ILSI Europe
Report Series

Guidance on Best Practices on the Risk Assessment of Non Intentionally Added Substances (NIAS) in Food Contact Materials and Articles

Report

ILSI Europe
International Life Sciences Institute

Commissioned by the ILSI Europe Packaging Materials Task Force

About ILSI / ILSI Europe

Founded in 1978, the International Life Sciences Institute (ILSI) is a non-profit, worldwide foundation that seeks to improve the well-being of the general public through the advancement of science. Its goal is to further improve the understanding of scientific issues relating to nutrition, food safety, toxicology, risk assessment, and the environment. ILSI is recognised around the world for the quality of the research it supports, the global conferences and workshops it sponsors, the educational projects it initiates, and the publications it produces. ILSI is headquartered in Washington, DC.

The European branch ILSI Europe was established in 1986. ILSI Europe fosters collaboration among the best scientists to provide evidence-based scientific consensus in the areas of nutrition, food safety, consumer behaviour and sustainability. ILSI Europe aims to build multi-stakeholder science-based solutions for a sustainable and healthier world. To deliver science of the highest quality and integrity, scientists collaborate and share their unique expertise in expert groups, workshops, symposia and resulting publications.

All ILSI Europe activities are conducted under the supervision of the Scientific Advisory Committee. Composed by a maximum of 20 experts with more than 50% coming from the public sector, the Scientific Advisory Committee plays an important role in reviewing all activities with respect to their scientific validity and coherence with ILSI Europe's programme. The Scientific Advisory Committee also provides scientific advice to the Board of Directors which must be composed of at least 50% public sector scientists, the remaining directors representing ILSI Europe's member companies.

This publication is made possible by support of the ILSI Europe Packaging Materials Task Force. Industry members of this task force, as well as the composition of the Board of Directors and the Scientific Advisory Committee are listed on the ILSI Europe website at www.ilsi.eu.

ILSI Europe
International Life Sciences Institute

GUIDANCE ON BEST PRACTICES ON THE RISK ASSESSMENT OF NON-INTENTIONALLY ADDED SUBSTANCES (NIAS) IN FOOD CONTACT MATERIALS AND ARTICLES

By Koster Sander, Bani-Estivals Marie-Hélène, Bonuomo Maurizio, Bradley Emma, Chagnon Marie-Christine, Garcia M. Leonor, Godts Françoise, Gude Thomas, Helling Rüdiger, Paseiro-Losada Perfecto, Pieper Gabriele, Rennen Monique, Simat Thomas, Spack Lionel.

REPORT

COMMISSIONED BY THE PACKAGING MATERIALS TASK FORCE

Published in July 2015
Version January 2016

For more information about ILSI Europe, please contact

ILSI Europe a.i.s.b.l.
Avenue E. Mounier 83, Box 6
B-1200 Brussels
Belgium
Phone: (+32) 2 771 00 14
Fax: (+32) 2 762 00 44
E-mail: info@ilsieurope.be
www.ilsi.eu

Printed in Belgium

D/2015/10.996/39

ISBN: 9789078637424

ISSN: 2294-5504

CONTENTS

Authors: Sander Koster, Nestlé (CH), Marie-Hélène Bani-Estivals, Danone (FR), Maurizio Bonuomo, Barilla G&R Fratelli (IT), Emma Bradley, FERA Science (UK), Marie-Christine Chagnon, University of Burgundy (FR), M. Leonor Garcia, The Coca-Cola Company *, Françoise Godts, Dupont de Nemours (BE), Thomas Gude, Swiss Quality Testing Services (CH), Rüdiger Helling, Saxon Institute for Public and Veterinary Health (DE), Perfecto Paseiro-Losada, University of Santiago de Compostela (ES), Gabriele Pieper, Tetra Pak (DE), Monique Rennen, TNO (NL), Thomas Simat, Technical University Dresden (DE), Lionel Spack, Nestlé (CH).
Coordinator: Jeroen Schuermans, ILSI Europe (BE).

* Chair of the Packaging Materials Task Force

1. INTRODUCTION

Food contact materials and articles are made of base materials, for example, plastics, metals or paper to which other materials might be added for different purposes, for example, adhesives, coatings, and printing inks to glue, protect, and impress base materials. Food contact materials are defined as the elements of objects and materials intended to come into direct or indirect contact with foodstuff, while food contact articles are defined as objects, being equipment, containers, packaging and various utensils which are clearly intended to be used for the manufacture, preparation, conservation, flow, transport or handling of foodstuffs or which are presented as such (Belgian Royal Decree, 1992). Food contact materials and articles are manufactured from chemicals intentionally used along the production process. These intentionally added substances (IAS) are essential in the manufacturing or use of the food contact material and article since they enhance, for example, the manufacturing, food contact material and article stability and/or mechanical properties or increase the shelf life of the packaged food. Examples of IAS are monomers, prepolymers, antioxidants, lubricants, and impact modifiers. In addition to these substances of known origin, the food contact materials and articles may contain substances that are non-intentionally added (NIAS) with sometimes unknown origin, for example, impurities present in the IAS or by-products created during the synthetic process. A non-exhaustive overview of the literature of NIAS is given in Appendix 1. Food contact materials and articles can therefore be considered to be materials containing a complex mixture of substances with known or unknown identity/origin. Food contact materials and articles are abbreviated as FCM in this guidance.

Depending on the physical/chemical parameters and the chemical composition of the FCM, and on the nature of the food, FCMs and articles may transfer their constituents (both IAS and NIAS) to foods. This mass transfer phenomenon is called migration. Migration may lead to too high exposure to certain chemicals, which might cause a risk for human health and so it must be evaluated and controlled. Furthermore, where migration brings about an unacceptable change in the composition of the food or brings about deterioration in the organoleptic properties of the food, this must be avoided.

In order to ensure that the use of FCMs and articles is safe, general requirements have been set up in the European Union (EU) in Regulation EU 1935/2004 on materials and articles intended to come into contact with food (known as the Framework Regulation) (EC, 2004). Additionally and referring to the above, the Regulation EU 2023/2006 on Good Manufacturing Practice for materials and articles intended to come into contact with food gives a general framework to ensure that the products at all stages of the manufacturing process of the FCMs and articles are consistently produced so that there is limited influence on the packaged food and that the safety of the consumers is ensured in the end (EC, 2006). The Framework Regulation allows for specific measures for different groups of materials (plastics, paper and board, metals and alloys, adhesives, printing inks, etc.) to be adopted at EU level. While there is indeed a specific measure for plastics, this is not the case for non-plastic FCMs (EFSA, 2012a) such as paper and board, rubbers, metals and alloys, coatings, adhesives, and printing inks. Some of those application fields are (partly) regulated by national legislation.

There are also specific EU Regulations dealing with certain substances, such as Regulation EU 1895/2006 on the restriction of use of certain epoxy derivatives in materials and articles intended to come into contact with food (EC, 2005), and Commission Directive 93/11/EEC concerning the release of N-nitrosamines and N-nitrosatable substances from elastomers or rubber teats and soothers (EEC, 1993). Regulation EU 10/2011 on plastic materials and articles intended to come into contact with

food is the first Regulation that introduces the term NIAS and clearly mentions the obligation for the manufacturer to conduct their risk assessment (Article 19) (EU, 2011). However, specific guidelines are not referred to.

Until recently, the term NIAS was not used in European legislation for non-plastic FCMs. Since NIAS not only occur in plastics but may also be present in non-plastic FCMs such as paper/board, coatings, metals, cork, etc, the term NIAS used in this document is assumed to be applicable for all types of FCM and not only for plastics.

The intention of this monograph is to provide a range of recommendations and a guideline to assess the safety of NIAS in all FCMs. To do so, this monograph will cover the following points:

- A definition/description of NIAS and IAS. The Regulation EU 10/2011 gives a definition of NIAS but it is not always straightforward or easy to classify substances of different origin (EU, 2011). This document intends to provide a guideline and practical examples that enable the classification of substances as NIAS or IAS.
- A reminder about good manufacturing practices, where the correct selection of raw materials and application of efficient processes could drastically decrease the presence of NIAS and lead to a better understanding of any NIAS that are formed during manufacture.
- Requirements for the exchange of information and sharing responsibilities along the supply chain.
- Approaches to predict the occurrence of NIAS in FCM. These approaches may help to predict NIAS or to determine the origin of a detected NIAS.
- Tools to determine NIAS in FCMs. Different approaches to determine NIAS have been described in the scientific literature.
- Strategies on how to assess the safety of NIAS. Some of the current approaches to assess the safety of NIAS have practical, ethical or economical drawbacks. As a result, alternative approaches may be sought. A guideline on how the safety of NIAS can be assessed is presented.

This monograph considers the state-of-the-art at the time of its preparation. As technologies continue to develop and legislation evolves, the reader should ensure that these changes are considered when risk assessing these substances. It should be noted that the intention of this monograph is not to provide guidance to detect, identify, and risk assess each and every NIAS present in the FCM but to provide a practical solution to assess their safety.

2. NIAS AND IAS: DEFINITION, CATEGORISATION AND EXAMPLES

2.1 General description of NIAS

Plastic materials and articles intended to come into contact with food are regulated by Regulation EU 10/2011. This Regulation comprises requirements for IAS and NIAS. For plastic FCMs and articles, the EU developed legislation is based on the principle of a 'positive list'. Annex 1 of Regulation EU 10/2011 contains the Union list of authorised monomers, other starting substances, macromolecules obtained from microbial fermentation, additives, and polymer production aids. These listed substances can be used to manufacture plastic materials, with the restrictions and specifications established in the list. This list is a closed list for monomers, starting substances, and additives. However, other substances like polymer production aids which are not included in Annex I may be used.

Many different interpretations and meanings have been used for the term NIAS. To be as clear and consistent as possible, the legal definition as given in Regulation EU 10/2011 will be adhered to in this document and the logic of the definition as described shall be applied to other FCMs beside plastics (EU, 2011). This document provides practical examples and guidance, and aims to give some practical advice to classify substances according to the legal NIAS definition.

In Article 3 of Regulation EU 10/2011, NIAS are defined as follows: "'*non-intentionally added substance' means an impurity in the substances used or a reaction intermediate formed during the production process or a decomposition or reaction product*" (EU, 2011).

Next to the regulatory definition of NIAS in Article 3 of Regulation EU 10/2011, some specific wording with regard to the presence of NIAS can be found in recital (18) and (20) as such, and should be considered in context with the definition itself:

Recital (18): "*Substances used in the manufacture of plastic materials or articles may contain impurities originating from their manufacturing or extraction process. These impurities are non-intentionally added together with the substance in the manufacture of the plastic material (non-intentionally added substance – NIAS). As far as they are relevant for the risk assessment the main impurities of a substance should be considered and if necessary be included in the specifications of a substance. However it is not possible to list and consider all impurities in the authorization. Therefore they may be present in the material or article but not included in the Union list*".

