation shall apply to materials and articles which are intended to be brought into contact with food, or are already in contact with food and were intended for that purpose or can reasonably be expected to be brought into contact with food or to transfer their constituents to food under normal or foreseeable conditions of use (Article 1).

According to Article 1 the Regulation (EC) No 1935/2004 does not apply to - materials and articles supplied as antiques; covering or coating materials which form part of the food and may be consumed together with the food; fixed public or private water supply equipment.

General requirement (Article 3) on materials and articles intended to come into contact with food laid down by this regulation state, that they 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: a) endanger human health; or b) bring about an unacceptable change in the composition of food; or c) bring about a deterioration in the organoleptic characteristics thereof.

On the basis of toxicological data the amount of a substance posing a danger to human health can be calculated (point a.)) and a possible deterioration of organoleptic characteristics of a food can be asserted in a sensory test by comparison of a reference food not in contact with the packaging material with the same food, which has been in contact with the material under specified conditions (point c.)). An unacceptable change in the composition of food defined under point b.) is more difficult to quantitate. In the specific directive concerning plastics (2002/72/EC) the term of "overall migration" has been defined in this context. (see 3.1.1)

The focus of Article 4 is on the special requirements for active and intelligent materials and articles. Given by the nature of active materials and articles they may bring about changes in the composition or organoleptic characteristics of food, but on the condition that the changes comply with the Community provisions applicable to food (e.g. provisions of Directive 89/107/EEC on food additives), or, if no Community provisions exist, with the national provisions applicable to food. Substances deliberately released into the food or the environment surrounding it shall be authorized and used in accordance with the relevant provisions applicable to food as well as in compliance with the Regulation (EC) No 1935/2004.

According to this Article these substances deliberately released from the active material into the food shall be treated as ingredients (within Article 6(4) of Directive 2000/13/EC). It also prohibits the use of active and intelligent materials in way that could mislead the consumer and puts requirements on the labeling.

Finally the Article 5 shall be mentioned here. This article on specific measures for groups of materials and articles refers to Annex I in which groups are listed where specific measures may be adopted or amended. Those measures may include lists of authorized substances for use in manufacturing, purity standards, specific and overall limits on migration, rules to ensure compliance etc. Annex I lists 17 groups, yet up to this date only for 3 of them – namely I).ceramics, II).plastics and III).regenerated cellulose - specific regulations exist. In future regulations for the remaining groups are expected.

3.1.1 Legislation related to food contact materials made of plastics – Directive 2002/72/EC

The European Commission decided that for reasons of clarity and rationality there was a need to consolidate the frequently amended Directive 90/128/EEC of 23 February 1990 relating to plastic materials and articles intended to come into contact with foodstuffs and so on 6th of August 2002 European Commission has adopted a specific Directive 2002/72/EC relating to the plastic materials and articles intended to come into contact with foodstuffs (further referred to as plastic directive) which presents the core for further regulation.

The original version of the Directive applied only to plastic materials and articles and parts thereof consisting exclusively of plastics or composed of two or more layers of materials, each consisting exclusively of plastic. It explicitly did not apply to materials and articles composed of two or more layers, one or more of witch does not consist exclusively of plastics, even if the one intended to come into direct contact with foodstuffs does consist exclusively of plastic (Article 1(4)). This caused some difficulties. For example it was demonstrated that plasticizers used in polyvinyl chloride (PVC) gaskets in lids may migrate into fatty foods in quantities that could endanger human health or bring about an unacceptable change in the composition of the foods, yet according to the definition the gasket did not fall under the scope of the plastic directive. This was one of the reasons for the Commission to adopt Directive 2007/19/EC amending Directive 2002/72/EC (plastic directive) to clarify that even if the gaskets are part of e.g. metal lids, they do fall under the scope of the plastic directive.

The plastic directive contains a list of monomers and other starting substances which may be used in the manufacture of plastic materials and articles (Annex II). It is a complete positive list excluding the use of any monomer or substance not included on the list. The list contains the following information:

- column 1 (Ref. No.): the EEC packaging material reference number of the substances on the list,
- column 2 (CAS No.): the CAS (Chemical Abstracts Service) registry number,
- column 3 (Name): the chemical name,
- column 4 (Restrictions and/or specifications): e.g. specific migration limit (SML), maximum permitted quantity of the substance in the finished material or article (QM), other restriction etc.

Annex III contains an incomplete list of additives which may be used in the manufacture of plastic materials and articles. This list is to be amended to introduce other additives evaluated by the EFSA and by decision of the Commission it shall become a positive list as from 1st of January 2010 (Directive 2008/39/EC).

The term "overall migration limit" (OML) is established as a measure of the inertness of the material which prevents an unacceptable change in the composition of the foodstuff. This is one of the general requirements on materials and articles in contact with foodstuffs laid down in the Regulation (EC) No 1935/2004 Article 3 point b.). It also aims to give effective control by reducing the need for a large number of specific migration limits.

It was agreed to set the overall migration limit to 10 mg/dm^2 of the food contact material or 60 mg/kg of the food itself. As far as these conventional limits are not exceeded and the migrating substances give no reason for concern from the toxicological or organoleptic point of view it can be reasonably assumed that no unacceptable change in the composition of the foodstuff occurs. (2)

Furthermore "specific migration limits" (SML) have been established for certain substances. While value of an OML is primarily given in mg/dm^2 of food contact material the SML's in Annex III are given in mg/kg foodstuff or food simulant (in some specified cases its values are to be expressed in mg/dm^2 (Article 7)). This difference stresses once more, that while the OML is a measure of the inertness of the material, the SML was derived based upon toxicological data and the predicted exposure of the consumer. From the amount of substances with an SML listed in the annexes of the Directive the regulatory difficulties are obvious.

Bradley et al. (3) investigated if overall migration test procedures could also be used to test for the migration of specific substances from plastics. They concluded that testing for specific migration by using overall migration methods is most applicable for polymers with a low intrinsic migration. For polymers with higher intrinsic migration the approach is only suitable for substances with high specific migration limits.

3.1.2 Food simulants and migration testing

Analytical determination of migrants from food packaging materials in real foodstuffs is often difficult, time consuming and expensive due to the complex matrix (variety of fat, protein, carbohydrate and water content as well as countless other constituents) and the necessity for measuring at very low concentration levels. As a result a system based on food simulants was established and used to conduct migration tests. (4) Introduction of food simulants as well as their use as defined in Council directives 82/711/EEC, 85/572/EEC and Directive 2007/19/EC was one of the steps towards better comparability of analytical results obtained in different laboratories, which is essential in the process of compliance testing.

Table 1 Food simulants

Simulant	Composition
A	Distilled water or water of equivalent quality
B	3 % acetic acid (w/v) in aqueous solution
C	15 % ethanol (v/v) in aqueous solution
D	Rectified olive oil*
E	50 % ethanol (v/v) in aqueous solution**

* in some cases mixture of synthetic triglycerides or sunflower oils may be used

** as defined in Directive 2007/19/EC (18)

Chapter I of Council Directive 82/711/EEC defines in general terms food types and the corresponding food simulants to be used. The simulants are classified by convention as having the character of one or more food types. More detailed list of foodstuffs covered by the use of a specific food simulant can be found in Council directive 85/572/EEC the annex of which also contains specific characteristics of the rectified olive oil, sunflower oils as well as composition and characteristics of the synthetic triglycerides which may be used in some cases. Based on new data Directive 2007/19/EC added the fifth simulant (50 % ethanol) to be used with some milk products which are defined in Annex VIII.

Basic rules for migration testing have been laid down by Council Directive 82/711/EEC, last amended by Commission Directive 97/48/EC. While choosing the right simulant for the migration testing requires the knowledge of the type of foodstuff that is intended to be in contact with the material, choosing the correct time/temperature test conditions requires the knowledge of the use of the article. It is necessary to know how long and at what temperature it is intended or can be foreseen to be in contact with the foodstuff. The test conditions are then selected from the table 3 in the Directive considering the worst foreseeable conditions of contact for the plastic material or article taking into account any labeling information on maximum temperature of use. In general the determination of migration should be restricted to the test conditions which are recognized to be the most severe. If no labeling or instructions are present depending on the food types, simulants A and/or B and/or C shall be used for 4 hours at 100°C or for 4 hours at reflux temperature and/or simulant D shall be used only at 2 hours at 175°C. If the materials or articles are intended for use at room temperature or below the test shall be carried out at 40°C for 10 days. A special case is the so called "hot fill". If the material or article may be employed at temperatures between 70°C and 100°C for less than 15 minutes and it is indicated by appropriate labeling or instructions, only the 2 hour test at 70°C shall be carried out. These are just some examples for migration testing. In cases where conventional conditions for migration testing are not adequately covered by the test contact conditions in the Directive, other contact conditions may be used which are appropriate provided they represent the worst foreseeable conditions of use.

Despite these Directives laying down the rules for testing migration of constituents of plastic materials and articles intended to come into contact with food it was observed, that laboratories did not always interpret these rules in the same way leading to different test procedures. It was therefore necessary to ensure that the laboratories responsible for official control analysis have the same interpretation of the test conditions for specific materials and articles. To address this problem the CRL-NRL network published in 2009 "Guidelines on testing conditions for articles in contact with foodstuffs – with a focus on kitchenware". These guidelines contain practical information that define the parameters that should be used to perform either an overall or a specific migration tests. In future the guidelines will evolve and expand into further editions.

4 Migration of substances from the packaging and various food contact materials into the foodstuffs

The main task of a food packaging is the protection and the quality assurance of the packed foodstuff. This includes prevention of any contamination of the foodstuff, not only from the environment but also from the packaging itself. The packaging and the foodstuff represent two systems. Due to a very close contact between these systems and the partially long shelf life of certain foodstuffs a migration of substances (in both directions) might take place.

Migration investigation with paper and board packaging materials in contact with foodstuffs and food simulants has been conducted by Zülch and Piringer (5). On the basis of the obtained results first model for the migration from paper and board into foods and simulants was developed. It was observed that for correct modeling of the experimental results in many cases paper and board must be regarded as a two-layer system and that the diffusion rate decreases with increasing molecular weight of the migrant.

Printed paper and board packaging materials were reported to be the source of contamination due to migration of printing ink components. Richter et al. (6) reported the migration potential of newly patented low migration offset printing ink. These inks based on a novel fatty acid ester as opposed to the mineral and vegetable oils broadly used in the printing inks.

For foods having a high affinity for the migrant, the partition equilibrium favours migration from the paperboard to the food. Migration will be controlled largely by diffusion of molecules within the board (7) and since diffusion coefficients are strongly temperature dependent (8), migration during microwave or other types of heating can be especially facile. It is notable however, that even at the low temperature of frozen storage, migration levels could approach those seen after microwave heating (e.g. frozen pizza contained 400μg/kg benzophenone after storage, after heating the concentration of benzophenone reached 645-700μg/kg). (9) Presumably the much longer time periods offset the lower temperature.

In the case of polycarbonate materials bisphenol A migration is often a reason for concern. Kubwabo et al. (10) conducted a migration study on bisphenol A from baby bottles. Residual bisphenol A leaching from the polycarbonate was observed and increased with temperature and time. Ehlert et al. (11) investigated the migration of bisphenol A from polycarbonate baby bottles into water during microwave heating. It was shown that during three microwave heating cycles of a baby bottle made of polycarbonate microwave radiation had no effect on the migration of bisphenol A into water. Maragon et al. (12) measured migration of bisphenol A from polycarbonate baby bottles under real use conditions. It was shown that the temperature was the crucial factor for the migration of bisphenol A from the plastic bottles to water. It was concluded that it is unlikely that additional bisphenol A is yielded throughout repeated treatment – sterilization and incubation with hot water – of the polycarbonate bottle. It was demonstrated that bisphenol A release during sterilization decreases throughout 12 cycles of use, indicating that polymer degradation does not occur during boiling in water for 10 minutes.

Bradley et al. (13) investigated the migration of melamine from melamine-formaldehyde plastics. Acidity of the foodstuff was shown to play a role in promoting migration. 3% acetic acid gave migration values about double those obtained using water under same time and temperature test conditions. Migration of melamine into fatty foods was not detectable, which was expected given the solubility properties of melamine. Very strong influence of time and especially temperature was manifest in the effects seen of microwave heating of food or beverage in melaware articles. Despite the short duration of hot contact, migration levels were similar to simulants used for longer periods. This was rationalized in terms of peak temperature achieved on microwave heating counterbalancing the shorter time period held hot. There was also evidence that when using melaware utensils in boiling liquids, the boiling action of circulating food/simulant can have an additional effect in promoting surface erosion, increasing the plastic decomposition and so elevating the melamine release. Lund et al. (14) measured migration of formaldehyde and melamine monomers from melamine plastics. At low test temperature the migration of formaldehyde and melamine was negligible while being measurable at higher test temperatures. It was confirmed that a continuing migration of monomers especially to hot acidic foods takes place from melamine plastics during the whole life time of the product. This phenomenon was attributed to a gradual breakdown of the polymer.

In a survey on formaldehyde migration from melamine-ware conducted in the United Kingdom in 2008 fifty samples were tested. (15) Formaldehyde was detected in the simulants exposed to 43 samples. Most levels found were below the limits set in the law such that 84% of the samples were compliant. The non-compliant samples exceeded the specific migration limit at six to 65 times.

Bueno-Ferrer et al. (16) investigated the migration of epoxidized soybean oil and other plasticizers in commercial lids for food packaging. Besides epoxidized soybean oil other plasticizers found included citrates, adipates and sebacates. Migration of epoxidized soybean oil and phthalates from twist closures into food was measured by Pedersen et al. (17) Nineteen samples of food in glass jars were tested. Epoxidized soybean oil was the principal plasticizer in 5 of the gaskets, in 14 it was phthalates. The conclusion of the test was that when the gaskets were used with fatty or oily food in small and medium sized glass jars the level of migration of epoxidized soybean oil or phthalates violated several times the overall migration limits in force at the time of sampling.

The mechanism and factors influencing the migration of plasticizers from gaskets used in metal lids were investigated by Graubardt et al. (18) Some of the factors included tightening the lid which has an effect on deformation of the gasket, the amount of oil adhering to the gasket as well as its proportion covered by oil. The nature of the plasticizer determines the saturation in the partitioning process between the gasket and the small amount of oil in contact with the gasket. Exchanging this oil, e.g. through shaking, is an important factor if saturation is reached rapidly. The data also suggested that migration accelerates after roughly one year storage.

Silicone used for production of baking mould was studied by Helling et al. (19) Foodstuffs in contact with silicone baking moulds were analyzed for siloxane. Meat loaf exhibited siloxane levels above the overall migration limit (60mg/kg), reaching values as high as 177mg/kg. It was shown there was no significant

formation of low molecular weight, potentially migrating siloxanes from the elastomer. Proper tempering of the moulds had a major influence on the migration properties.

Many cooking utensils are made of nylon, a material that may incorporate azodyes and where primary aromatic amines are the starting substances. Aromatic amines my also be present as technical impurities. Sendón et al. (20) tested 39 samples of kitchen utensils made of nylon for migration of primary aromatic amines. Approximately half the samples were tested non-compliant with current legislation concerning migration of the primary aromatic amines.

Also coating materials, e.g. non-stick materials used for cookware, are a possible source for migration. Bradley et al. (21) investigated the migration potential of such cooking materials from cookware products. 26 non-stick coated cookware samples were tested. The polymer coating were identified to be polyethersulfone, polytetrafluoroethylene (PTFE), bisphenol A/epichlorhydrin. There was no detectable release of perfluorochemicals. Levels of benzene were too low to give any detectable migration onto foods. The origin of many of the detectable substances in the coatings was considered to be by pick-up from the printed packaging materials in which the cookware was sold. However the migration values in general were low. Begley et al. (22) reported significantly higher migration of fluorochemicals originating from paper additives into emulsifier-in-oil systems compared to migration into pure oil. Paper with fluorochemical coatings/additives was suggested to represent the largest potential source of migratable fluorochemicals from food contact materials (23)

There are a number of food-packaging formats where direct contact between the food and the packaging is minimal, yet the potential for chemical migration still exists, for example through the vapour phase. The loss of volatiles from plastics and paper packaging into the vapour phase and subsequent uptake by food (or food simulant) has been noted by Terada, Naito (24) and Schwope, Reid (7) for the antioxidant BHT and also by Linssen et al. (25) and by Lehr et al. (26) for styrene monomer from polystyrenes.

The spectrum of migrating substances is broad as is the spectrum of various food contact materials. This was just a selection to exemplify some typical materials and substances, where migration tests are being conducted at present time. In this work one polymer film and two analytes were selected for the migration studies. One of the analytes belongs to the category of phthalates, which are discussed in chapter 5.2.

5 Selection of materials and substances

Scores of migration experiments at hand including varying foodstuffs and different time/temperature conditions need to be carried out with a well characterized, homogenous polymer film in order to be able to come to a conclusion about the influence of the varying factors on the migration kinetics. This film shall contain defined substances (model migrants) which should represent a wide range of potential migrants in food packaging while being measurable in a wide range of foodstuffs down to a concentration level of around 30 ppb with a straightforward analysis without the need for a laborious sample preparation. These substances

may neither be naturally present in the food nor as a food additive. As far as the chemical nature is concerned, these substances should cover a range of molecular weights, yet still have a propensity for migration. They should cover a range of chemical types and structures as well, should be highly stable in the food matrix and have a $D_P > D_F$. Ideally they should be relevant to food contact materials.

In order to cover all these requirements 4 films from the EU project G6RD-CT 2000-0411 "Feasibility study for production of CRM's for specific migration testing" (films were evaluated for $C_{P,0}$, D_P and specific migration into food simulants in a ring trial with 4 participating laboratories) and one film provided by the Fraunhofer Institute were chosen for the Foodmigrosure project.

The characteristics of the film used in this work, the one provided by Fraunhofer Institute, are summarized in the Table 2.

Table 2 Characteristics of the polymer film used for migration testing

Polymer type of the test film	Density (kg/m³)	Thickness (μm)	Model migrant no.	Chemical name of model migrant	Function	$C_{P,0}$ (mg/kg)	MW	PM / Ref No.
LDPE*	0,957 ±0,004	164±10	6	Benzophenone	Photo-initiator	458±21	182	38240
			7	Diphenyl phthalate	Not used	594±27	318	
			8	Bis(2-ethylhexyl)adipate (DEHA)			370	31920

* material from a national project provided by the Fraunhofer Institute

5.1 Benzophenone

Benzophenone (diphenyl methanone; phenyl ketone; diphenyl ketone) was one of the substances of interest in this work. It has been used as an ingredient of pharmaceuticals, insecticides, agricultural chemicals and fragrances in medicine/industry and as an ultraviolet light absorber in plastics/polymers for more than 30 years.
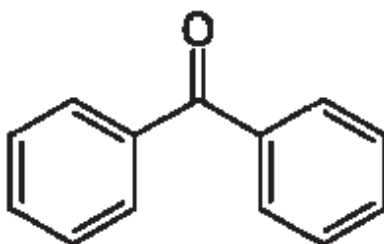
Figure 1 Structure of benzophenone

Benzophenone is also used as a photoinitiator. Ultraviolet (UV) inks are an alternative formulation system to the more usual paste or liquid inks (oils or solvent based) that dry mainly by evaporation or penetration into the printed substrate. Based on acrylic acid chemistry, UV inks dry – cure – by the process of photopolymerization. Their composition (acrylate monomers and oligomers together with photoinitiators) exposed to UV emission lamps enables very fast transformation of the printed ink layer into a tack-free film. (27)

Derivatives of benzophenones, designated benzophenone-1 to benzophenone-12, are widely used today in cosmetic products as photostabilizers, as a sunscreen in lotions and in sprays to protect the skin and hair from ultraviolet irradiation. (28)

Table 3 Properties of Benzophenone (29)

CAS Number	119-61-9
	$H_5C_6-CO-C_6H_5$
	$C_{13}H_{10}O$
Molecular weight	182,217 g/mol
Melting point	48°C
Boiling point	306°C ($1013x10^5$ Pa)
Solubility	Substance is not water soluble but shows good solubility in ether, alcohol, acetone, chloroform and benzene.
Partition coefficient	Log Pow = 3,18 (observed value)
Specific gravity	d_4^{18}= 1.1108

In a survey of 32 paper and board materials intended for food contact benzophenone has been identified as one of the substances having potential to migrate to foods. (30) It was also demonstrated that even a polyethylene coating layer does not act as a complete barrier against possible contaminants in papers when used for packaging for food. (31) In a later study using five surrogates, including benzophenone, the barrier properties of polypropylene (PP) film on the migration from paperboard packaging was studied. It was

concluded, that PP-coated paper generally is not acceptable construction for food packaging used at 100˚C for an extended period. (32) Extent of the migration can be however influenced by the thickness of the polymer film.

Pastorelli et al. (33) tested the barrier properties of different plastic films towards the migration of benzophenone into cake. Plastic films tested included conventional polypropylene used for cake packaging and two multilayer films – PET SiO$_X$/PE and PP/EVOH/PP. The results of the tests carried out at 70°C for 2 days as well as at 40°C for 10 days demonstrated significantly better barrier properties of both multilayer films tested.

Johns et al. (9) studied the migration of benzophenone in five different retail products packed in different styles of paperboard packaging (vegetable tortellini, vegetable lasagna, hamburger, potato chips, pizza). The concentrations measured in the foodstuffs after heating in the microwave ranged from 3-10 μg/kg (tortellini) to 600-700 μg/kg (pizza), which represented up to 8% transfer of benzophenone available in the packaging. The results indicated that benzophenone migration can occur by direct contact, by transient contact with splashed food, and by gas-phase diffusion through an air gap.

According to Feigenbaum (34) migrants with a molecular weight below 250 could migrate fast into any simulant and contamination of dry foodstuffs is possible.

Table 4 Benzophenone migrating from PP-coated paperboard spiked at 1mg/kg into 10% ethanol (M1) and 95% ethanol (M2) after migration testing at 100˚C for 2 hours for various PP-film thickness (32)

Film thickness [mm]	Replicates	Benzophenone concentration [μg/kg]	
		M1	M2
0,031	3	17,1 ± 0,4	18,9 ± 0,8
0,071	3	12,0 ± 0,4	14,5 ± 0,4
0,127	3	9,8 ± 0,5	11,0 ± 0,2

Washed and dried polyethylene terephthalate (PET) flakes obtained from curbside collection were analyzed for contaminants. Dichloromethane was used for extraction and GC-MS was used for identification and quantification. Altogether thirty-two semivolatile contaminants were extracted including benzophenone, which was present in the third highest concentration measured for the 32 contaminants (>800ppb). (35)

5.1.1 Food surveillance

In 1999 the UK Food standards agency conducted a survey to compare dietary intake of benzophenone with the tolerable daily intake (TDI) set by the European Commission Scientific Committee on Food at 0,01 mg/kg bodyweight/day. Benzophenone was detected at 0,01 to 7,3 mg/kg in 72% (51/71) of food

samples packaged in printed cartonboard. 143 samples of such packaging contained 0,05 to 3,3 mg benzophenone/dm^2. It was concluded that no health effects would be expected in an individual's lifetime from the levels found in this survey. Estimated intake of this substance was less than toxicological tolerable daily intake. The results confirmed once more that benzophenone can migrate from printed cartonboard to food. (36)

Woods (37) also reported the presence of benzophenone in the food and attributed the migration to the printed carton in which the food was packaged and heated.

5.1.2 Analytical determination of benzophenone and its derivatives

Analytical determination of benzophenone as a contaminant, predominantly from carton and board as a residue from UV-cured inks and lacquers used to print on the packaging, has been reported in numerous papers.

Anderson and Castle (38) used a modified version of a previously published method (Johns et al. 1995). A mixture of dichloromethane and acetonitrile was used for repeated extraction of foods. The extract was dried and re-dissolved in hexane and re-extracted with acetonitrile which was then analysed by GC-MS. Rtx-1 60m x 0.25mm x 0.25μm 100% dimethyl polysiloxane column was used for separation. Deuterated internal standard was used for quantitation. The calibration function was linear over the range 0.025-10μg/ml, corresponding to concentration of benzophenone in food of 0.05-20mg/kg. Method repeatability was good with an RSD=12% for five replicates of a pizza sample containing approximately 0.2mg/kg benzophenone. Recovery from a spiked corn oil sample at 1mg/kg had an average of 99%. The LOD for the analysis of benzophenone in food was 0.01mg/kg and the LOQ was 0.05mg/kg. (38)

Choi et al. employed gas chromatography separation with flame ionization detector to determine five surrogates, one of them being benzophenone, in water. n-hexane was used for extraction. After the extract has been separated it was dried with anhydrous natrium sulfate and analyzed by GC-FID. The recovery was reported to be >99%. It was demonstrated that a polyethylene coating layer should not be seen as a complete barrier against possible contaminants in papers when used for packaging (neither at room- nor at low temperatures). (31)

Papilloud and Baudraz (27) used a gas chromatograph equipped with an ion-trap mass spectrometric detector. Separation was achieved by an Optima Delta 6 column (30m x 0,25mm i.d.). It was possible to assess all the major photoinitiators commercially available including benzophenone. Above a concentration level of 100μg/l food simulant reproducible results were obtained (RSD <10%, based on five replicates). The recovery was generally close to 100%, and >70% in all the cases.

Johns et al. (9) analyzed food after it has been microwave heated in the packaging. The homogenated food was extracted twice with acetone and hexane (1:1 v/v). The extract was dried and the solvent removed. A portion of the extract (largely coextracted fat) was transferred into a vial with dichlormethane/cyclohexane (1:1 v/v). After a clean-up step involving two high performance size exclusion columns (300mm x 7,5mm

PLGel, 50A and 100A nominal pore size) the extract was analyzed on a GC equipped with a Chrompak CPSIL 5CB fused silica capillary column (27m x 0,25mm x 0,12μm) and coupled with a mass selective detector (MSD).

Reliable results in the detection of benzophenones are achieved with high performance liquid chromatography (HPLC). (39)

Also capillary electrochromatography (CEC), a hybrid separation technique combining the features of HPLC and capillary electrophoresis (CE), has been used for the simultaneous determination of hydrophobic benzophenones. Various polymeric monolithic columns such as acrylamide-, styrene-, and methacrylate ester-based monolithic columns have been developed and used as the separation column in CEC. Huang et al. examined the effect of mobile phase characteristics, of the composition of the methacrylate ester-based monolithic column as well as of the porogenic solvent ratio on benzophenone separation. The developed method demonstrated good separation of eight benzophenones. (39)

Benzophenone as a contaminant in washed and dried PET-flakes was extracted by Soxhlet extraction using dichloromethane. Gas chromatography with DB-5MS column (30m x 0,25mm x 0,25μm film thickness) was used for separation and mass spectrometer in SIM mode was used for quantification. Sufficient linearity of the calibration function ($R^2 > 0,99$) has been achieved (35)

5.1.3 In vitro toxicity test results

In binding assay using ligand binding domain of the human estrogen receptor, benzophenone did not bind to estrogen receptor up to 0.1 mM. (40) In yeast two-hybrid assay, benzophenone did not activate gene transcription. (41)

There exist the data suggesting that benzophenone per se did not activate gene transcription in reporter gene assay with HeLa cells (human cervix carcinoma cell line) incorporated with human or rat estrogen receptor expression plasmids and estrogen receptor responsive element, but 3-hydroxy-, 4-hydroxy-, 4,4'-dibromo-, 4,4'-dihydroxy-, 4-chloro-4'-hydroxy-, 2,4,4'-trihydroxy-, 4-fluoro-4'-hydroxy-, 2,4-dihydroxy- and 2,2',4,4'-tetrahydroxy-benzophenones exhibited binding affinity for human estrogen receptor in binding assays and activate transcription of reporter gene in reporter gene assay with HeLa cells transfected with human estrogen receptor expression plasmids and estrogen receptor responsive element. 2,3,4-trihydroxy-benzophenone and 2,3,4,4'-tetrahydroxy-benzophenone bound to human estrogen receptor but did not activate gene transcription in the reporter gene assay. (40)

In MCF-7 cells, estrogen-dependent human breast cancer cell line, benzophenone had no cell proliferative activity, whereas 4-hydroxybenzophenone induced cell proliferation at high concentrations (10-100microM). (42)

An assay by Suzuki et al. on estrogenic and antiandrogenic activities of benzophenone and 16 of its derivatives showed, that hydroxylated benzophenones exhibit estrogenic activity in human breast cancer cell line MCF-7, but their activities varied markedly. Benzophenone itself showed little activity in the assay. In

contrast, benzophenone and some related compounds showed significant inhibitory effects on the androgenic activity of dihydrotestosterone in rat fibroblast cell line NIH3T3. Benzophenone gave positive responses in uterotrophic assay using ovariectomized rats and 2,4,4'-trihydroxybenzophenone was positive in the Hershberger assay using castrated rats. The results suggested that a 4-hydroxyl group on the phenyl ring of the benzophenone derivatives is essential for high hormonal activities, and the presence of other hydroxyl groups markedly alters these activities. (43)

In reporter gene assay in yeast cells transfected with human progesterone receptor, benzophenone did not activate progesterone responsive element (PRE)-dependent gene transcription, nor did it antagonize gene transcription activation mediated by progesterone. (44)

5.1.4 In vivo toxicity test results in mammals

In uterotrophic assay (in accordance with the OECD draft guidelines), estrogenicity and anti-estrogenicity screening test, ovariectomized female Sprague-Dawley (SD) rats were exposed subcutaneously to benzophenone at concentrations ranging up to 500 mg/kg/day for 7 days. In the 500 mg/kg/day group, uterine weight increased slightly. Subcutaneous administration of benzophenone (from 50 mg/kg/day upwards) in combination with ethinylestradiol resulted in a slightly decreased uterine weight. (45)

In another uterotrophic assay, juvenile female SD rats were exposed subcutaneously to benzophenone at 0-200 mg/kg/day for 3 days, but uterine weight remained unchanged. (40)

Benzhydrol also did not cause change in uterine weight or histological changes at 400 mg/kg/day. On the other hand, subcutaneous administration of 4-hydroxybenzophenone, metabolite of benzophenone, at 0, 100, 200 and 400 mg/kg/day for 3 days resulted in a dose-dependent increase in uterine weight. (46)

In Hershberger assay (in accordance with the OECD draft guideline), androgenicity and anti-androgenicity screening test, the androgenic effect of benzophenone was assessed in castrated male SD rats by gavage with benzophenone up to 100 mg/kg/day for 10 days. Weights of the male accessory reproductive organs remained unchanged. Also benzophenone administration in combination with testosterone propionate (in order to assess the anti-androgenic effect) did not cause changes of male accessory reproductive organs. (45)

Results obtained in a study by Nakagawa et al. show that oral administration of benzophenone to ovariectomized SD rats causes estrogen-like effects inducing a proliferation of uterine luminal epithelium cells as well as cornified vaginal epithelium cells and an increase in uterine weight. (28)

5.1.5 Acute toxicity

In acute toxicity study male Swiss mice given benzophenone orally or intraperitoneally showed sedation, decreased motor activity, unstable gait, shivering and reduced respiration rate. (47) LD_{50} values after various dosing routes are reported for mice, rats and rabbits. (48)

Table 5 Result of acute toxicity (48)

	Mouse	Rat	Rabbit
Oral LD_{50}	2895 mg/kg	1900 mg/kg	-
Inhalation LD_{50}	-	-	-
Percutaneous LD_{50}	-	-	3535 mg/kg
Subcutaneous LD_{50}	727 mg/kg	-	

5.1.6 Repeated-dose toxicity

In a 28-day feeding study in which both sex of SD rats were given benzophenone in diet (up to 500 mg/kg/day), decrease in red blood cell count and hematocrit, increase in urea nitrogen, bilirubin, total protein and albumin, increases in kidney and liver weights as well as hepatocellular hypertrophy were observed in 100 mg/kg/day or higher groups, and decreases in hemoglobin and alkaline phosphatase and increase in glucose in 500 mg/kg/day group. In a 90-day dosed feeding study in SD rats exposed to benzophenone in diet at 0-20 mg/kg/day, no abnormal changes were observed. This would be equivalent to 1200 mg/day for a 60 kg human. Based on the calculated possible average daily intake of 0,33 mg/day, a safety factor of >3600 is demonstrated. The safety factor based on the more realistic per capita consumption of 0,32 microgram/day would be approximately 3,7 million. (49)

In a 14-week feeding study, both sex of F344 rats were given benzophenone up to 850 mg/kg in males and 1000 mg/kg in females. Body weight decrease was observed at the highest dose group. At one eighth of the highest dose body weight gain was suppressed. Already one sixteenth of the highest dose resulted in increased liver weight with hepatocellular hypertrophy and vacuolation, increased kidney weight, protein casts in tubular lumen. With rising concentration dose-related dilatation of renal tubules and renal papillary necrosis was observed. (48)

In a study in male guinea pigs given benzophenone intraperitoneally at 0 and 0,5 mg/kg/day for 15 days, liver was grossly enlarged, and histopathologically, liver showed hepatocellular degeneration and necrosis, connective tissue proliferation and proliferation of bile duct epithelial cells. (50)

5.1.7 Mutagenicity/genotoxicity and carcinogenity

Benzophenone is not mutagenic either in *in vitro* or *in vivo* assays. (51), (52)
Female Swiss mice (aged 7 weeks) were dermally given 0, 5, 25 and 50% benzophenone (acetone as solvent; 50 mice per group) twice weekly over their whole life, resulting in no benzophenone-related increase in tumor incidence. (53)

5.1.8 Fate and metabolism

Dermal absorption of benzophenone in a dermal toxicity study in Bengal monkeys is reported to be 44% and 69% of the dose (open and closed patch application).

The urinary excretion during a feeding study in rabbits was 41-61% of the dose. The carbonyl group of benzophenone was reduced to yield benzhydrol, which was then conjugated with glucuronic acid and excreted into urine. (51)

Benzophenone in isolated rat hepatocytes was enzymatically converted to at least three metabolites: benzhydrol, p-hydroxybenzophenone and a sulfate conjugate derived from p-hydroxybenzophenone. (28)

5.1.9 Adverse effects in humans

During a sensitization study (maximization test) of a 6% solution of benzophenone involving 25 volunteers, no positive reaction was observed. (54)

5.2 Diphenyl phthalate

Diphenyl phthalate was chosen representing the group of phthalate esters, which are widely used in food contact materials. This particular substance was considered appropriate as it is not one of the commonly used derivates (unlike e.g. DEHP, BBP etc.) which minimizes the risk of pre-contamination of the foodstuffs used for the migration testing.
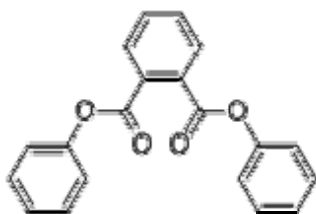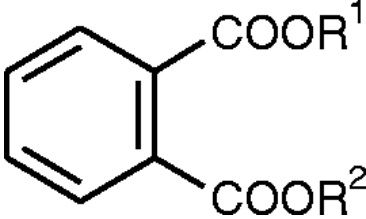

Figure 2 Structure of diphenyl phthalate

5.2.1 Use of phthalate esters

Structurally, phthalate esters (phthalates) consist of paired ester groups on a cyclohexatriene ring (benzenedicarboxylic acid). Phthalate esters are synthesized commercially by condensation of appropriate alcohols with phthalic anhydride. (55) Phthalates are widely used as plasticizers to alter the properties of plastics (flexibility, durability). To achieve the desired plasticizing effect on a material phthalates might be used in high concentrations (ranging up to 50 % w/w). (56) Phthalates act as plasticizers by embedding themselves between the polymer chains, yet no chemical bond is established between the phthalates and the polymer. Due to this reason phthalates can be rather easily extracted from the polymer and during the lifetime of the material they might, to some extent, migrate to the surrounding environment, which might be for example the air (plastics in cars), blood (medical blood bags) or food (packaging material).

Table 6 Common phthalate esters and their abbreviations (57)

Basic structure	Ester	Abbreviation
$COOR^1$ / $COOR^2$ structure on benzene ring	Dimethyl-	DMP
	Diethyl-	DEP
	Di(n-butyl)-	DNBP
	Diisobutyl-	DIBP
	Diallyl-	DAP
	Diisohexyl-	DIHP
	Dicyclohexyl-	DCHP
	Diphenyl-	DPP
	Di(n-octyl)-	DNOP
	Diisooctyl-	DIOP
	Di(2-ethylhexyl)-	DEHP
	Diisononyl-	DINP
	Diisodecyl-	DIDP
	Diundecyl-	DUP
	Ditridecyl-	DTDP
	Butyl benzyl-	BBP
	Butyl octyl-	BOP
	Butyl isooctyl-	BIOP

5.2.2 Analytical determination of phthalate esters

The analytical measurement of phthalate esters is often accompanied by difficulties with background contamination due to the ubiquitous character of these compounds. Therefore, special care needs to be taken to avoid contamination of the samples from the laboratory equipment as well as from the solvents used as stressed in countless studies concerning phthalate analytics. (58), (59), (60), (8), (61), (57), (62)

Commonly used practice involves washing laboratory glassware with phthalate-free solvents or heating of glassware for several hours prior to analysis thus evaporating volatile contaminants. It is also advised to check solvents for possible phthalate contamination. As many analytical procedures involve evaporating of solvents to increase analyte concentration, parallel increase in concentration of non-volatile contaminants originating from the solvent may cause analytical problems.

In order to minimize the risk of contamination, following precautionary measures should be undertaken: (I) any contact with plastic material should be avoided, (II) glassware should be properly cleaned and deactivated, (III) blank samples should be run for each series of samples, (IV) just one GC or LC injection of a sample extract should be made from the same vial (if several injections need to be made, several separate aliquots of the sample extract should be prepared). (62)

Ishida et al. (61) studied contamination by phthalates of the commercial reagents (water; organic solvents - e.g. ethanol, acetonitrile, benzene, acetone, dichloromethane etc.; either guaranteed reagent -GR- grade or for analysis of pesticide residue – PR; solid reagents of various grades – e.g. alumina powder, Na_2SO_4, NaCl, activated charcoal powder, ion-exchange resin etc.) and equipment used for the extraction and the chromatographic analysis of lipids (e.g. heavy-walled tubing, polyvinyl tubing, black rubber tubing, vial caps, filter-paper, glass-wool, aluminium foil etc.). Most of the solvents, reagents and materials examined contained dibutyl phthalate and/or de-2-ethylhexyl phthalate. The heavy-walled tubing contained over 60% (w/w) of DEHP. Also glass-wool and filter-paper contained measurable amounts of DBT and DEHP. Vitali et al. (63) determined contamination, coming from the use of sodium sulfate and glass-fiber filters in the water sample procedure, in a range from 10ng/l (DEP, DBP, BBP and DOP) to 100ng/l DEHP.

A set of eight phthalates (dimethyl-, diethyl-, diallyl-, diisobutyl-, dibutyl-, benzylbutyl-, diethylhexyl- and di-n-octyl phthalate) in butter and margarine samples has been analyzed by GC-FID and GC-MS. (58) DB-5 fused silica capillary column, 15m x 0.30mm x 0.25μm, has been used for separation previous to quantification by a flame ionization detector. DB-5 fused silica capillary column with chromatographic properties similar to those described has been also used in combination with a mass spectrometer. The recovery (%) and repeatability (±SD; n=6) for DBP, BBP and DEHP, the phthalate esters found in butter and margarine, ranged from 81,1% to 117,6% with standard deviations from 1,9 to 15,3.

Food samples in the UK, including cheese, pate, chocolate and confectionery products, meat pies, cake, quiches and sandwiches, have been analyzed for plasticizers, including phthalates. Homogenized samples were extracted repeatedly using acetone/hexane (1:1 v/v) followed by a solvent exchange to dichloromethane/cyclohexane (1:1 v/v) with subsequent fully automated size exclusion chromatography

enabling auto-injection. The phthalate plasticizers (dibutyl-, dicyclohexyl- and benzylbutyl phthalate) were analyzed by selected ion monitoring GC/MS using CP SIL 5CB fused silica column, 25m x 0,23mm ID.

Page and Lacroix (8) conducted analysis of phthalate esters in food packaging and a variety of foods ranging from aqueous foods, such as bottled water, soft drinks, vegetables and fruits; to foods with substantial lipid content, such as cheese, ground beef, butter or margarine; and to completely lipid foods such as vegetable oil. Therefore different extraction procedures were necessary prior to the gas chromatographic determination.

For non-fatty foods (e.g. fruits, vegetables, juices, drinks, wine, beer) acetonitrile-hexane partition has been employed. Samples have been blended with acetonitrile (and water if required). Filtration of the mixture was followed by extraction with hexane and dichloromethane (10+1). The hexane extract was washed with water, dried with anhydrous sodium sulfate, evaporated to dryness, made up to volume with hexane and analyzed by GC-FID. (8)

Fatty foods were extracted by dichloromethane. The samples were blended with sodium sulfate and dichloromethane. After filtration the solvent was evaporated to dryness. The extract was weighed. A defined quantity of the extract is then dissolved in 5ml of hexane. Plasticizers were isolated from the extracted lipid material or the lipid-hexane solution by sweep co-distillation, Florisil trapping and selective elution and subsequently analyzed by GC-FID. (8)

Milk and cream were blended with acetone-hexane. After the mixture has been centrifuged the hexane was removed and the extraction repeated with a fresh portion of hexane. The hexane extract was dried and the weighed lipid extract was treated as described above. Butter and margarine were liquefied at 70°C, the supernatant was mixed, decanted, filtered and centrifuged before analysis. (8)

GC equipped with a DB-5 fused silica capillary column (15m x 0,30mm i.d. x 0,25μm) and a flame ionization detector was used for determination of the plasticizers. (8)

Mortensen et al. (64) analyzed phthalate monoesters in milk. After liquid extraction followed by two-step solid-phase extraction (SPE), detection and quantification were accomplished by high-pressure liquid chromatography using a Betasil phenyl column (100mm x 2.1mm x 3μm) and triple tandem mass spectrometry (LC-MS-MS).

Petrovic et al. (62) reviewed recent GC-MS and LC-MS methods for determination of endocrine disrupting compounds, including phthalates, in environmental samples and drinking water. Phthalate esters were analyzed using LC-APCI-MS under PI conditions, with a C_{18} stationary phase was used for separation. If information on the molecular ion and quantitative analysis of phthalate esters is desired, LC-ESI-MS based on the formation of sodium adducts was found to be a reliable tool. It was also shown, that two major pathways of phthalate fragmentation in ESI(+)-MS-MS are similar to that in (EI)-GC-MS. One reaction is dominated by the loss of one of the substituents leading to the formation of monoester sodium adducts and another by the formation of sodiated phthalic anhydride ions $[(C_8H_4O_3)Na]^+$ with *m/z* 171. Also in combination with GC different detection techniques have been used (EI-MS, CI-MS with methane as the reagent gas either in the positive or negative mode, as well as tandem MS, under PCI conditions with

isobutane as reagent gas). For surface and drinking water on-line coupling of reversed-phase HPLC to GC-MS by the vaporizer/precolumn solvent split/gas discharge interface was proposed.

5.2.3 Food surveillance

260 samples of selected foods packed in materials with the potential to contribute plasticizers to the food, and 98 samples of available food composites were analyzed for phthalate plasticizers. Low levels of di-2-ethylhexyl phthalate (average in beverages 0,065μg/g; average in foods 0,29μg/g) associated with the use of DEHP-plasticized cap or lid seals, were found in a variety of glass-packaged foods. DEP was found in pies (average 1,8μg/g) as a migrant from the pie carton window. (8)

Retail samples of Canadian butter and margarine wrapped in aluminium foil-paper laminate were found to contain dibutyl-, butyl benzyl- and/or di-2-ethylhexyl phthalate (DBP, BBP, DEHP) as packaging migrants at levels up to 10.6, 47.8 and 11.9μg/g respectively. (58), (8)

In the United Kingdom a survey of plasticizer levels in retail foods (73 samples) wrapped in plasticized films or materials with plasticized coatings has been carried out. Foodstuffs analyzed included cheese, pate, chocolate and confectionery products, meat pies, cake, quiches and sandwiches. Dibutyl-, dicyclohexyl-, benzylbutyl phthalate and diphenyl 2-ethylhexyl phosphate (DBP, DCHP, BBP and DPOP) were found individually or in combination in confectionary, meat pies, cake and sandwiches, total levels from 0,5 to 53mg/kg. High levels were assumed to be a consequence of contact with butter or other fatty components. Source of DBP, DCHP, BBP and DPOP contamination was nitrocellulose coated regenerated cellulose film (the plasticized nitrocellulose film contained about 30-40% resulting in a plasticizer level of 0,5-1,5% on a total film-weight basis). Diethyl phthalate (DEP) was found in quinches. Source of DEP migration was a cellulose acetate window in cardboard box. Though the plasticized cellulose acetate was not intended to come into direct contact with the food product, there was the possibility of contact during transport. Levels of DEP found were higher than expected and ranged from 1,7 to 4,5mg/kg. It was suggested that a possible route of migration might be volatilization of DEP from the film without direct contact with the food or, alternatively, the plasticizer might be carried from the film to the food dissolved in aqueous condensate that falls from the cellulose acetate window onto the food. (59)

In a survey conducted parallel in Spain and the UK plasticizers in printing inks present in a selection of food packaging including confectionary, snacks, crisps, potatoes, chocolate bars and biscuits have been analyzed. Only samples packed in plasticizer free polypropylene were studied, so that the plasticizers found in the plastic bag would come from the printing ink. Several food samples were then analyzed for plasticizers. A correlation between the level of plasticizers in the plastic film and in the food was observed. The amount of the contaminants in the food was also very dependent on the surface contact between the film and food. Based on phthalate concentrations measured in food it was pointed out that, mainly in the case of children, the printing inks are not to be neglected as a source of food contamination and that the use of plasticizers in printing inks should be controlled. (60)

Food Safety Directorate of the Ministry of Agriculture, Fisheries and Food in the UK carried out several surveys on total and individual phthalate levels in fatty foods, infant formulae, paper and board.

All samples from a total diet study (samples consisting of retail food products, prepared as for consumption and combined in amounts reflecting their relative importance in the average UK diet) contained phthalates. Samples analyzed included carcass meat, meat products, offals, poltry, eggs, fish, fats and oils, milk and milk products. Estimates of average dietary intakes of total phthalates (measured and expressed as DMP) ranged from 0,1 to 0,8 mg/person/day and high level (97,5th percentile) dietary intakes of total phthalates ranged from 0,4 to 1,6 mg/person/day. As these were considerably below the tolerable daily intakes (TDI) set for some of the phthalates the Department of Health has advised that there are unlikely to be any health risks to consumers from these dietary intakes of individual phthalates.

Table 7 Tolerable daily intakes (TDI) for several phthalates set by the EC Scientific Committee for Food (SCF)

Phthalate	TDI (mg/kg bodyweight/day)
DEHP	0,05
BBP	0,1
DBP	0,05
DCHP	0,1
DEP	0,2

Due to lack of toxicological data for the remaining phthalates the SCF has recommended a "group restriction" for the sum of these remaining compounds of 0,05 mg/kg bodyweight/day.

The largest contribution to phthalate intakes was made by carcass meat (about 25%), eggs (about 15%), poltry (about 35%) and milk (about 10%). The most abundant individual phthalate in each sample was DEHP.

Castle et al. (66) measured DEHP levels in milk collected from a diary in Norway at various stages of the milking process in order to assess the extent of migration from plasticized tubing used in commercial milking equipment. Concentrations in the milking chambers for individual cows averaged 30μg/kg and rose to 50μg/kg in the central collecting tank. Retail pasteurized skimmed milk samples from Norway were found to contain 20μg/kg DEHP, and two retail cream samples contained 1200 and 1400μg/kg of DEHP, reflecting the association of plasticizer with the fat phase. Retail whole milks from the UK contained 35μg/kg of DEHP (this concentration was believed to originate from the environment as DEHP was not used in the milking equipment). In comparison control milk samples obtained by hand milking contained less than 5-10μg/kg of DEHP.

Di-(2-ethylhexyl)phthalate and total phthalate ester plasticizer levels were determined in milk, cream, butter and cheese samples from a variety of sources from the UK, Norway and Spain. Total phthalate levels (expressed as DEHP equivalents) in the raw milk samples from Norway contained between 0,12 and 0,28mg/kg. On processing the DEHP was concentrated in the cream at levels up to 1,93mg/kg, whereas low fat milk contained from <0,01 to 0,07mg/kg. Retail dairy products from Spain were contaminated with <0,01 to 0,55mg/kg of DEHP with a maximum total phthalate level of 3,0mg/kg in cream samples. UK pooled milk samples contained low levels of DEHP (<0,01-0,09mg/kg) and total phthalate (0,06-0,32mg/kg). The majority of cheese samples contained 0,6-3,0mg/kg DEHP (maximum level being 17mg/kg) and 4-20mg/kg total phthalates (maximum level being 114mg/kg). UK cream samples contained levels of 0,2-2,7mg/kg DEHP and 1,8-19,0mg/kg. The levels found in these products were too high to have resulted solely from milk concentration in the fat phase and must therefore have arisen in other ways. It was suspected these routes to be processing and/or packaging. Maximum level of DEHP found in butter was 7,4mg/kg. Infant formula dried milk samples contained less than 0,4mg/kg DEHP. (67)

5.2.4 Occurrence of phthalate esters in the environment and human exposure

Since the 1930s plasticizers have been used to impart flexibility to otherwise rigid polyvinylchloride (PVC) with di-(2-ethylhexyl)-phthalate being the most widely used plasticizer in PVC formulations. (68) Though DEHP was considered carcinogenic in rats and mice in the 1980s, it still accounted (together with related isomeric octyl phthalates) for over 50% of the phthalate plasticizers produced. (57) Hirayama et al. (69) observed a decline in the occurrence of cap-sealing resins for bottled foods containing phthalates between the periods of 1993-1995 and 1997-1999. However plasticizers leak out of the material and due to their massive application (annual world wide production of phthalates of about 2.7 million metric tons (56)) in daily use products ranging from plastics in food contact materials, medicine, toys, car industry as well as in cosmetics, pharmaceutical products etc. and their moderate resistance to degradation (65) they have consequently become ubiquitous environmental contaminants. The presence of phthalate esters in virtually all ecosystems and in the tissues of man, animals as well as in the food supply of man has been reported in numerous reviews since the 1970s. (70)

Samples of indoor air and dust from 120 homes analyzed for 89 organic chemicals identified as endocrine-disrupting compounds showed phthalates to be the most abundant compounds measured. In indoor air diethyl phthalate and di-n-butyl phthalate were present at the highest concentrations. These are the same phthalates observed to be most abundant in human urine samples reported for a cross-section of U.S. adults. In dust, diethylhexyl phthalate and benzyl butyl phthalate were the chemicals detected at the highest concentrations. (71)

Results of a survey conducted in the UK on plasticizers used in food contact materials were considered by governmental expert committees. Toxicological and epidemiological data available for 11

plasticizers found in foods were examined. The experts concluded it was very unlikely that there are any adverse health effects arising from the use of plasticizers in food packaging materials. (72)

Phthalates have several degradation pathways and therefore are not considered to be persistent chemicals. They are easily photodegraded in the atmosphere, and can be degraded by bacteria and actinomycetes. (73) Concentrations found in the environment may be explained by high fluxes due to considerable direct and expected indirect emissions. (56)

The exposure of man to phthalate esters has been addressed in countless reports. Jennifer J. Adibi et al. conducted a research on the prenatal exposure to phthalates among women. Four phthalates (DEP, DBP, DEHP, BBP) and their metabolites were measured in both personal air and urine. All were present in 100% of the air and urine samples. The results demonstrated considerable phthalate exposure during pregnancy among women in the regions of interest (New York, USA: Krakow, Poland) and indicated that inhalation is an important route of exposure which counters the general belief ingestion of contaminated food being the most significant exposure pathway. (74)

In utero exposure to di-(2-ethylhexyl) phthalate and its influence on duration of human pregnancy were the aims of a study carried out by Latini et al. Serum DEHP and its main metabolite MEHP were measured in the cord blood of 84 newborns. Detectable concentrations of DEHP and/or MEHP were found in 88.1% of the samples. The findings of the study confirmed that human exposure to DEHP can begin *in utero* and suggested that phthalate exposure is significantly associated with shorter pregnancy duration. (75)

Main K.M. et al. investigated phthalate monoester contamination of human breast milk (mono-methyl phthalate, mono-ethyl phthalate, mono-n-butyl phthalate, mono-benzyl phthalate, mono-2-ethylhexyl phthalate, mono-isononyl phthalate) and the influence on the postnatal surge of reproductive hormones in newborn boys. The results suggested that human Leydig cell development and the function may be vulnerable to perinatal exposure to some phthalates. (76)

Analysis of 36 human milk samples for 6 phthalate monoesters (mMP, mEP, mBP, mBzP, mEHP, mNP) showed, that all these phthalates were present, albeit at different concentrations (median values ranging from 0.11µg/l for mMP to 101 for mNP). In samples of consumer milk and infant formula only mBP and mEHP were detected. (64) One way or the other, the population is exposed to these substances daily.

5.2.5 Absorption

Within the population at large, the principal route of exposure to phthalates is via food. (77), (55) Some studies showed however that inhalation should not be neglected as a route of exposure either. (74), (71)

Due to the lipophilic nature of the phthalates dermal and pulmonary tissues would not be predicted to be major barriers to absorption. The extent of intestinal absorption has been estimated by monitoring urinary excretion of the compounds or their metabolites after administering a known amount of compound orally. DEHP and some other dialkyl phthalate esters were shown to be very well absorbed from the intestine over a

very wide concentration range. Both ester linkages of phthalate esters can be hydrolyzed, leaving phthalic acid as a product. Hydrolysis of a single ester group, however, occurs more readily than hydrolysis of the second. Esterases capable of generating the monoester metabolite are present in several mammalian tissues, including intestinal mucosal cells. (55)

5.2.6 Distribution

Fat, absorptive organs (gastrointestinal tract) and excretory organs (liver, kidney, gastrointestinal tract) are the major initial repositories for the dialkyl esters. Liver, kidney and gastrointestinal tract probably accumulate the phthalate esters as a mechanism of excretion (e.g. urine, bile) and may, therefore, be inappropriately labeled as repositories. Study of tissue accumulation of DEHP in fat and liver of rats after dietary exposure showed that steady-state concentrations in liver were achieved faster than those in fat. The difference in time to achieve a steady-state level was mirrored by a difference in rate of decline after removal of DEHP from the diet (reduction by 80% in liver within 1 week and below detection limit within 3 weeks whereas in fat the concentration was reduced to one third after 3 weeks). In contrast to high-dose, oral studies in rats, significant fractions of the cumulative dose of DEHP were reported to be retained for several months in the liver of rhesus monkeys infused (intravenously) repeatedly with very small amount of DEHP in blood. Virtually all DEHP in blood is protein-bound, approximately 80% to lipoproteins and the rest to albumin. (55)

5.2.7 Metabolism

Dialkyl phthalate esters are metabolized to the monoesters by enzymes present in many tissues, but only those in liver are capable of hydrolyzing DEHP completely to phthalic acid. (55)

While appreciable amounts of dimethyl phthalate are excreted as phthalic acid, only very small fractions of DEHP and other long-chain alkyl phthalates are converted to this product. Dimethyl phthalate and, to a lesser extent, DBP can be excreted in urine as the parent, unchanged compounds or as their monoester metabolites. Phthalate esters with longer chain lengths, however, such as DEHP, must undergo further modification after hydrolysis to the monoester to achieve sufficient polarity for renal excretion. Man form a glucuronide conjugate of mEHP. In addition to glucuronidation the residual alkyl chain is oxidized prior to urinary excretion of the metabolites. Generally, the metabolism of phthalate esters is qualitatively unaffected by the route of administration. (55)

A study of the disappearance of DEHP in human plasma following infusion of DEHP-laden platelet concentrates enabled its pharmacokinetic characterization. Upon other characteristics plasma disappearance half-life was determined to be 30±12min and a rapid conversion of DEHP in humans was demonstrated. (78)

5.2.8 Excretion

The major route of phthalate ester elimination in man is urinary excretion. Biliary excretion of DBP to the extent of 44% in 24 hours has been demonstrated in rats, yet only 5% of the same dose was eliminated in the feces (88% in urine), indicating extensive enterohepatic cycling. According to the results of further studies it appears that DEHP metabolites are excreted in bile to an unknown extent, reabsorbed from the intestine and ultimately eliminated in the urine. (55)

5.2.9 Biological effects

The attention focused on phthalate esters for decades is due to the suspected carcinogenic and estrogenic properties (79). Phthalates were judged to have a low order of acute and chronic toxicity after being evaluated in the 1940s and 1950s by classical toxicological criteria. It was not until the 1960s that the safety of the phthalates came into serious question and scattered reports in medical journals citing concern over the possible toxic effects of PVC plastic medical devices containing phthalate ester plasticizers appeared. In 1970s the concerns over phthalate toxicity hardened with reports that human blood stored in PVC plastic bags was being contaminated by di(2-ethylhexyl) phthalate (DEHP). Also PVC plastic catheters were reported to be responsible for DEHP accumulation in tissues of patients receiving transfusions. In the first half of the 1970s biological studies provided evidence that the phthalates could accumulate in mitochondria and that phthalates were teratogenic, mutagenic, toxic to various types of cultured cells, capable of placental transfer, capable of altering hepatic ultrastructure and capable of promoting hepatic lipid accumulation under certain conditions. Furthermore exposure of animals to phthalate esters can result in significant perturbation of normal metabolic patterns in liver, heart, testes, adrenal gland and brain and can affect blood lipids. (70) High concentrations of DEHP can cause functional hepatic damage. There is also a possibility for phthalates to interact with other xenobiotics during the pharmacokinetic phase. (80) DEHP is considered both a peroxisome proliferator and a hepatic carcinogen. (81)

In a NCI/NTP bioassay an observation of hepatocarcinogenic effect of DEHP in B6C3F$_1$ mice and Fischer 344 rats after both oral and intraperitoneal administration was reported. (82), (83) A study by Melnick and Schiller showed that phthalate esters affect isolated rat liver mitochondria by inhibiting succinate dehydrogenase activity. The phthalate esters that were examined vary in their potency in affecting these processes. (82)

Also testicular effect of phthalate esters was examined in numerous studies as summarized by Gangolli. It was shown that administration of phthalate esters can reduce the relative testicular weight, induce

testicular injury. Studies also showed that DBP or MBP treatment leads to increased urinary excretion of Zn as well as a decrease in Zn associated with testicular tissue. This was accompanied by a decrease in the activities of two enzymes containing zinc, namely, alcohol dehydrogenase and carbonic anhydrase. (84)

Effects of di-n-butyl phthalate (DBP) on the development of the reproductive system in male rats have been investigated by Foster et al. They concluded that the monoester is the active principle for induction of reproductive and developmental toxicity of specific phthalate esters. Thus, if humans produce very low levels of the monoester from an environmental exposure to the diester, the likelihood of any reproductive or developmental toxicity via the oral route appears extremely remote. (85)

Adverse effects on the development of the reproductive system in male offspring of rats were investigated by Ema and Miyawaki. (86), (87) The studies indicated that benzyl butyl phthalate (BBP) and monobutyl phthalate (MBP) introduced during pregnancy produced adverse effects on the development of the reproductive system in male offspring.

