Screening Tests for Visible and Non-Visible Set Off

Final Report

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Careful consideration was given to the use of trade names in this report. The alternative use of chemical names was subject to a number of difficulties. An inventory of reference calibration samples of ink components was obtained from industry. These ink components were labelled with the trade name and their purity was not established. Some trade names are mixtures and in many cases the pure chemical is not available from laboratory chemical suppliers. Where possible, permission was obtained from suppliers to use trade names. Where permission was not granted, the CAS number is used in this report. The use of the CAS number for ink components measured or used as calibrants in this report should not be taken as an indication of purity. In some cases, the chemical name is not in the public domain. In many cases there are numerous trade names for the same ink component. Where one trade name is used this was because It was either not possible to list all the alternatives, or the alternatives were not known to the author.

Abstract

Set off is defined as: 'the unintentional transfer of substances used in printing inks from the printed (outer) surface of packaging to the inner food contact surface'. There has been increased interest in Set off, as a result of the finding of 2-Isopropylthioxanthone (ITX), in drinks packaged in multi-layer cartons. This research fits into the Food Standards Agency's strategic aim of 'safe food and healthy eating for all' and its objective of improving food safety by ensuring that the food produced or sold in the UK is safe to eat, by tackling contamination in the food chain. The project will enable laboratories to identify printing ink components on unused food packaging, estimate worst case migration and thereby assist in the prevention of packaging transferring components to foods at undesirable levels.

There are no Specific Migration Limits for printing ink components in paper and board food packaging. However, as a component of a packaging material, printing inks must meet the more general requirements of Regulation (EC) No. 1935/2004. This requires that packaging materials do not transfer their components into food at levels which could either endanger human health, bring about an unacceptable change in the composition of food, or bring about deterioration in the taste, smell or texture of the food. Printing inks should also be manufactured in accordance with Regulation (EC) No. 2023/2006, on Good Manufacturing Practice.

The project was divided into two parts. The first part developed an optical scanner for measuring the total surface area of patches of visible set off on the food contact surface of packaging. The scanner further developed the technology produced in a previous Agency funded project (A03010/11/12), allowing a simple pass/fail test to be rapidly carried out on the packaging. It would be suitable as a quality control test for visual set off for regulators and the food packaging industry alike. The second part, in collaboration with industry, developed exposure techniques and analytical methods

which allowed the quantification of individual ink components on the food contact surface of packaging. The ink components considered in this project were photoinitiators and synergists. Photoinitiators are chemicals used in printing inks to speed up the drying process of the ink using ultra violet light. Synergists are chemicals which take part in the reaction involving the photoinitiator. A record of commonly used photoinitiators and synergists was established. This was used to create a library to enable the identification and quantification of the compounds that were looked for, as information on these compounds is not widely available within a single commercial reference library. Exposure techniques were developed that allowed measurement of non visible set off of individual chemical compounds. The procedure involved extraction of the food contact surface of the packaging into the solvents dioxane, iso-octane and 95 % ethanol, for time periods of up to five hours at 60 °C. Analytical methods (GC-MS, GC-FID, LC-UV and LC-MS) capable of measuring the photoinitiators and synergists in the selected extraction solvents were then developed using the inventory of reference samples.

Printed packaging films were supplied by industry with specially formulated ink compositions and these were used to test the set off measurement procedure. The measured set off was not always the same along the length of a roll of printed film. There was no significant effect on the set off results obtained after storage or in the different extraction solvents. Similarly, the application of pressure at 1.2 psi (at 40 °C for 10 days) had no significant influence on set off values. The analysis of polymer-based photinitiators and synergists was found to be particularly problematic. Many polymeric photoinitiators and synergists are not detectable using GC-MS, and many did not respond to other methods of analysis, either (LC-MS with electrospray ionisation (ESI) or Atmospheric Pressure Chemical Ionization (APCI). It is unlikely that the identification and measurement of set off of these substances will be possible by most laboratories without the disclosure of the ink formulation used.

The migration of ink components was measured from specially prepared test films into a variety of foodstuffs, namely soup, orange juice and breakfast cereal. These films contained known ink compositions at levels higher than would be expected in commercial applications. The objective was to promote set off to test the developed method and allow comparison of data. There was less of a difference in migration between all of the foods than was expected. High migration (often approaching 100 %) across a range of ink components into all the foods was observed. Significant migration (30 to 50 %) was also observed into ‘Tenax’, a simulant for dry food (such as cereal), over 10 days at 40 °C. Food simulants are used in the laboratory to mimic the characteristics and properties of foods. The set off measured using the developed procedure, which could be carried out within one working day, was found to be an accurate measure of the likely worst case migration for shelf life applications of six months or longer at room temperature. Comparable migration results to set off measurements were also observed using the European Union alternative fat tests.

List of abbreviations

ANOVA	Analysis of variance
APCI	Atmospheric chemical ionisation
CAD	Charged aerosol detector
DART	Direct Analysis Real Time (mass spectroscopy technique)
DEHP	Diethylhexyl phthalate
DCHP	Dicyclohexyl phthalate
DiTMPTA	di-trimethylolpropane tetra acrylate
DPGDA	dipropylene glycol diacrylate
EA	Epoxy acrylate
4-EDB	Ethyl-4-(dimethylamino)benzoate
EO-TMPTA	Ethoxylated trimethylolpropane triacrylate
EU	European Union
EuPIA	European Printing Ink Association
ESI	Electrospray Ionisation
FID	Flame Ionisation Detector
FSA	Food Standards Agency
GC-FID	Gas chromatography flame ionisation detection
GC-MS	Gas chromatography mass spectrometry
GPTA	Glyceryl propoxy triacrylate
GPTA	Glycerine propoxylate triacrylate
HDDA	Hexanediol diacrylate
HPLC	High performance liquid chromatography
HPLC-MS	high performance liquid chromatography mass spectroscopy
HPLC-UV	High performance liquid chromatography with ultra violet light detection
LC-MS	Liquid chromatography mass spectrometry
LC-UV	Liquid chromatography Ultra Violet light detection
MMEQ	monomethyl ether hydroquinone
MPPO	Modified polyphenylene oxide
MS	Mass spectroscopy
4-PBZ	4-phenyl benzophenone
PC	Personal computer
PE	Polyethylene
PSI	Pounds per square inch
RASFF	Rapid alert system for food and feed
RSD	Relative standard deviation
SML	Specific migration limit
TMPTA	Trimethylolpropane triacrylate
TPGDA	Tri(propylene glycol) diacrylate
UV	Ultra violet light

Table of contents

Chapter 4

Chapter 5

Chapter 6

Chapter 7

Chapter 8

Chapter 1

1.0 Introduction

In recent years, incidents of food contamination have been reported where chemicals used in the inks applied to the outer surface of food packaging have migrated into the packed food. These compounds were usually of unknown toxicity. Transfer of chemicals from the ink to the food can occur by contact of the printed surface with the food contact surface whilst in the stack or on the reel. This physical transfer of ink components from one surface to another contacting surface is termed “set off”. This definition of “set off” does not include transfer via migration through the bulk of the packaging structure. There are no widely agreed published test protocols for the measurement of set off. The purpose of this project is to develop procedures for measuring the extent of set off.

The project was split into two parts with the following objectives:

Part 1 - This was Objective 01 of the agreed work plan and this was to develop a scanner that would automate a test procedure for visualising set off on food contact surfaces of packaging along the lines described in FSA project A03010/11/12.

Part 2 - This was to develop exposure techniques and analytical methods suitable for the measurement of non visible set off. This was concerned with the measurement of specific chemicals used in inks.

Objective 02 of the work plan was the development of exposure techniques (use of test cells or pouches made from the test sample) for the food contact surface using a minimum number of optimum extraction solvents.

Objective 03 was to develop analytical methods to enable the identification and quantification of the ink/varnish components in the extraction solvents.

Objective 04 was the production of 6 reels of printed packaging printed with inks/varnish of known composition for which calibration samples of the individual components were available.

Objective 05 was to quantify the extent of set off for the individual ink/varnish components in all 6 reels of packaging.
Objective 06 was to quantify the extent of migration of selected ink/varnish components into foods.

Objective 07 was to develop a rapid screening method more suited to industry quality control check on set off, based upon the methods developed above.

A review of the scientific literature was carried out before commencing work. For part 1 of this project, the previously published Food Standards Agency funded research, Project A03010/11/12 February 2002, Bradley E [Ref 1], was used as the starting point for the work. FSA project number A03055 March 2007, Forrest M, [Ref 2] describes a range of chemicals used in packaging materials print and coatings. This range of chemicals is so wide that in part 2 of the project it was not possible to consider all these within the limited scope of this project. This meant that ink components such as pigments, solvents and additives such as chemical drying agents typically used in solvent based inks, (all of which may transfer by set off) were not considered in detail in this work. Packaging printed with UV cured inks has recently been the focus of attention of the media and industry because of the transfer of photinitiators. Part 2 of the project therefore focused on UV cured inks in developing the exposure techniques and analytical methods. Photoinitiators are a group of chemicals added to the ink which, under exposure to UV light, generate free radicals which initiate a series of chemical reactions which result in cure of the ink. Synergists are chemicals which take part in the chemical reaction involving the photoinitiator. As an example, benzophenone is a photoinitiator and under exposure to UV light, a proton from the benzophenone is abstracted by an amine synergist to form a free radical which in its turn reacts with the pre-polymers in the ink resulting in curing of the ink. The synergists are also termed “co-initiators”. The photoinitiators and synergists are a relatively large group of ink related chemicals which fall conveniently into groups, most of which can be quantified by a small number of methods. As a whole, they provide useful marker compounds to enable the development of test procedures (the selection of extraction solvents and test temperatures and sampling and exposure procedures) for set off. These procedures could be used to measure set off from non UV cured print with only the chemical analysis method remaining to be developed for specific chemical compounds.

PART 1
Chapter 2

2.0 Development of a scanner to estimate total visual set off

2.1 Introduction

The proposed method is intended to automate a process which allows the surface area of traces of visible set off to be measured on the food contact surfaces of packaging materials. The test uses a technique based on that developed by project A03010/11/12 to estimate set off observed by fluorescence and reflectance of ink components by optimising illumination and viewing wavelengths.

This report outlines the methodology developed to observe set off on packaging substrates and details the apparatus, procedures and principles of operation used. The limitations and conclusions are then discussed with reference to a range of test calibrations performed on selected packaging materials.

2.2 Process overview

The test apparatus uses a colour video camera and lighting system to capture images across and along the length of the packaging material, here after termed the “web”. This enables a composite assessment to be performed which is representative of the web’s repeating print image area.

The camera is mounted on a linear drive module enabling it to traverse across the width of the web. A controlling PC is able to position the camera, which acquires images from the area to be assessed. The PC then processes the images and determines any evidence of set off.

The image processing consists of a four stage procedure. This begins with softening of the image, to remove any bright or dark spots present in the image as a result of the texture/surface roughness of the substrate being inspected. ‘Histogram equalisation’ is used to enhance the image by optimising the brightness and contrast based on the detail present. This is particularly useful when the set off and background substrate are similar in appearance e.g. both dark or both light. Thresholding is then used to segment the image enabling any regions of set off to be highlighted against the background material. The thresholding operation can be performed on multiple threshold levels to detect different intensities/quantities of set off or performed in different ranges of the visible spectrum to identify different

sources of set off such as different coloured inks. The size of the regions can then be determined, which allows the equivalent surface area to be calculated. If the potential sources of set off are known, image processing can be optimised for the chemicals that have been deposited on the substrate.

To accurately quantify set off, the system must be calibrated for each potential set off ink using palettes prepared on the test substrate. This would include all the colours used in a multi coloured image. This information allows the selection of the 'thresholding' levels and the choice of excitation and viewing wavelengths to be optimised. If this calibration information is not available, the scanner may need to perform multiple scans using different illumination and viewing wavelengths to identify set off.

2.3 Apparatus

The scanner as shown in Figure 1 consists of the following components:

2.3.1 Digital camera

Images are captured using a Motic Moticam 2000, USB digital camera with a resolution of 2.0 Mega pixels.

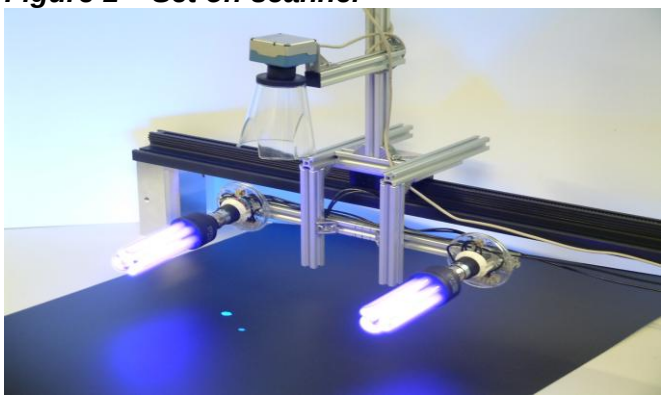
2.3.2 Lighting system

UV illumination is provided by a pair of commercially available fluorescent 'black lights'. The term 'black lights' is applied to commercially available light sources which emit only a small amount of visible light. White lighting and other configurations could be selected via the software interface.

2.3.3 Linear guide

The camera and lighting system are mounted on to linear guide using a custom frame. The Linear guide enables the scanner to inspect web materials with a width of up to 1m.

Figure 1 – Set off scanner



2.4 Test procedure

To improve the chances of detecting set off on the test substrate, the instrument should first be calibrated to optimise detection of the potential set off chemicals. Procedures for setting up, calibrating and performing tests are shown in Appendix 1.

2.5 Principles of operation

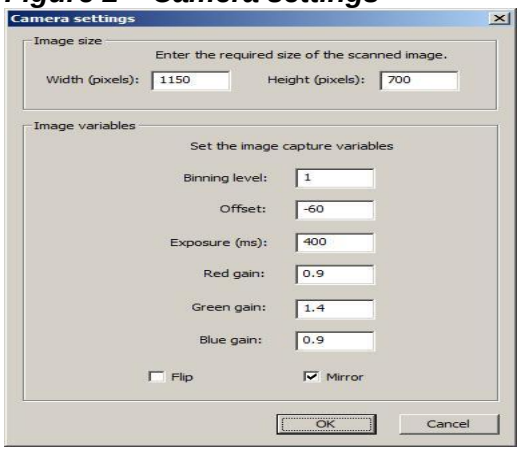
The detection of set off on any test substrate is dependent on the ability to observe contrast between the substrate and any region of contamination. To enhance this contrast the set off scanner provides a range of options for optimising images of the test material's surface.

The following subsections describe the optimisation of camera settings, image processing and illumination available to enhance the contrast between the test substrate and the region on the surface where set off material is present.

2.5.1 Camera optimisation

Settings for the camera can be adjusted to control the colour balance and sensitivity of the images recorded. The settings should be optimised to highlight regions of set off. However, this can result in long exposure times for certain combinations of film/ink set off. This might necessitate the scanner being used off line. Figure 2 shows the camera settings available.

Figure 2 – Camera settings



2.5.2 Image processing

To quantify set off and measure its area, images of the test substrate can be processed using an optimised routine which has been calibrated using specially prepared palettes. This technique is essential for inks or varnish which are difficult to observe on the test substrate. An example of this would be similar set off colours and backgrounds such as transparent varnish or white ink on white substrates. However, the surface area of set off with high

contrasts can be determined using non-optimised processing. Examples of these methods are presented in Figures 3 – 10.

Non-optimised processing

Figure 3 shows the transfer of a red and blue ink onto the food contact side of a foil laminate board viewed in 'white light'. Figure 4 shows the same image without any processing but thresholded to highlight the set off. While the transferred ink is clearly visible, the texture of the boards' surface adds a considerable amount of noise to the image which could be misinterpreted as set off during image analysis.

Figure 3 – Blue and red ink transfer

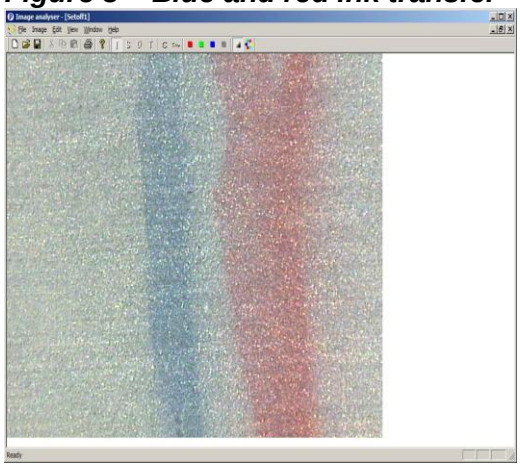
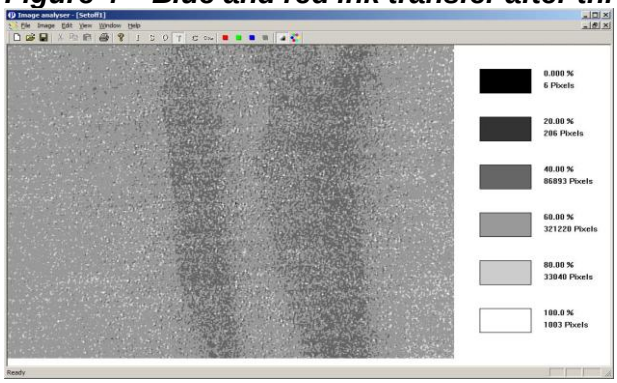


Figure 4 – Blue and red ink transfer after thresholding



Smoothing and Filtering

To reduce the effect of texture 'noise', in Figure 5, smoothing is applied to the original image to remove texture while retaining the colour detail. The image can also be digitally filtered for each colour before thresholding to enable the different transferred inks to be identified separately as shown in Figures 6 and 7.

Figure 5 – Blue and red ink transfer after softening

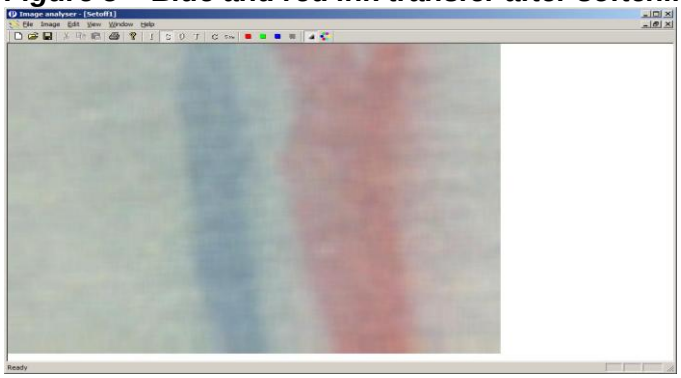


Figure 6 — Blue and red ink transfer after softening and filtered thresholding to identify blue transfer

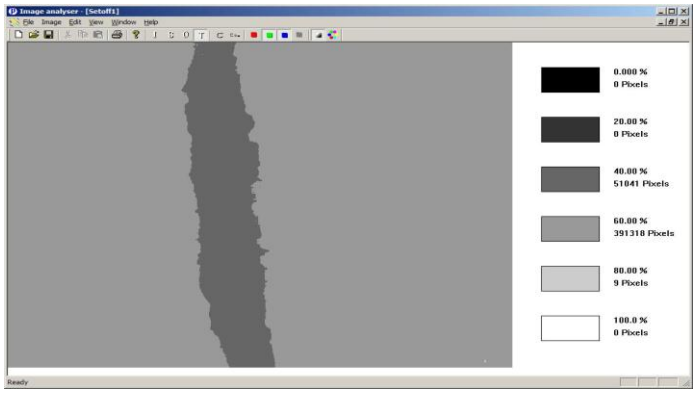


Figure 7 – Blue and red ink transfer after softening and filtered thresholding to identify red transfer

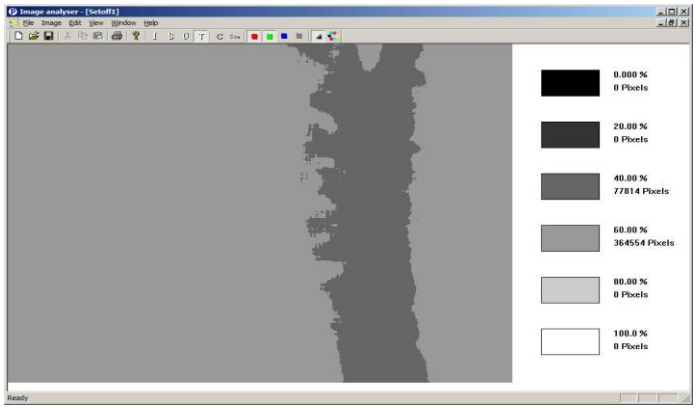


Image optimisation

Further optimisation of the image can be used if the colour and intensity of the potential set off is known. Figure 8 shows the controls for the colour optimisation. This type of optimisation is essential for low contrast, non-visible set off

Using this more advanced filtering method specific colours and intensities can be singled out within an image to identify and quantify set off.

Figure 8 – Colour optimisation controls

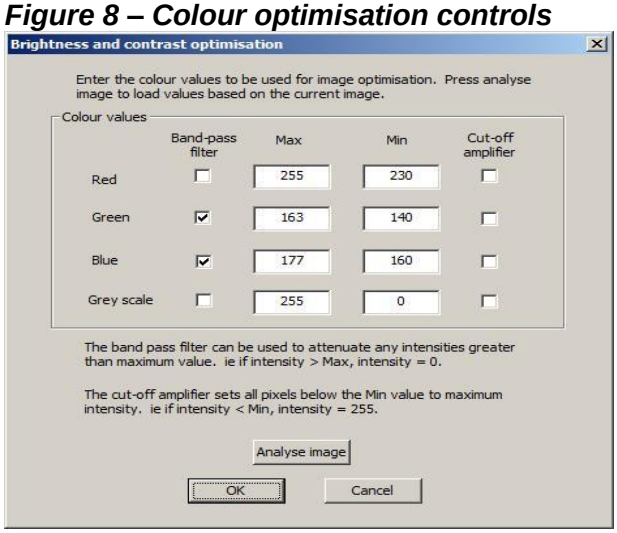


Figure 9 below shows a palette of 28 different colours which has been filtered and thresholded in Figure 10 to identify only pink.

Figure 9 – 28 colour palette

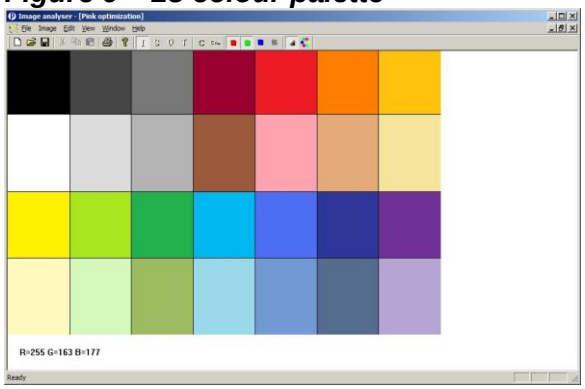


Figure 10 – 28 colour palette optimised for pink

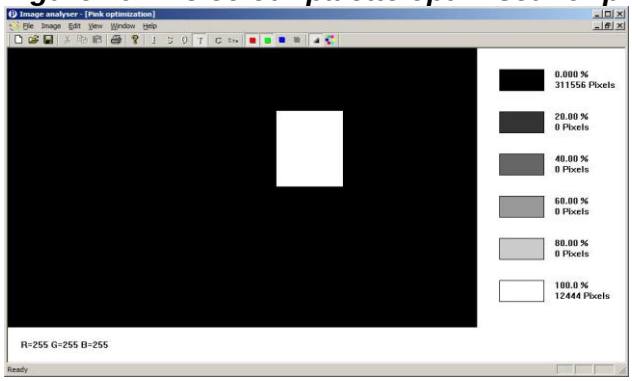


Image thresholding

Thresholding is then used to determine the surface area of any set off regions and can be used to determine the quantity of set off present. Figure 11 shows the threshold controls while Figure 12 shows an example of a thresholded image.