Recital (20): "*During the manufacture and use of plastic materials and articles reaction and degradation products can be formed. These reaction and degradation products are non-intentionally present in the plastic material (NIAS). As far as they are relevant for the risk assessment the main reaction and degradation products of the intended application of a substance should be considered and included in the restrictions of the substance. However it is not possible to list and consider all reaction and degradation products in the authorisation. Therefore they should not be listed as single entries in the Union list. Any potential health risk in the final material or article arising from reaction and degradation products should be assessed by the manufacturer in accordance with internationally recognised scientific principles on risk assessment*".

NIAS have to comply with the general safety requirements of Article 3 of Regulation EU 1935/2004 (EC, 2004) and are subject to a risk assessment by the business operator in accordance with Article 19 of Regulation EU 10/2011 (EU, 2011).

In the scope of this document and in addition to the above mentioned definition, certain categories of contaminants in FCMs linked to the production of the finished article should also be covered by the term NIAS. Contaminants are also non-intentionally added and need to be risk assessed if they have the potential to migrate.

Contaminants can be divided into two categories and are also considered to be NIAS:

- Process contaminants such as lubricants and contaminants from storage/transport. These are mostly known but the levels are typically unknown.
- Unknown and often unpredictable environmental contaminants that are adventitiously picked up by the FCM.

Oligomers are a special class of migrants, which might be known or unknown to the manufacturer and are mostly unknown to the user. The oligomers are tackled in more detail under point 2.3.

NIAS are also tackled in the Dutch Packaging and Utensils Decree[1], dated March 14th 2014. In a further revision of this decree, there will be a chapter dealing with the risk assessment of NIAS based on the threshold of toxicological concern (TTC) (Kroes *et al.*, 2004).

In terms of risk assessment, in most cases, only NIAS up to a molecular weight (MW) of 1000 Daltons (Da) have to be considered. This threshold of 1000 Da is important as the European Food Safety Authority (EFSA) has conventionally assumed in its assessments of plastics starting materials that above this molecular weight, substances are not absorbed by the body and therefore may be excluded from any calculations of migration and exposure (EFSA, 2008). Therefore, in the scope of this monograph, NIAS means only the part of NIAS which have a molecular weight below 1000 Da. It should however be noted that NIAS above this molecular weight can be a major part of the migrate (Grob *et al.*, 2006).

NIAS may also be formed as a result of an interaction between constituents of the FCM and the constituents of the food. To date, this has not been explored in detail, and is therefore not covered by this guidance document.

2.2 General description of IAS

In contrast to NIAS, IAS are specifically added during the production process of FCMs and have or had a function in either the manufacturing process or in the final product. Starting substances and monomers used to build the polymer, the main structural component of, for example, a plastic and a coating as well as additives, solvents, polymer production aids, aids to polymerisation, colourants and so on are considered to be IAS. On the other hand, raw chemicals used to synthesise additives or monomers are not IAS in the meaning of this monograph. The same principle applies to natural materials. Cotton fibres can be a starting substance but chemicals used to produce the cotton (to stimulate growth of the plant or to protect the plant) are NIAS despite the fact that they were intentionally added by the farmer. In addition, intentionally adding an IAS which contains known impurities does not cause the impurities to become IAS.

1. https://zoek.officielebekendmakingen.nl/stcrt-2014-8531.html

Referring to the plastic FCMs, Annex 1 of Regulation EU 10/2011 contains approximately 900 substances (IAS), being monomers, other starting substances, and additives (EU, 2011). The EFSA 'Note for Guidance' (EFSA, 2008) describes the general procedure for the authorisation of new substances to be included in this Annex. It differentiates between 'defined substances', 'defined mixtures' (mixtures obtained from a reproducible process with a limited number of components) and 'non-defined mixtures' (mixtures from natural sources, e.g. edible oils, rosin esters, and mixtures from reproducible processes showing a varying composition depending on the manufacturing process, e.g. diisononylphthalate, ethoxylated substances (EFSA, 2003). However, this monograph divides 'substances' into two main groups in a similar way to that used by both the European Chemicals Agency (ECHA, 2012) for Registration, Evaluation and Authorisation of Chemicals (REACH) substances, and the Environmental Protection Agency (EPA).

1. Well-defined substances:
   Substances with a defined qualitative and quantitative composition that can be sufficiently identified based on several chemical and physical parameters. Data on these substances are searchable in many computerised databases. In addition to mono-constituent substances (e.g. Ref. N°. 13480 Bisphenol-A, see below), multi-constituent substances (e.g. Ref. N°. 22331 Mixture of 1,6-diamino-2,2,4-trimethylhexane and 1,6-diamino-2,4,4-trimethylhexane) and substances defined by more than the chemical composition (e.g. Ref. N°. 58320 graphite, or nano- and non-nano substances) also belong to 'Well-defined substances'.
   From the ca. 900 substances listed in Annex I of the Regulation EU 10/2011, over 500 substances belong to this group of well-defined substances. Usually the complete information (physical and chemical properties, analytical details) for these substances is clear and easy to implement.

2. Substances of unknown or variable composition, complex reaction products or biological materials (UVCB Substances)[2]:
   Substances in this group comprise macromolecules (e.g. cellulose, Ref. N°. 14500), extracts from biological materials (e.g. carnauba wax, Ref. N°. 42720) and fermentation products (e.g. albumin, Ref. N°. 12310). UVCB may also comprise fractions or distillates (e.g. white mineral oils, Ref. N°. 95883), minerals (e.g. bentonite, Ref. N°. 37820), and complex reaction products (e.g. acids, fatty, unsaturated (C18), dimers, non-hydrogenated, distilled and non-distilled, Ref. N°. 10599, see below). While the variability of composition for 'well-defined substances' is specified by the upper and lower limit of the concentration range of the main constituents, for UVCB substances, the variability is relatively large and poorly predictable (ECHA, 2012).
   More than 300 UVCB substances are included in Annex 1 of the Regulation EU 10/2011. Complete information on the composition of these UVCBs is missing.
   For such complex mixtures, concepts such as 'pure chemical substance' and 'impurities' are diffuse and difficult to implement. Therefore, UVCB substances require other information with regard to the identification of their constituents. This may be achieved by using well-known reference samples or standards. Alternatively, a potential tool for generic information on the composition can be obtained by preparing chromatographic or spectroscopic fingerprints of the UVCBs.

2. The term UVCB is referred to in several legislative contexts: EPA, Office of Chemical Safety and Pollution Prevention, Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials: UVCB Substances. http://www.epa.gov/oppt/existingchemicals/pubs/tscainventory/uvcb.pdf; OECDE, Organisation for Economic Co-operation and Development (2014), Guidance on Grouping of Chemicals, 2nd edition, Series on Testing & Assessment, N°. 194. ENV/JM/MONO(2014)4. (http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2014)4&doclanguage=en)

Some well-defined substances and/or UVCBs are not included in the Union list but are authorised for use, and considered intentionally added. Among these are prepolymers, if the monomers or starting substances required to synthesise them are included in the Union list. Most of these prepolymers can be considered to be UVCB substances, such as phenolic resins, etc. The nature of these compounds remains difficult to determine for downstream users and the detection and identification of these chemicals in the plastic material can become a difficult task and may need risk assessment in case of migration into food or food simulants.

Of course, substances may also migrate from non-plastic FCMs. This section is therefore also applicable to non-plastic FCMs in an analogous way.

2.3 Examples of IAS and NIAS in plastic and non-plastic FCMs

2.3.1 Examples of IAS and NIAS in plastic materials

- Bisphenol-A is used as a monomer for the manufacture of plastics. The starting substances for the synthesis of Bisphenol-A are acetone and phenol and these may still be present as impurities in the Bisphenol-A used in the manufacture of the polymer. Since Bisphenol-A is regarded and listed as the monomer (starting substance), any remaining impurities of phenol and acetone as well as side reaction products of the Bisphenol-A synthesis are all considered to be NIAS. Although both acetone and phenol are listed within Regulation EU 10/2011 (EU, 2011), in this particular case, they are considered to be NIAS according to recital (18), as the starting substance used for the production of the polymer is Bisphenol-A. However, independent from these considerations (NIAS or IAS), the specific migration limits (SMLs) of acetone and phenol have to be respected.
- When trace levels of phenol are detected in a polymer made of the monomers phenol and formaldehyde, phenol is considered here to be an IAS, as it is the starting substance for the polymerisation process.
- Beside impurities, certain monomers, starting substances and additives need to be stabilised to prevent reaction or oxidation of the pure substance during storage. These stabilisers are intentionally added but not necessarily listed in the Union list. In applications for authorisation of monomers, starting substances and additives, the necessary stabilisers should be mentioned.
- Degradation products of additives are considered to be NIAS, even if they are an essential part of the effectiveness of the additive. For example, the oxidation product of an anti-oxidising agent is a NIAS (see also more detailed examples in Tables 1 and 2).
- In the sense of this document, oligomers are those low molecular weight fractions which are an integral part of the polymer formed during the polymerisation reaction.

"Oligomers" are defined here as substances consisting of a finite number of repeating units. Only oligomers which have a molecular weight of less than 1000 Da are considered here due to the very limited resorption of larger molecules. There are currently two categories of oligomers:

1. Oligomers that are an integral part of the polymer formed during the polymerisation reaction. These oligomers are often formed during a polymerisation reaction between monomers; they might contain side reaction products (e.g. cyclic oligomers) or might be present because the polymerisation process was incomplete.

2. Oligomers can intentionally be used as "prepolymers". These are reactive species that will be used as reacting blocks to manufacture polymers.

The main function of polymers is to make a FCM out of it based on the high molecular weight part of the polymer. Nevertheless, examples exist where the low molecular weight part of the polymer, i.e. the oligomeric fraction, provides indispensable properties to the material for its final application. If these oligomers were removed from the polymer, addition of IAS would frequently be necessary to make the material suitable for use as a packaging material.

As oligomers are not intentionally added, they are well within the scope of the NIAS definition. Of course, oligomers may be intentionally formed in some cases and hard to avoid in others, but nevertheless, they are an important part of the NIAS discussion. Either way, oligomers may contribute extensively to the overall migrate and, depending on the polymer type, they may migrate in much higher concentrations than other NIAS.

In recent years, oligomers formed from monomers that were petitioned with EFSA were included as part of the petition process taking into account the use of certain co-monomers and the manufacturing process.[3]

In contrast, no risk assessment for oligomers was done by EFSA in the case of older listings (e.g. styrene monomer) or if different processes are used which lead to different oligomeric patterns. It should be mentioned once again that classifying oligomers as IAS or NIAS says nothing about their legal status. Both intentionally and non-intentionally formed oligomers below 1000 Da need adequate risk assessment to ensure the safe use of the FCMs and articles.