Further reports related to biological effects of phthalate esters concerned pathological changes in the kidneys, atrophy of the testes (88), liver tumors (89), mutagenicity investigation (90), (91). Dybing et al. refers to studies indicating DEHP produces liver tumors in rats and mice by a non-DNA-reactive mechanism involving peroxisome proliferation. Yet the mechanism of hepatocellular tumors in rats and mice was not considered relevant to humans. On the contrary the testicular and kidney toxicity of the DEHP in PPARα knockout mice at a dose level which caused peroxisome proliferation in wild-type animals indicates that these effects are relevant for humans. (92)

Between 1998 and 2000a review of the information related to developmental and reproductive toxicity of seven phthalate esters (DBP, BBP, DnHP, DEHP, DnOP, DINP and DIDP) was conducted by an Expert Panel convened by the National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR). The panel concluded that reproductive risks were minimal to negligible in most cases although some specific uses were considered potentially more problematic. These cases included the exposure of young children to DINP through the use of toys or to DEHP from medical devices. Yet the responsible regulatory authorities concluded that the exposure to DINP from toys was well below effect levels in animals, and, therefore, there was no risk. On the other hand the exposure to DEHP from medical devices for some limited, intensive medical procedures could be similar to or greater than the NOAELs selected by the NTP-CERHR. The overall conclusion was that levels of concern are minimal to negligible in most situations. (93)

More recent study of the mono(2-ethylhexyl)phthalate indicated a genotoxic potential in human mucosal cells. (94)

Phthalates have also been mentioned as suspected endocrine disruptors (95), (96), (97) Information from *in vitro* testing, in particular, gives rise to this suspicion. Though different kinds of *in vitro* test do not always reveal the same picture and different authors, despite using comparable test systems, do not always report similar results, some phthalate compounds are considered to be able to act as xenoestrogens, and thereby as endocrine disrupters. (56)

The environmental impact of phthalate esters has also been the aim of many studies. The effect and toxicity of phthalate esters to hemocytes of giant freshwater prawn was studied by Hung-Hung Sung et al. All eight investigated phthalate esters (diethyl phthalate, benzyl butyl phthalate, di-n-butyl phthalate, di-(2-ethyl hexyl) phthalate, dicyclohexyl phthalate, diphenyl phthalate, dihexyl phthalate, dipropyl phthalate) were found to damage hemocytes and further influence the defense mechanism of prawn. (98) Further in vitro study of four phthalate esters also indicated a possibility of damage to the hemocytes and decrease in the cellular immunity of prawns. An in vivo study showed variability in the immune reactions of prawns. (99)

5.2.10   EFSA opinion

In 1994 the Scientific Committee on Food (SCF) set a TDI for many phthalate esters based on the NOEL for peroxisome proliferation in rat liver. In the following years however a general consensus has been agreed that rodents are highly sensitive to the phenomenon of peroxisome proliferation and that this particular effect should not be used for human risk assessment. Based on this new circumstance the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) published a statement on the possibility of allocating a group-TDI for BBP, DBP, DEHP, DINP and DIDP. The Panel reviewed recent literature on toxicological studies and considered that a group TDI for health protection should be employed if:

i)   exposure to several members of a structurally related series of chemicals is likely to occur frequently, and

ii) several members of the series have been demonstrated to have a common target organ(s) cellular target(s) and the same mode of action.

If the above criteria are met, an additive effect of the members of the series should be assumed. However, from the pivotal studies on a series of phthalates (BBP, DBP, DEHP, DINP and DIDP) it was concluded that these compound did not meet the criteria (either due to differences in mechanism, profile of effect or mode of action). Consequently a group-TDI cannot be allocated for BBP, DBP, DEHP, DINP and DIDP.

In 2005 the Scientific Panel (AFC) re-evaluated BBP, DBP, DEHP, DINP and DIDP and set TDI values based on observations of critical effects of these phthalates on the liver as well as their testicular and reproduction toxicity.

Based on the current literature on BBP testicular toxicity, the Panel allocated a TDI of 0,5 mg/kg bw, derived from a NOAEL of 50 mg/kg bw/day found in a multi-generation study in the rat and making use of an uncertainty factor of 100. The Panel noted that the dietary exposure to BBP (derived from packaging and other sources) may contribute up to about 1% of the TDI value. (100)

The TDI for DBP has been based upon critical observations of its effects related to reproduction in several studies. The Panel allocated a TDI for DBP of 0,01 mg/kg bw, based on a LOAEL of 2 mg/kg bw/day and making use of an uncertainty factor of 200. (101)

Based on the current literature on DEHP testicular toxicity, the Scientific Panel (AFC) allocated a TDI of 0,05 mg/kg bw, based on a No Observed Adverse Effect Level (NOAEL) of 5 mg/kg bw/day, and making use of an uncertainty factor of 100. Exposure to DEHP from food consumption was in the range of the TDI. As there are other sources which contribute to the overall human exposure to DEHP the Panel recommended that improved estimates of exposure to DEHP from all sources along with their relative importance should be provided in order to decide what proportion of the TDI can be allocated to food contact materials alone. (102)

The pivotal toxicological effect for DINP is considered to be the hepatic changes seen in various studies. The Panel agreed to use the NOAEL of 15 mg/kg bw/day for non-peroxisomal proliferation-related chronic hepatic and renal effects in establishing a TDI. Making use of this NOAEL and of an uncertainty factor of 100, a TDI of 0,15 mg/kg bw was derived. (103)

The lowest NOAEL of 15 mg/kg bw/day observed on the liver of dogs (a species considered as non-sensitive to peroxisome proliferation) has been considered and making use of an uncertainty factor of 100, a TDI of 0,15 mg/kg bw was derived. (104)

As DINP and DIDP are mixtures that overlap chemically with each other and cannot analytically be distinguished clearly if present in a mixture, the Panel proposed that a group restriction is established for migration from food contact materials. (103)

6 Selection of representative foodstuffs and test conditions for migration testing

To get a realistic view on the migration of substances with different molecular weights, solubility and boiling points from plastic packaging materials, it is important to determine migration in real foodstuffs instead of food simulants. Data obtained in this way shall form the basis for the development of a mathematic model of migration as a novel and cost efficient tool for estimation of consumer exposure from food contact materials.

The selection of representative foodstuffs must be done with particular care for covering a wide range of varying properties so that the migration experiments generate information of general character that can be applied to any other foodstuff of similar physico-chemical specifications.

From the view of the chemical composition of foodstuffs following parameters were selected as those having major influence on the migration and its kinetics:

- lipid and free fat content
- pH value, the concentration of acids
- water content
- concentration of ethanol

Also the question whether a food represents for example an emulsion (e.g. oil in water or water in oil), a suspension (e.g. sugar crystals in chocolate), a paste (e.g. purees), a powder (different particle sizes e.g. flour, sugar, starch, milk powder) or a gel type (e.g. some cheese or yoghurt) was considered during the selection of representative foodstuffs.

Within the scope of this work a database of over 110 foodstuffs was established containing primarily values on composition, pH values, storage conditions as well as state and structure. Detailed information such as density, viscosity, freezing and boiling point, microscopic structure and many more were gathered where available. Based on the data collected in the database the foodstuffs were divided into 3 major groups according to their properties:

I. Aqueous and acidic foods (see 6.1.)
II. Fatty foods (see 6.2.)
III. Dry foods (see 6.3.)

The final selection of representative foodstuffs for the experiments should comprise 15-20 foodstuffs. This means approximately 5-8 foodstuffs for each of the three groups. The individual foodstuffs were chosen with respect to the diversity of each group to cover the possible varieties as good as possible (e.g. in the group of aqueous and acidic foods the pH ranges from 3,1 in red currant nectar to 6,7 in milk; the carbohydrate content varies between 5 % in milk and 60 % in jam etc), However, overlapping of the specified groups is unavoidable, since some of the foodstuffs can not be strictly considered as explicitly aqueous, fat etc. (meat for example varies dramatically in its composition depending on its origin – not only the species, but also the part of animal body).

6.1 Aqueous and acidic foods

- **Orange juice, unsweetened**: representing fruit juices as aqueous foods (water content approximately 90%) with low pH value (3,6); low viscosity (liquid with suspended pulp); content of fatty substances around 0,1-0,2%. To consider the possible influence of suspended fruit flesh an orange juice with pulp was selected for the migration studies. Orange juice also appears to be a good choice considering its high consumption and availability on the markets within the EU.

- **Apple sauce**: representing not only from the consumption point of view the very important group of processed fruit products. Having a lower water content and higher carbohydrate content than the unsweetened juices, yet still low pH value (3,7) due to which the product can be considered as fairly aggressive towards the packaging. The viscosity of this suspension is considerably higher than that of juices.

- **Milk, UHT, min. 3,5% fat**: representing the essential group of dairy products. Compared to the previous products in the list the milk has much higher pH value (6,7). The viscosity is low (emulsion - fat, suspension – casein micelles), and it contains some fat (3,5%). This is expected to be a fundamental parameter for the migration of the lipophilic migrants from packaging into the food. Therefore the milk can be considered an interesting choice as a first step from aqueous- to fatty foods. Furthermore measuring migration into milk is of great interest also because of the broad consumption and its nutritional value. Furthermore consumers can choose from various milk products ranging from fresh milk, which requires low storage temperature and has a shelf life limited to several days, to UHT milk which need not be refrigerated during storage and has a shelf life of several month in an unopened packaging. These varying storage conditions were reflected in the test scheme.

- **Tomato ketchup**: ~ representing important group of processed vegetable products. Ketchup is a semi fluid food showing plastic behaviour (flow occurs after the yield stress has been exceeded), high viscosity (suspension), with lower water content, due to low pH value (3,65) highly aggressive, packaged in considerable amounts in plastic packaging. Used for various purposes, and as an ingredient in some cases also heated (as a single component of convenience foods tomato ketchup can be considered as a single representative for this more and more popular group of foods).

- **Carbonated beverages (Cola drink)**: sweetened drinks (homogenous liquid) containing carbon dioxide. The pH value is very low (3,4). Carbonated beverages are widely spread on the market and are very popular, resulting in high consumption. They are preferably packaged in plastic bottles.

- **Wine (white, alcohol content ~11%)**: representing alcoholic drinks; homogenous liquid with low viscosity. Chosen to analyze the influence of alcohol content on the migration of the substances from packaging. Preferred in the list compared with beer because of higher alcohol content. It was also

agreed to analyse migration into beer as well to see, if there is any difference in behaviour considering migration. Both will be linked and compared with carbonated beverages.

- **Beer:** representing alcoholic drinks. Low viscosity (homogenous liquid). Lower alcohol content than wine.

6.2 Fatty foods

- **Margarine (80% fat content):** representing foodstuffs with very high fat content (water in oil W/O emulsion), semi fluid food showing plastic behaviour (flow occurs after the yield stress has been exceeded), higher pH value (4,85), low water content. Very good contact between the foodstuff and the packaging results in good migration potential. Will be linked with mayonnaise to see if they behave in a similar way.

- **Cheese Gouda, 45 % fat content**: representing solid foodstuffs (composite material) with high fat content, also high in protein and still with considerable water content. Good migration potential.

- **Chocolate, dark, milk free, min. 40% cocoa content (30% fat)**: representing solid foodstuffs (dispersion of solid particles in fat). Due to the possibility of 'blooming', formation of fat crystals on the surface, and overall high fat content, lipophilic substances are likely to migrate into the chocolate. The blooming phenomenon can increase the potential for migration of lipophilic substances since pure fat gets into direct contact with the packaging material.

- **Soft cheese (~70% fat content in dry matter)**: 'Philadelphia' type cheese. Very extensive contact with the packaging material. Due to considerable amount of fat present in the matrix (yet lower than in the sauce type cheese) the migration of lipophilic substances can be expected.

- **Cheese sauce (~18,5% fat)**: the combination of fat content, smooth consistency providing extensive contact between the food and packaging, and the possibility of heat treatment while still in the packaging makes this product very interesting from the migration potential point of view. Due to this specific application possibility, this product can be also considered as a representative for the more and more popular group of convenience foods,

- **Cottage cheese (*Hytteost* according to www.lebensmittellexikon.de), fresh cheese, ~10% fat content (in dry substance)** representing dairy products with high water- and protein content. The considerable fat content increases the migration potential, while the microstructure is somewhat different from the previous types of cheese. It is not as smooth and the structure is porous. The fat content decreases in the following order: fresh cheese Philadelphia type → Cheese Gouda → Cheese sauce → cottage cheese. This should enable to determine the influence of different fat contents in one food category (cheese) on the amount of substances migrating from the packaging into the foodstuffs,

- **Mayonnaise (80% fat content)**: representing foodstuffs with very high fat content (a pasty O/W emulsion), semi fluid food showing plastic behaviour (flow occurs after the yield stress has been exceeded), due to the consistency good migration potential, low water content. The pH value (3,84) is lower than the pH of margarine while the fat content is similar. Therefore mayonnaise will be linked with margarine to compare their behaviour towards the migration.

- **Yoghurt drink, min. 3,5 % fat content**: dairy product, rough chemical composition similar to milk, but with lower pH value and higher viscosity, good contact with the packaging material.

- **Meat, lean pork (minced, fat content ≤5% plus 4 different fat contents by addition of loin – 10, 20, 30 and 50% fat)**: representing red meat, complex matrix with lower fat content, high water content. Lean meat was selected for the following reason: fresh meat can hardly be standardized (different animal individuals, physical condition etc.). Due to this fact it would be difficult to ensure even quality of meat for each partner of the project (even without the necessity of meats with different fat contents). Therefore lean pork meat was selected, with fat content of approximately ≤5 %. Separately, pork fat (75% fat) will be bought and mixed with the pork meat, resulting in meat with higher (and more exactly defined) fat content. This way, minced meat samples with fat contents between 5 – 50 % will be prepared and the influence of different fat contents will be determined. The assessed migration will be plotted against the fat content of the meat and the results will be compared with other types of meat (fish, chicken etc.) to see, if the fat content can be considered as the most relevant parameter responsible for migration.

- **Meat, pork neck (~10-20% fat)**: representing meat with medium fat content, complex matrix.

- **Chicken breast (low fat)**: representing meat with low fat content, complex matrix.

- **Fish (salmon with 13,6% fat)** (105): meat with high fat content and different fat composition than pork; also high water and protein content.

- **Whipping cream (~30% fat), UHT**: after mayonnaise, whipping cream represents another fluid product with high fat content (yet considerably lower than in the mayonnaise) and low carbohydrate and protein content. The water content is much higher than that of mayonnaise. In the group mayonnaise, whipped cream, condensed milk, and yoghurt drink the fat content decreases while all four foods having fluid character give a good media for migration processes.

- **Condensed milk**: high viscosity, but still fluid character ensures good contact with the packaging material. With a fat content at 10 % condensed milk represents a link between the whipping cream (30 % fat content) and yoghurt drink (3,5% fat content). The pH value of condensed milk is rather high (6,5).

- **Chocolate spread (25% fat)**: representing a food similar to chocolate but with lower fat content and paste-like consistence.

6.3 Dry foods

- **Butter toast (4% fat)**: chosen as a representative for bakery products (bread). Their porous structure favours the migration of volatile substances from the packaging into the foodstuff.
- **Wheat flour**: representing particulate dry foodstuffs [increased surface area, particle size >28μm (50%) and <28μm (50%)], high carbohydrate content, low water and fat content, rich in protein. Characteristic is the high surface area, just like by milk powder, but the fat content is much lower. This makes it possible to compare the influence of fat content on the migration into particulate foods with high surface area.
- **Rice**: representing dry solid foodstuffs, high carbohydrate content, rich in proteins and low water content, particulate character increases surface area. Vacuum packed in plastic bags – tight contact between the rice and packaging material. Rice in plastic bags suitable for cooking as a whole (rice within a plastic bag) is also widely spread on the market, making this particular foodstuff convenient for migration studies.
- **Honey**: representing semifluid high quality natural product with very high carbohydrate content, lower water content, no fat, high viscosity (depending on variety and temperature), intermediate pH value (3,9).
- **Milk powder**: widely used in various foods, also for baby-food, making this product interesting for the migration studies. Very high surface area and fat content exceeding 25 % promotes the migration. Lactose, as the main carbohydrate of milk powder, is present in an amorphous form (glassy state).

6.4 Selection of foodstuffs for benzophenone and diphenyl phthalate migration testing

The properties of a foodstuff have substantial influence on the migration potential of a substance from the food contact material. Depending on the substances of interest and their properties a final selection from the representative foodstuffs for the migration experiments was made. For benzophenone and diphenyl phthalate following foodstuffs were chosen.

Table 8 List of selected foodstuffs

Index	Foodstuff
1	Apple sauce
2	Beer
3	Butter toast (4% fat)
4	Cheese sauce (~18,5% fat)
5	Cola drink
6	Condensed milk
7	Fish (salmon with 13,6% fat)
8	Flour
9	Ketchup
10	Mayonnaise (80% fat content)
11	Meat, lean pork
	Meat, 10% fat
	Meat, 20% fat
	Meat, 30% fat
	Meat, 50% fat
12	Milk, UHT, min. 3,5% fat
13	Milk powder
14	Orange juice, unsweetened
15	Rice
16	Wine, (white, alcohol content ~11%)
17	Yoghurt drink, min. 3,5 % fat content

6.5 Selection of test conditions for migration testing

In order to be able to study the effect of various storage conditions on the migration, different time/temperature conditions for the experiments where defined. The test conditions were selected as being most appropriate for conditions in use that the representative foodstuff would normally be exposed to during storage. In many cases two or more temperatures were used. Foodstuffs which are supposed to be refrigerated were tested at lower temperatures whereas foodstuffs which might also be heated (e.g. in an oven) were tested at higher temperatures. Where a foodstuff can have a relatively long shelf life at ambient temperature experiments were continued up to 180 days. Detailed test conditions are summarized in the Table 9.

Table 9 Experimental work – test conditions

Foodstuff	Temperature [°C]	Storage time
Apple sauce	25	1, 2, 4, 10, 20 days
Beer	25	1, 2, 4, 10, 20 days
Butter toast (4% fat)	25	1, 2, 4, 10, 20 days
Cheese sauce (~18,5% fat)	5	2, 4, 10, 20, 30 days
	90	5, 10, 30, 60, 120 minutes
Cola drink	25	1, 2, 4, 10, 20 days
	40	1, 2, 4 , 7, 10 days
Condensed milk	25	1, 2, 4, 10, 20 days
Fish (salmon; 13,6% fat)	5	8 hours; 1, 2, 5 days
Flour	25	2, 4, 10, 20, 60, 180 days
	40	1, 2, 4, 7, 10 days
	70	8, 24 hours
Ketchup	25	1, 2, 4, 10, 20 days
	70	2, 4, 8, 16, 24 hours
Mayonnaise (80% fat content)	5	2, 4, 10, 20, 30 days
	25	1, 2, 4, 10, 20 days
Meat, lean pork (plus different fat contents)	5	1, 2, 4, 10 days
	25	8, 48 hours
Milk, UHT, min. 3,5% fat	5	2, 4, 10, 20, 30 days
	25	1, 2, 4, 10, 20 days
	40	1, 2, 4 , 7, 10 days
Milk powder	25	2, 4, 10, 20, 60, 180 days
	40	1, 2, 4, 7, 10 days
Orange juice, unsweetened	5	2, 4, 10, 20, 30 days
	25	1, 2, 4, 10, 20 days
	40	1, 2, 4 , 7, 10 days
Rice	25	2, 4, 10, 20, 60, 180 days
	40	1, 2, 4, 7, 10 days
Wine, (white, alcohol content ~11%)	25	1, 2, 4, 10, 20 days
Yoghurt drink, min. 3,5 % fat content	5	2, 4, 10, 20, 30 days

6.6 Analyte stability testing

One of the main tasks of a food packaging is to prolong the shelf life of the packed foodstuff. Depending on the type of the packaging, the packed food and the storage conditions the shelf life may vary from a few days to several months (even years). Based on the expected real storage conditions the experimental design has foreseen storage times ranging from hours to several weeks as well as different temperatures of storage (ranging from 5 to 90°C).

Plotting of migration kinetics curves requires the substances of interest to be stable over the entire time periods of storage at given temperatures. Furthermore they need to be stable in the foodstuffs which may be quite aggressive from the chemical point of view (e.g. low pH value of an orange juice). Therefore the stability of diphenyl phthalate and benzophenone in different foodstuffs under varying time/temperature conditions was investigated to prove their stability during the experiments. The results of the stability tests are summarized in the Table 10.

Table 10 Analyte stability during storage

Foodstuff	Storage time (days)	Storage temperature (°C)	Stability in terms of recovery (%) after the storage	
			Benzophenone (spike at 4ppm)	Diphenyl phthalate (spike at 4ppm)
Apple sauce	20	25	99	95
Beer	20	25	97	97
Butter toast	20	25	84	87
Cheese sauce	30	5	90	87
Cola	10	40	89	100
	20	25	100	98
Condensed milk	20	25	98	97
Fish	5	5	92	95
Wheat flour	1	70	89	97
	10	40	92	83
	60	25	90	93
Tomato ketchup	1	70	89	90
	20	25	92	89
Mayonnaise	20	25	94	84
Pork minced meat	2	25	95	94
	10	5	91	93
Milk	10	40	101	87
	20	25	106	88
	30	5	98	115
Milk powder	10	40	95	97
Orange juice	10	40	91	91
	20	25	96	89
	30	5	95	94
Rice	10	40	97	90
	60	25	92	86
Wine	20	25	93	87
Yoghurt drink	30	5	100	103

7 Experimental part

7.1 *Materials and methods*

An analytical method was developed for the determination of migration levels of diphenyl phthalate and benzophenone into foodstuffs. This method is appropriate for the quantitative determination of diphenyl phthalate and benzophenone in foodstuffs in approximate analyte concentration range of 0.01 to 5 mg/kg foodstuff.

The determination is based on solvent extraction of the analytes from aqueous foodstuffs with dichloromethane at room temperature. The separated organic phase is dried, filtered and submitted for analysis by GC-MS.

If necessary, foodstuffs containing higher amount of fat and protein are pretreated with hydrochloric acid. The extraction is carried out using diethyl ether and petroleum benzine with subsequent solvent exchange to acetonitrile and hexane. Determination is carried out by means of GC-MS. Internal standards are used for quantification.

Note: All glasware was washed thoroughly with acetone and dichloromethane before use to avoid cross-contamination.

7.1.1 Standards and reagents

Following standards were used:

Diphenyl phthalate, purity 99.9%, Sigma-Aldrich, Product 36617, Batch 1296X

Benzophenone ‚sublimed‘, purity 99+%, Aldrich, Product 427551

Internal standards:

4-Methylbenzophenone, 99%, Aldrich, Cat. No.: M2,995-9; Lot.: 33035-034

Benzyl Butyl Phthalate, 97.4%, Riedel-de Haën, Product 36927

Dioctyl phthalate, purity ≥98%, Fluka, Product 80153, Lot&Filling code 381893/1 11899

Reagents and solvents shall be, unless otherwise stated, of recognized analytical grade.

Acetone, p.a., purity ≥99.5%, Riedel-de Haën

Acetone, p.a., purity ≥99.8%, Rotipuran, Carl Roth

Acetone, C.R.

Acetonitrile for HPLC, min. 99.9%, Riedel-de Haën

Acetonitrile for HPLC, min. 99.8%, LGC Promochem

Dichloromethane, p.a., Riedel-de Haën

Dichloromethane, p.a., purity ≥99.8%, Merck

Dichloromethane, ROTISOLV Pestilyse, purity ≥99.9%, ROTH

Diethyl ether, GR, min. 99.5%, Merck

Distilled water

Hexane for pesticide residue analysis, Promochem

Hydrochloric acid, GR, 32%, Merck

Hydrochloric acid, p.a., 25%, Riedel-de Haën

Petroleum benzene, boiling range 40-60ºC min. 95%, Riedel-de Haën

Sea sand, GR, Merck, purified by acid and calcinated

7.1.2 Solutions

Stock solution of Diphenyl phthalate in acetone (1.0 mg/mL)

Weight, to the nearest 0.1 mg, 100 mg Diphenyl phthalate in a 100 mL volumetric flask. Fill volumetric flask up to the mark with acetone and mix.

Calculate the exact concentration of the substance in mg/mL.

Note: The solution should be stored protected from light in a refrigerator (4 - 6ºC).

Intermediate standard solutions of Diphenyl phthalate in acetone (0.1 mg/mL)

Pipette 10 ml of the standard stock solution into a 100 mL volumetric flask and fill the flask up to the mark with acetone.

Calculate the exact concentration of Diphenyl phthalate in µg/ml.

Stock solution of Benzophenone in acetone (1.0 mg/mL)

Weight, to the nearest 0.1 mg, 100 mg Benzophenone in a 100 mL volumetric flask. Fill volumetric flask up to the mark with acetone and mix.

Calculate the exact concentration of the substance in mg/mL.

Note: The solution should be stored protected from light in a refrigerator (4 - 6ºC).

Intermediate standard solutions of Benzophenone in acetone (0.1 mg/mL)

Pipette 10 ml of the standard stock solution into a 100 mL volumetric flask and fill the flask up to the mark with acetone.

Calculate the exact concentration of Benzophenone in µg/ml.

Stock solutions of the internal standards Benzylbutyl phthalate and 4-methylbenzophenone in acetone (1.0 mg/mL)

Weight, to the nearest 0.1 mg, 100 mg of benzylbutyl phthalate and 4-methylbenzophenone into a 100 mL volumetric flask. Fill volumetric flask up to the mark with acetone and mix.

Calculate the exact concentrations of the substances in mg/mL.

Note: The solution should be stored protected from light in a refrigerator (4 - 6ºC).

Stock solution of the internal standard Dioctyl phthalate in acetone (2.0 mg/mL)

Weight, to the nearest 0.1 mg, 200 mg of dioctyl phthalate into a 100 mL volumetric flask. Fill volumetric flask up to the mark with acetone and mix.

Calculate the exact concentration of the substance in mg/mL.

Note: The solution should be stored protected from light in a refrigerator (4 - 6ºC).

Intermediate internal standard solution of Dioctyl phthalate in acetone (0.5 mg/mL)

Pipette 5 ml of the standard stock solution into a 20 mL volumetric flask and fill the flask up to the mark with acetone.

Calculate the exact concentration of Dioctyl phthalate in µg/ml.

7.1.3 Apparatus

In addition to ordinary laboratory apparatus the following equipment was used:

Centrifuge able to reach 4500rpm
Round bottom glass centrifuge tubes (100 mL)
Vacuum rotary evaporator

Separation of the analytes was achieved by means of a gas chromatograph equipped with a capillary column capable of delivering reproducible peaks of diphenyl phthalate, benzophenone and the internal

standards and capable to separate this peaks from interference peaks originated from samples used. Parameters found suitable for the analysis are given below:

Gas chromatograph: HP 5890 Series II

Analytical Column: Optima δ-3, 30m x 0,25mm I.D. x 0,5μm film

Carrier gas and pressure: Helium at 70kPa

Injetor temperature: 275°C

Injection volume: 1μl

Mode: splitless

Total flow: 25,1ml/minute

Oven temperature program: 90°C, 1 minute -> 40°C/minute -> 320°C, 5,25minute

Detection was carried out by a mass selective detector HP 5970 in SIM mode.

Table 11 GC-MS chromatography data

	Target ion	Qualifier ion	Retention time (minutes)
Benzophenone	105	182	6.07
Diphenyl phthalate	225	153	9.25
4-methylbenzophenone	105	182	6.46
Benzyl butyl phthalate	149	206	8.14
Dioctyl phthalate	149	105	9.48

7.1.4 Migration tests – general principles

Regardless of the properties of the foodstuff a duplicate test was set up for each time-point e.g. for the migration tests with ketchup a separate jar with a specified amount of the sample was sealed with a lid containing a fresh plastic film for each time-point.

Stability tests were carried out in duplicate alongside the migration tests. The tests were performed in the same "test cell" as the actual migration tests by fortifying the foodstuffs with the analytes at a concentration level of 4 ppm. Other than the migration tests the stability test samples were analysed only at the longest time-points at each temperature.

Also a sample of blank food was stored alongside the migration tests and treated in the same way as the samples.

Exposure types include -   wrap

                         wide mouth jar with film placed in the lid

                         total immersion

Wrap – appropriate for solid food. Plastic film of specified size was layed on the butter toast, the whole was packed tightly in aluminium foil and light pressure was applied to ensure intimate contact between the foodstuff and the plastic film. This arrangement was considered best for solid foods such as the above mentioned butter toast.

Wide mouth jar – appropriate for example for liquids, viscous liquids, powders etc. The plastic film is cut out to fit on the inside of the screw cap of the jar. The foodstuff is then weighed in the jar, closed with the screw cap containing the plastic film on the inside and rotated upside down to expose the foodstuff to the plastic film. Advantage of this approach is its applicability to most fluid, semifluid and even some dry foods as well as powders. The main drawback of this arrangement is the possibility of leaks due to sealing difficulties of the plastic film on the inside of the lid.

Total immersion – appropriate for liquids. Plastic film of a specified area was cut out and totally immersed in the sample. This way both sides of the film as well as the cutting edges (their contribution to the migration might not be negligible for thicker films) were exposed to the food sample. Advantage of this approach is that the flask or jar containing the sample can be sealed easily without the risk of leaks (leaks being a problem in the wide mouth jar approach with the film in the lid).

In general single side exposure was preferred. It represents best the kind of exposure encountered in food packaging and there are no effects of cutting edges of the plastic film, which might have significant influence on the results of the migration when testing thicker films.

The EU conventional exposure ratio of 6 dm^2 of film to 1 kg of foodstuff was desired in the migration test design. Therefore all the tests regardless of the exposure type (wrap, wide mouth jar or total immersion) were arranged in such a way as to meet this criteria as closely as possible.

7.1.4.1 *Migration into aqueous and acidic foods foodstuffs – testing procedures*

7.1.4.1.1 **Orange juice**

Exposure type: total immersion

Sample amount: 0,1 litre

Exposure area: 0,6 dm^2

Test substances: Benzophenone [BP], Diphenyl phthalate [DPP]

Internal standards: Benzyl Butyl phthalate [BBP], Dioctyl phthalate [DOP]

Sample handling: 100ml of sample are put into a flask and 0,6dm^2 of the plastic film are immersed in the sample (contact between the whole area of the film and the sample must be ensured).

After storage the sample is homogenised and 50ml are transferred into a separation funnel. Add the internal standard in acetone (50µl) with a micro syringe. Subsequently 10ml of dichloromethane are added, the funnel is closed and hand-shaken for 2 minutes. Mixture is transferred into a centrifuge tube and the funnel washed with 2ml dichloromethane which are added into the centrifuge tube. Sample is centrifuged at 4500rpm for 10minutes at 17°C. Lower (organic) layer is separated into an Erlenmeyer flask (containing 1-2g of anhydrous sodium sulphate). The centrifuge tube is washed with the aqueous phase from the funnel which is then transferred back into the funnel. The centrifuge tube is then washed with 10ml of fresh dichloromethane, which is also transferred into the funnel and the extraction is repeated (altogether the extraction is repeated three times). The obtained extracts are pooled together in an Erlenmeyer flask with anhydrous sodium sulphate, filtered through a glass filter (porosity G4). The Erlenmeyer flask is washed 3 times with 1ml dichloromethane and finally the filter is washed with 2ml of the solvent. The extract is transferred into a 100ml round bottom flask and the solvent evaporated using a vacuum rotary evaporator (under nitrogen at 40°C, no vacuum applied) to a volume less than 10ml. The final extract is transferred into a 10ml volumetric flask (the round bottom flask is washed with dichloromethane), filled up to the marker and analysed.

7.1.4.1.2 Apple sauce

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 g

Exposure area: 0,0855 dm^2

Test substances: Benzophenone [BP], Diphenyl phthalate [DPP]

Internal standards: Benzyl Butyl phthalate [BBP], 4-Methyl benzophenone [4MBP]

Sample handling: 14,25g of sample are weighed into wide mouth jars. The jars are closed using screw caps containing plastic foil on the inner side and stored upside down.

After storage the jars are opened, the ISTD solutions are added and the sample is thinned with 20ml water (apple sauce residues on the plastic foil are washed off with this water) and the sample transferred into a separation funnel. The jars are washed with 5 ml of fresh distilled water and finally with 10ml dichloromethane. The funnel is closed and hand-shaken for 2 minutes. Mixture is transferred into a centrifuge tube and the funnel washed with 2ml dichloromethane which are added into the centrifuge tube. Sample is centrifuged at 4500rpm for 10minutes at 17°C. Lower (organic) layer is separated into an Erlenmeyer flask (containing 1-2g of anhydrous sodium sulphate). The centrifuge tube is washed with the aqueous phase from the funnel which is then transferred back into the funnel. The centrifuge tube is then washed with 10ml of fresh dichloromethane, which is also transferred into the funnel and the extraction is repeated (altogether the extraction is repeated three times). The obtained extracts are pooled together in an Erlenmeyer flask with

anhydrous sodium sulphate, filtered through a glass filter (porosity G4). The Erlenmeyer flask is washed 3 times with 1ml dichloromethane and finally the filter is washed with 2ml of the solvent. The extract is transferred into a 100ml round bottom flask and the solvent evaporated using a vacuum rotary evaporator (under nitrogen at 40°C, no vacuum applied) to a volume less than 10ml. The final extract is transferred into a 10ml volumetric flask (the round bottom flask is washed with dichloromethane), filled up to the marker and analysed.

7.1.4.1.3   Milk, UHT, min. 3,5% fat

Exposure type: one sided using wide mouth jar

Sample amount: 15 ml

Exposure area: 0,0855 dm^2

Test substances: Benzophenone [BP], Diphenyl phthalate [DPP]

Internal standards: Benzyl Butyl phthalate [BBP], 4-Methyl benzophenone [4MBP] (also used as internal standard for DPP during tests at 40°C because of inconsistent results using BBP)

Sample handling: 15ml of sample are weighed into wide mouth jars. The jars are closed using screw caps containing plastic foil on the inner side and stored upside down.

After storage the jars are opened, the ISTD solutions are added and the sample is transferred into a separation funnel (use 20ml of distilled water to wash out the jar and quantitatively transfer the sample into the funnel). Subsequently 20ml of diethyl ether/ petroleum benzine (1:1 v/v) are added and the funnel is hand-shaken for 2 minutes. Sample is transferred into a centrifuge tube, the funnel is washed with 2ml of diethyl ether and sample is centrifuged at 4500rpm for 10 min. 5ml of the upper layer are evaporated to dryness at 35°C for 15 minutes. 2ml hexane and 2ml acetonitrile are added and vortexed for 30 seconds. The lower (acetonitrile) phase is taken for GC-MS analysis.

7.1.4.1.4   Ketchup

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 g

Exposure area: 0,0855 dm^2

Test substances: Benzophenone [BP], Diphenyl phthalate [DPP]

Internal standards: Benzyl Butyl phthalate [BBP], 4-Methyl benzophenone [4MBP]

Sample handling: 14,25g of sample are transferred into a glass jar used for migration studies. The jars are closed using screw caps containing plastic foil on the inner side and stored upside down.

After storage the jars are opened, the ISTD solutions are added and the plastic foil washed with 5 ml of distilled water and the sample transferred into a separation funnel. The jars are washed with 5 ml of fresh distilled water and finally with 10ml dichloromethane. The funnel is closed and hand-shaken for 2 minutes.

Mixture is transferred into a centrifuge tube and the funnel washed with 2ml dichloromethane which are added into the centrifuge tube. Sample is centrifuged at 4500rpm for 10minutes at 17°C. Lower (organic) layer is separated into an Erlenmeyer flask (containing 1-2g of anhydrous sodium sulphate). The centrifuge tube is washed with the aqueous phase from the funnel which is then transferred back into the funnel. The centrifuge tube is then washed with 10ml of fresh dichloromethane, which is also transferred into the funnel and the extraction is repeated (altogether the extraction is repeated three times). The obtained extracts are pooled together in an Erlenmeyer flask with anhydrous sodium sulphate, filtered through a glass filter (porosity G4). The Erlenmeyer flask is washed 3 times with 1ml dichloromethane and finally the filter is washed with 2ml of the solvent. The extract is transferred into a 100ml round bottom flask and the solvent evaporated using a vacuum rotary evaporator (under nitrogen at 40°C, no vacuum applied) to a volume less than 10ml. The final extract is transferred into a 10ml volumetric flask (the round bottom flask is washed with dichloromethane) and the extract is filled up to the marker.

7.1.4.1.5 Cola drink

Exposure type:	one sided using wide mouth jar
Sample amount:	15 ml
Exposure area:	0,0855 dm^2
Test substances:	Benzophenone [BP], Diphenyl phthalate [DPP]
Internal standards:	Benzyl Butyl phthalate [BBP], 4-Methyl benzophenone [4MBP]
Sample handling:	The sample is de-carbonated by shaking and short heating up to 90°C prior to analysis.

15ml of sample are transferred into a glass jar used for migration studies. The jars are closed using screw caps containing plastic foil on the inner side and stored upside down.

After storage the jars are opened, the ISTD solutions are added and the plastic foil washed with 5 ml of distilled water and the sample transferred into a separation funnel. The jars are washed with 5 ml of fresh distilled water and finally with 10ml dichloromethane. The funnel is closed and hand-shaken for 2 minutes. Mixture is transferred into a centrifuge tube and the funnel washed with 2ml dichloromethane which are added into the centrifuge tube. Sample is centrifuged at 4500rpm for 10minutes at 17°C. Lower (organic) layer is separated into an Erlenmeyer flask (containing 1-2g of anhydrous sodium sulphate). The centrifuge tube is washed with the aqueous phase from the funnel which is then transferred back into the funnel. The centrifuge tube is then washed with 10ml of fresh dichloromethane, which is also transferred into the funnel and the extraction is repeated (altogether the extraction is repeated three times). The obtained extracts are pooled together in an Erlenmeyer flask with anhydrous sodium sulphate, filtered through a glass filter (porosity G4). The Erlenmeyer flask is washed 3 times with 1ml dichloromethane and finally the filter is washed with 2ml of the solvent. The extract is transferred into a 100ml round bottom flask and the solvent evaporated using a vacuum rotary evaporator (under nitrogen at 40°C, no vacuum applied) to a volume less

than 10ml. The final extract is transferred into a 10ml volumetric flask (the round bottom flask is washed with dichloromethane) and the extract is filled up to the marker.

7.1.4.1.6 Wine and beer

Exposure type: one sided using wide mouth jar

Sample amount: 15 ml

Exposure area: 0,0855 dm^2

Test substances: Benzophenone [BP], Diphenyl phthalate [DPP]

Internal standards: Benzyl Butyl phthalate [BBP], 4-Methyl benzophenone [4MBP]

Sample handling: Beer is shaken repeatedly and let stand open in a wide beaker for couple of hours prior to analysis. 15ml of sample are transferred into a wide mouth jar. The jars are closed using screw caps containing plastic foil on the inner side and stored upside down.

After storage the jars are opened, the ISTD solutions are added and the plastic foil washed with 5 ml of distilled water and the sample transferred into a separation funnel. The jars are washed with 5 ml of fresh distilled water and finally with 10ml dichloromethane. The funnel is closed and hand-shaken for 2 minutes. Mixture is transferred into a centrifuge tube and the funnel washed with 2ml dichloromethane which are added into the centrifuge tube. Sample is centrifuged at 4500rpm for 10minutes at 17°C. Lower (organic) layer is separated into an Erlenmeyer flask (containing 1-2g of anhydrous sodium sulphate). The centrifuge tube is washed with the aqueous phase from the funnel which is then transferred back into the funnel. The centrifuge tube is then washed with 10ml of fresh dichloromethane, which is also transferred into the funnel and the extraction is repeated (altogether the extraction is repeated three times). The obtained extracts are pooled together in an Erlenmeyer flask with anhydrous sodium sulphate, filtered through a glass filter (porosity G4). The Erlenmeyer flask is washed 3 times with 1ml dichloromethane and finally the filter is washed with 2ml of the solvent. The extract is transferred into a 100ml round bottom flask and the solvent evaporated using a vacuum rotary evaporator (under nitrogen at 40°C, no vacuum applied) to a volume less than 10ml. The final extract is transferred into a 10ml volumetric flask (the round bottom flask is washed with dichloromethane), filled up to the marker and analysed.

7.1.4.2 Migration into fatty foodstuffs – testing procedures

7.1.4.2.1 Cheese sauce (~18,5% fat)

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 g

Test substances: Benzophenone [BP], Diphenyl phthalate [DPP]

Internal standards: Benzyl Butyl phthalate [BBP], 4-Methyl benzophenone [4MBP]

Sample handling: 14,25g of sample are weighed into wide mouth jars. The jars are closed using screw caps containing plastic foil on the inner side and stored upside down under specified conditions.

After storage the jars are opened and the ISTD solutions are added. The sample is transferred into an extraction flask and 30ml of 25% HCl are added (use the acid also to wash out the wide mouth jar. The flask is then placed into a boiling water bath for 30 minutes. Extract is cooled and 25ml diethyl ether and petroleum benzine respectively are added (after each addition, the extract is shaken for half a minute). The organic and the aqueous phase are allowed to separate. 5ml of the upper phase are evaporated to dryness at 37°C for 15 minutes. 2ml hexane and 2ml acetonitrile are added. Sample is vortexed for 30 seconds and the lower (acetonitrile) phase is taken for GC-MS analysis.

7.1.4.2.2 Mayonnaise (80% fat content)

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 g

Exposure area: 0,0855 dm^2

Test substances: Benzophenone [BP], Diphenyl phthalate [DPP]

Internal standards: Benzyl Butyl phthalate [BBP], 4-Methyl benzophenone [4MBP]

Sample handling: 14,25g of sample are weighed into wide mouth jars. The jars are closed using screw caps containing plastic foil on the inner side and stored upside down.

After storage the jars are opened, the ISTD solutions are added and the sample is transferred into a round-bottom flask. 20ml hexane are added and the flask is shaken for 1 hour. Upper layer is transferred into a 50ml volumetric flask. The residue is extracted with 20ml of fresh hexane by shaking for 1 hour. The upper layer is pooled with the first extract in the 50ml volumetric flask, which is filled up to the marker with solvent.

10ml acetonitrile are added to 10ml of the extract. Extract is vortexed for 30 seconds and the lower (acetonitrile) phase is taken for GC-MS analysis.

7.1.4.2.3 Yoghurt drink

Exposure type: one sided using wide mouth jar

Sample amount: 15 ml

Exposure area: 0,0855 dm^2

Test substances: Benzophenone [BP], Diphenyl phthalate [DPP]

Internal standards: Benzyl Butyl phthalate [BBP], 4-Methyl benzophenone [4MBP]

Sample handling: 15ml of sample are weighed into wide mouth jars. The jars are closed using screw caps containing plastic foil on the inner side and stored upside down.

After storage the jars are opened, the ISTD solutions are added and the sample is transferred into a separation funnel (use 20ml of distilled water to wash out the jar and quantitatively transfer the sample into the funnel). Subsequently 20ml of diethyl ether/ petroleum benzine (1:1 v/v) are added and the funnel is hand-shaken for 2 minutes. Sample is transferred into a centrifuge tube, the funnel is washed with 2ml of diethyl ether and sample is centrifuged at 4500rpm for 10 min. 5ml of the upper layer are evaporated to dryness at 35°C for 15 minutes. 2ml hexane and 2ml acetonitrile are added and vortexed for 30 seconds. The lower (acetonitrile) phase is taken for GC-MS analysis.

7.1.4.2.4 Meat, lean pork (minced, fat content ≤5% plus 4 different fat contents by addition of loin – 10, 20, 30 and 50% fat)

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 g

Exposure area: 0,0855 dm^2

Test substances: Benzophenone [BP], Diphenyl phthalate [DPP]

Internal standards: Benzyl Butyl phthalate [BBP], 4-Methyl benzophenone [4MBP]

Sample preparation: Minced lean pork meat and pork fat was bought in a local retail store. The pork fat was then mixed with the lean pork meat to give a final fat content desired for the experiments. The fat was blended thoroughly into the minced meat to ensure a homogenous distribution in the sample.

Sample handling: 14,25g of the sample is put into a wide mouth jars. The jars are closed using screw caps containing plastic foil on the inner side and stored upside down under specified conditions.

After storage the jars are opened and the sample is transferred into a porcelain grinder. From the fish fillet a whole piece is cut. 14.25g of the sample are put into a porcelain grinder (sample amount chosen with respect to the EU conventional ratio foodstuff / plastic film ratio = 1kg food to 6dm^2 and with respect to our test cell with plastic film area of 0,0855dm^2). Subsequently the standards, sea sand and 30ml of 25% HCl are added and the sample is ground (approximately 5 minutes, until the tissue disintegrates). Sample is transferred into a round-bottom flask, the grinder is washed twice with 10ml of fresh HCl. Flask is equipped with a reflux cooler, put into a water bath and heated up to >90°C for 30 minutes. Sample is transferred into an extraction flask while still warm and cooled. Round-bottom flask is washed with 25ml petroleum benzine (which is added to the sample) and 25ml diethyl ether (also pooled with the sample). After each addition, sample is shaken for half a minute. 5ml of the upper phase are evaporated to dryness at 40°C. 2ml hexane and 2ml acetonitrile are added and vortexed for 30 seconds. The lower (acetonitrile) phase is taken for GC-MS analysis.

7.1.4.2.5 Fish (salmon with 13,6% fat)

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 g

Exposure area: 0,0855 dm^2

Test substances: Benzophenone [BP], Diphenyl phthalate [DPP]

Internal standards: Benzyl Butyl phthalate [BBP], 4-Methyl benzophenone [4MBP]

Sample handling: A piece of fish fillet weighing 14,25g is cut and put into a wide mouth jars. The jars are closed using screw caps containing plastic foil on the inner side and stored upside down under specified conditions.

   After storage the jars are opened and the sample is transferred into a porcelain grinder. Subsequently the standards, sea sand and 30ml of 25% HCl are added and the sample is ground (approximately 5 minutes, until the tissue disintegrates). Sample is transferred into a round-bottom flask, the grinder is washed twice with 10ml of fresh HCl. Flask is equipped with a reflux cooler, put into a water bath and heated up to >90°C for 30 minutes. Sample is transferred into an extraction flask while still warm and cooled. Round-bottom flask is washed with 25ml petroleum benzine (which is added to the sample) and 25ml diethyl ether (also pooled with the sample). After each addition the sample is shaken for half a minute. The organic and the aqueous phase are allowed to separate. 5ml of the upper phase are evaporated to dryness at 40°C. 2ml hexane and 2ml acetonitrile are added and vortexed for 30 seconds. The lower (acetonitrile) phase is taken for GC-MS analysis.

7.1.4.2.6 Condensed milk

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 g

Exposure area: 0,0855 dm^2

Test substances: Benzophenone [BP], Diphenyl phthalate [DPP]

Internal standards: Benzyl Butyl phthalate [BBP], 4-Methyl benzophenone [4MBP]

Sample handling: 14,25g of sample are weighed into wide mouth jars. The jars are closed using screw caps containing plastic foil on the inner side and stored upside down under specified conditions.

   After storage the jars are opened and the ISTD solutions are added. The sample is transferred into an Erlenmeyer flask. 20ml of water are used to thin the sample and transfer it quantitatively into the flask.Subsequently 20ml of diethyl ether / petroleum benzine (1:1 v/v) are added and the flask is hand-shaken for 2 minutes. Sample is transferred into a centrifuge tube, the flask is washed with 2ml of diethyl ether and sample is centrifuged at 4500rpm for 10 min. 5ml of the upper layer are evaporated to dryness at 35°C for 15

minutes. 2ml hexane and 2ml acetonitrile are added. Sample is vortexed for 30 seconds and the lower (acetonitrile) phase is taken for GC-MS analysis.

7.1.4.3   Migration into dry foodstuffs – testing procedures

7.1.4.3.1   Butter toast (4% fat)

Exposure type: one sided, wrap

Sample amount: 25 g

Exposure area: 0,1440 dm^2

Test substances: Benzophenone [BP], Diphenyl phthalate [DPP]

Internal standards: Benzyl Butyl phthalate [BBP], 4-Methyl benzophenone [4MBP]

Sample handling: Plastic foil with an area of 0,144 dm^2 is put in intimate contact with 25g of sample, wrapped tightly in an aluminium foil and stored under specified conditions. Sample is then cut to small pieces into a 500ml conical flask. Standards and 200ml diethyl ether are added, flask is closed and shaken for 24 hours.

Sample is filtered through a glass filter (porosity G3) into a 500ml round-bottom flask. Subsequently the filter is washed with 10ml of fresh solvent. The residue is extracted with 100ml of diethyl ether by shaking for 24 hours.

Sample is filtered through a glass filter (porosity G3) into the 500ml round-bottom flask from previous day (=extracts are pooled together). The residue is extracted with 50ml of diethyl ether by shaking for a minute, afterwards filtered and the filter washed with small amount of fresh solvent.

The solvent from the pooled extracts is evaporated on a vacuum rotary evaporator at 40°C (no vacuum applied) to a volume <100ml. Sample is transferred into a 100ml volumetric flask. The round-bottom flask is washed with fresh solvent and the volumetric flask filled up to the marker. 2ml are evaporated to dryness and the residue extracted with 2ml hexane and 2ml acetonitrile. Sample is vortexed for half a minute and the lower (acetonitrile) phase is taken for analysis.

7.1.4.3.2   Flour

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 g

Exposure area: 0,0855 dm^2

Test substances: Benzophenone [BP], Diphenyl phthalate [DPP]

Internal standards: Benzyl Butyl phthalate [BBP], 4-Methyl benzophenone [4MBP]

<u>Sample handling</u>   14,25g of sample are weighed into wide mouth jars. The jars are closed using screw caps containing plastic foil on the inner side and stored upside down under specified conditions.

After storage the jars are opened and the ISTD solutions are added. The sample is tranferred into an extraction flask, 20ml hexane are added and the sample is vortexed. After 1 hour the sample is filtered through a glass filter of porosity G5 and the extraction flask is washed with 5ml of solvent. The residue in the filter is then transferred back into the extraction flask and the extraction is repeated with 20ml of fresh solvent. After the sample is vortexed for half a minute, stand for a few minutes and filter. Extraction flask is washed again with 5ml of solvent. Filtered extract is transferred into a round bottom flask and evaporated on a vacuum rotary evaporator to less than 10ml volume. Extract is transferred into 10ml volumetric flask and filled to the marker with hexane. 2ml of this extract are evaporated to dryness. 2ml of hexane and 2ml of acetonitrile are added to the residue and vortexed for half a minute. The lower (acetonitrile) phase is taken for GC-MS analysis.

7.1.4.3.3   Rice and milk powder

<u>Exposure type:</u>   one sided using wide mouth jar

<u>Sample amount:</u>   14,25 g

<u>Exposure area:</u>   0,0855 dm^2

<u>Test substances:</u>   Benzophenone [BP], Diphenyl phthalate [DPP]

<u>Internal standards:</u>   Benzyl Butyl phthalate [BBP], 4-Methyl benzophenone [4MBP]

<u>Sample handling</u>   14,25g of sample are weighed into wide mouth jars. The jars are closed using screw caps containing plastic foil on the inner side and stored upside down under specified conditions.

After storage the jars are opened and the ISTD solutions are added. The sample is transferred into an extraction flask, 20ml dichloromethane are added and the sample is vortexed. After 1 hour the sample is filtered through a glass filter of porosity G5 and the extraction flask is washed with 5ml of solvent. The residue in the filter is then transferred back into the extraction flask and the extraction is repeated with 20ml of fresh solvent. After the sample is vortexed for half a minute, stand for a few minutes and filter. Extraction flask is washed again with 5ml of solvent. Filtered extract is transferred into a round bottom flask and evaporated on a vacuum rotary evaporator to less than 10ml volume. Extract is transferred into 10ml volumetric flask and filled to the marker with dichloromethane. 2ml of this extract are evaporated to dryness. 2ml of hexane and 2ml of acetonitrile are added to the residue and vortexed for half a minute. The lower (acetonitrile) phase is taken for GC-MS analysis.

8 Results

8.1 Calculations regarding the maximum possible migration levels

In order to obtain comparable results on migration a plastic film with known and well defined properties had to be used. The plastic film used in this work for the migration studies was provided by the Fraunhofer Institute in Germany and was produced with the specific demands in mind to be appropriate for migration studies. The properties of the plastic film were as follows:

Material:	LDPE
Thickness:	$164 \pm 8\mu m$
Weight per area:	$1,50 \pm 0,05g/dm2$
Benzophenone concentration:	$450 \pm 11mg/kg$
Diphenyl phthalate concentration:	$561 \pm 31mg/kg$

Based on this data following calculations were made:

Benzophenone per $1dm^2$ of the plastic film $= 0,675 \pm 0,017$ mg
Diphenyl phthalate per $1dm^2$ of the plastic film $= 0,842 \pm 0,047mg$
These represent the total amounts available to migrate into the food sample.