Figure 11 – Threshold controls

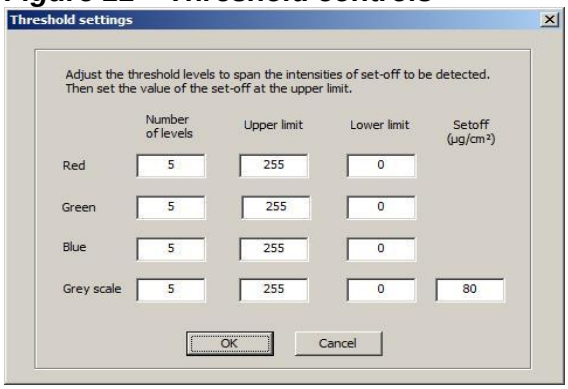
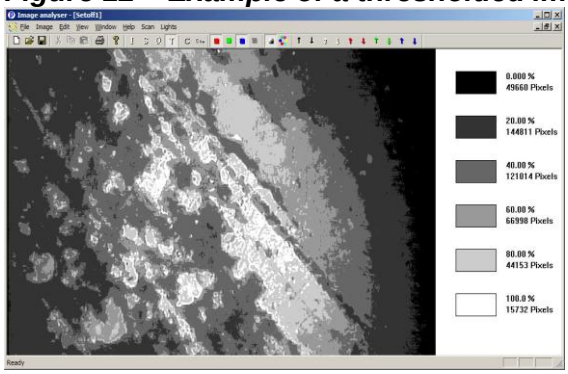


Figure 12 – Example of a thresholded image



2.5.3 Lighting

A range of lighting configurations have been evaluated to identify the most effective systems for the scanner. In addition to visible wavelengths, a number of dark lighting systems (~340nm) have been tested as shown in Figures 13 and 14 below. These show a calibration palette before and after optimised thresholding.

Figure 13 – UV fluorescent calibration palette

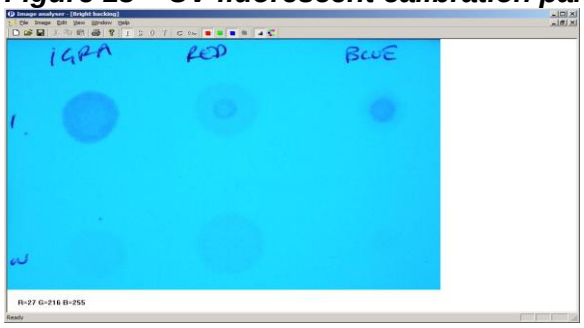
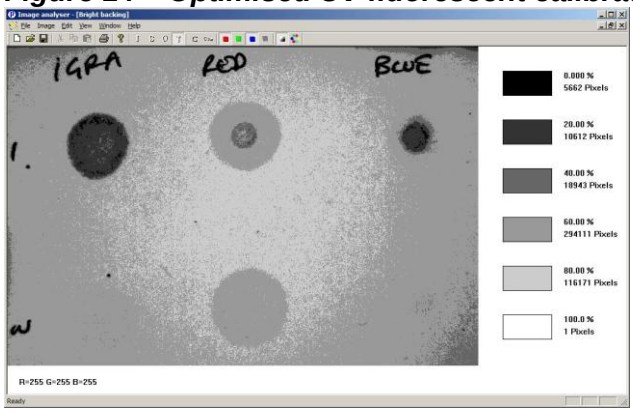


Figure 14 – Optimised UV fluorescent calibration palette



The UV illuminated images in Figures 15 and 16 show a calibration palette which includes the photoinitiator Irgacure 379 (coded IRGA on the palette) which appears transparent under visible light.

Figure 15 – Non-fluorescent calibration palette observed with dark light excitation

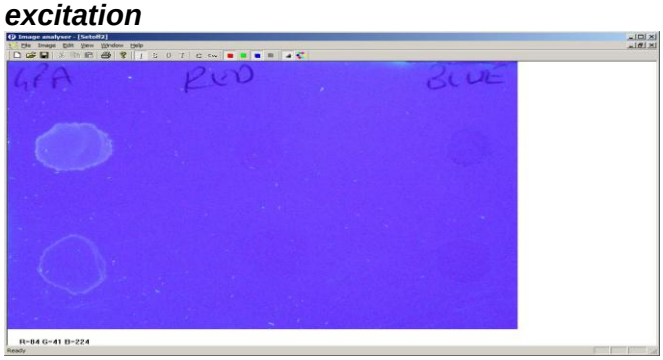
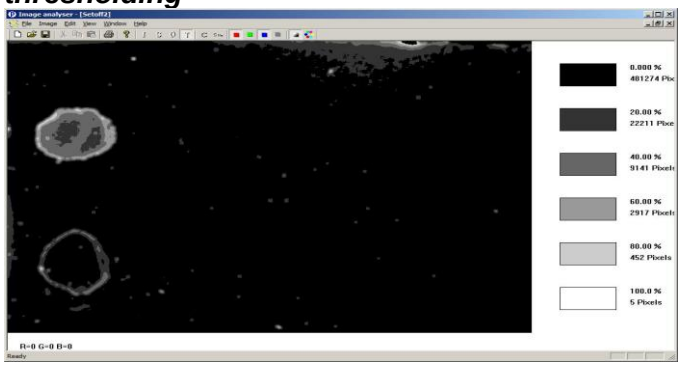


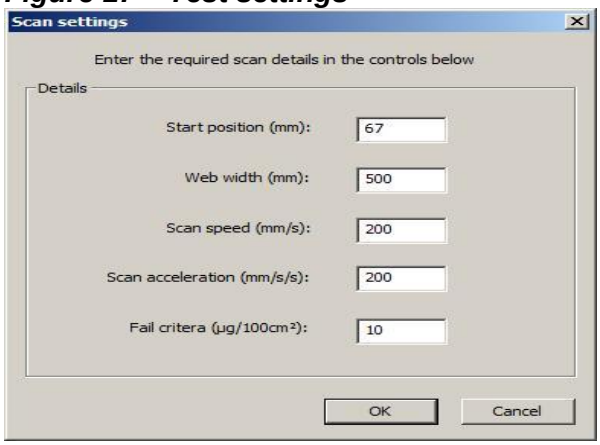
Figure 16 – Non-fluorescent calibration palette after optimisation and thresholding



2.5.4 Scanning tests

To assess set off on web based substrates the scanner's linear guide can be used to traverse across the width of the material. Figure 17 shows the test settings which can be entered to automate this process. When the test is started, the scanner records the total area scanned together with the amount of set off observed.

Figure 17 – Test settings



2.6 Discussion

Examples of the results and limitations of the scanner are now discussed.

2.6.1 Test samples

To assess the performance of the scanner, tests were performed on 6 different materials using a solution containing IRGACURE 379. This particular UV photoinitiator was selected as it represents a challenging source to detect – it is less-visible and provides a low level of UV fluorescence.

The selection of materials tested was as follows:

1. Coated paper – Highly UV fluorescent
2. Red plastic film – Dark non fluorescent
3. Uncoated paper – Contained bright fluorescent fibres
4. White plastic film – Mild print show-through
5. Thin white plastic film – Severe print show-through
6. Foil laminate – Reflective surface

Images illustrating the samples under UV illumination and after optimised processing are presented in Appendix 2 together with the optimisation settings required to calibrate the scanner for these substrates.

With the exception of the samples 5 and 6 it was possible to optimise the image and observe set off in each case. However, contrast from the print show-through in sample 5 was greater than the contrast between the substrate and the contaminant. In the case of sample 6 the set off could not be clearly observed using the current experimental setup. The reason for this was that the illumination light reflected back off the substrate and saturated the image with an uneven distribution of light over the image.

2.6.2 Observations and limitations

To achieve optimum results it has been found that the illumination must be consistent and even across the inspected surface. Good results were achieved with commercial lighting arrays used in a laboratory dark room; however sensitivity would be improved with the use of a more bespoke solution. Two possibilities are line scanning or use of a collimated light source. With line scanning, illumination is carried out with a narrow strip of light just wider than the camera observation area rather than illuminating the entire test area. With collimated light, the light is focused with reflectors so that all the light is applied from a single direction.

The test substrate needs to be flat and undamaged to prevent reflections being observed by the camera. The material also needs to be dust free.

It may be possible for printers to select the printing components based on the substrate material which allows the greatest chance of detecting the set off.

Off-line testing of the complete web surface should be achievable with equipment costing less than £10,000.

On-line scanning is more difficult but is believed to be possible, particularly on intermittent motion machines, providing set off can be detected with compatible scan parameters. This means where the exposure time can be related to the line speed. Where there is not a brief pause in motion of the web (such as on packing machines), the camera must be able to move at the same speed as the web for sufficient time to collect sufficient data. The scanning may be used to examine a representative fraction of the total web area, specifically targeted to known problem areas or the entire web surface. Depending on the test parameters required and web motion profile, testing should be achievable with equipment costing in the same budget region as for off line testing.

2.7 Conclusions

To have the greatest application to industry, the scanner will need to be installed on-line and be capable of operating at economical production speeds (line speeds that do not have adverse cost implications by slowing the production process down). However this would require image capture and processing to be performed at a rate which cannot be achieved within the budgetary requirements defined by this project due to lighting, camera and processing limitations. It is therefore proposed that the scanner is used as a periodic off-line quality control tester. For some intermittent motion

applications it may be possible to scan a representative proportion of the substrate on-line.

It has been found that some combinations of substrate and contaminant cannot be effectively assessed due to difficulties in obtaining sufficient contrast between the two regions to enable detection. It may, therefore, be possible for printers to match printing components to the substrate in order to give the best chance of detection.

Transparent and some translucent substrates have also been found to be unsuitable for set off detection due to show-through of inks from the printed side. However, it may be possible to overcome this issue at the image processing stage using a suitable 'digital mask'. This would increase the complexity of the test and is beyond the scope of this project.

The overall capability of the scanner is dependent on the combinations of substrate and set off to be analysed. The speed of testing is also dictated by the surface area of the smallest region of set off which is to be detectable by the system and the amount of light required for its observation. Even and consistent lighting is also critical for obtaining good results.

The operation of the scanner is described in Appendix 1 on page 92.

Chapter 3

PART 2

3.0 Measurement of non visible set off

3.1 Introduction

The chemicals used in UV cured inks, for which set off measurements are likely to be of interest, were identified from a literature and internet search. The project industrial partners were consulted, enabling a list of chemicals to be made and calibration samples obtained.

Exposure techniques were investigated using a selected set of extraction solvents that were suitable for a range of packaging materials such as printed plastic and paper board supplied on the reel, or in a stack, and containers supplied in stacks. This was carried out to establish whether, for example, it was possible to form pouches from carton board sheet, or whether it was possible to use single sided cells clamped over the test substrate without leakage of the solvents. Solvents which soften or dissolve the polyethylene layer of a laminate may result in leakage of the cells.

Analytical methods were then developed using the inventory of calibration reference samples which were capable of measuring the photoinitiators and synergists in the selected extraction solvents. Packaging materials were provided by the project industrial partners; these were printed with test inks on reels which had been stored under typical production and supply conditions. The set off from these reels was then measured using the developed test procedures. These data were then used to assess the performance of the procedures. Finally, migration tests using the test packaging were carried out into food using the established EU rules for migration testing, (EN13130-1:2004) [Ref 3]. These results allowed a comparison to be made between the set off results obtained from the test films and migration into foods.

3.2 Selection of ink components requiring measurement

UV cured inks incorporate a resin binder system which undergoes a chemical cure reaction resulting in a dry polymer layer. The curing reaction is initiated with ultra-violet light or less commonly with an electron beam. A wide range of chemicals are used, they are usually involatile and may migrate depending upon the substrate and the extent of cure. There has been continual development of the technology of UV inks with the object of reducing the extent of migration of chemicals to the food. Initially development focused on the use of pre-polymers, larger molecular weight compounds, rather than

single molecule monomers and reactive diluents that become incorporated in the molecular structure of the cured polymer rather than solvents. Due to the concern of contamination of food from photoinitiators (the chemicals used to initiate cure) developments in recent years have focused on increasing the molecular weight of the photoinitiator or adding reactive functional groups to bind the photoinitiators into the cured ink resin. An increasing range of photoinitiators, synergists and other additives have therefore been developed. Information in the public domain on these ink components (such as chemical structure) is often limited for commercial reasons. The curing chemical reaction usually gives rise to a complex mixture of chemical compounds. The complex chemical composition of the cured inks and the wide range of additives used means that monitoring for the transfer of these compounds to food is difficult for laboratories that are independent of the ink suppliers. Mass spectra of the ink components may not be available in commercial libraries. Small quantities of the pure ink components are required in order to calibrate analytical instruments and to develop and validate analytical methods. These are often only available from ink suppliers.

The text book [Ref 4] Crivello J, provides a comprehensive overview of the chemistry of UV cured inks. This lists many of the photoinitiators in common use and their chemical structures. Assistance was provided by the project partners to obtain calibration samples of commonly used photoinitiators and synergists. In addition, the project partners provided printed test packaging which was designed specifically for the project to have a range of set off values for different ink components used in the ink formulations. These test films enabled analytical methods to be developed which were capable of quantifying the components known to have been used in the inks. The ink components used were agreed with the project industrial partners and could be grouped into two categories. Firstly, those that are widely used which would be expected to give relatively easily measurable and significant set off. Secondly, polymeric photoinitiators and synergists which would be expected to give low set off values and which would be expected to be more difficult to measure.

Whilst the project was in progress, legislation relevant to printing inks has come into force in Switzerland. The Swiss Ordinance on materials and articles in contact with food (SR 817.023.21), [Ref 5] lists in Annex 6 the permitted photoinitiators and synergists with specific migration limits. It is reasonable to assume this list includes all the chemical compounds in common use. Whilst not directly applicable within the EU, it is a useful guide in interpreting the requirements of the EU legislation Regulation (EC) No 1935/2004 because of the toxicological evaluation that has been carried out in order to provide the specific migration limits.

3.3 Consideration of exposure techniques

3.3.1 Initial treatment of packaging samples prior to set off measurements

The extent to which set off of ink components occurs to the food contact surface of packaging stored in the stack on the reel depends upon numerous factors. These include:

i) Storage conditions of temperature and time.
ii) Pressure applied to the ink in contact with the food contact surface
iii) Extent of ink cure
iv) Composition of the packaging substrate
v) Composition of the ink such as plasticisers, diluents and pre-polymers

It is desirable that, where possible, variables that may be controlled in the laboratory should be specified so that consistent reproducible results are obtained by different laboratories. In some cases test portions may have to be taken from large stacks or reels of packaging where it is impractical or uneconomic to send a complete reel or stack. In these cases, therefore, it may be necessary to specify a test contact pressure and time duration.

Wells Carter J and Jupina M "Cationic UV ink migration and safety assessment, RadTech 1997 [Ref 6], used storage conditions of 1.2 psi at ambient temperature for 7 days. The European Printing Inks Association (EuPIA) have published the "EuPIA Guideline on Printing Inks applied to the non-food contact surfaces of food packaging materials and articles" September 2009, [Ref 7] the link is provided below for convenience. http://www.eupia.org/EPUB/easnet.dll/ExecReq/Page?eas:template_im=10008E&eas:dat_im=05048E. This provides in Appendix 2, page 11, guidelines for storage/conditioning conditions for printed packaging to be exposed to prior to migration measurements. For reel fed plastic films the applied pressure is 80 kg/cm^2, for reel fed paper the pressure is 40 kg/cm^2. Exposure time is 10 days at 25 °C. These reel pressures are stated in the EuPIA guideline as being typical for printed packaging. Attempts were made to obtain data on pressures typically found in reels by contacting some selected clients in Industry. No data were available as this seems not to be routinely monitored, with reliance placed instead on other measures such as the Schmidt hammer which measures the rebound of a spring loaded mass against the reel surface. The rebound is dependent upon the hardness of the reel surface, which is in turn affected by, amongst other factors, the reel tension. For a practical laboratory test area of 0.7 dm^2 , weights of 2.8 and 5.6 tonnes for reel fed paper and plastic films respectively, are required. Reducing the surface area of the test portion is not desirable as the variation in replicate test results increases due to the variation in set off over the surface. Even

using a 0.3 dm^2 test area the weights required are 1.2 and 2.4 tonnes. Guided weights are required for safety reasons and care is required to ensure a perfectly flat base. Ordinary hydraulic presses are not suitable because of the problem of pressure bleed over time. Screw type presses with a reliable pressure, which do not impart a rotational sheer force, are not easily sourced. Compression between metal plates held together with bolts is a possible approach, but uniform pressure and the need for a pressure read out from a load cell device, or accurately known torque setting for the bolts, is required to ensure the test pressure is achieved and maintained during test. Microprocessor controlled presses are commercially available and the advantage of these is that they continually maintain the pressure over the required test period. However, the cost of this equipment is of the order of £10,000 per press. Such presses are too heavy to be placed in an oven so a temperature controlled room or specially constructed temperature controlled enclosure would be required.

The storage pressures for test films given in the EuPIA guideline could not be achieved with equipment available at Pira. It is unlikely that such equipment would be available in many laboratories and a lower test pressure would have to be accepted that is within the range of practical laboratory weights. For the purposes of this project, a storage test pressure of 1.2 psi was used on individual test specimens.

3.3.2 Exposure techniques

In most cases printed packaging will be from production batches. In the case where test samples from a reel or stack are not available (such as when an ink supplier is formulating an ink), two approaches to testing are possible. Printed test sheets may be stacked print side to food contact side and the test storage pressure applied. The food contact surface is then tested for set off. This is the approach used in the project to compare set off with and without the application of a storage pressure of 1.2 psi described on page 58. The test sheets were placed on a flat surface and covered with a steel plate. A metal container was placed upon the plate and filled with a volume of water, calculated in combination with the weight of the metal container and metal plate, to give the required pressure. Alternatively the print surface could be placed in contact with an inert test surface such as aluminium foil and the test pressure applied. The aluminium surface in contact with the print is then tested for set off by immersion in a solvent or food simulant. This approach was not used in the project, but it is seen in some published work (Wells Carter J, Jupina M [Ref 6]). This approach may not be representative of actual or even worst case conditions and it is also contrary to Technical Document No 3 of the Council of Europe Guidelines on test conditions for packaging inks, [Ref 8] which states that “the ink to be tested should be printed on the

same substrate and by the same procedure that is intended for the final product". This technical document is available via the internet web site of the Council of Europe and is one of a collection of documents relating to printing inks issued under a policy statement of 21 December 2006.

The food contact layer in laminates is usually polyethylene and migration of ink components towards the middle of the polyethylene layer bulk from the food contact surface may be significant in comparison to the aluminium foil surface. Ink components transferred by set off which have migrated into the polyethylene food contact layer may or may not then migrate back out into the food simulant. The amount of set off may vary depending upon the chemical nature of the surface in contact with the ink. Quite different results might therefore be obtained using aluminium foil as the food contact surface. As a general principle, the monitoring of ink set off should be treated, as far as possible, in accordance with the rules already established for migration testing. This means that the food contact surface of the actual packaging should be tested for set off.

The methodology for exposure of the food contact surface of packaging is generally well established due to the requirements for single sided migration testing in the food contact plastics legislation [Ref 3]. Migration test cells are widely applicable to a range of substrates for surface areas up to 2.5 dm^2. Carton board can be exposed in this manner when the food contact surface is polyethylene, although it was found that leakage can occur if the cell is positioned on a crease. A reduced volume of extraction solvent was found to be generally required compared to the EU accepted convention of 6 dm^2 to 1 kg of simulant, in order to provide adequate detection limits (10 ppb equivalent) by GC-MS and HPLC. Sensitivity of detection by LC-MS in the scan mode for some polymeric photoinitiators and synergists was found to be poor (see pages 45, 70 and 71). For a surface area of 0.71 dm^2, the volume of solvent or food simulant was reduced to 25 ml for compounds freely soluble in the simulants or solvent that exhibited poor LC-MS response. This was in order to remain in compliance with the EU test requirements for migration testing and achieve adequate detection limits for set off measurements. For polymeric photoinitiators and synergists such as Speedcure 7010 and 7005, evaporation of the exposed set off measurement test mixtures to 0.5 ml gave detection limits of 7 $\mu g/dm^2$. The lowest detection limit required for set off measurements is 1.7 $\mu g/dm^2$ (or 10 ppb equivalent in the food), to allow the set off value to be compared to migration into the food, (assuming comparison against a 10 ppb migration limit, 100 % migration and the 6 dm^2 to 1 kg of food conventional EU surface area to volume test ratio). For set off measurements of the polymeric photoinitiators and synergists with poor analytical sensitivity and specific migration limits of 10 ppb, future

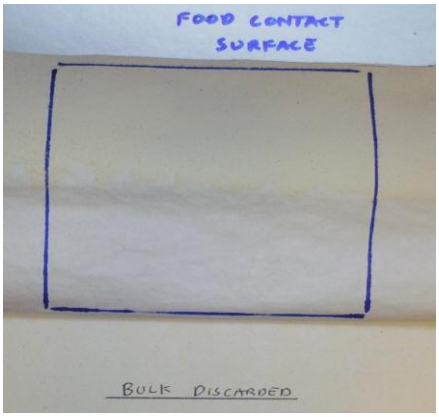
consideration could be given to exposing four test portions to the extraction solvents and combining these to give a single test replicate. This would provide a four times improvement on the detection limit.

Carton board used in drinks cartons with a foil barrier and an inner polyethylene food contact layer, were formed into pouches in the following manner. The bulk of the outer paperboard layer was peeled off and discarded. This was to allow adequate heat transfer for heat sealing. An area of 1 dm^2 was marked on two portions of the remaining paper board outer surface. These 1 dm^2 areas were cut out and placed on top of each other and the polyethylene food contact surfaces heat sealed together along the marked lines. The corner of the pouch was snipped with scissors, a glass funnel inserted and the extraction solvent poured in. The corner was then closed with a metal staple or by heat sealing.

Example photographs of the pouch making process are shown below.

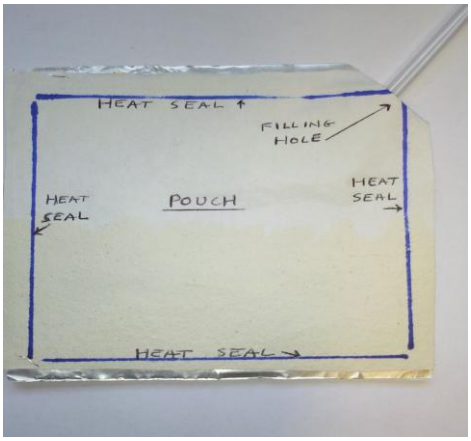
1) Tear off backing bulk

2) Draw 1 dm^2 test area



3) Repeat for second portion and overlay

4) Heat seal together to form pouch



Pouches can be made from larger polymer laminate sheet if required, depending upon the construction of the laminate. Larger exposure areas

require support and therefore need to be laid flat during exposure. This can take up valuable storage space in ovens. Conducting tests on surface areas less than 0.7 dm^2 was not considered to be sufficiently reliable, as below this area it becomes difficult to achieve the required detection limits. In addition, the variation between replicate test results increases due to the variation in the extent of set off across the food contact surface.

The above investigation of exposure techniques demonstrated that pouches or single sided test cells were suitable for the purpose of measuring set off on printed plastic and paper board packaging in the various forms it is commonly provided in. This included packaging provided on the reel or in a stack and also containers supplied in nested stacks.

3.3.3 The selection of extraction solvents and extraction times

The set off measurement is an estimate of the ink component present on the food contact surface and is dependent upon the solubility of the ink components in the extraction solvent. The value does not allow for migration through the bulk of the packaging structure. Solvents that swell or penetrate the food contact surface may not be desirable on thin films where this could result in extraction of ink components from the outer print layer. It is sensible to select the existing EU substitute fat test simulants, 95 % ethanol and iso-octane as extraction solvents. The reasons for this are:

i) Most ink components will have good solubility in the concentration range of interest in one or other of these
ii) They are sufficiently volatile to be easily concentrated by evaporation
iii) They are established fatty food simulants selected by the EU
iv) They are suitable solvents for injection for gas chromatography Analysis
v) Set off values may if required be conveniently compared with migration values

A third solvent may be required however, as some ink components may have a poor solubility in iso-octane and may also react with 95 % ethanol. An aliphatic ether is likely to dissolve a wide range of ink components and have low reactivity with them. Dioxane was selected because it has a boiling point similar to 95 % ethanol and iso-octane and because of its ability to dissolve polar and non polar compounds.

An initial series of experiments were carried out to establish whether the above extraction solvents were suitable.

Two printed films were obtained from the industrial partner described as;

Film A	PET/ LLDPE	printed UV flexo ("green")
Film B	Paper/ PE/ foil/ PE	Morri white sauce printed UV offset ("brown")

The ink composition of these was not known and therefore, it was necessary to extract the print surface. In this case dichloromethane was deliberately chosen as an aggressive solvent, to maximise extraction from the print surface. Portions (2 dm^2) of each film was shaken briefly with warm dichloromethane, and then filtered. Approximately 2 ml of chloroform was added and the extract was reduced in bulk by evaporation to about 2 ml under nitrogen. The extracts were analyzed by GC-MS (see Appendix 10 page 320 for equipment and operating conditions). Comparison of the chromatograms with the Wiley 7n library identified the following compounds:

Film A Irgafos 168, irgafos 1076, 4-phenyl benzophenone, 2-ethylhexyl-4-(dimethylamino)benzoate, possible unidentified acrylates, 2,4-bis-(1,1-dimethylethyl)phenol, triacetin, 4-methoxyphenyl acetate, 2,4,6-trimethylbenzaldehyde

Film B 4-phenyl benzophenone, 2-ethylhexyl-4-(dimethylamino)benzoate, benzophenone, BHT, triacetin, possible unidentified acrylate.