In addition, prepolymers which remain in the final product may be seen as NIAS (Dutch Packaging and Utensils Decree)[4].

A general overview for all plastic FCMs can be found in Tables 1 and 2 on the type of substances that are defined as IAS (Table 1) and the type of substances that are considered to be NIAS (Table 2).

---

3. Monomer 1,3-bis(isocyanatomethyl) benzene, CAS N°. 3634-83-1, FCM Substance N° 988: http://www.efsa.europa.eu/en/efsajournal/pub/2824.htm
   Monomer methacrylic acid, 2-hydroxypropyl ester (synonym: 2-hydroxypropyl methacrylate - HPMA), FCM Substance N° 995: http://www.efsa.europa.eu/en/efsajournal/pub/2745.htm

4. https://zoek.officielebekendmakingen.nl/stcrt-2014-8531.html (Official version in Dutch) Translation: Prepolymer used as monomer: a polymer often with a relatively low molecular weight, and often an intermediate product between the monomer and the final polymer. A prepolymer used as a monomer must consist of at least two monomer units of each of the monomers used. A prepolymer should be fully incorporated into the polymer chain, and will be deemed not to be present in the final product.

Table 1: Examples of IAS for plastic FCM.

Type of IAS	Explanation	Examples
Monomers	Monomers are the building blocks of a polymer and are the repeating unit in polymers and thus form the polymer backbone.	Styrene for the production of polystyrene. Bisphenol-A for the production of polycarbonate and epoxy resins.
Starting substances	Starting substances together with monomers are the building blocks of a polymer. Other starting substances can be used to modify a polymer with e.g. side-chains or end-caps. The term "other starting substances" also covers natural macromolecules which are being chemically modified.	Lauric acid, a mono carboxylic acid to be used as an end capper.
Additives	Additives are added to FCMs to achieve a physical or chemical effect during processing of the plastic or in the final material or article. Additives are intended to be present in final materials or articles.	Calcium stearate added to plastics as a release agent.
Polymer production aids	Polymer production aids are substances used to provide a suitable medium for polymer or plastic manufacturing. They may be present, and are intentionally added, but are neither intended to be present in the final materials or articles nor have a physical or chemical effect in the final material or article.	Anti-foam reagents/degassing agents necessary during the manufacturing process, anti-cluster, nucleating agents.
Aids to polymerisation	Aids to polymerisation are substances which initiate polymerisation and/or control the formation of the macromolecular structure.	Accelerators, catalysts, chain scission reagents, chain transfer or extending agents, photoinitiators.
Colourants	A colourant is a substance added to a product to give a certain colour. Colourants can be dyes or pigments.	Carbon black, titanium dioxide.
Solvents	A solvent is a medium that dissolves a solid, liquid, or gaseous substance without any chemical reaction between solvent and that substance.	Acetone, methyl acetate, ethyl acetate, toluene, …
Prepolymers	According to IUPAC, a prepolymer is a polymer or oligomer molecule capable of entering, through reactive groups, into further polymerisation, thereby contributing more than one monomeric unit to at least one chain of the final molecule. 1,1,1-trimethylolpropane ethoxylated, reaction product of monomers being 1,1,-trimethylolpropane and ethylene oxide.	Polyurethane prepolymers, reaction product of a polyol-component and a polyisocyanate-component.

Table 2: Examples of NIAS for plastic FCM.

Type of NIAS	Explanation	Examples
Impurities	Impurities present in authorised substances.	Trace amounts or residues of substances used during the production of substances. Also reaction by-products from the synthesis of monomers, additives, etc. Primary aromatic amines as impurities in azo-pigments.
Oligomers	In the scope of this document, "oligomer" means a substance consisting of a finite number of repeating units. We speak about dimers, trimers, tetramers and higher oligomeric fractions. These can be linear or cyclic in form. Due to their gastrointestinal absorption properties, they are generally considered up to a molecular weight of 1000 Da.	Polyolefin oligomeric saturated hydrocarbons (POSH) for polyolefins/ oligopeptides, being di-peptides, tri-peptides, etc. Cyclic oligomers from polyamides or polyesters. Oligomers in polystyrene and styrene-acrylonitrile polymers.
Reaction intermediates (including remaining prepolymers)	A reaction intermediate is a molecular entity that is formed from the reactants (or preceding intermediates) and reacts further to give the directly observed products of a chemical reaction. Most chemical reactions are stepwise, that is they take more than one elementary step to complete. An intermediate is the reaction product of each of these steps, apart from the last one, which forms the final product.	An alkyl alcohol which is ethoxylated and then transformed into an acrylate. The reaction intermediate here is the ethoxylated alcohol, which can be present as an impurity in the final acrylate, which is used as a monomer. Residual polyadipate in the end-capped polyadipate.
Contaminants	Contaminants are unintended substances, not already mentioned before, present in another substance coming from, for example, the production process, residual chemicals from a previous production batch, environmental contaminants and so on. Another source of contaminants is from storage or transportation of a raw material or a finished product.	Phthalates being released from tubes, belts or lids of a machine used in FCM manufacture or being a cross-over contaminant from a previous FCM production batch. Baby bottles made from polypropylene contaminated via gas phase transfer from the packaging (recycled board, printing inks) and instruction leaflets, respectively.
By-products / Unintended reaction products	By-product is a product which is usually produced in an industrial or biological process in addition to the expected main product. Something produced in the making of something else.	In the synthesis of p-xylene, o-xylene is formed as a by-product. In the production chains of polyurethanes, phenyl and 4-methylphenyl isocyanide dichlorides are formed. Cyclo-di Bisphenol-A diglycidyl ether (BADGE) formed in the production of Bisphenol-A (BPA) epoxy resins.
Degradation products	Degradation products are, for example, decomposition products due to, for example, thermal instability, instability to light, oxidation reactions and so on.	Azoinitiators can degrade and form recombination products without the azo group, e.g. tetramethylsuccinonitrile from 2,2'-dimethyl-2,2'-azodipropiononitrile (CAS# 78-67-1), chlorohydrins of epoxy compounds.

Based on examples from plastic materials and articles, an explanation of how to classify substances as NIAS or IAS and how to define the implications for the supply chain is given in Table 3. This table describes the supply chain, starting from a substance producer, through the polymer producer and up to the final plastic material or article in contact with food.

Although the concept of "unknown migrants" (NIAS or IAS) is the same at the different stages of the supply chain, the nature of the migrants depends very much on the operator's position within this chain. As highlighted in Table 3, at each stage of the supply chain, there might be processing steps that reduce the level of NIAS coming from the previous step. For example, the produced polymeric filaments, coming out of the extruder, are going through a water bath for cooling. This process can indeed eliminate some NIAS from the surface of the polymer strings. Residual traces of volatile components may be removed as a consequence of a further thermal processing step.

Depending on the position in the supply chain, the type and number of unknown migrants (NIAS or IAS) can be different but once a substance is an IAS at the raw material producer level, this substance will also be an IAS for the end user. The communication of information throughout the supply chain respecting proprietary information will be discussed in Chapters 3 and 4.

Here are specific typical examples where NIAS should be considered in industrial practices:

- In the application for authorisation of a new substance, the applicant describes the purity of the substance together with details of the main impurities present. These impurities, although evaluated during the petition process, are in many cases not mentioned in the authorisation of the substance. Therefore, the user of the substance has already to face NIAS which might be known impurities for the manufacturer of the substance.

  A positive listing refers only to the substance, not to the manufacturing process. The type and quantity of impurities may therefore vary between different suppliers, depending on the individual process. As a consequence, impurities cannot be considered or evaluated in a general way for a given listed substance but need individual assessment with regard to the supplier and their production process.

- A plastic article can contain a degradation product which was generated during conversion and was not present in the plastic that was used as a raw material.

- A residual solvent (IAS) can become an unknown IAS at the level of the plastic manufacturer, while, during the additional processing steps, the solvent may be evaporated and will not be present any more in the plastic article.

- An IAS can become an unknown migrant in the next steps of the supply chain but can never become a NIAS. If a solvent is used in the manufacturing of the polymer, the residual amount of this solvent found in final articles made of this plastic will be an unknown migrant but not NIAS. Note that clear communication of IAS and NIAS composition throughout the supply chain is an absolute necessity to enable distinction between IAS and NIAS. More details on this can be found in Chapter 5.

Until now, there have been very few examples of NIAS which are subject to specific legal limits.
Within the Regulation EU 10/2011, Annex II, a limit for the sum of released primary aromatic amines (PAA) is given apart from those PAA which are positive listed in Annex I (monomers) (EU, 2011). PAAs are an important part of the current NIAS discussion because of their toxicological properties (Chung, 2000; Sinsheimer *et al.*, 1992; IARC; Baan *et al.*, 2008; Ohsawa *et al.*, 2000). They may be a cleavage product or impurity of azo-pigments or colourants, respectively, or formed by the hydrolysis of aromatic isocyanates.

Some NIAS are regulated together with the authorised substance in Annex I of the Regulation EU 10/2011. Examples are:

- Chlorodifluoromethane (Ref. N°. 43680) with restriction of the impurity chlorofluoromethane to 1 mg/kg of substance.
- Carbon black with a limit of benzo(a)pyrene (max. 0.25 mg/kg) as an impurity.
- Bis(2,6-diisopropylphenyl)carbodiimide (Ref. N°. 13303) where the SML is applicable to the sum of the substance itself and the hydrolysis product 2,6-diisopropylaniline.
- Some phosphite-based antioxidants include in their SML the corresponding oxidation product (e.g. Ref. N°. 68145, 74010 and 38810). For the antioxidant bis(2,4-dicumylphenyl)pentaerythritol-diphosphite (Ref. N°. 95270), the SML covers the substance itself as well as the corresponding phosphate and the hydrolysis product tris(tert-butyl)phenol.

But taking into account all ca. 900 substances regulated under Regulation EU 10/2011, these are rare examples.

The following examples give a better understanding of the definition of NIAS for plastics, see Appendix 1 for a literature overview:

2.3.2. Examples of NIAS in non-plastic materials

As mentioned before, the Regulation EU 10/2011 was the first legislative text mentioning NIAS (EU, 2011). Until recently, the principle of NIAS was not explicitly mentioned in legislative texts other than this Regulation. Attention to NIAS can also be found back in some Council of Europe Resolutions[5] (e.g. degradation products) and industry guidelines, e.g. "Good Manufacturing Practices for the Manufacture of Paper and Board for Food Contact[6]".

