Safety Assessment of Food Contact Materials: The Role of High-resolution Mass Spectrometry in the Comprehensive Analysis of the Total Migrate

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Overview

This white paper describes the issue of chemicals migrating into food from packaging materials, the need to assess the safety of those chemicals that migrate, and the role that high-resolution mass spectrometry has to play in the related analysis.

Introduction

Nearly all the food and drink we buy and then consume is packaged in some way. The main functions of food packaging are to protect and preserve the food, to maintain its quality and safety, and to reduce food waste. There can be a downside however, and this is the possibility of chemical contamination of the food via migration from the packaging. Food packaging materials are the most obvious example of the more general group of food contact materials (FCMs) and articles, which also includes food processing equipment, storage containers, kitchen utensils, etc that may come into contact with ("touch") the food.

There are many hundreds of substances used to make food packaging materials. For example, Table 1 summarises the number of substances in the various European inventory lists for different plastics and paper/board. A similar number and variety of substances are used worldwide in different geographical, economic and/or legislative areas. In addition to the main packaging materials, other products such as inks, adhesives and coatings (on metal substrates) are used to make the finished packaging. Note that there is duplication in these lists when substances are used by two or more sectors. Also, not all of these substances are currently in use today. Nevertheless, there are several hundreds of substances used to make today's food packaging materials.

Table 1. Number of substances in various inventory lists for different food packaging materials (European industrial sectors).

Plastics	Paper/board	Inks	Adhesives	Coatings on Metal
862	285	5721	370	592

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The many substances in FCMs are used in different ways; the two main classes are monomers and additives. Monomers, along with other starting substances, are used to make the packaging material. An example is styrene used to make polystyrene. There may be residual levels of unreacted styrene monomer in the polystyrene, but any such residue is incidental and the residual monomer does not serve any useful function in the polymer. In contrast, additives are used to serve a function in the packaging material and so they are present intentionally. In the polystyrene example, the polymer may contain a few hundred parts-per-million (ppm, mg/kg) of an antioxidant to protect the polymer, or even a small percentage (by weight) of mineral oil as a flow promoter. In addition to the main classes of monomers and additives, there are many other minor but still important classes such as catalysts, aids to polymerisation, processing aids, etc used. Whatever the purpose of the substances, there can be a transfer (also called migration) of these chemicals to the food when packaging materials touch the food. If they can migrate and so contaminate the food, then they all have to be assessed to ensure they pose no risk to consumer health. See Box 1.

Box 1. This white paper discusses food contact materials, including food packaging. The same challenges and technical solutions apply to the related fields of pharmaceutical packaging and materials used in fixed supplies for drinking water (e.g. pipes, fittings and the like). These fields have evolved as distinct disciplines with little, if any, scientific or technical crossover. This is unfortunate because, although the regulatory backgrounds do differ, the basic mass-transfer processes whereby contamination can arise are the same — in food packaging it is termed migration whereas for pharma and water it tends to be called *extractables* and/or *leachables*.

The Unwanted and the Unexpected

A proper safety assessment must go further than simply testing for known ingredients used to make FCMs. This includes an assessment of what have become known as non-intentionally added substances (NIAS). In Europe, Regulation (EU) No. 10/2011 on plastic materials and articles intended to come into contact with food includes the explicit requirement to assess the safety of all potential migrants, including the NIAS. These are the impurities, oligomers, degradation and/or reaction products of the intended ingredients. Substances not specifically regulated by name must be subjected to a risk assessment by the business operator according to internationally recognised scientific principles on risk assessment.

Detection, identification and quantification of the NIAS have been facilitated by significant advances in the capabilities of analytical instruments in the recent past (see Box 2). Gas chromatography-mass spectrometry (GC-MS) evolved into a powerful tool quite early on. More recently, the use of liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) has advanced for both targeted and non-targeted analysis of packaging materials, food simulants (model foods, see Box 3) and foodstuffs themselves. Mass spectrometry, especially HRMS, is now one of the preeminent tools for chemical analysis by food safety experts.

Box 2. Put simply, we are finding more and more, of less and less. Our analytical coverage is greater, and the sensitivity of the instruments that we use is greater too. This presents both opportunities and challenges.