Table 12   Maximum possible migration from plastic film

Substance	Calculated maximum possible migration from the plastic film in mg/dm^2
Benzophenone	0,675
Diphenyl phthalate	0,842

8.2 Aqueous and acidic foods

8.2.1 Orange juice

Test conditions:

Temperature: 5°C

Exposure time: 2, 4, 10, 20 and 30 days

Exposure type: total immersion

Sample amount: 0,1 litre

Exposure area: 0,6 dm^2

Table 13 Migration of benzophenone into orange juice at 5°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,285	0,277	41,63
4	0,316	0,337	48,37
10	0,466	0,426	66,07
20	0,420	0,468	65,78
30	0,466	0,489	70,74
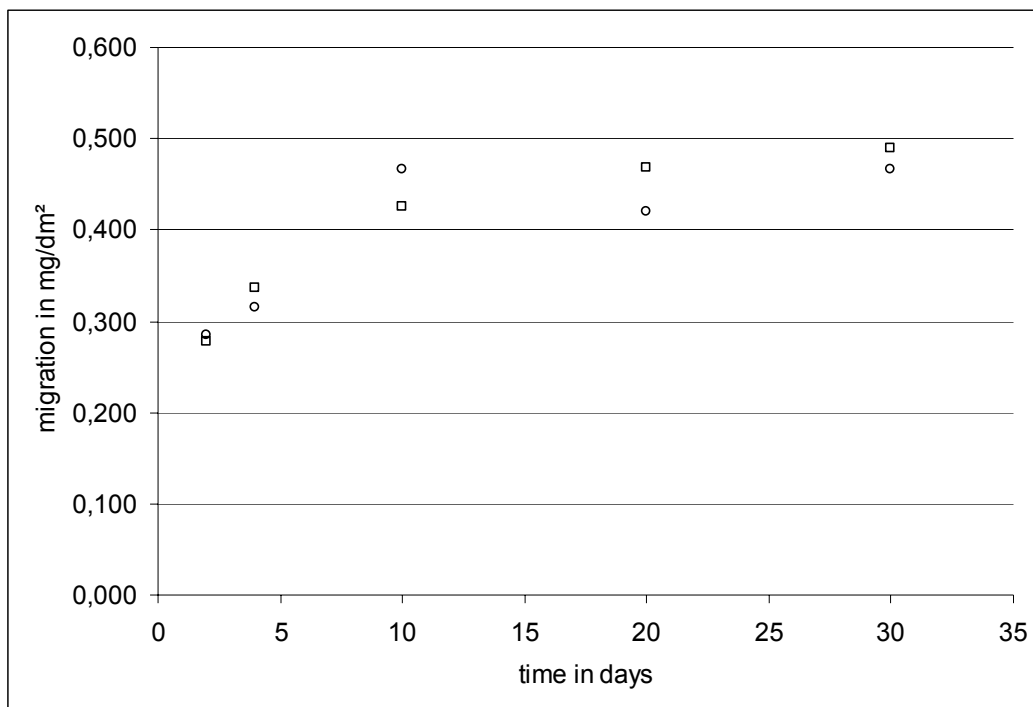
Figure 3 Migration of benzophenone into orange juice at 5°C

Test conditions:

Temperature: 5°C

Exposure time: 2, 4, 10, 20 and 30 days

Exposure type: total immersion

Sample amount: 0,1 litre

Exposure area: 0,6 dm^2

Table 14 Migration of diphenyl phthalate into orange juice at 5°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,059	0,058	6,95
4	0,058	0,061	7,07
10	0,095	0,090	10,99
20	0,107	0,097	12,11
30	0,102	0,102	12,11
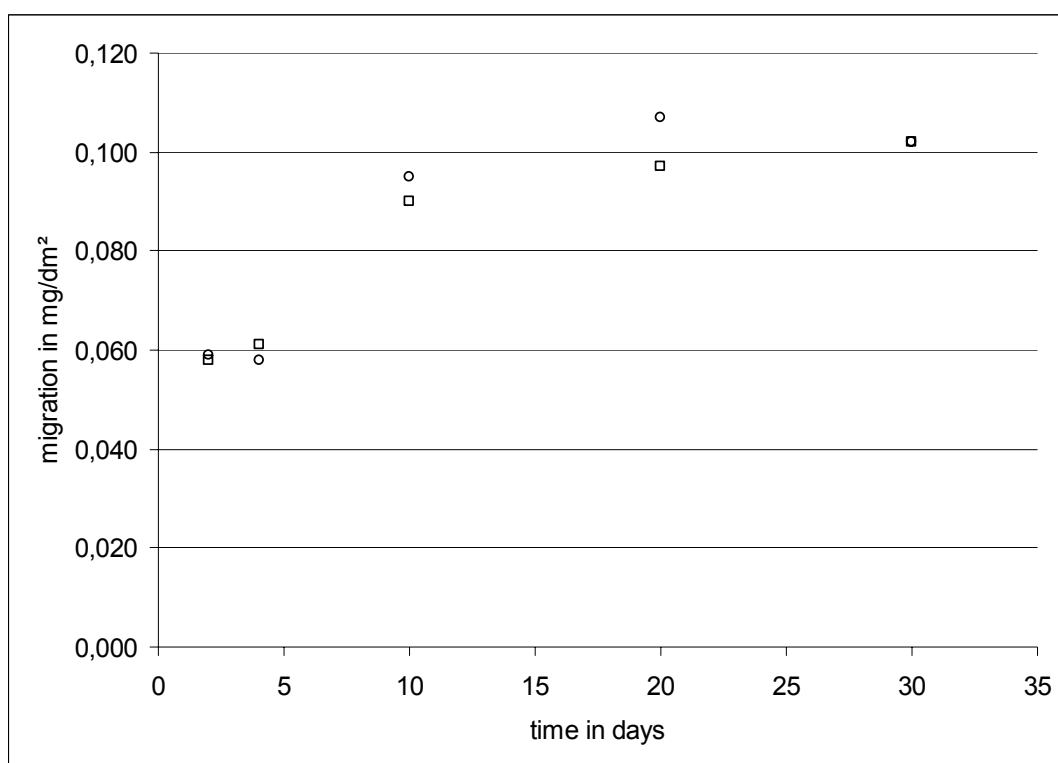
Figure 4 Migration of diphenyl phthalate into orange juice at 5°C

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: total immersion

Sample amount: 0,1 litre

Exposure area: 0,6 dm^2

Table 15 Migration of benzophenone into orange juice at 25°C

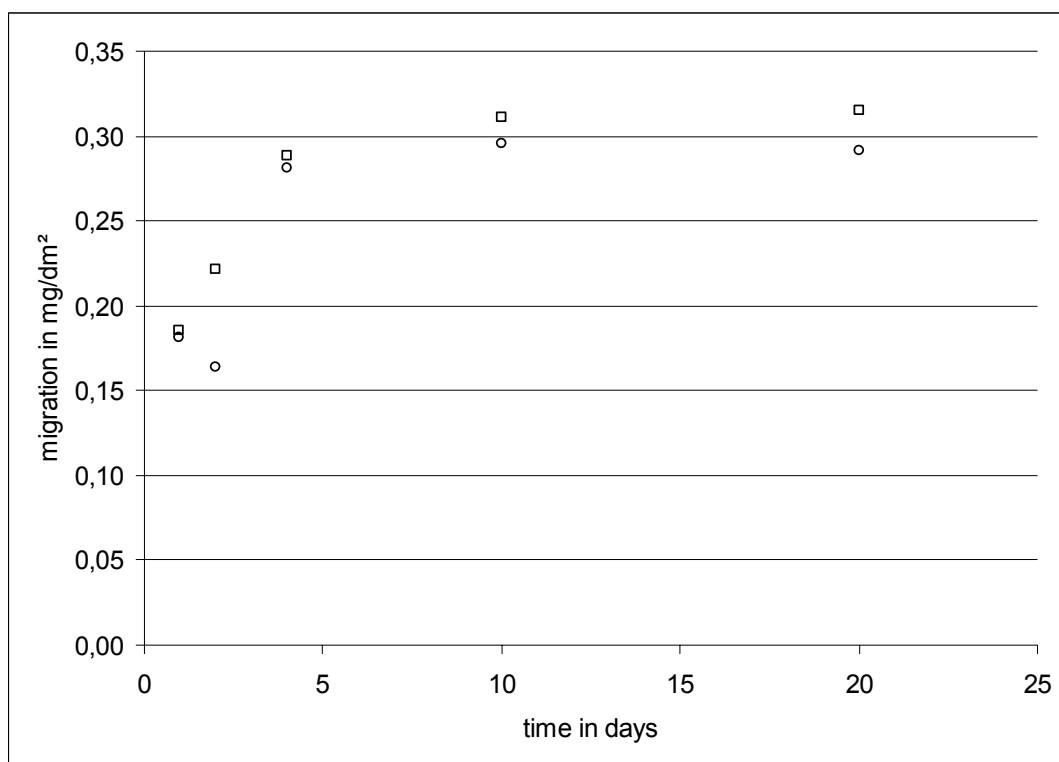| Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,514	0,421	69,26
2	0,521	0,671	88,30
4	0,589	0,594	87,63
10	0,658	0,657	97,41
20	0,532	0,512	77,33
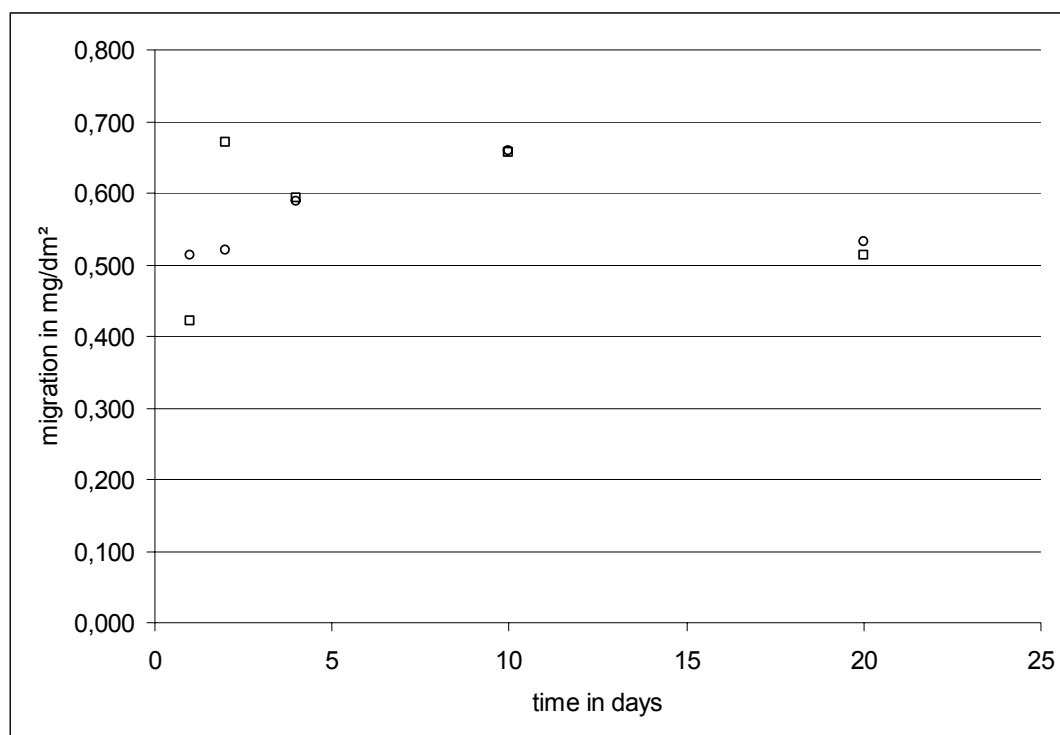
Figure 5 Migration of benzophenone into orange juice at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: total immersion

Sample amount: 0,1 litre

Exposure area: 0,6 dm^2

Table 16 Migration of diphenyl phthalate into orange juice at 25°C

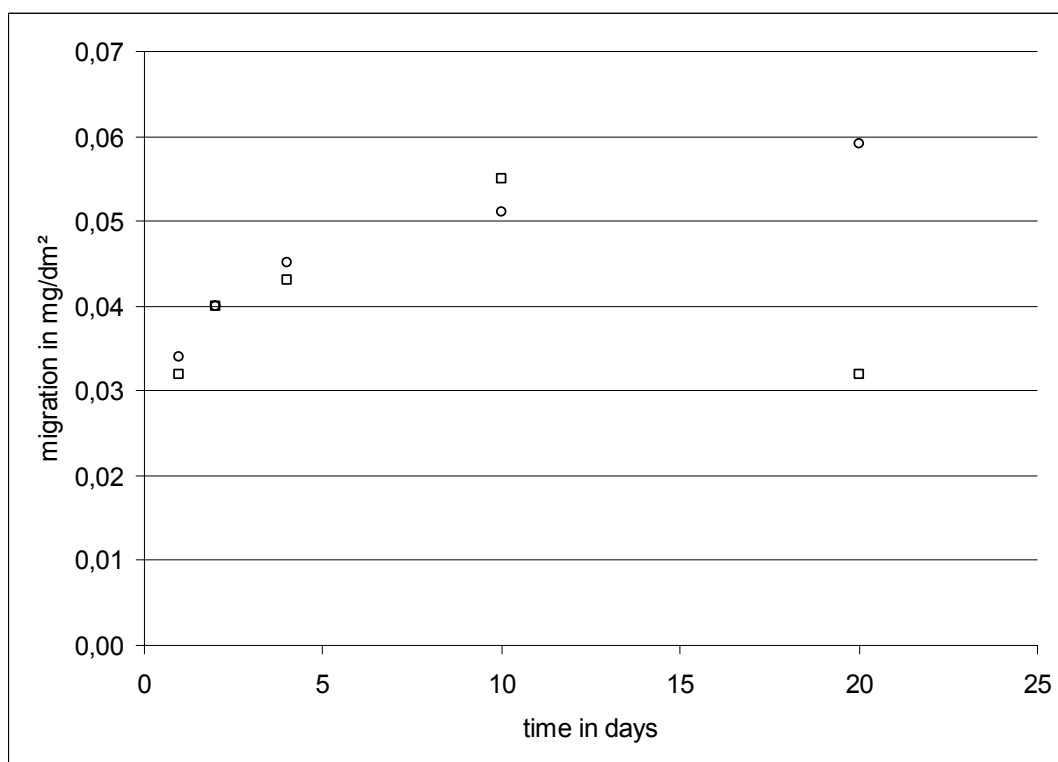| Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,141	0,103	14,49
2	0,157	0,197	21,02
4	0,222	0,213	25,83
10	0,385	0,419	47,74
20	0,437	0,376	48,28
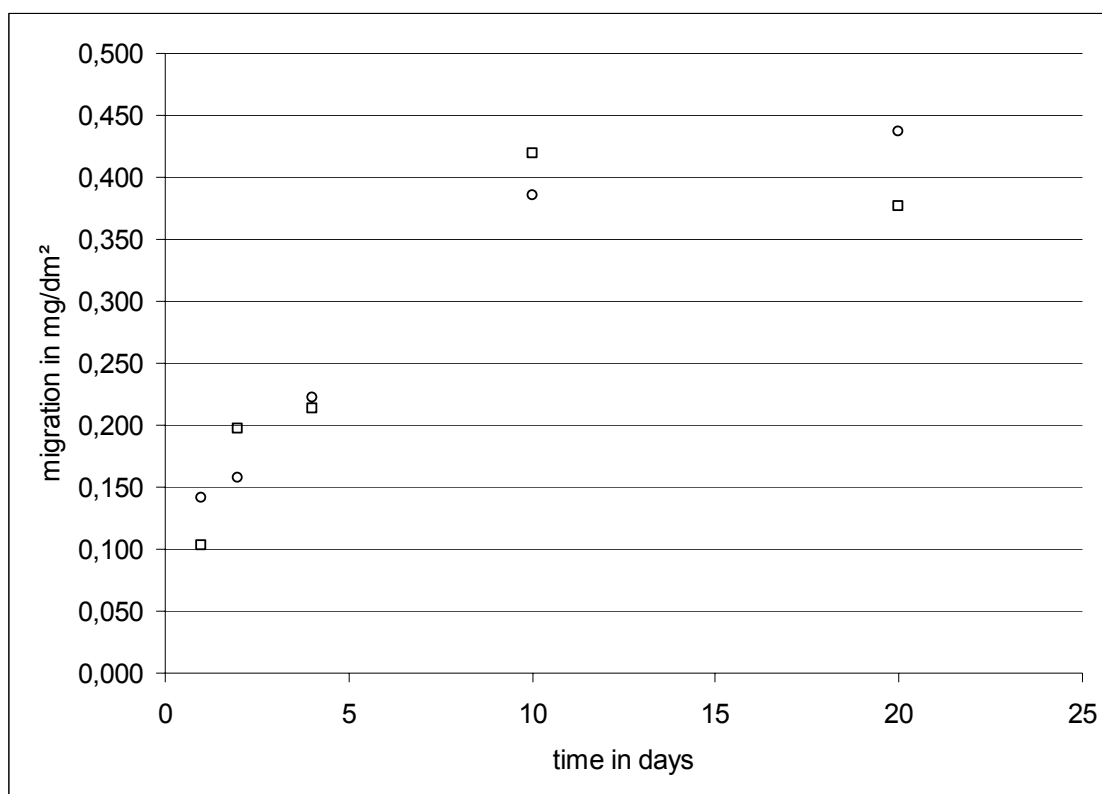
Figure 6 Migration of diphenyl phthalate into orange juice at 25°C

Test conditions:

Temperature: 40°C

Exposure time: 1, 2, 4, 7 and 10 days

Exposure type: total immersion

Sample amount:0,1 litre

Exposure area: 0,6 dm^2

Table 17 Migration of benzophenone into orange juice at 40°C

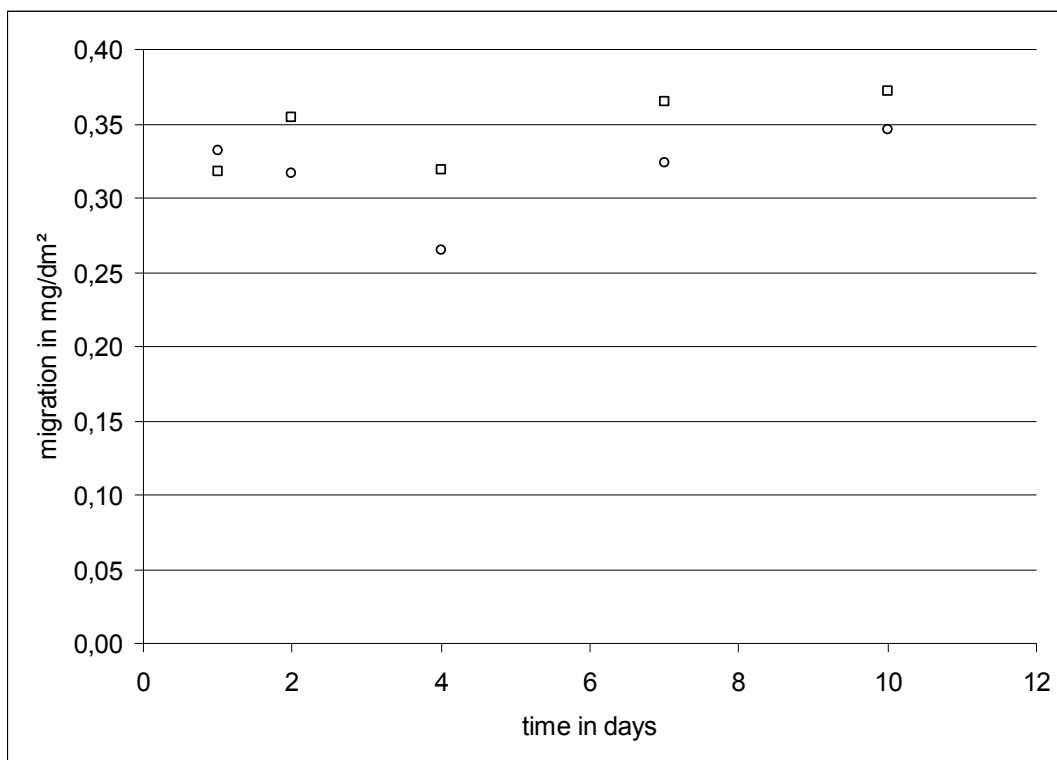| Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,673	0,669	99,41
2	0,742	0,756	110,96
4	0,761	0,665	105,63
7	0,746	0,719	108,52
10	0,588	0,616	89,19
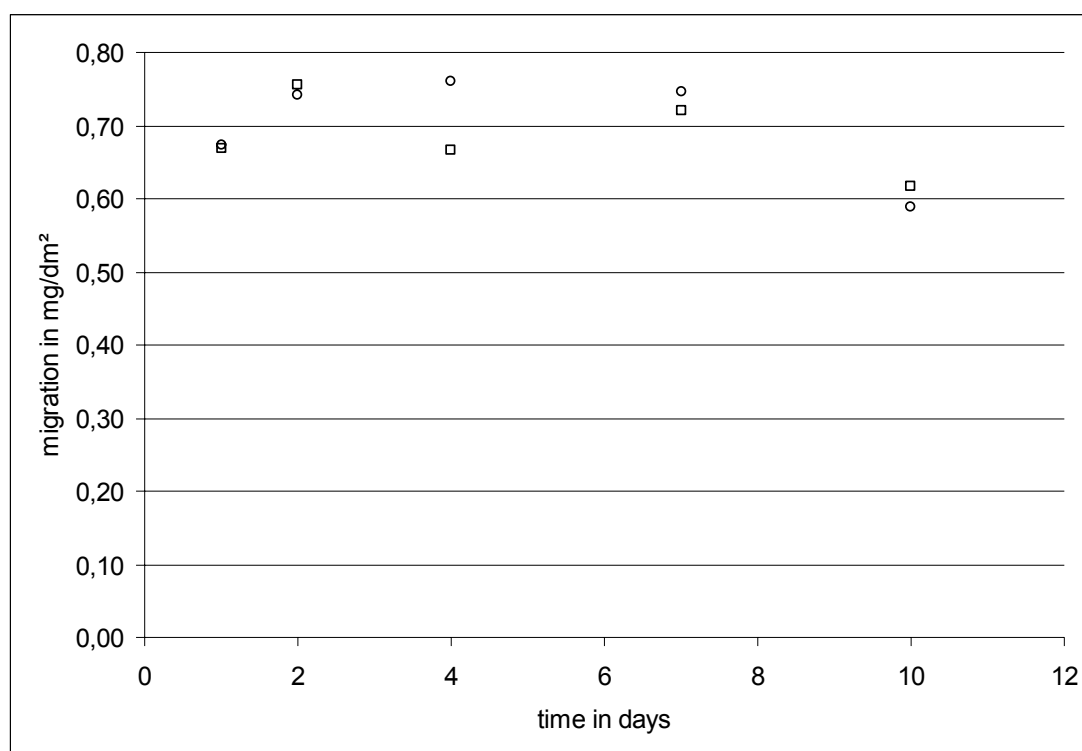
Figure 7 Migration of benzophenone into orange juice at 40°C

Test conditions:

Temperature: 40°C

Exposure time: 1, 2, 4, 7 and 10 days

Exposure type: total immersion

Sample amount:0,1 litre

Exposure area: 0,6 dm^2

Table 18 Migration of diphenyl phthalate into orange juice at 40°C

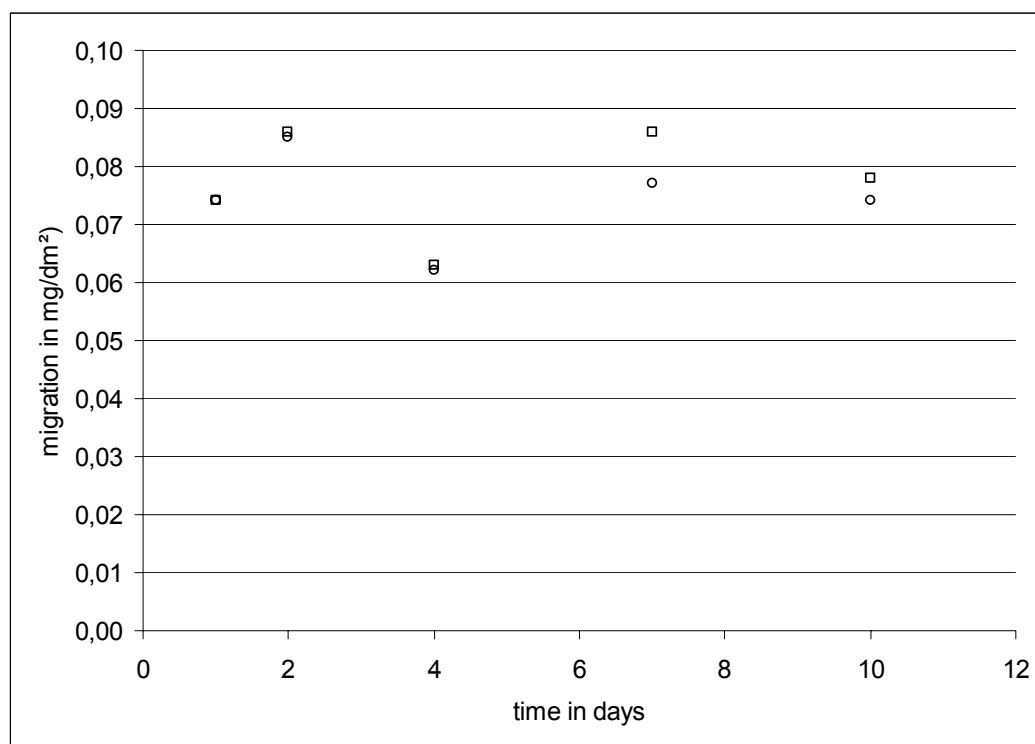| Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,279	0,235	30,52
2	0,467	0,453	54,63
4	0,596	0,579	69,77
7	0,671	0,663	79,22
10	0,527	0,491	60,45
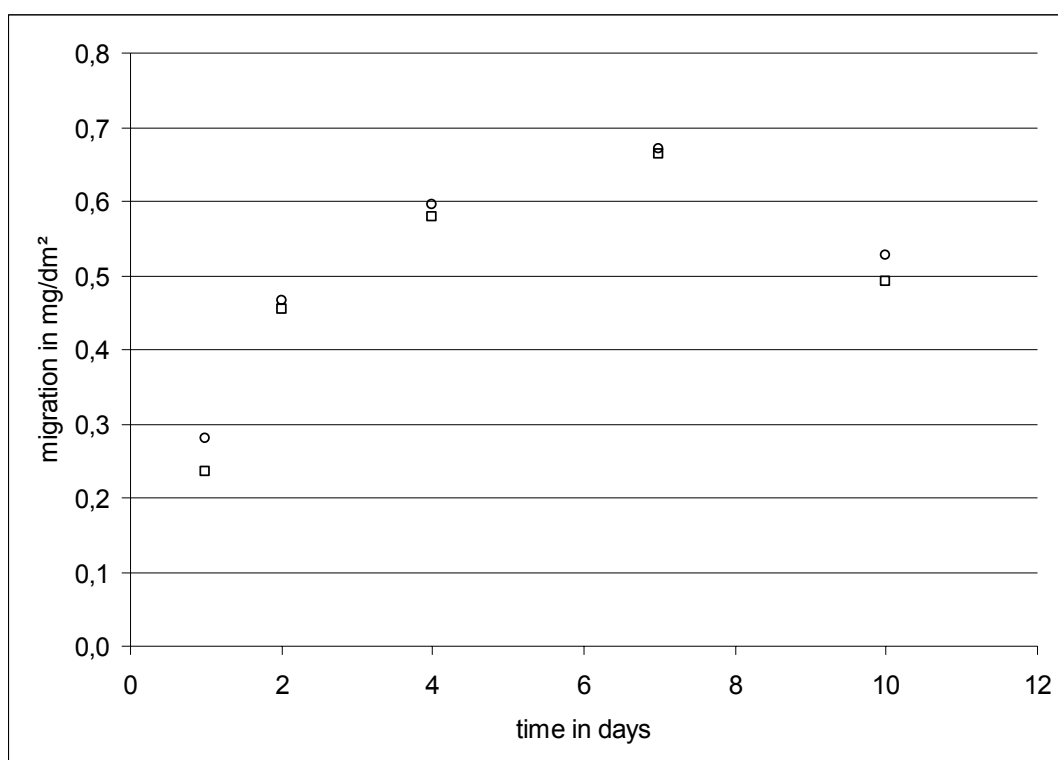
Figure 8 Migration of diphenyl phthalate into orange juice at 40°C

8.2.2 Apple sauce

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 19 Migration of benzophenone into apple sauce at 25°C

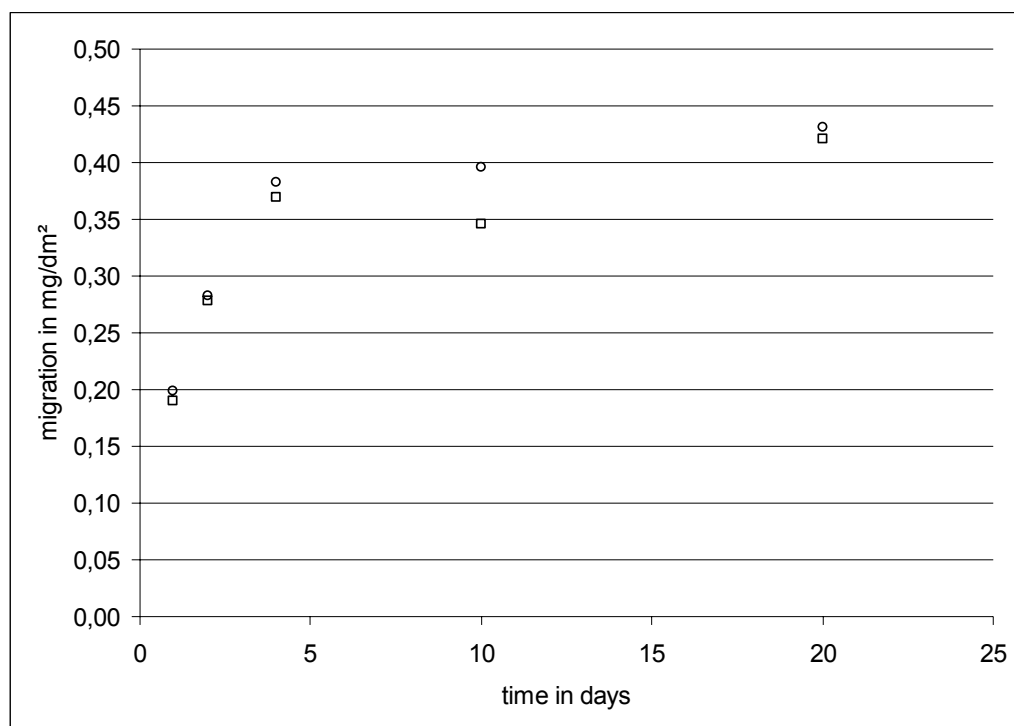| Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,216	0,155	27,48
2	0,302	0,298	44,44
4	0,415	0,413	61,33
10	0,599	0,307	67,11
20	0,529	0,636	86,30
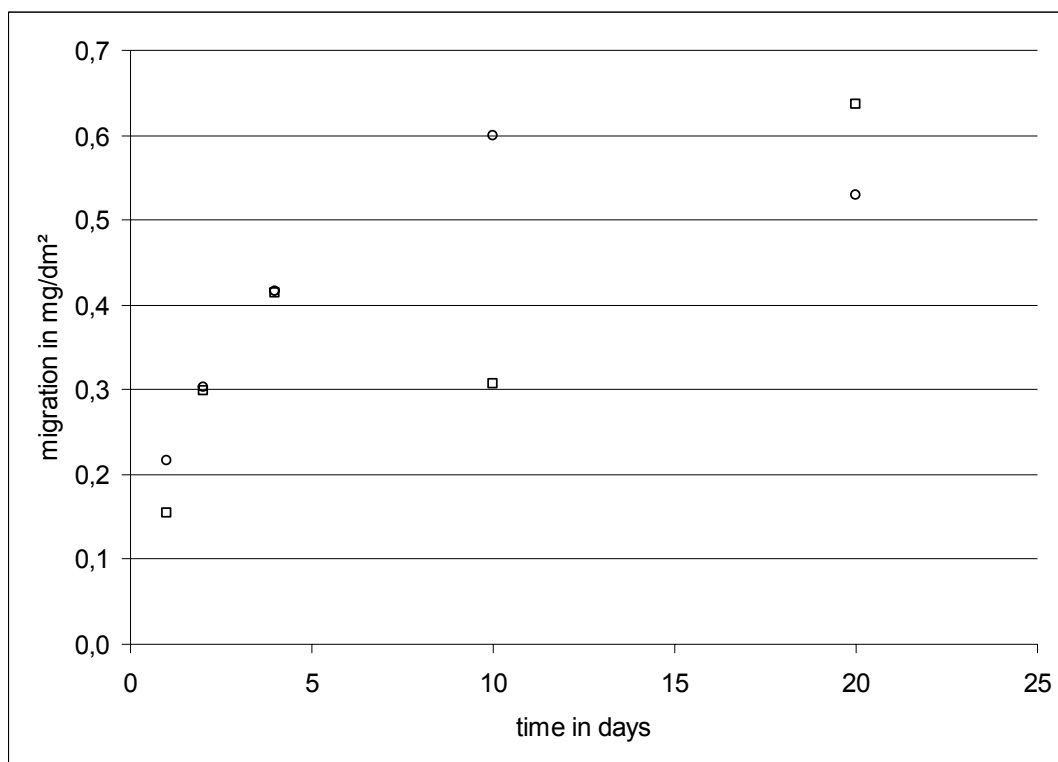
Figure 9 Migration of benzophenone into apple sauce at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 20 Migration of diphenyl phthalate into apple sauce at 25°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,044	0,041	5,05
2	0,061	0,064	7,42
4	0,081	0,079	9,50
10	0,155	0,138	17,40
20	0,220	0,213	25,71
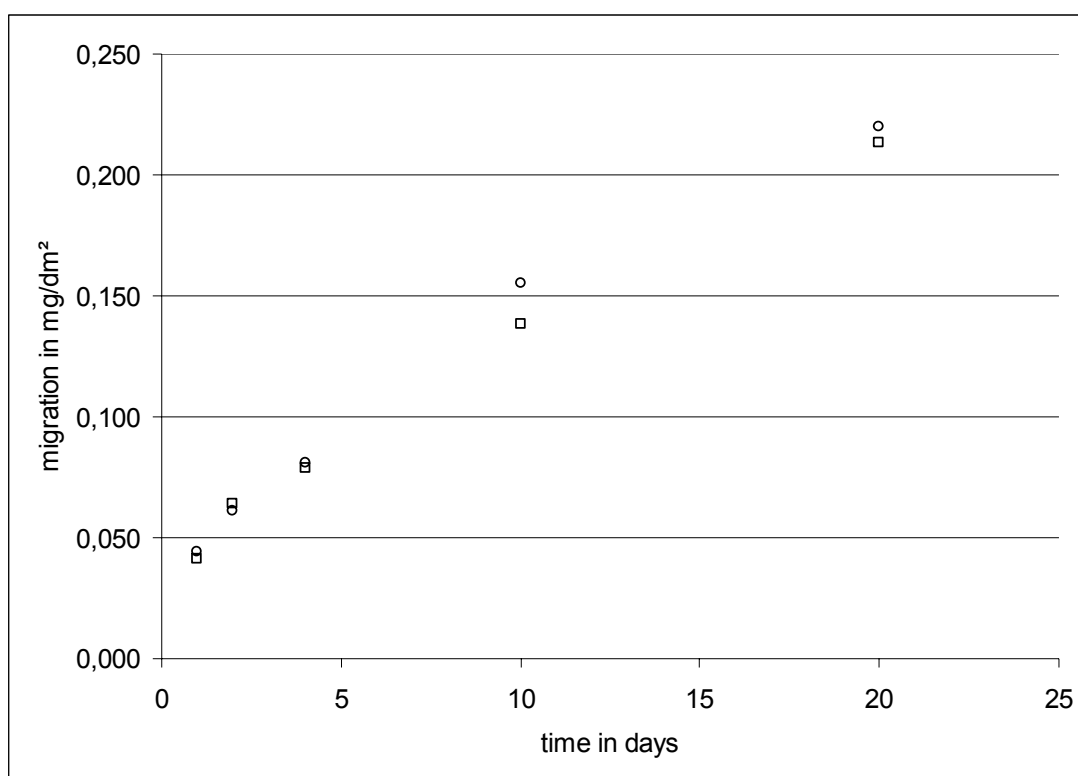
Figure 10 Migration of diphenyl phthalate into apple sauce at 25°C

8.2.3 Milk, UHT, min. 3,5% fat

Test conditions:

Temperature: 5°C

Exposure time: 2, 4, 10, 20 and 30 days

Exposure type: one sided using wide mouth jar

Sample amount: 15 millilitres

Exposure area: 0,0855 dm^2

Table 21 Migration of benzophenone into milk at 5°C

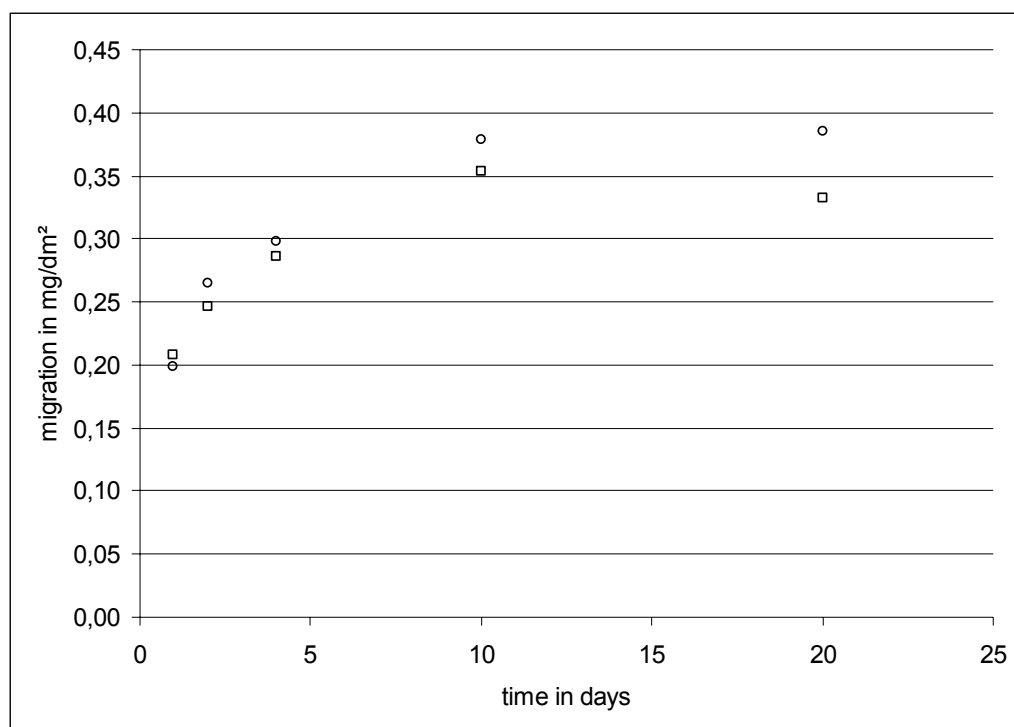| Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,070	0,079	11,00
4	0,123	0,131	18,79
10	0,212	0,191	29,83
20	0,281	0,278	41,44
30	0,373	0,437	59,98
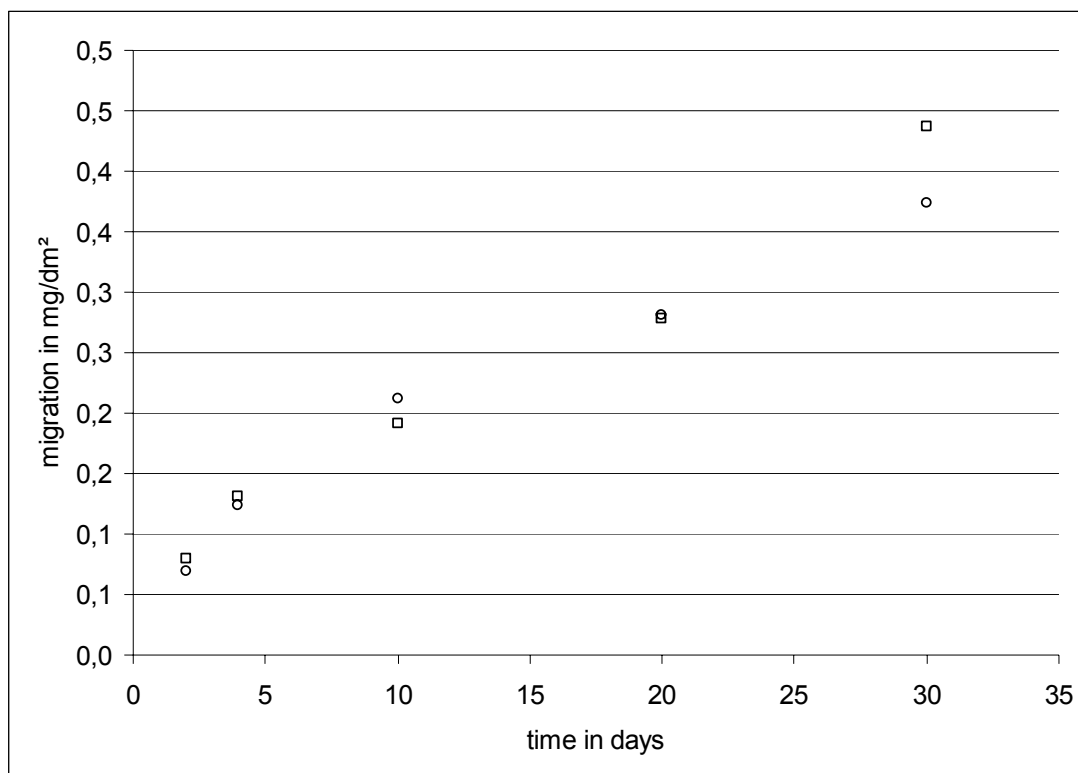
Figure 11 Migration of benzophenone into milk at 5°C

Test conditions:

Temperature: 5°C

Exposure time: 2, 4, 10, 20 and 30 days

Exposure type: one sided using wide mouth jar

Sample amount: 15 millilitres

Exposure area: 0,0855 dm^2

Table 22 Migration of diphenyl phthalate into milk at 5°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,018	0,019	2,20
4	0,026	0,025	3,01
10	0,038	0,033	4,22
20	0,049	0,049	5,84
30	0,055	0,065	7,16
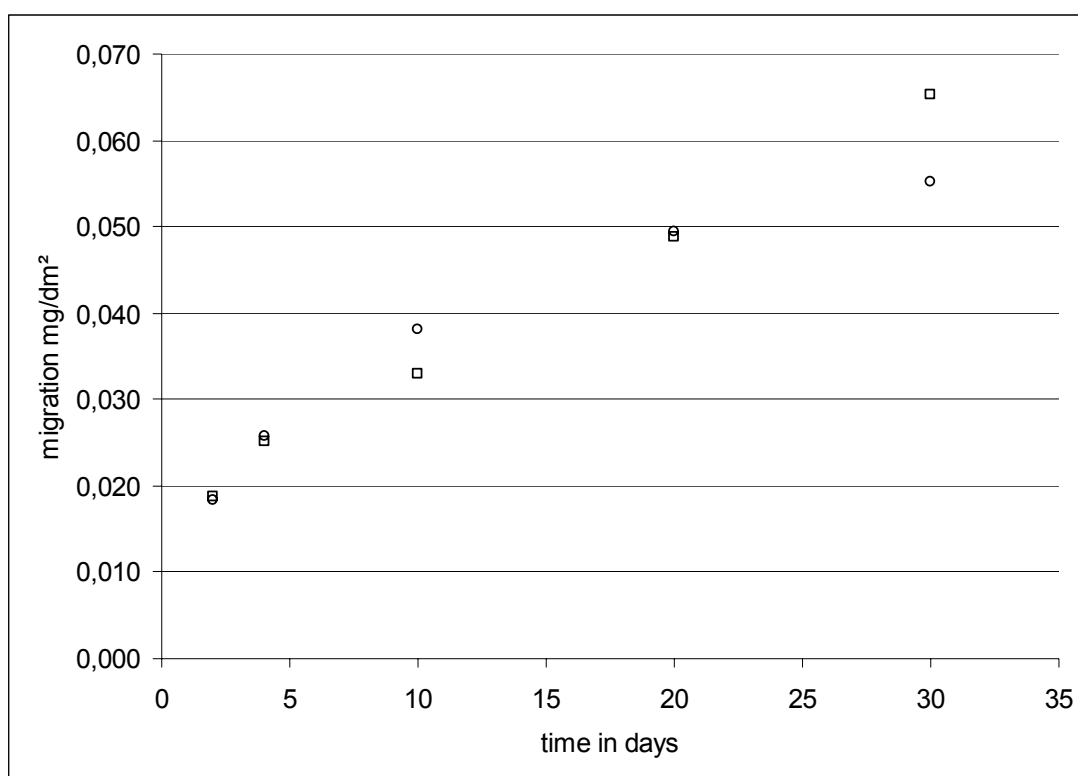
Figure 12 Migration of diphenyl phthalate into milk at 5°C

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided using wide mouth jar

Sample amount: 15 millilitres

Exposure area: 0,0855 dm^2

Table 23 Migration of benzophenone into milk at 25°C

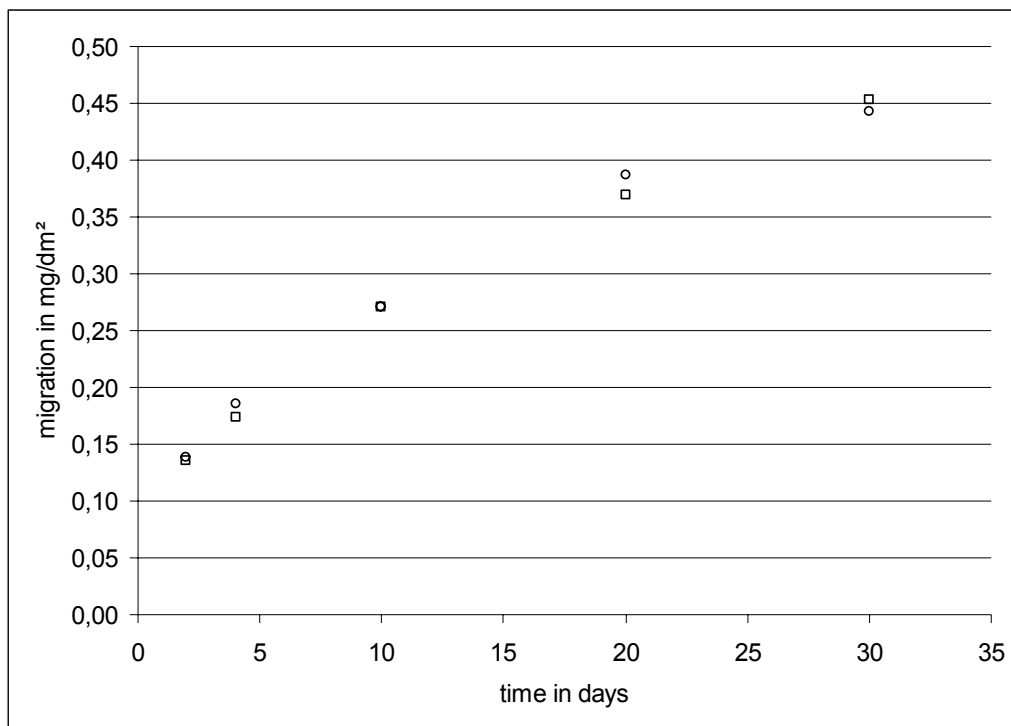| Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,223	0,216	32,47
2	0,331	0,341	49,77
4	0,472	0,453	68,56
10	0,608	0,649	93,12
20	0,451	0,518	71,75
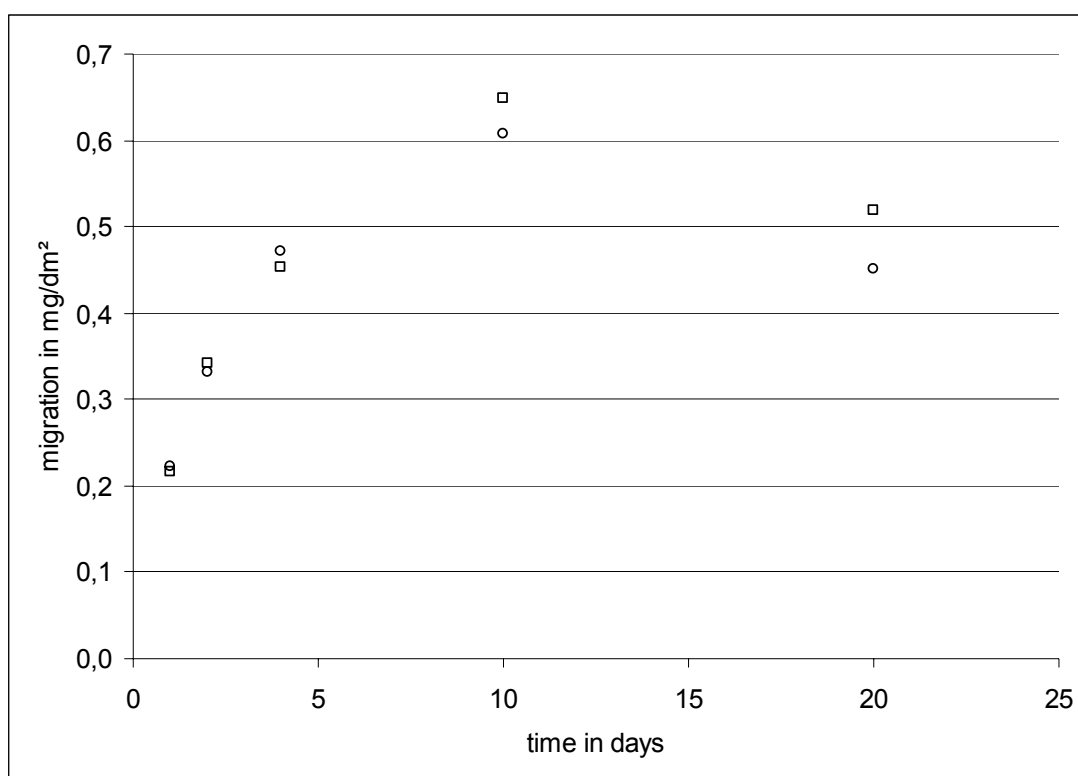
Figure 13 Migration of benzophenone into milk at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided using wide mouth jar

Sample amount: 15 millilitres

Exposure area: 0,0855 dm^2

Table 24 Migration of diphenyl phthalate into milk at 25°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,072	0,100	10,24
2	0,122	0,105	13,52
4	0,140	0,107	14,69
10	0,317	0,156	28,07
20	0,296	0,267	33,46
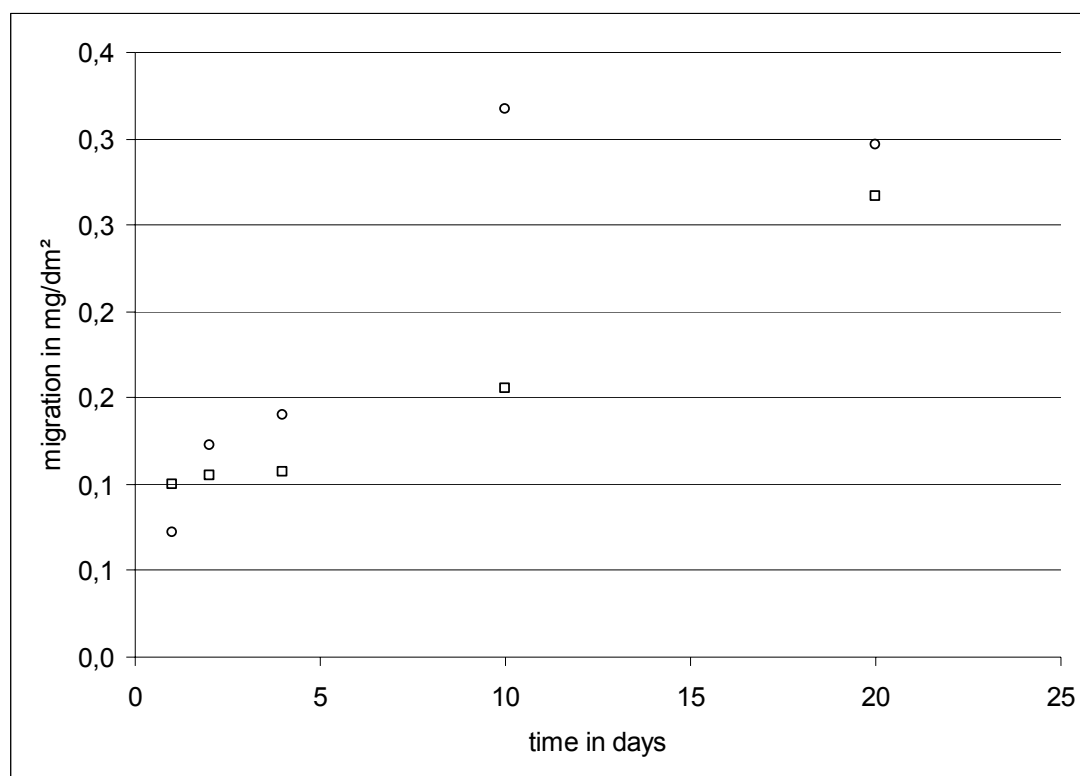
Figure 14 Migration of diphenyl phthalate into milk at 25°C

Test conditions:

Temperature: 40°C

Exposure time: 1, 2, 4, 7 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 15 millilitres

Exposure area: 0,0855 dm^2

Table 25 Migration of benzophenone into milk at 40°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,441	0,363	59,55
2	0,579	0,594	86,89
4	0,585	0,644	91,08
7	0,546	0,669	90,03
10	0,743	0,774	112,32
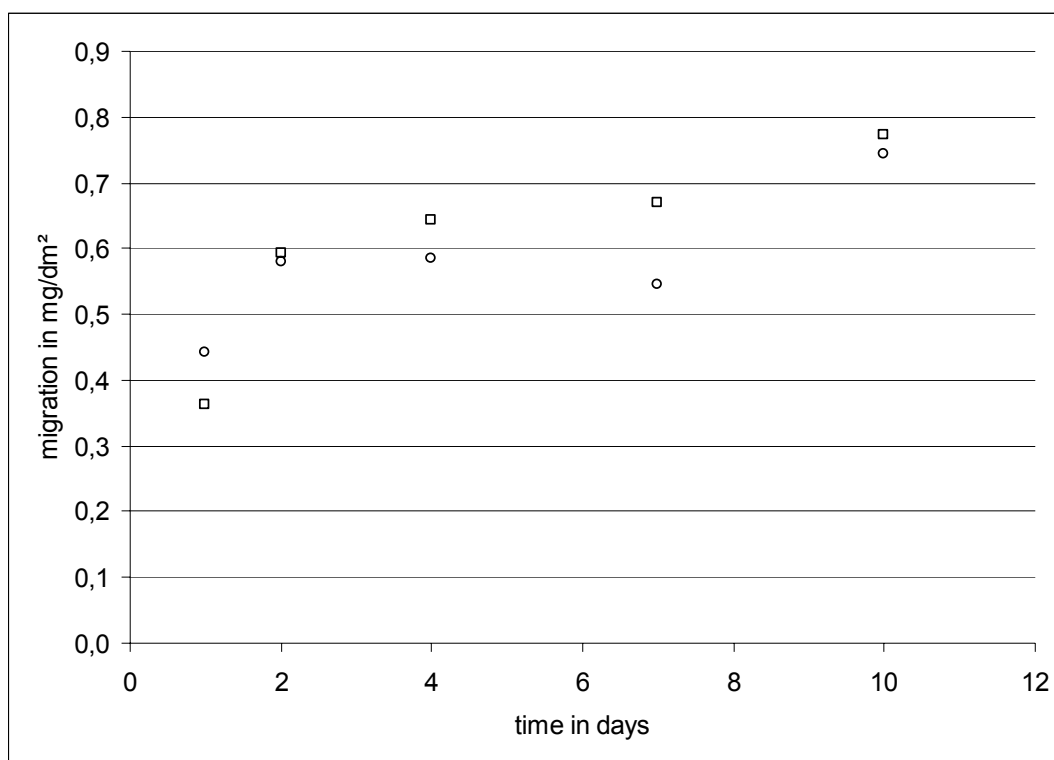
Figure 15 Migration of benzophenone into milk at 40°C

Test conditions:

Temperature: 40°C

Exposure time: 1, 2, 4, 7 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 15 millilitres

Exposure area: 0,0855 dm^2

Table 26 Migration of diphenyl phthalate into milk at 40°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,117	0,128	14,55
2	0,127	0,141	15,93
4	0,264	0,334	35,48
7	0,225	0,563	46,79
10	0,263	0,298	33,34
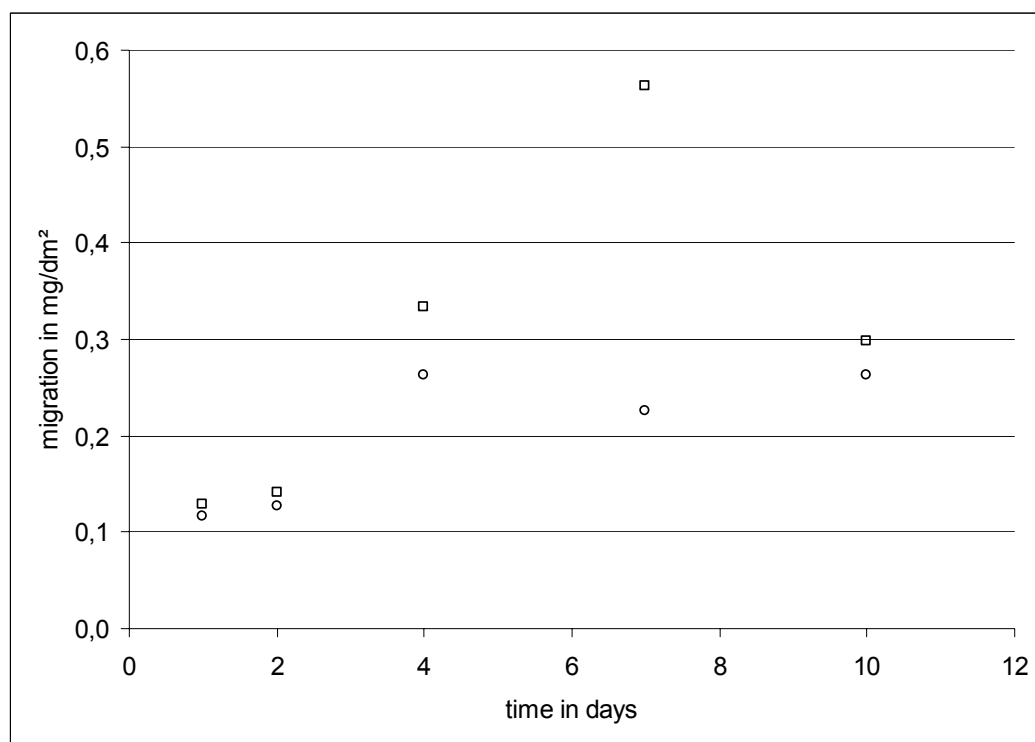
Figure 16 Migration of diphenyl phthalate into milk at 40°C

8.2.4 Ketchup

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 27 Migration of benzophenone into ketchup at 25°C

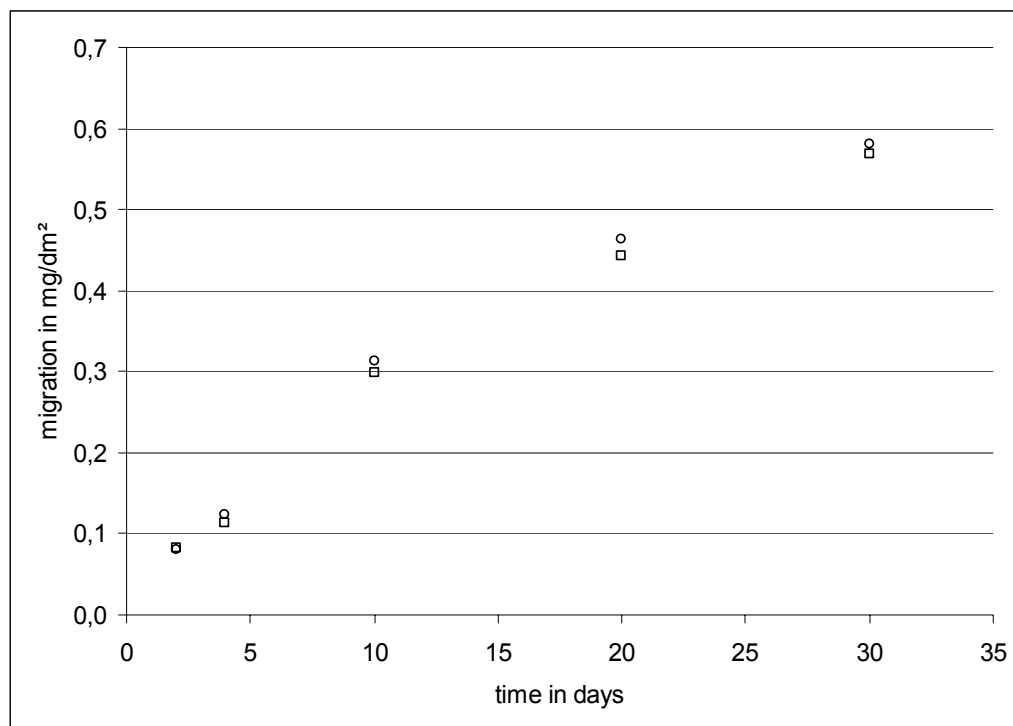| Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,134	0,170	22,52
2	0,280	0,287	42,00
4	0,412	0,412	61,04
10	0,590	0,603	88,37
20	0,664	0,697	100,81
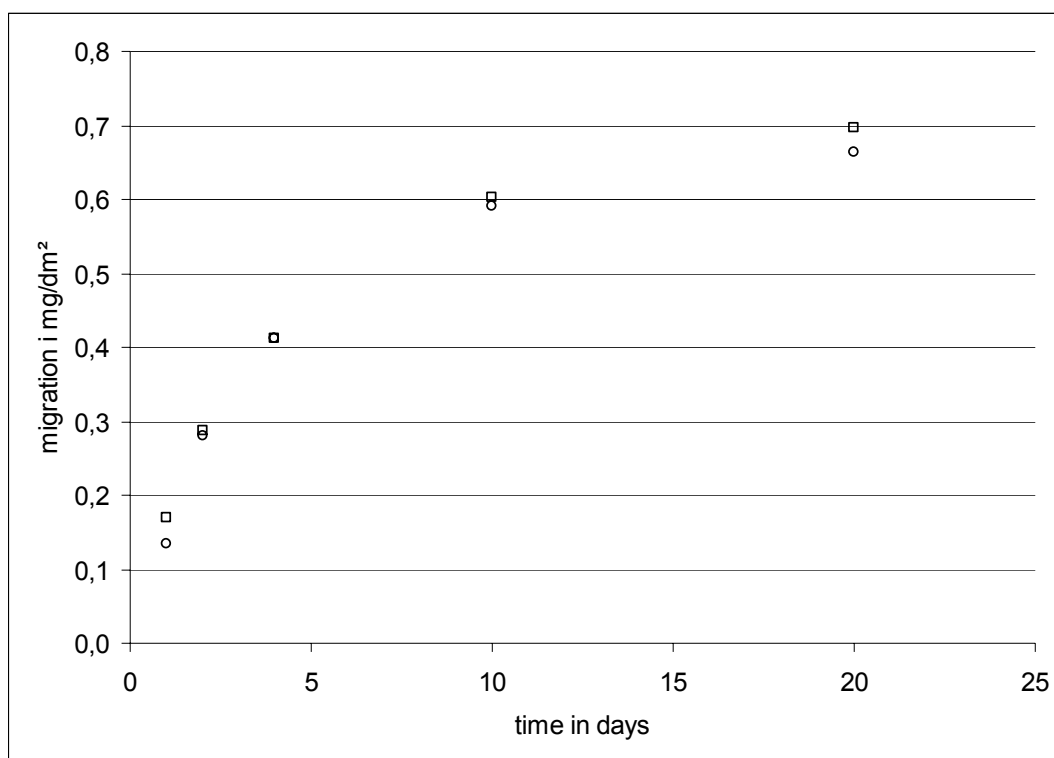
Figure 17 Migration of benzophenone into ketchup at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 28 Migration of diphenyl phthalate into ketchup at 25°C

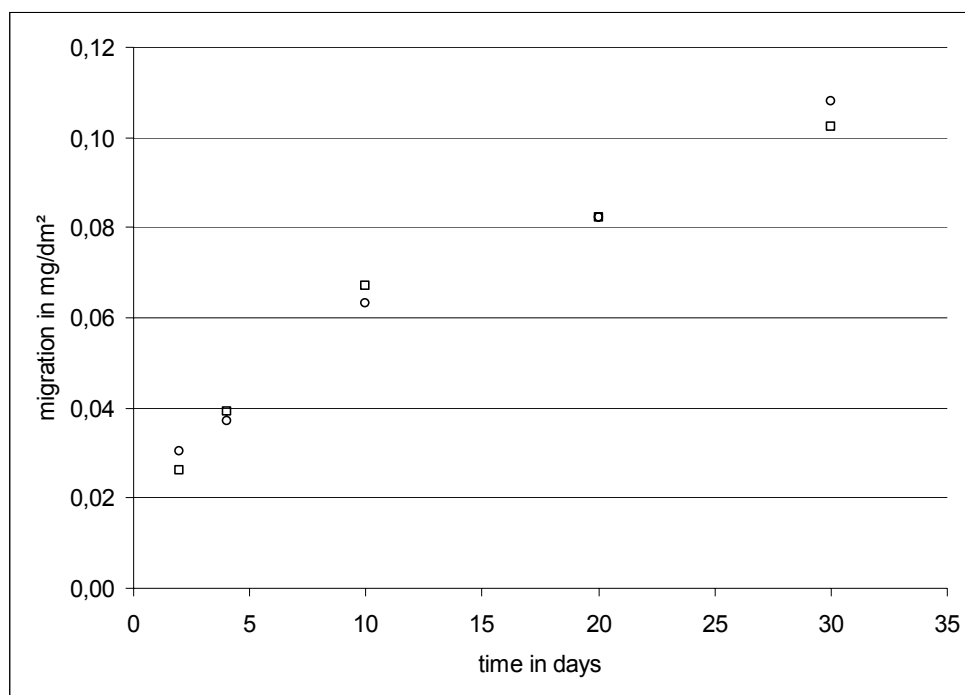| Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,039	0,040	4,69
2	0,055	0,059	6,77
4	0,081	0,082	9,68
10	0,134	0,138	16,15
20	0,176	0,210	22,92
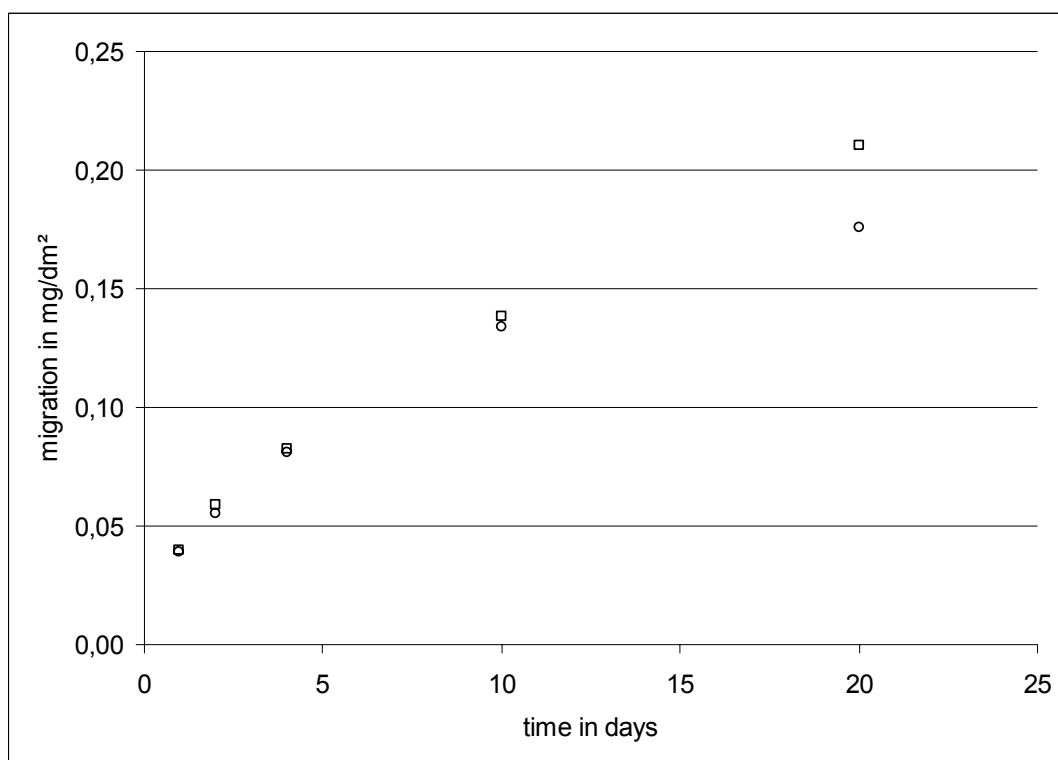
Figure 18 Migration of diphenyl phthalate into ketchup at 25°C