Film A was selected for further investigation as it had the greatest proportion of 2-ethylhexyl-4-(dimethylamino)benzoate and 4-phenyl benzophenone present in the ink. This was most likely to give high set off values which would be required for comparing the extraction ability of the extraction solvents. Test portions of film A were mounted in single sided test cells and triplicate migration experiments were performed using the following conditions:

- Exposure to isooctane at ambient temperature and at 60˚C
- Exposure to 95% ethanol at ambient temperature and at 60˚C
- Exposure to dioxane at ambient temperature and at 60˚C

Ambient and elevated temperatures were chosen to investigate whether extraction of the set off ink components was influenced by temperature. The 60 °C temperature was selected as the upper temperature for the following reasons:

i) The use of higher temperatures might distort the test film and give rise to leaks from the test cell.

ii) Higher temperature is likely to be undesirable due to possible thermal degradation of ink components.

iii) Test temperatures of 60 °C are specified in Regulation (EU) No 10/2011 “on plastic materials and articles intended to come into contact with food” for contact times longer than 30 days at room temperature or below. Results may be conveniently compared to migration tests. The test may be conveniently carried out using the same ovens in use for migration testing.

In every case, 0.7dm^2 of film was exposed to 40ml of solvent for 5 hours. A set off measurement should, in principle, be obtainable by washing of the food contact surface with a brief contact of the extraction solvent. However, it is possible that the ink component on the food contact surface is present within a fragment of the ink matrix and a time period is required for complete extraction from the ink matrix to occur. A judgement has to be made on the optimum time period to use, which may vary depending upon the ink and the packaging substrate. Time periods of more than a few hours may introduce a contribution to the results from degradation of the ink components in the solvent and or migration into or from the packaging. The five hours was selected as a convenient maximum exposure time available in a 7 ¼ hour working day, leaving sufficient time to prepare the extracts for GC-MS or LC-MS analysis with automatic injection overnight. Shorter exposure time periods were investigated in Chapter 6.

The test portions were all cut from areas of solid green print to minimize sample to sample variation. At the end of the exposure period, 100µl of a 1.132mg/ml solution of d_{10} benzophenone in chloroform was added to each cell. The cell contents were mixed by swirling and immediately transferred to vials. To each extract was added 2 ml of propan-2-ol to act as a keeper solvent. The extracts were then reduced in volume to approximately 2 ml using gentle heating under nitrogen. Precipitated polymer was visible in the isooctane and dioxane extracts that had been incubated at 60˚C. This was filtered off using a syringe filter. The concentrated extracts were then analysed by GC-MS in the SIM mode (see Appendix 10 page 320 for equipment and operating conditions) looking specifically for 4-phenyl benzophenone and 2-ethylhexyl-4-(dimethylamino)benzoate. Calibration standards were prepared in dioxane and run alongside the test samples.

Results obtained are shown in Table 3-1. The difference in results between extraction solvents and extraction temperatures was not so marked in later work described in Chapter 6 page 56. This may be because the time required for extraction from the ink resin matrix in which set off of the individual chemical compounds varies between ink types, extent of ink cure and other factors such as ink composition. The choice of 5 hours at 60 °C is likely to

ensure the most reliable set off value, assuming the ink components are stable under these test conditions.

Table 3-1 Comparison of set off results obtained in different solvent extracts of the food contact surface

Solvent		2-ethylhexyl-4-(dimethylamino)benzoate		4-phenyl benzophenone	
		µg /0.7dm^2		µg /0.7dm^2	
		5hrs@ ambient	5hrs@ 60˚C	5hrs@ ambient	5hrs@ 60˚C
95% ethanol	1	14.5	11.4	40.0	163.8
	2	12.5	31.8	46.2	144.3
	3	11.9	8.8	36.0	85.5
	Mean	**13**	**17**	**41**	**131**
	RSD %	10	73	13	31
dioxane	1	6.9	62.9	54.8	232.9
	2	6.6	74.0	40.6	316.1
	3	19.3	41.3	90.7	234.4
	Mean	**11**	**59**	**62**	**261**
	RSD %	66	28	42	18
isooctane	1	39.5	24.6	115.8	109.7
	2	30.2	42.0	104.9	154.2
	3	41.3	45.0	129.9	166.7
	Mean	**37**	**37**	**117**	**144**
	RSD %	16	30	11	21

Extraction with dioxane at 60 °C for 5 hours gave the highest results. Dioxane offers the advantage of having a sufficiently high enough boiling point to allow exposure at 60 °C whilst being sufficiently volatile to allow concentration of the extracts by evaporation under nitrogen. It also can be injected for analysis by HPLC and by GC. Dioxane would not be expected to react with a wide range of compounds of interest, although stability studies for each ink component would need to be carried out to confirm this.

3.3.4 Set off extraction solvent choice compared to migration test food simulants

Chemical reaction of the ink/varnish compounds of likely toxicological interest with olive oil is likely for a wide range of these compounds. Most of the compounds would reasonably be expected to migrate to a greater extent into fats than aqueous foods, as most are insoluble in water but soluble in

solvents such as ethanol and isooctane. The alternative fat simulants 95 % ethanol and isooctane are appropriate food simulants likely to represent worst case migration. For the majority of flexible packaging 10 days exposure at 40 °C would be chosen for olive oil. This would mean 2 days at 20 °C for isooctane and 10 days at 60 °C for 95 % ethanol would be selected. Alternatively, it could be considered that maximum migration will occur at elevated temperatures of use. If there was a hot fill temperature, this would be unlikely to exceed 2 hours at 175 °C for the majority of packaging films, for which, 4 hours at 60 °C for isooctane and 6 hours at 60 °C for 95 % ethanol would be selected. Some printed cartons are retorted at 121 °C but this would require less severe conditions of 2.5 hours for isooctane and 4.5 hours for 95 % ethanol both at 60 °C. These tests could conveniently be completed in one day. Chemical reaction would be expected for some of the photoinitiators with 95 % ethanol. Isooctane would be expected to be the most suitable food simulant, although Directive 97/48/EC specifies both to be used and the worst case results compared against migration limits.

Thus from the above considerations it is reasonable to propose measuring set off using iso-octane and 95 % ethanol, because these are used in EU substitute fat tests. Test conditions of up to 5 hours at 60°C is also consistent with current rules on migration testing, bearing in mind that sufficient test time is only required to dissolve ink components on the food contact surface, as migration through the packaging is not relevant to set off measurements. Dioxane provides an alternative test solvent for ink components where iso-octane or 95 % ethanol are not suitable because of the analytical procedure required (for example reverse phase liquid chromatography where the extraction solvent must be miscible with water) or low stability of the ink component.

3.4 Proposed set off exposure procedure

The following set off exposure procedure is proposed.

3.4.1 Selection of samples for testing

It can not be assumed that the same ink components are present in each colour applied to the print design. If this assumption is made and portions of packaging cut randomly from a reel, it is likely that an incorrect assessment of set off will be made, particularly if the print is not continuous. In most cases, the position of a printed image will not overlay that of another image after winding on the reel. To measure set off reproducibly, it is necessary for each test replicate, to select a region of the food contact surface that has been in contact with the same region of the printed image above it in the reel. These test replicates should be at identical printed image repeat distances along the packaging length.

Table 3-2 below illustrates how the concentration of ink components was found to vary depending upon the colour used. Regions with red, yellow and brown ink only were cut out from test repeat reel 3 described on page 60 (last paragraph). These were extracted with chloroform overnight at room temperature and the extracts filtered and injected for analysis by GC-MS.

Table 3-2 Concentrations of ink components in different colour regions of an image mg/kg of printed film

Colour	***Benzophenone***	***Ethyl-4-(dimethylamino) benzoate***	***CAS 0071868-10-5***	**4-phenyl benzophenone**
	mg/kg	***mg/kg***	***mg/kg***	**mg/kg**
Red 1	860	150	80	1200
Red 2	850	130	70	1100
Mean	**860**	**140**	**75**	**1200**
Yellow 1	280	50	30	310
Yellow 2	310	60	30	330
Mean	**300**	**55**	**30**	**320**
Brown 1	510	100	50	570
Brown 2	500	100	50	550
Mean	**510**	**100**	**50**	**560**

Results are rounded to two significant figures. The table above shows that if set off is measured in a region of the food contact surface which has been in contact with predominantly yellow ink, the set off value obtained could be quite different to a region that has been in contact with red ink.

The following procedure is proposed for sampling sub portions for testing taken along the length of the packaging material unwound or cut as a slab from a reel. The position of the start of a print design in contact with the food contact surface is marked on the food contact surface. The lengths of the print design and the gap before the start of the next print design are measured. These are summed together to give the repeat length of the image. This value is multiplied by an arbitrary number for example 10 to give a starting distance if we wish to ensure that we do not test at the very start of the reel. If a complete reel is sent for testing this procedure is started after having peeled off a slab of the reel corresponding to at least one quarter of the reel thickness measured from the core. Discarding the first quarter of the reel would ensure set off measurements are representative of the whole reel as set off may be influenced by a higher pressure nearer the centre of the reel. This starting distance is then measured off on the food contact surface as the reel is unwound and a line marked across the film. The length of the image is

measured from this line and another line drawn. The region between these two lines is the region of test corresponding to one complete image. The length of the gap between images is measured and a line drawn. The length of the image is marked from this line and the region in between corresponds to a second test region. This process is repeated to give replicate test portions. If the image is greater than the surface area that can be exposed to extraction solvent in the test cell, then representative sub portions of the food contact surface should be taken corresponding to regions of different colour in the image which has been in contact with on the reel. The mean value of the replicates is taken as the set off value.

3.4.2 Exposure of the test portions

The packaging test portions are clamped into a single sided cell or pouches made by heat sealing and the food contact side exposed to the exposure media tabulated below. Containers are filled with the media and exposed to the test time and temperature as tabulated below.

Table 3-3 Exposure conditions for set off measurement

Packaging type	***Exposure medium***	***Test conditions***
Carton board	Tenax TA	10 days at 60 °C or 30 days at 40 °C
Cartons with a PE coating, polymeric films, laminates, containers	Isooctane or 95 % ethanol or 1,4-dioxane	5 hours at 60 °C

3.4.3 Analysis of the test extracts

After exposure, the exposed media are concentrated by evaporation to 25 ml by evaporation under vacuum or on a hot plate under a stream of nitrogen. The exposed test mixtures are injected for GC-MS analysis and LC-UV-MS or LC-UV-CAD. Results are reported in units of µg/dm^2 and these values multiplied by 6 to give µg/kg. In the case of Tenax TA, this is then extracted with diethylether and the extracts concentrated after the addition of propan-2-ol or dioxane and then injected for GC-MS and LC-MS-UV. The analytical methods for the solvent extracts have been found to allow a detection limit equivalent to 10 ppb in the food.

3.5 Summary

Care is required in sampling from the packaging to ensure that the food contact surface tested has been in contact with exactly the same region of the print design as different types and concentrations of ink components may have been used in different colours.

Most packaging may be tested by the use of single sided cells or by forming pouches. Single sided cells of area 0.7 dm^2 are likely to be the most practical and widely applicable exposure method. It is possible to achieve a detection limit of 1.7 $\mu g/dm^2$ (10 ppb) by use of a reduced volume of solvent and concentration of the extraction solvent to approximately 2 ml followed by selected ion monitoring or UV detection.

Isooctane and 95 % ethanol were selected as test extraction solvents as they are specified as the EU substitute fat test for migration testing. Dioxane was included to provide an alternative for ink components that might be expected to react with 95 % ethanol and also to allow the choice of direct injection for both GC-MS and HPLC. Most photoinitiators and synergists would be expected to be soluble in these solvents in the likely concentration range in the food contact surface. Test temperatures up to 60°C for 5 hours were used to ensure extraction of ink components that may have moved into the polymer bulk of the food contact surface. The time period is sufficiently long to ensure extraction from the surface and from the ink resin matrix, the vehicle in which set off has occurred, and short enough to allow set off to be measured in one working day.

For dry food applications and paperboard where liquid media are not suitable, modified polyphenylene oxide (MPPO) is best used following the test requirements set out in CEN EN 14338 Paper and Board intended to come into contact with foodstuffs [Ref 9].

Chapter 4

4.0 Development of Analytical methods

An inventory of 71 calibration samples of photoinitiators and synergists were obtained (some of which were the same compound but from different batches or suppliers). Solutions of all these compounds (in the concentration range 100 to 250 ppm) were then prepared in acetonitrile and injected for GC-MS and HPLC with UV and MS detection. This enabled reference chromatograms and UV and mass spectra to be obtained. The HPLC and GC equipment and operating conditions are given in Appendix 10 on pages 321 and 322.

The compounds are tabulated in Table 4-3 on pages 44 to 46 at the end of this chapter, with the trade name, CAS number and molecular weight, where it was possible to obtain them. Where the compounds were not included in commercial GC-MS mass spectra libraries available at Pira, these are shown with page references to the GC-MS mass spectra. Those that were subjected to LC-MS and LC-UV are shown with page references to the spectra.

4.1 Investigation of photolysis products

It was apparent from the literature that decomposition of photoinitiators may be expected during cure of the ink. This then raises the requirement that the analytical methods are capable of identifying and quantifying the photolysis products, since comparison of set off or migration values against migration limits ought to include photolysis and decomposition products as well as the starting substance. To develop methods applicable to photolysis products it is therefore necessary to expose the photoinitiator calibration samples to UV light and characterize the photolysis products. The possibility of identifying the photolysis products of photoinitiators using an ink mini-cure UV equipment available at Pira was investigated. This work cannot be carried out with ordinary laboratory UV lamps as they are not sufficiently intense. Photoinitiators divide into two basic types: Norrish type I and Norrish type II. The Norrish type I cleave to give a whole series of photolysis products upon exposure to UV light. Norrish type II initiators do not cleave and the significance for this project is that these Norish type II photoinitiators can be expected to be found essentially unaltered in the cured ink. Benzophenone and substituted benzophenones and xanthones such as ITX are all examples of Norrish type II initiators.

Five photoinitiators were selected and concentrated solutions prepared in acetonitrile. These solutions were then exposed to intense UV light in a print curing machine. This machine was a laboratory test machine with a moving conveyor belt designed to replicate conditions on a printing line. The solutions

were injected for GC-MS analysis using the equipment and operating conditions given in Appendix 10 on page 321. No photolysis products were observed for ITX, substituted ITX or 4-phenyl benzophenone (Norish type II). Irgacure 819 and 369 (Norrish type I) both decomposed to give a series of photolysis products, nearly all of which having mass spectra which were not on the Wiley 7 n library. The photoinitaors that have been the subject of rapid food alerts (RASFF) are predominantly type II where the photoinitiator remains unaltered in the ink after cure and is relatively easy to detect and identify. The most likely reason why type I initiators are rarely the subject of alerts is because they decompose during cure to a multitude of compounds that are not easily identifiable. Transfer of type I initiators and their photolysis products to food is likely to be similar to the type II but at present the analytical methods and necessary spectra are not widely available to allow measurement of these.

Some example chromatograms are appended to this report. The chromatogram of Irgacure 819, a type I initiator, after UV exposure is shown in Appendix 8 page 174. Apart from 2,4,6-trimethyl benzaldehyde, the other photolysis products did not match to library spectra. Page 186 of Appendix 8 shows the chromatogram of Irgacure 369 after UV exposure. Irgacure 369 is not amenable to GC-MS, after exposure to UV light, photolysis products were detectable. Apart from a good match for benzyl ethyl ketone, the photolysis products did not match to library spectra. Examination of a number of type I photoinitiators where cleavage would be expected, revealed that exposure of solutions of photoinitiators in pure solvents such as acetonitile to intense UV light was not a viable method of obtaining mass spectra of photolysis products. Apart from the fact that different photolysis products may result in the actual ink, in most cases (the phosphine oxides are exceptions), no photolysis products are detectable, presumably due to recombination of the free radicals produced.

Portions of approximately 0.01g of CAS 0000947-19-3 and CAS 0071868-10-5 were weighed into separate glass vials together with approximately 0.08g of HDDA. Acetonitrile (5 ml) was added and the solutions injected for GC-MS. The solutions were then passed 10 times through the UV curing machine with the conveyor belt on the slowest setting. The resulting solutions were injected for GC-MS analysis to identify the photolysis products given in table 4-1 below.

Table 4-1 Photolysis products of photoinitiators in solution with HDDA only

Photoinitiator	Photolysis products
CAS 0000947-19-3	Cyclohexanone Benzaldehyde Acetophenone Benzoic acid 1-oxa-spiro[4,5]decan-2-one
CAS 0071868-10-5	N-formylmorpholine 4-acetyl-morpholine 4-methylthio benzaldehyde 4-methylthiobenzoic acid

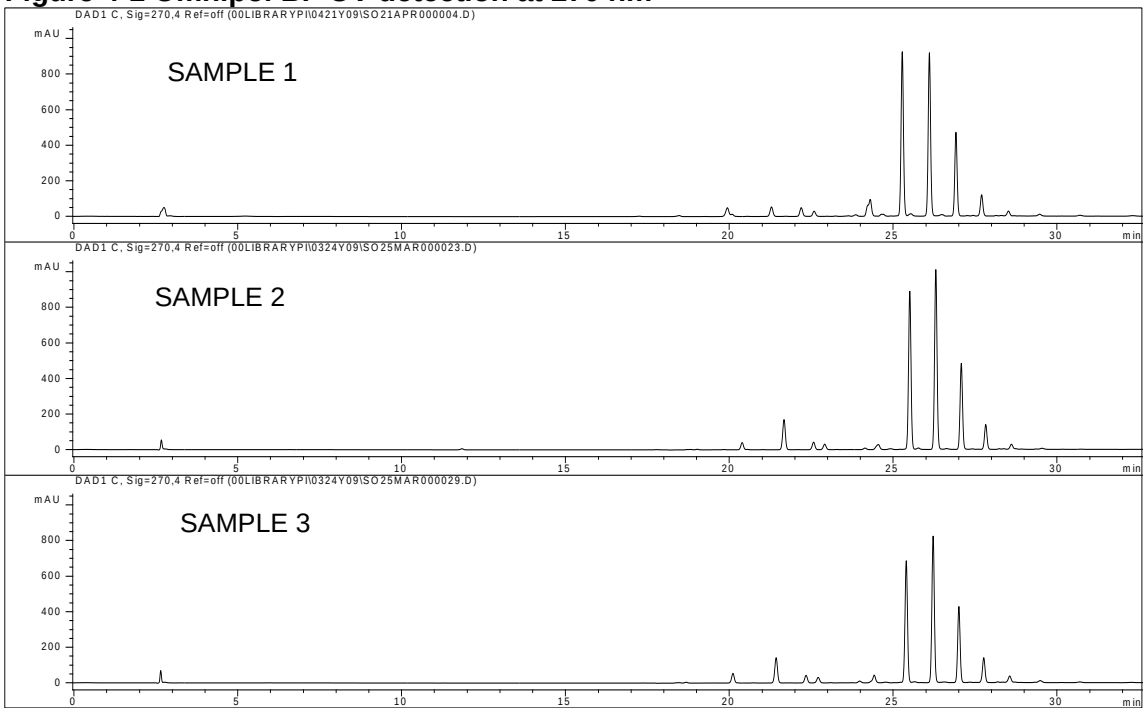
Page 65 of chapter 6 of this report describes the production of a set of test films used to evaluate the set off measurement procedures developed in this project. One of these films (Film 7 printed with ink series X) which had been printed with sub optimum printing press operating parameters selected deliberately to give under cure of the ink, was chosen to investigate the presence of photolysis products. A 1 dm^2 portion of the film was extracted by immersion in approximately 20 ml of chloroform and the extract injected for GC-MS analysis. No photolysis products were detected. Ethyl benzoate, presumably a photolysis product, was detected in the extract. Although under cured, the ink was dry and therefore had undergone at least partial cure. This experiment suggests that photolysis products are likely to be present at relatively low concentration in the printed surface, particularly if printed under sub optimum press operating conditions. They are likely to be identifiable by GC-MS but there are numerous possible photolysis products to monitor for.

4.2 Development of analytical methods for polymeric photoinitiators and synergists

An HPLC gradient elution program was developed to enable separation and detection by mass spectroscopy of a wide range of ink/varnish compounds (see Appendix 10 page 321 for equipment and operating conditions). The compounds in the Pira inventory of ink components were dissolved in acetonitrile (250 ppm) and injected for analysis by HPLC with UV and MS detection. Figures 4-1 and 4-2 show the chromatograms and spectra obtained for Omnipol BP. This is an example of a “polymeric” photoinitiator. The term “polymeric” is somewhat of a misnomer with the molecule actually being a benzophenone terminated polyether. These are all type II initiators and do not cleave to photolysis products upon exposure to UV light. The chromatograms in figures 4-1 and 4-2 below illustrate the problem of a multitude of peaks, some detectable by UV others by mass spectroscopy. The relative size of the

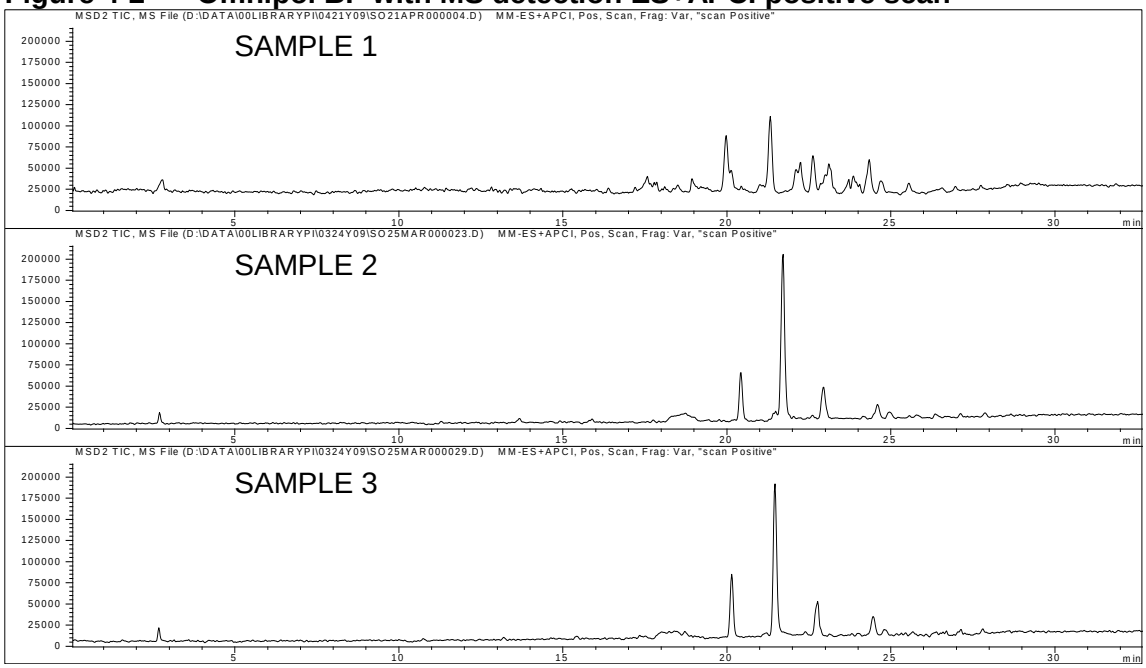
peaks may vary between batches, introducing an error in the measurement of the compound.

Figure 4-1 Omnipol BP UV detection at 270 nm



These show consistent chromatograms with a small variation in relative peak ratios.