Box 3. Simulants are simplified model foods intended to mimic the migration behaviour of real foods. Exact specifications and recommendations differ worldwide, but some generally-recognized food simulants are: 10% ethanol solution to mimic non-acidic aqueous foods, 3% acetic acid solution to mimic acidic foods, and vegetable oil, 95% ethanol or heptane to mimic fatty foods, among others.

Simulants were introduced at a time when instrumentation and analytical methods were not available to test foods for all the substances of interest at detection levels of mg/kg to µg/kg. Simulants also provide a means to test for broad food categories rather than having to test individual food items. Consequently, most routine tests conducted worldwide, and especially tests to demonstrate compliance with Regulations, use food simulants. However as methodology and instrumentation have advanced, our ability to measure migration into foods has evolved rapidly.

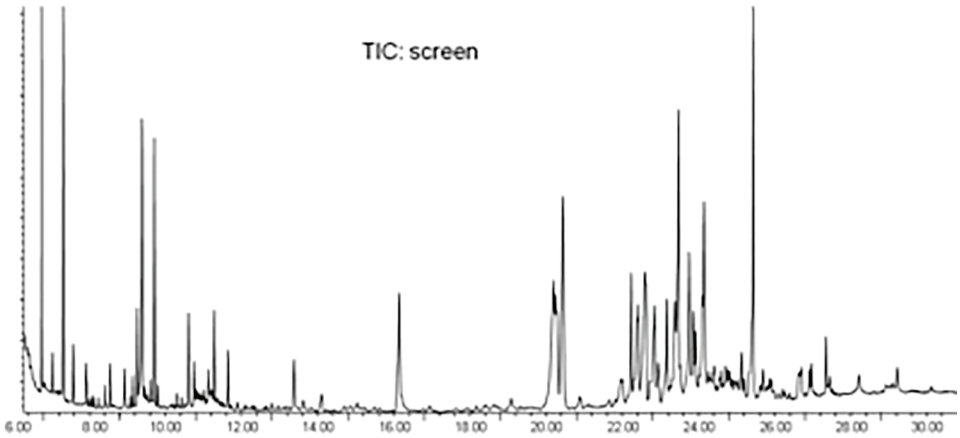

Figure 1. Typical NIAS study. GC-MS TIC of an ethanol extract of a cartonboard FCM.

Figure 1 shows a typical GC-MS total ion chromatogram (TIC) of a simple ethanol extract from a sample of cartonboard used for food packaging. It shows a large number of peaks. Very few (if any?) of these peaks correspond to chemicals used in papermaking. So what are they? How does the responsible producer of the cartonboard or the responsible user (the food packer) check that they are all safe?

There is an important legal distinction between the intentionally added substances (IAS) and the NIAS, viewed in the context of existing EU Regulations on plastics in particular but also the general provisions of the Framework Regulation (EC) No. 1935/2004 that covers all types of materials used in contact with foods (see Table 2).

Table 2. Typical classes of IAS and NIAS.

IAS: Intentionally Added Substances	NIAS: Non-intentionally Added Substances
Monomers & other starting substances	Impurities
Additives	Degradation products
Polymer production aids	Oligomers
Aids to polymerisation	Reaction intermediates
Colourants	By-products
Solvents	Contaminants
Pre-polymers	

From a risk assessment viewpoint, the distinction is a moot one. Attention has focussed increasingly on the NIAS in the last few years, but the requirement to evaluate all substances that migrate from FCMs is certainly not a new idea. Over 20 years ago, in a letter to the journal Nature entitled 'Multiplicity of migrants', Katan observed that scientific resources were being needlessly wasted with the emphasis only on those substances used rather than those that actually migrate.[1]

An assessment of the NIAS should have three components:

- Identification of the substances present in the material
- Estimation of their migration level leading to an estimate of possible consumer exposure
- Risk assessment which considers the potential human exposure to any hazard (nature and potency) posed by the chemicals

This process can be further subdivided, for convenience, into 8 steps:

1. Prediction
2. Detection
3. Identification
4. Migration levels
5. Consumer exposure
6. Hazard characterisation
7. Risk assessment
8. Assessment (yes/no) and conclusions

Each of these steps will be summarised in turn with amplified (but still simplified and abbreviated) sections specifically on the role of HRMS.