Test conditions:

Temperature: 70°C

Exposure time: 2, 4, 8, 16 and 24 hours

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 29 Migration of benzophenone into ketchup at 70°C

Time of exposure in hours	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,130	0,104	17,30
4	0,280	0,292	42,37
8	0,156	0,170	24,15
16	0,353	0,429	57,93
24	0,135	0,199	24,74
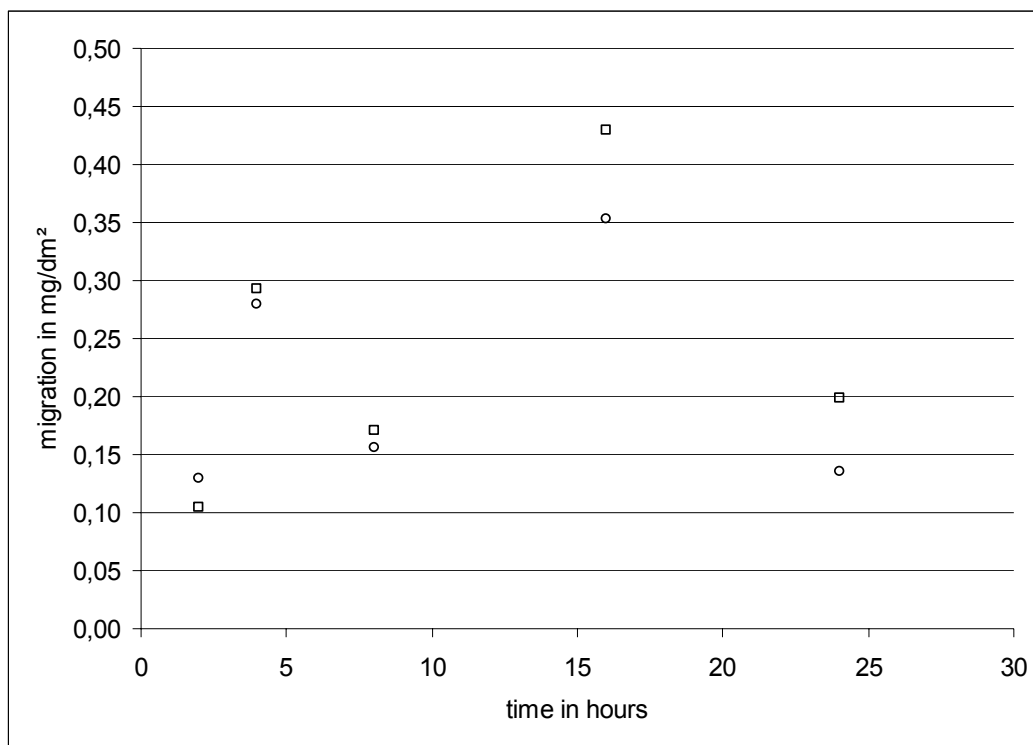
Figure 19 Migration of benzophenone into ketchup at 70°C

Test conditions:

Temperature: 70°C

Exposure time: 2, 4, 8, 16 and 24 hours

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 30 Table Migration of diphenyl phthalate into ketchup at 70°C

Time of exposure in hours	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,064	0,062	7,48
4	0,095	0,085	10,69
8	0,101	0,127	13,54
16	0,209	0,186	23,46
24	0,228	0,214	26,25
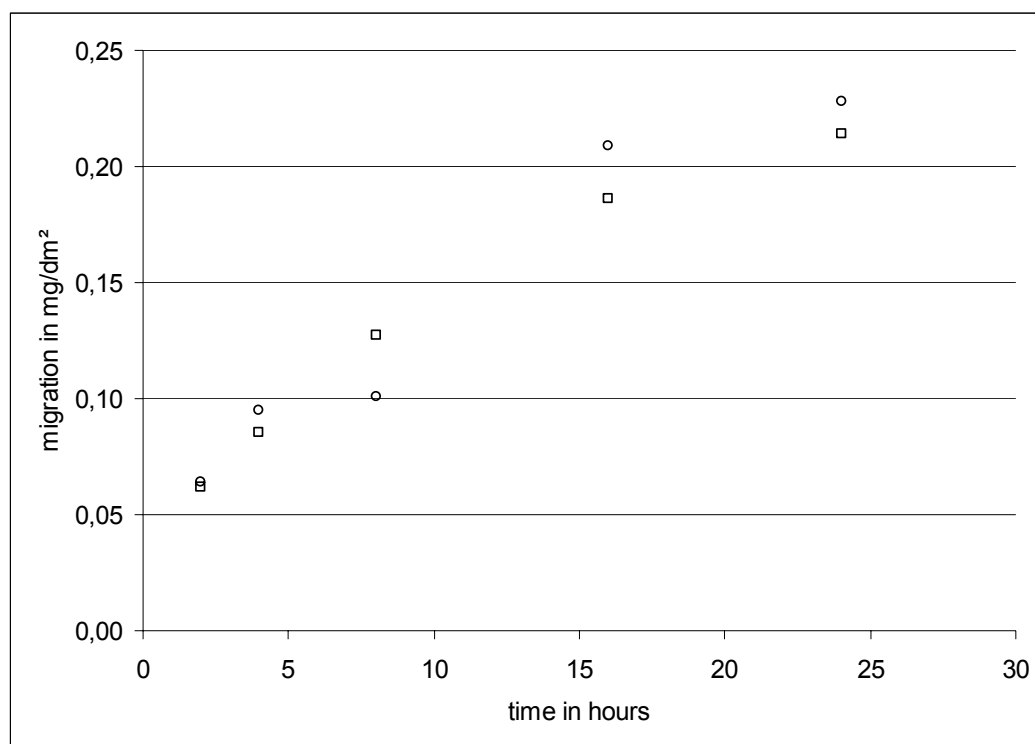
Figure 20 Migration of diphenyl phthalate into ketchup at 70°C

8.2.5 Cola drink

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided using wide mouth jar

Sample amount: 15 millilitres

Exposure area: 0,0855 dm^2

Table 31 Table Migration of benzophenone into cola drink at 25°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,181	0,185	27,11
2	0,164	0,221	28,52
4	0,281	0,288	42,15
10	0,295	0,311	44,89
20	0,291	0,315	44,89

Figure 21 Migration of benzophenone into cola drink at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided using wide mouth jar

Sample amount: 15 millilitres

Exposure area: 0,0855 dm^2

Table 32 Migration of diphenyl phthalate into cola drink at 25°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,034	0,032	3,92
2	0,040	0,040	4,75
4	0,045	0,043	5,23
10	0,051	0,055	6,29
20	0,059	0,032	5,40

Figure 22 Migration of diphenyl phthalate into cola drink at 25°C

Test conditions:

Temperature: 40°C

Exposure time: 1, 2, 4, 7 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 15 millilitres

Exposure area: 0,0855 dm^2

Table 33 Migration of benzophenone into cola drink at 40°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,332	0,318	48,15
2	0,316	0,354	49,63
4	0,265	0,319	43,26
7	0,324	0,365	51,04
10	0,346	0,372	53,19

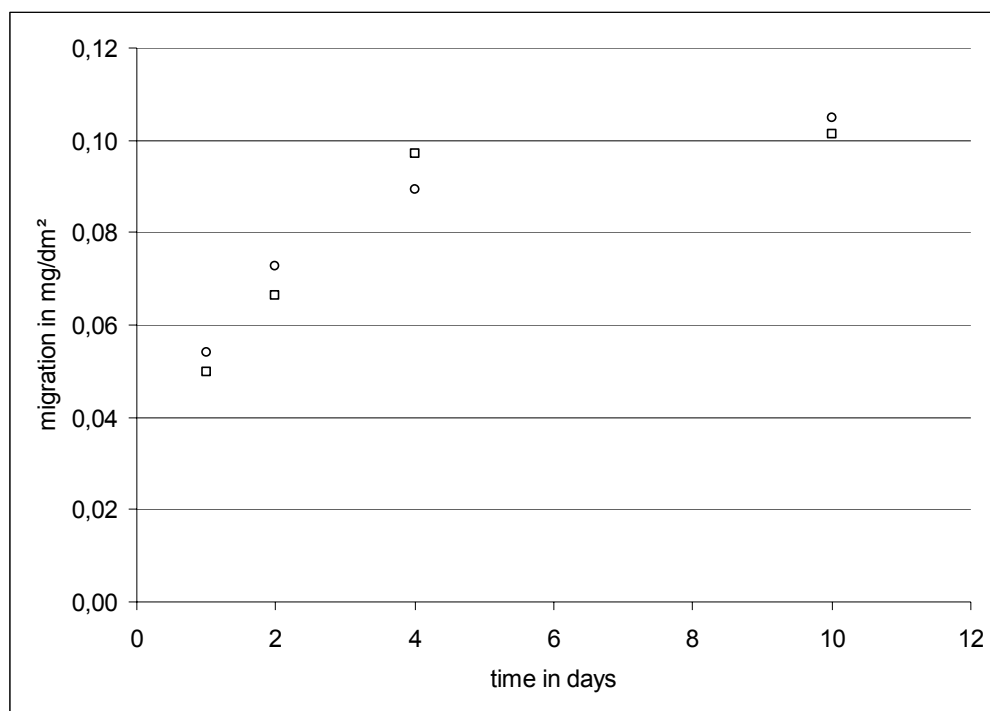**Figure 23 Migration of benzophenone into cola drink at 40°C**

Test conditions:

Temperature: 40°C

Exposure time: 1, 2, 4, 7 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 15 millilitres

Exposure area: 0,0855 dm^2

Table 34 Migration of diphenyl phthalate into cola drink at 40°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,074	0,074	8,79
2	0,085	0,086	10,15
4	0,062	0,063	7,42
7	0,077	0,086	9,68
10	0,074	0,078	9,03

Figure 24 Migration of diphenyl phthalate into cola drink at 40°C

8.2.6 Wine

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided using wide mouth jar

Sample amount: 15 millilitres

Exposure area: 0,0855 dm^2

Table 35 Migration of benzophenone into wine at 25°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,198	0,190	28,74
2	0,283	0,278	41,56
4	0,383	0,369	55,70
10	0,395	0,345	54,81
20	0,431	0,421	63,11

Figure 25 Migration of benzophenone into wine at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided using wide mouth jar

Sample amount: 15 millilitres

Exposure area: 0,0855 dm^2

Table 36 Migration of diphenyl phthalate into wine at 25°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,040	0,039	4,69
2	0,040	0,040	4,75
4	0,055	0,055	6,53
10	0,064	0,065	7,66
20	0,071	0,076	8,73
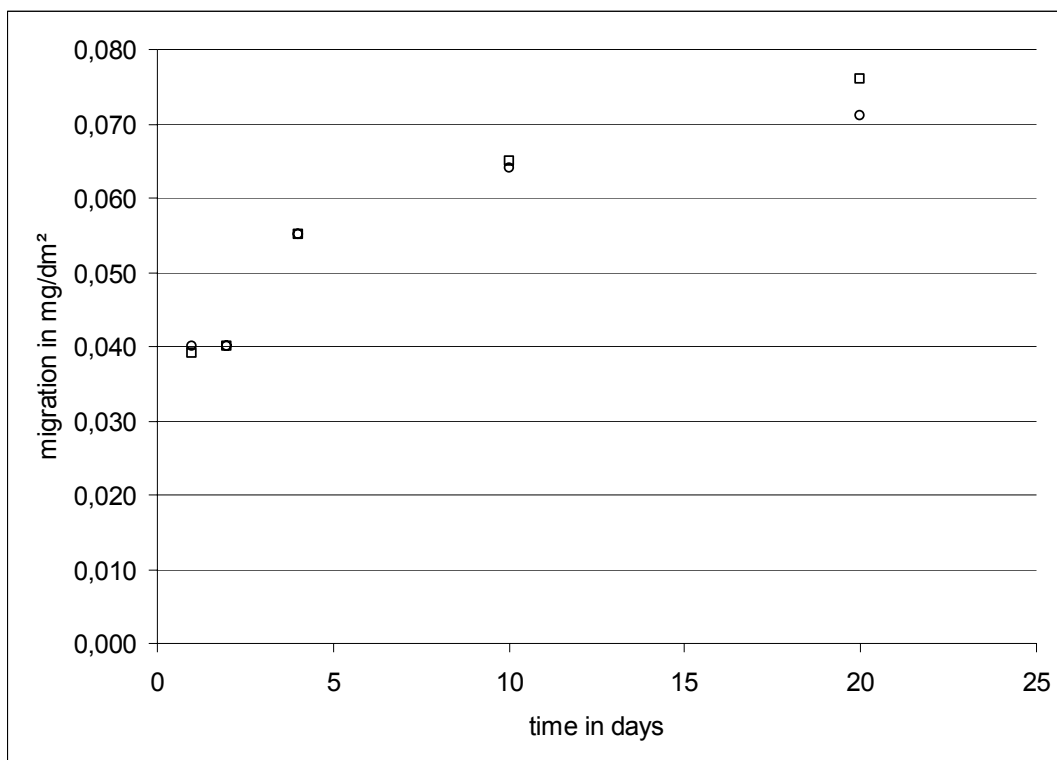
Figure 26 Migration of diphenyl phthalate into wine at 25°C

8.2.7 Beer

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided using wide mouth jar

Sample amount: 15 millilitres

Exposure area: 0,0855 dm^2

Table 37 Migration of benzophenone into beer at 25°C

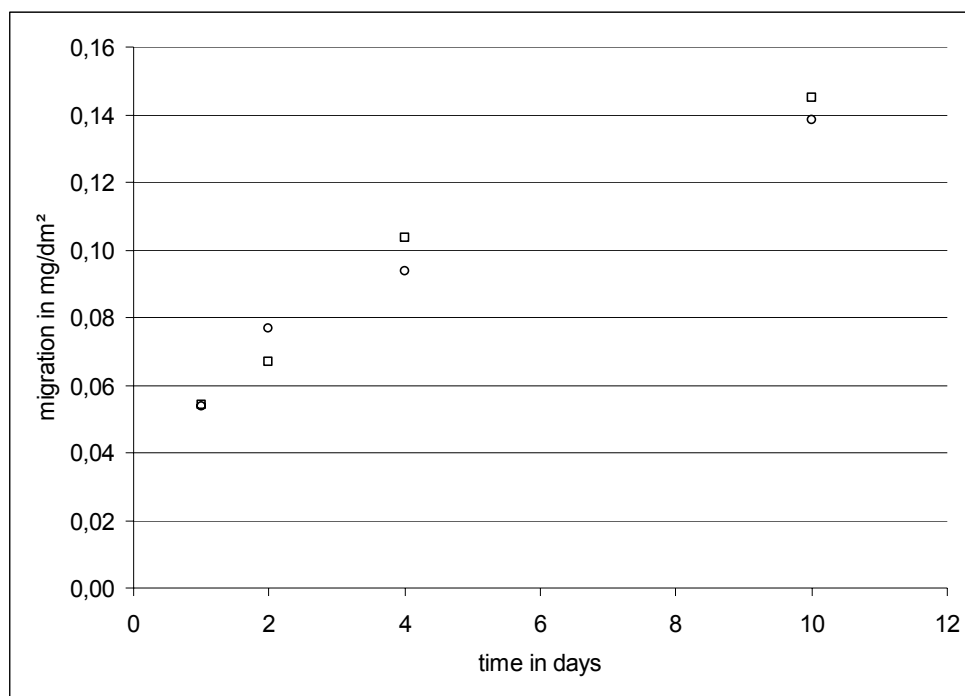| Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,198	0,208	30,07
2	0,265	0,246	37,85
4	0,298	0,286	43,26
10	0,378	0,354	54,22
20	0,385	0,332	53,11

Figure 27 Migration of benzophenone into beer at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided using wide mouth jar

Sample amount: 15 millilitres

Exposure area: 0,0855 dm^2

Table 38 Migration of diphenyl phthalate into beer at 25°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,037	0,035	4,28
2	0,047	0,043	5,34
4	0,056	0,048	6,18
10	0,055	0,052	6,35
20	0,054	0,058	6,65
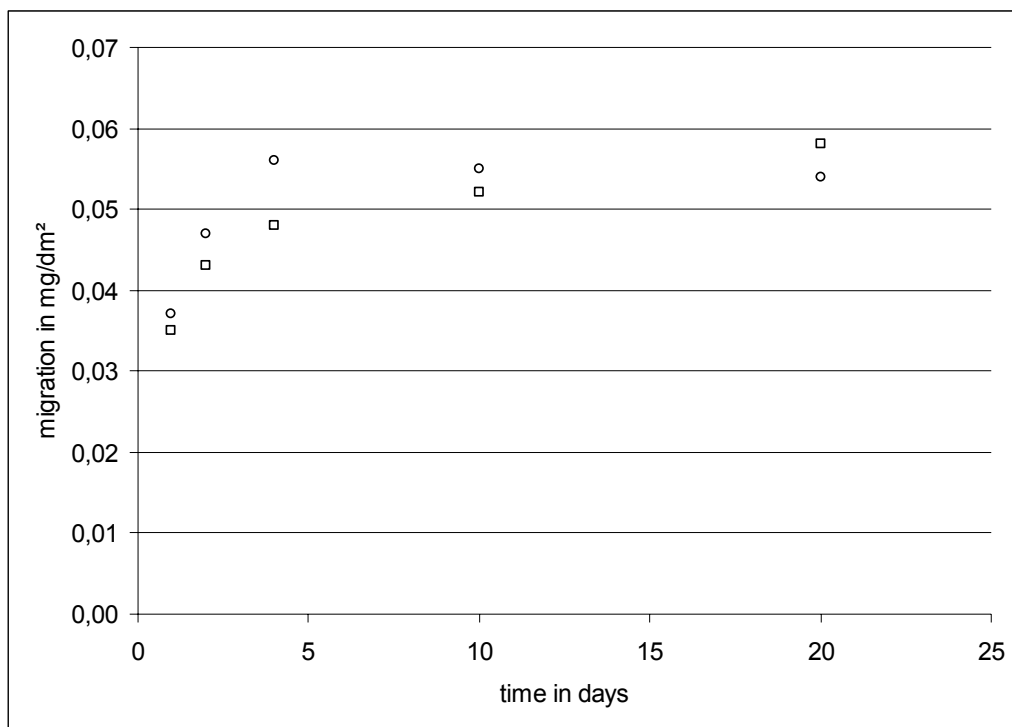
Figure 28 Migration of diphenyl phthalate into beer at 25°C

8.3 Fatty foodstuffs

8.3.1 Cheese sauce (~18,5% fat)

Test conditions:

Temperature: 5°C

Exposure time: 2, 4, 10, 20 and 30 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 39 Migration of benzophenone into cheese sauce at 5°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,138	0,135	20,21
4	0,185	0,174	26,59
10	0,271	0,270	40,07
20	0,386	0,369	55,90
30	0,443	0,453	66,39

Figure 29 Migration of benzophenone into cheese sauce at 5°C

Test conditions:

Temperature: 5°C

Exposure time: 2, 4, 10, 20 and 30 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 40 Migration of diphenyl phthalate into cheese sauce at 5°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,057	0,053	6,55
4	0,062	0,064	7,49
10	0,072	0,072	8,53
20	0,087	0,086	10,27
30	0,104	0,097	11,92
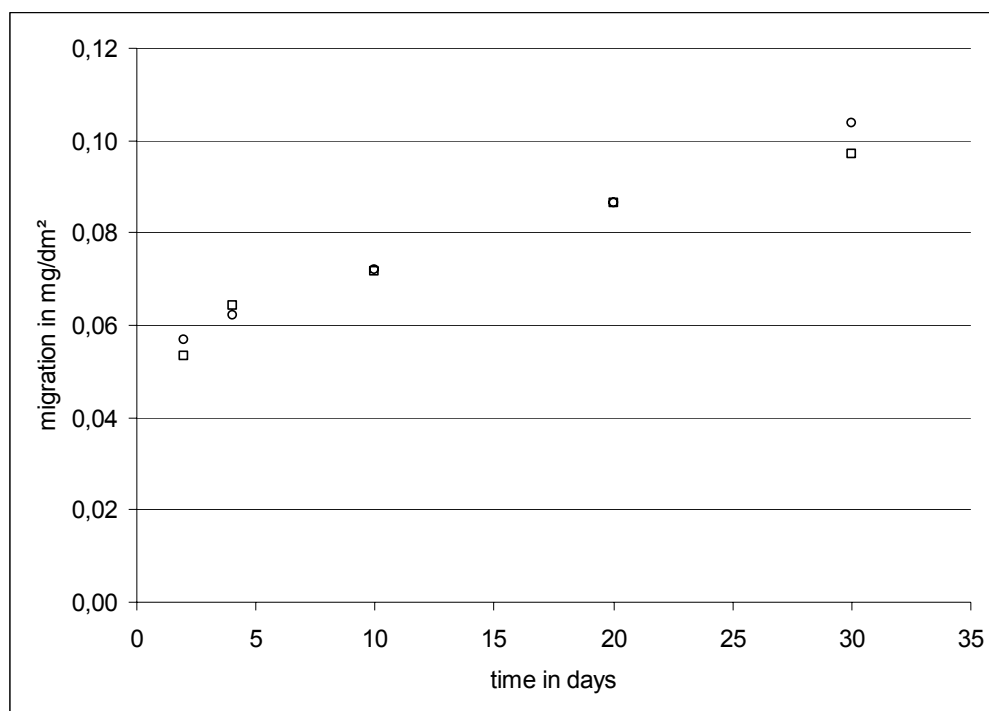
Figure 30 Migration of diphenyl phthalate into cheese sauce at 5°C

Test conditions:

Temperature: 90°C

Exposure time: 5, 10, 30, 60 and 120 minutes

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 41 Migration of benzophenone into cheese sauce at 90°C

Time of exposure in minutes	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
5	0,148	0,118	19,73
10	0,163	0,140	22,47
30	0,259	0,289	40,55
60	0,351	0,319	49,61
120	0,325	0,367	51,30
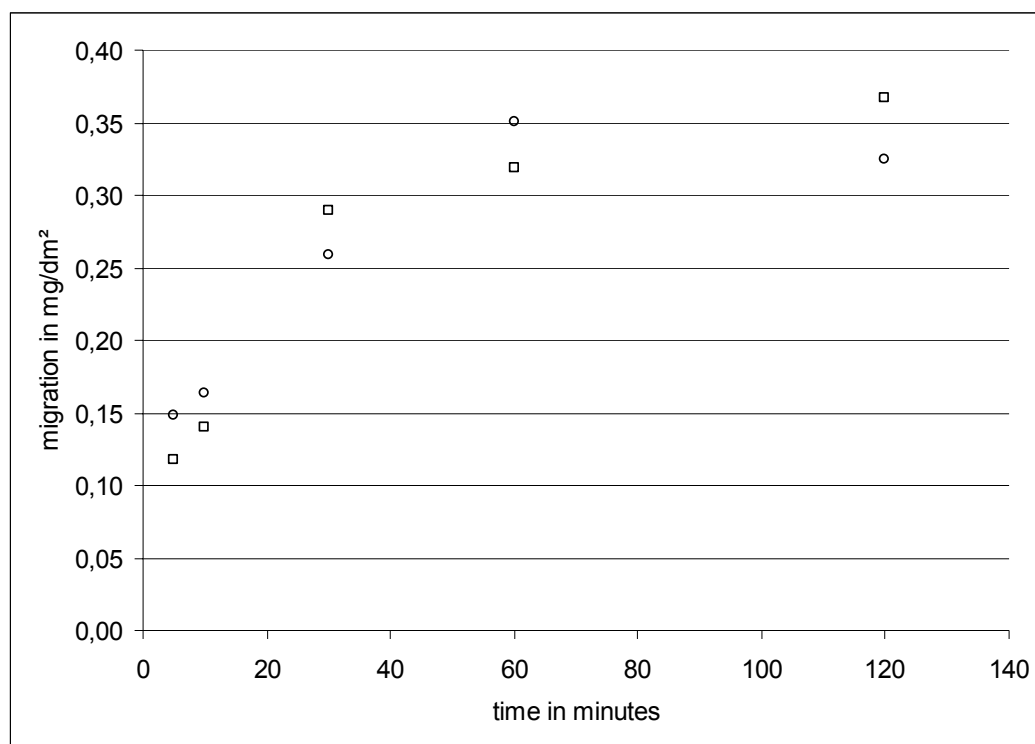
Figure 31 Migration of benzophenone into cheese sauce at 90°C

Test conditions:

Temperature: 90°C

Exposure time: 5, 10, 30, 60 and 120 minutes

Exposure type: one sided using wide mouth jar

Sample amount:14,25 gram

Exposure area: 0,0855 dm^2

Table 42 Migration of diphenyl phthalate into cheese sauce at 90°C

Time of exposure in minutes	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
5	0,118	0,101	13,01
10	0,149	0,113	15,52
30	0,238	0,210	26,61
60	0,326	0,415	43,99
120	0,577	0,503	64,15
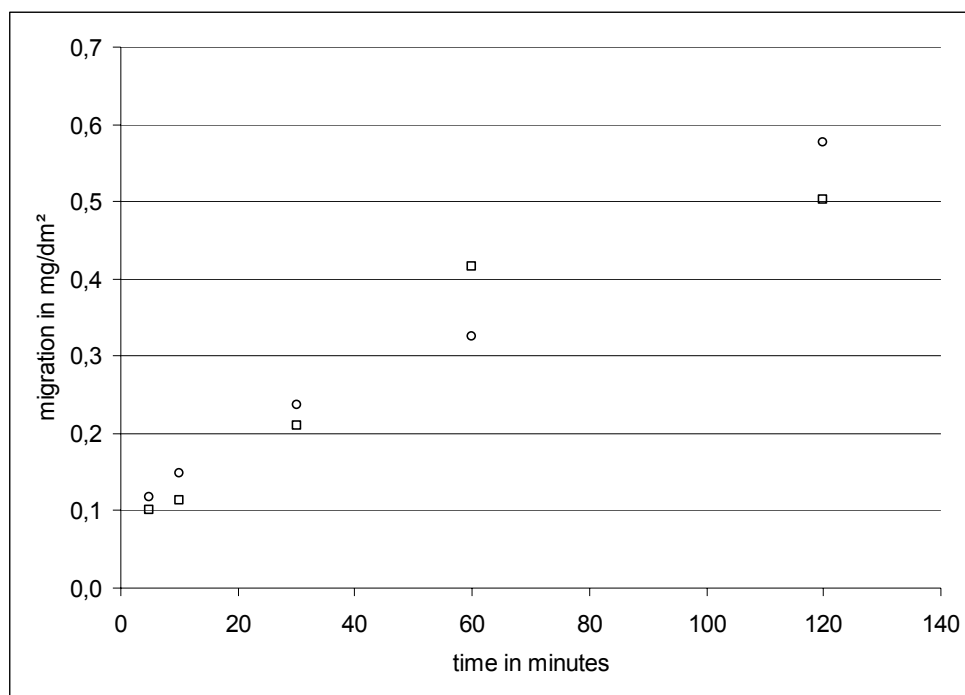
Figure 32 Migration of diphenyl phthalate into cheese sauce at 90°C

8.3.2 Mayonnaise (80% fat content)

Test conditions:

Temperature: 5°C

Exposure time: 2, 4, 10, 20 and 30 days

Exposure type: one sided using wide mouth jar

Sample amount:14,25 gram

Exposure area: 0,0855 dm^2

Table 43 Migration of benzophenone into mayonnaise at 5°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,081	0,082	12,09
4	0,123	0,112	17,46
10	0,313	0,298	45,27
20	0,463	0,442	66,99
30	0,580	0,568	85,00

Figure 33 Migration of benzophenone into mayonnaise at 5°C

Test conditions:

Temperature: 5°C

Exposure time: 2, 4, 10, 20 and 30 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 44 Migration of diphenyl phthalate into mayonnaise at 5°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,030	0,026	3,35
4	0,037	0,039	4,52
10	0,063	0,067	7,74
20	0,082	0,082	9,75
30	0,108	0,102	12,48

Figure 34 Migration of diphenyl phthalate into mayonnaise at 5°C

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 45 Migration of benzophenone into mayonnaise at 25°C

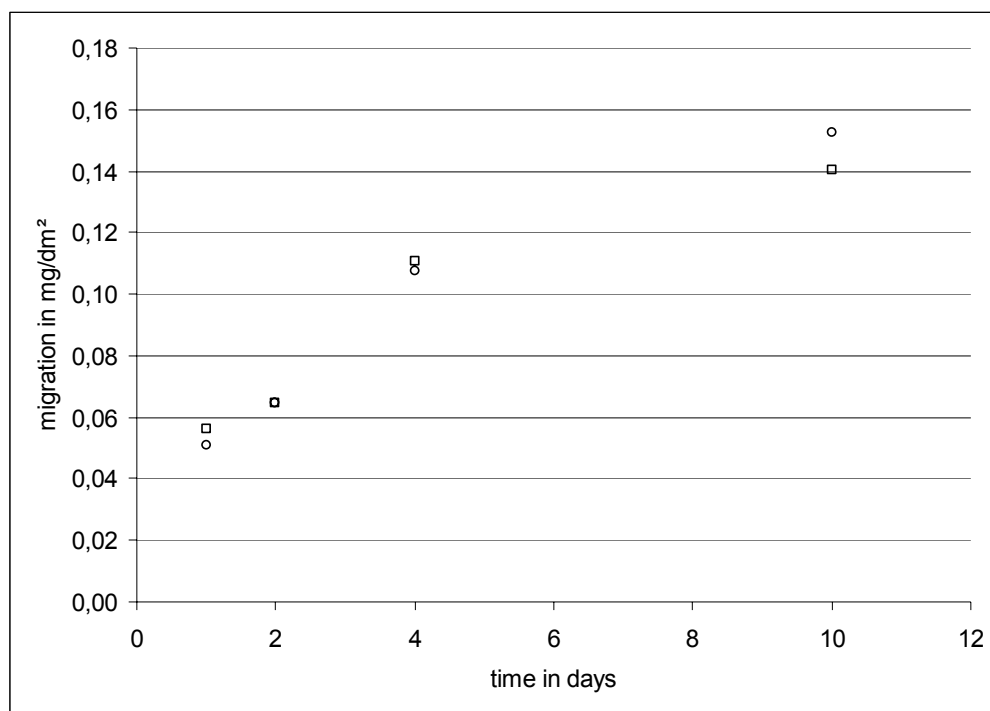| Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,272	0,272	40,29
2	0,344	0,357	51,90
4	0,452	0,510	71,27
10	0,608	0,675	95,04
20	0,785	0,696	109,72
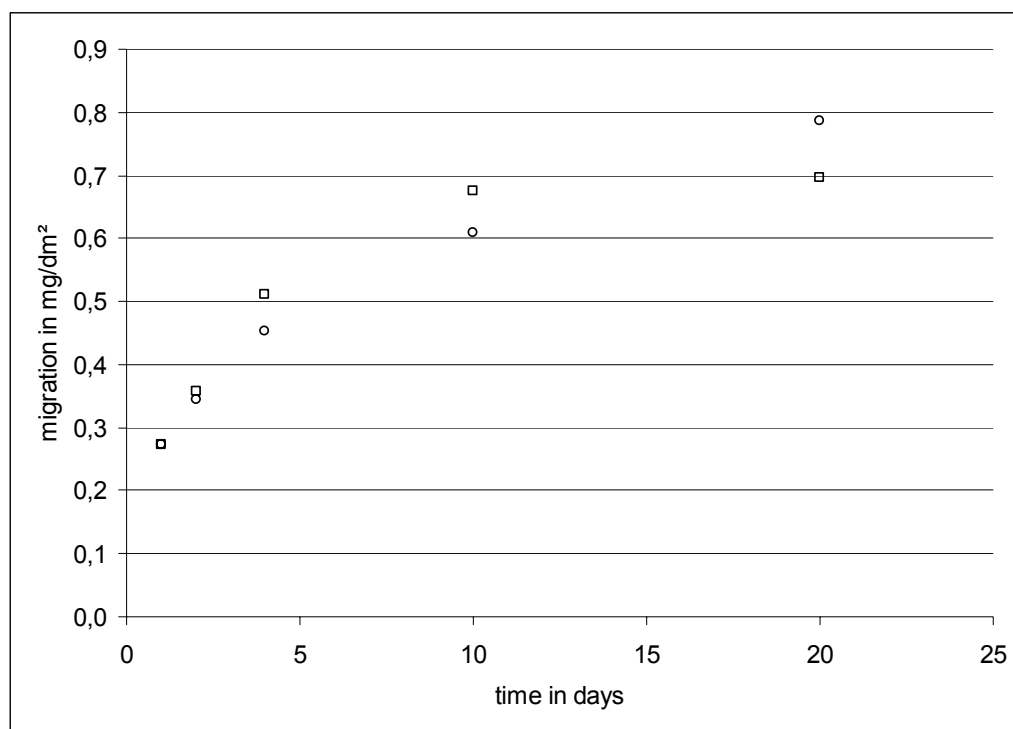
Figure 35 Migration of benzophenone into mayonnaise at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 46 Migration of diphenyl phthalate into mayonnaise at 25°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm² in sample A	Diphenyl phthalate migration in mg/dm² in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,088	0,087	10,38
2	0,134	0,128	15,57
4	0,168	0,180	20,72
10	0,307	0,294	35,69
20	0,440	0,383	48,86
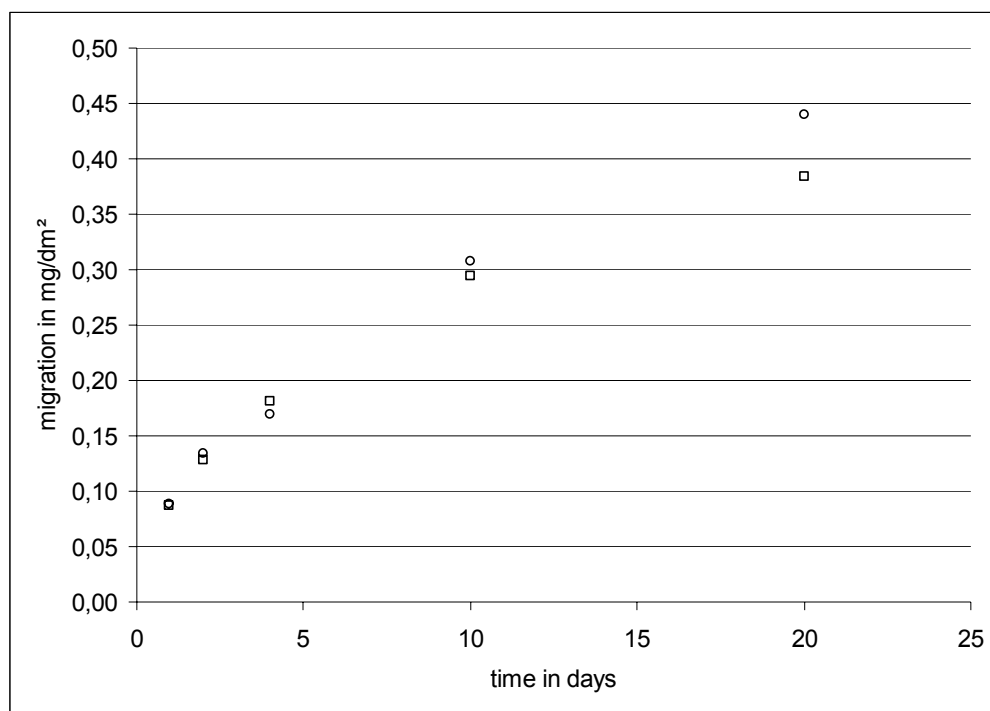
Figure 36 Migration of diphenyl phthalate into mayonnaise at 25°C

8.3.3 Yoghurt drink

Temperature: 5°C

Exposure time: 2, 4, 10, 20 and 30 days

Exposure type: one sided using wide mouth jar

Sample amount: 15 millilitres

Exposure area: 0,0855 dm^2

Table 47 Migration of benzophenone into yoghurt drink at 5°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,078	0,072	11,11
4	0,109	0,115	16,57
10	0,178	0,163	25,28
20	0,270	0,253	38,73
30	0,285	0,225	37,77
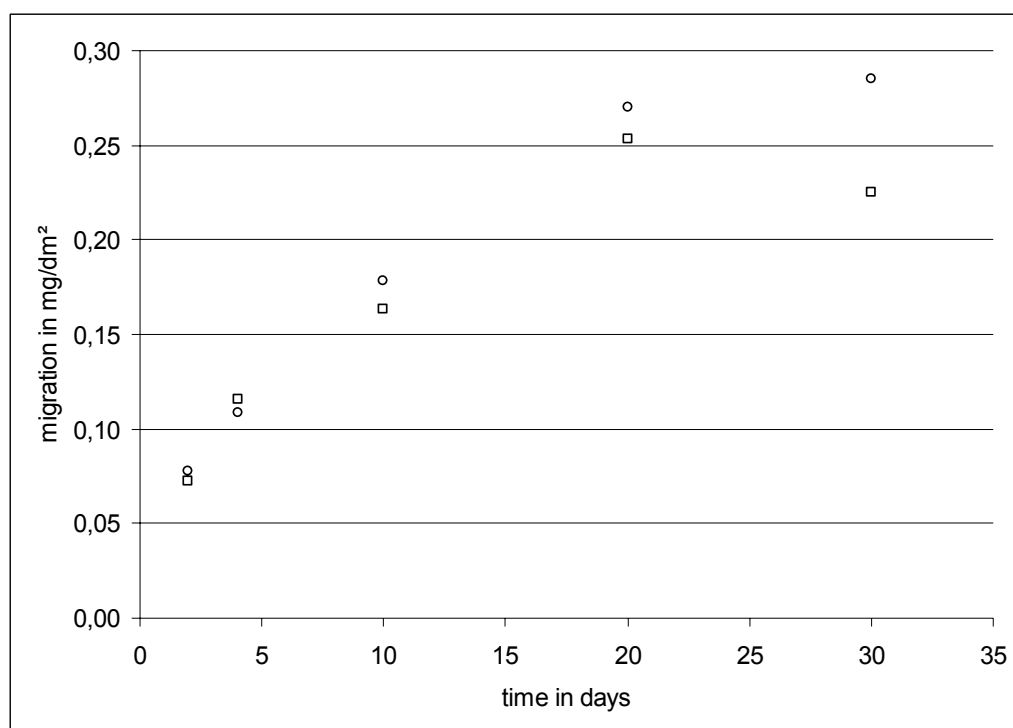
Figure 37 Migration of benzophenone into yoghurt drink at 5°C

Temperature: 5°C

Exposure time: 2, 4, 10, 20 and 30 days

Exposure type: one sided using wide mouth jar

Sample amount: 15 millilitres

Exposure area: 0,0855 dm^2

Table 48 Migration of diphenyl phthalate into yoghurt drink at 5°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,028	0,021	2,92
4	0,028	0,032	3,55
10	0,036	0,034	4,18
20	0,056	0,054	6,49
30	0,059	0,059	7,00
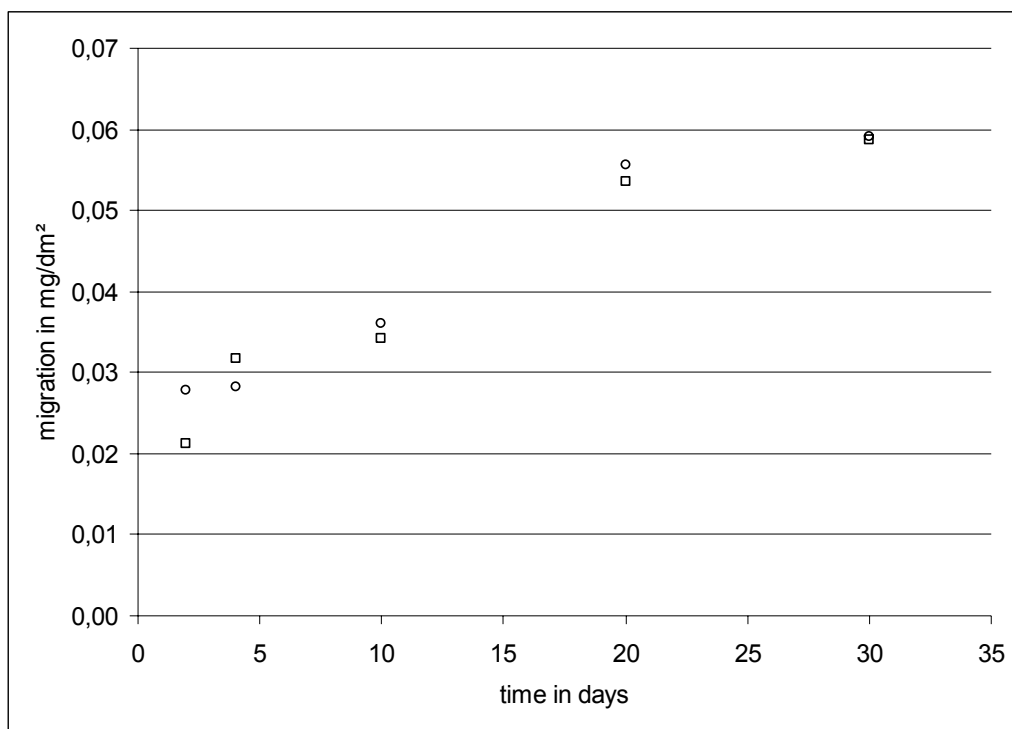
Figure 38 Migration of diphenyl phthalate into yoghurt drink at 5°C

8.3.4 Meat, lean pork (minced, fat content ≤5%)

Test conditions:

Temperature: 5°C

Exposure time: 1, 2, 4 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 49 Migration of benzophenone into minced meat (<5% fat) at 5°C

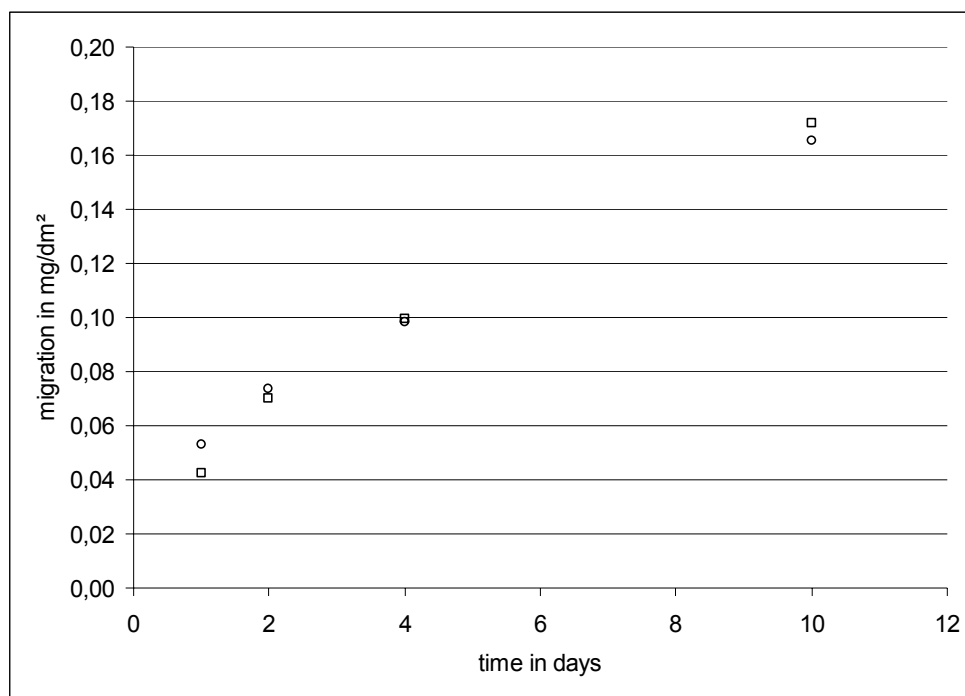| Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,054	0,050	7,67
2	0,073	0,066	10,28
4	0,089	0,097	13,82
10	0,105	0,101	15,28

Figure 39 Migration of benzophenone into minced meat (<5% fat) at 5°C

Test conditions:

Temperature: 5°C

Exposure time: 1, 2, 4 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount:14,25 gram

Exposure area: 0,0855 dm^2

Table 50 Migration of diphenyl phthalate into minced meat (<5% fat) at 5°C

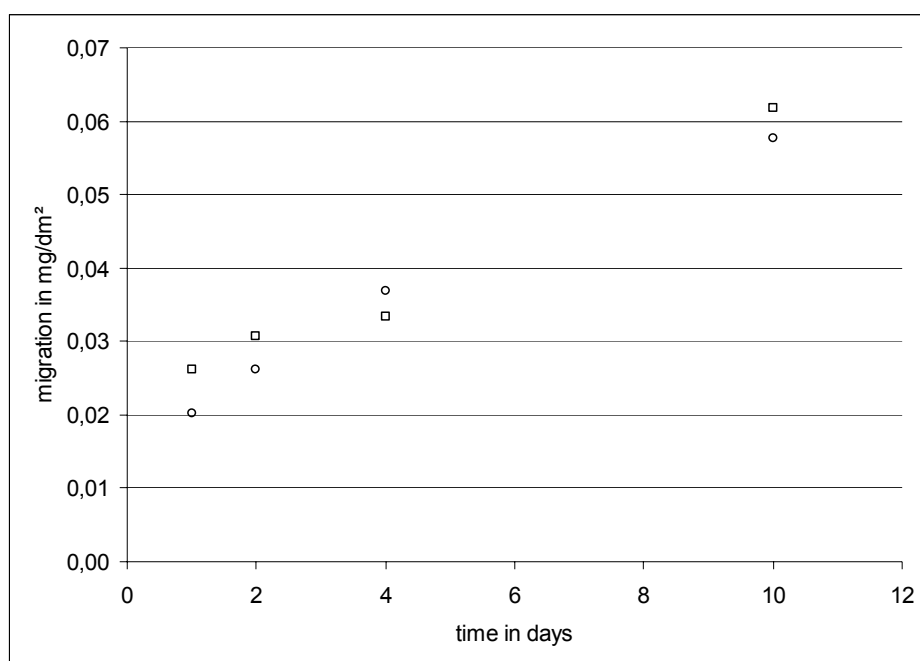| Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,045	0,022	3,98
2	0,020	0,043	3,74
4	0,025	0,025	2,97
10	0,019	0,018	2,20
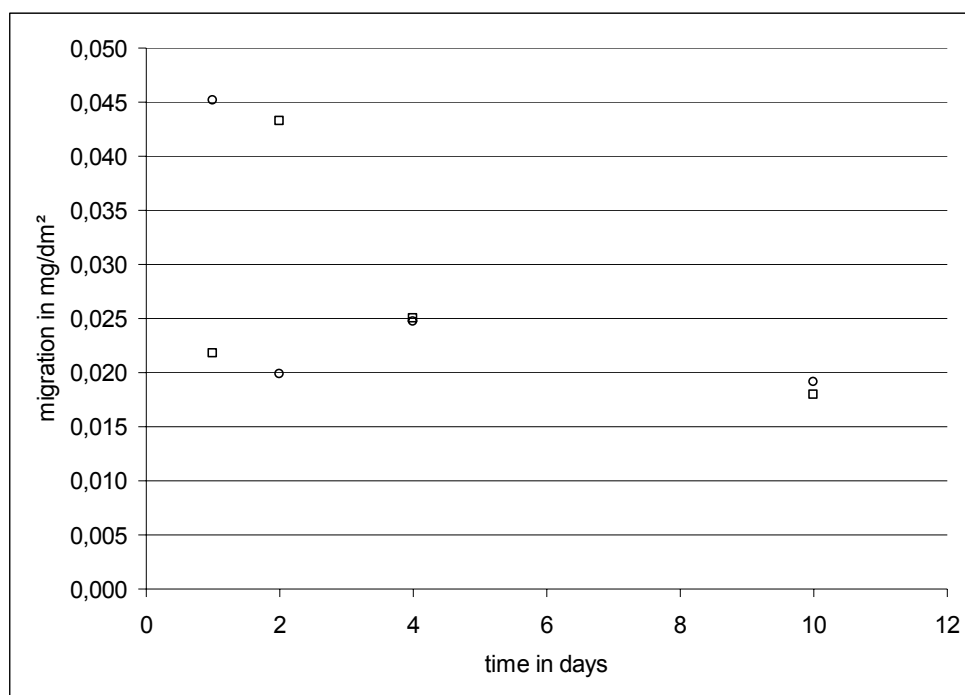
Figure 40 Migration of diphenyl phthalate into minced meat (<5% fat) at 5°C

Test conditions:

Temperature: 25°C

Exposure time: 8 and 24 hours

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 51 Migration of benzophenone into minced meat (<5% fat) at 25°C

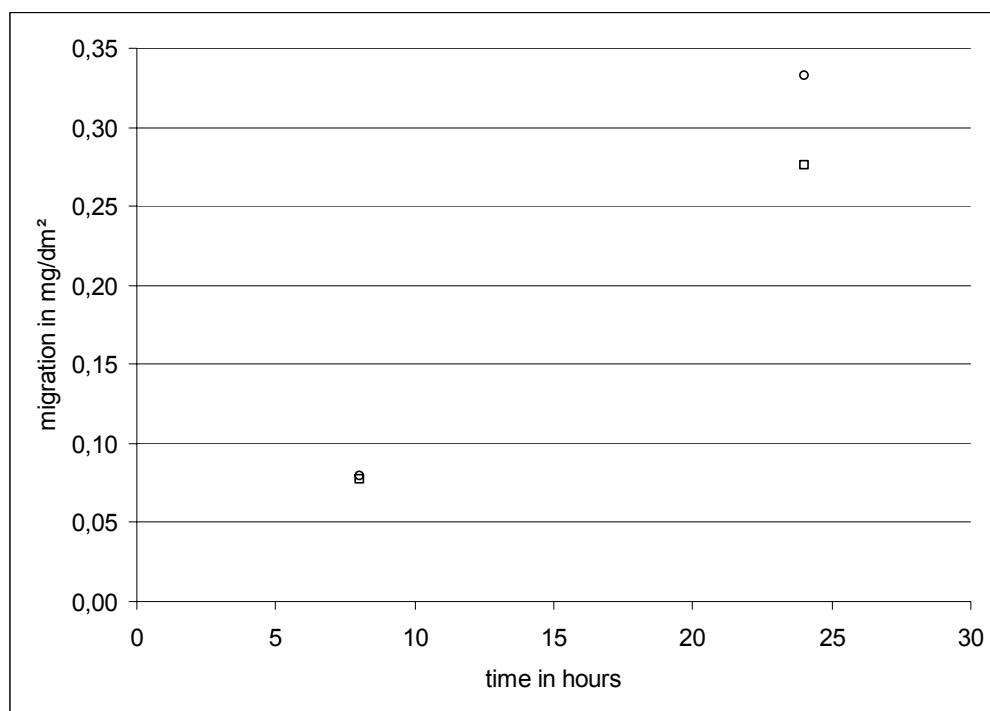| Time of exposure in hours	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
8	0,060	0,056	8,64
24	0,240	0,254	36,58
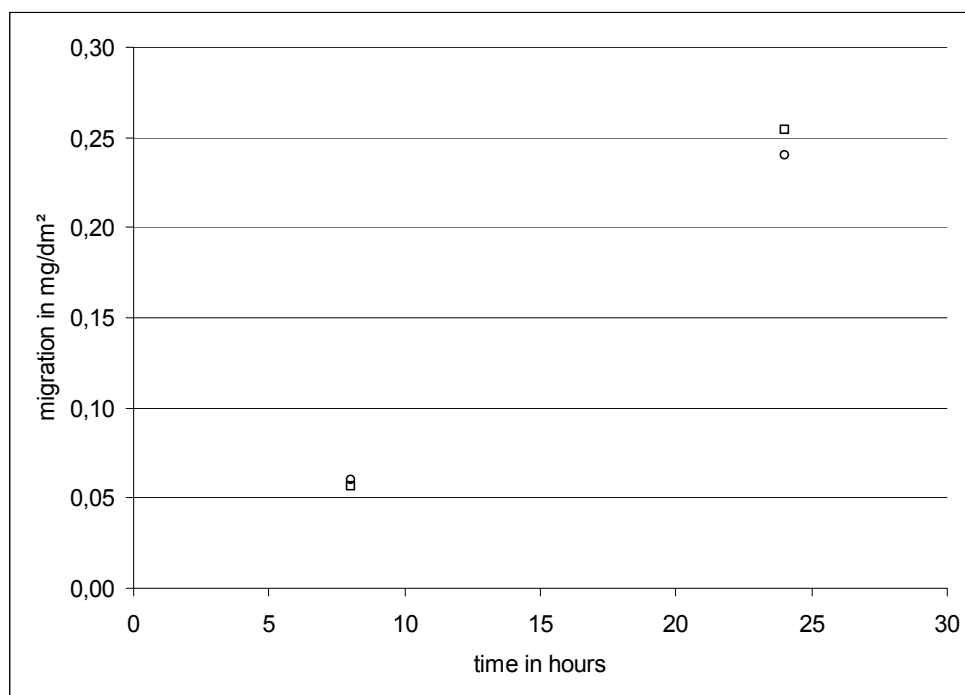
Figure 41 Migration of benzophenone into minced meat (<5% fat) at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 8 and 24 hours

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 52 Migration of diphenyl phthalate into minced meat (<5% fat) at 25°C

Time of exposure in hours	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
8	0,042	0,044	5,11
24	0,046	0,053	5,85
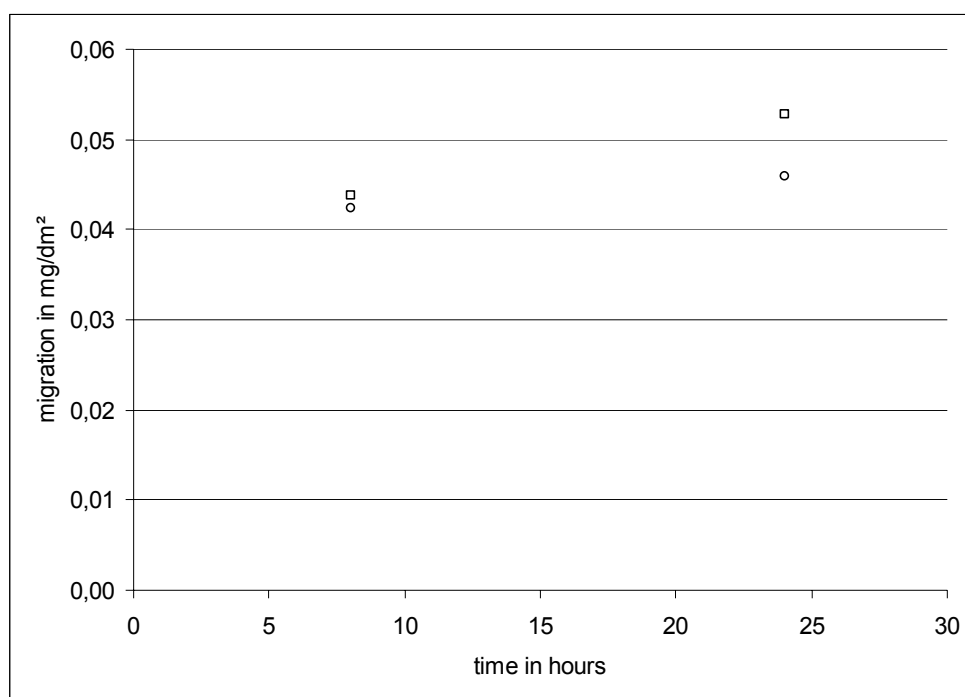
Figure 42 Migration of diphenyl phthalate into minced meat (<5% fat) at 25°C

8.3.5 Meat, lean pork (minced, fat content approx. 10%)

Test conditions:

Temperature: 5°C

Exposure time: 1, 2, 4 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 53 Migration of benzophenone into minced meat (approx. 10% fat) at 5°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,054	0,054	7,97
2	0,076	0,067	10,62
4	0,094	0,104	14,60
10	0,139	0,145	21,00

Figure 43 Migration of benzophenone into minced meat (approx. 10% fat) at 5°C

Test conditions:

Temperature: 5°C

Exposure time: 1, 2, 4 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount:14,25 gram

Exposure area: 0,0855 dm^2

Table 54 Migration of diphenyl phthalate into minced meat (approx. 10% fat) at 5°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,017	0,016	1,96
2	0,018	0,025	2,53
4	0,025	0,025	3,00
10	0,025	0,029	3,17
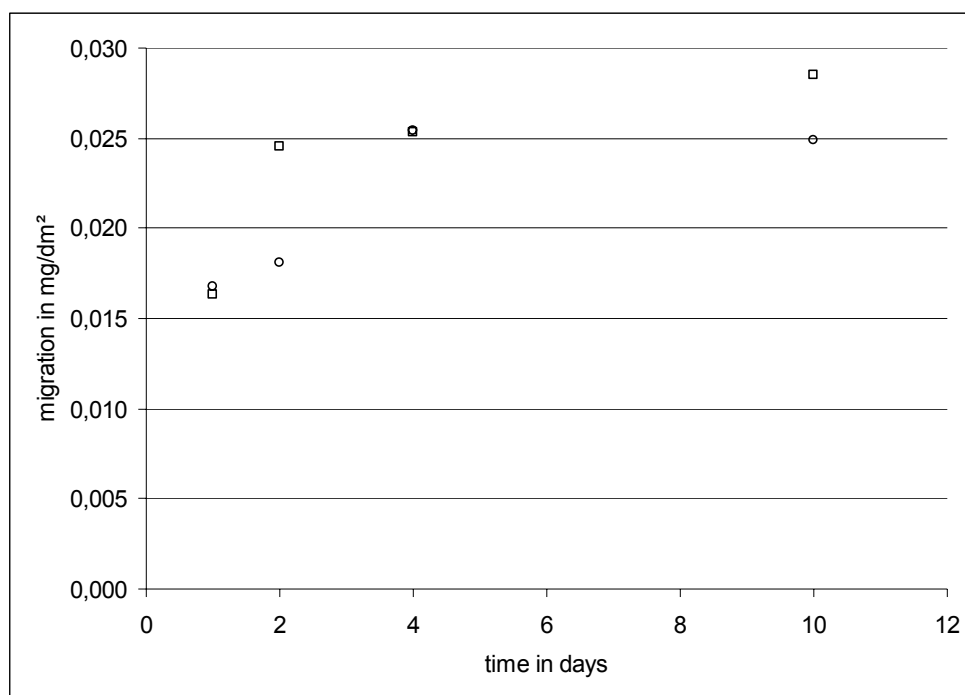
Figure 44 Migration of diphenyl phthalate into minced meat (approx. 10% fat) at 5°C

Test conditions:

Temperature: 25°C

Exposure time: 8 and 24 hours

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 55 Migration of benzophenone into minced meat (approx. 10% fat) at 25°C