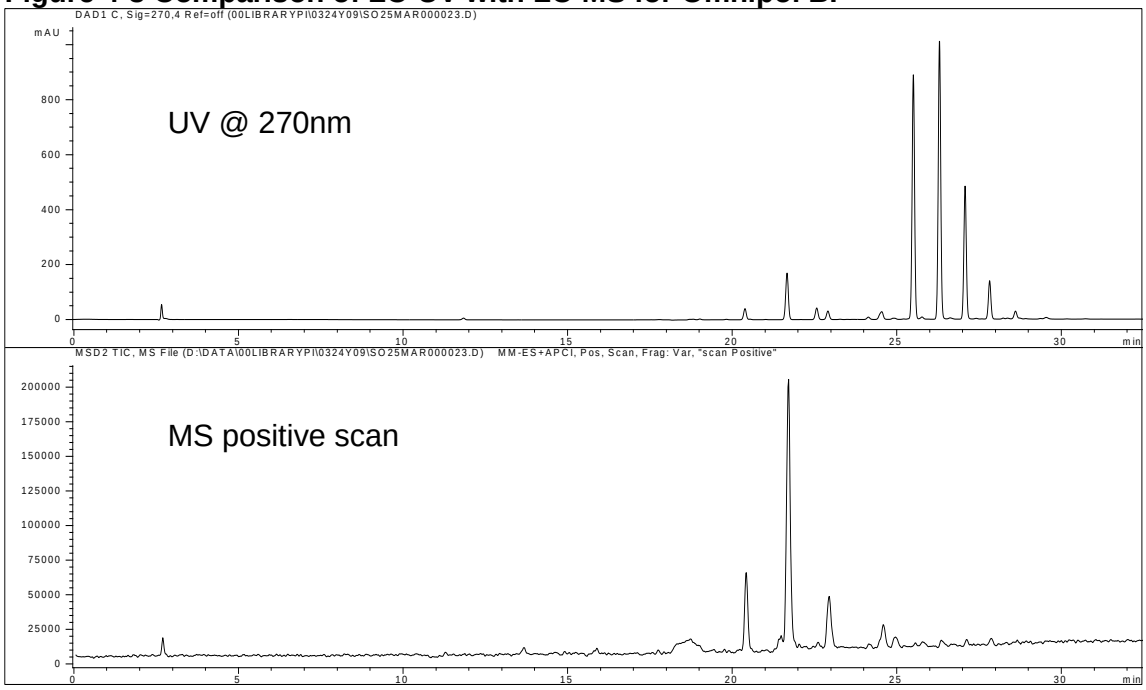
Figure 4-2 Omnipol BP with MS detection ES+APCI positive scan



Samples 2 and 3 showed a similar response. The mass spectrometer was easily affected by operating conditions such as contamination on the

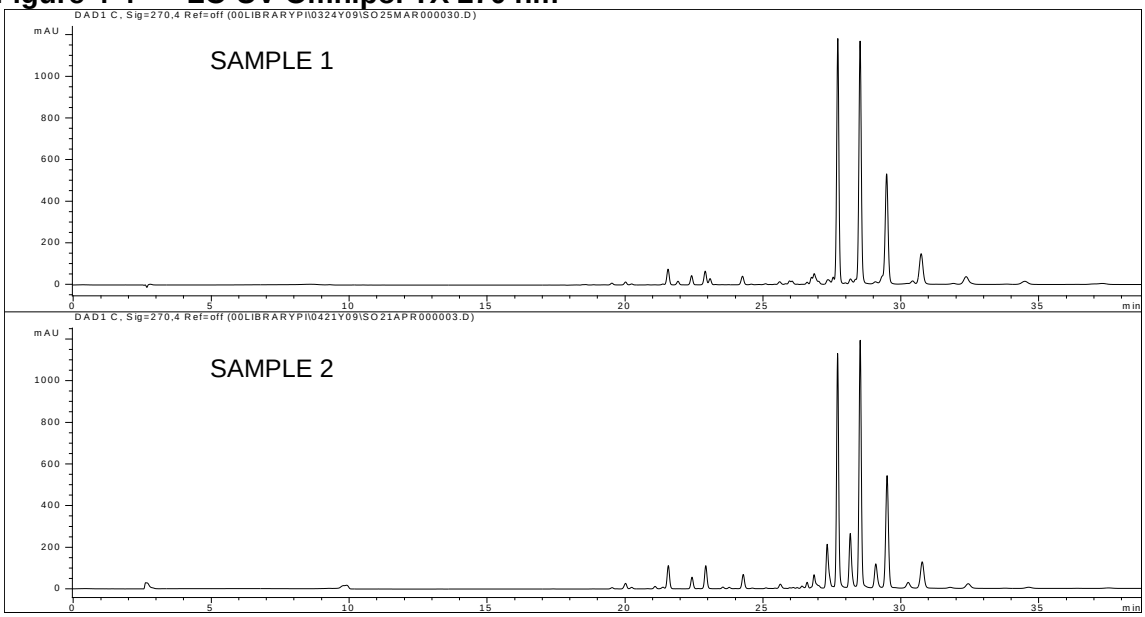
ionisation chamber from mobile phase residues. This might be the reason for the reduced sensitivity apparent in sample 1 in Figure 4-2. Figure 4-3 below shows a comparison of the LC-UV chromatogram with the LC-MS chromatogram. This shows that the mass spectrometer responds to what are in fact relatively minor components of the Omnipol BP (assuming the UV response to be equal for all components).

Figure 4-3 Comparison of LC-UV with LC-MS for Omnipol BP



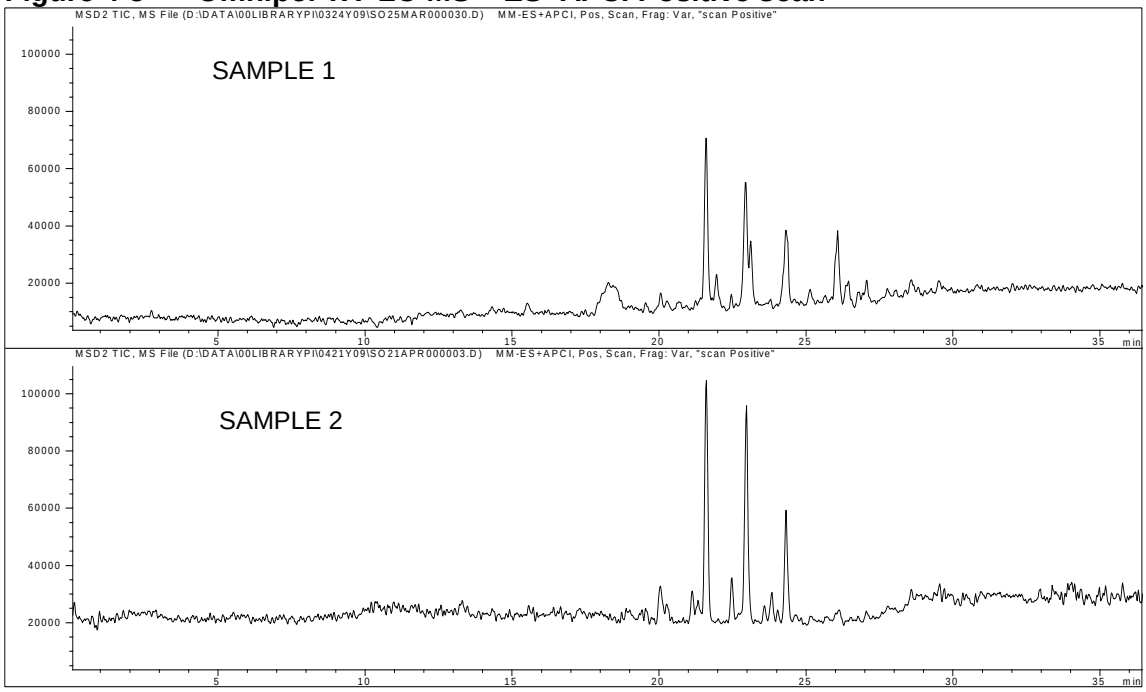
A further example, Omnipol TX was investigated in the same manner. Figure 4-4 shows the LC-UV chromatograms of two batches.

Figure 4-4 LC-UV Omnipol TX 270 nm



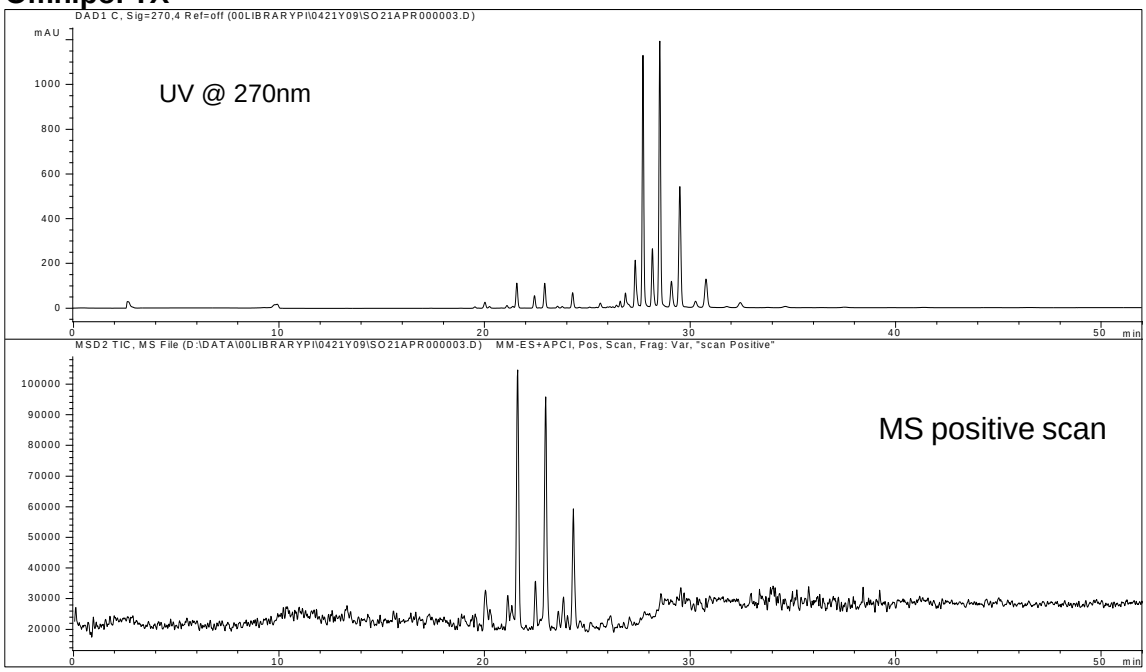
Differences between batches in the LC-UV chromatogram are relatively minor. There was a greater variation in the LC-MS traces in Figure 4-5 below.

Figure 4-5 Omnipol TX LC-MS ES+APCI Positive scan



The chromatograms may also be compared with the chromatogram shown in Figure 5-2 on page 50 which was obtained using the mass sensitive CAD detector (different retention times are due to different operating conditions). This showed a similar response to the LC-UV.

Figure 4-6 Comparison of LC-UV and LC-MS chromatograms for Omnipol TX



These show again that the mass spectrometer was not responsive to the major components of Omnipol TX.

This leads to the conclusion that the error in measurement of the polymeric photoinitiators such as Omnipol BP and Omnipol TX is likely to be larger by LC-MS than LC-UV if a different batch to that used to print the packaging sample is used for calibration This is because the mass spectrometer responds to a relatively minor component of the mixture of oligomers.

The complexity of ink formulations and the absence of spectra in commercial libraries, or the poor amenability of an ink component to GC-MS or LC-MS, means that it is difficult to identify all the components used in an ink by solvent extraction and analysis of the print surface. There may be photolysis products which may provide a clue to the presence of a photoinitiator which is not itself detectable, for example due to lack of volatility for GC-MS. However, the presence of these photolysis products may be difficult to interpret manually because:

i) A commercial photoinitiator product may be a blend of several photoinitiators and therefore, the chromatogram of the starting photoinitiators may contain numerous peaks, each having a mass spectrum.

ii) Each photolysis product arising from a starting photoinitator may give rise to multiple peaks in the UV exposed photoinitiator chromatogram, each one of which will have its own mass spectrum.

iii) A photolysis product on its own may not be characteristic of a starting photoinitiator.

It was recognized that if the collected data (mass spectra, retention times and relative proportions of components in blends) were collected in a database this would allow a more rapid computer search to be made. A computer program has been written and the mass spectra loaded as a database. This work was not funded by this project and the software development was not complete by the time of this reports publication. The analytical methods and computer database are intended to be used in the following manner. The printed side of the packaging is extracted with chloroform and the extract injected for GC-MS and HPLC-MS analysis. The chemicals present in the extract are identified as far as is possible using existing proprietary instrument software and mass spectral libraries. It is probable at this stage that many type I photoinitaors and polymeric photoinitiators will not be identifiable as

their mass spectra will not be on commercial spectral libraries. The mass spectra of compounds that cannot be identified (probable photolysis products of the photoinitiator) is printed off. The relative abundances to the base ion are calculated using a ruler and the relative abundances entered into the project database program. The program will compare the relative abundances with the reference spectra obtained from UV exposed photoinitiators. It will output the best match to the starting photoinitiator, the names and CAS numbers of the photolysis products (if known) and the mass spectra of these to allow a check on the degree of fit. If the names of the photoinitators used in the ink are known, then if these are entered into the program, the program will output the mass spectra and names and CAS numbers (if known) of all the photolysis products that have to be monitored in the set off measurement. It may then not be necessary to carry out a prior extraction of the packaging. The database could in principle be applicable to other ink components which are not photoinitiators, for example plasticizers and monomers.

4.3 Development of analytical methods for acrylates

It was envisaged in the project plan that an analytical procedure would be developed for the quantification of acrylate monomers based upon the chemical derivatisation of the acrylates to the corresponding methyl ester followed by quantification and comparison against the SML of 6 mg/kg for the acrylates listed in Regulation EC No 10/2011. This approach was described by Ruter M et al “UV-printing inks in food contact materials – migration and set off problems” RadTech 2005, [Ref 10]. However, the difficulty with this approach is the fact that this limit is not applicable to the individual acrylates found to be in common usage in inks and varnishes. It was agreed with the FSA not to investigate this approach. It was found that the individual acrylates could be quantified by HPLC or GCFID-GC-MS. HPLC and GC methods were developed to allow this.

Analytical recovery data were obtained for the acrylates tabulated below to demonstrate adequate method recovery. The acrylates were added to absolute ethanol with and without the presence of monomethyl ether hydroquinone (MMEQ) as a stabilizer. The use of MMEQ was investigated as it was learnt from consultation with industry that some acrylates polymerise during analysis without the addition of the inhibitor. The ethanol mixtures were concentrated on a rotary evaporator to 25 ml and the extracts then injected for HPLC analysis. Simultaneous UV and CAD detection was used and the results compared.

Table 4-2 Analytical recovery of acrylates using the analytical procedure

Acrylate	*UV detection*		*CAD detection*	
	With MMEQ	**Without MMEQ**	**With MMEQ**	**Without MMEQ**
	%	**%**	**%**	**%**
EO-TMPTA replicate 1	94	95	102	100
EO-TMPTA replicate 2	96	97	101	101
GPTA replicate 1	104	85	101	101
GPTA replicate 2	96	100	100	102
EA replicate 1	100	100	113	116
EA replicate 2	101	100	115	117
TMPTA replicate 1	103	101	98	101
TMPTA replicate 2	102	103	98	100
TPGDA replicate 1	102	100	98	98
TPGDA replicate 2	105	102	100	99

The addition of MMEQ appears from the data obtained to be unnecessary for these particular acrylates. The inclusion of MMEQ had no detrimental affect. However, when set off was measured for a range of printed test films in chapter 6 without the addition of MMEQ, GPTA recovery (tabulated in Table A5-3 of Appendix 5 page 136) was found to be less than 1% in iso-octane, 95% ethanol and dioxane after 5 hours at 60 °C. This suggests that the acrylates are not stable under the set off test extraction conditions of 5 hours at 60 °C.

4.4 Summary

An inventory of photoinitiators and synergists used in food packaging inks and varnishes has been compiled. GC-MS and LC-UV and LC-MS methods have been established for these compounds (See Appendix 4 and Appendix 10). Some of the photoinitiators were not amenable to GC-MS even with on column injection. UV and mass spectra have been collected for these compounds. LC-MS chromatograms were obtainable for most ink components in the positive APCI mode. The chromatograms of polymeric photoinitiators were in some cases quite different to the LC-UV chromatograms obtained from the same ink component, with the mass spectrometer responding only to minor components. Variations in molecular weight distributions between batches of polymeric photoinitiars judged by LC-UV appeared relatively small.

Variations in LC-MS response between batches of polymeric photoinitiators would be expected to be significant due primarily to the response depending upon minor components. Significant calibration error is expected if the

calibration of the LC-MS is made using a polymeric photoinitiators to that used in the test film. This error could be reduced by creating a calibration standard from a number of batches of each polymeric ink component by combining equal quantities from as many batches of the components as are available This would allow for an average calibration response to be obtained which has a greater chance of lying near to the response obtained from the ink from a packaging sample. In many cases it may not be possible to identify all the photoinitiators used in the ink by solvent extraction and analysis of the print surface without prior knowledge of the ink formulation. This is particularly so if polymeric photoinitiators have been used.

Acrylate monomers were found to be quantifiable in the set off extraction solvents by HPLC with UV or CAD detection.

Table 4-3 below tabulates some of the ink components in the inventory of reference chemicals with information useful for analytical purposes (where it could be established) such as their trade name, CAS number, molecular weight and page references to chromatograms and spectra provided in Appendix 8 and Appendix 9. The chromatograms were obtained using more than one GC method (See Appendix 10 pages 321 and 322), so retention times may not be comparable for different compounds.

Table 4-3 Ink components trade names, CAS numbers and molecular weight information

Chemical name	Trade name	CAS number	Molecular weight	Chromatogram and spectra page ref
4-methyl benzophenone	4MBP	0000134-84-9	196.2	
2-Hydroxy-2-methyl-1-phenyl-1-propanone	0007473-98-5	0007473-98-5	164	166, 304-305
Diphenyl (2,4,6-trimethylbenzoyl)-phosphine oxide	Lucirin TPO	0075980-60-8	348.4	169,180,181, 301-303
Di-(Trimethylolpropane) tetraacrylate	di-TMPTA	0094108-97-1	466.5	
unknown	Ebercryl 3420	unknown	unknown	
Polymeric benzophenone derivative	Ebercryl P39	unknown	700	158,251-253
Ethyl-4-dimethylaminobenzoate	EDB, Genocure EPD, Speedcure EDB	0010287-53-3	193	
Ethoxylated 1,6-hexane diol diacrylate	EO-HDDA	0006606-59-3	314	
Ethoxylated trimethylol propane triacrylate	EO-TMPTA	0028961-43-5	unknown	
Mixture of less 3-(4-(2-Hydroxy-2-methylpropionyl)phenyl)-1,1,3-trimethylindan-6-yl 2-hydroxyprop-2yl ketone and 3-(4-(2-Hydroxy-2-methylpropionyl)phenyl)-1,1,3-trimethylindan-5-yl 2-hydroxyprop-2-yl ketone	Esacure 1	0164578-07-8	unknown	161,162,238-240
2-Ethylhexyl 4-(dimethylamino)benzoate	Escalol 507, Quantacure EHA, Speedcure EDB	0021245-02-3	277	148,224,225

4-phenylbenzophenone	Genocure PBZ; Speedcure PBZ	0002128-93-0	258	226,227
Polymeric amino benzoate	Genopol AB1	unknown	860	258-260
Polymeric version of benzophenone	Genopol BP1	unknown	960	232-234
Glyceryl propoxylated triacrylate	GPTA	0052408-84-1	428.5	
1,6-hexane diol diacrylate	HDDA; Sartomer SR238B	0013048-33-4	226	
2-Hydroxy-1-(4-(4-(2-hydroxy-2-methylpropionyl)benzyl)phenyl-2-methyl-2-propanone	Irgacure 127	0474510-57-1	340.4	151,172,173, 289-291
1-hydroxy-cyclohexyl phenyl ketone	CAS 0000947-19-3	0000947-19-3	204.3	201,202
not disclosed but a Phosphine oxide	Blend CAS 84434-11-7 & 162881-26-7	unknown	unknown	150,168 298-300
2-Benzyl-2-(dimethylamino)-1-[4- (4-morpholinyl) phenyl]-1-butanone Alternative name : 2-Benzyl-2-(dimethylamino)-4'-morpholinobutyrophenone	Irgacure 369	0119313-12-1	366.5	186-191, 281-283, 315-317 222,223
2-Dimethylamino-2-(4-methyl-benzyl)-1-(4-morpholin--4-yl-phenyl)-butan-1-one	Irgacure 379	0119344-86-4	380.5	284-286
Mixture 50%/50% of 1-hydroxy-cyclohexyl phenyl ketone with benzophenone	CAS 0000947-19-3 & benzophenone	CAS 0000947-19-3 & benzophenone	Not applicable	154,155
2,2-Dimethoxy-2-phenylacetophenone	CAS 0024650-42-8	0024650-42-8	256.3	306,307
mixture of oxy-phenyl-acetic acid 2-[2 oxo-2-phenyl-acetoxy-ethoxy]-ethyl ester CAS 442536-99-4 and Oxy-phenylacetic acid 2-[2-oxo-2-phenyl-acetoxy-ethoxy]-ethyl CAS 21150-16-6	Blend CAS 0211510-16-6 & 0442536-99-4	unknown	unknown	170,171 295-297
Bis(2,4,6-trimethylbenzoyl)-phenylphosphineoxide	Irgacure 819	0162881-26-7	418.5	174-179 311-314 220-221
Phosphine oxide, phenyl bis(2,4,6-trimethyl benzoyl) 45% dispersion in water	Irgacure 819 DW	unknown	unknown	193-195 308-310
2-Methyl-1[4-(methylthio)phenyl]-2-morpholinopropan-1-one	CAS 0071868-10-5	0071868-10-5	279.4	164,199,200
Alpha, alpha-dimethoxy-alpha-phenylacetophenone	*PIRA CODE 659	unknown	unknown	
9H-Thioxanthen-9-one,2-(1-methylethyl)-	ITX, Quantacure ITX	0075081-21-9	254.35	
2,4,6-Trimethylbenzoylphenylphosphine acid ethylester	Lucirin TPO L (liquid)	0084434-11-7	316	
2,4,6-Trimethylbenzoyldiphenylphosphine oxide	Lucirin TPO; Esacure TPO Speedcure TPO	0075980-60-8	348.3	216,217
Methyl-2-benzoylbenzoate	MBB/OMB	0000606-28-0	240.3	147
Acrylate amine	unknown	unknown	unknown	206,207
Amine synergist	unknown	unknown	unknown	157, 208-210
Epoxy acrylate monomer	unknown	unknown	unknown	203 - 205
unknown	Omnipol 52	unknown	unknown	
unknown	Omnipol 910	unknown	1039	
Poly (oxy-1,2-ethanediyl),alpha-(4-(dimethylamino) benzoyl)-omega-((4-(dimethylamono)benzoyl)oxy)-(9cl)	Omnipol ASA	0071512-90-8	510	241-243
Di-ester of carboxymethoxy benzophenone and polytetramethyleneglycol 250	Omnipol BP	0515136-48-8	730	196-198, 235,237, 292-294,
unknown	Genopol BP1			232-234
Unknown	Omnipol BP	unknown	unknown	196-198

Poly(oxy-1,4-butanediyl), .alpha.-[2-[(9-oxo-9H-thioxanthenyl)oxy]acetyl]-.omega.-[[2-[(9-oxo-9H-thioxanthenyl)oxy]acetyl]oxy]-	Omnipol TX	813452-37-8	unknown	269-271
Benzophenone	Omnirad BP	0000119-61-9	182.2	
Multifunctional acrylate	Omnirad CI 250		unknown	228-231
Octyl dimethyl para amino benzoic acid	OPABA	0058817-05-3	277.4	
Pentaerythritol tri-,tetracrylate	PETA	0004986-89-4	352.34	
unknown	Poly Q 9368	unknown	unknown	244-246
Propoxylated neopentyl glycol diacrylate	PO-NPGDA	0084170-74-1	unknown	
4-(4-Methylphenylthio)benzophenone	Quantacure BMS; Speedcure BMS	0083846-85-9	304.4	149
Quantacure EPD	Quantacure EPD	unknown	unknown	
Octyl/decyl acrylate	Sartomer SR484	unknown	unknown	
Mixture polymeric benzophenone	Speedcure 7005	1003567-82-5 1003557-16-1	c.1216	264-266
unknown	Speedcure 7010	1003567-83-6	c.1839	
A mixture of: 1,3-di({a\-4-(dimethylamino)benzoylpoly[oxy(1-methylethylene)]}oxy)-2,2-bis ({£\-4-(dimethylamino)-benzoylpoly[oxy(1-methylethylene)]}oxymethyl) propane and{ƒÑ-4-(dimethylamino)benzoylpoly(oxyethylene)-poly[oxy(1-methylethylene)]-poly(oxyethylene)} 4-dimethyl-amino)benzoate	Speedcure 7040	1003567-84-7 1003557-17-2	c.1039	267-268
1-Chloro-4-propoxy-9H-thioxanthen-9-one	Speedcure CPTX	0142770-42-1	272	160,214,215
Trimethylol propane triacrylate	TMPTA; Omnimer EM231	0015625-89-5	296.3	
di ester of carboxymethoxy benzophenone and polyethylene glycol 200	Unknown	0011536-49-9	unknown	
2-Hydroxy-4-(2-hydroxyethoxy)-2-methylpropiophenone	Irgacure 2959 Darocur 2959	0106797-53-9	224.25	152,159,163, 165, 167,218, 219,287,288, 318,319
Butanediol monoacrylate	Unknown	0001070-70-8		
4,4-Bis[2-(1-propenyl)phenoxy]benzophenone,	Unknown	0109423-33-8	446.54	153, 211-213
unknown	* Pira code 659	unknown	unknown	156
Difunctional alpha hydroxyl ketone	Esacure 1	unknown	408	161,162
2-hydroxy-2-methyl-1-phenyl propanone	Irgacure 1173	7473-98-5	164.2	166
1-propanone, 1-[4-[(4-benzoylphenyl)thio] phenyl]-2-methyl-2-[(4-methylphenyl)sulphonyl]	Esacure 1001M	272460-97-6	510	247-250
Polymeric thioxanthone derivative	Genopol TX	unknown	820	254-257
Mixture of oligo [2-hydroxy-2-methyl-1-[4-(1-methylvinyl) phenyl] propanone] (75%) and tripropyleneglycoldiacrylate, (25 %)	Esacure KIP 75LT	unknown	unknown	261-263
unknown	Omnipol AB1	unknown	unknown	272,273
Polyethylene glycol(200)di(4-pacetlyphenyl piperazine) propionate	Omnipol S2	unknown	1039	274-276
Diester of carboxymethyl thioxanthone and polytetramethylene glycol 250	Omnipol TX1	unknown	790	277-280

* Trade name was not disclosed and no alternative CAS number available
Where a CAS number is used in place of a trade name, permission was not granted to use the trade name.