Step 1. Prediction

We have to accept the fact that despite how clever we think we are as chemists, our ability to predict the formation of NIAS will never be complete. For some additives, such as phenolic or phosphite antioxidants, the major transformation products may be predictable. For simple condensation polymers such as polyesters, the main oligomers are predictable. However, for more complex reactions such as free-radical polymerisations, the low molecular weight oligomers can be far more complex. Even for simple phenolic antioxidants, the free radical pathways (including scission, recombination, disproportionation, etc.) can give rise to a plethora of reaction products that are difficult to rationalise even post-hoc let alone to predict in advance.

As well as transformation products, non-reactive impurities in the starting substances may persist and migrate, which can amplify their importance. So 99% or even 99.9% purity of an original substance may still give rise to impurities recognised as NIAS with significant migration potential.

A level of interest in the parts-per-billion (ppb, µg/kg) concentration range means needing to understand reaction pathways that may have only a very low yield - perhaps as low as 0.001%. Reactions may be energetically unfavourable, mechanistically complex, difficult or impossible to predict, and yet could be "significant". A parallel can be drawn with food chemistry where the initial discovery of, for example, ethyl carbamate in distilled spirits, 3-monochloropropandiol in processed foods and, most famously, acrylamide in heat-treated foods, was a complete and shocking surprise.

Step 2. Detection

There is a wide range of powerful analytical techniques available for the detection of the NIAS.

- GC-MS with headspace- (HS) or purge-trap sampling for volatiles
- Solvent extraction followed by GC-MS for semi-volatiles
- LC-MS for polar and non-volatile substances
- Inductively coupled plasma (ICP)-MS for trace elements

Investigation would normally start with analysis of the FCM itself, or a solvent extract of it, since the substances exist there in higher concentrations than in foods or food simulants after migration. Screening tests can make use of a non-selective detector such as the flame ionisation detector (FID) fitted to GC or an ultra-violet detector (UV) coupled to an LC, but most laboratories prefer to use the extra selectivity and sensitivity offered by MS-based detection. This is non-targeted analysis so optimisation of the analytical conditions is a compromise; it is not known which chemicals may be present. This calls for use of, for example, generic LC conditions with a C18 column and a simple low-to-high organic solvent gradient. Similarly, whereas electron ionisation and positive ion monitoring are fairly reliable for GC-MS analysis, the atmospheric pressure ionisation in LC-MS - such as electrospray (ES) or atmospheric pressure chemical ionisation (APCI) — is quite variable. Extracts should be tested with both positive and negative ionisation modes to detect substances with different chemical properties. Although modern instruments are very sensitive and the detection of a range of NIAS is quite straightforward, it is still rather difficult to prove that the suite of techniques is fully comprehensive and substances have not been missed. This difficulty has implications in making declarations of compliance.

Step 3. Identification

With the use of MS, the same techniques used for the detection of NIAS can also give information leading to the identification of those NIAS. For example, the electron impact mass spectra from GC-MS analysis can be compared with library spectra. Unfortunately, although the commercial MS libraries contain many thousands of entries, they have significant limitations in the area of FCM substances due to a lack of coverage. As an aside, it might also be mentioned that the skill of interpreting a mass spectrum - from first principles to elucidate the structure of an unknown — seems to have been largely lost with the dependence on commercially available libraries. Many laboratories are investing in HRMS instruments such as GC-HRMS (Thermo Scientific™ Orbitrap™ or time-of-flight) for tasks such as pesticide residue analysis. Such instruments can also be used to help in the identification of NIAS.

For the identification of NIAS that originate as impurities in the ingredients used to formulate the FCM, analysis of the ingredients help to pinpoint the origin of the NIAS. The NIAS will still need to be identified, but knowing the origin can help by suggesting possible structures that fit the MS data obtained.

The limitations of LC-MS techniques are well known. The spectra obtained often show little diagnostic fragmentation unless steps are taken to induce in-source fragmentation in single-stage HRMS instruments or use of the collision cell in quadrupole hybrid instruments. The dependence of fragmentation on the instrument settings means that the spectra vary from instrument to instrument, hindering the sharing of library spectra. Many analysts therefore use the accurate mass measurement of the pseudomolecular ion. The accurate mass information for the NIAS in/from the FCM can be compared with a user-prepared database that should also contain the oligomers and reactions products predicted from the known ingredients and their impurities. This often requires close partnerships with manufacturers and their suppliers. It also requires a good level of chemical knowledge coupled with as much detailed information as possible on the formulation details and the manufacturing process of the FCM.