Time of exposure in hours	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
8	0,060	0,055	8,53
24	0,243	0,247	36,27
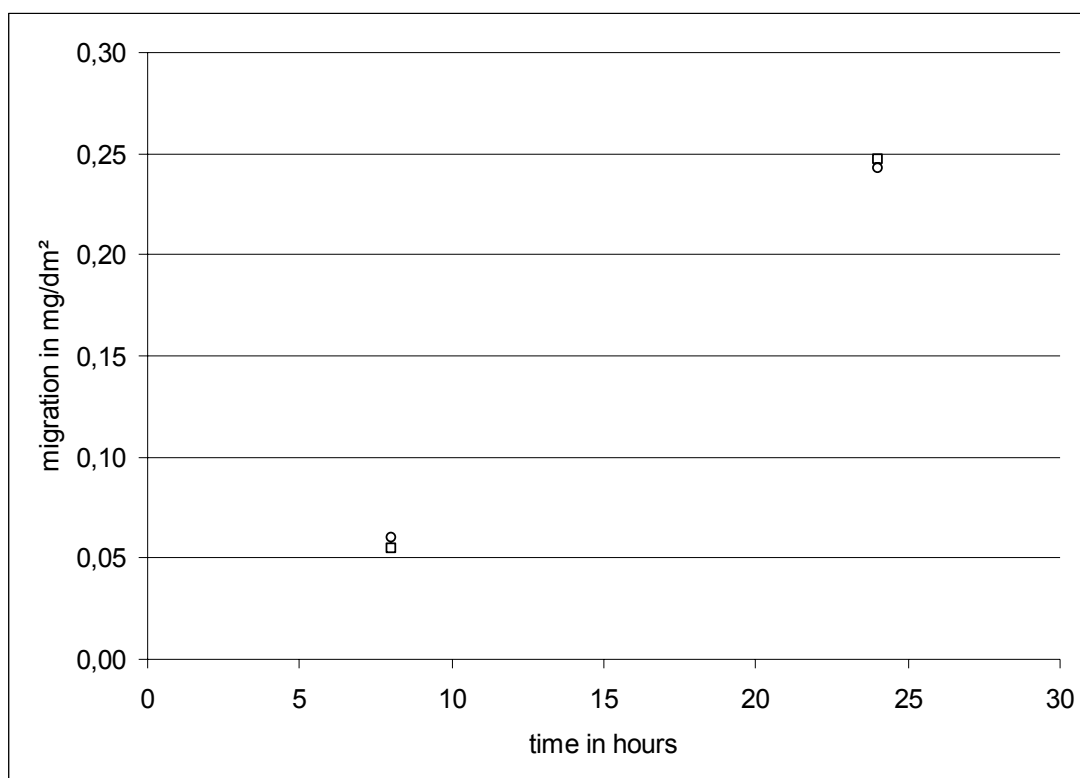
Figure 45 Migration of benzophenone into minced meat (approx. 10% fat) at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 8 and 24 hours

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 56 Migration of diphenyl phthalate into minced meat (approx. 10% fat) at 25°C

Time of exposure in hours	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
8	0,040	0,039	4,68
24	0,073	0,067	8,34
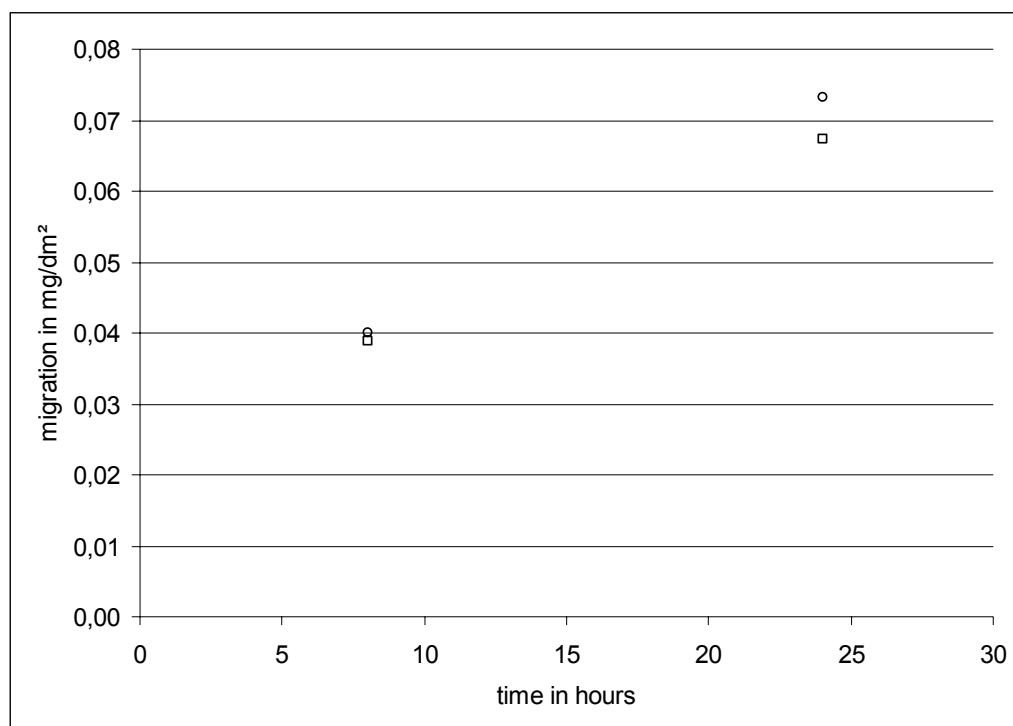
Figure 46 Migration of diphenyl phthalate into minced meat (approx. 10% fat) at 25°C

8.3.6 Meat, lean pork (minced, fat content approx. 20%)

Test conditions:

Temperature: 5°C

Exposure time: 1, 2, 4 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 57 Migration of benzophenone into minced meat (approx. 20% fat) at 5°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,052	0,051	7,62
2	0,073	0,072	10,71
4	0,089	0,109	14,69
10	0,134	0,141	20,34
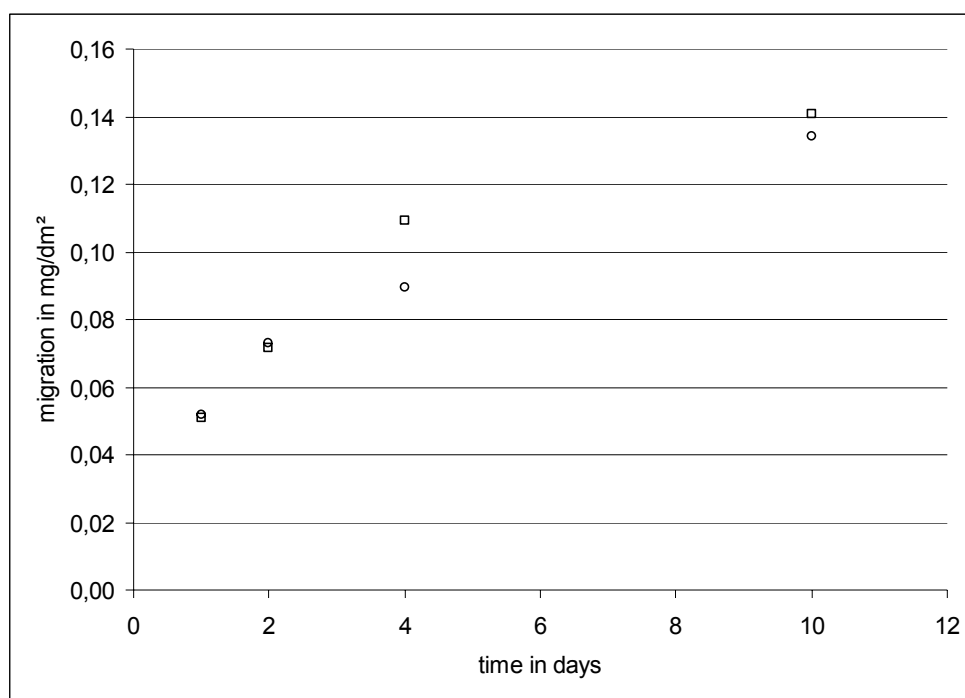
Figure 47 Migration of benzophenone into minced meat (approx. 20% fat) at 5°C

Test conditions:

Temperature: 5°C

Exposure time: 1, 2, 4 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 58 Migration of diphenyl phthalate into minced meat (approx. 20% fat) at 5°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,016	0,016	1,90
2	0,025	0,023	2,85
4	0,039	0,030	3,56
10	0,041	0,031	4,29
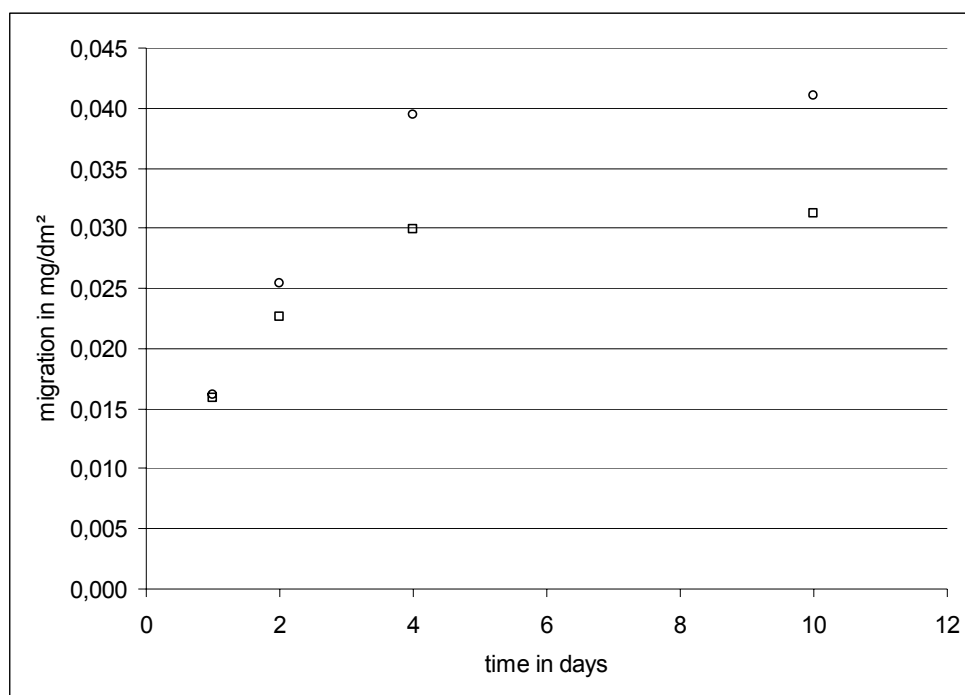
Figure 48 Migration of diphenyl phthalate into minced meat (approx. 20% fat) at 5°C

Test conditions:

Temperature: 25°C

Exposure time: 8 and 24 hours

Exposure type: one sided using wide mouth jar

Sample amount:14,25 gram

Exposure area: 0,0855 dm^2

Table 59 Migration of benzophenone into minced meat (approx. 20% fat) at 25°C

Time of exposure in hours	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
8	0,060	0,075	10,00
24	0,291	0,253	40,28
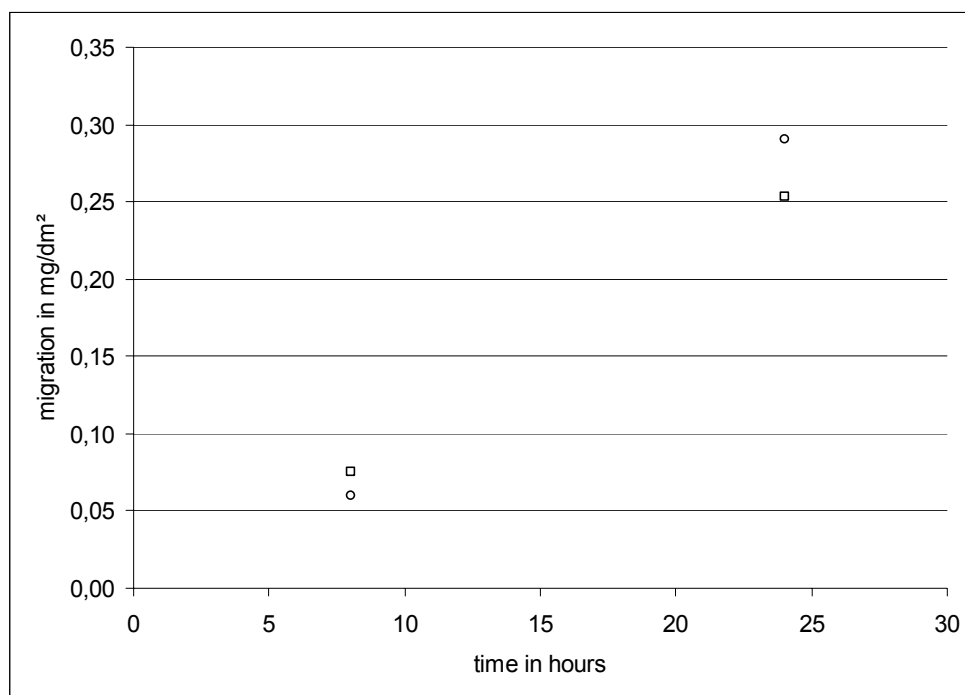
Figure 49 Migration of benzophenone into minced meat (approx. 20% fat) at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 8 and 24 hours

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 60 Migration of diphenyl phthalate into minced meat (approx. 20% fat) at 25°C

Time of exposure in hours	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
8	0,055	0,063	6,98
24	0,152	0,161	18,60
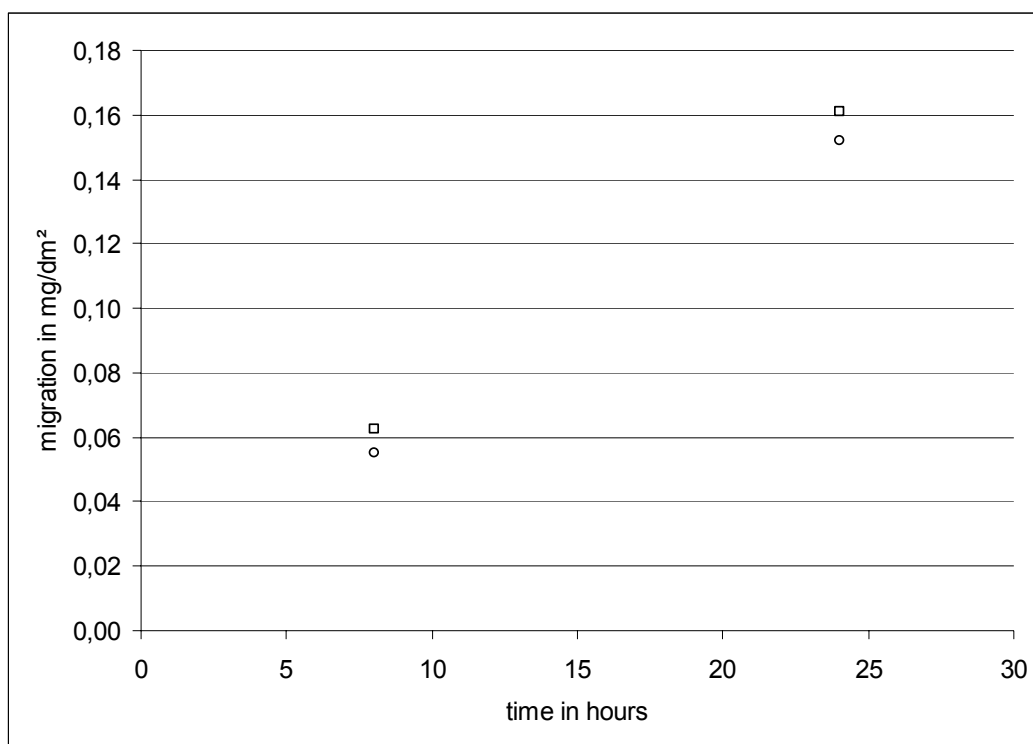
Figure 50 Migration of diphenyl phthalate into minced meat (approx. 20% fat) at 25°C

8.3.7 Meat, lean pork (minced, fat content approx. 30%)

Test conditions:

Temperature: 5°C

Exposure time: 1, 2, 4 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 61 Migration of benzophenone into minced meat (approx. 30% fat) at 5°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,051	0,056	7,92
2	0,064	0,065	9,58
4	0,108	0,111	16,17
10	0,153	0,140	21,70

Figure 51 Migration of benzophenone into minced meat (approx. 30% fat) at 5°C

Test conditions:

Temperature: 5°C

Exposure time: 1, 2, 4 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 62 Migration of diphenyl phthalate into minced meat (approx. 30% fat) at 5°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,018	0,019	2,24
2	0,034	0,032	3,87
4	0,030	0,033	3,92
10	0,046	0,040	5,07
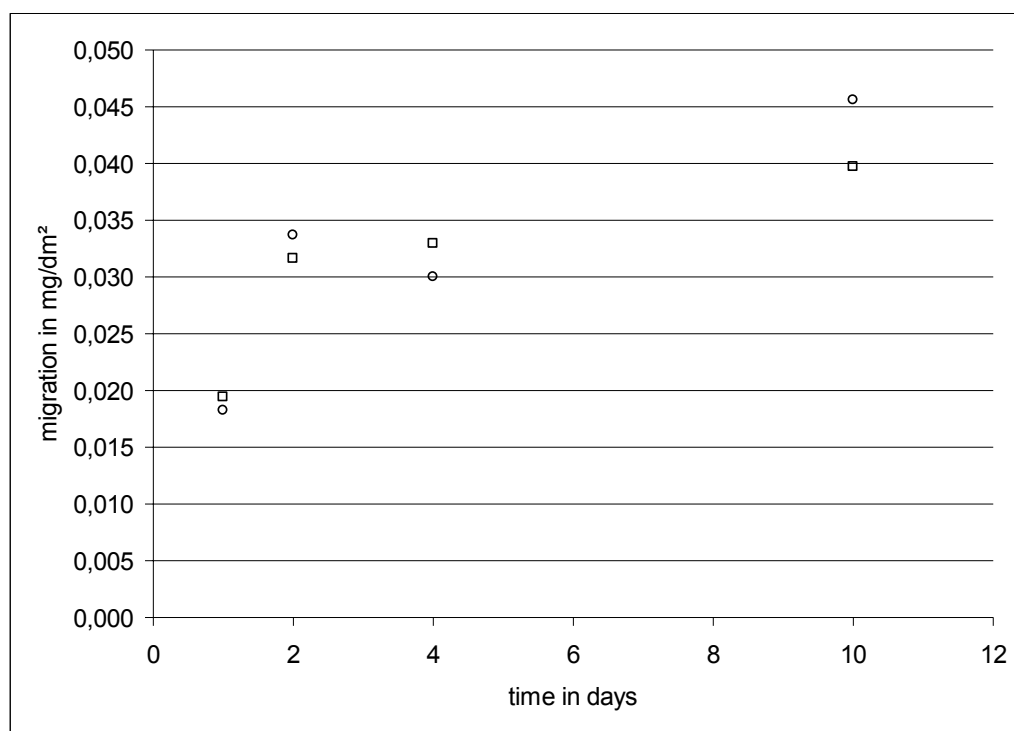
Figure 52 Migration of diphenyl phthalate into minced meat (approx. 30% fat) at 5°C

Test conditions:

Temperature: 25°C

Exposure time: 8 and 24 hours

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 63 Migration of benzophenone into minced meat (approx. 30% fat) at 25°C

Time of exposure in hours	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
8	0,073	0,070	10,66
24	0,267	0,294	41,57
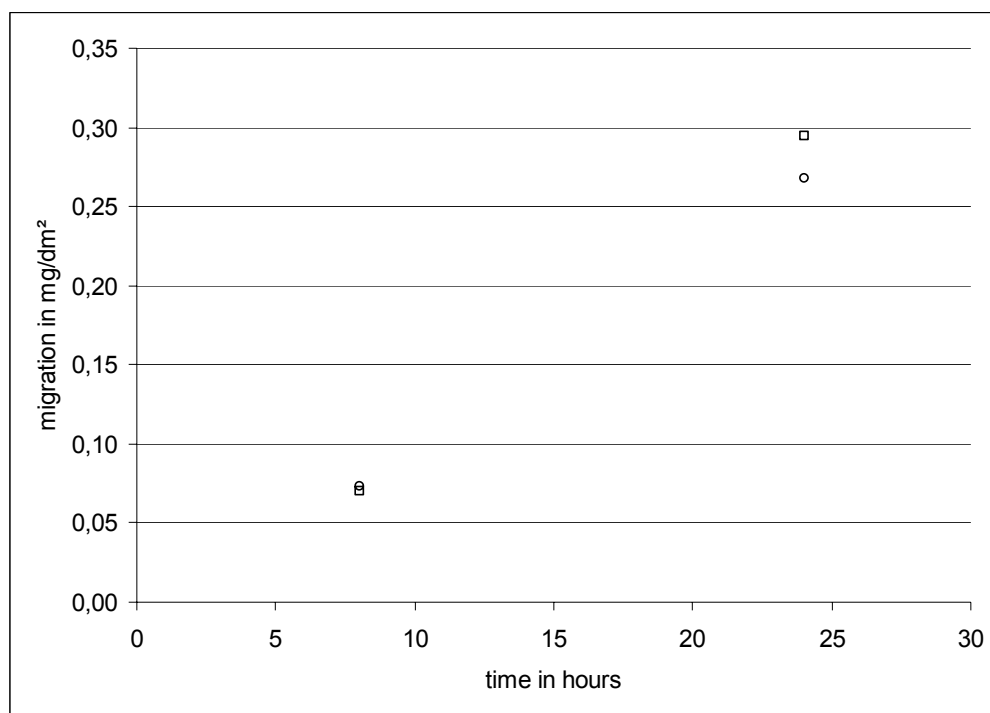
Figure 53 Migration of benzophenone into minced meat (approx. 30% fat) at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 8 and 24 hours

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 64 Migration of diphenyl phthalate into minced meat (approx. 30% fat) at 25°C

Time of exposure in hours	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
8	0,077	0,074	8,97
24	0,177	0,184	21,42
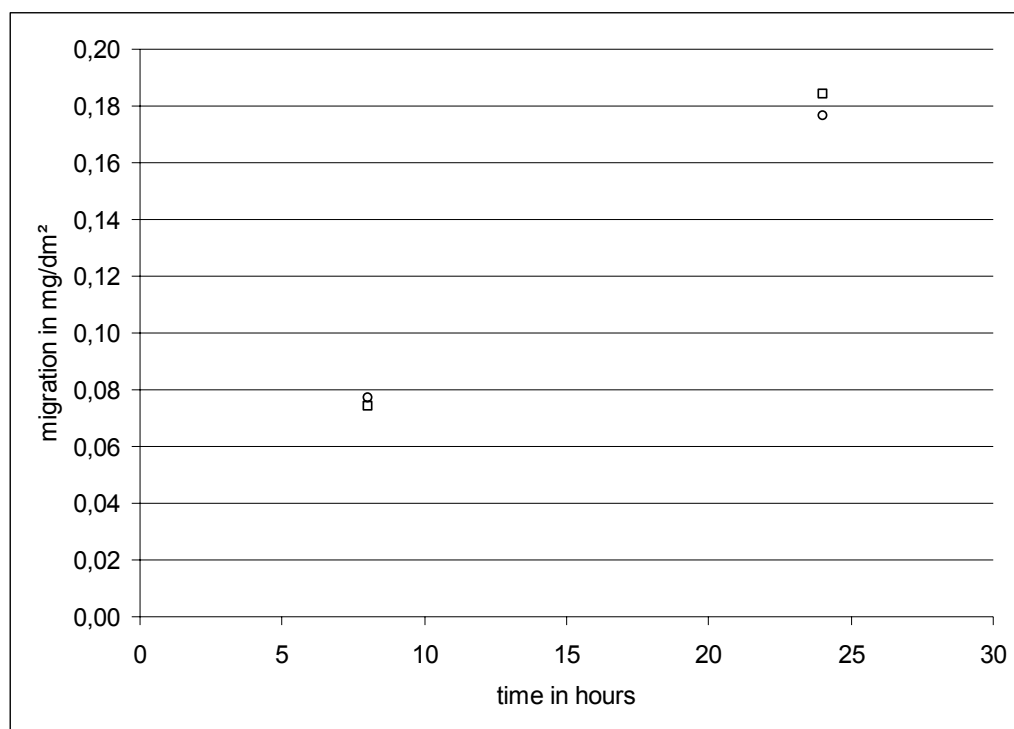
Figure 54 Migration of diphenyl phthalate into minced meat (approx. 30% fat) at 25°C

8.3.8 Meat, lean pork (minced, fat content approx. 50%)

Test conditions:

Temperature: 5°C

Exposure time: 1, 2, 4 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 65 Migration of benzophenone into minced meat (approx. 50% fat) at 5°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,053	0,042	7,08
2	0,074	0,070	10,64
4	0,098	0,099	14,61
10	0,165	0,172	24,96

Figure 55 Migration of benzophenone into minced meat (approx. 50% fat) at 5°C

Test conditions:

Temperature: 5°C

Exposure time: 1, 2, 4 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 66 Migration of diphenyl phthalate into minced meat (approx. 50% fat) at 5°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,020	0,026	2,75
2	0,026	0,031	3,37
4	0,037	0,033	3,96
10	0,058	0,062	7,08

Figure 56 Migration of diphenyl phthalate into minced meat (approx. 50% fat) at 5°C

Test conditions:

Temperature: 25°C

Exposure time: 8 and 24 hours

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 67 Migration of benzophenone into minced meat (approx. 50% fat) at 25°C

Time of exposure in hours	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
8	0,079	0,077	11,58
24	0,333	0,276	45,09

Figure 57 Migration of benzophenone into minced meat (approx. 50% fat) at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 8 and 24 hours

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 68 Migration of diphenyl phthalate into minced meat (approx. 50% fat) at 25°C

Time of exposure in hours	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
8	0,078	0,075	9,07
24	0,226	0,191	24,75
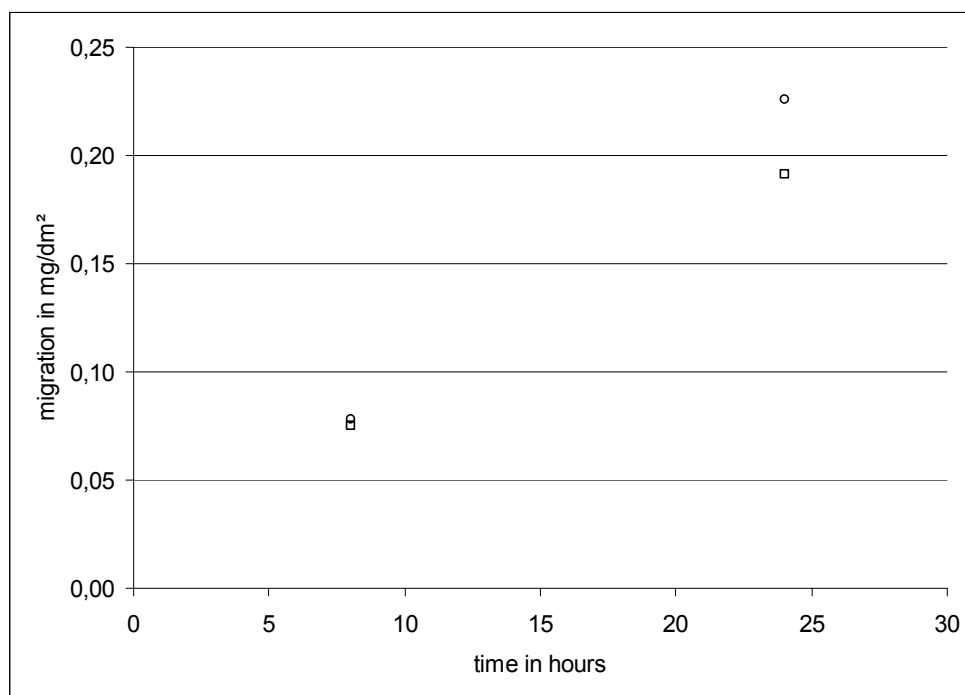
Figure 58 Migration of diphenyl phthalate into minced meat (approx. 50% fat) at 25°C

8.3.9 Fish (salmon with 13,6% fat)

Test conditions:

Temperature: 5°C

Exposure time: 0,33; 1, 2 and 5 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 69 Migration of benzophenone into fish at 5°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
0,33	0,028	0,027	4,06
1	0,037	0,038	5,55
2	0,051	0,054	7,75
5	0,116	0,118	17,36
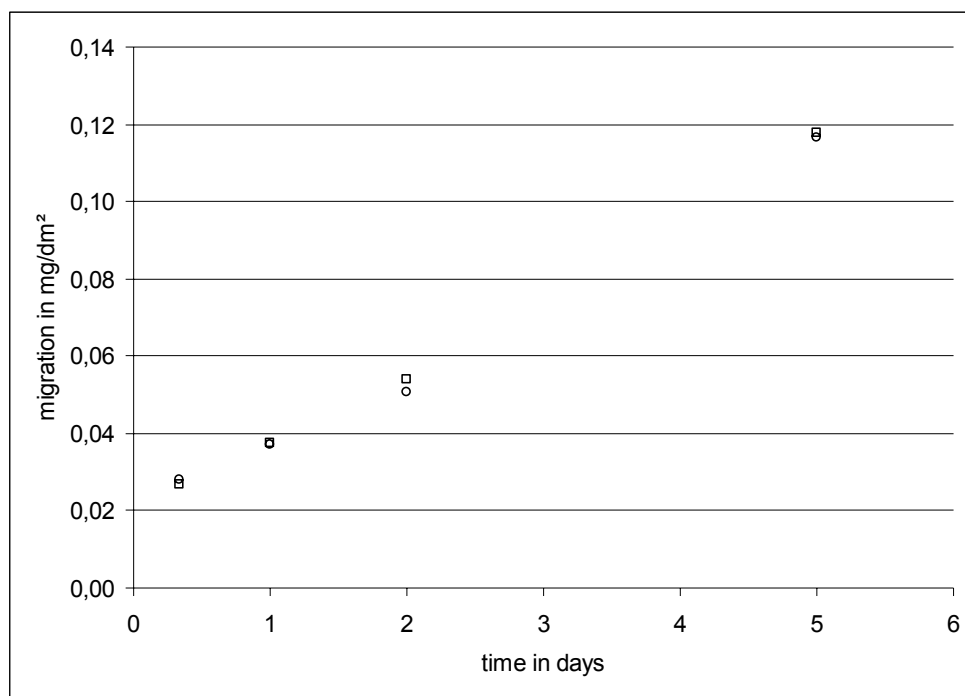
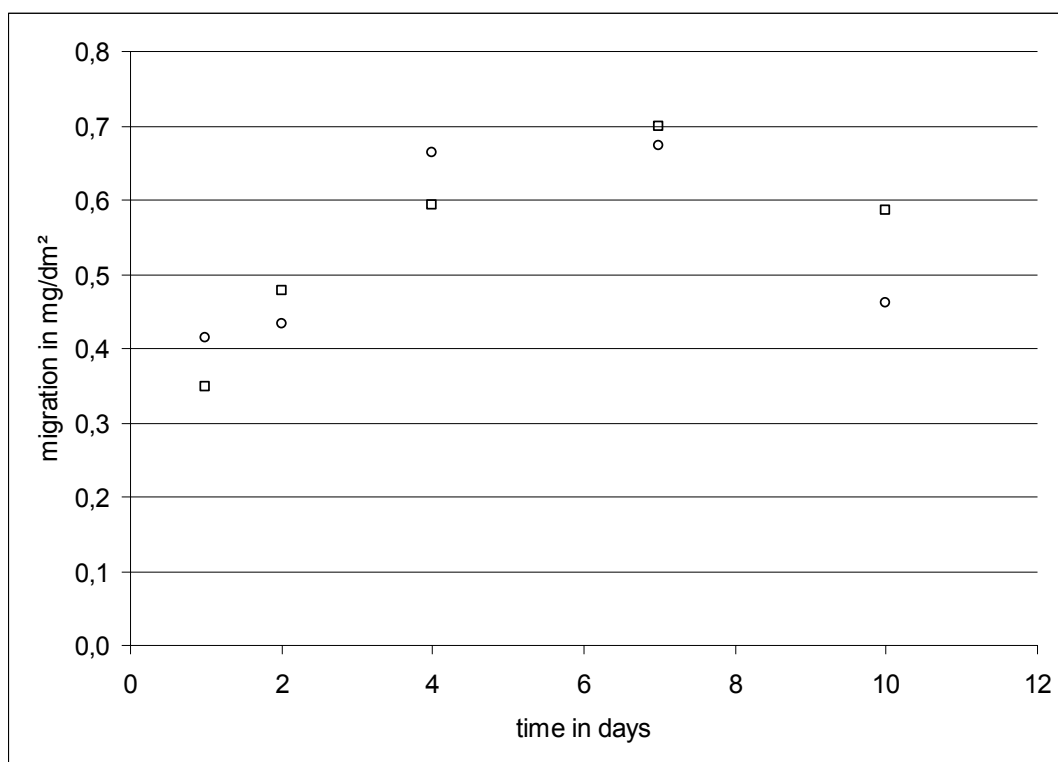**Figure 59 Migration of benzophenone into fish at 5°C**

Test conditions:

Temperature: 5°C

Exposure time: 0,33; 1, 2 and 5 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 70 Migration of diphenyl phthalate into fish at 5°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
0,33	0,004	0,004	0,45
1	0,006	0,012	1,04
2	0,004	0,004	0,49
5	0,015	0,014	1,75
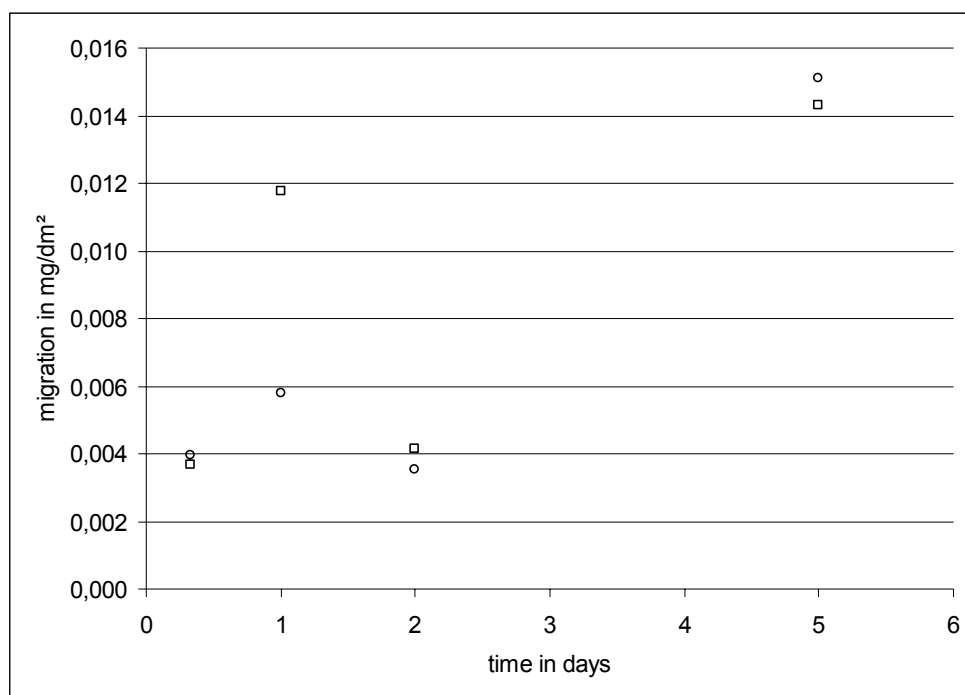
Figure 60 Migration of diphenyl phthalate into fish at 5°C

8.3.10 Condensed milk

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 71 Migration of benzophenone into condensed milk at 25°C

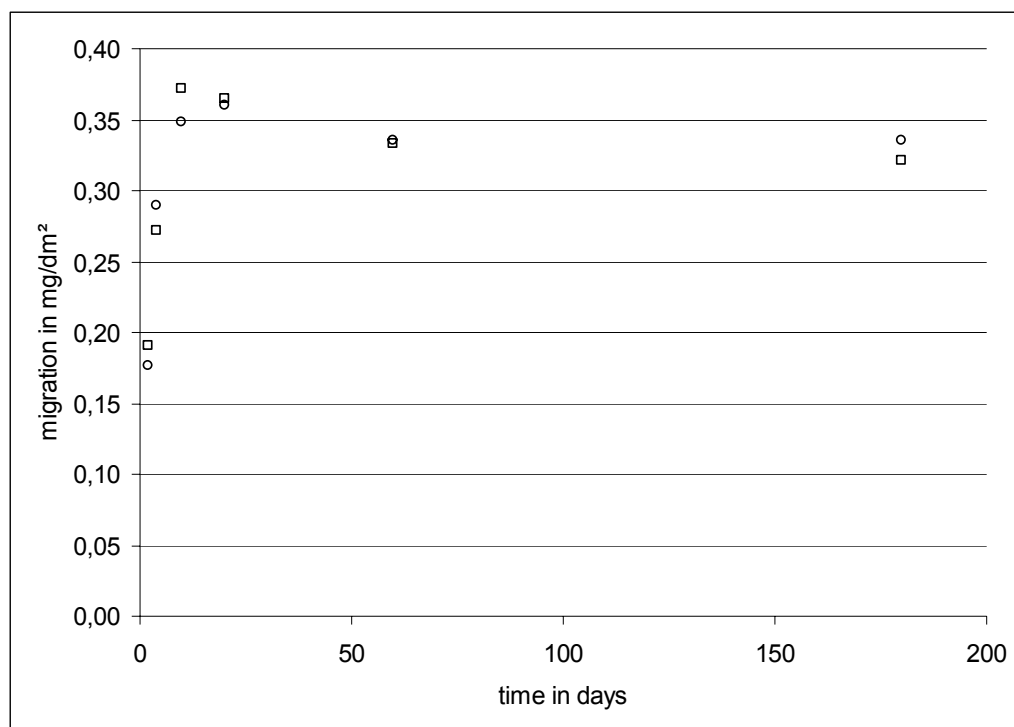| Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,133	0,124	19,03
2	0,225	0,219	32,87
4	0,252	0,267	38,43
10	0,408	0,418	61,19
20	0,429	0,447	64,87
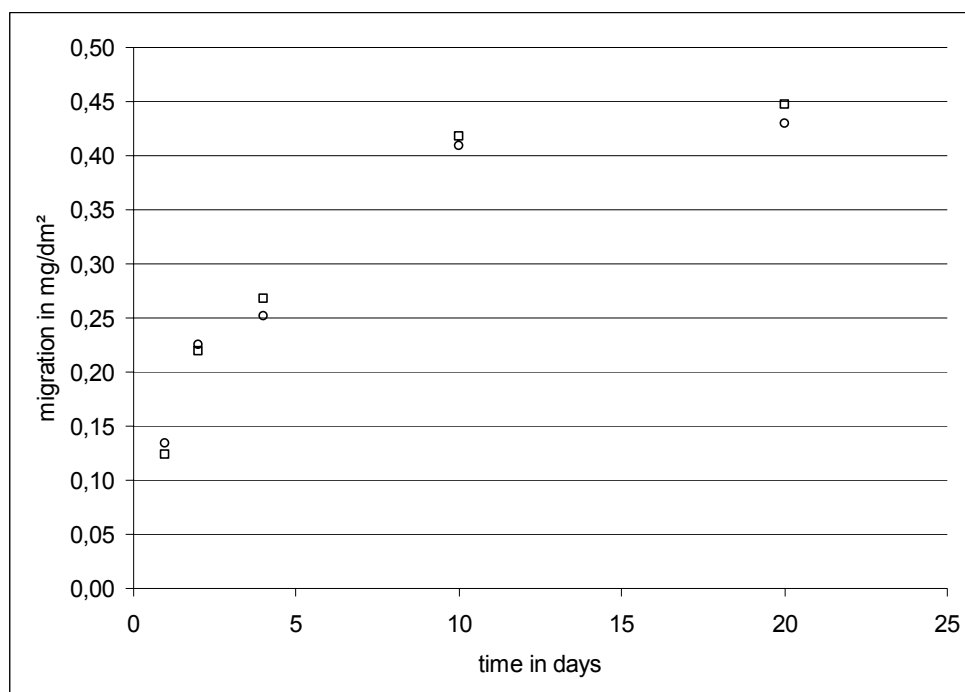
Figure 61 Migration of benzophenone into condensed milk at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 72 Migration of diphenyl phthalate into condensed milk at 25°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,043	0,042	5,05
2	0,066	0,062	7,58
4	0,088	0,082	10,14
10	0,140	0,150	17,20
20	0,213	0,219	25,63
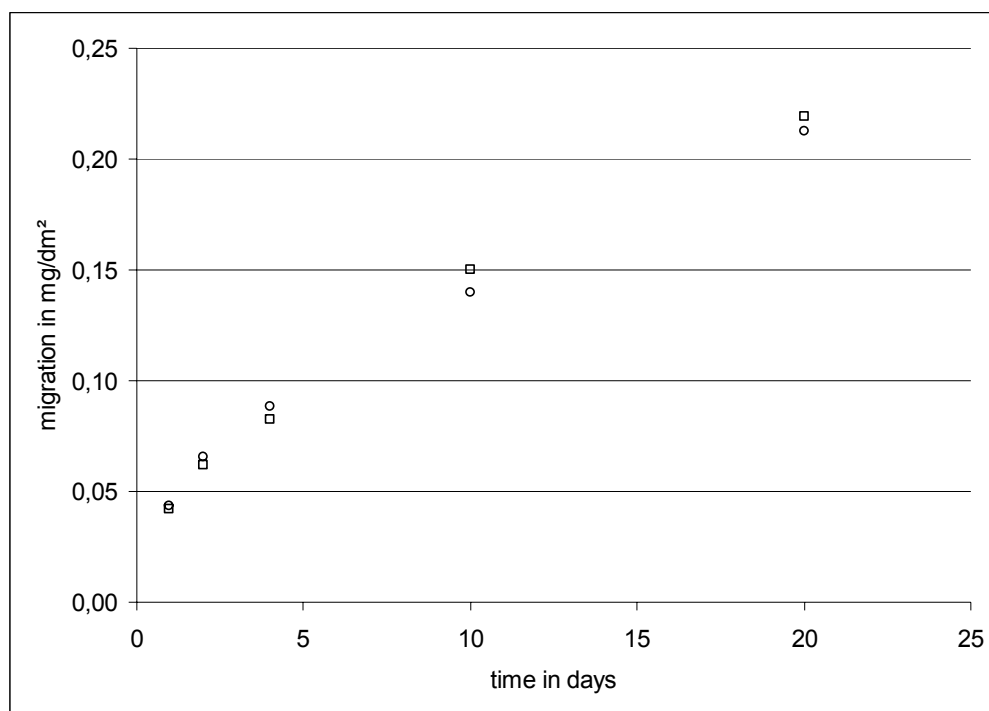
Figure 62 Migration of diphenyl phthalate into condensed milk at 25°C

8.4 Dry foodstuffs

8.4.1 Butter toast (4% fat)

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided, wrap

Sample amount: 25 gram

Exposure area: 0,1440 dm^2

Table 73 Migration of benzophenone into butter toast at 25°C

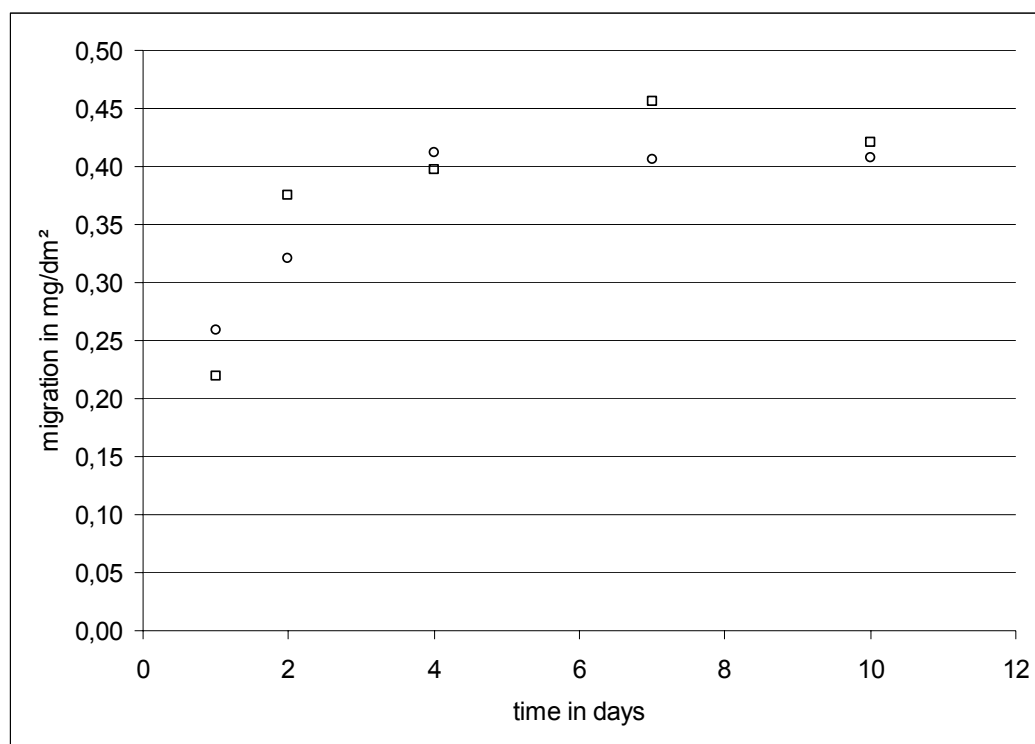| Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,189	0,193	28,31
2	0,261	0,269	39,23
4	0,339	0,360	51,77
10	0,439	0,443	65,35
20	0,481	0,464	70,02
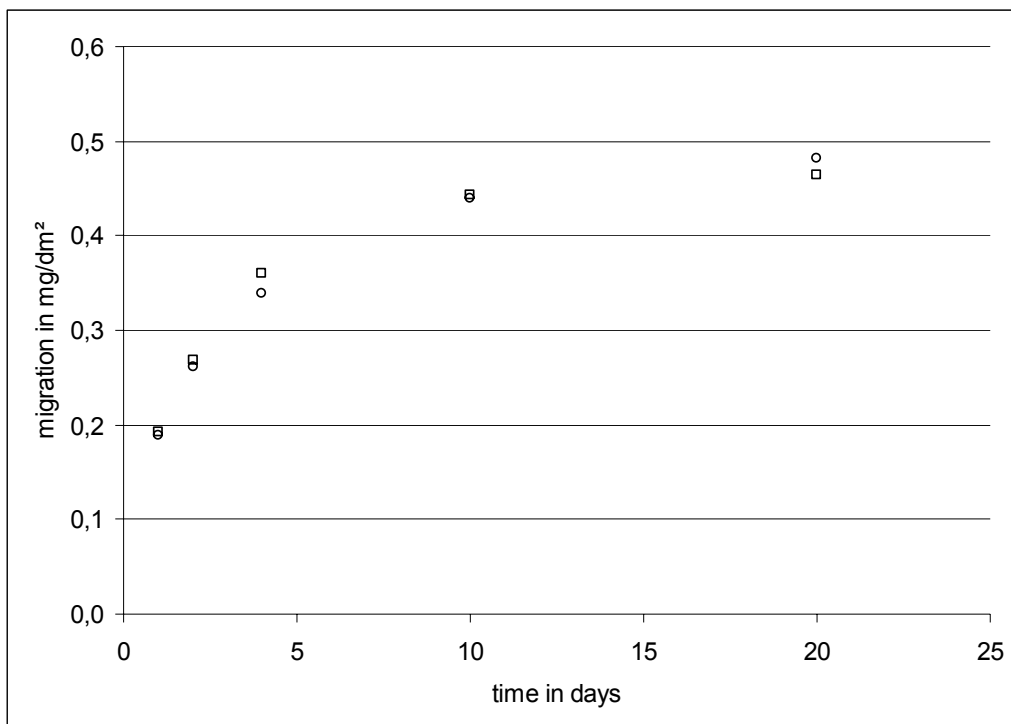
Figure 63 Migration of benzophenone into butter toast at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 1, 2, 4, 10 and 20 days

Exposure type: one sided, wrap

Sample amount: 25 gram

Exposure area: 0,1440 dm^2

Table 74 Migration of diphenyl phthalate into butter toast at 25°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,013	0,012	1,45
2	0,018	0,022	2,40
4	n.d.	0,033	3,96
10	0,046	0,040	5,12
20	0,087	0,069	9,27
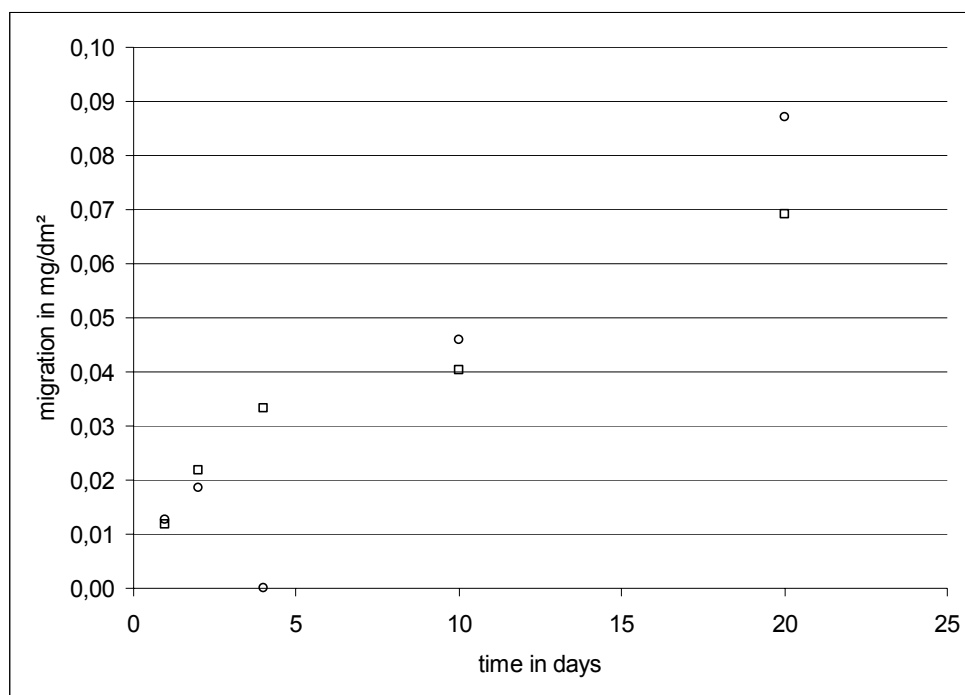
Figure 64 Migration of diphenyl phthalate into butter toast at 25°C

8.4.2 Flour

Test conditions:

Temperature: 25°C

Exposure time: 2, 4, 10, 20, 60 and 180 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 75 Migration of benzophenone into flour at 25°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,199	0,224	31,28
4	0,311	0,281	43,82
10	0,405	0,422	61,27
20	0,370	0,346	53,04
60	0,152	0,146	22,09
180	0,083	0,077	11,86
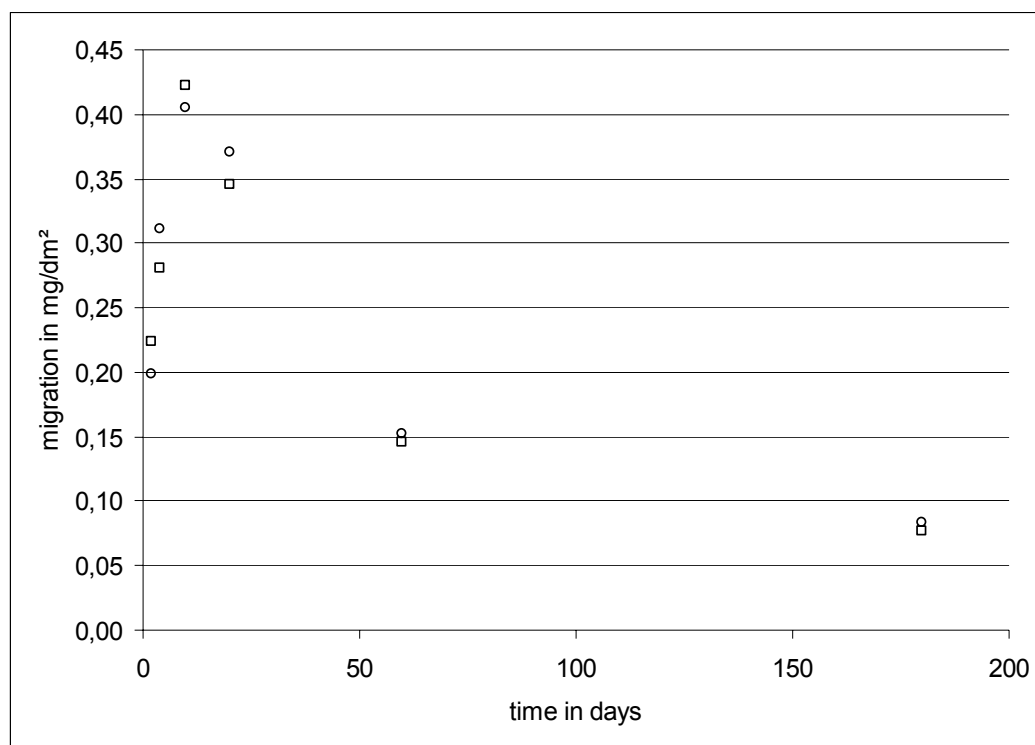
Figure 65 Migration of benzophenone into flour at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 2, 4, 10, 20, 60 and 180 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 76 Migration of diphenyl phthalate into flour at 25°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,040	0,039	4,74
4	0,051	0,053	6,15
10	0,105	0,095	11,87
20	0,180	0,175	21,08
60	0,228	0,260	28,94
180	0,465	0,477	55,92
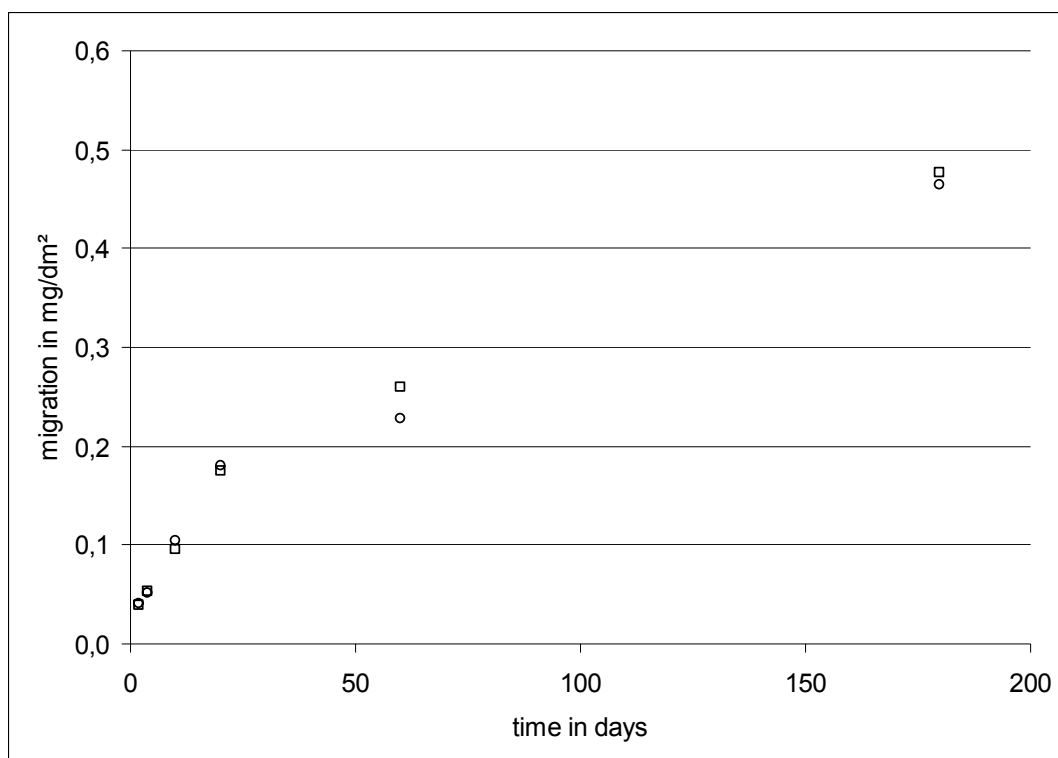
Figure 66 Migration of diphenyl phthalate into flour at 25°C

Test conditions:

Temperature: 40°C

Exposure time: 1, 2, 4, 7 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 77 Migration of benzophenone into flour at 40°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,260	0,273	39,47
2	0,387	0,365	55,74
4	0,415	0,438	63,16
7	0,371	0,350	53,39
10	0,373	0,405	57,64
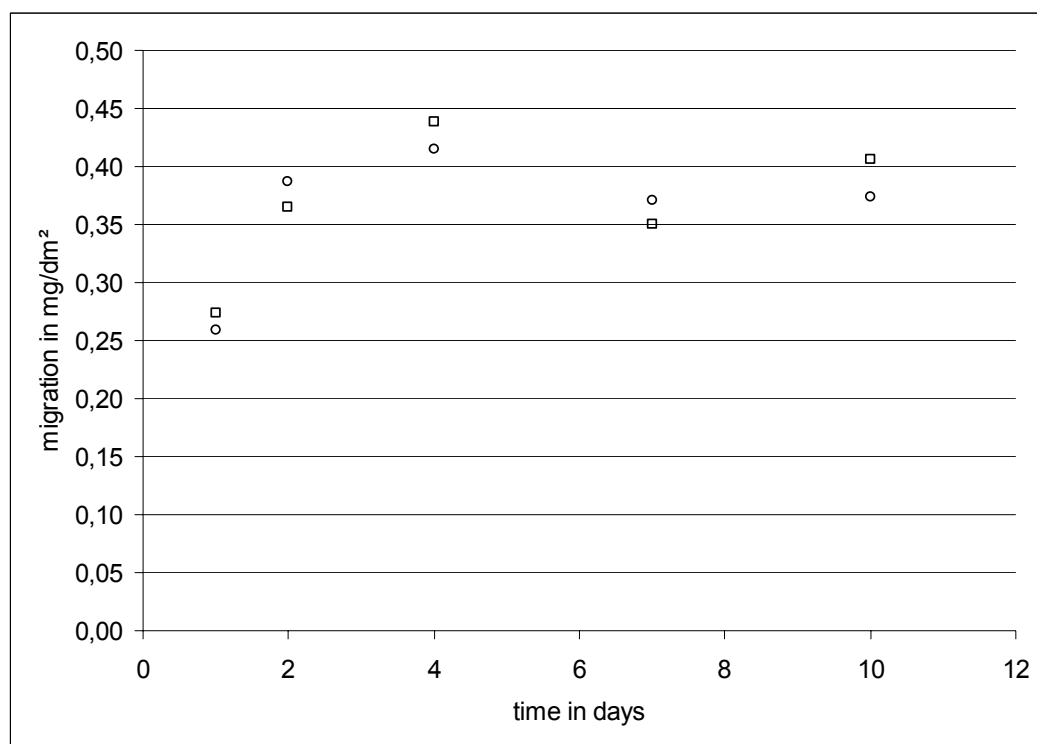
Figure 67 Migration of benzophenone into flour at 40°C

Test conditions:

Temperature: 40°C

Exposure time: 1, 2, 4, 7 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 78 Migration of diphenyl phthalate into flour at 40°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,076	0,078	9,17
2	0,107	0,129	14,07
4	0,180	0,178	21,27
7	0,250	0,240	29,09
10	0,296	0,283	34,37
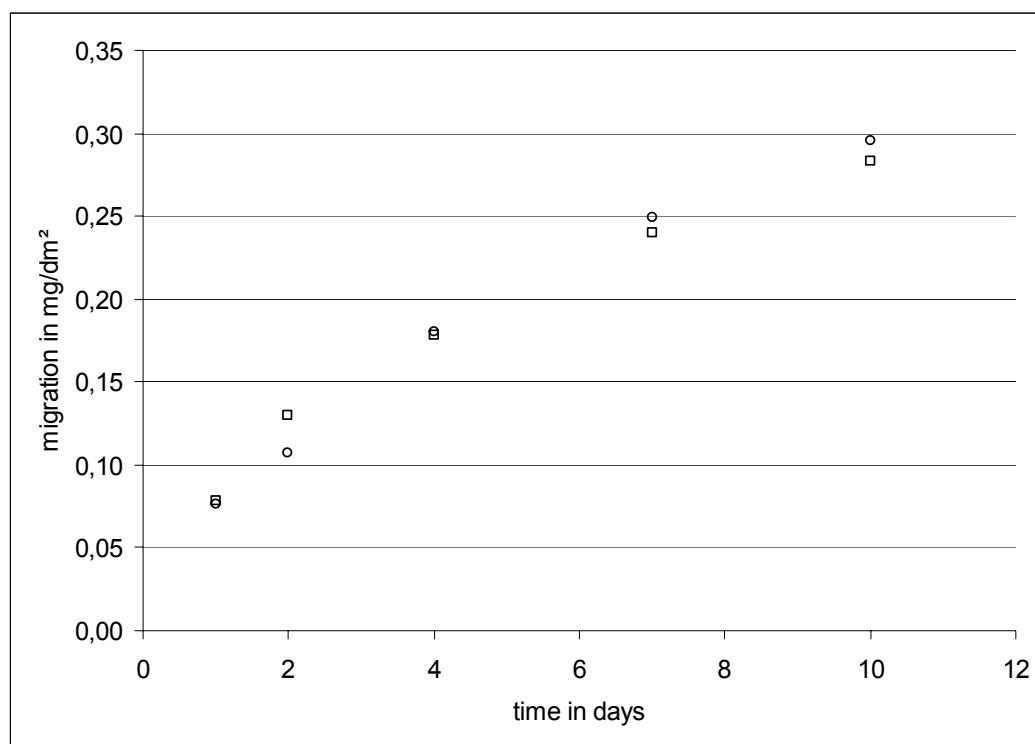
Figure 68 Migration of diphenyl phthalate into flour at 40°C