In this monograph, it is assumed that the definition of NIAS is also applicable to non-plastic FCM such as rubbers, textile products, glass, epoxy polymers, adhesives, ceramics, regenerated cellulose, coatings, cork, wood, paper/board, metals, and recycled materials. Specific examples of NIAS in non-plastic FCM are:

- Chlorohydrins and hydrates HCl/H_2O of Bisphenol-A diglycidyl ether (BADGE) used in epoxy-based coatings with their limits set out in Regulation EU 1895/2005 (EC, 2005).
- N-nitrosamines migrating from rubber teats are another example of NIAS being considered early in the legislation (Directive 93/11/EEC) (EEC, 1993).
- Waste paper is used for the production of recycled paperboard. The IAS to be recycled is the fibre and the additives used in this recycling process are also IAS. Any contaminant being adsorbed onto a wooden fibre and reaching the final product through the recycling process is a NIAS, e.g. mineral oil components (mineral oil saturated hydrocarbons and mineral oil aromatic hydrocarbons[7]) or plasticisers (diisobutylphthalate[8]).

5. http://www.coe.int/t/e/social_cohesion/soc-sp/public_health/food_contact/PS%20PAPER%20AND%20BOARD%20Version%204%20E.pdf
6. http://www.cepi.org/system/files/public/documents/publications/foodcontact/2010/Good%20Manufacturing%20Practice%20(GMP).pdf
7. These are mixtures of chemically similar substances with similar physicochemical properties.
8. http://www.bfr.bund.de/cm/349/di_isobutyphthalate_in_food_contact_paper_and_board.pdf
DiBP listed in the last version of the CEPI guidelines: http://www.cepi.org/system/files/public/documents/publications/foodcontact/2012/Industry%20guideline-updated2012final.pdf

Table 3: Types of IAS used at the different steps in the supply chain for plastic FCM and source and type of NIAS formed.

Stages in the process	GMP or adequate purity of starting materials	GMP quality assurance of manufacturing process		Potential outcomes of process
Material	IAS	Processes generating NIAS*	Process reducing NIAS*	Potential types of migrants (including NIAS and IAS) in addition to those coming from previous step
Substance	Monomers, other starting substance including prepolymers, additives, polymer production aids (PPA), aids to polymerisation (AP)	Choice of reaction process – manufacturing facility	Clean up – extraction process. Choice of reaction process	Impurities Residues of IAS used in production process (e.g. solvent, antioxidant present in a monomer)
Polymer	Monomer, other starting substance including prepolymer, PPA, AP	Reaction temperature and time, catalysts used Lubricants in processing equipment, cleaning, …	Reaction temperature and time, catalysts used, … Processing temperatures	Oligomers, side reaction products, reaction/ degradation products of PPA, AP, monomer, impurities, contaminants, residues cleaning agent/lubricants
Plastic	Polymer, additives, PPA	Processing temperature Processing time Lubricants in processing equipment, incomplete curing, cleaning, ...	Post treatment, e.g. washing, extraction process, … Processing temperatures	Reaction/degradation products of PPA, additives, polymer residual monomer, contaminants, residues, cleaning agent/ lubricants
Plastic article	Plastics, additives, PPA, printing inks, coatings, adhesives	Processing temperature Processing time Lubricants in processing equipment, cleaning, …	Post treatment, e.g. post curing Processing temperatures, extrusion process	Reaction products between /Degradation products from PPA, additives, polymer-residual monomer, contaminants, residues, cleaning agent/lubricants, set-off
Plastic article in contact with food	Plastic articles, foods	Packaging operations Food treatment in the packaging, cleaning		Reaction products between /Degradation products from PPA, additives, polymer-residual monomer, reaction products with food components, residues, cleaning agent, set-off

GMP, good manufacturing practice.

*The indications provided in those two columns are only indicative. A process reducing NIAS might also introduce other NIAS. Example: a washing step can eliminate several NIAS but can (depending on the operating conditions and washing solvent) potentially transfer chemical(s) into the product.

- Azo-pigments for printing inks are made of aromatic amines and e.g. β-naphthol. Both substances are present as impurities in the pigment and also in the final ink formulation. The pigment is intentionally used to formulate the ink. For such substances, aromatic amines, naphthol or naphthol derivatives, PCBs and dioxins, can occur as NIAS in pigments.
- Textiles for food contact used in a bakery are made of cotton. Although the cotton fibre is a natural material of varying composition, any contaminant within the material is a NIAS, e.g. pesticide residues.

Tables 4 and 5 give some examples which are specific to non-plastic food contact applications.

Other examples of studies on NIAS in non-plastic FCM described in the literature are included in Appendix 1.

Table 4: Examples of IAS/NIAS for non-plastic FCM.

Type of application	Types of IAS and examples	Types of NIAS and examples
Paper and board	Basic starting material: Cellulose fibres, natural occurring minerals such as calcium carbonate, and natural polymers such as starch	Transformation products of sizing agents
Rubbers	Vulcanisation agents: Sulphur and accelerators such as zinc oxide	Reaction compounds from vulcanisation; residual solvents
Inks	Photo-initiators for UV/EB inks: 2-Isopropyl thioxanthone, benzophenone, …	Decomposition products of the photo-initiators
Adhesives	Tackifiers; Rosins, Paraffinic oils	Residual solvents, residues of natural compounds, prepolymers/ oligomers
Coatings	Thermoset coating: epoxy resin, cured with phenolic resin, amino resin and/or anhydride resin	Oligomers of epoxy, Cyclo-di-BADGE, oligomers from polyester resins
Pigments and Colourants	Orange Pigments, Violet Pigments	Residue of PCB, residue of dioxins, PAH in carbon black
Metals	Passivation	Residues of metals from alloys originating from impurities such as As, Sb
Textile products	Anti-grease agents	

Rubbers, textile products, glass, epoxy polymers, adhesives, ceramics, regenerated cellulose, coatings, cork, wood, paper/board, metals, recycled materials

EB, electron beam; UV, ultraviolet; BADGE, Bisphenol-A diglycidyl ether; PCB, polychlorinated biphenyl; PAH, polycyclic aromatic hydrocarbon

Table 5: Types of IAS used at the different stages in the paper and board supply chain and the source and type of NIAS formed.

Stages in the process	GMP or adequate purity of starting materials	GMP quality assurance of manufacturing process		Potential outcomes of process
Material	IAS	Processes generating NIAS*	Process reducing NIAS*	Potential types of migrants (including NIAS and IAS) in addition to those coming from previous step
Substance	Process chemicals in stock preparation, size press and coating	Impurities in process chemicals (1), contamination from manufacturing of previous grade(2) and lack of pest control (3)	1. Declaration of Compliance (DoC) and material safety data sheets (MSDS) on all process chemicals. 2. Risk analysis with critical control points (CCP) and proper cleaning of production facilities 3. A pest control system in place	Residual solvents in raw chemicals. Presence of residual mineral oil. Transformation products, e.g. ketones from alkyl ketene dimer (AKD) sizing agents
Cellulose	Logs with bark	Process variation from debarking, digesting and bleaching (=chemical pulp)	Process equipment in good condition, with CCPs and proper screening after digester and bleaching. In addition, also well-educated personnel	
Paper/Board	1: Dry market pulp 2: Wet pulp from integrated pulp mill	1: Improper storage of bales, contamination from wrapping paper and steel wires. 1+2: Contaminated storage tanks and/or no pulp screening	1: Clean storage rooms indoors 1+2: Cleaning of equipment (from risk analysis), proper screening of the pulps, and well-educated personnel	
Printed board				Residual solvents in offset inks or varnishes such as alpha-methyl styrene or ethyl hexyl acrylates
Dye cut and pre-glued board				Residual solvents in adhesives, mainly water-based emulsion or hotmelts used in pre-glued cardboards

GMP, good manufacturing practice.

*The indications provided in those two columns are only indicative. A process reducing NIAS might also result in the formation of other NIAS. Example: a washing step can eliminate several NIAS but can (depending on the operating conditions and washing solvent) potentially transfer chemical(s) into the product.

2.4 References outside the European Union

2.4.1. Food Contact Notification (FCN) process in USA and NIAS

Analogous to the European petition process, the chemical characterisation part of the FCN process asks for:

- Chemical formula for known or likely side reactions occurring during the manufacture of the food contact substance (FCS), including catalyst degradation reactions.
- Concentrations of all major impurities (e.g. residual starting materials, including all reactants, solvents, and catalysts, in addition to by-products and degradation products) together with supporting analytical data and calculations. In the case of polymers, concentrations of residual monomers should be included.

As far as the authors are aware, there are no guidelines on how to perform a screening for NIAS/ impurities for FCNs.[9]

The difference between the European system and the FCN process is that the FCNs are proprietary to the submitter and its customers. This means that only the impurity profiles which are part of the FCN have been evaluated and the submitter has to guarantee that the substance being used in FCM manufacture matches the purity profile of the FCN.

2.4.2. Food Contact Notification process in Latin America and NIAS

There are no specific regulations in Latin America which include measures to handle NIAS. Some regulations which could describe the NIAS are:

- Chile: Regulation 977 Articles 123–126 which include a description and limits of some substances that could be considered to be NIAS.[10]
- Peru: the Regulation of Sanitary Surveillance and Control of Food and Beverages in its article 119 includes impurities that must not be present according to established limits.[11]

2.4.3. Food Contact Notification process in Asia Pacific and NIAS

To the best of the authors' knowledge, the concept of NIAS has not yet been introduced in the legislation on FCMs and articles in the Asia Pacific region. Nevertheless, some of these countries, such as Malaysia, are following closely the legislation in USA and Europe and so it may be expected that future updates will include the concept of NIAS.

9. http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/ucm081818.htm
10. http://juridico1.minsal.cl/977_de_1996.doc
11. http://bpa.peru-v.com/documentos/Vigilancia_y_control_de_alimentos.pdf

3. GOOD MANUFACTURING PRACTICE

One important aspect of the management of NIAS is directly linked to good manufacturing practices (GMP). With regard to compliance with the Regulation EU 1935/2004, the selection of starting materials implies that impurities, by-products and contaminants in the raw materials are taken into consideration, at least as far as they have potential to migrate (EC, 2004). In addition, more attention has to be given to the production operations, where further creation of NIAS in each processing step from the starting material to the final article has to be considered. The scope of this chapter is to remind the reader about the basic principles of good manufacturing practices and the requirements of the EU Regulation EU 2023/2006 (EC, 2006).

3.1 Good Manufacturing Practice Regulation EU 2023/2006

From a legislative point of view, Europe has implemented two overarching regulations which are applicable to all materials and articles intended to come into contact with food. The Regulation EU 1935/2004 of the European Parliament and the Council lays down the general rules for all food contact materials and articles (EC, 2004). In particular, it requires that migration from food contact materials has to be safe in its intended application. Additionally and referring to the above, the Regulation EU 2023/2006 demands that products are consistently produced so that there is no migration into food which is not in compliance with the Framework Regulation and that the safety of the consumer in the end is ensured (EC, 2006).