If the accurate mass determined for a given NIAS peak in the chromatogram is not in the user-prepared database, then the HRMS software proposes molecular formulae for each of the accurate masses detected. The number of acceptable fits depends on the mass resolution of the instrument. Even with the best instruments, it is normal for several possible formulae to be an acceptable match with the experimental accurate mass. Deciding on which is the most likely formula requires insight and judgement. Interrogation of the HRMS data such as isotope patterns and any fragments present, as well as the accurate mass of adducts formed, leads to the best empirical molecular formulae. The formulae can then be searched against internet databases too, e.g. ChemSpider, to allow identities for the NIAS to be proposed. But again, the empirical formulae may give many possible structures, so further detective work and rationalisation of the more likely structure(s) is necessary. This can be very time-consuming if the analysis detects many NIAS peaks — it is not uncommon to be faced with 200 or more — if every peak in the chromatogram is considered at first.

Step 4. Migration levels

Where the identity of a migrant/extracted substance is known and an analytical standard can be purchased or synthesised, the concentration can be determined in the normal way. If however, a standard is not available or the identity remains unknown, other approaches have to be taken for quantitation.

a) Using a universal detector in which the response of all substances is essentially the same as the internal standard(s) used. The classic example is the FID. However, HS-GC analysis for volatiles is complicated by volatilisation/vapour phase equilibration issues.

b) For detectors that do not give a uniform response, this approach can still be adopted but the consequent uncertainty in the concentrations must be reported. For example, using LC-HRMS it would be necessary to assume that different substances have the same ionisation efficiencies (as each other and as the standard(s) used to compare the response), and that the response of a given substance in negative ionisation mode is the same as that in positive ionisation mode (when comparing to a standard that only responds in one ionisation mode).

c) If the NIAS is known to contain a chemical feature, or if the industry is wanting to estimate all NIAS derived from a known starting substance, the chromophore of that substance — if present — (e.g. a phthalic acid moiety or a bisphenol A (BPA) moiety) can be used. The LC-UV or LC-fluorescence (FLD) chromatogram can then be interpreted accordingly using, e.g. dimethyl terephthalate or BPA as the calibrants.

d) Another approach is to determine the overall migration or the total solvent extractables from the packaging material, and then subject the residue from that experiment to gel permeation chromatography (GPC) analysis. Determine the low molecular weight fraction (LMWF) of interest (i.e. below 1000 Daltons), and then assign that proportionately to all the NIAS present in the sample. As a simple example, if the overall migration value is 10 mg/kg, the LMWF is 50% w/w, and analysis by LC-HRMS detects four substances in the total ion chromatogram in the ratio 1 : 3 : 4 : 2, then the concentrations would be calculated to be 0.5, 1.5, 2 and 1 mg/kg respectively. The same assumptions and associated uncertainties given above in (b) would have to be declared in the test report.

Step 5. Consumer Exposure

The extent of human dietary exposure should determine the nature and amount of toxicity data needed to establish safety-in-use. A useful guidance document on the assessment of exposure to substances migrating from FCMs was published by ILSI Europe.[2] Most recently, good progress has been made on soundly-based exposure models using migration levels, food consumption statistics and packaging usage factors with the Flavourings, Additives, and food Contact materials Exposure Tool (FACET).[3,4] Depending on the nature and origin of a NIAS, it may not be possible to assume or to prove the material under test is the only source of exposure. In those cases, a very refined estimation of exposure could be misleading, and it would be better to make a more conservative estimate.

Step 6. Hazard characterisation

Many, and probably most of the NIAS found will not have an existing health-based reference value such as a specific migration limit (SML) or a tolerable daily intake (TDI) value. Linked to that, for most NIAS there will not be experimental toxicity data available. The question arises: is experimental data on chemicals always needed; is it always feasible — or even justified — taking account of time, costs, resources, the use of animals, etc? A useful and scientifically sound approach for the NIAS is to apply the threshold of toxicological concern (TTC) approach. Constituents can be divided into classes based on structure. Those substances having structural alerts for genotoxicity, or for which structural identity is uncertain, could be evaluated using the lowest threshold. The other structures can be assigned to one of the three Cramer Classes and an acceptable threshold level of exposure can be assigned for that substance (see Table 3). For more information, including the recommendation to merge Cramer Classes II and III, see the EFSA guidance document on application of the TTC concept.[5]

Table 3. Some example TTC values.