Chapter 5

5.0 Development of rapid set off tests

A rapid test procedure would be useful for quality control laboratories and enforcement laboratories. Test procedures that do not require expensive sophisticated equipment would be of use in laboratories where this equipment is not available. Two low cost detectors expected to have a similar response to a wide range of compounds were investigated. The applicability of these is summarised in Appendix 4 page 128. In addition a direct food contact surface measurement technique, not requiring sample preparation and solvent extraction was also considered.

5.1 Evaluation of a charged aerosol detector (CAD) as a universal detector

This detector was evaluated for two reasons. A detector with a universal response would overcome the problem of calibration arising for polymeric photoinitiators and pre-polymers where a series of peaks on the chromatogram appear because of the compounds having a molecular weight distribution. These peaks can vary in relative proportions between batches, introducing an unknown degree of error in the measurement of set off. Such a detector would also provide a low cost test suitable for quality control laboratories where sophisticated and expensive GC-MS and LC-MS may not be available and where a single compound can be used as calibrant for a wide range of test compounds.

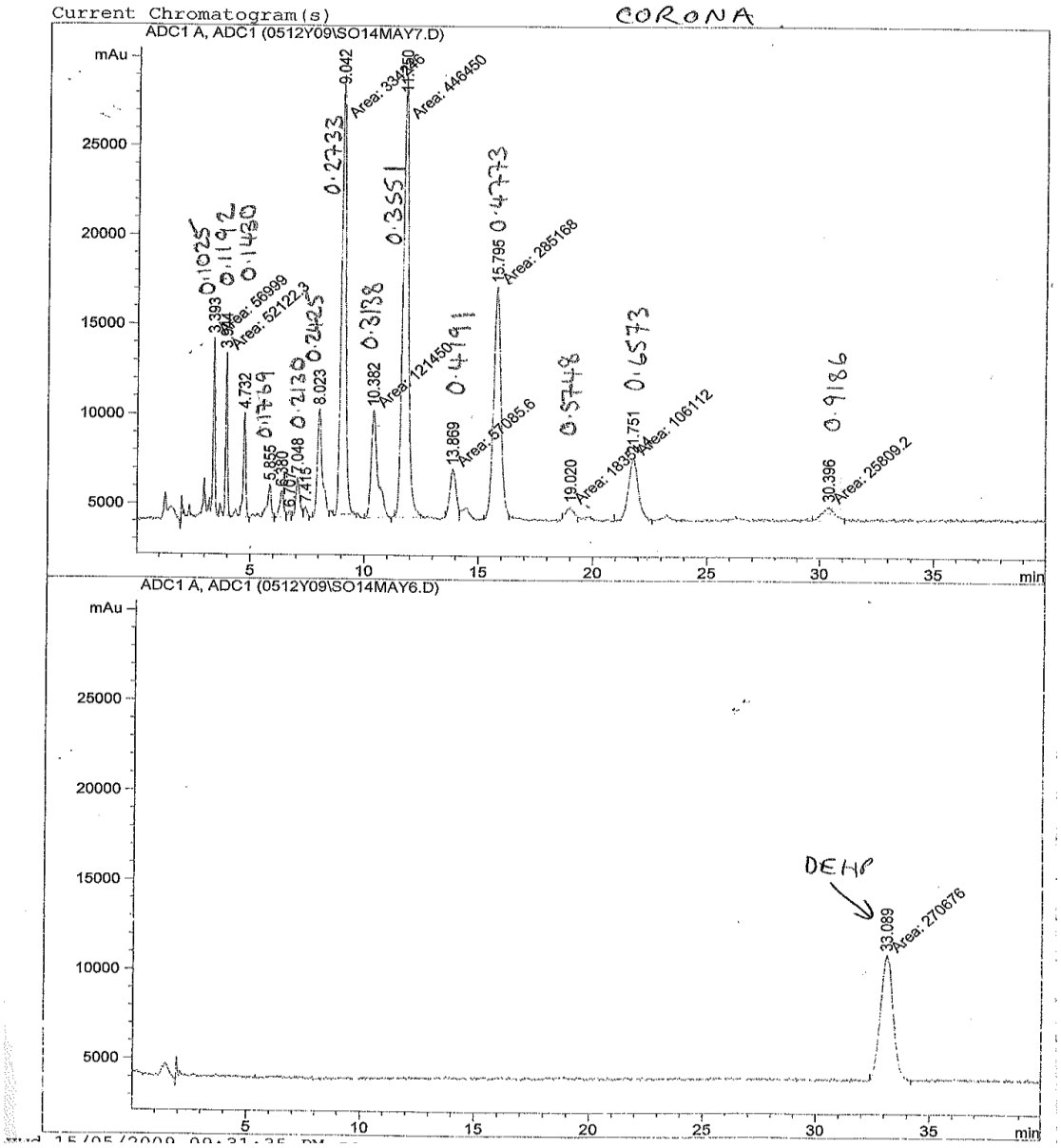
The ESA Biosciences Corona CAD detector is designed for use with HPLC. The eluent from the analytical column enters the detector where the mobile phase is evaporated off under a stream of nitrogen and the electrical current from the charged particles measured. The response of the detector is claimed to be approximately proportional to the square root of analyte mass. The theoretical basis of the detector is explained in the manufacturers manual [Ref 11], ESA Biosciences and by Dixon R “Development and testing of a detection method for liquid chromatography based on aerosol charging”, 2002, [Ref 12].

A comparison of the detector was made with UV and MS detection by preparing a set of solutions of polymeric photoinitiators and acrylates and injecting these for analysis by HPLC using two chromatographs. One of these was fitted with a mass selective detector and diode array detector in series and the other chromatograph was fitted with the Charged Aerosol Detector (CAD). The same stationary phase and column dimensions were used on

both instruments. The operating parameters of the equipment are given in Appendix 10 page 322.

Figure 5-1 below show example chromatograms obtained from the HPLC UV instrument and CAD respectively for Omnipol TX polymeric photonitiator. The relative retention times of the peaks to DEHP are indicated on the chromatograms since the use of different instruments means that retention times will differ.

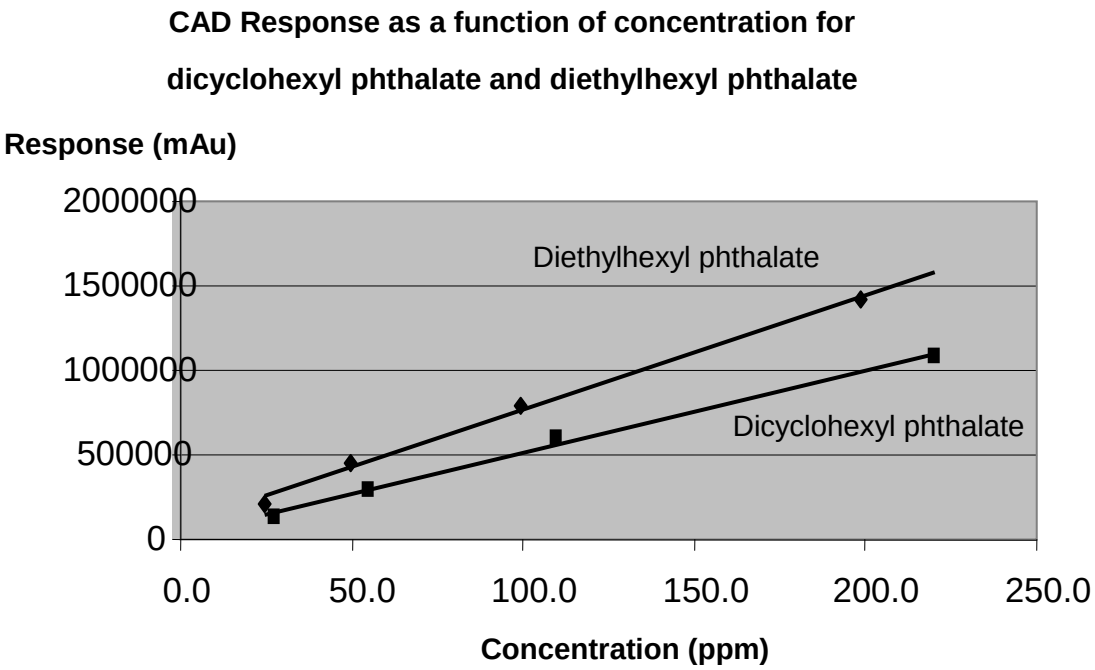
Figure 5-1 LC-CAD Chromatograms for Omnipol TX



Similar chromatograms were obtained by UV and CAD both in terms of relative retention time and relative response of the individual components in the polymeric photoinitiator. The CAD was found to have sufficient sensitivity

to be suitable for use in the likely concentration range required for migration testing.

The response of the CAD to a number of compounds was compared. Solutions of DEHP and dicyclohexylphthalate (DCHP) were prepared in the range 25 to 200 ppm and injected for analysis by HPLC with CAD. Graphs were plotted in Excel, with concentration against response as shown below.



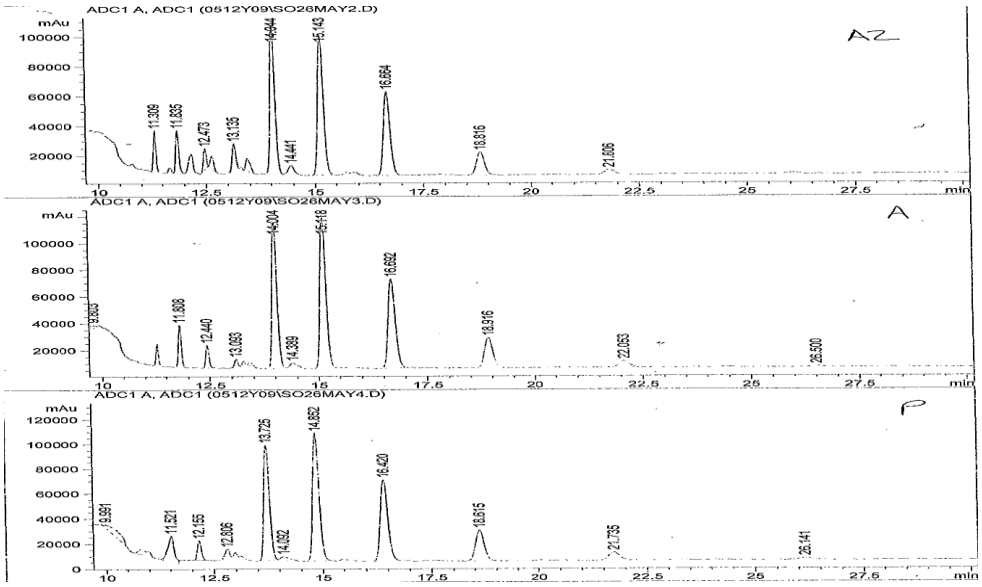
The graphs were found to curve towards the X axis, (although the regression lines of best fit appear linear in the above graph). More significantly, the gradients were different to each other so that the lines diverged with increasing analyte concentration. Table 5-1 below shows the error in the concentration of a solution of each polymeric photonitiator when the CAD was calibrated with DCHP or DEHP.

Table 5-1 Comparison of error obtained using different calibrants

	DCHP calibration %	*DEHP Calibration %*
Omnipol TX	+ 78	+ 24
Omnipol BP	+ 34	- 7

Three batches of Omnipol BP polymeric photoinitiator were compared using the CAD. The chromatograms obtained are shown in Figure 5-2 below which shows the batches to be very similar in composition to each other.

Figure 5-2 LC-CAD chromatograms of three different batches of Omnipol BP



The following concentration errors were obtained when concentrations of the Omnipol BP batches were calculated from a single calibration injection of a 99 ppm solution of DEHP.

Table 5-2 between batch comparison of Omnipol BP calibration with DEHP

Batch code	*Weighed out concentration ppm*	*Measured concentration ppm*	*Error %*
az	500	450	- 10
a	564	470	- 17
p	568	488	- 14

The data shows that a similar response was obtained from the three different batches of Omnipol BP and that this response was somewhat lower than DEHP resulting in an under estimate of Omnipol BP.

A series of acrylate solutions were prepared and injected for analysis by HPLC with CAD detection. The responses obtained are tabulated below in

Table 5-3. The values were obtained by dividing total peak area by concentration in ppm.

Table 5-3 Responses obtained for a range of acrylates by CAD

Acrylate	*Concentration µg/ml (ppm)*	*Response*
Craynor CN3715	1014	2113
Epoxy acrylate	930	8603
Acrylated amine	1024	3261
3-dimethylaminopropyl acrylate	1124	25
Tri(propyleneglycol)diacrylate	1136	1270
Trimethylolpropane triacrylate	1292	3241
Trimethylolpropane ethoxylate (7/3 EO/OH) triacrylate	1280	6070
Glycerol propoxylate (1PO/OH)triacrylate	1184	7248
Hexamethylene diacrylate	964	639
Soybean oil epoxidised acrylate	1112	165
Phosphoric acid 2-hydroxyethyl methylacrylate ester	920	7015

The data shows that a wide range in response of the CAD is obtained for different acrylates. This shows that the CAD does not produce a similar response for acrylates. A range in response was also observed for polymeric photoinitiators as shown in Table 5-4 below.

Table 5-4 Responses obtained for polymeric photoinitiators by LC-CAD

Photoinitiator	*Concentration*	*Response factor*	*Increase in response relative to Esacure 1001 M*
	ppm	*area/ppm*	*%*
Esascure 1	968	4627	17
Omnipol ASA	940	5436	37
Omnipol TX	984	7943	101
Esacure 1001 M	932	3959	-
Genopol TX1	1124	5855	48
Esacure KIP 75 LT	936	5894	49
Florstab UV2	1200	4294	8

Table 5-4 shows the response can vary by a factor of up to 2.

Table 5-5 Comparison of UV detection with CAD detection for acrylates

Acrylate	*Concentration ppm*	*UV response*	*CAD response*
Trimethylolpropane ethoxylate (7/3 EO/OH) triacrylate	143	61	2558030
Glycerol propoxylate 1PO/OH)triacrylate	149	353	3270750
Epoxy acrylate	73	240	3583230
Trimethylolpropane triacrylate	137	1456	1966820
Tri(propyleneglycol)diacrylate	139	147	878029

The CAD detector appears from the tabulated integrated peak areas (Table 5-5) to be more sensitive compared to the UV. The CAD chromatogram has a greater base line noise so that signal to noise ratio would have an adverse effect on actual limits of detection. It was found that when the calibration graphs for the CAD were not forced through zero, the correlation coefficients of the graphs improved as a curvature was apparent when the regression line was forced through zero. Correlation coefficients were then in all but one case better than 0.996. The monomeric photoinitators, Irgacure 369 and Irgacure 819, were selected which gave a single peak in the chromatogram. These were injected for analysis on two different days to observe whether a consistent response was obtained between days.

Table 5-6 Response factors for Irgacure 369 and Irgacure 819 obtained on different days with CAD detection

Time hours	*Irgacure 369 concentration ppm*	*Irgacure369 Response factor area/ppm*	*Irgacure 819 concentration ppm*	*Irgacure 819 Response factor area/ppm*	*Total peak area Response factor area/ppm*
0	972	726	1008	738	765
24	972	608	1008	723	768

The CAD response was consistent between the two test days. The solutions were observed to have decomposed after 24 hours, decomposition of Irgacure 369 was apparent with a reduction in the response factor whilst the total peak area response (summed peak areas of all peaks in the chromatogram divided by the total starting concentration) remained constant. This might allow the convenient estimation of set off for an ink component as the sum of the ink component and its decomposition products without the need to calibrate for all the decomposition products separately.

The CAD detector was found not to give a similar response for different ink components. The use of a single calibration solution suitable for a range of ink

components was therefore not possible. The CAD detector was a useful and reliable detector when calibrated with the individual ink component.

5.2 Evaluation of gas chromatography flame ionisation detection (GC-FID)

There are two reasons for considering this technique. Firstly GC-MS may not be available in some laboratories. Secondly, the ink components to be measured may not be available as calibration standards or the ink components may not be identifiable. Use can then be made of the approximately similar response of the FID for a wide range of compounds. If both GC-MS and GC-FID are available then the following approach may be considered. The print side is extracted with an aggressive solvent such as chloroform. The extract is injected for GC-MS and the spectra of peaks examined to identify potential set off components of interest. These are likely to be peaks with spectra which if not available on a reference library are obviously aromatic in character, so that they stand out from non photoinitiator related compounds. If GC-MS-FID is not available on one instrument, some suppliers software provide the capability of retention time locking which enables a carrier gas pressure to be calculated by the instrument such that peaks on both instruments elute at the same retention time. If such software is not available then a carrier gas pressure could be calculated from a calibration graph of pressure against retention time, plotted from five injections of the same solution of a marker compound obtained with known carrier gas pressures. The gas chromatographs would need to have electronic pressure control to two decimal places and be operated with a constant carrier gas pressure (Instrument suppliers provide an alternative option where the instrument calculates a carrier gas pressure to maintain a constant flow rate through the column as the oven temperature is raised, this will prevent retention time locking being achieved). Recalibration of the carrier gas pressure would be necessary if any changes to the instrument configuration were made, such as changes to the length of the capillary column. A single set off calibration graph could then be used to read off the mass of all the ink components detected on the food contact surface by solvent extraction, followed by GC-FID, integrating the peak areas of only those peaks on the GC-FID chromatogram found to be of interest on the GC-MS chromatogram. A marker internal standard could be added at 10 ppb equivalent and any peaks at or below this height ignored.

The repeat film 3 was selected for demonstration of this approach. Table 5-7 below shows the set off measured on a printed test films exhibiting significant set off (repeat Film 3 see bottom paragraph page 60) by extraction into dioxane for 5 hours at 60 °C and measured by GC-MS and GC-FID. The same solutions were injected for GC-MS and GC-FID analysis.

Table 5-7 Set off by GC-MS and GC-FID repeat Film 3

GC detector	***EDB***	***Benzophenone***	***CAS 0071868-10-5***	**4-phenyl benzophenone**
	ug/dm^2	ug/dm^2	ug/dm^2	ug/dm^2
GC-MS	62	280	24	110
GC-MS	63	250	24	110
GC-MS	67	260	27	130
GC-MS	67	250	26	110
Mean	**65**	**260**	**25**	**120**
RSD %	4	5	6	9
GC-FID	59	350	25	110
GC-FID	59	350	25	120
GC-FID	59	350	27	120
GC-FID	58	340	26	120
Mean	**59**	**350**	**26**	**120**
RSD %	**< 1**	**1**	**4**	**4**

Both GC-MS and GC-FID instruments were calibrated with the ink component being measured, so similar results are to be expected from both instruments. The difference between GC-MS and GC-FID results given in table 5-7 for benzophenone suggests an interference with the measurement of benzophenone by GC-FID. The non selective nature of the detector means that there is a risk of an over estimate of set off if an interfering additional chemical compound is present that is not resolved from the ink component of interest. The GC-FID results were re-calculated by calibrating in turn with each of the other ink components. This generated the data in Table 5-8 below. The numbers in brackets show the comparison to the true result obtained by GC-FID calibrating using the actual ink component being measured, expressed as a percentage.

Table 5-8 Errors in set off values obtained using different calibrants

Measured ink component	**Calculated using the calibrants below**			
	EDB	Benzophenone	CAS 0071868-10-5	4-phenyl benzophenone
EDB	59 (100)	42 (72)	79 (140)	44 (75)
Benzophenone	490 (140)	340 (100)	640 (190)	360 (110)
CAS 0071868-10-5	18 (70)	13 (52)	26 (100)	14 (53)
4-phenyl benzophenone	160 (140)	110 (95)	210 (180)	115 (100)

Significant error was observed when structurally different compounds were used for calibration. CAS 0071868-10-5 is probably a worst case choice as this contains both a nitrogen and a sulphur atom which are not present in benzophenone or 4-phenyl benzophenone. The presence of sulphur, oxygen and nitrogen lowers the FID response. This results in an over estimate when used to calibrate for compounds where there are less of these atoms present. When more structurally similar compounds are used for calibration, a reasonable agreement with the true result was obtained. For example, when

benzophenone was measured calibrating using 4-phenyl benzophenone the result was 110% of the true result. When 4-phenyl benzophenone was measured using benzophenone as the calibrant, the result was 95 % of the true result. This is also supported by similar results obtained for EDB and CAS 0071868-10-5 calibrated using benzophenone or 4-phenyl benzophenone.

Recovery data was obtained using the analytical method in Appendix 4 page 134 for acrylates as well as other ink components; these are appended in Appendix 5 pages 135-137.

5.3 Direct analysis in real time (DART) Measurements

Direct Analysis in Real Time (DART) is a mass spectroscopy technique whereby the surface of a material can be subjected to mass spectroscopy analysis in a sample chamber without prior sample preparation such as solvent extraction. Samples of test film Ink series X and Ink series Z described on page 67 together with calibration samples of the photoinitiators used were sent to the Center for Food Safety and Applied Nutrition, Food and Drug Administration in the USA for evaluation using this technique. A summary report is included in Appendix 7 page 141 of this report. The report shows that some but not all photoinitators were detectable on the food contact surface.

5.4 Summary

GC-FID was found to give significant error when calibrated using structurally different ink components. Similar response cannot be assumed for all ink components and set off may be over estimated as a result of the presence of interferences. If GC-MS is available, GC-FID is only likely to be of use if the ink component is not available for calibration but a structurally similar one is.

The CAD was found not to provide a similar response for all chemical compounds. It is however, a useful alternative detector when calibrated with the ink component of interest. Indeed, some polymeric photoinitiators such as KIP 150 do not respond to LC-MS, are not amenable to GC and have little absorbance in the UV, making the CAD a useful alternative detector. The CAD appears to be more sensitive than UV detection for compounds which do not absorb significantly in the UV. The CAD can be used as an alternative detector for HPLC and this may be an advantage for acrylates that do not absorb strongly in the UV. Calibration is required for each analyte using individual calibration samples of the analyte. Calibration graphs are best plotted as the square root of concentration or mass and not forced through zero, (the response for blank extraction solvent should be plotted and not assumed to be zero).

Chapter 6

6.0 Measurement of set off from test films

The purpose of obtaining set off measurements from these test films was to assess the performance of the procedures described in chapters 3, 4 and 5. The inks used were specially formulated for the purposes of the project to give a range of set off values.

6.1 Production of printed packaging reels of test samples

A set of test samples were printed by a project participant. The substrate chosen was a paper laminated to aluminium foil with a polyethylene food contact surface. The choice of inks was carefully arranged to cover the range of ink types in use. Two of the test films were printed using inks formulated by a project participant so that the composition of the inks was known. As the supplier of the inks used on the other test films was not willing to disclose the ink formulation, the composition of the inks was not known beyond the basic ink type (solvent based, monomeric or polymeric photoinitiators). The objective was to produce test films with a high degree of set off that would allow comparison of data obtained at different positions within a reel and also with migration tests. The test films were as detailed below, print coverage was 100 % on all reels. Reels were stored for 4 weeks or more at ambient humidity and temperature before testing.