For the purpose of both Regulations, the following definition of Article 3 Regulation EU 2023/2006 shall apply:

"*Good Manufacturing Practice (GMP) means those aspects of quality assurance which ensure that materials and articles are consistently produced and controlled to ensure conformity with the rules applicable to them and with the quality standards appropriate to their intended use by not endangering human health or causing an unacceptable change in the composition of the food or causing a deterioration in the organoleptic characteristics thereof*".

The primary objective of the GMP Regulation is to assure a consistent FCM manufacturing process in compliance with the safety and inertness provisions of the Framework Regulation and, by that, prevent transfer of substances from FCMs which impair their compliance. The manufacturing of FCMs from raw material to the finished article gives room for the formation or introduction or elimination of NIAS at every stage of the supply chain as highlighted for plastics in Table 3.

For example, raw materials may have varying impurity profiles which might not be fully characterised and which may have an impact on the final product. At the converting stage, NIAS might be formed or introduced by the process itself, and additionally contamination can occur by cleaning or lubricating production equipment. At the final stage (food manufacturing and packaging), NIAS can also be formed or introduced by the production or filling equipment as well by any kind of material which comes into contact with the packaged foodstuff.

As NIAS are unavoidable, they should be risk assessed. For those NIAS identified as posing a risk following the risk assessment, attention should be paid to the reduction in the occurrence of those NIAS. Table 3 gives an overview on possible formation or introduction of NIAS during the complete production process of food contact materials. This table is not exhaustive and needs to be extended case-by-case based on individual experiences.

Not all possible sources of NIAS listed in Table 3 lead automatically to an unacceptable migration of those substances into food. In order to evaluate this, the migration potential of the individual substances has to be assessed. In addition, where migration does occur, then the toxicological profiles of the migrating substance(s) need to be considered to assess the risk to the consumer.

It is important to realize that the production of FCMs can never be free of NIAS. At an industrial level, raw materials are rarely 100% pure and each process might create additional artefacts and introduce additional contaminants. As a consequence, NIAS have to be assessed and their presence consistently controlled. An appropriate GMP would imply that, in the case of a relevant change having an effect on NIAS (e.g. supply or process), a reassessment of the NIAS is conducted. Therefore, besides choosing appropriate starting materials and processing conditions, GMP is mainly a tool to ensure a stable production environment in all sectors of manufacture, processing, and distribution of food contact materials and articles.

One of the main questions is how to achieve conditions which are in compliance with GMP. The GMP Regulation itself gives only a framework for tools which should be implemented. Details are missing and have to be worked out individually by each company. The list of substances to be considered for risk assessment as shown in Table 3 should trigger a case-by-case evaluation by the responsible company.

Overall validation of the production process should also have been implemented and documented. Validation means to identify which substances are created in the production process within the operation windows and to evaluate the risk associated with these substances (presence in the final article, migration, exposure, toxicity, etc, depending on the position in the supply chain). Analytical fingerprints may be a potential qualitative tool in every step of the value chain to monitor process stability.

Relevant assessments of NIAS analogous to Table 3 should be performed at relevant stages of the supply chain and should form one part of the company's supporting documentation:

Production of chemical substances, including prepolymers:

- Assessment of the suitability and purity level of starting materials (see Table 1), with regard to e.g. contaminants, by-products, artefacts, side-reaction products, impurities.

Production of intermediates plastic and non-plastic

- Assessment of the purity level of starting materials and additives with regard to e.g. contaminants, by products, artefacts, side-reaction products, impurities.
- Assessment of the production process with regard to e.g.o contaminants, oligomers as part of material including batch-to-batch variability and dependency on back up supply, residual levels of substances which should not be present in the finished product (e.g. solvents), artefacts, side-reaction products, impurities.

Production of food contact materials and articles:

- Assessment of impact of convertion on the content of NIAS by creation of new NIAS (degradation products or reaction products), elimination of NIAS from previous production step (degradation, evaporation), creation of NIAS due to gluing, printing/setoff or other contamination.

Use of food contact materials at food packer level:

- Verifying, if any compliance work was delegated.
- Assessment of potential NIAS from storage/filling/transport.

3.2 Stating compliance with GMP

Stating compliance with GMP covers, in particular, the following aspects:

- The starting materials are selected and comply with pre-selected specifications that ensure the compliance of the finished article with the Framework Regulation. Information on the selection criteria applied to starting materials (such as identity, purity, toxicological profile) is relevant for all substances, not just for those not subject to authorisation under EU or national legislation.
- The operations are carried out in accordance with pre-established instructions and procedures to ensure the compliance of the finished article with the Framework Regulation. In particular, information on operating procedures is relevant for reaction and degradation products and contaminants.
- Quality assurance and quality control systems are established and adhered to.

Responsibility for those NIAS which were identified during the production validation step as possible substances with migration behaviour can either be delegated to the next step in the value chain for the necessary risk assessment at this stage or assessed by the company performing the validation. In the case of delegation, at least the identity of the identified substances and the need for a specific risk assessment have to be communicated as part of the Declaration of Compliance (DoC).

All information generated in the quality assurance and quality control systems needs to be documented and available to authorities on request.

The GMP Regulation is a tool and a prerequisite to be able to claim compliance with Regulation EU 1935/2004 (EC, 2004). It becomes obvious that good manufacturing practice includes a risk assessment on the production process and its possible impact on the creation of NIAS.

4. INFORMATION TRANSFER THROUGHOUT THE SUPPLY CHAIN

As already outlined in Chapter 2 in the definition of NIAS, it is sometimes difficult to distinguish between "real" NIAS and IAS if insufficient information is available to the downstream user. The exchange of information along the supply chain is therefore of high importance. The objective of this chapter is to provide examples of practical approaches for an efficient exchange of information along the supply chain.

For plastics, the Regulation EU 10/2011 as regards information in the supply chain[12] describes in detail the obligation and roles of the different business operators (EU, 2011). This Guidance document covers information to be generated and exchanged in the supply chain, as required in the context of compliance with Regulation EU 10/2011. Although the Guidance document is intended for plastic materials, it recommends the same principles for non-plastic business operators, i.e. for manufacturers of adhesives, printing inks, and coatings.

Based on companies' predefinitions, the final GMP documentation and, if applicable, the DoC should clearly state the compliance with GMP. Next to the statement declaring that quality management standards have been met and the work was done according to the GMP Regulation, it is important to clarify if parts of the compliance work are still to be done by the downstream user and, if so, detailed information about the substances to be evaluated must be given. For downstream users to rely on the risk assessments made in the previous production step(s), relevant information should be passed on, which clarifies which parts of NIAS have already been safety assessed and which parts have not been safety assessed.

4.1 Exchange of information in the supply chain

The following is taken from the aforementioned EU Guidance document[11]. The compliance of the final food contact material and article with EU provisions can only be ensured if, along the supply chain, relevant information exchange takes place between the supplier and the customer and vice versa. The information given has to be clear and distinct. Information should relate to the actual composition of the material.

The DoC and the Adequate Information are a confirmation of the compliance work performed by the business operator issuing the documents. Compliance work covers a risk assessment, including the assessment of the hazard of substances added, generated or present in the material, together with their potential to migrate into the food. The compliance work that can be performed is dependent on the position of the business operator in the supply chain and the information that is available to that business operator. The roles and obligations of the different business operators, as far as relevant for issuing a DoC, are explained in detail in the Union Guidelines[12]. The same document explains which information needs to be provided in the DoC based on the position of the business operator in the supply chain.

A key problem of complex manufacturing processes is that usually no single stage can perform the complete compliance work: information on chemical composition, presence of NIAS such as impurities and degradation products, plastic processing conditions, composition of the food, storage and contact conditions, among others, are not all known at every step of the supply chain. Therefore, an optimised exchange of information is key to ensure the compliance of the final article. In other words, two-way communication in the supply chain can help to identify relevant information that allows suppliers and customers to adequately perform their own compliance work. It also helps to build trust, which is essential, as the DoC does not include all of the information contained in the supplier's Supporting Documents.

12. http://ec.europa.eu/food/food/chemicalsafety/foodcontact/docs/guidance_reg-10-2011_en.pdf

The following principles for sharing compliance work throughout the production chain (taken from the Union Guidelines for plastics) should be followed as much as possible, and also for non-plastic materials:

1. Avoid duplication of compliance work

Producers performing the same compliance work on the same material should be avoided. In order to minimise duplication and costs, as much compliance work as possible should be concluded at an early stage.

2. Responsibility of business operators for their manufacturing step with a view to compliance of the finished article under the intended or foreseeable uses

The compliance of the finished article can only be ensured if all business operators in the chain, from the manufacturer of starting substances to the food packer, assume the necessary responsibility for their manufacturing step, with a view to the compliance of the finished article. This follows from the obligation that the whole manufacturing process respects GMP. It means that only components suitable for use in food contact materials can be used. This also excludes the possibility that a business operator can transfer to his customer all responsibility for compliance work arising from his manufacturing step (general disclaimers).

3. Responsibility of the business operator that introduces or generates a substance in the manufacturing process

A business operator introducing or generating a substance in a product (raw material, intermediate or finished material or article) is responsible for compliance of this substance. This includes the impurities of the substance and degradation and/or decomposition products linked to its intended use which may be formed at this or a later manufacturing step under the specified use.

All aspects of compliance work linked to the introduction or generation of a substance may not be finalised at the manufacturing stage at which the substance is introduced. Therefore, the DoC or Adequate Information serves as a means to inform on the aspects of compliance work that have been performed by the business operator issuing the DoC or Adequate Information and on which aspects still need to be performed by the downstream business operators.

4. Conclude compliance work as early as possible in the manufacturing chain

Compliance work should be concluded as early in the manufacturing chain as possible. As an example, in the case of addition of a small quantity of a substance with a high SML, it may be possible at the plastic manufacturing stage to ensure compliance and conclude that part of the compliance work, e.g. based on the calculation that, even with complete migration, the SML would not be reached. However, in particular in multilayers, it has to be taken into account that a substance can originate from several layers and compliance has to be ensured for the final article, taking into account the contribution from all layers.

5. Information from customer to supplier on intended use

Through communication between customer and supplier, the customer may already provide the necessary information to his supplier that will enable the supplier to complete the compliance work at this stage. For example, if the plastic converter informs the plastic manufacturer on the exact shape or size, food contact conditions and contacting food of his final article, the plastic manufacturer may already conclude the relevant aspects of the compliance work.

6. Specific description of compliance work transferred to the customer

The description of the compliance work that is transferred to the customer must be specific and allow the customer to perform the compliance work.

7. Responsibility of compliance work not transferred to the customer

A business operator automatically accepts responsibility for compliance work if he is not providing a specific description of compliance work transferred to the customer.