Category	Threshold in µg/person/day
Genotoxicity Alert[6]	0.15
FDA Threshold of Regulation[7]	1.5
Cramer Structural Class III[5]	90
Cramer Structural Class II	540
Cramer Structural Class I	1800

Step 7. Risk assessment

The estimate of exposure is compared with the health-based reference value. Frequently, analysis reveals a series of related substances such as hydrocarbon oligomers from polymers. If it is considered possible that they exert any toxicity by a common mode of action, then the estimates of exposure can be summed and the resulting sum compared with the reference value.

Step 8. Assessment (yes/no) and conclusions

The full elucidation and evaluation of the NIAS remains problematic, but the analytical tools available nowadays help greatly in the process. The challenge of identifying the NIAS was the topic of a recent mini-review[8] by university researchers in Zaragoza, Spain. The review revealed that working closely with the industry and analytical instrument manufacturers greatly helps the detection, quantification and identification of the NIAS. Consensus is needed so there can be general agreement between regulators, control authorities and industry on how far a responsible industry needs to go to fulfil the requirements for product safety. Brand owners are looking for better partnerships to share more technical information on the composition of packaging materials, thereby allowing early identification and elimination of chemical contaminants such as the NIAS.

Analytical Method Example

Testing for NIAS: A Polyester Can Coating

Procedure: Investigation would normally start with analysis of the packaging material itself, or a solvent extract of it, since the substances exist there in higher concentrations than in the food or food simulants after migration. Identification of the potential migrants is achieved through the application of a suite of analytical methods, focussing on the analysis of substances with molecular weight below 1,000 Dalton. This molecular weight cut-off is chosen in view of toxicological significance — larger molecules tend not to be absorbed in the stomach or the gastrointestinal tract. Analysis of a packaging material is made using:

- GC-MS with headspace- or purge-trap sampling for volatiles
- Solvent extraction followed by GC-MS for semi-volatiles
- Solvent extraction followed by LC-MS for polar and non-volatile substances
- Acid digestion followed by ICP-MS for trace elements

The following outlines the procedure used for one of these 4 complementary approaches: using LC-MS. Samples of tinplate with a polyester coating are cut into 0.5 × 0.5 cm pieces and randomized. Samples (200 cm^2, in triplicate) are extracted by total immersion in acetonitrile (100 mL) for 18 hours at room temperature. The extract is concentrated ten-fold under a gentle stream of nitrogen gas at 40 °C. A procedural blank (solvent alone, no coated panel) and coated panels overspiked with an oligomer standard at 1.7 µg/100 cm^2 are prepared in the same way. The extracts are analysed by LC-HRMS using a C18 column, a water/methanol gradient, and with the HRMS operated in positive and then negative mode electrospray to maximise detection coverage.

Results

The TIC of the extract run in positive ionisation mode is shown in Figure 2. Simple visual inspection showed a large number of peaks with much co-elution. The data was processed using proprietary qualitative software for substance identification revealing more than 200 chemical entities.

For quantification, estimated concentrations were calculated by comparison of extracted ion chromatogram (EIC) peak areas of the substances detected to the peak area of the representative oligomer standard overspiked into the acetonitrile extracts. In the absence of authentic standards, this approach assumes equal substance response. See Box 4.

Box 4. The details of data processing depend on the exact vendor software, but all follow similar aims if not the exact same approaches. Following HRMS analysis, the data generated are processed using software that employs algorithms to automatically identify all the detectable substances or molecular features in accurate mass data, even when analysing very complex mixtures. This generates a list of molecular features with retention time, neutral mass and ion abundance. All of the related ions of a molecular feature (isotopes, charge states, adducts and multimers) are grouped together, and areas of noise are removed. Then the possible molecular formulae for the HRMS peaks are proposed using accurate mass, isotope spacing, and mass peak abundance information to decrease the number of potential formulae generated. These are then listed in order of likeliness using a scoring system.

The accurate mass information for the substances detected is compared with a user-prepared database from the analysis of substances used in the manufacture of the coating (including the impurities); this requires working together with manufacturers and their suppliers. The user-prepared database can also contain the oligomers and reactions products predicted from the known ingredients and their impurities. This requires a good level of chemical knowledge coupled with as much detailed information as possible on the formulation details and the manufacturing process.