Test conditions:

Temperature: 70°C

Exposure time: 8 and 24 hours

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 79 Migration of benzophenone into flour at 70°C

Time of exposure in hours	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
8	0,311	0,299	45,20
24	0,355	0,334	50,98
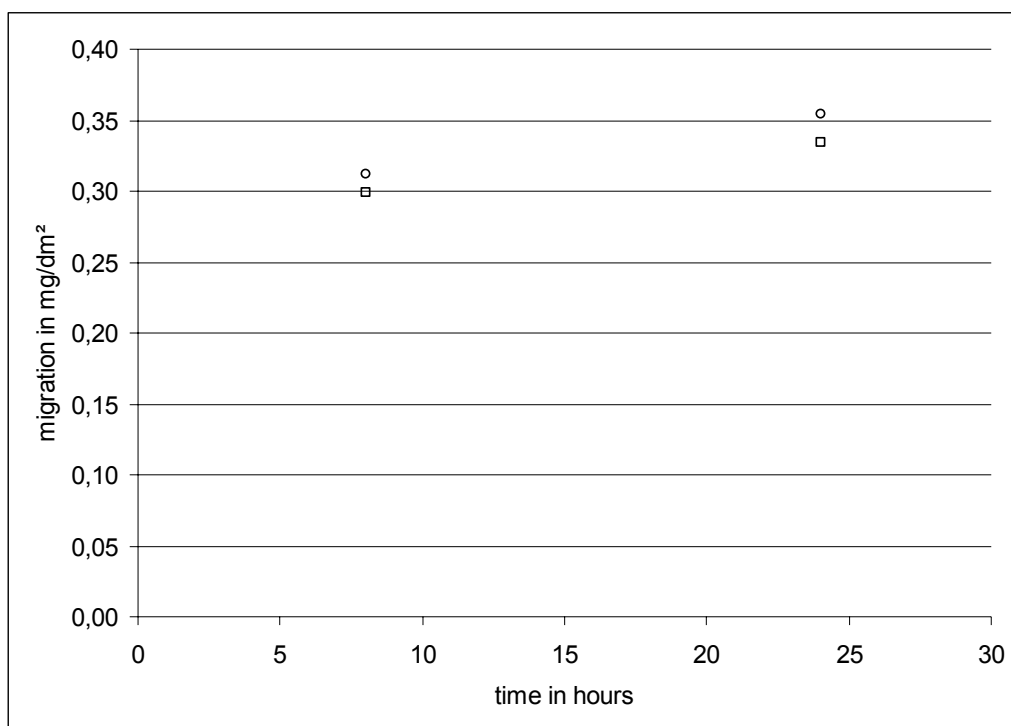
Figure 69 Migration of benzophenone into flour at 70°C

Test conditions:

Temperature: 70°C

Exposure time: 8 and 24 hours

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 80 Migration of diphenyl phthalate into flour at 70°C

Time of exposure in hours	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
8	0,284	0,272	33,02
24	0,407	0,418	48,97
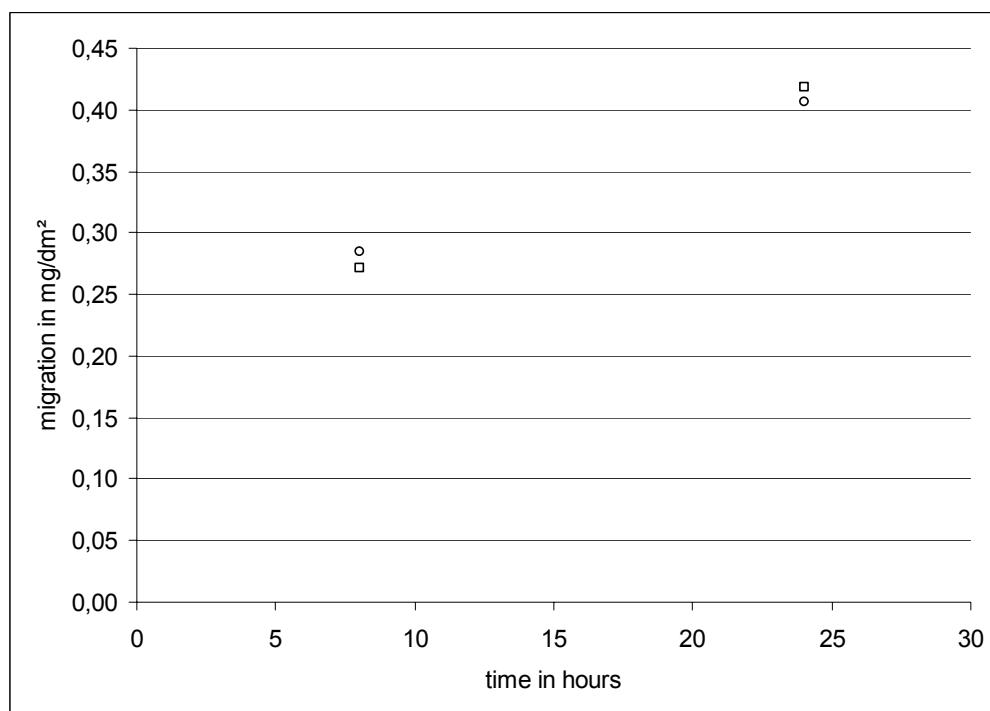
Figure 70 Migration of diphenyl phthalate into flour at 70°C

8.4.3 Milk powder

Test conditions:

Temperature: 25°C

Exposure time: 2, 4, 10, 20, 60 and 180 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 81 Migration of benzophenone into milk powder at 25°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,286	0,298	43,21
4	0,409	0,417	61,17
10	0,514	0,518	76,44
20	0,634	0,519	85,37
60	0,501	0,711	89,76
180	0,512	0,698	89,63
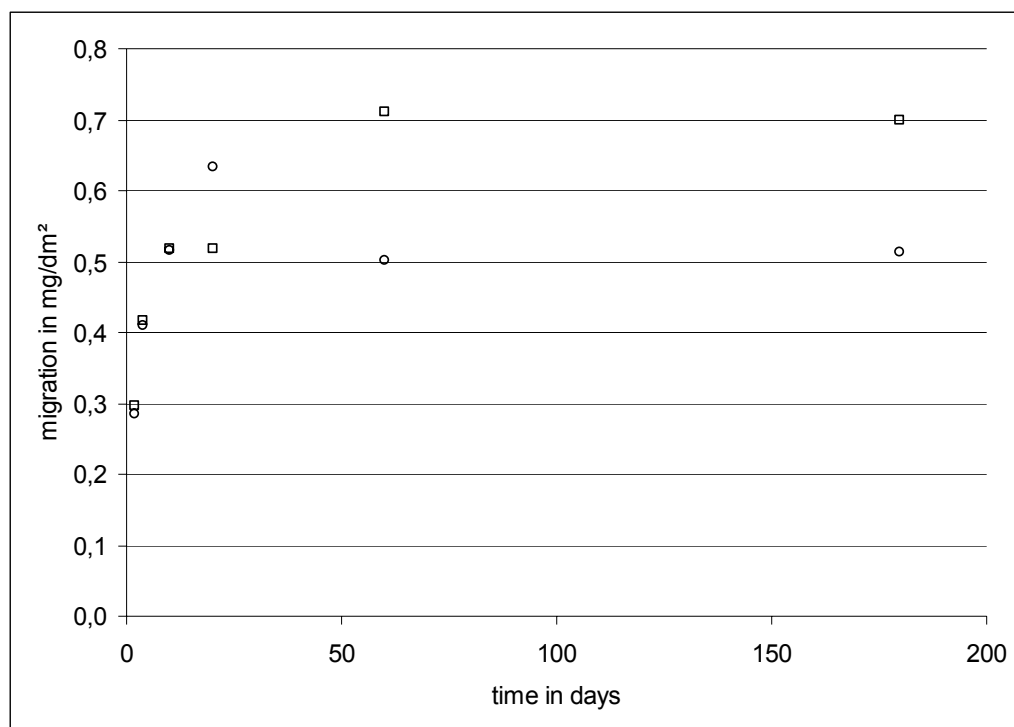
Figure 71 Migration of benzophenone into milk powder at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 2, 4, 10, 20, 60 and 180 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 82 Migration of diphenyl phthalate into milk powder at 25°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,039	0,033	4,27
4	0,046	0,046	5,49
10	0,123	0,107	13,68
20	0,131	0,120	14,91
60	0,293	0,329	36,95
180	0,488	0,520	59,80
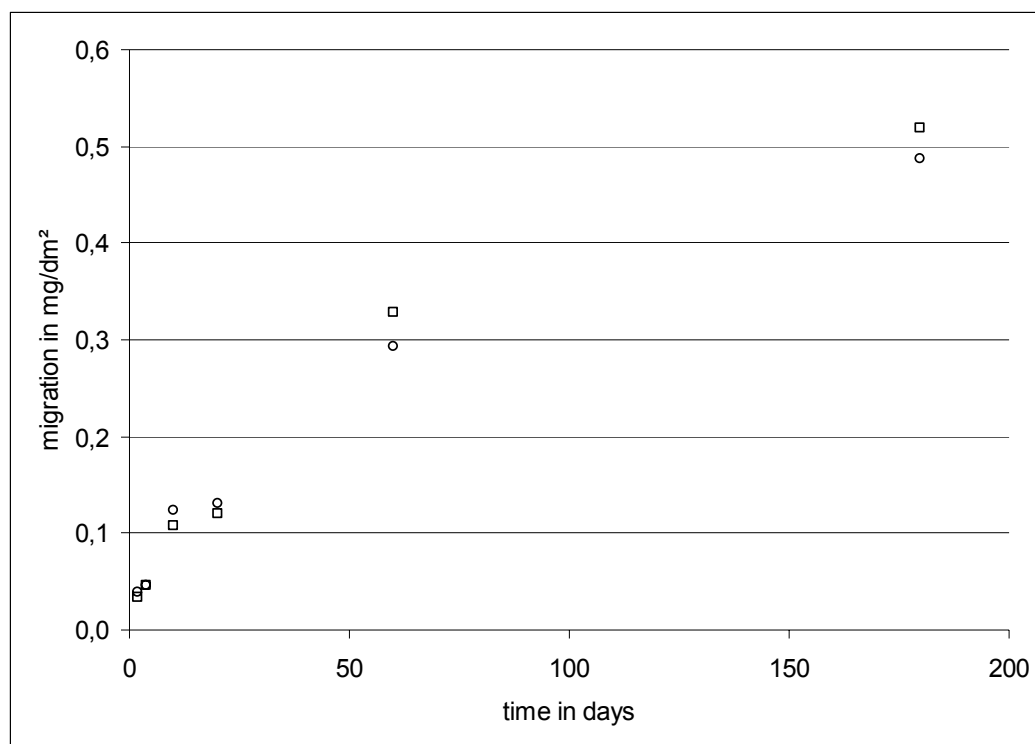
Figure 72 Migration of diphenyl phthalate into milk powder at 25°C

Test conditions:

Temperature: 40°C

Exposure time: 1, 2, 4, 7 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 83 Migration of benzophenone into milk powder at 40°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,414	0,348	56,45
2	0,432	0,478	67,44
4	0,663	0,594	93,10
7	0,672	0,699	101,60
10	0,461	0,586	77,59

Figure 73 Migration of benzophenone into milk powder at 40°C

Test conditions:

Temperature: 40°C

Exposure time: 1, 2, 4, 7 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 84 Migration of diphenyl phthalate into milk powder at 40°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,064	0,068	7,79
2	0,110	0,109	13,05
4	0,170	0,162	19,68
7	0,229	0,215	26,39
10	0,380	0,381	45,20
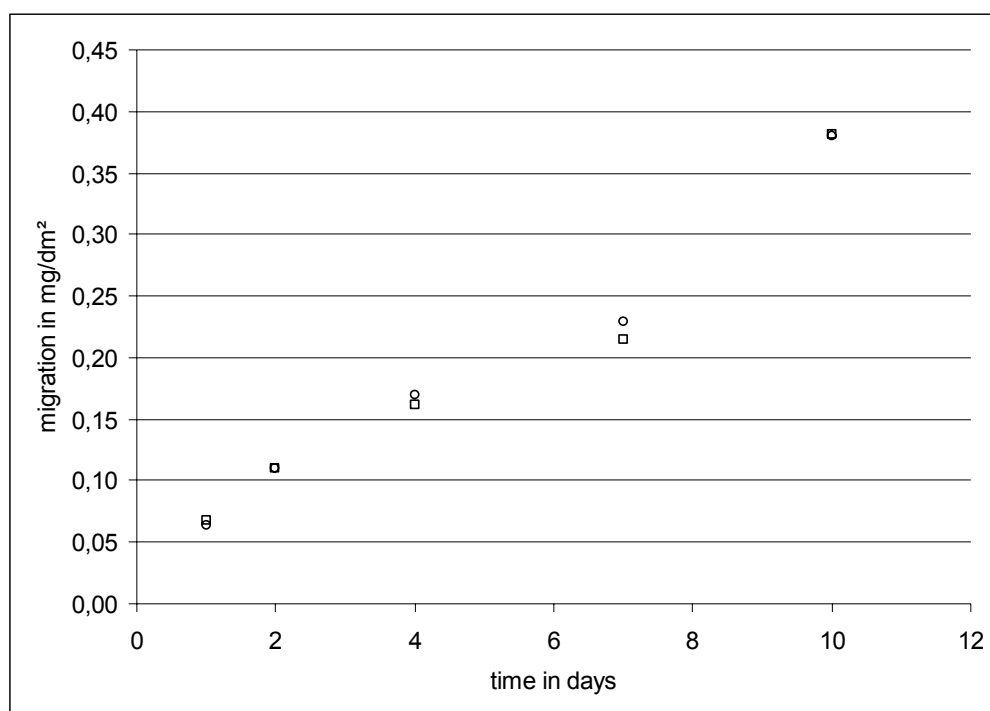
Figure 74 Migration of diphenyl phthalate into milk powder at 40°C

8.4.4 Rice

Test conditions:

Temperature: 25°C

Exposure time: 2, 4, 10, 20, 60 and 180 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 85 Migration of benzophenone into rice at 25°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,177	0,191	27,26
4	0,289	0,272	41,55
10	0,348	0,372	53,33
20	0,360	0,364	53,67
60	0,335	0,333	49,52
180	0,336	0,321	48,66

Figure 75 Migration of benzophenone into rice at 25°C

Test conditions:

Temperature: 25°C

Exposure time: 2, 4, 10, 20, 60 and 180 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 86 Migration of diphenyl phthalate into rice at 25°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
2	0,005	0,005	0,57
4	0,010	0,009	1,13
10	0,021	0,022	2,58
20	0,037	0,037	4,39
60	0,080	0,089	10,03
180	0,19	0,17	21,39
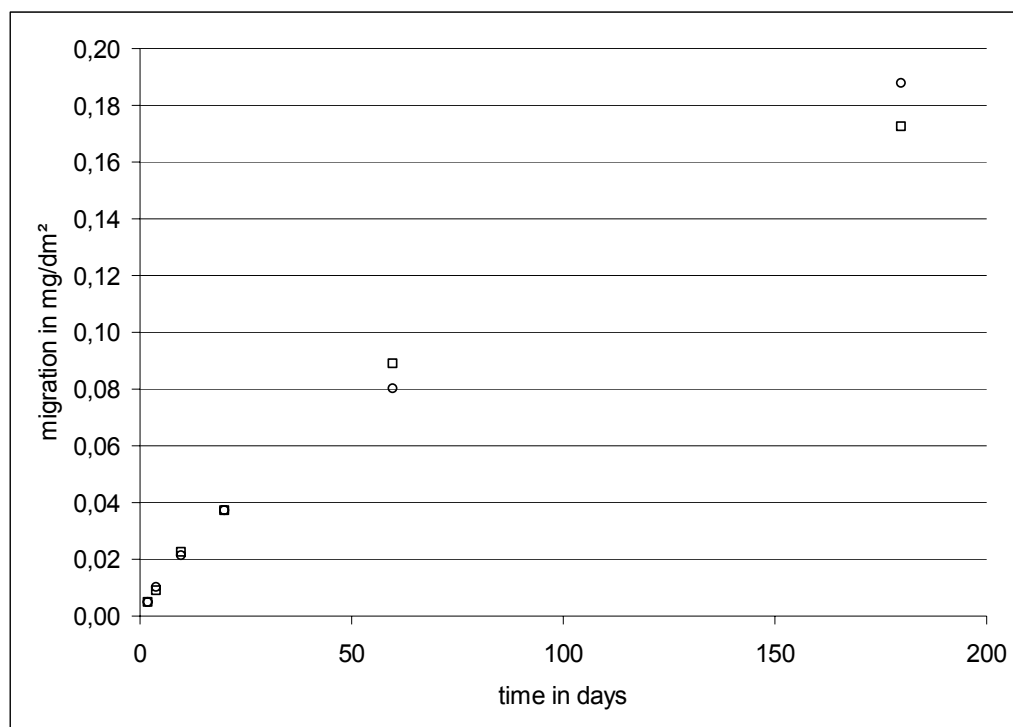
Figure 76 Migration of diphenyl phthalate into rice at 25°C

Test conditions:

Temperature: 40°C

Exposure time: 1, 2, 4, 7 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 87 Migration of benzophenone into rice at 40°C

Time of exposure in days	Benzophenone migration in mg/dm^2 in sample A	Benzophenone migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,259	0,220	35,45
2	0,320	0,375	51,45
4	0,412	0,397	59,96
7	0,406	0,456	63,82
10	0,407	0,421	61,32

Figure 77 Migration of benzophenone into rice at 40°C

Test conditions:

Temperature: 40°C

Exposure time: 1, 2, 4, 7 and 10 days

Exposure type: one sided using wide mouth jar

Sample amount: 14,25 gram

Exposure area: 0,0855 dm^2

Table 88 Migration of diphenyl phthalate into rice at 40°C

Time of exposure in days	Diphenyl phthalate migration in mg/dm^2 in sample A	Diphenyl phthalate migration in mg/dm^2 in sample B	Mean value of migration in % of the calculated maximum possible migration
1	0,017	0,014	1,90
2	0,032	0,031	3,72
4	0,055	0,052	6,36
7	0,076	0,077	9,03
10	0,086	0,088	10,34
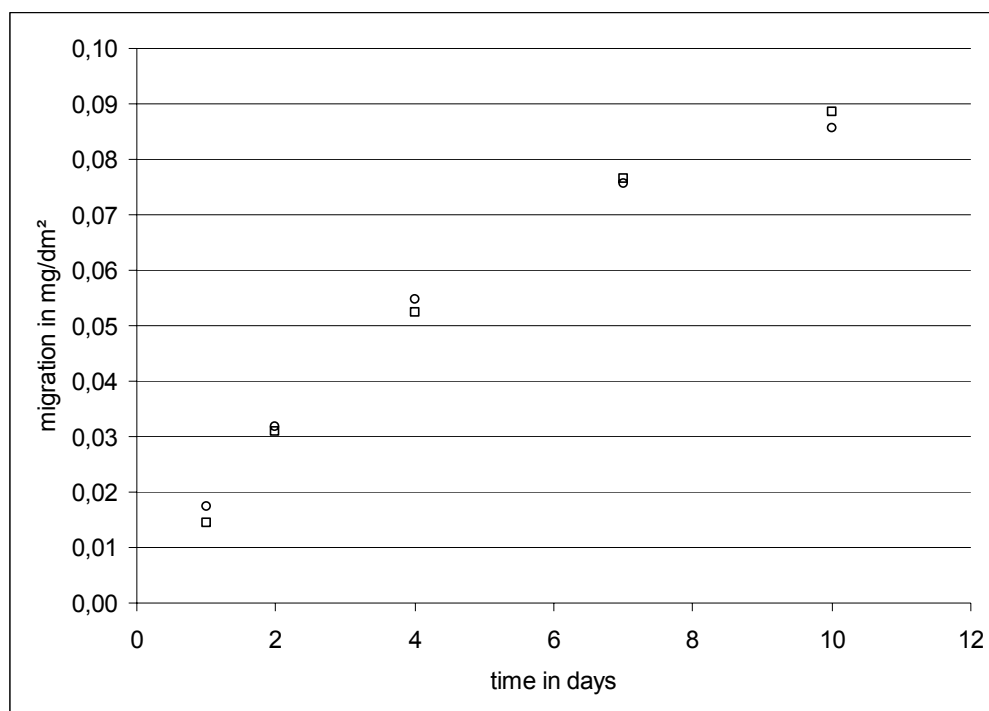
Figure 78 Migration of diphenyl phthalate into rice at 40°C

9 Discussion

Migration of two different analytes – benzophenone and diphenyl phthalate – from an LDPE-film into 17 various foodstuffs under different time/temperature conditions was studied. The aim of this work was to develop methods for rapid determination of analyte concentration in different foodstuffs and measure their migration under various storage conditions.

Foodstuffs used were divided into 3 groups based on their characteristics considered most likely to be the predominant factors influencing the mass transfer from a packaging material into the foodstuff itself. These groups were:

- aqueous and acidic foods
- fatty foodstuffs
- dry foodstuffs

In these groups the influence of further factors – like alcohol content, fat content, temperature etc. – was analyzed.

Use of a standardized plastic film in the migration studies as well as defined storage conditions during exposure allows us to compare the migration into different foodstuffs and observe the influence of changing foodstuff composition on the migration as well as the influence of varying storage conditions.

9.1 Influence of different food components on the migration

9.1.1 Influence of the alcohol content on the migration of benzophenone and diphenyl phthalate

Solubility of a substance is a major aspect influencing the migration into a certain media. Benzophenone is easily soluble in alcohol, whereas it's solubility in pure water is very low. The phthalates vary in their solubility, yet as can be seen from Table 89, with increasing chain length of the corresponding alcohol in the ester the partition coefficient is moving towards the higher values hence reflecting the hydrophobic character of the substance. (29)

Table 89 Solubility of phthalate esters (29)

Phthalic ester	Solubility in water at 20°C [mg/L]	Partition coefficient $logP_{OW}$
DMP	4300	1,53
DEP	Approx. 950	2,35
DBP	10	4,57
DEHP	0,05-0,5	4,88

Due to the solubility of both benzophenone and diphenyl phthalate it was assumed that higher alcohol content of a foodstuff would promote their migration from the test film into the foodstuff. To verify this assumption cola drink, beer and wine were included in the migration tests.

As can be seen from Figure 79 the migration of both test substances at the same test conditions was highest into wine followed by beer and the lowest migration occurred into cola drink. After 20 days storage at 25°C compared with wine the migration into beer was 16% lower for benzophenone and 24% lower for diphenyl phthalate. Migration into the cola drink compared with the migration into the wine was 29 % lower for benzophenone and 38% lower for diphenyl phthalate.
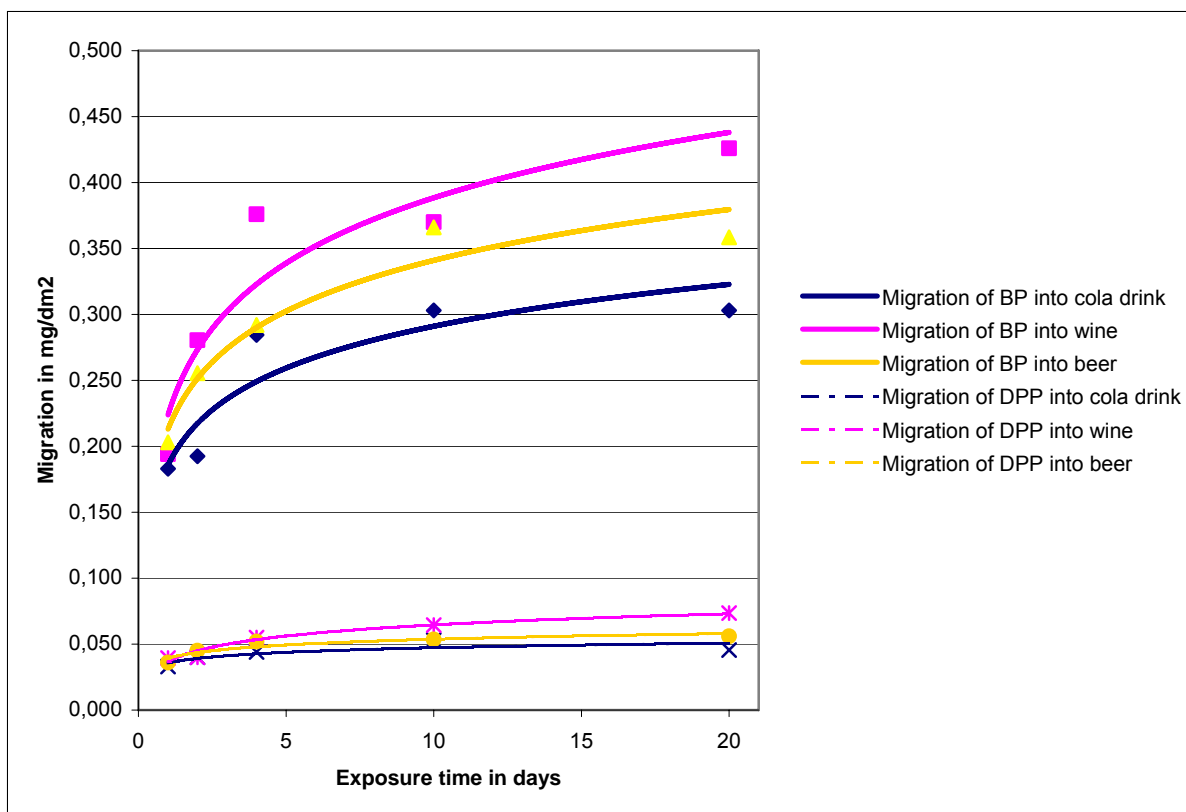
Figure 79 Comparison of migration of BP and DPP into cola drink, wine and beer at 25°C

The influence of the alcohol content on the migration of benzophenone and diphenyl phthalate is obvious from these results, thus confirming the assumption that higher alcohol content of a foodstuff promotes the migration of BP and DPP from the packaging into the foodstuff.

9.1.2 Influence of the fat content on the migration of benzophenone and diphenyl phthalate into meat

The hydrophobic character of benzophenone and diphenyl phthalate was expected to result in higher migration levels into fatty foodstuffs compared to low fat foods. Meat was selected as the foodstuff on which the correlation between the fat content and the migration levels was studied. For this purpose lean pork loin was mixed with lard to obtain mixtures with defined fat contents. This way the samples varied in the fat content due to different additions of lard. The basis, lean pork loin, was the same for all the samples. This enabled us to study the influence of fat content of the samples on the migration levels of benzophenone and diphenyl phthalate.
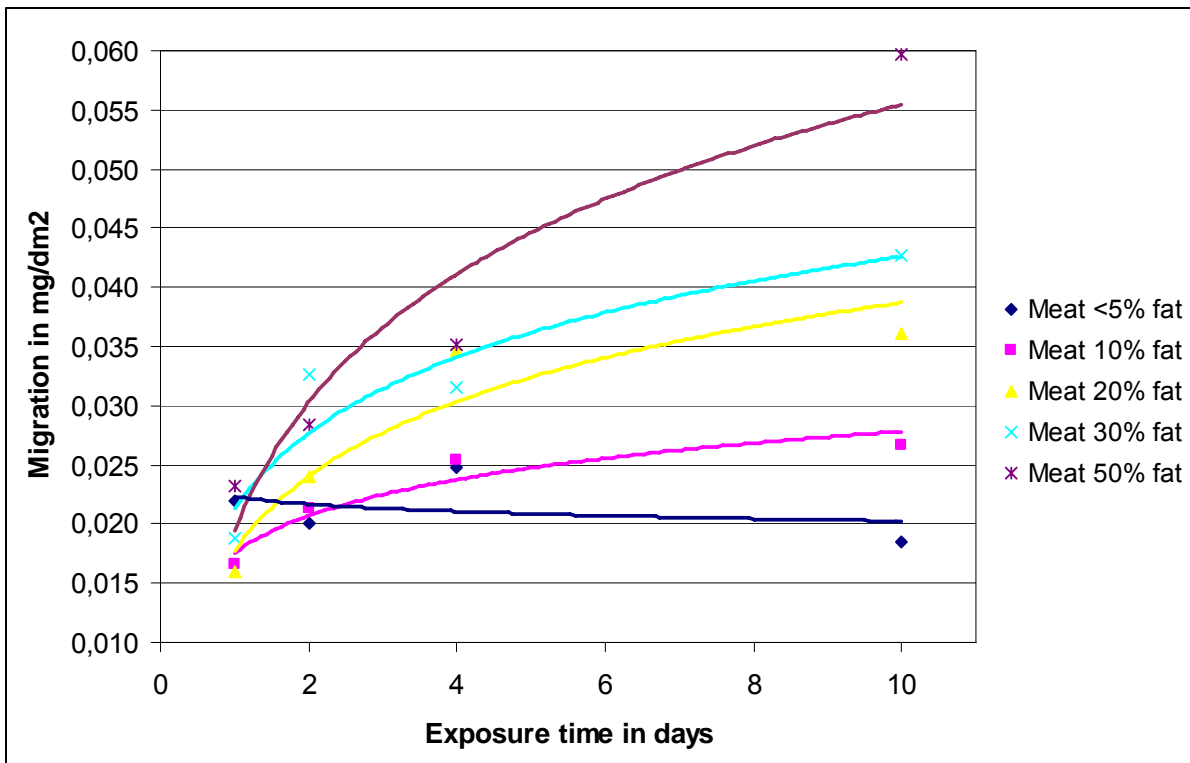
Figure 80 Migration of diphenyl phthalate at 5°C into meat with various fat contents

Figure 80 shows the migration curves of diphenyl phthalate into meat with various fat contents at 5°C. The higher the fat content of the meat, the higher the migration level of diphenyl phthalate. The migration into meat with 20% and 30% fat was twice as high and into meat with 50% fat three times as high as into lean meat (<5% fat). Due to variation in the repeated determination of concentration of diphenyl phthalate in the lean meat samples for day 1 and 2 (Table 50) the higher values were ommited and only the lower values were taken into account as the extracts were obviously contaminated during handling.

Looking at Figure 81 it is a similar situation for the migration of benzophenone at the same test conditions. The migration of benzophenone is significantly higher than the migration of the diphenyl phthalate at given conditions as the smaller molecules of benzophenone migrate more readily, but the relative differences between meats with different fat content are not as big as for diphenyl phthalate. Yet the same correlation between the fat content and the migration level was observed here also, which means the higher the fat content the higher the migration.
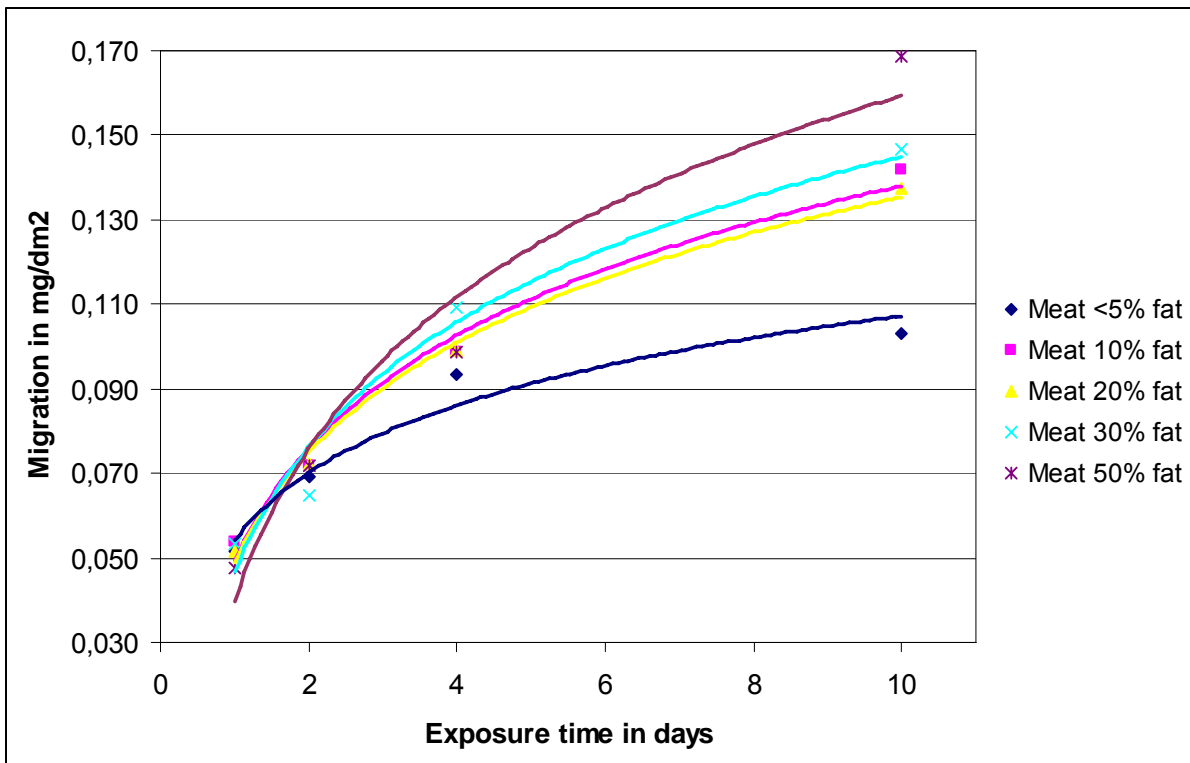
Figure 81 Migration of benzophenone at 5°C into meat with various fat contents

At higher temperature the same behaviour was observed (Figure 82 and Figure 83). Test at 25°C showed the same pattern of higher migration values at higher fat content for both substances. Due to the nature of the sample these test were limited to 24 hours storage time with just two setpoints at 8 and 24 hours. At these conditions the fat content has more significant influence on the migration of the diphenyl phthalate which can be seen on the rise of the slope with higher fat content of the sample. Whereas the migration of benzophenone after 24 hours into meat with 50% fat reaches just about 120% of the value measured after the same exposure time in meat with <5% fat, for diphenyl phthalate the difference is more than 400%. In other words the migration of diphenyl phthalate into meat with 50% fat was more than 4 times higher than into meat with <5% fat. And even the migration into meat with 20% fat was more than 3 times the migration into meat with <5% fat after 24 hours at 25°C.
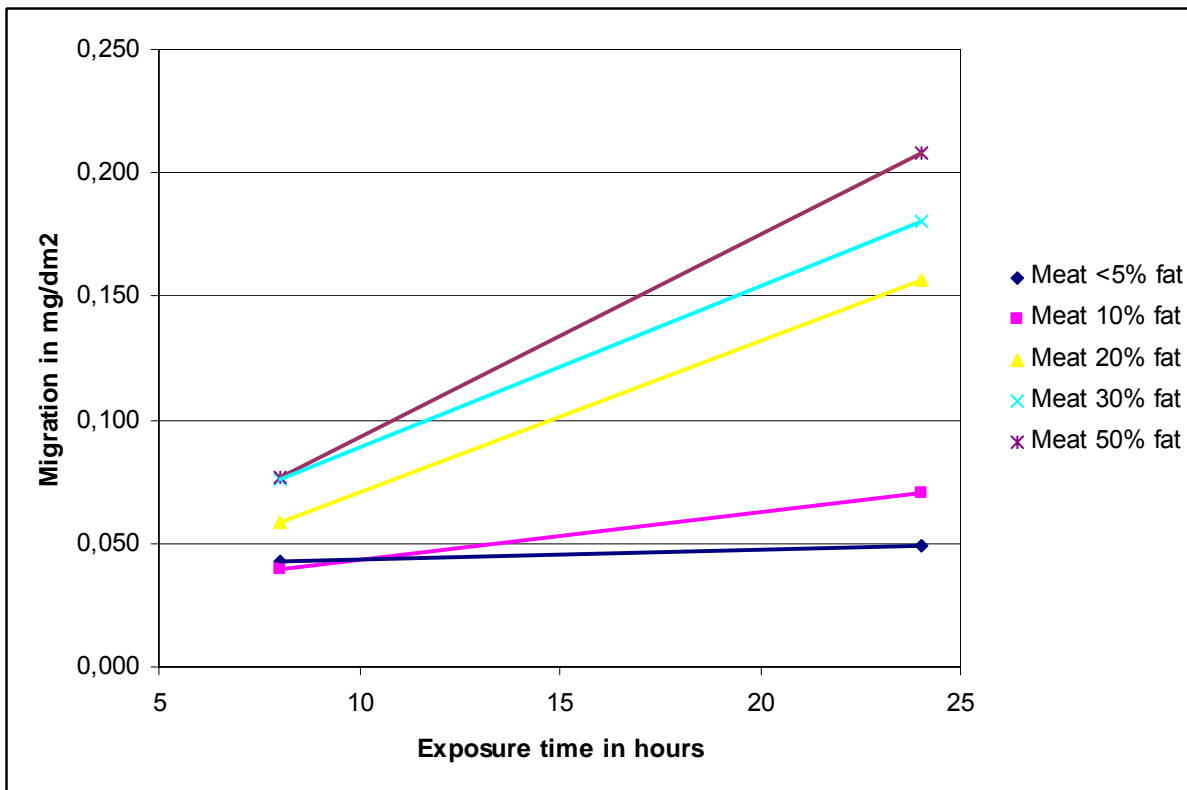
Figure 82 Migration of diphenyl phthalate at 25°C into meat with various fat contents
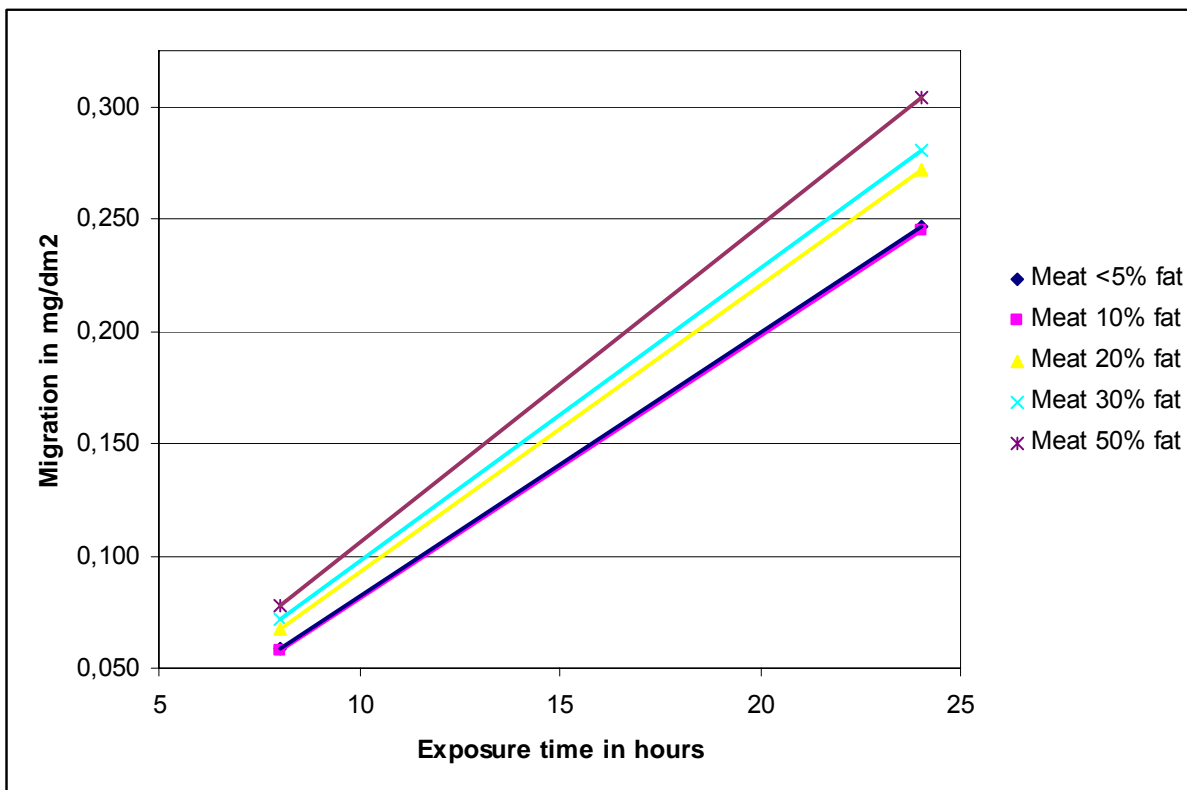
Figure 83 Migration of benzophenone at 25°C into meat with various fat contents.

9.2 Influence of the temperature on migration

The temperature is one of the crucial parameters having an effect on the extent of the migration. This has been shown also in other studies focused on migration (10),(12),(14). Higher temperature also means higher kinetical energy of molecules which further results in higher migration.

From the range of foodstuffs included in the migration tests some were tested at two or even three different temperatures. In the following chapters the effect of different temperature on the migration of benzophenone and diphenyl phthalate into selected foodstuffs is demonstrated. Also all the foodstuffs tested at three different temperatures were selected here for comparison: orange juice, milk, flour.

9.2.1 Influence of the temperature on the migration into orange juice
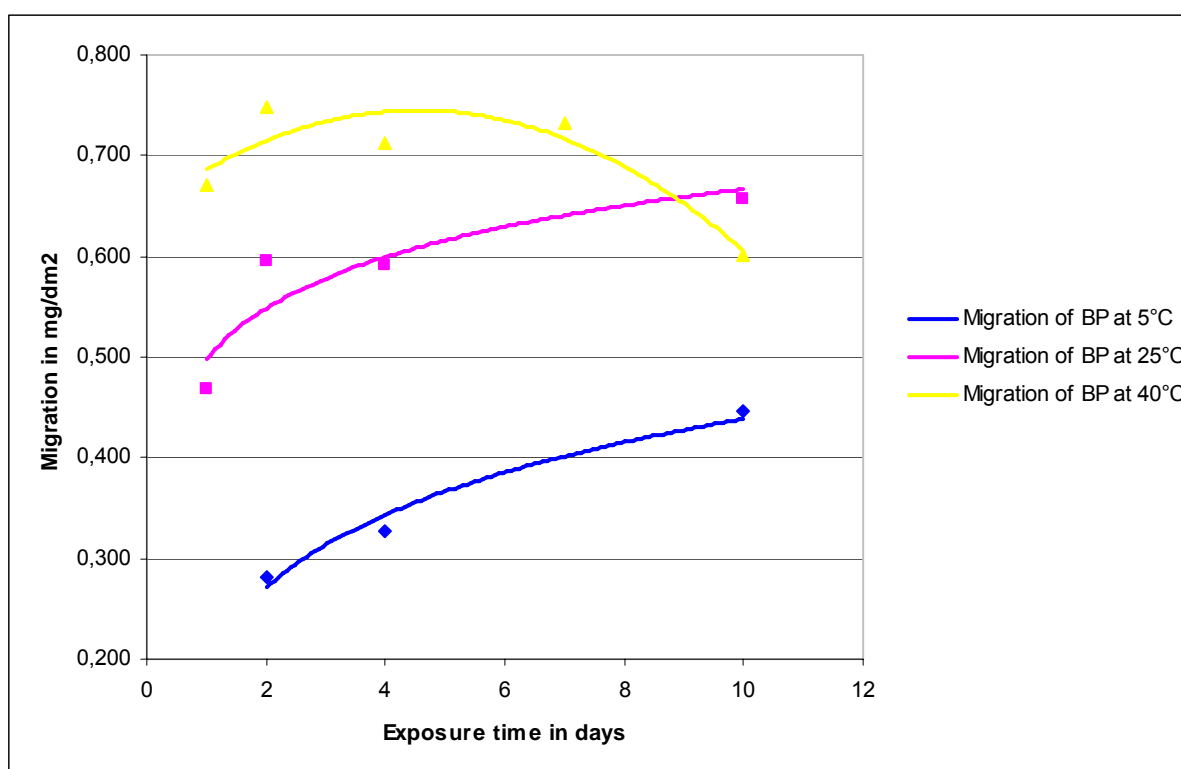
Figure 84 Migration of benzophenone into orange juice at different temperatures

In Figure 84 the effect of varying temperature on the migration of benzophenone into orange juice is illustrated. It can be seen that after just 2 days storage the migration at 25°C is approximately twice the migration observed at 5°C and if the temperature is raised to 40°C the migration is even more rapid. The bending of the yellow curve describing migration at 40°C is different to those at lower temperatures. The

reasons for this might be the very fast leaching of the benzophenone from the plastic film to the orange juice combined with the instability of the substance in this particular foodstuff – see also Table 10. As can be seen from Figure 84 almost all the benzophenone present in the plastic film migrated into the orange juice after just 1 day exposure. Due to the almost total migration of benzophenone from the plastic film into the orange juice during the first 24 hours of exposure the whole amount of the analyte available was subject to interaction with the orange juice for almost the entire time of storage. During the stability tests the recovery for benzophenone in orange juice after 10 days storage at 40°C was 91%. Assuming maximum possible migration of benzophenone from the plastic film (given the composition of the plastic film it was calculated to be $0,675mg/dm^2$) and 91% recovery after 10 days storage at 40°C, the expected migration value would be $0,614mg/dm^2$. The measured value was $0,602mg/dm^2$. It was therefore concluded that the stability of the benzophenone in the orange juice together with the measurement uncertainty were responsible for the observed drop in migration values at these storage conditions.

The deviation between the two repeated determinations is less than 5% eliminating the possibility of film inhomogeneity.
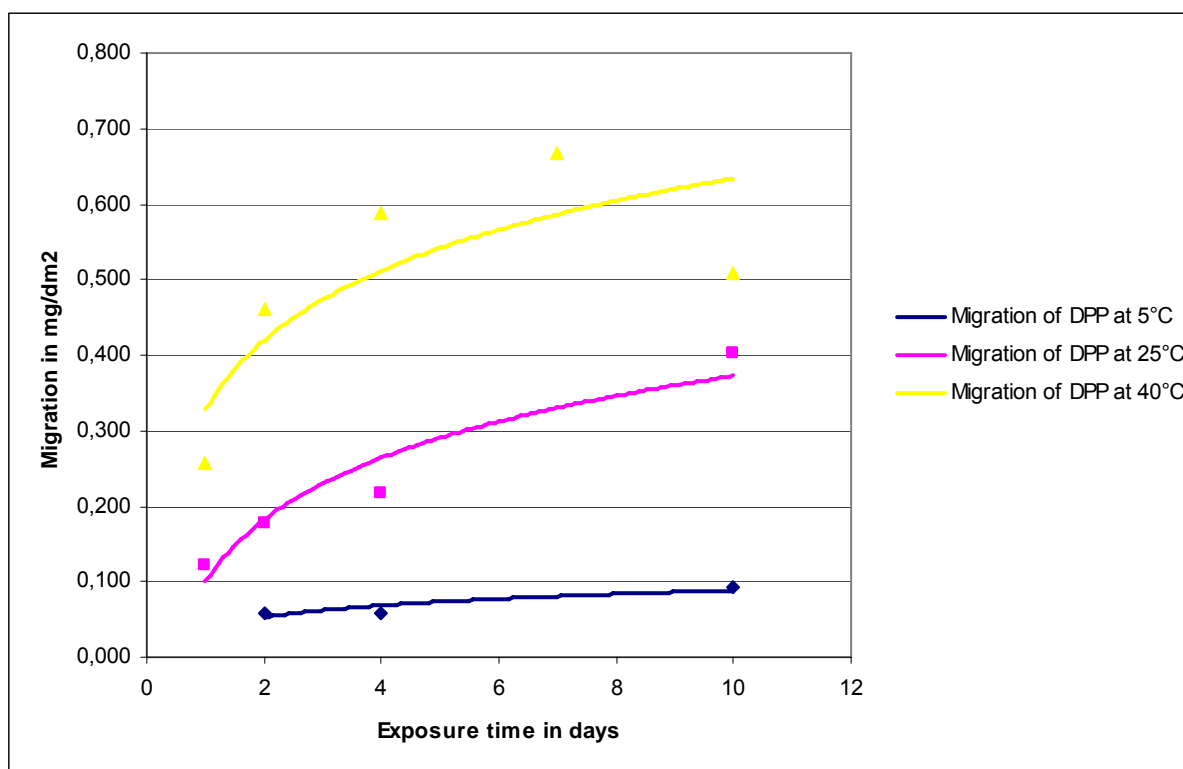
Figure 85 Migration of diphenyl phthalate into orange juice at different temperatures

Similar observation was made with diphenyl phthalate. The migration after two days at 25°C was 3 times higher than it was the case at 5°C. At 40°C the increase of the migration was even more distinctive at more than seven fold the migration value at 5°C. After 10 days of storage the migration at 25°C was 4 times the value measured at 5 °C. At 40°C an observation similar to that with benzophenone was made, as the

measured concentration of diphenyl phthalate on the 10th day of storage was lower than on the 4th and 7th day. This might be partially the result of the stability of the substance tested, but other processes are suspected to be involved too. Yet film inhomogeneity is unlikely as the correlation between the two repeated determinations is good with a standard deviation of less than 3%.

9.2.2 Influence of the temperature on the migration into milk
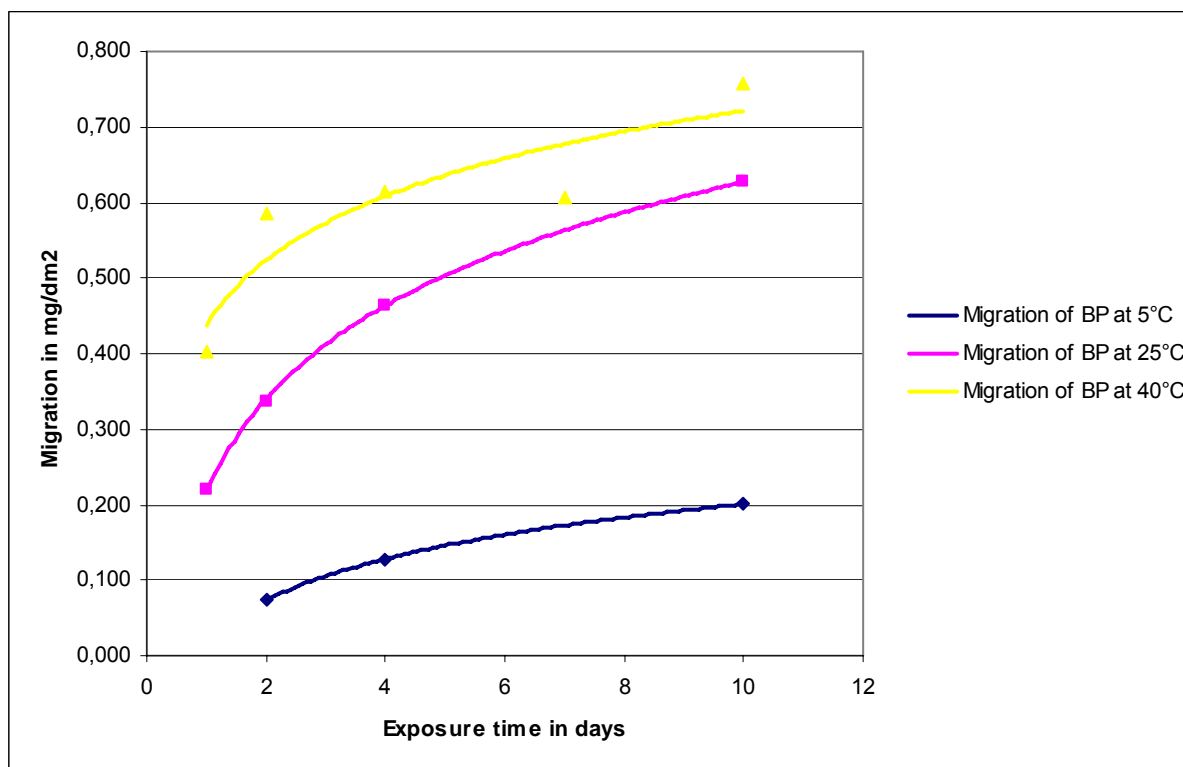
Figure 86 Migration of benzophenone into milk at different temperatures

Measuring the migration behaviour into milk has been of great interest not only because of the broad consumption and its nutritional value, but also as the milk represents an aqueous foodstuff yet with a considerable amount of fat, which could have great influence on the migration of hydrophobic substances from materials coming into contact with milk.

Figure 86 illustrates the extent of migration of benzophenone into milk at different temperatures. Even at the lower temperature of 5°C almost 30% of benzophenone present in the contacting plastic film transferred into the foodstuff after 10 days storage. At higher temperatures, 25°C and 40°C likewise, the migration was beyond 90% after the same storage time.
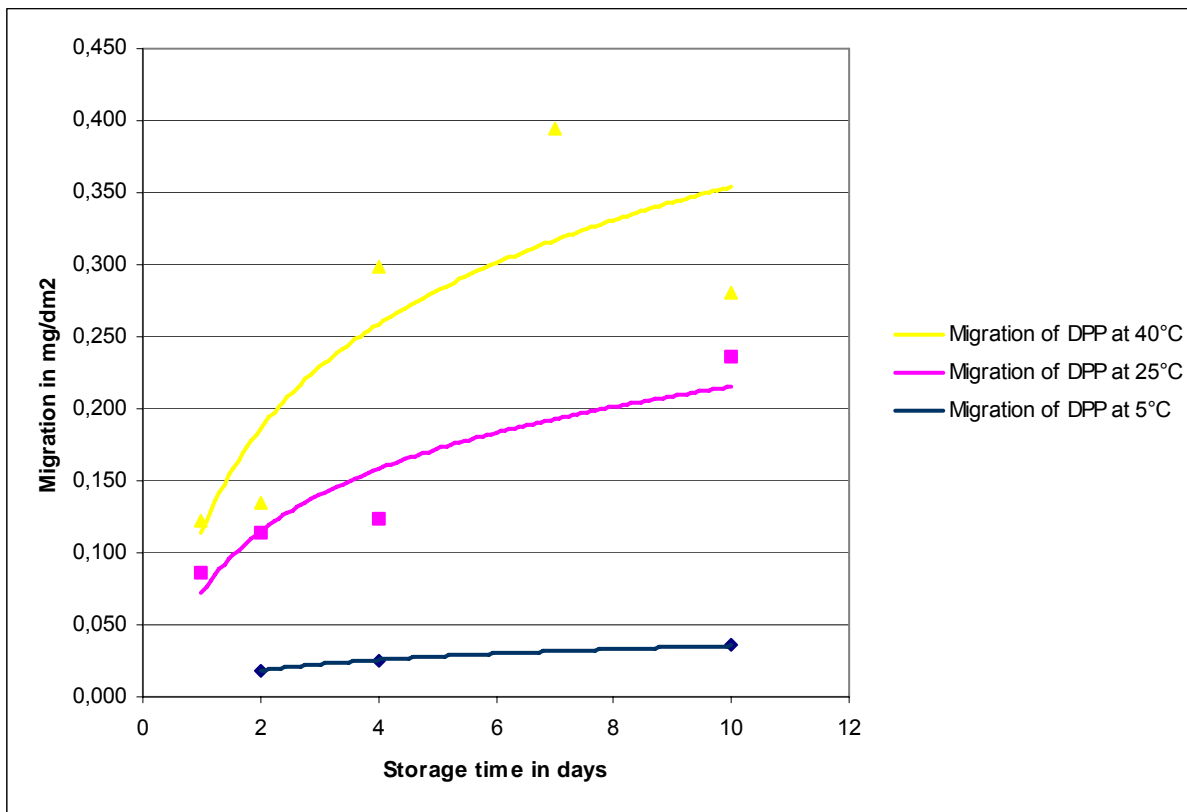
Figure 87 Migration of diphenyl phthalate into milk at different temperatures

The measured migration values for diphenyl phthalate were lower than those of benzophenone, yet the run of the migration curves is similar. The curve describing migration at 5°C is flat but the migration increases consistently with rising temperature. Migration of DPP at 25°C after 10 days of storage was more than 6 times the value measured at 5°C. At 40°C the migration increased even more to almost 8 times the value measured at 5°C. It has to be noted though that the analytical determination of DPP concentration in milk involved some difficulties including the choice of internal standard and recovery. The stability of the analyte as well as the choice of a suitable internal standard proved to be problematic while measuring migration at higher temperatures after several days of storage. After 7 days of storage at 25°C or 40°C the measured values started to scatter. This was not the case at 5°C where the values of the repeated determinations showed good correlation even after 30 days of storage. Yet even with these value variations the trend is visible and the migration rises with higher temperature.