There is no specific or recommended standard for the transfer of information in the supply chain, it depends very much on the business model of the partners and on the competences available at each stage. If and how much information is or, better, must be shared also depends on the question of who takes responsibility for the final product. Nevertheless, the above mentioned principles should always be respected.

Three possible scenarios are mapped:

Scenario 1. Full responsibilities at each stage in the supply chain.

This scenario assumes that each partner in the supply chain takes full responsibility for its own products and operates following GMP rules. This is the preferred default approach.

Example: A polymer supplier whose pellets are the granulate used in a packaging material converting operation, e.g. in extrusion coating, cast film or blown film production.

The polymer supplier can claim **full responsibility** for the pellets and will document this in a DoC to the downstream user. As part of his compliance work, the supplier will perform the safety assessment of all IAS and all NIAS, including oligomers, present in his polymer. In this case, the information shared with the downstream user might only include information and confirmation required by legislation and relevant to the restrictions or limitations applicable to the conditions of use of the polymer. This would especially be in accordance with the Union Guidelines:

Confirmation that reaction intermediates, decomposition or reaction products comply with the relevant requirements of the Framework Regulation and that a risk assessment in accordance with Article 19 of Regulation EU 10/2011 has been performed.

As an example, information related to the final use should be given (e.g. not suitable for fatty food or microwave heating).

In addition, reference might be made, e.g. in the case of pellets or granules, to a technical frame or upper limit for parameters such as temperature, pressure, processing time, humidity, etc, under which further processing needs to be performed in order for the customer to ensure safe use of the material in accordance with Article 3 of the Framework Regulation. When test results are reported, it is recommended that these process conditions are disclosed if they are relevant for the compliance work already performed.

The same principle applies for non-plastic materials. The mandatory risk assessment is now based on the GMP Regulations Regulation EU 2023/2006 (EC, 2006) instead of Article 19 of Regulation EU 10/2011 (EU, 2011). From a practical point of view, the necessary compliance work is the same.

As an example, a paper manufacturer declares compliance with a national law recommendation, i.e. German BfR XXXVI. Taking full responsibility also for NIAS would mean that NIAS which were introduced as impurities or might occur during the paper making process and may migrate into food, would need to be identified and safety assessed.

Scenario 2. Shared responsibility of at least two business partners in the supply chain.

The shared responsibility is an option that would work at all stages in the supply chain, but is in particular suitable for set-ups, where the compliance of the raw material will depend very much on the downstream user. Good examples of this are the applications of inks and coatings where the formulation of the raw material is as important as the processing set-up in the converting plant. In this case, parts of the formulation including relevant information about NIAS in upstream raw materials is shared with the downstream user and both would jointly evaluate the suitability in the intended application. The same applies, for example, in the case of plastic pellets sold without any compliance work covering further processing.

The information to be provided according to the EU guideline would differ from the scenario described under n°1 in the following points:

- Confirmation that the plastic intermediate material complies with relevant requirements of Regulation EU 10/2011 and the Framework Regulation, as described below:

- Confirmation that reaction intermediates, decomposition or reaction products comply with the relevant requirements of the Framework Regulation and that a risk assessment in accordance with Article 19 of Regulation EU 10/2011 has been performed. If further steps of the risk assessment in accordance with Article 19 of Regulation EU 10/2011 have to be performed by the downstream operator, the identity of the substance (chemical name and CAS number) together with relevant information for the risk assessment have to be provided.

- Depending on the material, additional specific information may be needed to enable the downstream user to perform his part of the compliance work. Compliance work may only be delegated if a specific description of the remaining task is transferred to the customer.

- As such, the downstream user (customer) might approach the raw materials supplier to seek his help for the assessment of an unidentified/identified migrant found when the product is applied in his processing conditions. Nevertheless, it is the responsibility of the raw material supplier to inform about the compliance assessment that had been done and the parts of it still missing.

- Some parts of the responsibility for compliance might still remain with the supplier and some might also remain with the customer, but a major part is evaluated and certified jointly. For this set-up, it is necessary that both supplier and customer share competence and know-how which might be proprietary and might need to be protected by confidentiality agreements.

- Comment: For some products, the delegation principle might be the selection of choice, but needs an intensive communication and the willingness of both involved parties to agree that tasks can be specifically delegated and the receiving partner will be able to do the final assessment.

Scenario 3. Full responsibility transferred to a downstream user.

The third scenario transfers full responsibility for the legal compliance of the upstream material or article to the customer. This model might work in cases, where the supplier of the raw material has no experience/competence in food contact applications or in cases where the converter does not know the final application (use) of the material. In this case, the responsibility for safe use is transferred to his customer. Full disclosure of composition including impurities and minor components, agreed specification of the raw materials together with full transparency of the supplier with regard to possible NIAS is needed to enable the customer to meet the legal requirements. Analytical testing alone will never be sufficient.

Comment: Full delegation might be possible at an early stage of the supply chain, i.e. raw material supplier. At a later stage, it might not be the solution of choice due to its complexity. Instead, the main principles to avoid duplication of compliance work (i.e. not every customer should do the same job) and concluding relevant aspects of compliance work as early as possible should be followed. This scenario, although possible, should remain the exception.

With all three scenarios, it would be possible to meet the Framework Regulation requirements with regard to NIAS and all other aspects, but as outlined above, this would require a completely different information transfer in the supply chain. It should be noted that GMP implementation (in the meaning of Regulation EU 2023/2006 cannot be delegated and has to be ensured at all stages.

An example for a non-listed substance is given next.

Example for solvents

Solvents can be used at different steps in the manufacturing of a food contact material (i.e. for the production of a polymer, in the formulation of coating, ink, adhesive, …). In some cases, they might even serve dual functions like solvent and reactant but overall, they are not intended to remain in the finished material or article that comes into contact with the food.

When selecting a solvent, it is therefore mandatory to ensure that it will be removed by one (or more) of the subsequent production steps or that residual amounts are acceptable with regard to the inertness and safety requirements. The same applies to its impurities, stabilisers and/or additives which do not necessarily have the same physical properties (volatility, polarity, stability, …) and could potentially be retained in the material after removal of the solvent. The possibility of reaction with other components of the material also needs further consideration.

To conduct the safety evaluation of a solvent by the user of the solvent, the following information (not exhaustive) is required:

- Chemical identity of the solvent components.
- Identity and content of impurities, stabilisers and/or other additives.
- Expected residual levels of the solvent, impurities, additives, side reaction products, etc, left in the final article as a result of its use.
- Physico-chemical properties such as boiling point, solubility in water and other solvents.

- Tolerable daily intake (TDI) values.
- If no TDI data are available, genotoxity data from toxicity tests or by modelling to determine the acceptable residual levels. Considerations with regard to genotoxicity are addressed in section 5.5.

The label "food grade solvent" is a good starting point, because it implies a certain level of safety confidence. However, a risk assessment of the use of the solvent in a particular application is still needed.

5. RISK ASSESSMENT OF NIAS

As outlined in the previous chapters, NIAS may be present in FCM but should be subject to risk assessment using scientifically recognised principles. Risk assessment generally involves four steps. For NIAS, the order of these steps may be different depending on the problem:

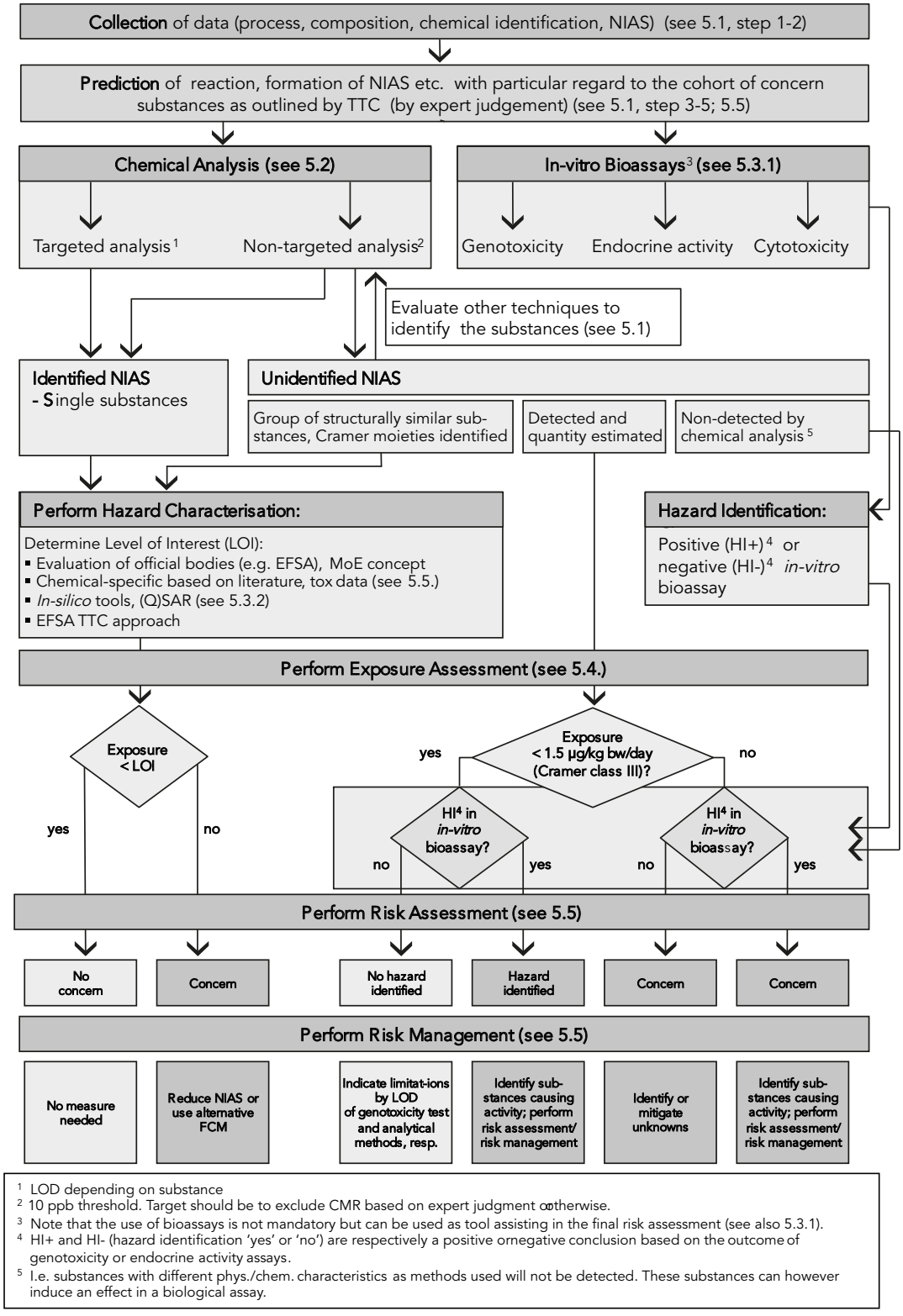
- Hazard identification: an evaluation of the adverse health effects a chemical substance is capable of causing, e.g. liver damage.
- Hazard characterisation: determination of how much of a chemical is required to cause a toxic effect, and prediction of exposure levels at which risk is likely to be negligible or non-existent.
- Exposure assessment: determination of daily exposure to a chemical substance under various conditions such as use of the substance or product in different consumer products.
- Risk characterisation: an integration of the pertinent information from the preceding steps to characterise the risks to the exposed population. In other words, what is the likelihood that there will be an increased health risk in a consumer population exposed to a particular contaminant via food, air or skin contact? The risk characterisation also includes an explicit description of the assumptions and uncertainties that go into the risk assessment, and the overall confidence in the results of the analysis. It is important to note that even for very toxic chemicals, if the exposures are low enough, the risks may be very low or non-existent. The principle that "the dose makes the poison" (first announced by Paracelsus 1493–1541) is a basic tenet of toxicology and is best described by the following equation:

$$\text{RISK} = \text{HAZARD} \times \text{EXPOSURE}$$

This formula indicates that an exposure to a hazardous substance with a toxic threshold may not be a safety concern as long as the exposure is low. Also, exposure to substances may be high without a health risk, in cases where the substance is not very hazardous.