Table 6-1 Test film descriptions

Film	***Description Reel diameter 5 cm, production date 01/03/10***
1	Standard solvent based 1 colour no varnish
2	Monomeric known photoinitiators no varnish
3	Unknown polymeric, known monomeric photoinitiators no varnish
4	Known monomeric photoinitiators no varnish
5 A	Unknown polymeric photoinitiators no varnish
5 B	Unknown polymeric photoinitiators with varnish
6	Known polymeric photoinitiators no varnish

6.2 Measurement of set off of individual ink/varnish components

Measurement of set off was made using the procedure given in Appendix 4 on page 134. There was no measurable set off from film 1 and this was not considered further. In the case of films 4 and 6, the composition of the inks was known and set off measurements were made by monitoring for these compounds. In the case of the other reel samples, a 1 dm^2 portion of the test films was extracted overnight at room temperature in 40 ml of chloroform. The extract was then concentrated by evaporation under a stream of nitrogen to approximately 5 ml, filtered through a syringe membrane filter and injected for

GC-MS and LC-MS analysis. The library of mass spectra and retention times compiled in Table 4-3 on pages 44 to 46 was used to identify compounds for set off measurements. Set off measurements were then made (using the procedure in Appendix 4 page 134) in the extraction solvents 95 % ethanol, iso-octane and dioxane on the test films as received and after application of 1.2 psi pressure at ambient temperature for 10 days. These results are presented in Appendix 2 page 99. Example bar charts of the data are shown below. The sampling positions on the reel are referred to as the start, middle and end of the reel. The mean set off was plotted to provide an overview of the range of results. A statistical treatment of the results was carried out as in Appendix 3 (on page 114). The purpose of this was to decide whether there was a statistically significant difference between the controlled factors (choice of solvent, test pressure and sampling position) and the combined variation arising from the experimental error and the in-homogeneity of the set off. No useful data was obtained from Film 1 (solvent based ink). The data for film 2 (monomeric photoinitiators), is shown in Table A2-1 in Appendix 2 on page 99. Only compounds with the largest detectable set off are considered in the following charts. “Start” and “end” refer to the beginning and end of the reel.

Chart 6-1 Set off from film 2 as a function of sampling position on the reel iso-octane 5 hours at 60 °C

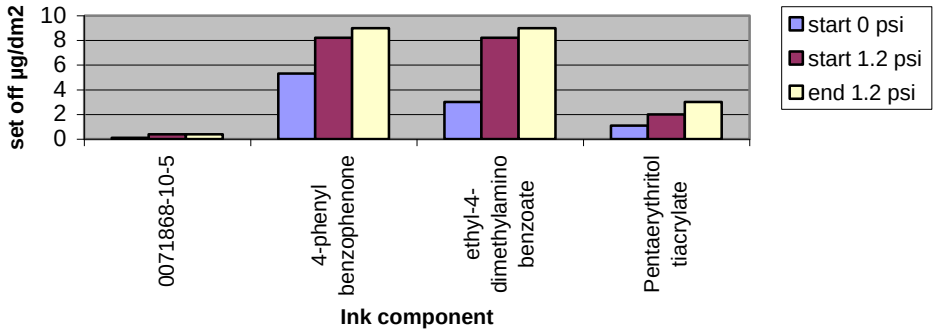
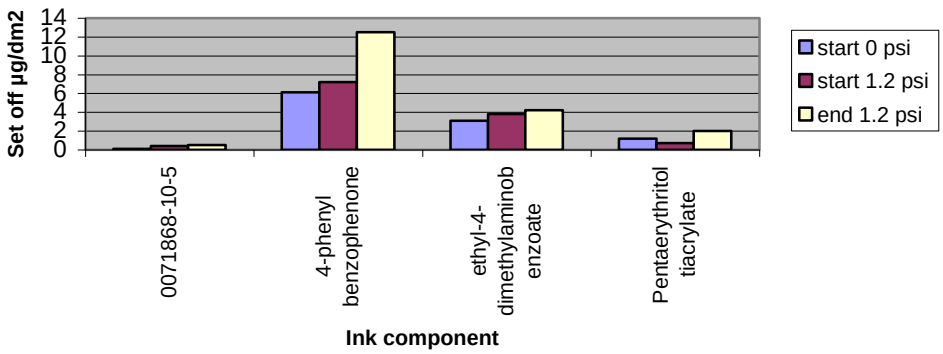
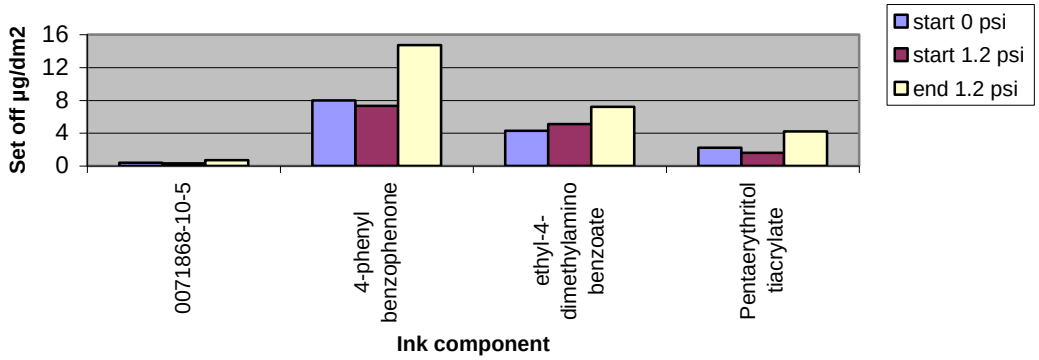


Chart 6-2 Set off from film 2 as a function of sampling position on the reel Dioxane 5 hours 60 °C



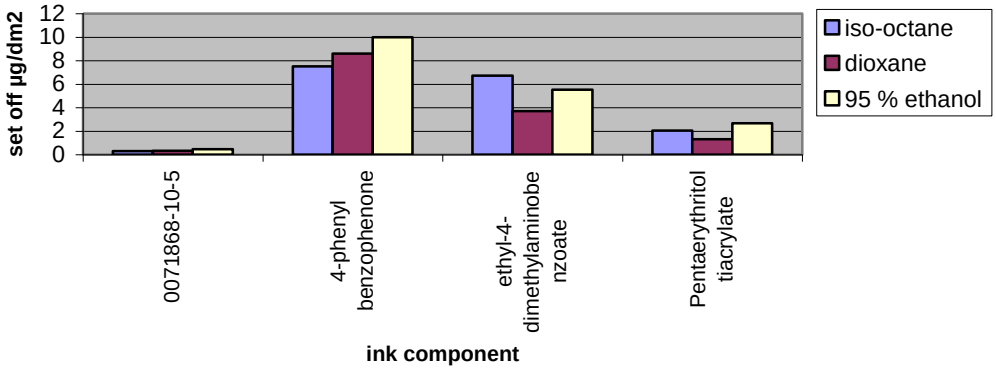
The variation due to the in-homogeneity of the set off may be greater than the variation due to the controlled factors, particularly where set off values were relatively small, such as for pentaerythitol triacrylate. In chart 6-2 it appears, at first sight, that increasing pressure reduces set off for pentaerythitol triacrylate. However, such a comparison of means does not allow a reliable conclusion to be drawn, because it does not separate the variation due to set off homogeneity from the variation due to the controlled factors. Charts 6-1 to 6-3 show that, for the same applied pressure, the end of film 2 gave consistently higher set off values than the start. This suggests that the set off is not homogenous along the length of the film. The statistical interaction study summarised in Table 6-4 on page 68, was taken to be a more reliable assessment of the effect of the controlled factors upon set off than a simple comparison of the means given in the charts presented in this chapter.

Chart 6-3 Set off from film 2 as a function of sampling position on the reel 95 % ethanol 5 hours at 60 °C



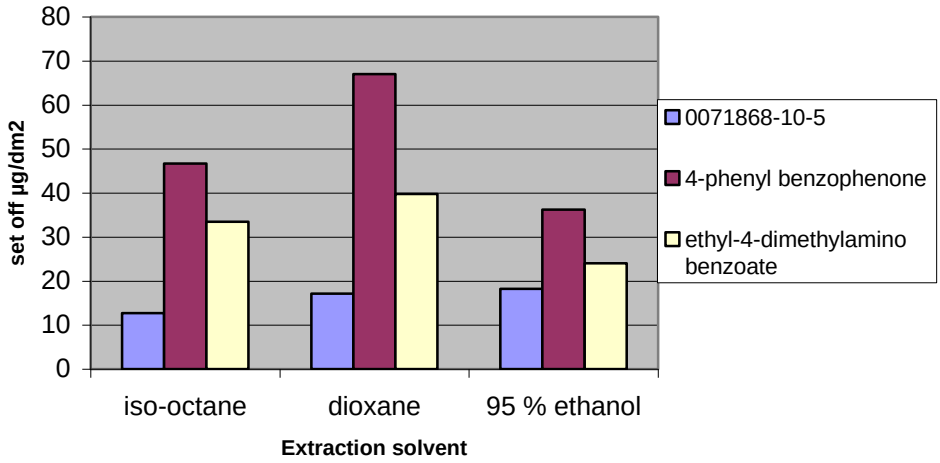
The statistical treatment of the results (on page 68) showed no significant effect upon set off values from the test pressure of 1.2 psi. Similar set off results were obtained for film 2 in all three solvents as demonstrated in the chart below. Only ink components exhibiting a measurable set off were considered.

Chart 6-4 Comparison of set off from film 2 in different solvents



The data in the chart below was obtained from test Film 3 (unknown polymeric photoinitiators with known monomeric photoinitiators). The measurements were made at the start of the reel after application of a pressure of 1.2 psi for 10 days at 20 °C. The data is tabulated in Appendix 2 Table A2-2 on page 99.

Cart 6-5 Comparison of set off in different solvents 5 hours at 60 °C film 3

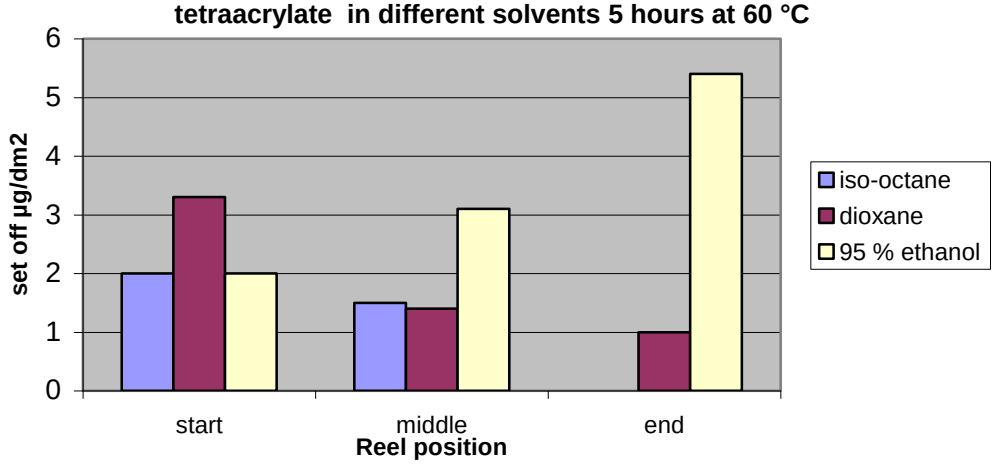


It was not possible to identify the polymeric photoinitiators used from the chromatograms obtained. The reason for this was that the supplier of the inks was not able for commercial confidentiality reasons to disclose which polymeric photoinitiators had been used. There was no available library of LC-MS mass spectra to compare against. In addition, it was discovered that not all polymeric photoinitiators give a response with LC-MS. The statistical treatment of results showed no significant difference in set off between extraction solvents. Whilst the chart of means (Chart 6-5) suggests there is a difference, this did not take into account the variation in replicates for each mean, emphasising the need for a statistical treatment of results.

Film 4 (known monomeric photoinitiators expected to give high set off) gave little measurable set off in all three solvents. The results are included in Table A2-3 and Table A2-4 of Appendix 2 on page 100 and 101 respectively. Only two ink components could usefully be compared. It is apparent from the table of data that there was a difference in set off at the start of the reel compared to the middle and end. Since the data at the start was obtained using a different test pressure, it was difficult to statistically compare these data with the data from the other reel positions. Considering all the data for Film 4, there appeared to be a difference in set off between the start of the reel and the middle and end positions, assuming that test pressure had no effect. This

assumption was made on the basis that, where data could be compared, a test pressure of 1.2 psi had no significant effect on the results obtained.
Set off measurements were obtained for three representative acrylates (di-trimethylolpropane tetra acrylate (DiTMPTA), Glycerine propoxylate triacrylate (GPTA) and dipropylene glycol diacrylate, (DPGDA) included in the ink formulation for film 4. These results are appended to this report in tables A2-5 to A2-7 in Appendix 2 (pages102- 103). There was no significant measured set off (< 1.7 µg/dm^2 or 10 µg/kg equivalent assuming the conventional exposure ratio of 6 dm^2 to 1 kg of food). Set off values were all less than the 10 ppb equivalent for films 5A and 5B (unvarnished and varnished unknown polymeric photoinitiator inks). The results are tabulated in tables A2-8 and A2-9 of Appendix 2 on page 104. Set off for film 6 (known polymeric photoinitiators) was low for all ink components measured. Only one compound had measurable set off and this is shown in Chart 6-6 below.

Chart 6-6 Set off from film 6 of di(trimethylolpropane) tetraacrylate in different solvents 5 hours at 60 °C

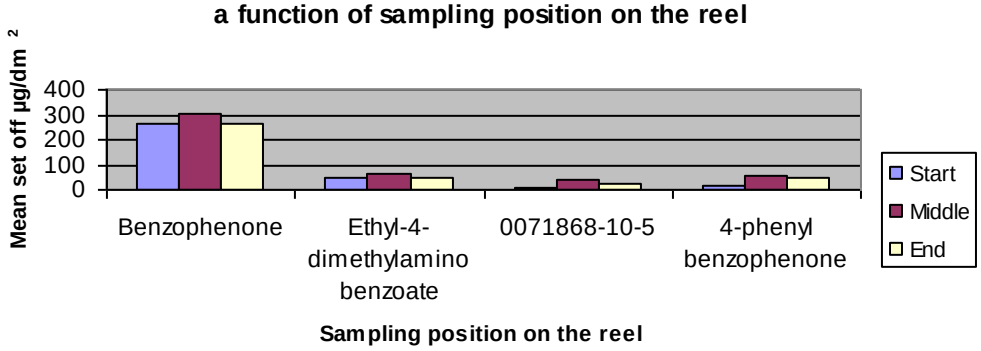


The mean for the 95 % ethanol was affected by a single high result. A statistical comparison of the effect of solvent choice and test pressure was made and no significant effect observed for these two factors on the set off results. There were no data obtained for the end position in iso-octane to allow a similar comparison of reel position.

The set off measured from all the above test films was in most cases generally low. An additional test reel was obtained (approximate reel diameter was 30 cm, production date was October 2010, the reel was stored for more than 4 weeks at ambient temperature before testing was started), referred to here as repeat film 3. This was believed to correspond to Film 3 (unknown polymeric photoinitiator ink with unknown monomeric photoinitiators) which although printed with polymeric photoinitiators was found to contain significant amounts of monomeric photoinitiators. The set off of these was measured on the food contact surface of the reel (for method see Appendix 4 page 134). The reel was cut vertically through to the core to produce a stack of film in

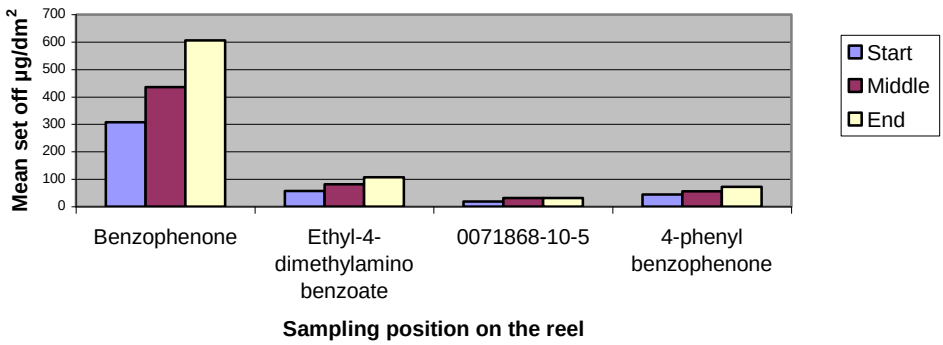
which position down the reel length could be preserved. These results are presented in Table A2-11 Appendix 2 on page 106. The results are shown in Chart 6-7 below for iso-octane.

Chart 6-7 Comparison of mean set off obtained in iso-octane as a function of sampling position on the reel



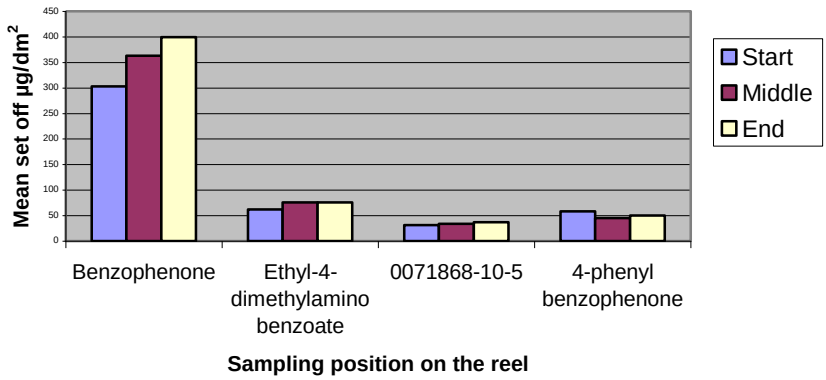
Results from Table A2-11 page 106 obtained in dioxane for repeat film 3 are shown in Chart 6-8 below.

Chart 6-8 Comparison of mean set off obtained in dioxane as a function of sampling position on the reel



Results for repeat film 3 in Table A2-11 on page 106 obtained in 95 % ethanol are shown in Chart 6-9 below.

Chart 6-9 Comparison of mean set off obtained in 95 % ethanol as a function of sampling position on the reel



The data obtained from the repeat Film 3 (unknown polymeric photoinitiators with known monomeric photoinitiators), are also presented below as a series of charts. This was to allow a comparison to be made between mean set off values obtained in the different extraction solvents. The charts 6-10 to 6-12 indicate that similar results are obtained in the three extraction solvents. The statistical comparison of the results in Appendix 3 on page 114 showed no significant effect on the results from reel position or extraction solvent.

Chart 6-10 Comparison of mean set off obtained at the start of the reel in different extraction solvents

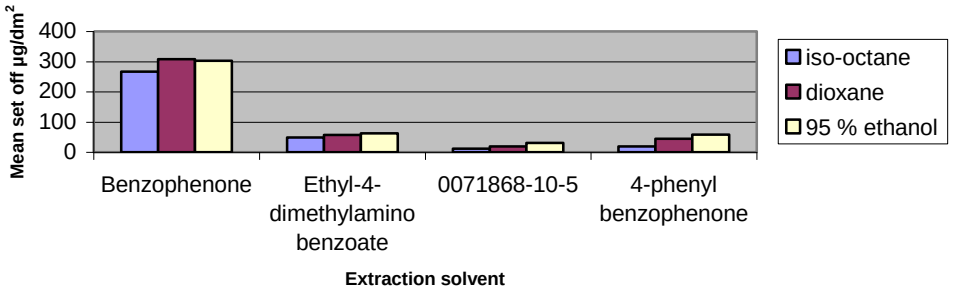


Chart 6-11 Comparison of mean set off obtained at the middle of the reel in different extraction solvents

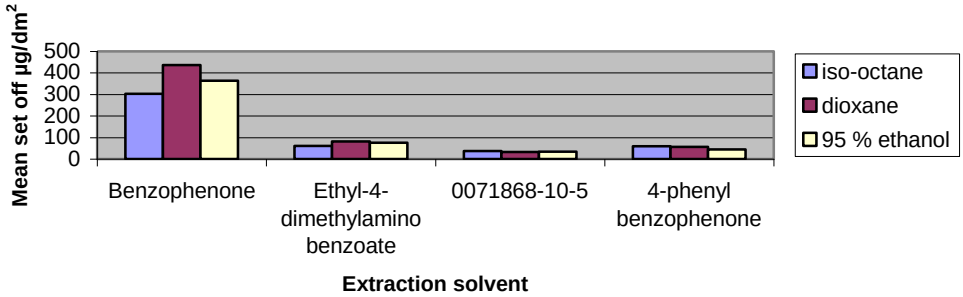
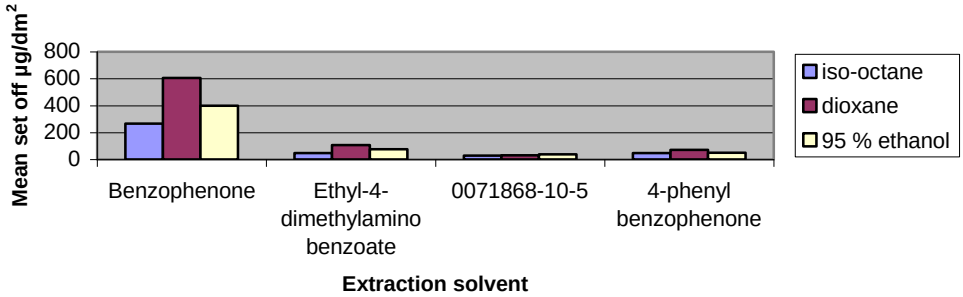


Chart 6-12 Comparison of mean set off obtained at the reel end in different extraction solvents



More set off data was obtained from the repeat film 3 (unknown polymeric photoinitiators known monomeric photoinitiators), in iso-octane and 95 % ethanol by sampling several metres further along the web from the nominal positions of “start”, “middle” and “end”. This data is presented in tables A2-12

and A2-13 in Appendix 2 on pages107 and 108. These results are presented as bar charts by plotting the means from table A2-11, A2-12 and A2-13 in charts 6-13 and 6-14. The third and fourth data points for all four ink components from the start replicate 2 in 95 % ethanol (column 3 in Table A2-12) were eliminated as outliers.

Chart 6-13 Comparison of mean set off obtained in 95 % ethanol as a function of sampling position on the reel

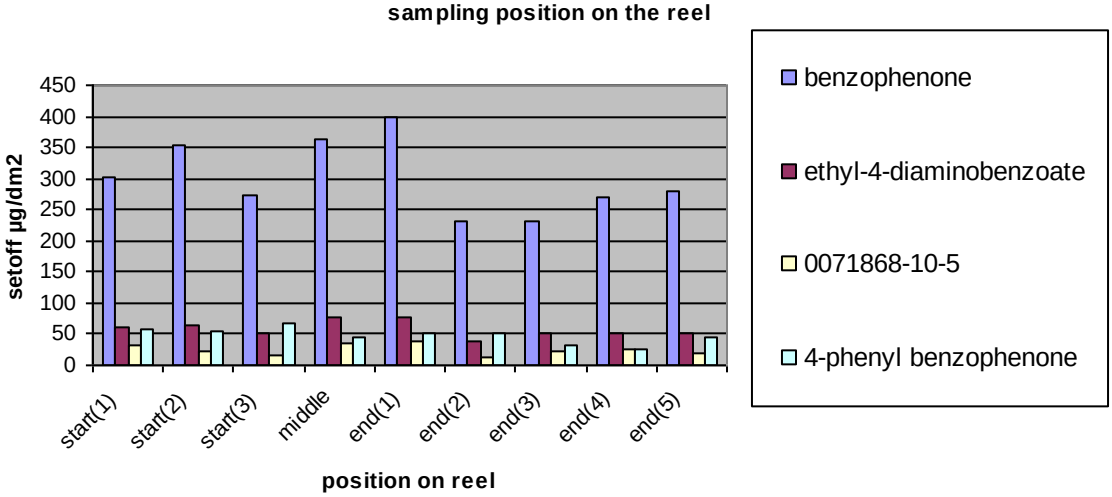
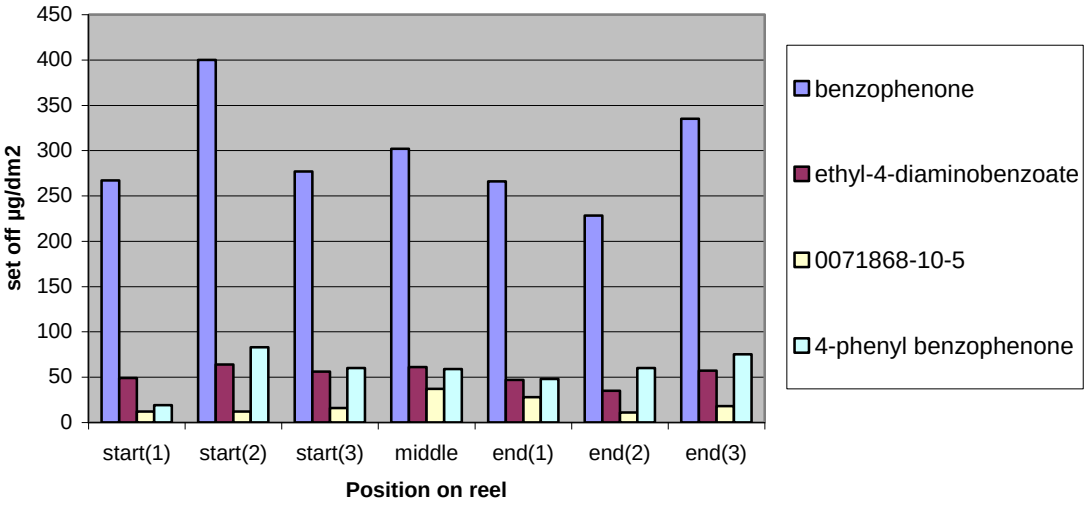


Chart 6-14 Comparison of mean set off obtained in iso-octane as a function of sampling position on the reel



These results show that set off was variable along the length of the reel. This variation was more apparent for benzophenone because of the greater set off compared to the other ink components. This underlines the importance of conducting the analysis on sufficiently large test surface areas and obtaining a sufficient number of replicates. Test surface areas less than 0.7 dm^2 are not desirable. The pooling of more than one test cell prior to analysis, or the calculation of an overall mean from a sufficiently representative number of

replicates, would enable the estimation of set off which is more representative of the whole reel. A single set of four replicates made at a single reel position may underestimate the maximum set off in the reel. Taking the data in Tables A2-11, A2-12 and A2-13 in Appendix 2 pages 106, 107 and 108 as examples, Tables 6-2 and Table 6-3 below show the range in mean set off measured expressed as a percentage of the maximum mean.