9.2.3 Influence of the temperature on the migration into flour
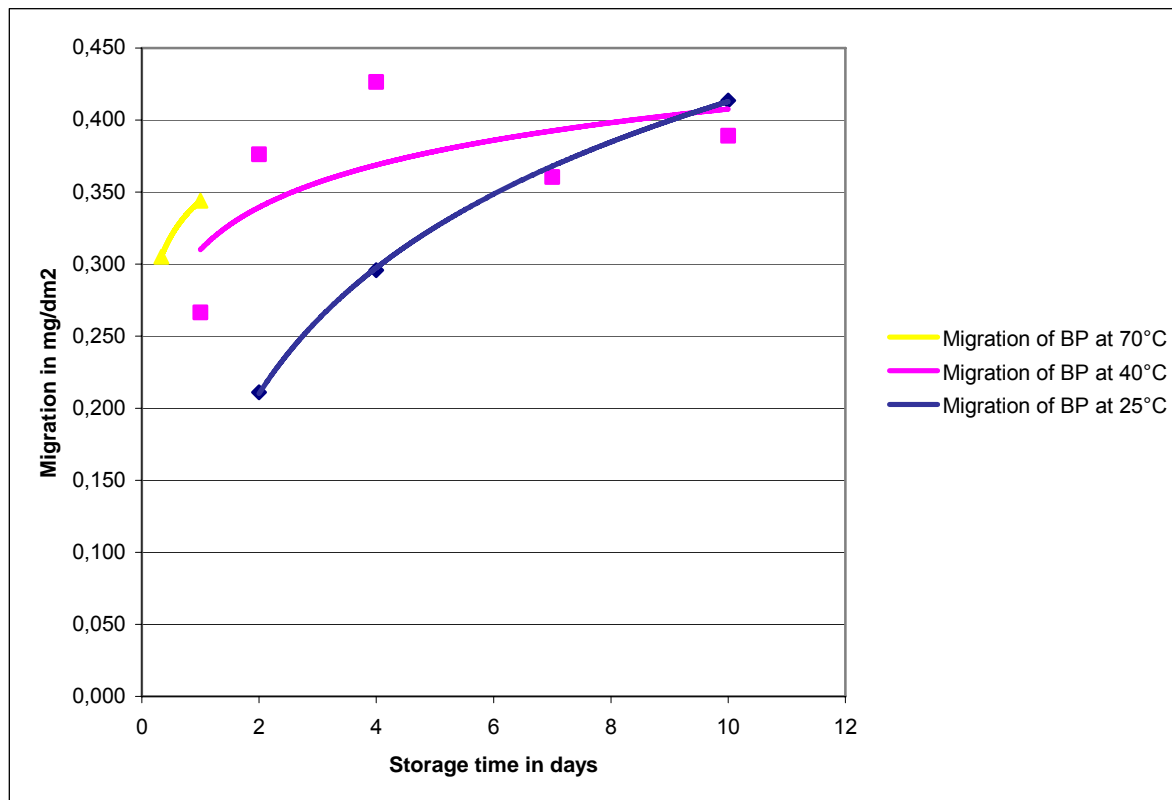
Figure 88 Migration of benzophenone into flour at different temperatures

In the case of flour the effect of temperature elevation up to 70°C on the migration of BP and DPP was studied. The dramatic influence of varying temperature on the extent of the migration has already been shown on the examples of orange juice and milk. Similar observation has been made in flour. Figure 88 illustrates the influence of elevated temperature on the migration of BP into flour. The concentration of BP in the flour after approximately 6 days storage at 25°C is reached just after 2 days at 40°C and after 24 hours at 70°C. Moreover, it takes 4 days at 25°C to reach the same concentration of BP in the foodstuff that was measured after just 8 hours at 70°C.
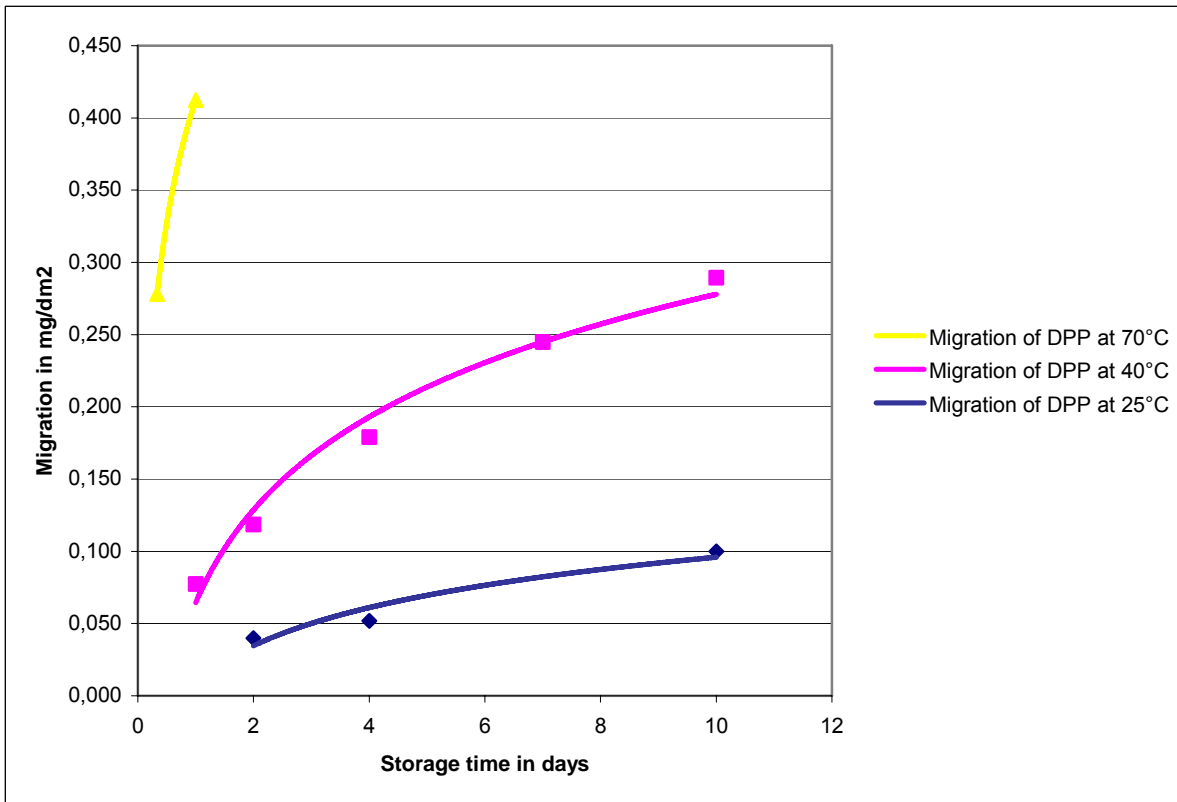
Figure 89 Migration of diphenyl phthalate into flour at different temperatures

Compared with BP the migration of DPP is more temperature dependant. Figure 89 illustrates the trend in the migration of DPP into flour at different temperatures. Elevating the temperature from 25°C to 40°C results in more than twice the concentration value of DPP in the foodstuff after just 2 days and the difference increases with longer storage time. Raising the temperature to 70°C accelerates the migration dramatically. It takes 10 days at 40°C and more than 60 days at 25°C to reach the same concentration of DPP in the foodstuff as after just 8 hours at 70°C. The concentration of DPP measured in the foodstuff after 24 hours storage at 70° reached 87% of the value measured after 6 months at 25°C.

9.2.4 Influence of the temperature on the migration into cheese sauce
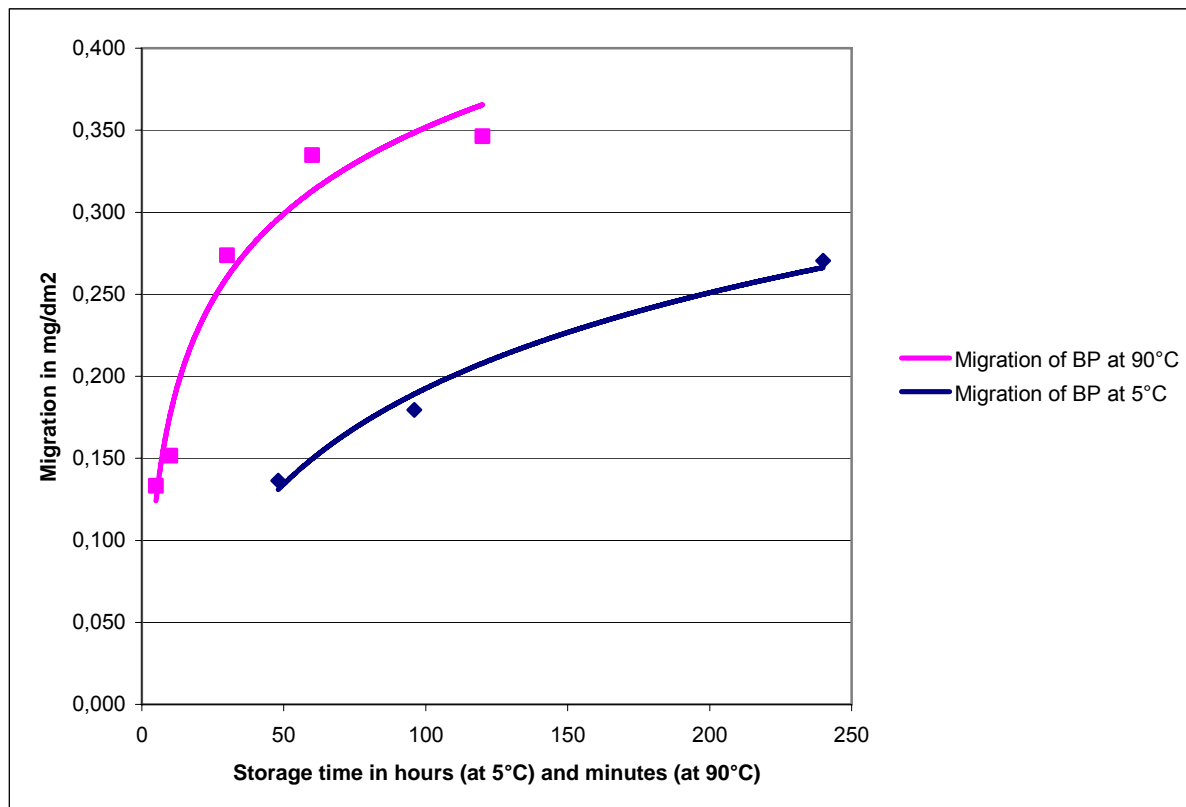
Figure 90 Migration of benzophenone into cheese sauce at different temperatures

In Figure 90 the migration of BP into cheese sauce is illustrated. It should be pointed out, that the x-axis has to be read in hours for the migration curve describing the migration at 5°C and in minutes for the migration curve at 90°C. The same level of BP migrated into the cheese sauce after 5 minutes at 90°C and after 48 hours at 5°C. It takes 10 days at 5°C for the BP to reach the same concentration in the foodstuff as it does after just 30 minutes at 90°C.
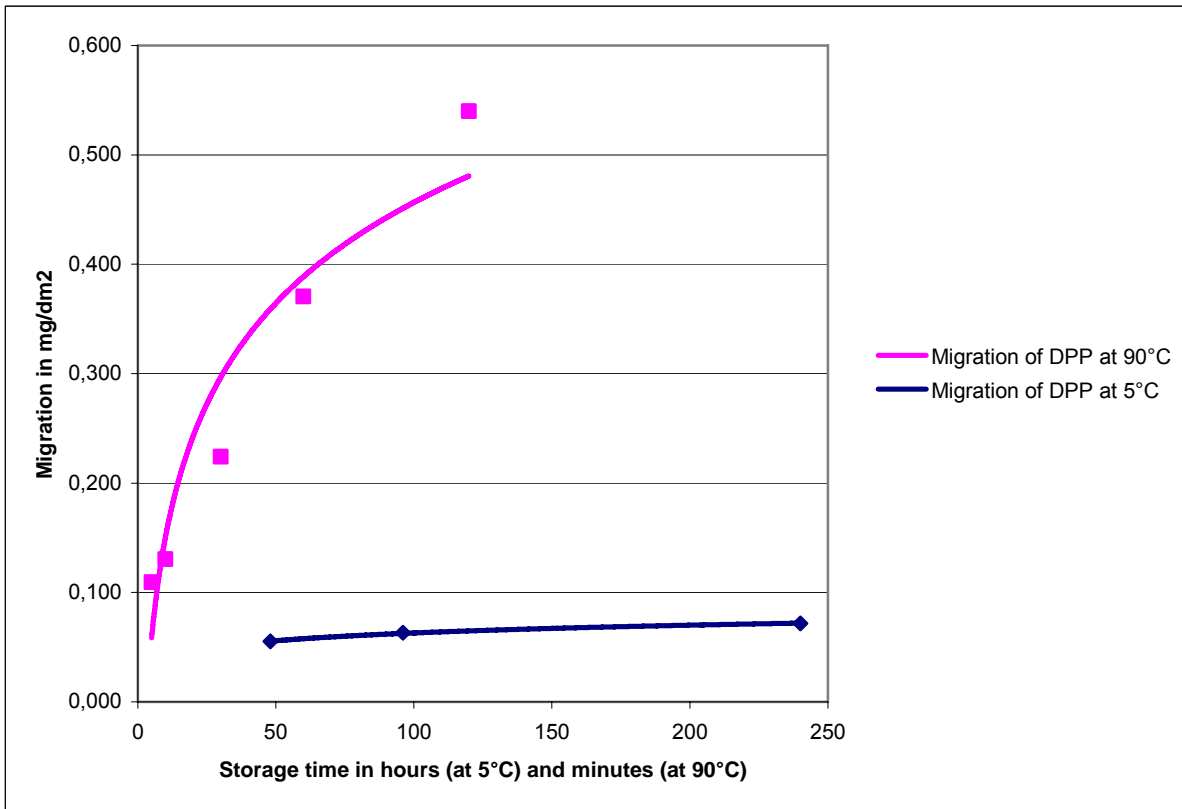
Figure 91 Migration of diphenyl phthalate into cheese sauce at different temperatures

Also concerning Figure 91 it should be pointed out, that the x-axis has to be read in hours for the migration curve describing the migration at 5°C and in minutes for the migration curve at 90°C. Once again the dependency of the migration of DPP on the temperature is demonstrated. At 5°C the first concentration value measured after 48 hours is relatively low and increases only slowly during storage. On the other hand the concentration of DPP in the cheese sauce measured after just 5 minutes at 90°C is already higher than the final concentration measured after 30 days at 5°C.

9.2.5 Influence of the temperature on the migration into mayonnaise
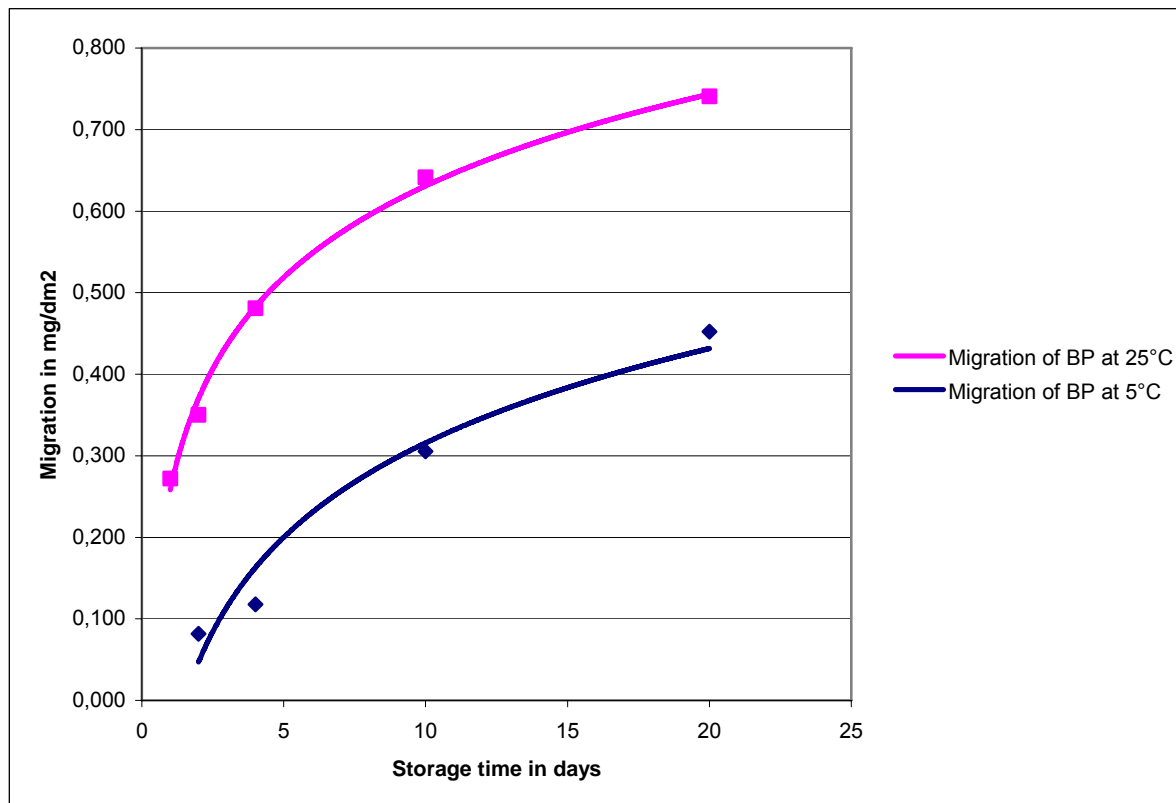
Figure 92 Migration of benzophenone into mayonnaise at different temperatures

Figure 92 demonstrates the influence of varying temperature on the migration of BP into mayonnaise. The mayonnaise proved to be a potent extracting agent as even at low temperatures considerable amount of BP transferred into the foodstuff. After 10 days at 25°C almost all the BP present in the plastic film migrated into the mayonnaise and even at 5°C it was more than 66% after 20 days storage and reaching 85% after 30 days. On average the migration at 25°C was 2-3 times higher than at 5°C at each time-point.
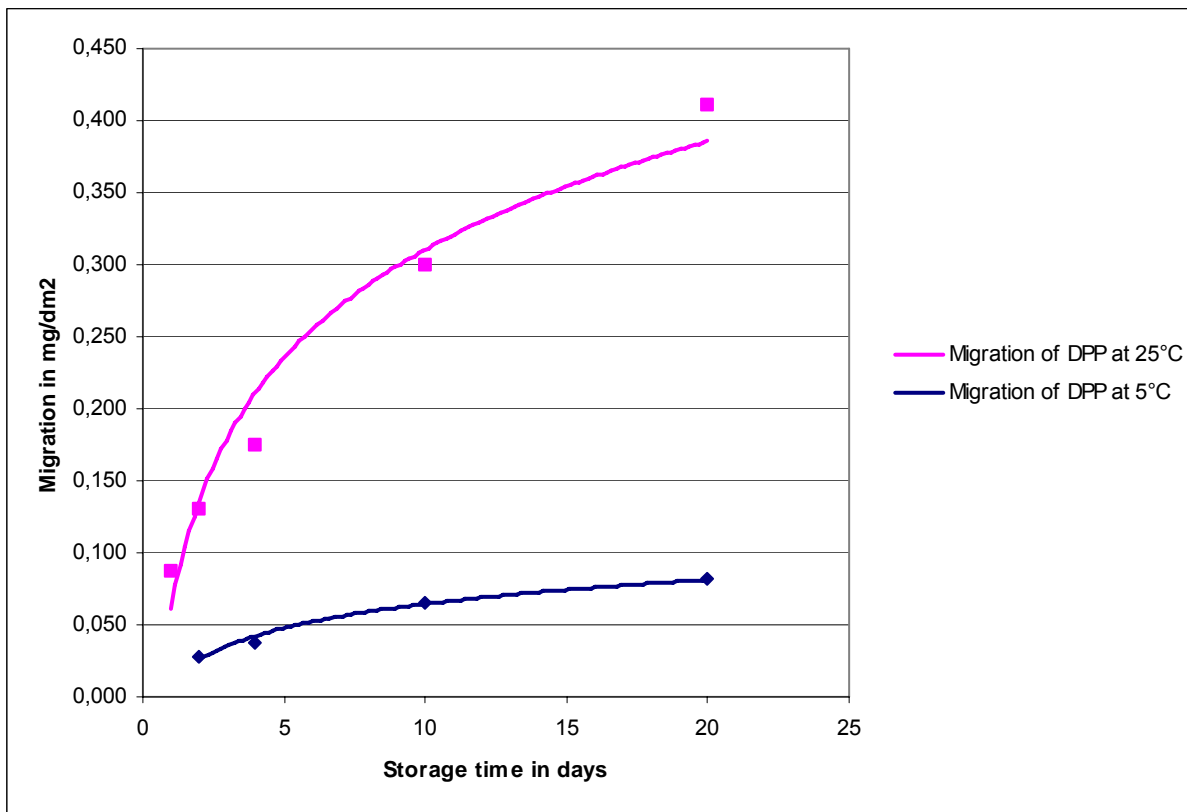
Figure 93 Migration of diphenyl phthalate into mayonnaise at different temperatures

Figure 92 and Figure 93 enable us to compare the extent to which elevated temperature influences the migration of BP and DPP respectively. Whereas BP migrates into mayonnaise in considerable amount even at temperature as low as 5°C, DPP migration at the same conditions is much slower. Yet with temperature elevated to 25°C the migration of DPP is accelerated and reaches similar values to those of BP at 5°C. 49% of the DPP present in the plastic film transferred into the mayonnaise after 20 days at 25°C, whereas at 5°C it was only 10% under the same conditions.

9.2.6 Influence of the temperature on the migration into ketchup
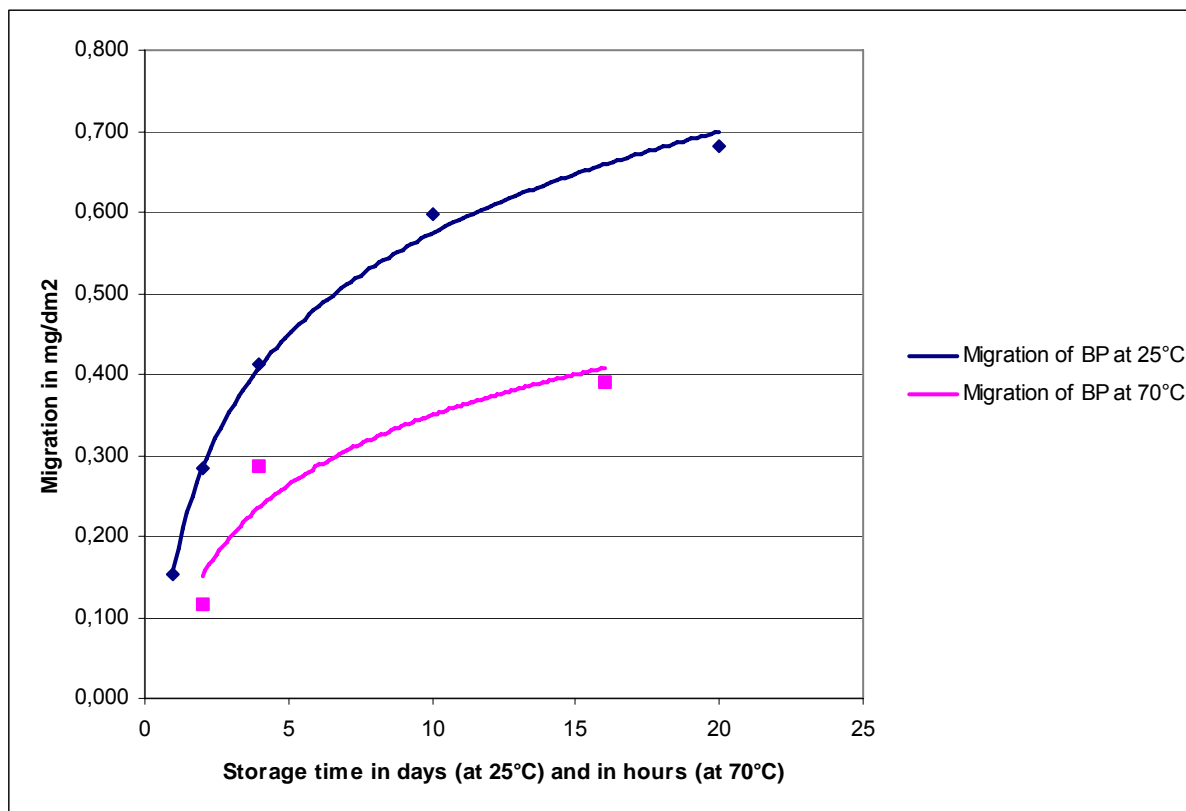
Figure 94 Migration of benzophenone into ketchup at different temperatures

Figure 94 illustrates the migration of BP into ketchup at different temperatures. The curve describing migration of BP at 70°C omits the values measured after 8 and 24 hours. Based on the experience with migration of BP into other foodstuffs and looking into the migration of DPP into ketchup under the same conditions (see Figure 95) the concentration of BP measured in the ketchup after 8 and 24 hours is considerably lower than expected (Table 29). Concerning the unusual variation in measured BP concentration during the migration testing at 70°C it should be noted that each point on the graph represents an average value of two independent measurements, including the whole migration testing procedure beginning with the storage in two separate jars, subsequent extraction of the foodstuff and finally analytical determination of the analyte in the extracts. The duplicate determinations after both 8 and 24 hours storage showed good correlation within the determinations itself, yet did not fit into the overall trend of the curve describing the migration. This error was limited to BP only as the measured concentrations of DPP in the same extracts did not show any abnormality. The reason for the variation in the BP concentration is suspected to be an inhomogeneous distribution of the analyte in the plastic film.

From the data in Figure 94 it can be seen that after 20 days storage at 25°C all of the BP present in the plastic film migrated into the ketchup, after 16 hours at 70°C it was 58% which was approximately the same amount as after 4 days at 25°C.
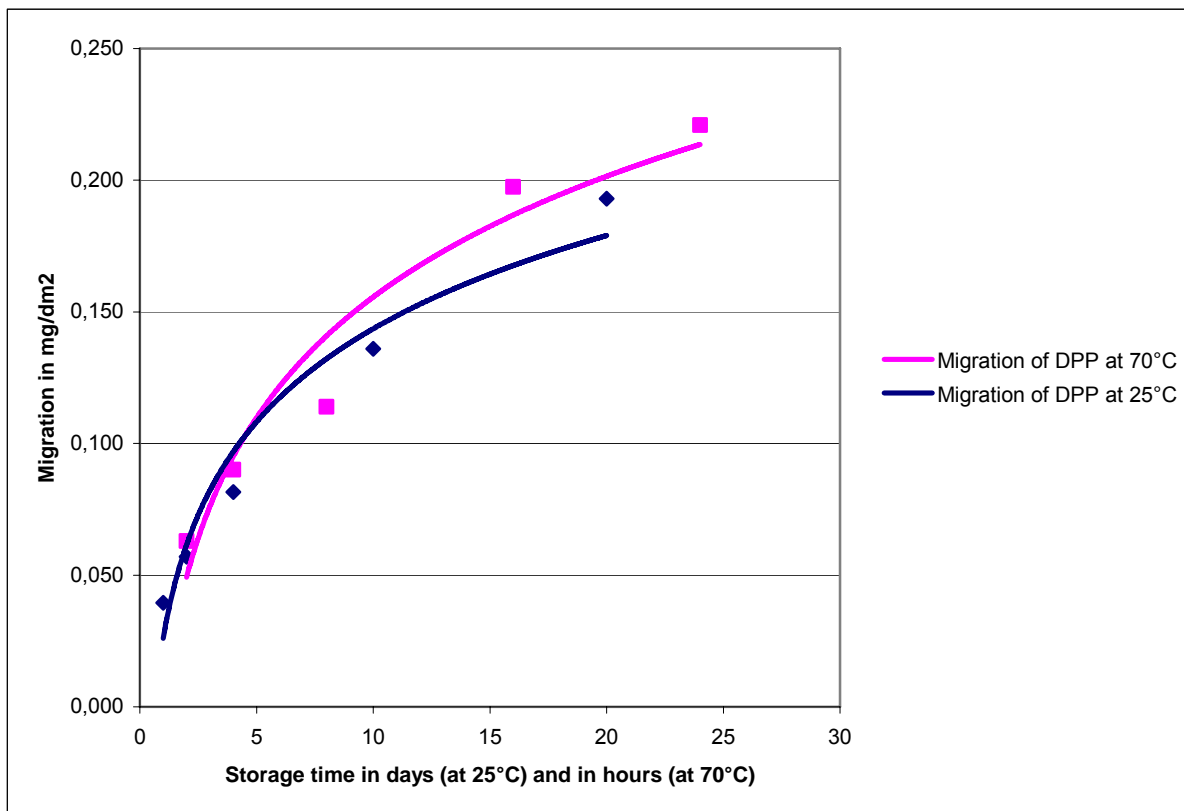
Figure 95 Migration of diphenyl phthalate into ketchup at different temperatures

The trends in migration of DPP into ketchup are illustrated in Figure 95. Once again the scale of the x-axis has to be read in days for the migration curve at 25°C and in hours for the migration curve at 70°C. It is clear that the migration at the elevated temperature is much more rapid. After 24 hours storage at 70°C about 26% of the DPP transferred from the plastic film into the ketchup whereas after 20 days at 25°C it was approximately 23%.

9.2.7 Influence of the temperature on the migration into milk powder
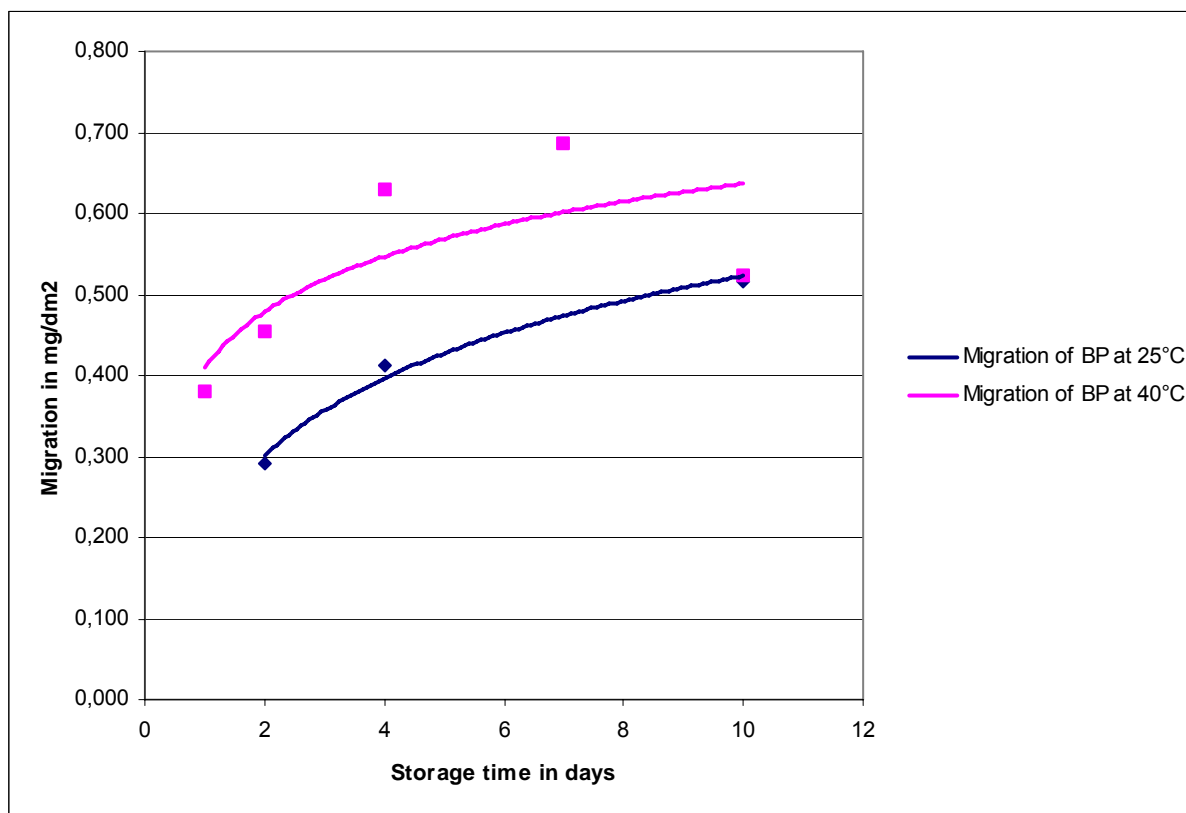
Figure 96 Migration of benzophenone into milk powder at different temperatures

Dry foodstuffs in general were considered to give less concern about migration compared with liquid and fatty foodstuffs. It therefore was surprising to see the extent of migration occurring during our test. Figure 96 shows the migration of BP into milk powder at different temperatures. It can be seen that at 40°C the migration approaches its maximum, which was calculated to be $0,675mg/dm^2$, after just a few days storage and all of the BP present in the plastic film migrates into the milk powder after less than 10 days. And even at 25°C the migration reached 76% of the maximum possible value after 10 days of storage.

With DPP the situation was not as dramatic, but there was still considerable migration, especially at 40°C. Figure 97 illustrates the migration of DPP into milk powder at different temperatures. After 10 days at 40°C more than 45% of the DPP migrates from the plastic film into the milk powder. At 25°C the migration is clearly lower, after 10 days about 14% of the DPP migrated into the foodstuff. Prolonging the storage at 25°C up to 180 days the migration of DPP reaches 60%.
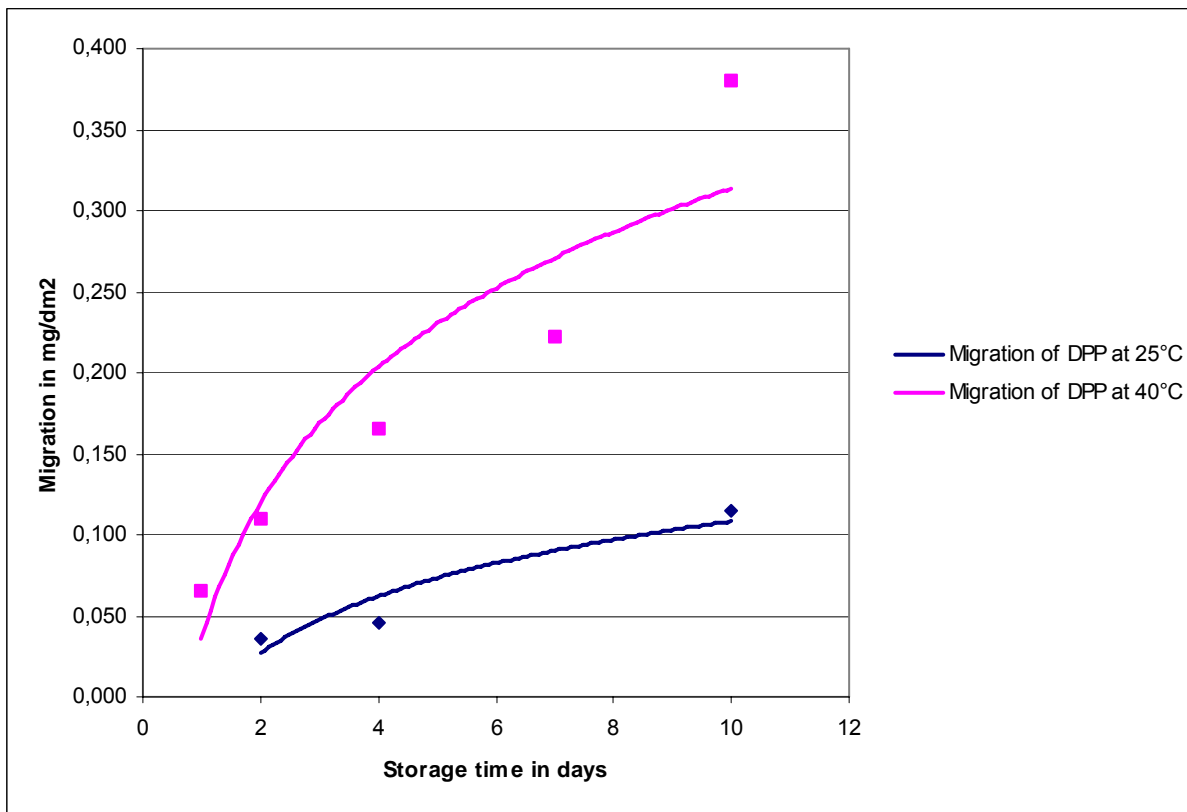
Figure 97 Migration of diphenyl phthalate into milk powder at different temperatures

9.2.8 Influence of the temperature on the migration into rice
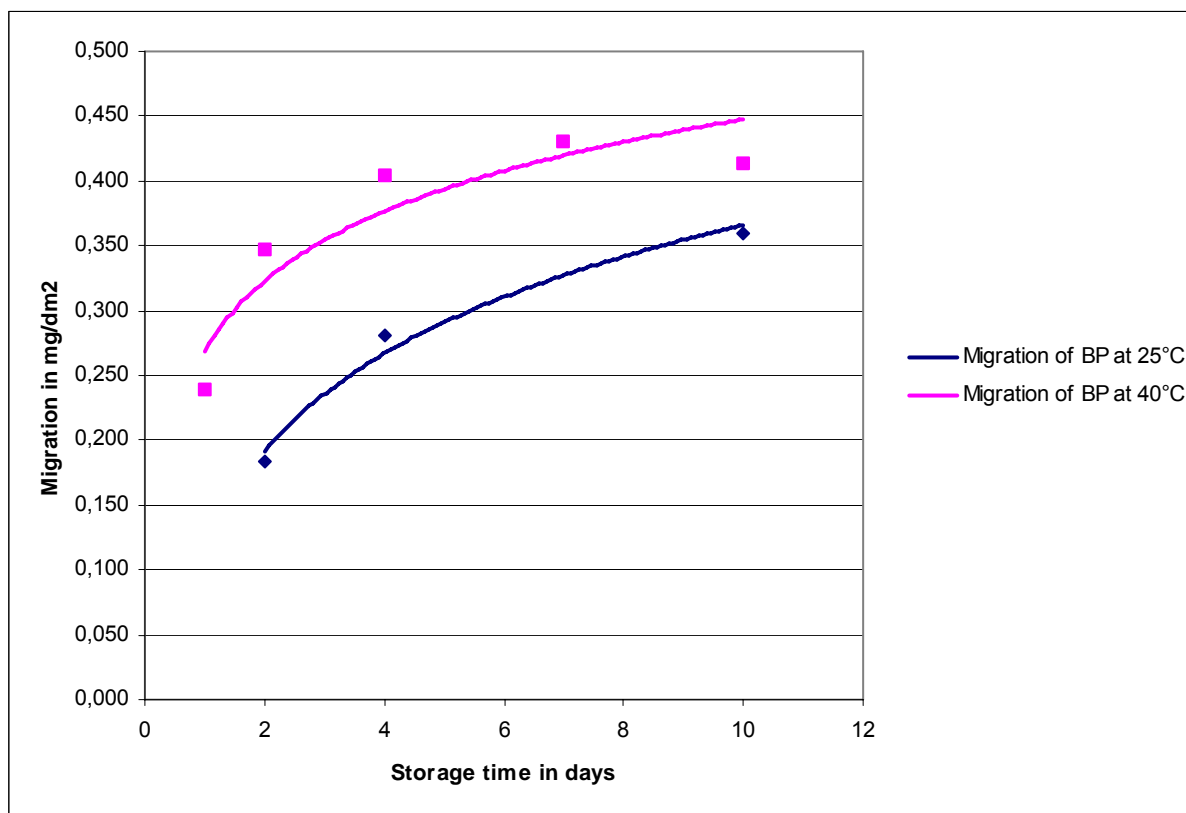
Figure 98 Migration of benzophenone into rice at different temperatures

The influence of different temperatures on the migration of BP into rice is illustrated in Figure 98. Elevating the temperature from 25°C to 40°C results in a rise in migration as expected. After 2 days storage the migration at 40°C is almost twice as high as at 25°C but after 10 days storage the concentrations of BP measured in the foodstuff differ only around 15%. The migration of BP at 25 °C reaches its maximum after 10 days storage (at this point 53% of the BP present in the plastic film migrated into the foodstuff) and remains approximately at this level even until 180th day of storage.

The influence of elevated temperature is more obvious in the case of DPP as can be seen from Figure 99. The absolute values of DPP concentrations measured in the rice are considerably lower than those of BP but the influence of the different temperatures is more obvious. After 2 days storage the migration at 40°C is 6 times higher than at 25°C and after 10 days it is still 4 times as high. It takes 60 days at 25°C to reach the same level of migration as after 10 days at 40°C. In contrast to BP the DPP continues to migrate at 25°C even up to 180th day of storage at which point it reaches $0,180 mg/dm^2$, which constitutes approximately 21% of the DPP present in the plastic film.
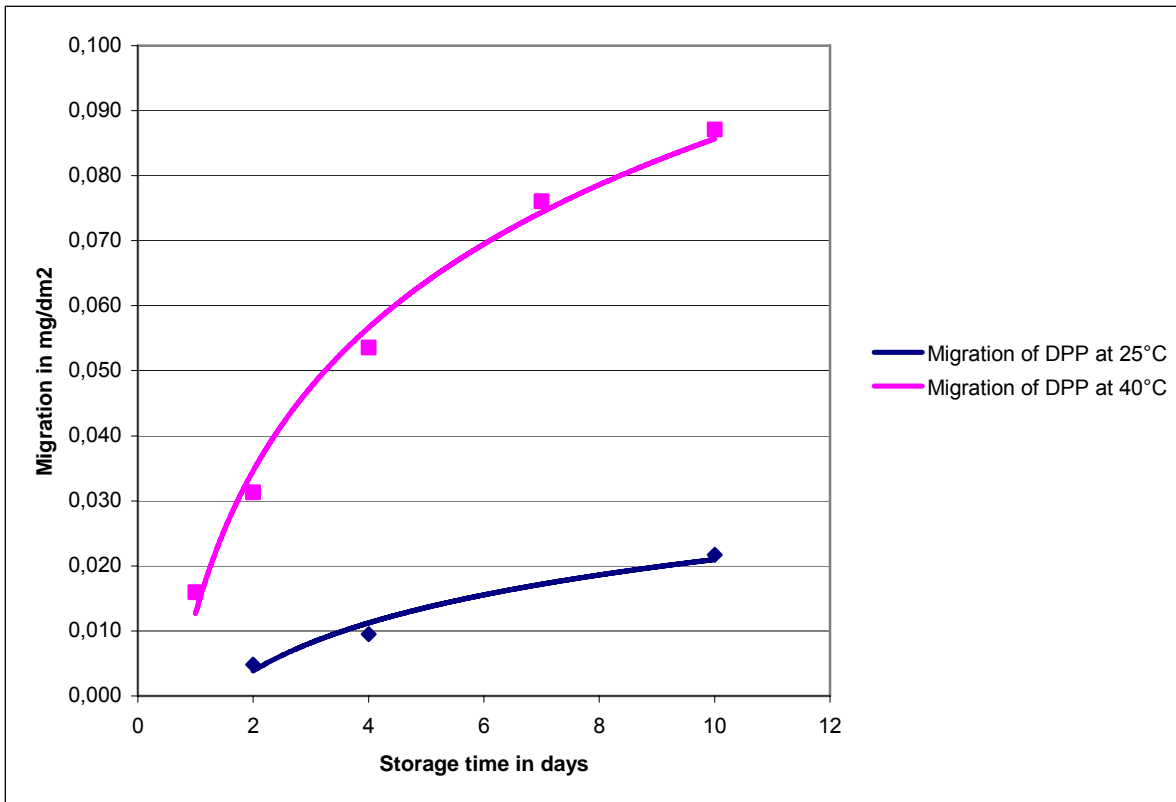
Figure 99 Migration of diphenyl phthalate into rice at different temperatures

9.2.9 Influence of the temperature on the migration into meat

From Figure 100 and Figure 101 the influence of elevated temperature on the migration of benzophenone and diphenyl phthalate into meat is obvious. Pork meat with 5 various fat contents has been tested. The influence of rising temperature on the migration is even more noticeable with rising fat content of the foodstuff, especially for DPP. The migration of DPP into meat with 50% fat content after just 24 hours at 25°C is almost 10 times higher than at 5°C.

In the case of BP the migration at 25°C is 5 to 6 times higher than at 5°C throughout all the different fat contents of the meat samples. But also here a slight rise in the migration with the rising fat content at 25°C was observed.
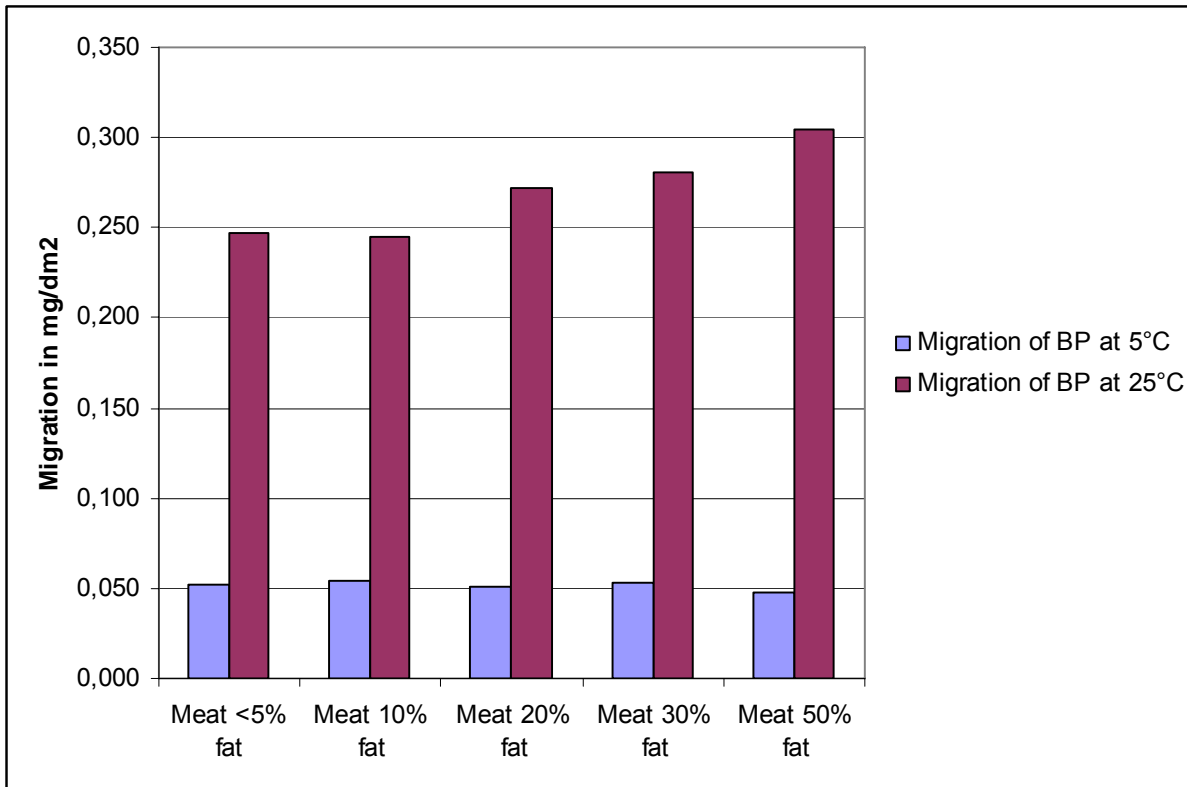
Figure 100 Migration of benzophenone into meat after 24 hours at different temperatures
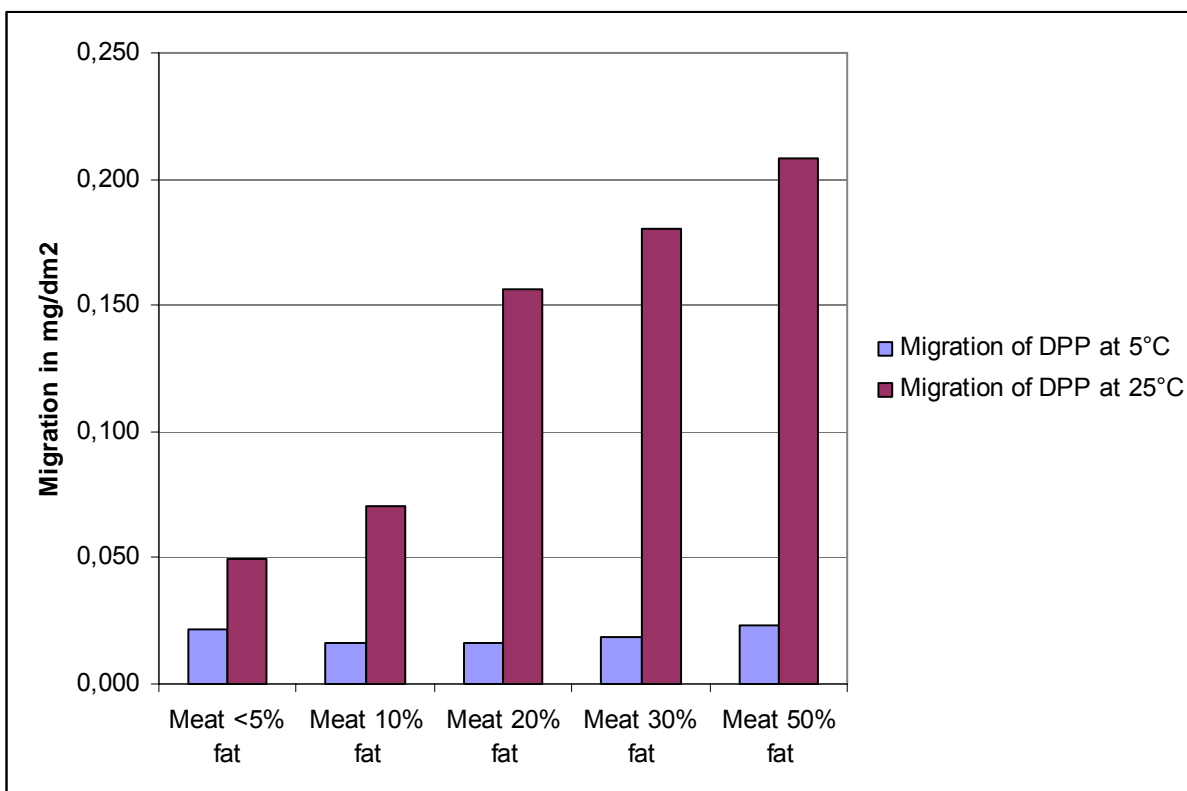
Figure 101 Migration of diphenyl phthalate into meat after 24 hours at different temperatures

Based on the results of the experiments, which have been illustrated in the previous comparison graphs it can be concluded that the storage conditions, in this case the temperature, has a substantial influence on the extent of the migration. Elevating the temperature for even a short time might promote the migration dramatically. In some cases migration levels which would normally require month of storage are reached within hours if the temperature is raised. This emphasizes the importance of appropriate storage conditions for packed foodstuffs regarding not only the possibility of microbial spoilage or changes of the organoleptic properties but also the possibility of undesirable changes in the composition of the foodstuff caused by the packaging material itself.

9.3 Comparing migration within the individual food categories

9.3.1 Migration into dry foodstuffs – flour, milk powder, rice and butter toast

As has been shown before, dry foodstuffs like flour and milk powder showed unexpected high levels of BP and DPP migrating from a contacting plastic film. Despite the solid state and the particulate character of these foodstuffs which could lead to the false assumption that the migration of substances from the packaging material does not require as much attention as in aqueous foodstuffs for example, the flour and the milk powder feature very high specific areas, which in combination with an adsorbtion process on the surface of the foodstuff can result in significant migration through the gas phase of the packaging. As the migration curves in the following figures - Figure 102, Figure 103 , Figure 104 and Figure 105 - demonstrate, the highest levels of migration were observed in the milk powder, followed by flour and rice.

From the total amount of BP present in the plastic film following amounts migrated into the dry foodstuffs during a storage period of 180 days at 25°C -> the highest migration was observed into milk powder with 90%, second highest migration level was measured in flour with a peak of 61% and the lowest migration exhibited rice with a still considerable 54%. Possible explanation of the atypical trend of migration into flour might be starch ageing. In presence of humidity starch ageing may occur and result in poor analyte recovery.

During 10 days at 40°C the results were as follows -> the highest migration was once again observed into milk powder with 100% BP migrating into the foodstuff followed by rice and flour both with peaks around 63%.

From the total amount of DPP present in the plastic film following amounts migrated into the dry foodstuffs during a storage period of 180 days at 25°C -> the highest migration was observed into milk powder with 60%, second was the flour with a maximum of 56% and the lowest migration was measured into rice with 21%. Similar picture with the migration during 10 days at 40°C -> the highest migration was again measured in milk powder with 45%, second was the flour with a maximum of 34% and the lowest migration was observed in rice with 10% of DPP transferring into the foodstuff.
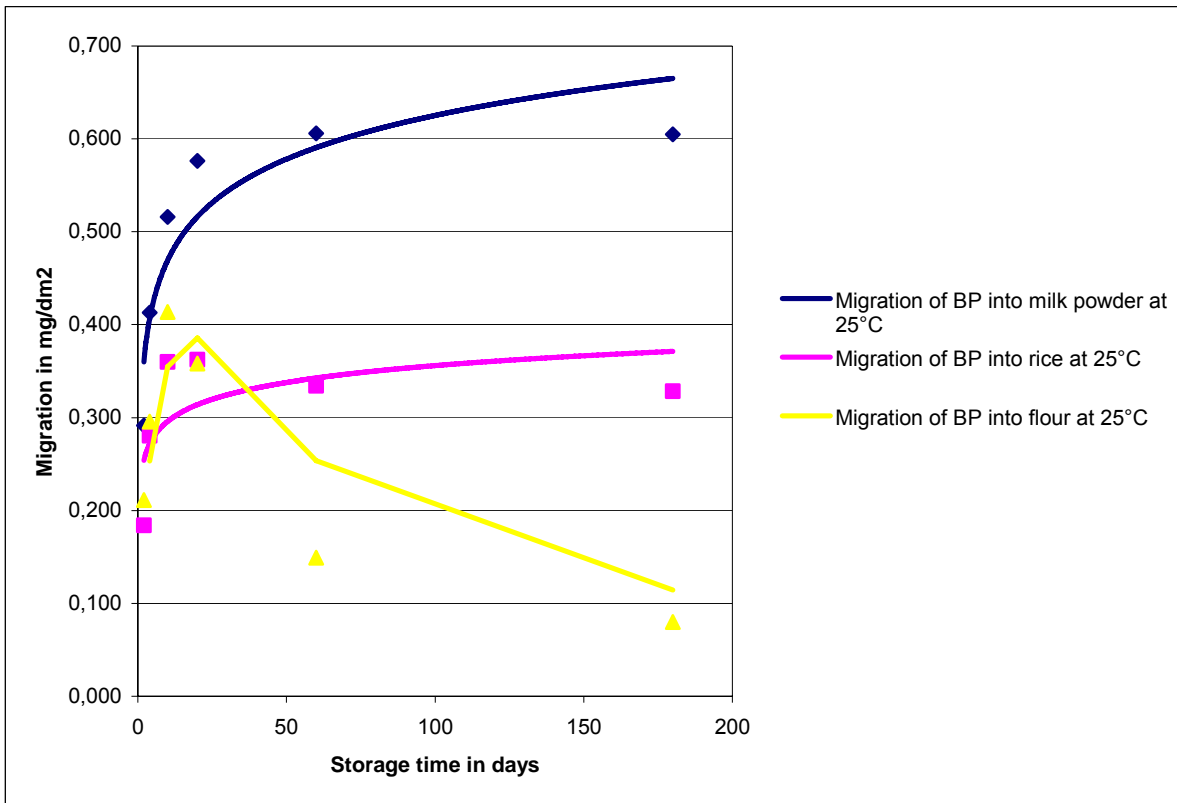
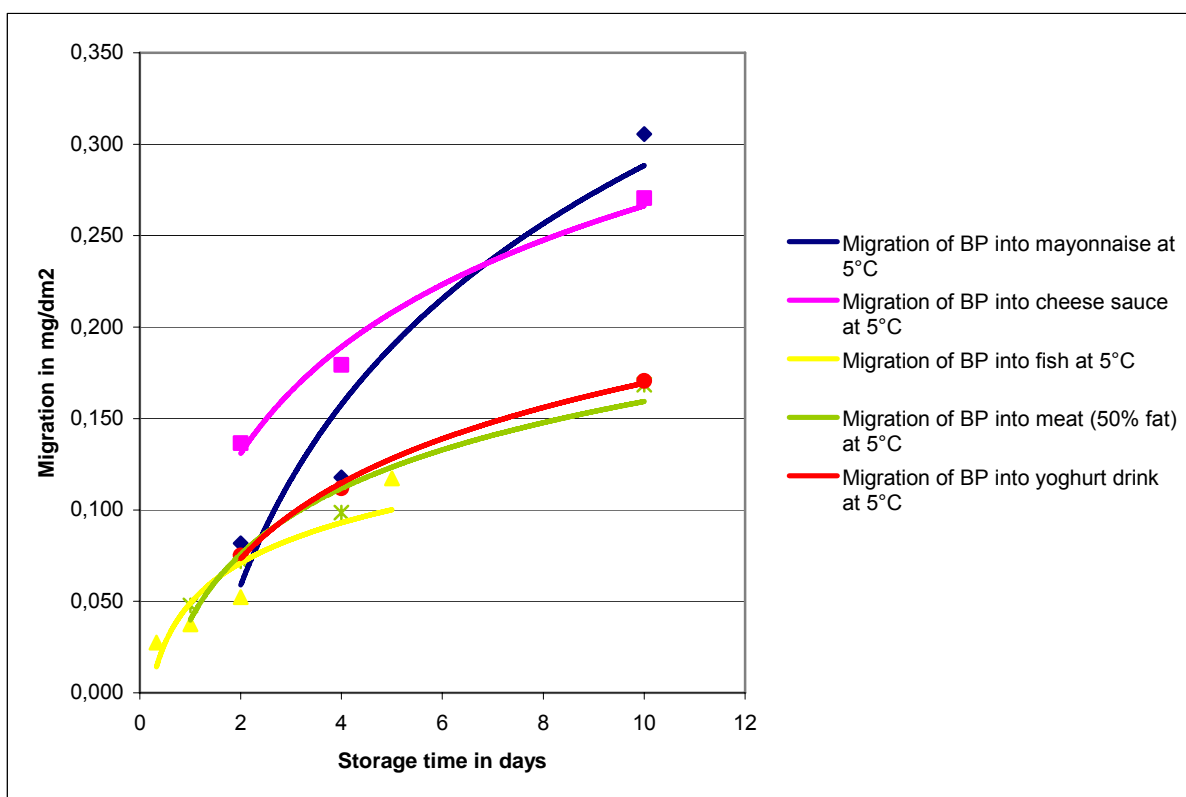Figure 102 Migration of benzophenone into dry foodstuffs at 25°C
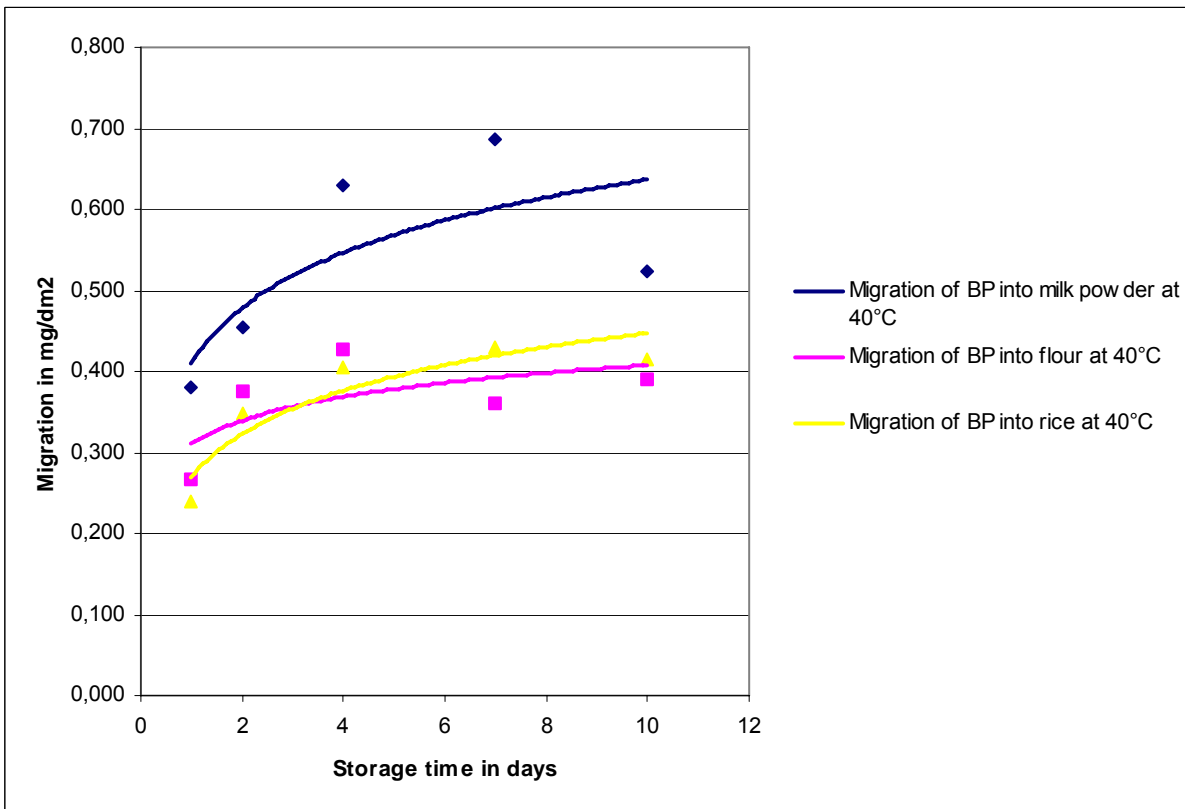
Figure 103 Migration of benzophenone into dry foodstuffs at 40°C
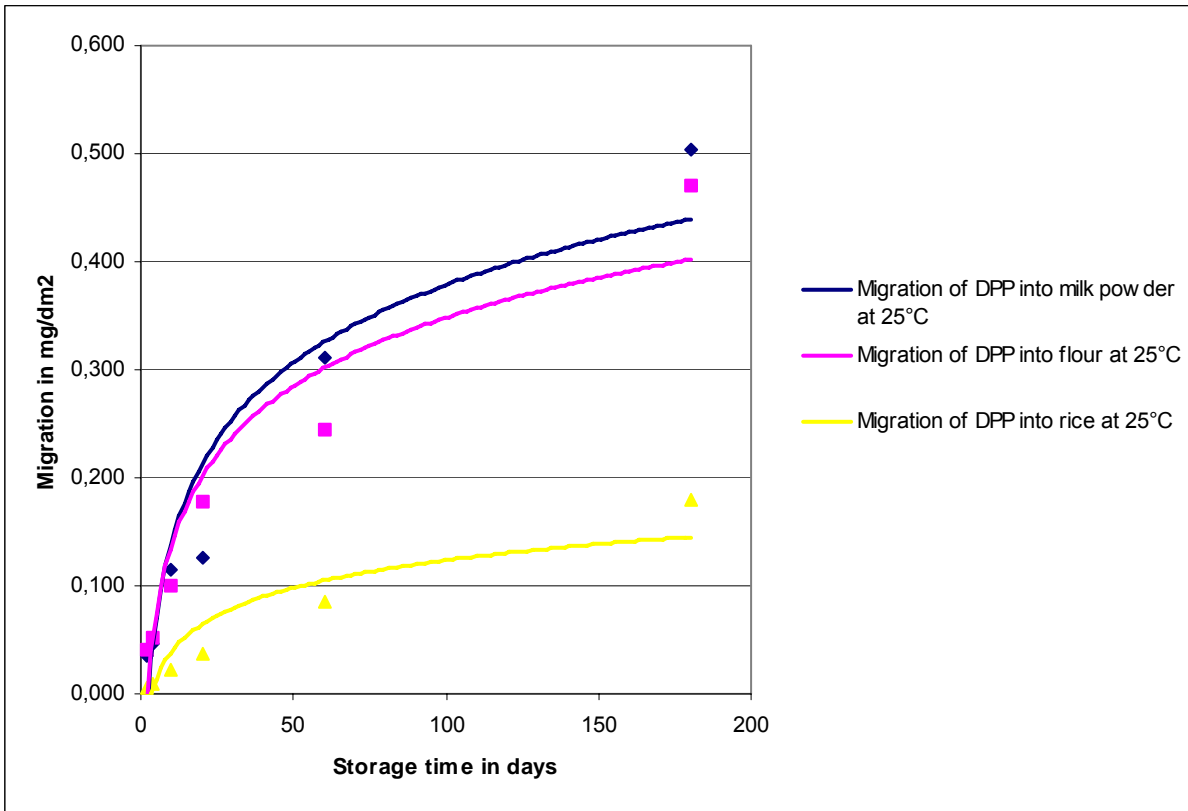
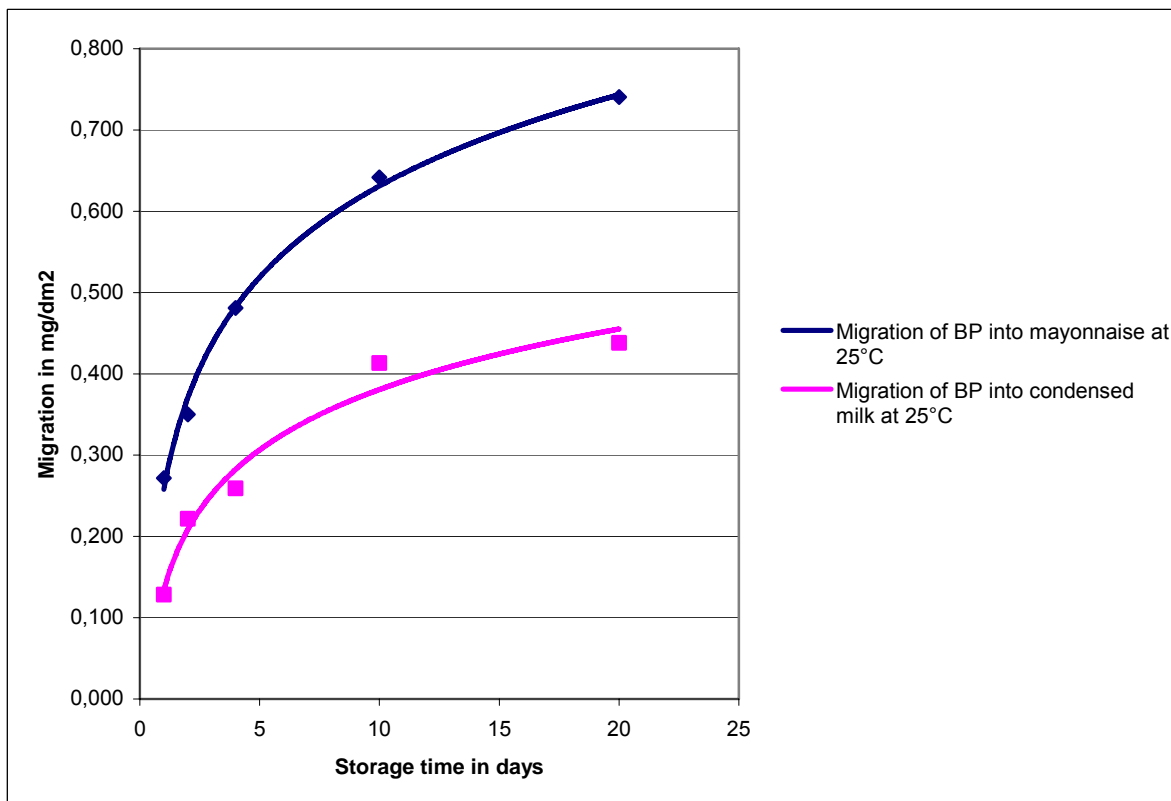Figure 104 Migration of diphenyl phthalate into dry foodstuffs at 25°C
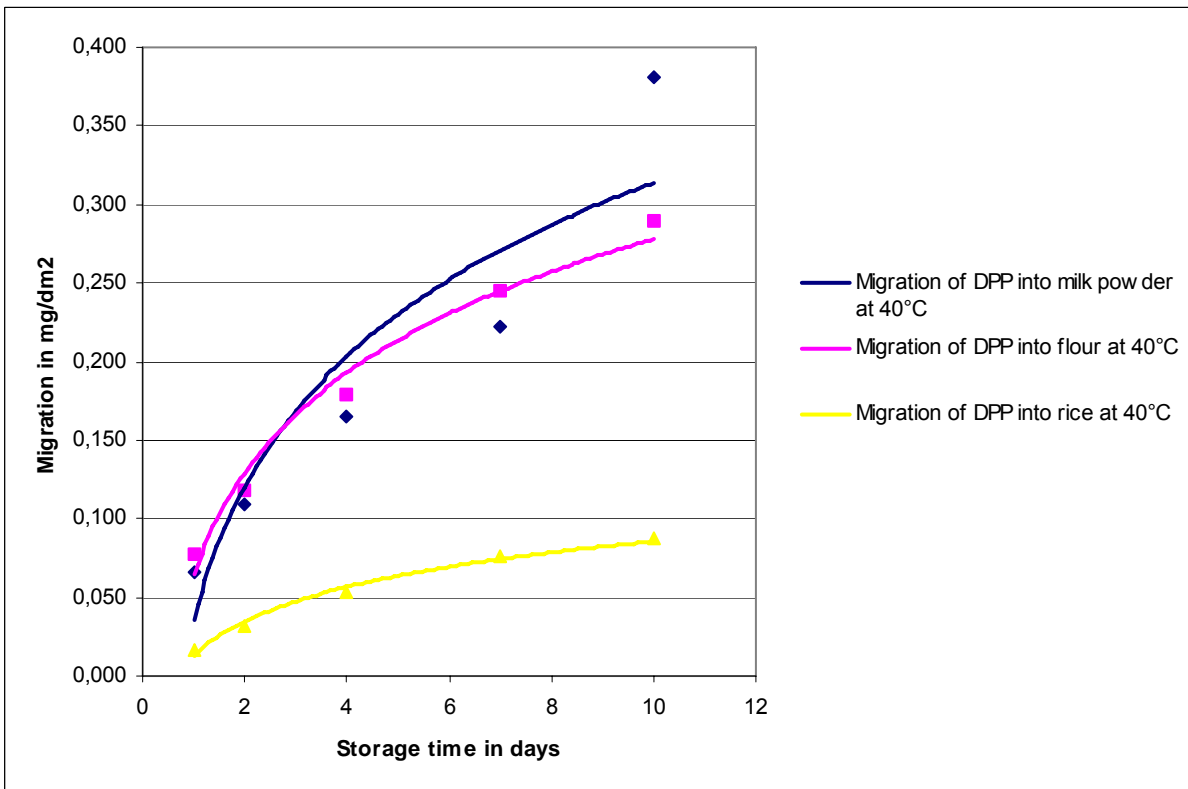
Figure 105 Migration of diphenyl phthalate into dry foodstuffs at 40°C