Figure 1 illustrates the risk assessment strategy as proposed by the ILSI Europe Expert Group on NIAS for both the identified and unidentified NIAS. Additional details for each step are given in the corresponding sub-chapters. The use of *in-vitro* biological assays is not mandatory but can be used to provide additional information to support the final risk assessment.

Figure 1: Flowchart for the risk assessment of NIAS (may also apply to substances other than NIAS).



[1] LOD depending on substance
[2] 10 ppb threshold. Target should be to exclude CMR based on expert judgment otherwise.
[3] Note that the use of bioassays is not mandatory but can be used as tool assisting in the final risk assessment (see also 5.3.1).
[4] HI+ and HI- (hazard identification 'yes' or 'no') are respectively a positive or negative conclusion based on the outcome of genotoxicity or endocrine activity assays.
[5] I.e. substances with different phys./chem. characteristics as methods used will not be detected. These substances can however induce an effect in a biological assay.

5.1. Collection of information on NIAS

NIAS will always represent a small part of the full composition of a FCM. A FCM consists of the following type of materials/substances:

- The base material of the FCM. This frequently has a molecular weight far above 1000 Da, for example, the high molecular weight polymer in a plastic/rubber FCM or the cellulose fibres in a paper/board FCM. However, the release of substances from the base materials due to degradation reactions such as hydrolysis (e.g. polycondensates: Bisphenol-A from polycarbonate), oxidation (Sn(II) from tinplate), etc. cannot be excluded.
- The IAS which are regulated at EU level may be used in plastics materials including monomers, additives (such as antioxidants, UV absorbers, etc.) and, not exhaustively, polymer production aids Regulation EU 10/2011 for plastic FCM) (EU, 2011). Also, IAS used in regenerated cellulose are regulated at EU level (EU Directive 2007/42/EC) (EC, 2007). For most food contact materials, no EU legislation is available. There, national legislation or national recommendations may be applicable.
- The predicted NIAS. This type of substance may be predicted based on:
    - o knowledge of the chemistry of the IAS and base materials present,
    - o the processing conditions,
    - o condition of use,
    - o what is found in the literature,
    - o what is known from experience.

  For example, mineral oils in recycled paperboard, set-off of degradation products of UV-initiators in printed FCM, oxidised antioxidants, cyclic oligomers formed during polymerisation and impurities in starting materials.
- The unpredicted NIAS. NIAS whose occurrence could not be predicted. For example, NIAS related to the very complex chemistry of the FCM (e.g. coatings, rubbers and adhesives), contamination of FCM with substances used for the production of non-FCMs (e.g. contamination of FCM by non-FCMs during transport and storage), and printing ink components on information leaflets in baby bottles made from polypropylene (PP) (Simoneau *et al.*, 2012).

The assessment of predicted/unpredicted NIAS is performed in different ways by different laboratories. To foster a common approach, a stepwise approach is proposed as described below. Steps 1 and 2 are typically performed when demonstrating compliance of IAS in a FCM. Steps 3-5 focus on NIAS assessment which should also be carried out.

Step 1. Characterisation of the samples

The composition of the FCM has to be clarified (e.g. for a plastic laminate: identity of the different layers, use of adhesives, lacquers, printing inks and varnishes), migration barriers and the possibility of set-off during storage have to be considered. The manufacturing process to make the starting material(s) and final FCM may also influence the chemistry of the mixture of base materials, IAS and NIAS. This includes processing temperatures (e.g. plasma and radiation treatment) and techniques and also drying time/temperature. A good characterisation of the FCM is therefore of utmost importance for defining what type of NIAS may be expected and which analytical techniques should be used to determine their migration. Relevant questions that can be of help include: is it a multilayer material, are adhesives used, is the sample

bleached or coated, are the samples printed, is there a reason to assume that set-off may be relevant or are there other sources of potential contamination, what are the intended time/temperature conditions, etc? Also, it is important to define which Regulations are applicable for the FCM under investigation.

Step 2. What are the IAS in the FCM or starting materials/substance(s)?
Information on IAS present in the FCM should be found in the accompanying documentation (DoC, specifications, material safety data sheets, etc.). In order to improve the sharing of information, it is important to strengthen communication between supplier and customer and to agree between the involved parties how much information is needed to enable adequate risk assessment of NIAS.

Relevant questions are: Do these IAS respect their specific migration limits for listed substances (e.g. plastics) from the FCM? Are the IAS in non-regulated materials such as adhesives of potential health concern? What are the impurities present? At what percentage is the IAS used in the manufacture of the final FCM? Which side reaction or degradation products are typically known to be formed during manufacturing of the IAS?

Step 3. Predicted NIAS, being, for example, reaction/breakdown products formed from the IAS and base materials
Relevant questions are: Which reaction and breakdown products (predicted NIAS) may be formed from the IAS and base material? What are the processing conditions? This should be assessed theoretically and monitored by analytical screening of migrate/extracts.

Identification of predicted NIAS from knowledge of the composition.

The identity of predicted NIAS present in a FCM can be determined by an efficient exchange of information. Predicted NIAS can be analysed by targeted analysis of the known substance(s).

Example: Known degradation product of a photo-initiator in UV-curing inks, chloropropanols, acrylamide or information about the substance provided by the ink manufacturer, amongst other additives used and/or potential impurities known to be present. These predicted NIAS can be detected by targeted analysis performed by the downstream user.

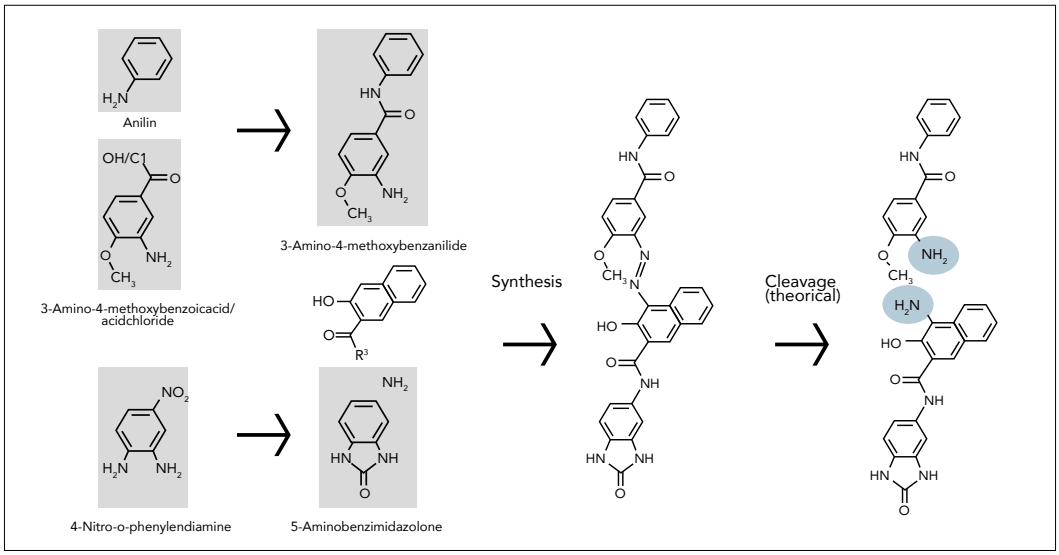
Hypothesis of composition by expert judgment and evaluation of predicted NIAS.

It has been demonstrated and well explained for several plastic materials (Bradley *et al.*, 2007) that many NIAS can be predicted based on theoretical chemistry, analytical experience and literature search. Seen from a quantitative perspective, the most relevant substances such as oligomers and the main impurities or degradation products of additives were predicted correctly for several materials. Only the major degradation pathways of the additives itself were considered in this theoretical exercise and a more complete prediction would be possible if all impurities of additives were known and were also considered. Hence, prediction of these NIAS depends on the information base used as a starting point, including both substance information such as impurities, and process conditions such as temperature, pressure, air contact or moisture. Information exchange is crucial; this was also highlighted in the GMP chapter.

An example of the importance of foreseeing predicted NIAS is given for the azo-pigment PR 176, which is used in colourants for plastics as well as for printing inks applied to paper and board substrates. If analysing for primary aromatic amines (PAAs), five structures have to be considered based on the synthesis of the pigment and one additional structure might become relevant if reductive cleavage of the azo bond in the dye can occur (Figure 2). Recent analytical approaches for PAAs are based on a photometric sum method or targeted methods by LC-MS/MS. Referring to the example of PR 176, in migration studies, both intermediates from the synthesis of 3-amino-4-methoxybenzanilide and 5-aminobenzimidazolone were found to migrate in food simulants.

For a full predicted NIAS assessment of the pigments used and also napthoeic acid derivatives, the coupling agent and impurities of the starting materials have to be considered as well as the PAAs.

Figure 2: PAA as relevant migrants for the azo-pigment PR (pigment red) 176.



Prediction tools for simulation of formation of NIAS

To the best of the authors' knowledge, computerised models do not exist to predict the formation/occurrence of predicted NIAS. A literature search, knowledge of organic chemistry and knowledge of the product are the main sources of information to predict which NIAS may be formed.