Table 6-2 Ranges in the mean set off for repeat film 3 in 95% ethanol

Ink component	*Lowest mean set off* $\mu g/dm^2$	*Highest mean set off* $\mu g/dm^2$	Variation %
Benzophenone	230	440	48
Ethyl-4-dimethylamino benzoate	39	84	54
CAS 0071868-10-5	13	29	55
4-phenyl benzophenone	25	69	64

Table 6-3 Ranges in the mean set off for repeat film 3 in Iso-octane

Ink component	*Lowest mean set off* $\mu g/dm^2$	*Highest mean set off* $\mu g/dm^2$	Variation %
Benzophenone	230	400	43
Ethyl-4-dimethylamino benzoate	35	66	45
CAS 0071868-10-5	11	18	39
4-phenyl benzophenone	60	83	28

These two tables indicate that if by chance the lowest mean value was measured, it could have been of the order of up to 60% less than the highest mean set off obtained.

A more reliable estimate of the maximum set off on the reel is likely to be achieved by calculating an overall mean from a smaller number of replicate measurements made at large distances apart on the reel length. Set off may vary significantly along the length of a reel. Duplicate measurements made at 5 or 6 positions evenly spaced along the reel length are likely to be preferable to a larger number of replicates at a smaller number of positions on the reel.

6.3 Production of a further set of test reels

Six further test reels were manufactured with inks as described below. Production date 16/09/2010, reel thickness approximately 3 cm, stored at ambient temperature for 4 weeks before testing.

Film 7 Ink series X (expected to give high set off)
Amine modified polyether acrylate
HDDA
GPTA
DiTMPTA
CAS 0000947-19-3
CAS 0071868-10-5
Irgacure 369
Irgacure 379
TPO
Phenyl benzophenone

Film 8 Ink series Y (formulated to minimise set off)
EDB
Irgacure 2959
Irgacure 369
Genopol BP-1
Irgacure 379
TMP(eo)TA and Di TMPTA

Film 9 Ink series Z (exclusively polymeric amines and photoinitiators for minimum set off)
Omnipol 910
Speedcure 7010
Speedcure 7005
TMP(eo)TA
Amine modified polyether acrylate

These inks were printed using a single design onto a PE/aluminium foil/PE laminate with press operating conditions set to deliberately under cure the inks (setting 40/20) and again to ensure fully cured ink (setting 80/40). This generated the six further test films. These were then subjected to set off measurements (see method in Appendix 4 page 134).

6.4 Set off measurements for film 7 under cured

The results obtained in iso-octane, dioxane and 95 % ethanol at 60 °C at the extraction times of ½ hour, 1 hour and 5 hours are presented in Table A2-16 in Appendix 2 on page 111. The results are shown in charts 6-15 to 6-17 below respectively.

Chart 6-15 Set off as a function of extraction time in Iso-octane at 60 °C

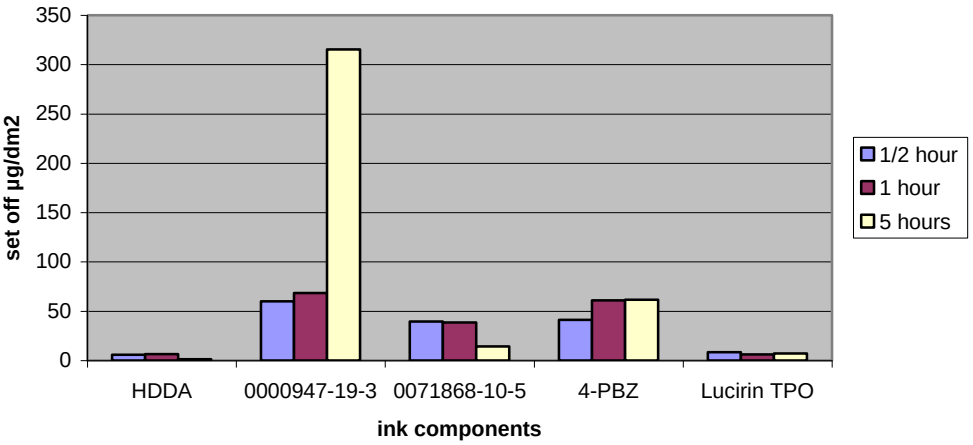


Chart 6-16 Set off as a function of extraction time in dioxane at 60 °C

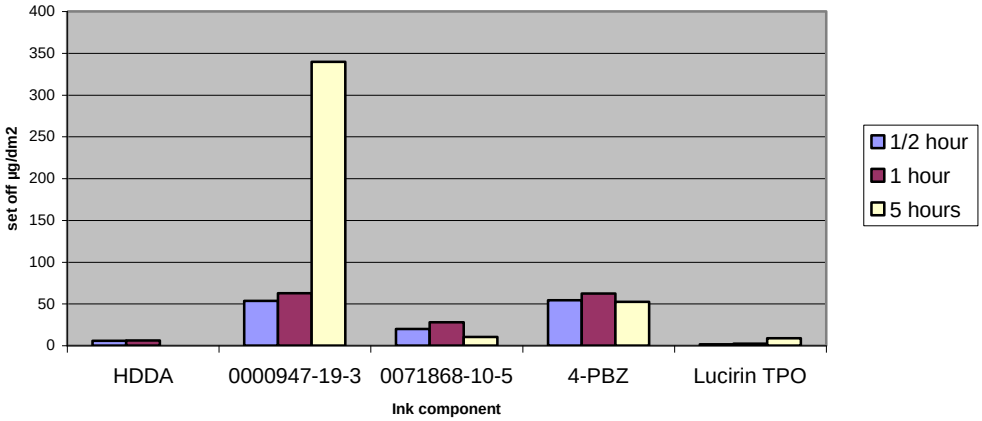
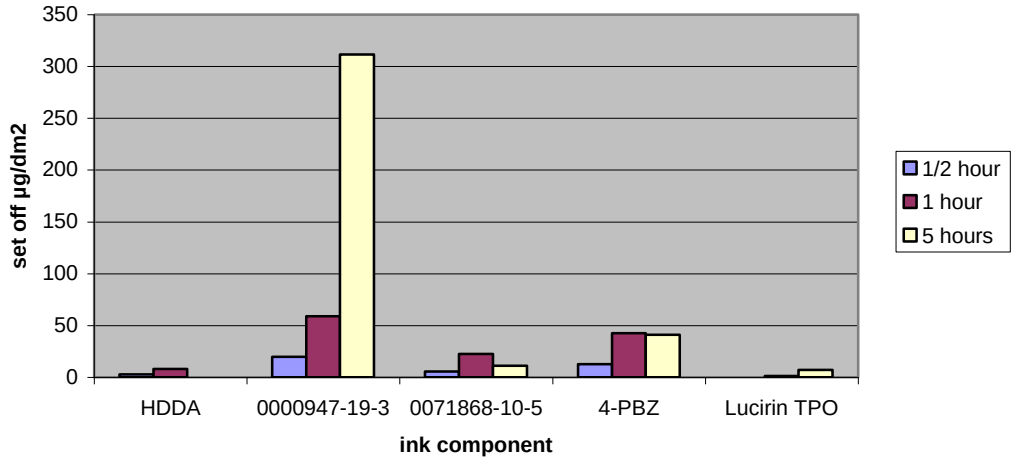


Chart 6-17 Set off as a function of extraction time for 95 % ethanol at 60 °C



The results for the fully cured film 7 (monomeric photoinitiators formulated to give high set off) are tabulated in Table A2-17 in Appendix 2 on page 112. These gave similar results for iso-octane, dioxane and 95 % ethanol.
Set off of ink components was lower in the fully cured film for all compounds except 4-phenyl benzophenone. This is consistent with 4-phenyl benzophenone being a Norrish type II initiator which remains unaltered irrespective of the extent of cure.

6.5 Set off measurements for the test film 8 Ink series Y

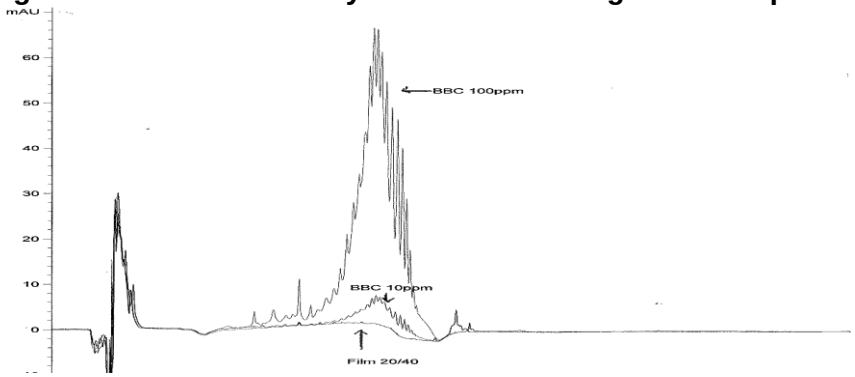
The results obtained are shown in Tables A2-18 and A2-19 Appendix 2 on page 113. Irgacure 369 and Irgacure 379 were not measured in test Film 7 so a comparison of these, which are common to both inks, can not be made. The ethyl-4-dimethylamino benzoate gave consistently high set off demonstrating the persistence of the intact amines after cure.

Results for the under cured Test film 8 (monomeric photoinitiators expected to give low set off) obtained in dioxane 5 hours at 60 °C are shown in Table A2-19 of Appendix 2 on page 113. A greater degree of set off of the photoinitiators Irgacure 369 and Irgacure 379 was observed consistent with under cure, but the ethyl-4-aminobenzoate set off was lower than the fully cured. More data is required to reliably compare cured and under cured set off.

6.6 Set of measurements for the test film 9 Ink series Z

There was no significant set off detected for Speedcure 7010 and Speedcure 7005 from test film 9 (exclusively polymeric photoinitiators and synergists expected to give low set off). The limit of detection was estimated to be 7 $\mu g/dm^2$ for these polymeric photoinitiators. It was not possible to achieve adequate detection limits for Omnipol 910 or the amine modified polyether acrylate. No judgement could be made on the extent of set off of these two compounds. Figure 6-1 shows a chromatogram overlay for Speedcure 7010 (coded BBC on the chromatogram) showing the limit of detection and the absence of detectable set off. The lower trace is the test mixture (dioxane solvent) from the 5 hour extraction at 60 °C of test film 9. The extract was concentrated to approximately 0.5 ml prior to injection. The middle trace shows a standard solution of Speedcure at 10 ppm in dioxane. The upper trace is a 100 ppm standard solution of Speedcure in dioxane.

Figure 6-1 Trace overlay LC-UV Chromatograms for Speedcure 710



6.7 Summary of the set off data from the test films

Using Analysis of Variance (ANOVA), the effects of the controlled factors, extraction solvent, extraction time, test pressure and sampling position on the reel could be separated from the combined variation of analytical error and localised set off homogeneity (which could not be separated). These calculations are shown in Appendix 3 (pages 114 to 127). The outcome of these is tabulated below. Some caution has to be placed on these statistical conclusions. The data was assumed to be normally distributed. The groups compared may also have different distributions (variances) and more than three replicates are really required to obtain a reliable measure of this. Such a detailed statistical study was outside the scope of this project.

Table 6-4 Summary of statistical interaction study

Film	Interaction	conclusion	Page
2	Test pressure	No effect	114
3	Extraction solvent	No effect	115
Repeat 3	Solvent and reel position	No effect	118
Repeat 3	Reel position tested with 95 % ethanol	significant	120
Repeat 3	Reel position tested with iso-octane	significant	122
4	Extraction solvent& reel position (mid and end only)	No effect	116
5	No useful data	none	-
6	Extraction solvent and pressure	No effect	117
7 Series X under cured	Extraction time and solvent	Time significant for CAS 0000947-19-3, solvent significant for 4-phenyl benzophenone	124
7 fully cured	Extraction solvent	Significant for CAS 0071868-10-5 only	126
8 Ink series Y	solvent	Just significant	127

For most ink components and films tested, the choice of extraction solvent was not significant. The effect of prior storage of the reel under 1.2 psi was also not significant. For repeat reel 3 there was more data obtained than for

the other films and these results showed that when a larger set of data was obtained a significant variation in set off according to sampling position on the reel became noticeable.

6.8 Comparison of set off values with migration measurements

Migration tests were carried out on the repeat film 3 (unknown polymeric photoinitiators known monomeric photoinitiators) according to the EU rules on migration testing. The test conditions were 10 days at 40 °C for 95 % ethanol and 2 days at 20 °C for iso-octane. The results are shown in Table 6-5 below.

Table 6-5 Repeat film 3 migration into EU substitute fat test simulants

Simulant	Benzophenone µg/dm^2	ethy-4-(dimethylamino)benzoate µg/dm^2	CAS 0071868-10-5 µg/dm^2	4-phenyl benzophenone µg/dm^2
10 days 40 °C in 95 % ethanol				
Replicate 1	260	48	25	25
Replicate 2	220	37	20	17
Replicate 3	240	39	22	22
Replicate 4	320	71	34	37
Replicate 5	250	39	23	24
Mean	**260**	**47**	**25**	**25**
RSD %	15	30	22	30
2 days at 20 °C in Iso-octane				
Replicate 1	200	30	7.5	9.6
Replicate 2	210	32	8.8	7.9
Replicate 3	230	35	11	11
Replicate 4	210	26	4.1	7.1
Replicate 5	230	23	9.0	7.6
Mean	**220**	**29**	**8.0**	**8.8**
RSD %	**6**	**16**	**32**	**17**
2 days at 20 °C in Iso-octane				
Replicate 1	220	34	9.0	15
Replicate 2	204	30	8.2	13
Replicate 3	210	28	6.0	14
Replicate 4	200	31	9.3	16
Mean	**210**	**31**	**8.1**	**15**
RSD %	**4**	**8**	**18**	**9**

An overall mean was calculated from the mean set off data in Table A2-14 Appendix 2 (page 109) and compared against the migration results.

Table 6-6 Comparison of set off with migration tests with 95 % ethanol

Ink component	*Mean set off 95 % ethanol µg/dm^2*	*Migration 95 % ethanol 10 days 40 °C µg/dm^2*	Set off Over estimate of migration %
Benzophenone	300	260	13
Ethyl-4-(dimethyl amino) benzoate	59	47	20
CAS 0071868-10-5	25	25	0
4-phenyl benzophenone	47	25	47

Table 6-7 Comparison of set off with migration tests with iso-octane

Ink component	*Mean set off Iso-octane* $\mu g/dm^2$	*Migration Iso-octane 2 days at 20 °C* $\mu g/dm^2$	Set off over estimate of migration %
Benzophenone	300	210	29
ethy-4-(dimethylamino)benzoate	53	30	43
CAS 0071868-10-5	19	8.0	58
4-phenyl benzophenone	58	12	80

The set off procedure was found to be a worst case compared to migration testing. The results suggest a loss of 4-phenyl benzophenone in the migration experiments. The above is based upon a small number of migration tests; a greater number of migration tests are desirable because of the variability in set off. It is possible that the difference between the results is due to an insufficient number of migration tests to establish the true mean migration.

Migration tests were carried out into Tenax 10 days at 40 °C using printed test film from the same region on the reel as tested in section 5.2 on page 53 (last paragraph) for which the results in Table 5-7 page 54 had been obtained. The results are shown in Table 6-8 below.

Table 6-8 Migration into Tenax 10 days at 40 °C

Replicate	*Benzophenone* $\mu g/dm^2$	*ethyl-4-(dimethyl amino) benzoate* $\mu g/dm^2$	*CAS 0071868-10-5* $\mu g/dm^2$	*4-phenyl benzophenone* $\mu g/dm^2$
1	110	23	16	28
2	120	22	12	32
3	150	24	14	39
4	150	27	13	40
Mean	130	24	14	35
RSD	16	9.0	12	17
Migration %	51	37	56	32

These results show that up to approximately 50 % of the measured set off migrates into Tenax after 10 days at 40 °C. Film 3 and repeat Film 3 were both intended to be printed with polymeric photoinitiators and synergists to give low set off. The presence of the monomeric photoinitiators and synergists suggests that the wrong ink has been used, or that the ink has been contaminated either during production of the ink or on the printing press. It is quite possible that such a situation may occur generally within the packaging industry. Therefore, it is probable that such levels of set off and migration may occur with production batches of film.

The analytical recovery obtained using the methods given in Appendix 4 for the set off measurements in solvents and the migration measurements in foods are given in Appendix 5 and Appendix 6. Known amounts of the ink components were added to glass vials containing the extraction solvents and

stored for 10 days at 40 °C. Test cells containing known amounts of the ink components and extraction solvents were stored for 5 hours at 60 °C. The food was fortified with known amounts of the ink components in test cells stored for 10 days at 40 °C. The recoveries were within acceptable ranges given that in some cases (for example Table A5-6 on page 137) the fortifying masses of ink components were small i.e. did not exceed 10 µg.

6.9 Summary

A series of printed test films were manufactured and the set off for selected ink components measured by extraction into dioxane, iso-octane and 95 % ethanol, for time periods of up to 5 hours at 60 °C.

Set off was found not to be homogenous on the printed packaging reels, with significant variation set off across the width and down the length. In order to obtain set off results that are statistically representative of the set off of the entire reel, a relatively large number of replicate tests are required down the length of the reel. In order to obtain the most reproducible results, care is required to ensure that replicates are obtained from identical regions in contact with the print image. There was no significant effect on the set off results obtained after storage at 20 °C for 10 days at 1.2 psi. Taken as a whole, there was no significant difference in set off results obtained in iso-octane, dioxane or 95 % ethanol for a range of ink components across the different printed films.

The stabilities of a selected range of ink components were measured under the most severe test conditions of 5 hours at 60 °C. These data are tabulated in tables A5-1 to A5-6 (Appendix 5 pages 135 to 137). Some ink components (such as acrylates) were not stable under the test conditions. This highlights the need to perform stability measurements in parallel with set off measurements and to use the results, where appropriate, to correct the set off values.

Set off measurements obtained using the proposed set off procedure were found for some ink components (but not all) to over estimate migration into 95 % ethanol and iso-octane. The problem of the in-homogeneity of set off, (producing a large uncertainty in the value for the set off on the actual test specimen used for migration testing), gave a wide range in this over estimate of migration. On balance, it was concluded that set off measurements may be used as a reasonable worst case estimate of migration into the EU food simulants.

Chapter 7

7.0 Migration measurements into food

The migration of selected ink components from selected test films was measured into three foods. These were orange juice with pulp, cream of tomato soup (3 % fat) and cereal containing 23 % fat. This cereal was a mixture of crushed nuts, oats and sugar syrup that had been baked to aggregated clumps of the food product. The migration tests were carried out by single sided exposure of the food contact surface in migration test cells. The cereal was blended to a small particle size with a food blender before transferring to the test cells. Exposure of the food was 10 days at 40 °C. Some migration results were also obtained in 3 % acetic acid to enable a comparison of migration into orange juice. Results were not corrected for recovery given in Tables A6-1, A6-2 and A6-3 in Appendix 6 pages 138-140.

7.1 Migration from Repeat film 3 into food

Results for repeat film 3 are shown in Table 7-1 below.

Table 7-1 Repeat film 3 Migration into orange juice 10 days at 40 °C

Compound	*Migration µg/dm^2*	*Migration µg/kg*
Ethy-4-(dimethylamino)benzoate		
Replicate 1	53	320
Replicate 2	51	310
Replicate 3	49	300
Replicate 4	57	340
Mean	53	320
RSD (%)	7	5
Benzophenone		
Replicate 1	130	750
Replicate 2	120	710
Replicate 3	120	700
Replicate 4	140	820
Mean	130	750
RSD (%)	8	7
4-phenyl benzophenone		
Replicate 1	53	320
Replicate 2	51	300
Replicate 3	51	310
Replicate 4	58	350
Mean	53	320
RSD (%)	6	7
CAS 0071868-10-5		
Replicate 1	32	190
Replicate 2	33	200
Replicate 3	32	190
Replicate 4	33	200
Mean	33	200
RSD (%)	2	3

Table 7-2 Repeat film 3 Migration into soup 10 days at 40 °C

Compound	*Migration µg/dm²*	*Migration µg/kg*
Ethy-4-(dimethylamino)benzoate		
Replicate 1	65	390
Replicate 2	59	360
Replicate 3	63	380
Replicate 4	68	410
Mean	64	390
RSD (%)	6	5
Benzophenone		
Replicate 1	240	1400
Replicate 2	210	1300
Replicate 3	230	1400
Replicate 4	220	1300
Mean	230	1400
RSD (%)	6	4
4-phenyl benzophenone		
Replicate 1	80	480
Replicate 2	80	450
Replicate 3	67	400
Replicate 4	69	410
Mean	74	440
RSD (%)	9	9
CAS 0071868-10-5		
Replicate 1	37	220
Replicate 2	29	170
Replicate 3	30	180
Replicate 4	31	190
Mean	32	190
RSD (%)	11	11

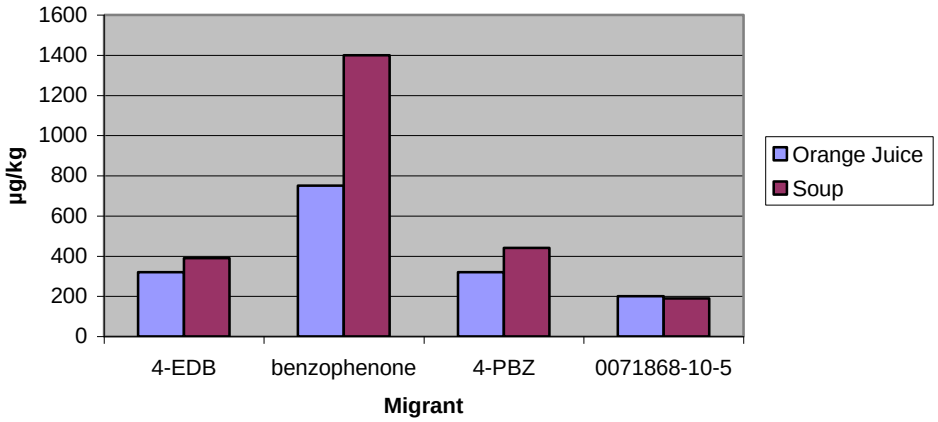
The set off was measured from the same position on the reel as subjected to the migration tests and the mean set off used to compare the proportion of set off that migrates into the food. This is shown Table 7-3 below.