Migration of benzophenone into butter toast was also higher than expected. Despite the fact that the porous structure of the butter toast does not allow as intimate contact between the foodstuff itself and the plastic film as in the case of fluids or even particulate foodstuffs like flour or milk powder, the migration of BP into butter toast was still considerable as illustrates Figure 106. Under the same storage conditions the migration into butter toast after 20 days storage was second highest of all dry foodstuffs tested, exceeded only by milk powder. This example also proves that the migration through the gas phase can not be neglected even if the area of contact between the foodstuff and the plastic film is minimal. This is especially the case for volatile substances with low molecular weight. In comparison, DPP which has a higher molecular weight and is also less volatile than BP showed overall lower migration values. Comparing Figure 106 and Figure 107 some conclusions about the effect of volatility of a substance as well as the structure of a foodstuff on the migration can be drawn. BP as the more volatile substance shows consistently higher migration values. The extent of direct contact between the foodstuff and the plastic film is less crucial for volatile substances from the migration point of view. These substances do migrate readily through the gas phase of a packaging and provided they are soluble in the foodstuff they readily migrate into it as well. This is well illustrated by the butter toast in Figure 106. In the case of less volatile substances more intimate contact between the foodstuff and the plastic film is necessary for migration to take place. The migration of DPP into butter toast in Figure 107 illustrates this very well. Compared with the migration of BP in Figure 106 which was second highest into butter toast, in case of DPP the migration into butter toast was third highest and well below the migration measured into milk powder and flour.
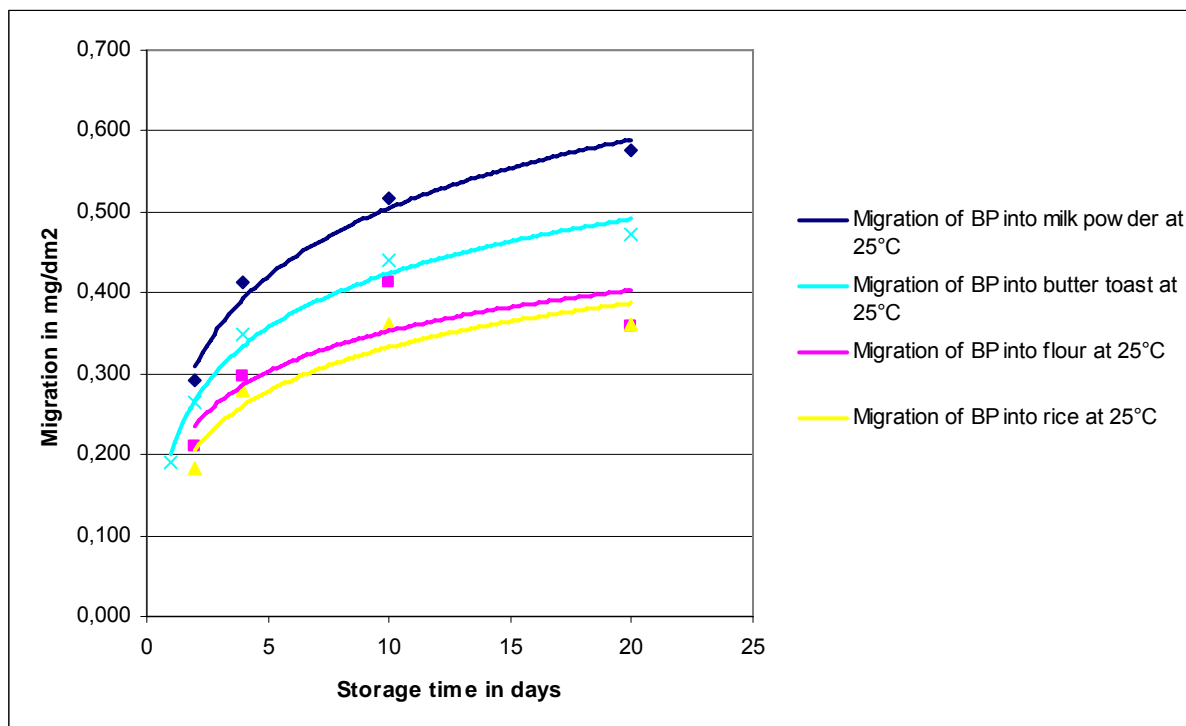
Figure 106 Migration of benzophenone into milk powder, butter toast, flour and rice at 25°C
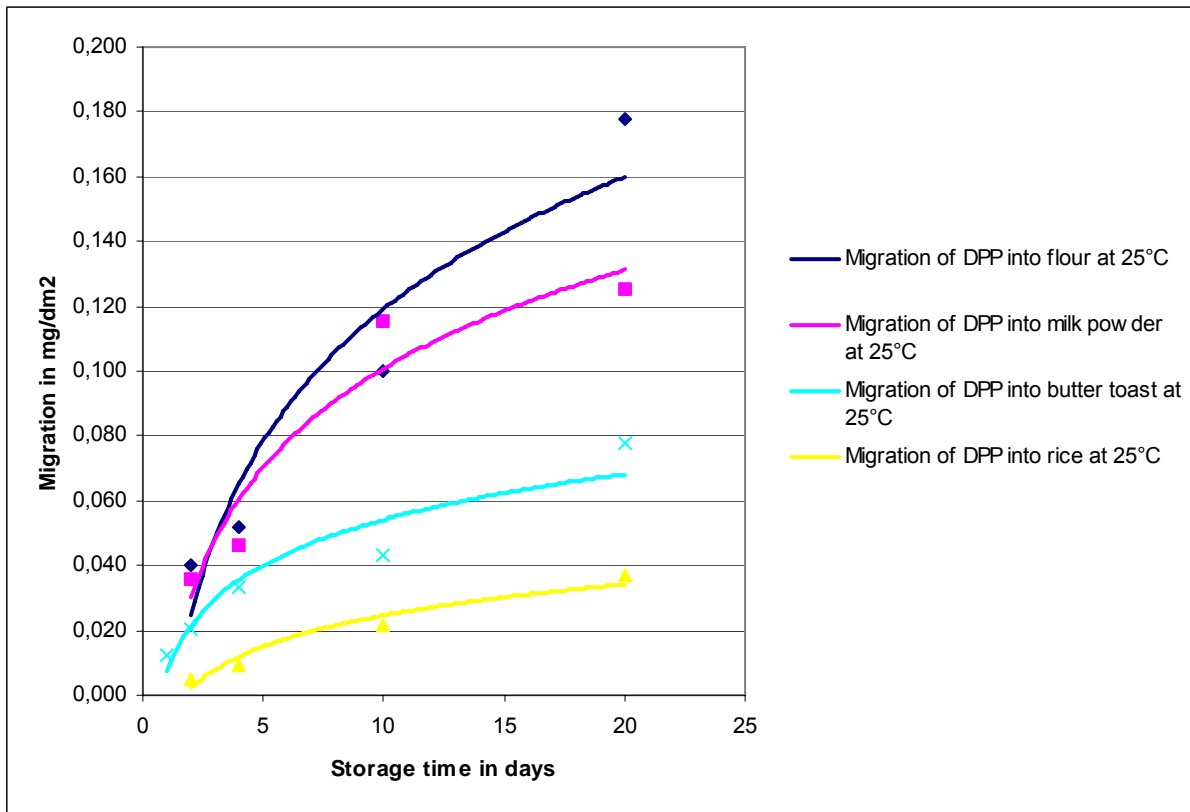
Figure 107 Migration of diphenyl phthalate into milk powder, butter toast, flour and rice at 25°C

9.3.2 Migration into aqueous and acidic foodstuffs – apple sauce, beer, cola drink, ketchup, milk, orange juice and wine

Figure 108 and Figure 109 illustrate the migration of BP and DPP into various aqueous and acidic foodstuffs. In this comparison the lowest migration values for both BP and DPP were measured in purely aqueous and acidic foodstuffs with no fat content - cola drink followed by beer and wine. All other foodstuffs tested in this group contained very little amount of fat (<1% with the exception of milk with min. 3,5% fat).

From the total amount of BP present in the plastic film after 20 days storage at 25°C approximately 45% transferred into the cola drink, 53% into beer and 63% into wine. In the case of DPP at the same conditions it was approximately 5% into the cola drink, 7% into beer and 9% into wine.

Looking at the migration curves of DPP the next higher migrations exhibited ketchup (max. migration of 23%) and apple sauce (max. migration of 26%) followed by milk (max. migration of 33%). From this data it is obvious that the migration increases with the rising fat content of the foodstuff, even if the actual fat content is still very low. Foodstuffs with no fat content at all gave consistently the lowest migration values and the migration did not increase dramatically with storage time as opposed to the foodstuffs containing some amount of fat. Concerning the values for orange juice (max. migration of 48%) it has to be stressed that contrary to all other migration experiments which were conducted as one-sided exposure the migration into

orange juice was determined by total immersion of the plastic film. Nevertheless the measured values show an extensive migration of DPP into orange juice which is assumed to take place due to adsorption on the pulp present in the juice as well as the oils originating from the orange fruit.

The differences in the migration of BP are not as distinctive as it was the case with DPP. The next higher migration was measured in apple sauce (max. migration of 86%) followed by milk (max. migration of 93%), orange juice (max. migration of 97%) and ketchup in which case all of the BP present in the plastic film transferred into the foodstuff under the specified storage conditions. Also here the effect of fat present in the foodstuff is obvious, though not as dramatic as in the case of DPP.
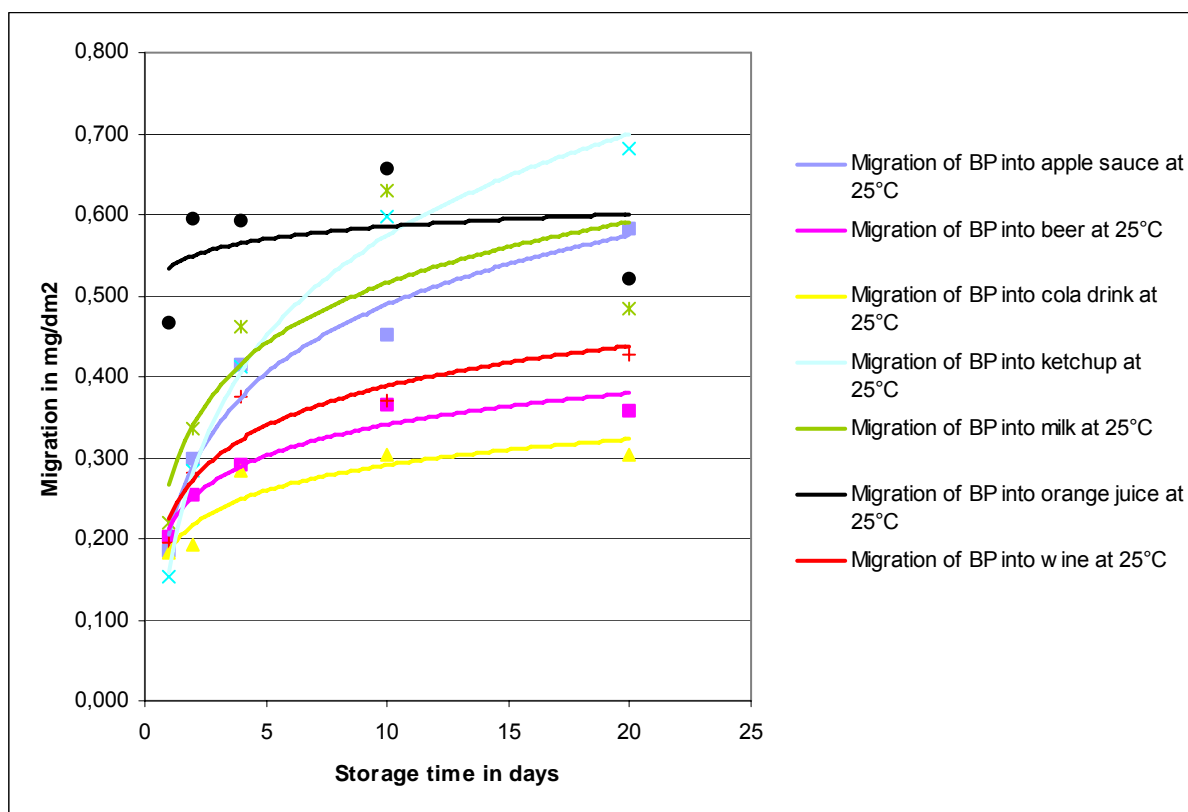
Figure 108 Migration of benzophenone into aqueous and acidic foodstuffs at 25°C
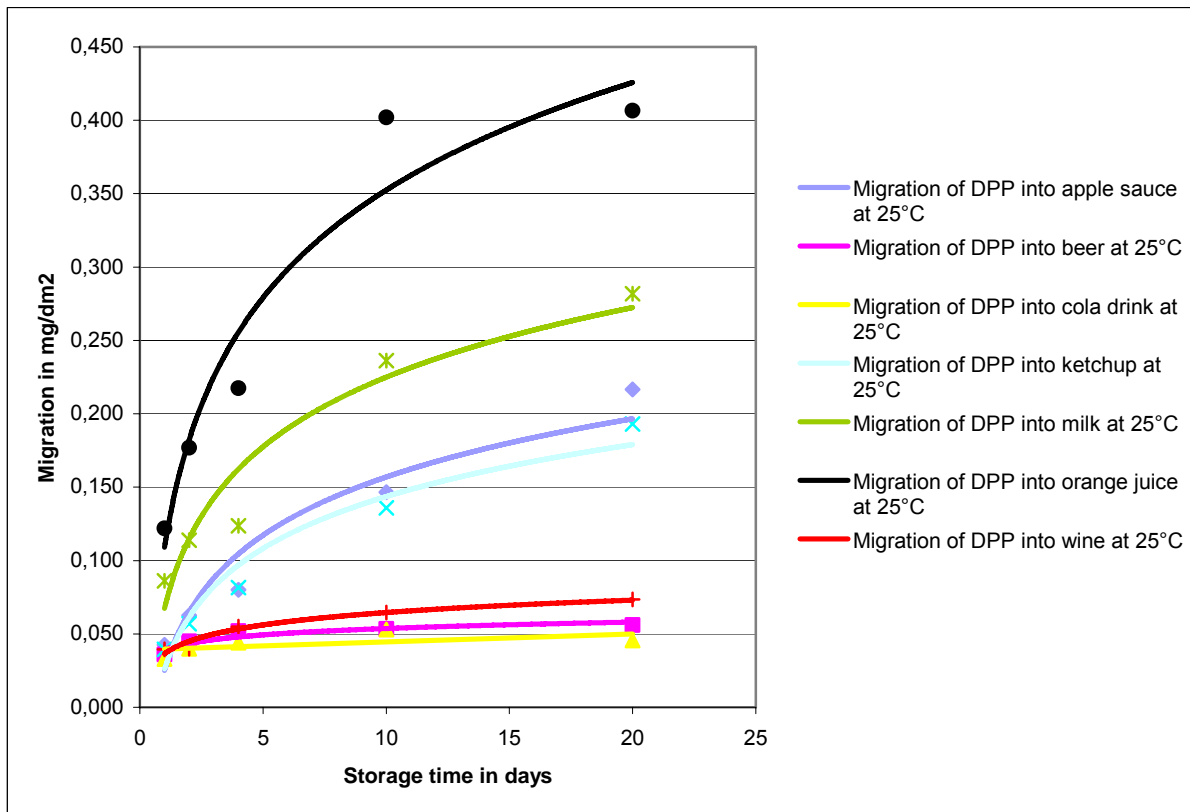
Figure 109 Migration of diphenyl phthalate into aqueous and acidic foodstuffs at 25°C

9.3.3 Migration into fatty foodstuffs – cheese sauce, condensed milk, fish, mayonnaise, meat (50% fat) and yoghurt drink

Figure 110, Figure 111, Figure 112 and Figure 113 illustrate the migration measured into various fatty foodstuffs at 5°C and 25°C.

At 5°C the migration into all fatty foodstuffs has been determined, the only exception being condensed milk which has not been tested at this temperature. From the migration curves for both BP and DPP is obvious, that the lowest migration values were measured in fish (max. migration after 5 days - 17% BP and 2% DPP). Somewhat higher migration was measured in yoghurt drink (max. migration after 10 days - 25% BP and 4% DPP) and meat with 50% fat content (max. migration after 10 days - 25% BP and 7% DPP). The migration of DPP into meat with 50% fat content was almost as high as into mayonnaise with 80% fat. For both substances BP and DPP the highest migration values were measured in cheese sauce (max. migration after 10 - days 40% BP and 9% DPP) and mayonnaise (max. migration after 10 - days 45% BP and 8% DPP). It is interesting to see the migration into cheese sauce with 18,5% fat content being comparable to the migration into the mayonnaise (which had a fat content of 80%) and being considerably higher than into meat (with 50% fat content). This exemplifies that while the fat content of the foodstuff certainly is one of the

crucial parameters influencing the migration of benzophenone and diphenyl phthalate other factors like the structure of the foodstuff and other components also play an important role in the migration process.

On the opposite at 25°C only condensed milk, mayonnaise and meat have been tested. Whereas condensed milk and mayonnaise have been tested over a period of 20 days, meat has only been tested for 24 hours due to its limited storage life under these conditions. Because of the different time scales between the tests conducted with meat and the other fatty foodstuffs tested at 25°C, meat has been ommited in the figures Figure 112 and Figure 113. The migration curves show the expected trend and for both BP and DPP the migration into mayonnaise (max. migration after 20 days – all of the BP and 49% of DPP migrated into the foodstuff) is significantly higher than into condensed milk (max. migration after 20 days – 65% BP and 26% DPP).

Figure 110 Migration of benzophenone into fatty foodstuffs at 5°C
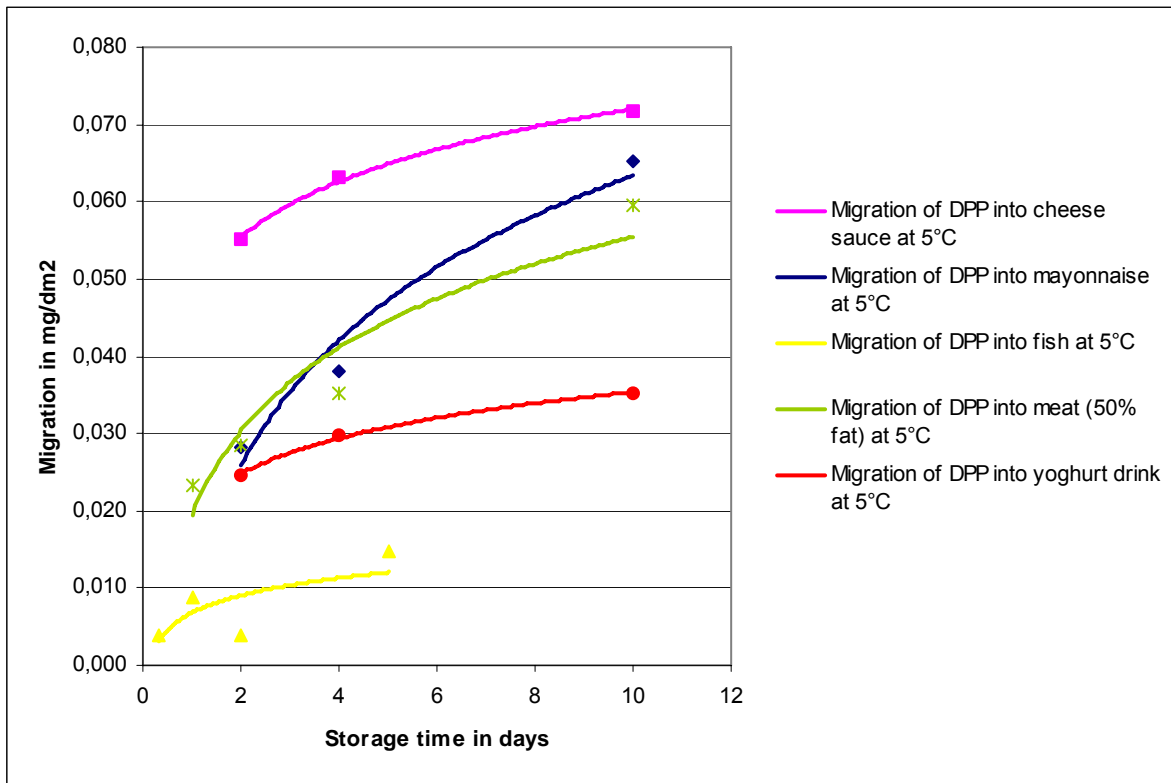
Figure 111 Migration of diphenyl phthalate into fatty foodstuffs at 5°C

Figure 112 Migration of benzophenone into fatty foodstuffs at 25°C
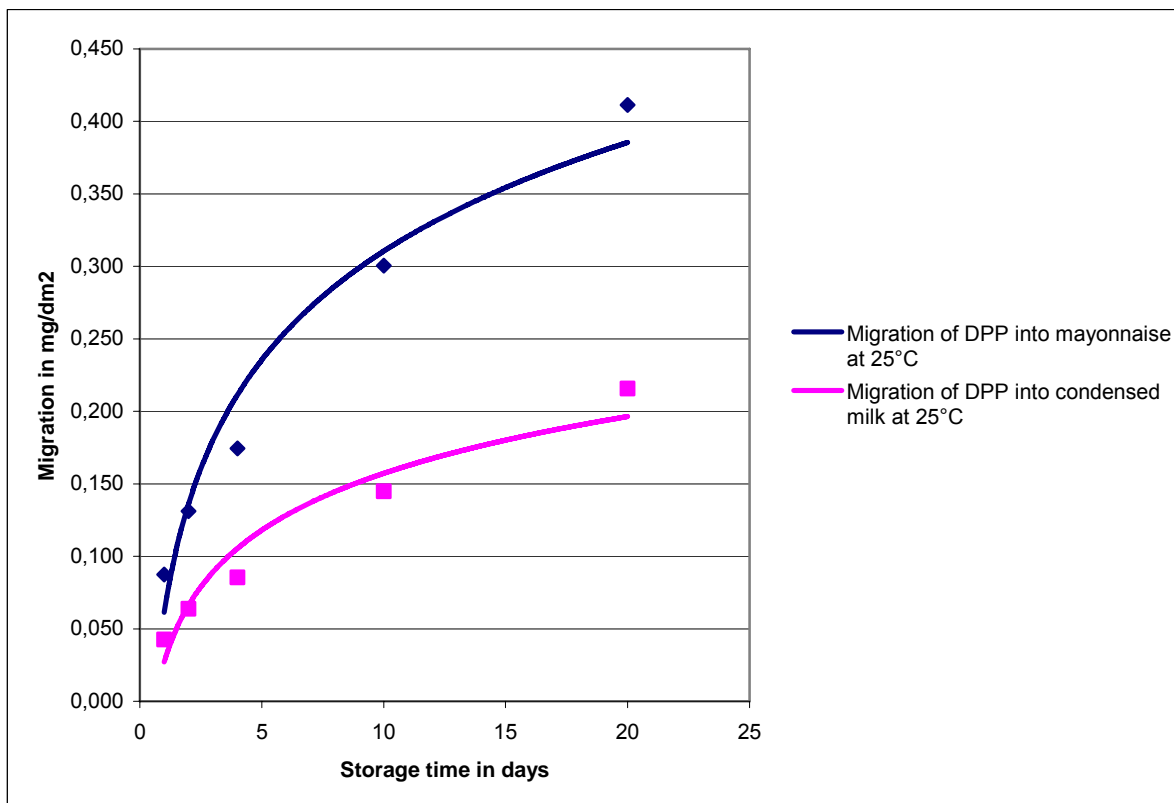
Figure 113 Migration of diphenyl phthalate into fatty foodstuffs at 25°C

9.4 Migration into different food categories – aqueous and acidic, fatty and dry foodstuffs

In order to get an overview about the migration into various foodstuff categories figures Figure 114 and Figure 115 show the migration curves of all foodstuffs tested at 25°C over a period of time of 20 days. Individual foodstuff categories are marked in different colours to highlight trends in the migration.

From both figures also the influence of the molecular weight of the migrating substance on the migration is apparent. The benzophenone being a substance with the lower molecular weight migrated readily into all tested foodstuffs. In general it can be seen that the higher migration values were measured in fatty and some aqueous and acidic foodstuffs (those with little fat content like milk or ketchup). Dry foodstuffs as well as purely aqueous and acidic foodstuffs exhibited somewhat lower concentrations of migrating BP, yet the trend is not as clear as it is the case with DPP.

Whereas in the case of BP the differences in migration between various foodstuff categories are not clearly visible, in the case of DPP the differences are much more obvious. DPP migrated readily into fatty foodstuffs – two of four foodstuffs in figure Figure 115 with the highest migration values were fatty foodstuffs - as well as aqueous and acidic foodstuffs containing some fat. On the other hand the lowest migration values for DPP were measured in dry foodstuffs as well as in purely aqueous and acidic foodstuffs.

The migration curves describing the migration of DPP into dry and purely aqueous and acidic foodstuffs can all be found at the bottom of the chart.

It can be therefore concluded that benzophenone due to its lower molecular weight migrated more readily to the tested foodstuffs at given test conditions than diphenyl phthalate, but the DPP allowed us to better study and identify trends in the migration between the various foodstuff categories. The migration of a substance strongly depends on its physico-chemical properties, DPP showing affinity to fatty foodstuffs and substantially lower migration values into dry or purely aqueous foodstuffs with no fat. On the other hand lower molecular weight substances with higher mobility such as BP show overall higher migration values with less distinction of the character of the foodstuff.
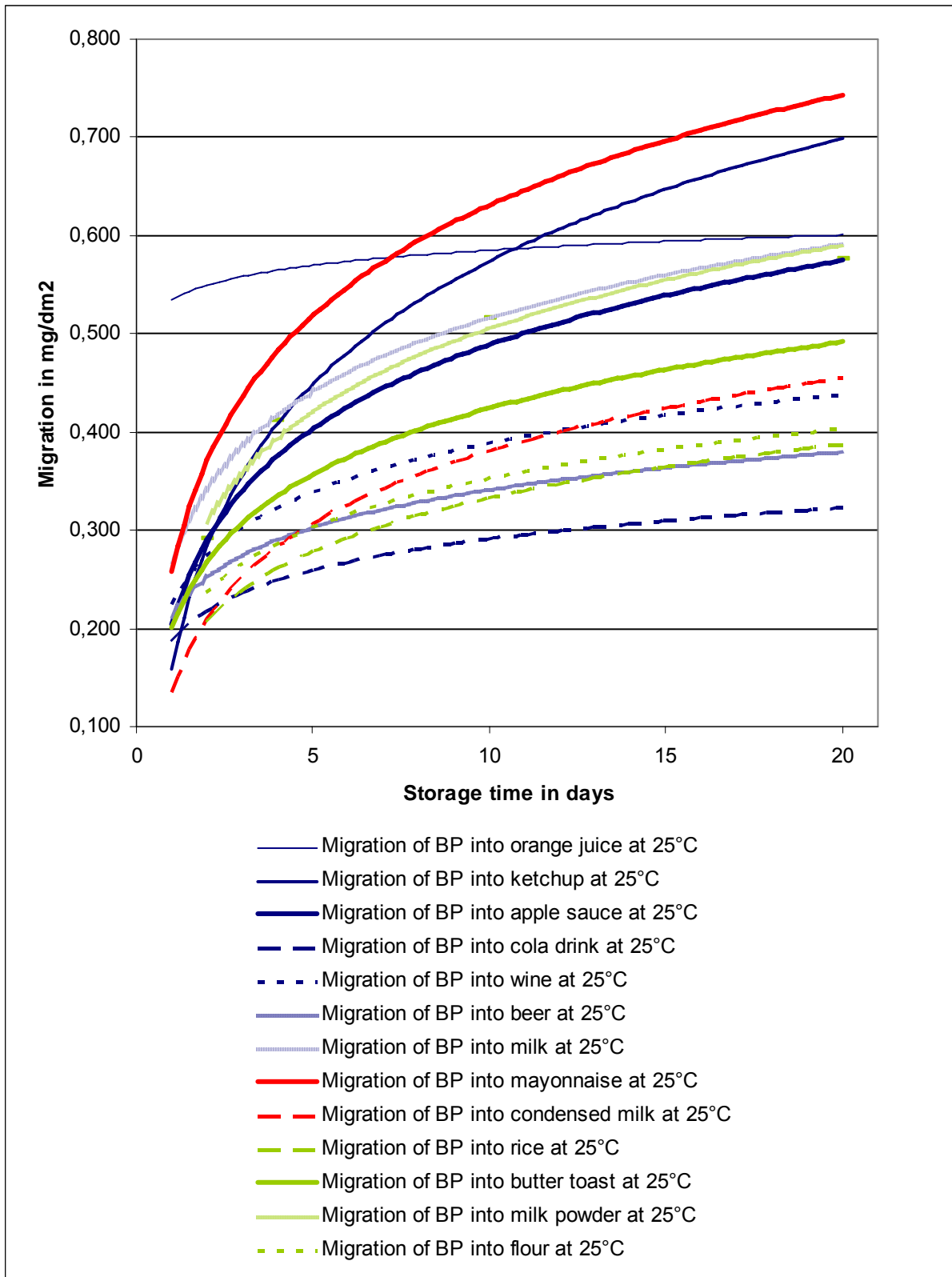
Figure 114 Migration of benzophenone into various foodstuffs at 25°C (**blue** = aqueous and acidic foodstuffs; **red** = fatty foodstuffs; **green** = dry foodstuffs)
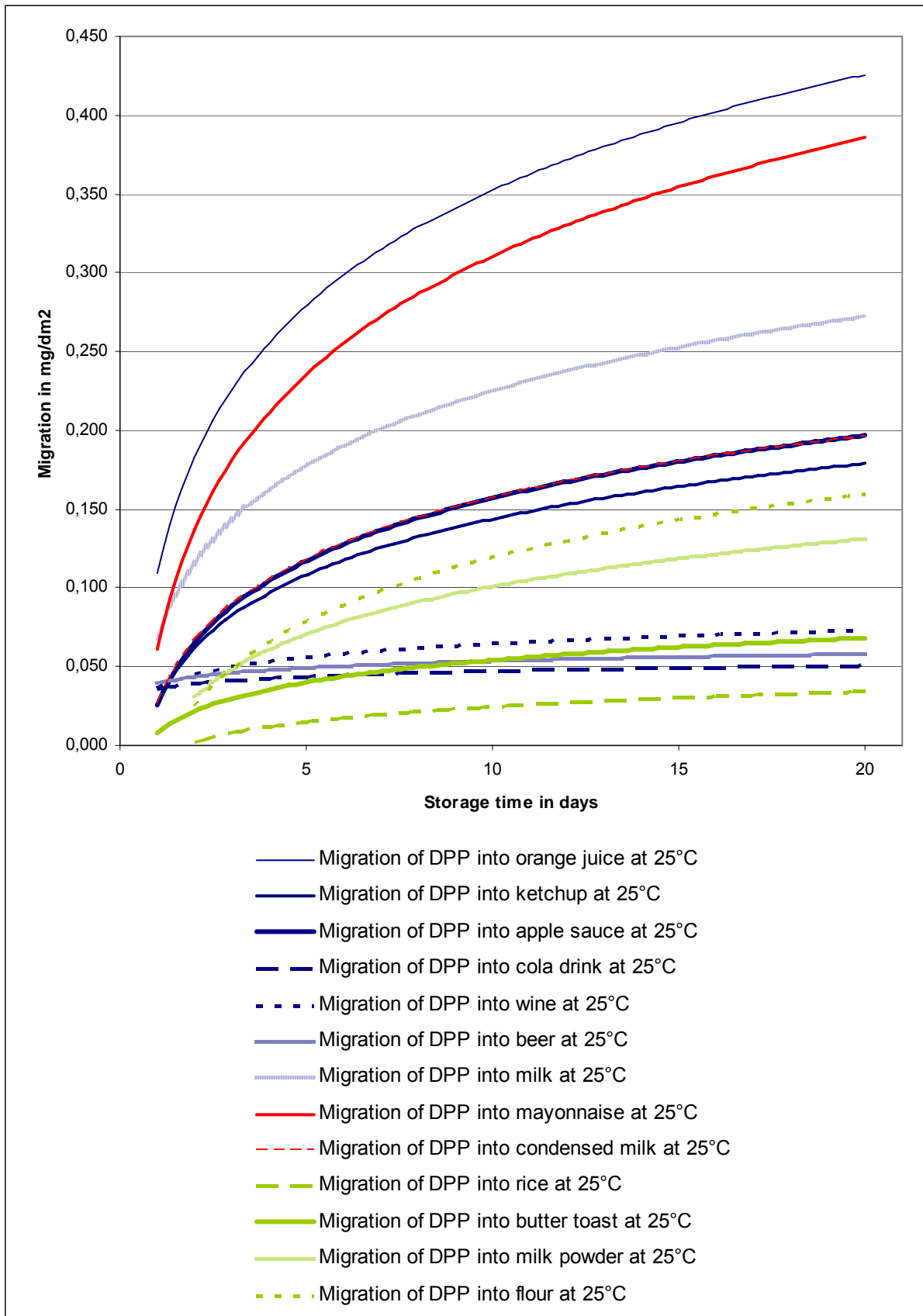
Figure 115 Migration of diphenyl phthalate into various foodstuffs at 25°C (**blue** = aqueous and acidic foodstuffs; **red** = fatty foodstuffs; **green** = dry foodstuffs)

9.5 Influence of the structure of the foodstuffs on the migration

Additional to the composition of a foodstuff also its microscopic as well as molecular structure influence the migration. In the case of dry foodstuffs the particle size, porosity, specific surface area or surface structure might influence the migration to a higher or lesser extent. In the case of emulsions the type of the emulsion determines whether the migration of hydrophilic or hydrophobic substances will be promoted. Milk for example consists up to 87-89% of water (105) and is an O/W – oil in water – type of emulsion stabilized by phospholipids absorbed on fat globules. Basic structure elements of milk are fat globules, casein micels, globular proteins and lipoprotein particles. The diameter of the fat globules varies from around 100 to 10 000nm and may have a surface area of $70m^2/dm^3$. The lipoproteins dispersed in the milk have an area of $10m^2/dm^3$. (106) Despite the predominantly aqueous character of the milk from the chemical composition point of view, during the migration tests at 25°C milk exhibited the third highest migration of DPP and the fourth highest migration of BP of all foodstuffs tested at these conditions. The reason is suspected to be the emulsion of fat which promotes migration of hydrophobic substances such as DPP.

In the case of dry foodstuffs the structure of the particles also effects the migration behaviour. Porous particles with a high surface area might promote the migration of volatile substances due to adsorption processes. Specific surface area of dried milk particles range from $0,05m^2/g$ to $0,56m^2/g$. (107) Also fat present on the surface of the particles can be a crucial factor influencing not only technical functionality like moistening and dispersability but migration also. (108) From all the dry foodstuffs tested at 25°C milk powder exhibited the highest final migration values for BP and DPP respectively. This is contributed to the particulate character with high surface area and the fat present on the surface of the powder. In the case of butter toast high values of BP migration were measured, yet the migration of DPP was low. This reflects the structure of the butter toast which does not allow an intimate contact between the foodstuff and the plastic film thus hindering the migration of low volatile substances. As a result higher migration values were obtained with BP which migrates more readily through the gas phase than the less volatile DPP.

All these results point up, that beside the knowledge of chemical properties of a foodstuff also the microscopic and molecular structure play an important role in the process of migration.

9.6 Conclusions

Based on the results of this study it can be concluded that the food composition and storage conditions have an essential effect on the migration of plastic packaging constituents into the packed foodstuff. It is therefore important that the consumers are aware of the possible interactions between the plastic packaging material and the packed foodstuff. Plastic packaging is not an inert material and under inappropriate circumstances it can cause serious deterioration of the foodstuff. In general following basic

recommendations for handling foodstuffs in contact with plastic packaging materials can be given to the consumer:

- Elevated temperature accelerates the migration. It is therefore advisable not to heat up foodstuffs in plastic packaging unless it has been designed and approved for such a purpose by the producer. In case of doubt it is safer to transfer the food onto a glass plate for example before heating it up in a microwave oven. But also during storage appropriate temperature should not be neglected. Due to the longer contact times between the foodstuff and the plastic material also slightly elevated temperature can have an effect on the amount of migrating substances.

- The longer the contact between the plastic material and the foodstuff the higher the amount of substances migrating into the foodstuff. This should be considered during prolonged storage. A lower storage temperature can serve as an effective countermeasure to keep the migration as low as possible.

- Furthermore the consumers should always consider that the various plastic materials employed in the food packaging industry differ in their properties and these have to be taken into account when handling the packed foodstuffs.

The migration tests conducted in this study showed that the migration is strongly determined by the properties of the migrating substance like solubility, volatility and the properties of the foodstuff into which the migration takes place. The data obtained also suggests that the structure of the foodstuffs affects the extent of the migration and might be studied more thoroughly to bring more understanding into which properties are of special interest from the migration point of view. But mostly the time of contact and above all the temperature have crucial influence on the migration.

All together the data provided by this study can not only be used as the basis for a mathematical model describing the process of migration but also to illustrate the importance of appropriate storage conditions and handling of plastic packed foodstuffs which can increase the awareness of the consumers towards the phenomenon of packaging/foodstuffs interactions and so increase the food safety by reducing the cases of inappropriate handling of plastic packaging materials for foodstuff.

10 Summary

The migration of benzophenone and diphenyl phthalate from a plastic film made from LDPE into a selection of 17 real foodstuffs under various time/temperature conditions has been studied. The goal during the selection of foodstuffs was to cover in the best possible way the broad spectrum of foodstuffs available on the market where the migration from a plastic packaging might be of interest while considering also the aspect of consumption within the EU. The foodstuffs selected for the migration testing were then divided into three main groups according to their predominant characteristics expected to have a substantial influence on the migration of substances from a plastic material into the foodstuff:

-aqueous and acidic foodstuffs

-fatty foodstuffs

-dry foodstuffs

Based on the analytical results the influence of varying test conditions and different food properties were evaluated.

As expected and shown in previous studies temperature has a considerable influence on the migration. Most of the foodstuffs were tested at two or even three different temperatures (orange juice, milk, flour). These experiments clearly illustrated the influence of the temperature on the migration. Elevating the temperature from 5°C to 25°C generally resulted in a 2 to 4-times higher migration values. When the temperature was elevated from 5°C to 40°C the migration rose up to 7-times (migration of diphenyl phthalate into orange juice during 2 days storage). The effect high temperature on the migration was demonstrated on the example of cheese sauce. The migration of diphenyl phthalate measured after 5 minutes exposure at 90°C was already higher than the values obtained after 30 days of storage at 5°C.

The extent of the migration at a specified temperature strongly depends on the migrant itself. The less volatile phthalate requires higher temperature to migrate than benzophenone. The smaller molecules of benzophenone migrate readily even at temperatures as low as 5°C. For this reason the influence of the elevated temperature (within the intervals of the experiments) seems in many cases more obvious with diphenyl phthalate than with benzophenone. Altogether based on the obtained data it can be pointed out, that elevated temperature might promote the migration dramatically, so that in certain cases migrations levels which would require months of storage at low temperatures are reached within hours if the temperature is raised to a high enough level.

It was expected that the migration of both benzophenone and diphenyl phthalate will correlate with the fat content of the foodstuff considering the hydrophobic character of both substances. Results of the migration experiments confirmed this expectation. Above all the migration experiments conducted with pork meat mixed with various amounts of lard demonstrated the influence of different fat content on the migration. The meat with 50% fat content exhibited after 24 hours storage at 25°C 4-times higher migration of diphenyl

phthalate than meat with less than 5% fat content under the same storage conditions. With benzophenone the influence under the same conditions is not as distinctive, nevertheless the migration into the meat with 50% fat content was 20% higher. From all the fatty foodstuffs tested the highest migration was measured in cheese sauce and mayonnaise followed by meat with 50% fat content, yoghurt drink and fish.

The category of dry foodstuffs exhibited surprisingly high levels of migrating benzophenone as well as diphenyl phthalate. Due to their particulate nature (rice, flour, milk powder) or their porous structure (butter toast) the contact between the foodstuff and the plastic film during the migration tests was considerably less intimate than for example with fluids. Despite the limitation of the direct contact between these foodstuffs and the plastic film after 20 days storage at 25°C milk powder exhibited the fifth highest migration of benzophenone from all foodstuffs tested. Among the dry foodstuffs tested at 25°C diphenyl phthalate exhibited the highest migration into milk powder during 180 days storage. At elevated temperature of 40°C the same was observed as the highest concentrations were measured again in the milk powder. Based on the observations of the relation between fat content of a foodstuff and the migration of benzophenone and diphenyl phthalate the explanation for these high concentrations measured in milk powder can be found in the fat contained in the milk powder particles together with the particulate character of the foodstuff, resulting in a large surface which favours the migration. As opposed to milk powder, rice exhibited very low migration values for both substances tested, which can be attributed to the structure of rice grains as well as the considerably smaller area per weight unit compared to flour or milk powder.

In the category of aqueous and acidic foodstuffs the lowest migration values for both benzophenone and diphenyl phthalate were measured in purely aqueous foodstuffs with no fat content. In this respect cola drink gave the lowest migration values followed by beer and wine. The highest values on the other hand were observed in apple sauce, ketchup, milk and orange juice. The reason for the significant migration of diphenyl phthalate into the orange juice is assumed to be the presence of the oils originating from the orange fruit together with the pulp, to with the analyte might be adsorbed to. Concerning the influence of the alcohol content on the migration the values observed in the cola drink, beer and wine have been compared. It was shown, that the migration of both substances rose with the amount of alcohol present in the foodstuff. The lowest migration in this group was measured in the cola drink, the highest on the other hand in wine.

It was also concluded that besides the chemical composition of a foodstuff including parameters like for example water content, fat content or alcohol content also the structure of the foodstuff, like particle size or porosity, plays an important role influencing the migration. The influence of these parameters on the migration of a certain substance strongly depends on the properties of the migrating substance itself, on its volatility, solubility or its mobility in the polymer. These parameters together with time and temperature at which the process of migration occurs as well as the properties of the polymer from which the migrating substances are released determine the extent of the migration and have to be considered comprehensively.

Results obtained in this study further point up the importance of proper storage conditions from the food safety point of view. The data can be used to increase consumer awareness of possible interactions of plastic packaging and foodstuffs as well as of the consequences of inappropriate packaging use.

11 Zusammenfassung

Die Migration von Benzophenon und Diphenylphthalat aus einer LDPE-Folie in 17 ausgewählte Lebensmittel unter verschiedenen Lagerbedingungen (Zeit, Temperatur) wurde untersucht. Die Zielsetzung bei der Auswahl der Lebensmittel war es eine repräsentative Gruppe der am europäischen Markt erhältlichen Lebensmittel zusammenzustellen, die ebenfalls den Verbrauch durch die Konsumenten berücksichtigt. Die Lebensmittel wurden in drei Kategorien unterteilt, die auf den für die Migration ausschlaggebenden Eigenschaften der Lebensmittel beruhen:

-wässrige und saure Lebensmittel,

-fetthaltige Lebensmittel,

-trockene Lebensmittel.

Anhand der Analysenergebnisse wurde der Einfluss der Lagerbedingungen und der unterschiedlichen Lebensmitteleigenschaften untersucht.

Wie erwartet und bereits in anderen Studien beobachtet hat die Temperatur einen beachtlichen Einfluss auf die Migration. Die meisten Lebensmittel wurden bei zwei oder sogar drei unterschiedlichen Temperaturen getestet (Orangensaft, Milch, Mehl). Diese Versuche belegten eindeutig den Einfluss der Temperatur auf die Migration. Bei einer Temperatursteigerung von 5°C auf 25°C stieg die Migration im Durchschnitt auf das Zwei- bis Vierfache. Als die Temperatur von 5°C auf 40°C erhöht wurde, stieg die Migration von Diphenylphthalat im Orangensaft nach zwei Tagen um das Siebenfache. Der Effekt von hoher Temperatur auf die Migration wurde am Beispiel der Käsesauce untersucht. Die gemessene Migration von Diphenylphthalat nach nur 5 Minuten bei 90°C war bereits höher als nach 30-tägiger Lagerung bei 5°C.

Das Ausmaß der Migration bei einer bestimmten Temperatur hängt stark von der migrierenden Substanz ab. In diesem Fall erfordert die Migration von weniger flüchtigem Diphenylphthalat eine höhere Temperatur als bei dem leichter flüchtigen Benzophenon. Die kleineren Moleküle des Benzophenons migrieren bereitwillig sogar bei Temperaturen von nur 5°C. Aus diesem Grund scheint der Einfluss der höheren Temperatur auf die Migration von Diphenylphthalat deutlicher zu sein als bei Benzophenon. Basierend auf den experimentellen Daten kann der Schluss gezogen werden, dass eine Erhöhung der Temperatur die Migration dramatisch beschleunigen kann, sodass in bestimmten Fällen bei hoher Temperatur bereits nach wenigen Studen Migrationswerte auftreten, die bei einer niedrigen Temperatur erst nach einigen Monaten erreicht worden wären.

Es wurde erwartet, dass die Migration von Benzophenon und Diphenylphthalat mit dem Fettgehalt der Lebensmittel korrelieren wird, da beide Substanzen einen hydrophoben Charakter aufweisen. Die Ergebnisse der Migrationsversuche bestätigten diese Annahme. Versuche mit Schweinefleisch gemischt mit unterschiedlichen Mengen Schmalz veranschaulichen den Einfluss von Fett auf die Migration.

Schweinefleisch mit 50% Fett wies nach 24 Stunden bei 25°C eine vierfach höhere Konzentration an Diphenylphthalat auf als Schweinefleisch mit weniger als 5% Fett unter denselben Bedingungen. Bei Benzophenon war der Einfluss unter den selben Bedingungen nicht so markant, die Migration war beim Schweinefleisch mit 50% Fett um 20% höher verglichen mit Schweinefleisch mit weniger als 5% Fett. Die höchste Migration unter allen fetthaltigen Lebensmitteln wurde in der Käsesauce gemessen gefolgt von der Mayonnaise, Schweinefleisch mit 50% Fett, Joghurtgetränk und Fisch.

In der Kategorie der trockenen Lebensmittel wurden überraschend hohe Konzentrationen an migrierendem Benzophenon und Diphenylphthalat beobachtet. Wegen ihrer Partikelförmigkeit (Reis, Mehl, Milchpulver) oder ihrer poröser Struktur (Buttertoast) ist der Kontakt zwischen der Kunststofffolie und dem Lebensmittel während des Migrationstests bei weitem nicht so eng wie zum Beispiel bei flüssigen Lebensmitteln. Trotz dieser Einschränkungen beim Kontakt zwischen dem Lebensmittel und der Kunststofffolie wurde im Milchpulver nach 20 Tagen bei 25°C die fünfthöchste Konzentration an Benzophenon von allen getesteten Lebensmitteln gemessen. Unter den trockenen Lebensmitteln wurde bei 25°C die höchste Konzentration an Diphenylphthalat im Milchpulver nach 180 Tagen gemessen. Bei 40°C wurde derselbe Trend beobachtet und die höchsten Konzentrationen wurden ebenfalls im Milchpulver gemessen. Der Grund dafür ist einerseits das im Milchpulver enthaltene Fett zusammen mit der großen Fläche, die sich aus der Partikelstruktur des Pulvers ergibt. Auf der anderen Seite wies Reis sehr geringe Mengen an migrierendem Benzophenon und Diphenylphthalat auf. Der Grund ist in der Struktur der Reiskörner zu sehen zusammen mit der deutlich geringeren Fläche pro Masseeinheit verglichen mit Mehl oder Milchpulver.

In der Kategorie der wässrigen und sauren Lebensmittel wurden die niedrigsten Werte an Benzophenon und Diphenylphthalat in rein wässrigen Lebensmitteln gemessen. Die niedrigste Migration wurde in Colagetränk gemessen gefolgt von Bier und Wein. Die höchsten Werte wurden wiederum in Apfelsauce, Ketchup, Milk und Orangensaft gemessen. Es wird angenommen, dass der Grund für die hohe Migration im Orangensaft in dem Gehalt an Ölen aus der Frucht und dem Fruchtfleisch, an dem die Analyten adsorbiert werden, liegt. Der Einfluss des Alkoholgehaltes auf die Migration wurde anhand von Wein, Bier und Colagetränk als alkoholfreies Lebensmittel zum Vergleich untersucht. Es wurde gezeigt, dass die Migration sowohl von Benzophenon als auch von Diphenylphthalat mit dem Alkoholgehalt des Lebensmittels korreliert.

Neben der chemischen Zusammensetzung eines Lebensmittels, die Parameter wie Wassergehalt, Fettgehalt oder Alkoholgehalt umfasst, ist auch die Struktur für die Migration von Bedeutung. Die Partikelgröße oder Porosität spielen ebenfalls eine wichtige Rolle bei der Migration. Dabei müssen auch die Eigenschaften der migrierenden Substanzen berücksichtigt werden wie die Flüchtigkeit, Löslichkeit oder ihre Mobilität im Polymer aus dem heraus sie migrieren. Diese Parameter zusammen mit der Zeit und Temperatur, bei der die Migration stattfindet, sowie die Eigenschaften des Polymers, aus dem die Substanzen migrieren, bestimmen letztendlich das Ausmaß der Migration und müssen als Ganzes beachtet werden.

Die Ergebnisse dieser Arbeit verdeutlichen die Bedeutung geeigneter Lagerbedingungen aus Sicht der Lebensmittelsicherheit. Mit den gesammelten Daten kann das Bewußtsein der Konsumenten hinsichtlich der möglichen Interaktionen von Kunststoffverpackung und Lebensmittel gestärkt werden und die Konsequenzen unsachgemäßen Verpackungsgebrauchs verdeutlicht werden.

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13 Annex

13.1 List of tables

13.2 List of figures

13.3 Abbreviations

4MBP	4-methyl benzophenone
ACPI	Atmospheric pressure chemical ionization
AFC	Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
BBP	Butylbenzyl phthalate
BIOP	Butylisooctyl phthalate
BOP	Butyloctyl phthalate
BP	Benzophenone
CAS	Chemical abstract service
CE	capillary electrophoresis
CEC	electrochromatography
CI	Chemical ionization
$C_{p,0}$	Concentration of a substance in the plastic film
CRL	Community reference laboratory
CRM	Certified reference material
DAP	Diallyl phthalate
DCHP	Dicyclohexyl phthalate
DEHP	Di(2-ethylhexyl) phthalate
DEP	Diethyl phthalate
DIBP	Diisobutyl phthalate
DIDP	Diisodecyl phthalate
DIHP	Diisohexyl phthalate
DINP	Diisononyl phthalate
DIOP	Diisooctyl phthalate
DMP	Dimethyl phthalate
DNBP	Di(n-butyl) phthalate
DNOP	Di(n-octyl) phthalate
D_p/D_f	Diffusion coefficient in polymer/foodstuff
DPP	Diphenyl phthalate
DTDP	Ditridecyl phthalate
DUP	Diundecyl phthalate
EC	European Community

EI	Electron impact
ESI	Electrospray ionization
EU	European Union
EVOH	Ethylene vinyl alcohol
FID	Flame ionization detector
GC	Gas chromatography
HeLa	Human cervix carcinoma cell line
HPLC	High performance liquid chromatography
ID	Internal diameter
ISTD	Internal standard
LD_{50}	Lethal dose (50%)
LDPE	Low density polyethylene
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
Log P_{ow}	Partition coefficient
LOQ	Limit of quantitation
mBP	Monobutyl phthalate
mBzP	Monobenzyl phthalate
MCF-7	Michigan Cancer Foundation – 7 - Breast cancer cell line
mEHP	Monoethylhexyl phthalate
mEP	Monoethyl phthalate
mM	Milli Mol
mMP	Monomethyl phthalate
mNP	Monononyl phthalate
MS	Mass spectrometry
MSD	Mass selective detector
MW	Molecular weight
NCI	National cancer institute
NOAEL	No observed adverse effect level
NRL	National reference laboratory
NTP	National Toxicology Program
NTP-CERHR	National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction
OECD	Organisation for Economic Co-operation and Development
OML	Overall migration limit
PCI	positive ion chemical ionization
PET	Polyethyleneterephthalate

PP	Polypropylene
PTFE	Polytetrafluoroethylene
PVC	Polyvinyl chloride
QM	Maximum permitted quantity of the substance in the finished material or article
RSD	Relative standard deviation
SCF	Scientific Committee on Food
SD	Sprague-Dawley rat
SIM	Single ion monitoring
SML	Specific migration limit
SPE	Solid-phase extraction
TDI	Tolerable daily intake
UHT	Ultra high temperature
UK	United Kingdom
v/v	Volume/volume percent
w/v	Weight/volume percent
w/w	Weight/weight percent