If the accurate mass determined for a given peak in the chromatogram is not in the user-prepared database, then the LC-HRMS software proposes molecular formulae for each of the accurate masses detected. The number of acceptable fits depends on the mass resolution of the instrument. Even with the best instruments, it is normal for several or even many possible formulae to be an acceptable match with the experimental accurate mass. Deciding on which is the most likely formula requires insight and judgement.

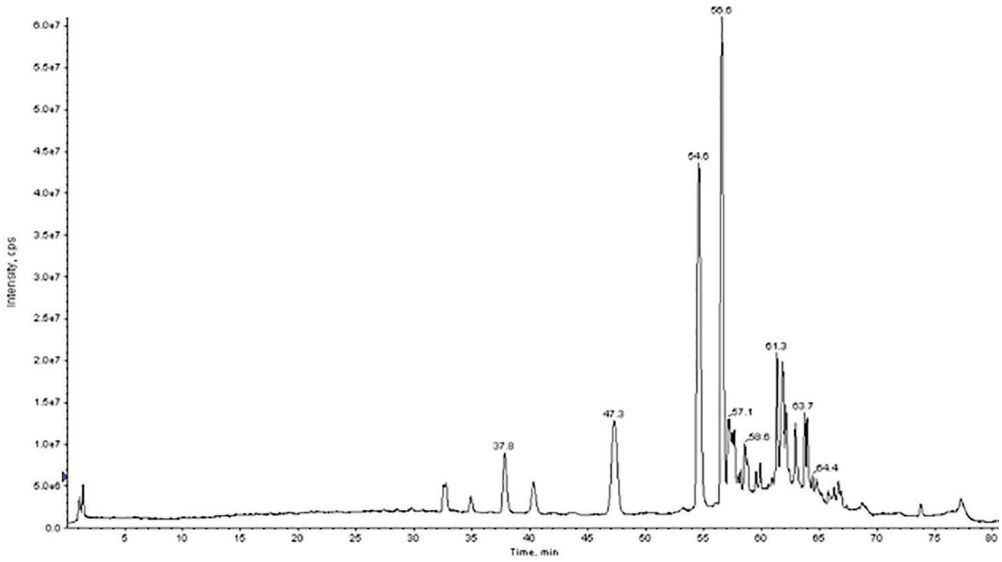

Figure 2. LC-HRMS total ion chromatogram for a solvent extract of a polyester coating.

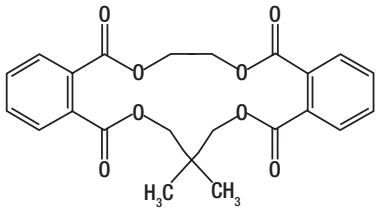

Figure 3. Proposed structure for the peak at a retention time of 47.3 minutes in Figure 2.

Interpretation:

Taking the peak at retention time 47.3 minutes as an example (Figure 2) from the mass spectrum of this peak, the three most diagnostic ions observed were at masses 444.1648 (assigned $[M+NH4]^+$), 449.1203 ($[M+Na]^+$) and 875.2507 ($[2M+Na]^+$). From this the mass of the substance measured was 426.1303. ChemCalc (http://www.chemcalc.org/mf_finder) found 49 formulae within 3 ppm of the experimental accurate mass of 426.1303. Eliminating the possibility of halogens (leaving just CHNO) still gave 4 possibilities: $C_{21}H_{20}N_3O_7$, $C_{20}H_{14}N_{10}O_2$, $C_{22}H_{16}N_7O_3$ and $C_{23}H_{22}O_8$. Eliminating the possibility of nitrogen left just the last. ChemSpider (http://www.chemspider.com/Search.aspx?) found 136 known structures for $C_{23}H_{22}O_8$. Knowing that the sample was a polyester and knowing the ingredients, allowed the structure in Figure 3 to be assigned.

As in this example, it is not unusual to find 200-400 ‘peaks’ (unique species) in LC-HRMS analysis of a food contact material extract. Clearly attention must focus first on the largest peaks but a full elucidation can be very time consuming. Although modern instruments are very sensitive and the detection of a range of NIAS is quite straightforward, it is in contrast rather difficult to prove that the suite of techniques is fully comprehensive and that no substance or class of substance has not been missed. This has implications in making declarations of compliance; namely the impossibility of proving a negative - the absence of any substance that is either hazardous or is simply not authorised on a positive list of permitted ingredients.

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