Table 7-3 Proportion of set off ink component migrating into food

Compound	*Set off µg/dm²*	*Migration Soup %*	*Migration Orange juice %*
Benzophenone	260	86	47
Ethy-4-(dimethylamino)benzoate	65	98	82
4-phenyl benzophenone	110	66	48
CAS 0071868-10-5	25	130	130

The data in Tables 7-1 and 7-2 are shown in Chart 7-1 below.

Chart 7-1 Comparison of mean migration values for different food types for repeat film 3



7.2 Migration from Film 7 under cured ink series X into food

Tables 7-4, 7-5, 7-6 and 7-7 below show the migration from film 7 into soup, orange juice, 3 % acetic acid food simulant and cereal.

Table 7-4 Film 7 Migration in soup 10 days 40 °C

Compound	*Migration µg/dm²*	*Migration µg/kg*	*RSD %*
1,6 Hexanediol diacrylate			
Replicate 1	< 1	< 6	
Replicate 2	< 1	< 6	
Replicate 3	< 1	< 6	
Mean	< 1	< 6	-
Di-(trimethylolpropane) tetraacrylate			
Replicate 1	< 1	< 6	
Replicate 2	< 1	< 6	
Replicate 3	< 1	< 6	
Mean	< 1	< 6	-
CAS 0000947-19-3			
Replicate 1	58	350	
Replicate 2	56	340	
Replicate 3	58	350	
Mean	57	350	2
4-phenyl benzophenone			
Replicate 1	58	350	
Replicate 2	54	320	
Replicate 3	55	330	
Mean	56	330	5
CAS 0071868-10-5			
Replicate 1	22	130	
Replicate 2	12	74	
Replicate 3	18	110	
Mean	17	100	30

Table 7-5 Film 7 Migration into orange juice 10 days at 40 °C

Compound	*Migration µg/dm²*	*Migration µg/kg*	*RSD %*
1,6 Hexanediol diacrylate			
Replicate 1	< 1	< 6	
Replicate 2	< 1	< 6	
Replicate 3	< 1	< 6	
Mean	< 1	< 6	-
Di-(trimethylolpropane) tetraacrylate			
Replicate 1	< 1	< 6	
Replicate 2	< 1	< 6	
Replicate 3	< 1	< 6	
Mean	< 1	< 6	-
CAS 0000947-19-3			
Replicate 1	77	463	
Replicate 2	80	480	
Replicate 3	68	405	
Mean	75	450	9
4-phenyl benzophenone			
Replicate 1	44	270	
Replicate 2	41	250	
Replicate 3	41	250	
Mean	42	260	4
CAS 0071868-10-5			
Replicate 1	32	190	
Replicate 2	31	190	
Replicate 3	27	160	
Mean	30	180	10

Table 7-6 Film 7 Migration into 3% acetic acid 10 days at 40 °C

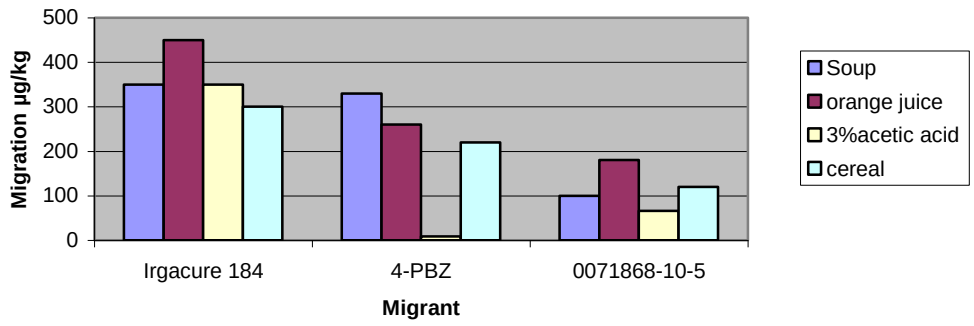
Compound	*Migration µg/dm²*	*Migration µg/kg*	*RSD %*
1,6 Hexanediol diacrylate			
Replicate 1	< 1	< 6	
Replicate 2	< 1	< 6	
Replicate 3	< 1	< 6	
Mean	< 1	< 6	-
Di(trimethylol propane) tetraacrylate			
Replicate 1	< 1	< 6	
Replicate 2	< 1	< 6	
Replicate 3	< 1	< 6	
Mean	< 1	< 6	-
CAS 0000947-19-3			
Replicate 1	55	330	
Replicate 2	72	430	
Replicate 3	47	280	
Mean	58	350	22
4-phenyl benzophenone			
Replicate 1	1.7	10.4	
Replicate 2	1.5	9.2	
Replicate 3	1.2	7.0	
Mean	1.5	8.9	19
CAS 0071868-10-5			
Replicate 1	11.2	67	
Replicate 2	13.2	79	
Replicate 3	8.8	53	
Mean	11.1	66	20

Table 7-7 Film 7 Migration into cereal 10 days at 40 °C

Compound	*Migration µg/dm²*	*Migration µg/kg*	*RSD %*
1,6 Hexanediol diacrylate			
Replicate 1	5.7	34	
Replicate 2	5.5	33	
Replicate 3	7.9	47	
Mean	6.4	38	21
Di-(trimethylolpropane) tetraacrylate			
Replicate 1	< 1	< 6	
Replicate 2	< 1	< 6	
Replicate 3	< 1	< 6	
Mean	< 1	< 6	-
CAS 0000947-19-3			
Replicate 1	48	290	
Replicate 2	57	340	
Replicate 3	50	300	
Mean	52	310	9
4-phenyl benzophenone			
Replicate 1	36	220	
Replicate 2	38	230	
Replicate 3	36	210	
Mean	37	220	5
CAS 0071868-10-5			
Replicate 1	18	110	
Replicate 2	21	130	
Replicate 3	21	120	
Mean	20	120	9

The data in Tables 7-4 to 7-7 are shown in Chart 7-2 below.

Chart 7-2 Comparison of migration values for different food types



CAS 0000947-19-3 showed a similar migration into orange juice as into 3 % acetic acid, whereas 4-phenyl benzophenone showed a lower migration into 3 % acetic acid. This was consistent with the more polar CAS 0000947-19-3 and CAS 0071868-10-5 being more soluble in 3 % acetic acid than 4-phenyl benzophenone and all three being more soluble in orange juice due to the presence of essential oils from the orange.

The data presented in Appendix A2 Table A2-16 for set off concentrations present on the food contact surface showed a variation that was attributed to the in-homogeneity of set off. To calculate the proportion of the ink components present on the food contact surface that migrates into the food, a reliable estimate of the set off is required. The set off data in Table A2-16 was combined and used to calculate an overall mean set off value in the following manner. The mean set off data obtained at 1 and 5 hours in the extraction solvents, 95 % ethanol, dioxane and iso-octane were averaged, or where a mean value was zero, the highest set off value taken as the overall mean. These means were again averaged to give a single overall mean set off for each ink component. This is quoted in column 2 of Table 7-8 below as the set off value. This value was used to calculate the proportion of ink component that had migrated into each food type and these are tabulated in Table 7-8 below.

Table 7-8 Proportion of set off ink component migrating into food

Ink component	***Set off***	***Migration***			
		Soup	***Orange juice***	***3 % acetic acid***	***Cereal***
	µg/dm²	***%***	***%***	***%***	***%***
1,6 Hexanediol diacrylate	6	< 10	< 10	<10	110
Di-(trimethylolpropane) tetraacrylate	< 1	<10	<10	<10	<10
CAS 0000947-19-3	190	30	40	30	30
4-phenyl benzophenone	50	100	80	<10	70
CAS 0071868-10-5	20	80	150	60	100

Table 7-8 shows that, at the concentrations present on the food contact surface, solubility of the ink components in the food is sufficient for a significant proportion to migrate into the food. The proportion of 1,6-hexanediol diacrylate and CAS 0071868-10-5 migrating into cereal and orange juice respectively, was greater than 100 %. The most likely reason for this was that the actual concentration present on the food contact surface was greater than the overall mean set off, due to in-homogeneity of set off.

7.3 Migration from Film 8 under cured ink series Y into food

Tables 7-9, 7-10 and 7-11 presented below show the migration from Film 8 into soup, orange juice and cereal after storage for 10 days at 40 °C.

Table 7-9 Film 8 Ink series Y Soup 10days at 40°C

Ink component	migration µg/dm^2	migration µg/kg	RSD %
Irgacure 2959 Replicate 1	5.9	35	
Replicate 2	6.3	38	
Replicate 3	6.4	39	
Mean	**6.2**	**37**	**6**
Ethyl-4-dimethylamino benzoate			
Replicate 1	35	210	
Replicate 2	30	180	
Replicate 3	27	160	
Mean	**31**	**180**	**14**
Irgacure 369 Replicate 1	6.2	37	
Replicate 2	6.0	36	
Replicate 3	6.6	40	
Mean	**6.3**	**38**	**5**
Irgacure 379 Replicate 1	2.1	13	
Replicate 2	2.0	12	
Replicate 3	2.5	15	
Mean	**2.2**	**13**	**12**

Table 7-10 Film 8 Ink series Y Orange Juice 10days at 40°C

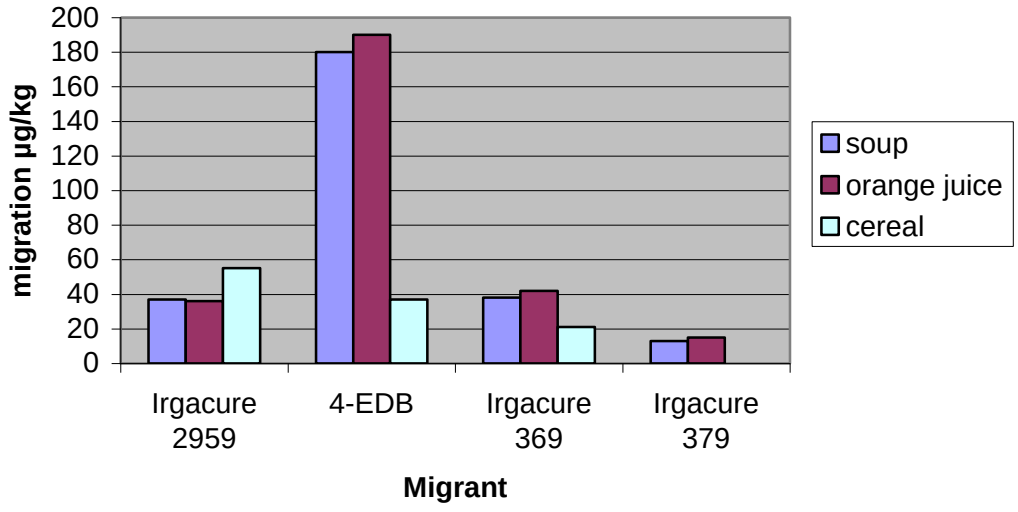
Ink component	Migration µg/dm^2	Migration µg/kg	RSD %
Irgacure 2959			
Replicate 1	5.2	31	
Replicate 2	7.8	47	
Replicate 3	5.3	32	
Mean	**6.1**	**36**	**25**
Ethyl-4-dimethylamino benzoate			
Replicate 1	31.6	190	
Replicate 2	36.1	220	
Replicate 3	28.7	170	
Mean	**32.1**	**190**	**13**
Irgacure 369			
Replicate 1	6.9	41	
Replicate 2	7.3	44	
Replicate 3	6.9	42	
Mean	**7.0**	**42**	**4**
Irgacure 379			
Replicate 1	2.5	15	
Replicate 2	2.6	16	
Replicate 3	2.5	15	
Mean	**2.5**	**15**	**4**

Table 7-11 Film 8 Ink series Y Cereal 10days at 40°C

Ink component	Migration µg/dm^2	Migration µg/kg	RSD %
Irgacure 2959			
Replicate 1	9.7	58	
Replicate 2	9.0	54	
Replicate 3	9.0	54	
Mean	**9.2**	**55**	**4**
Ethyl-4-dimethylamino benzoate			
Replicate 1	5.9	35	
Replicate 2	6.3	38	
Replicate 3	6.4	38	
Mean	**6.2**	**37**	**5**
Irgacure 369			
Replicate 1	3.7	22	
Replicate 2	3.4	20	
Replicate 3	3.4	20	
Mean	**3.5**	**21**	**5**
Irgacure 379			
Replicate 1	1.3	<10	
Replicate 2	1.1	<10	
Replicate 3	1.0	<10	
Mean	**1.1**	**<10**	**-**

The data in Tables 7-9, 7-10 and 7-11 are shown in Chart 7-3 below.

Chart 7-3 Comparison of mean migration in different food types



The mean proportion of the set off migrating into the food was calculated by dividing the mean migration measured in the food by the overall mean set off from Table A2-19 in Appendix 2 page 113 and multiplying by 100 to express this as a percentage. These values are tabulated in Table 7-12 below

Table 7-12 proportion of migrants from under cured test film 8 in food

Ink component	*Set off*	*Migration*		
		Soup	*Orange juice*	*Cereal*
	µg/dm²	*%*	*%*	*%*
Irgacure 2959	1.0	600	600	900
Ethyl-4-dimethylamino benzoate	20	60	160	30
Irgacure 369	4.0	160	180	90
Irgacure 379	4.5	50	60	20

Table 7-12 shows that a high proportion of the ink components present on the food contact surface transfer to the food, in agreement with the results obtained with the Film 7 ink series X ink film. For this film, the set off measured was relatively low (13 ppb equivalent in the food). Small variations in the actual amount of set off on the migration test samples results in the proportions migrating exceeding 100 % of the mean overall set off. Choosing Irgacure 2959 as an example, the amount of set off on the migration test samples must have been much greater than the mean set off calculated from the data obtained in Chapter 6. The migration concentration in the food was in the range 40 to 60 ppb. These are small concentrations at or near the limit of quantification for the migration testing. Where the set off is relatively high compared to the variation due to in-homogeneity of the set off, a more reliable estimate of the proportion migrating was obtained (for example, the ethyl-4-dimethylamino benzoate). The conclusion from Table 7-12 is that it may be assumed that a high proportion of set off ink components will transfer into aqueous, acidic and fatty foods. More set off data are required to enable a more reliable estimate of the proportion of set off migrating into food, because of the inhomogeneity of the set off.

Migration from the test film 9 series Z ink into food was not measured because set off from both the cured and under cured film was low. Migration (assuming 100 %) would not exceed guide limits in the food.

7.4 Comparison of migration into different foods

In general, there was less of a difference in migration between all of the foods than was expected. The soup contained less than 3 % fat, whilst the orange juice contained essential oils and the cereal 23 % fat which would be expected to increase the solubility of the ink components. The migration

results show that there is sufficient solubility even in the soup, for a high proportion of the set off to transfer to both aqueous and fatty foods.

7.5 Summary

Migration tests in 3 % acetic acid gave lower migration results than those obtained in orange juice. This was attributed to the presence of essential oils in the pulp improving the solubility of the ink component. Similar migration results were obtained in orange juice, cereal and soup.

The results in chapter 6 demonstrated that set off was not homogenous along the length of packaging reels. This in-homogeneity meant that a constant set off value could not be measured at different points on the reel. This complicates the comparison of set off with migration because a statistical approach is required, with testing of a sufficient number of samples. Comparison of the mean set off and mean migration values does however, show that migration of ink components, present on the food contact surface, into packed foods is likely to approach 100 %. Most of the data in this report were obtained from films expected to give high set off. However, it is not the magnitude of the set off or migration values that are of significance, it is the finding that, if there is set off, all of it is likely to transfer into both aqueous and fatty foods.

The migration studies presented in this chapter, demonstrate that measurement of set off by extraction with 95 % ethanol, iso-octane or dioxane at 60 °C for 5 hours will give a reliable indication of worst case migration into real foods at the end of their shelf life. This means that a set off measurement which can be completed within a day is a reliable quality control test procedure to ensure compliance with migration limits. The choice of dioxane, iso-octane or 95 % ethanol extraction solvent can be made according to anticipated reactivity with the ink components and also to allow compatibility with the analysis technique, either GC or HPLC.

Chapter 8

8.0 General conclusions

8.1 Part 1 Development of a scanner to estimate total visual set off

A scanner was developed that allowed the measurement of the total surface area of visual set off on a range of food contact substrates. It was possible to set simple pass fail criteria for the total surface area of set off on the food contact surface. The scanner would be suitable for quality control of visual set off when the web is rewound by the converter to correct width for use or by the packer filler immediately prior to use.

Some combinations of substrate were problematic due to difficulties in obtaining sufficient contrast between the substrate and the set off region. Transparent and some translucent substrates were also problematic due to show through from the print side.

8.2 Part 2 Measurement of non visual set off

Exposure techniques were developed that allowed measurement of non visual set off of individual chemical compounds. Exposure by pouches or single sided cells was possible on packaging substrates such as polymer films and laminates and even coated carton board. Surfaces areas of at least 0.7 dm^2 are desirable due to variations in the homogeneity of set off. The optimum test conditions for set off measurement were extraction of the food contact surface in dioxane, iso-octane or 95 % ethanol for 5 hours at 60 °C.

To obtain reproducible results, it is essential to test exactly the same region of the food contact surface in contact with the same corresponding region of the print image. This can be achieved by stepping off the repeat distance of the image on the food contact surface immediately below in the reel or stack.

Analytical methods were developed to allow the quantification of a wide range of ink photoinitiators and synergists. Many of the monomeric photoinitiators were amenable to GC-MS on non polar or intermediate polar stationary phases. The exceptions were the morphilino and phosphine oxide classes of photoinitiators. Polymeric photoinitiators were only amenable to HPLC, most by UV detection. Some polymeric photoinitiators exhibited low sensitivity to UV detection and some did not respond to LC-MS. The presence of the low UV absorbing polyethylene glycol chain in the molecule reduces the proportion of the molecule with the aromatic, higher UV absorbing groups, such benzophenone or thioxanthone. The poor LC-MS response is indicative of weak ionisation of these molecules in the LC-MS ion source. The polymeric

ink components were not pure discrete chemical compounds. LC-MS response was in some cases to minor components of the polymeric ink component mixture. Variations in LC-UV response between batches did not appear to be large. Variations in LC-MS response between batches of the same photoinitiator were, however, observed. These minor components may be lower molecular weight fractions or impurities, which are more easily ionised than the principal polymeric ink component. Significant error by LC-MS is, therefore, to be anticipated if the same batch used in the ink is not available for calibration. If the molecular ion (or molecular adduct ion) is not available (either not known or not generated in the ion source), the extreme situation could arise where calibration is based upon an impurity or lower molecular weight fraction. If this is present at different concentration in the ink compared to the calibration sample (or worse not present at all), the set off values will be incorrect.

A general method for acrylates was not developed as it is theoretically not possible to compare the result obtained with any legislation limit. Individual acrylates could be quantified in the extraction solvents by GC-MS and GC-FID using the methods described in Appendix 4.

Rapid methods using low cost chromatography detectors were investigated for GC and HPLC. The charged aerosol mass detector did not give a similar response for all compounds but was found to be a useful detector when calibrated with the individual compounds. Similar response was obtained for different batches of the same polymeric photoinitiator and it was more sensitive than UV detection for some polymeric photoinitiators. GC-FID could be used to measure set off from printed packaging. On modern equipment with carrier gas pressure control, retention times can be set to a specified value from a calibration plot of pressure against retention time. This provides the ability of matching peaks identified with a GC-MS. Calibration using a single calibrant or a simple pass/fail test by comparison of peak height against a 10 or 50 ppb equivalent marker calibrant is possible. Large errors in measurements were observed if compounds with additional nitrogen or sulphur atoms were assumed to have equal responses to compounds with carbon, oxygen and hydrogen atoms only. Set off measurements are therefore possible using the methodology in laboratories not equipped with GC-MS or LC-MS using lower cost LC-UV or LC-CAD and GC-FID. It may be possible to calibrate the GC-FID with a single structurally similar ink component in a set of ink components. Suggested calibrants are given in Table 8-1 in Appendix 10 page 324.

Set off was measured on printed test films by extraction into iso-octane, 95 % ethanol or dioxane for up to 5 hours at 60 °C. Testing using pressures recommended by EuPIA can not in practice be carried out in most laboratories. Storage under a pressure of 1.2 psi had no effect on set off. For most samples likely to be tested, the most important sampling factor was found to be obtaining a statistically representative sub-sample of the whole reel, as significant variation in set off was found along the length of the web. The reasons for this variation may include:

i) Different colour inks in the printed image have different concentrations of ink components and the food contact surface has been in contact with different colours.
ii) Set off is in the form of patches of ink of irregular surface area and thickness.
iii) Localised differences in the ink cure chemistry arising from varying UV light absorption (ink colour or printing press lamp related)
iv) Printing press related parameters such as variation in the coat weight of the ink applied, line speed, ink viscosity.

Migration from the test films for a range of ink components into the EU simulants, iso-octane (2 days at 20 °C) and 95 % ethanol (10 days at 40 °C) was comparable or lower than the measured set off using the set off test conditions of 5 hours at 60 °C. The set off measurement procedure provides a rapid and reliable worst case estimate of migration in the EU simulants. Set off measurements can be completed within a single working day.

Migration measurements in food were made of selected ink components from the printed test packaging for which set off had been measured. The foods were tomato soup, orange juice with bits and breakfast cereal. These migration tests showed that it was possible for all the set off ink components to transfer into all three foods after 10 days at 40 °C. The measured set off was therefore, found to be a reliable worst case estimate of migration into foods.

This finding has implications with respect to food contact packaging legislation compliance. Many ink components in use have no toxicological safety evaluation. Therefore, for the purposes of compliance with Regulation (EC) 1935/2004 and in the absence of harmonised EU legislation on printing inks, the Swiss national legislation migration limit of 10 ppb in the food is a reasonable limit to apply. The migration studies presented in this report demonstrate that, in order to comply with Regulation (EU) 1935/2004, set off should not exceed 1.7 µg/dm^2 for these individual ink components. This is a

demanding set off limit for a printer to achieve. The set off data obtained for Film 8 (expected to give low set off) shows that a set off limit of 1.7 µg/dm^2 is difficult to achieve with monomeric photoinitiators and synergists. Unless monomeric photoinitiators and synergists are used for which a 50 ppb limit in the food may be applied, (or polymeric photoinitiators and synergists) it is probable that it will not be possible to demonstrate by set off measurement that the packaging is in compliance with Regulation (EC) No 1935/2004 with respect to migration of some or all of these ink components.

If the ink composition is not known, a complete safety assessment will not be possible. It is currently not possible to guarantee the detection and identification of all the polymeric ink photoinitiators and synergists that might be used.

The procedure developed in this project for measuring non visible set off is summarised in Appendix 4 on page 134.

Chapter 9

9.0 Recommendations and suggested further work from Pira

9.1 Recommendations by Pira

The large number of photoinitiators and synergists in common use means that surveillance screening of a selected range is not a reliable approach. It is inevitable that some may be missed. A better approach for future surveillance screening is to extract the print surface of the packaging to identify the ink components and then monitor for these in the food.

Toxicological assessments of a greater number of the ink components in common use could be of benefit to industry.

It is useful for food packers and converters to consider carefully new ink formulations in order to maximise the margin for compliance against Regulation (EC) No 1935/2004 before proceeding with set off or migration measurements. A possible way this could be done is to incorporate numerous photoinitiators at lower concentrations to arrive at the same total photoinitiator concentrations. It is also beneficial if photoinitiators with higher migration limits listed in the Swiss Ordinance 817.023.21 Annex 6 are selected in preference to those with lower limits. Pira is of the opinion that the use of polymeric photoinitiators or mixtures of these with monomeric photoinitiators provide the best approach for ensuring compliance with Regulation (EC) No 1935/2004.

9.2 Further work

At present, it is very difficult to detect and identify the polymeric ink components, if there is no prior knowledge that they have been used. Further work could be carried out to develop suitable methods of analysis for set off measurements and migration testing in foods or food simulants for all the polymeric photoiniators and synergists in current use. Some of these pose significant difficulties in achieving the required selectivity and sensitivity for measuring set off. The CAD detector was found to be a useful detector particularly where UV and MS response was low, but other mobile phase and stationary phase combinations need to be investigated.

More could be done to establish the photolysis products of the photoinitiators in common use, so that these may be screened for in migration testing and set off measurements. In principle, the set off and migration values should be the sum of the starting photoinitiators and all its photolysis and decomposition products. Very little published work appears to have been done on this.

The stability of the photoinitiators in EU food simulants could be investigated so that this may be taken into consideration when comparing migration values with specific migration limits. The stability of individual ink components (that have not been considered in this report) needs to be measured in the proposed set off extraction solvents, iso-octane, 95 % ethanol and dioxane under the proposed test conditions. This needs to be in the likely set off concentration range and not at excessively high concentration.

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