Step 4. Predicted NIAS detected previously in FCM/starting substance(s)

NIAS already reported in certain FCM and/or IAS are also taken into account as predicted NIAS within the definition in this guideline. The predicted NIAS that have been found in the past in the FCM or starting substance(s) under investigation has to be considered. For example, mineral oils in recycled paper/board, BADGE-HCl/–H_2O adducts in coatings, and monochloropropanediol in paper/board. These predicted NIAS should be considered. The NIAS that may be present should be evaluated by a specific migration study or by applying expert judgment. Based on information available from the literature, it may be possible to propose which NIAS may be relevant for the FCM/starting substance(s) under investigation. A non-exhaustive literature overview is given in Appendix 1.

Another source of information with regard to NIAS may be from the analytical laboratory of the FCM/starting substance(s) manufacturer or an external laboratory that has experience with the FCM/starting substance(s) under investigation. These laboratories will most likely have analytical data such as peak patterns of the starting substances used. These can be fairly complex, for example, chromatograms for prepolymers consisting of dozens/hundreds of peaks. This type of information should be part of GMP, see Chapter 3, and can be used to see which of the substances detected are also present in the starting materials. Also, these profiles may be used to see whether quality between production batches has changed.

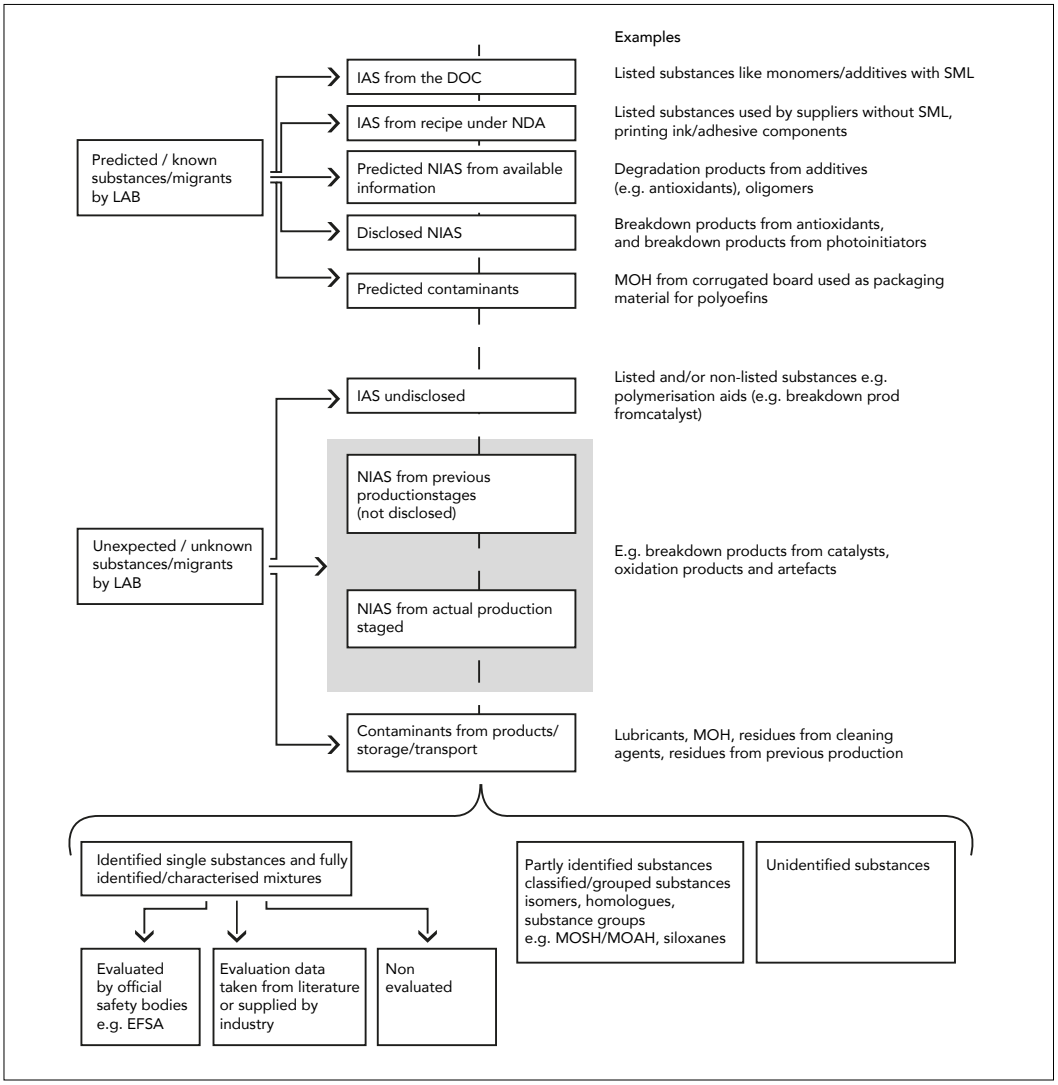
Step 5. Assessment of unpredicted NIAS not detected earlier and difficult to predict

This step concerns the analytical assessment of unpredicted NIAS that have not been detected before and could not be predicted based on considerations of the chemistry of the system. These NIAS can, for example, be contaminants and reaction products present in products made by complex chemistry such as, for example, coatings, rubbers and adhesives. Non-targeted screening analytical approaches should be used to assess these unpredicted NIAS.

Good examples of unpredicted NIAS (in the scope of this document) that are difficult to predict are process contaminants. These NIAS should be evaluated using non-targeted analytical screening techniques. Since the NIAS is unpredicted, the non-targeted analytical screening approaches applied should ensure that substances with a wide range of physical-chemical properties will be detected, see section 5.1.2.

Figure 3 shows the types of migrants and their classification as known or unknown from a migration testing point of view. It allows, on one hand, the identification of migrants based on information that is typically found in DoCs (known migrants by the laboratory performing the tests) and, on the other hand, other migrants. Additional information can be gathered by the testing institute together with the manufacturer of, for example, a final FCM (unknown migrants by the laboratory performing the tests).

Figure 3: Identification of the migrants found in migration testing.



5.2. Chemical analysis of NIAS

For the analysis of the predicted and unpredicted NIAS described in section 5.1, two main types of analytical methods may be considered (see Figure 4):

1. Targeted analytical methods, for the analysis of predicted NIAS. The methodology may also be applied such that unpredicted NIAS are analysed simultaneously. A targeted approach may consist of both targeted analytical methods and screening methods.

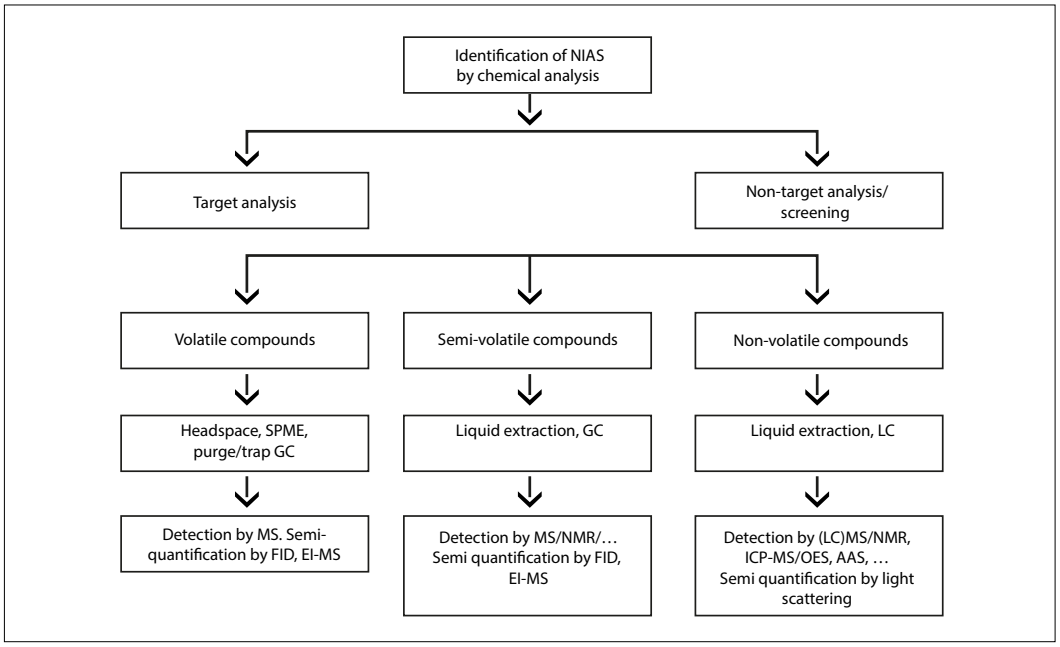
2. Non-targeted analytical methods or screening methods to analyse substances with a wide range of physical/chemical properties. This concerns mainly the detection of unpredicted NIAS, but if present, predicted NIAS and IAS from previous production stages may also be detected.

In the framework of NIAS analysis, both groups of methods should have the capabilities to detect and quantify or estimate the quantity of traces of compounds in a migration solution or solvent extract. In Regulation EU 10/2011, the non-listed substances used in layers behind a functional barrier should not be detectable (<10 µg/kg food) for non-carcinogenic, mutagenic or reprotoxic (CMR) substances. This means that the scope of both the targeted and screening analysis should be at the low µg/kg food level. It should be realized that all FCM will release NIAS during migration experiments at a detectable level. It is therefore unrealistic to demand that NIAS should not migrate. As a pragmatic tool, the 10 µg/kg food migration level or a Level of Interest (LOI, see section 5.4.4) for NIAS may also be used for non-barrier materials. Important in the (estimated) quantitative analysis to determine exposure is to take into account the fact that exposure to the same migrants may occur via different foods (for example, packaged in materials that use the same starting materials). Before the (estimated) quantitative analysis is performed, the exposure (see section 5.4) should be taken into account and a realistic and sufficient limit of detection (LOD) should be defined.

In addition to targeted and non-targeted analytical methods, situations exist where information on the sum of similar molecules that migrate may give important information. For example, polyester oligomers may be hydrolysed artificially into small molecules to mimic the hydrolysis process that will occur in the human body after exposure to these oligomers. This enables to estimate the sum and type of molecules to which one is exposed. The same procedure may give a global overview of the composition of the oligomers with respect to type and quantity of monomers used and end-group functionalities.

Another tool that may help to gain more insight into the migration of NIAS is to determine the overall migrate of a FCM. Comparing the overall migrate to what was found with targeted and non-targeted analytical methods using a mass balance will give an indication on the portion of NIAS that was and was not detected using the targeted and non-targeted analytical methods. Note that this procedure will be prone to a large analytical error.

Figure 4: Targeted and non-targeted analytical approaches for NIAS.



It should be noted that not necessarily all analytical techniques will need to be applied when evaluating a food contact material and article. The choice for the analytical techniques applied will in many situations be on a case-by-case basis.

5.2.1 Targeted analysis for quantification of predicted NIAS

Targeted analysis can be performed for the predicted NIAS. For this evaluation, an internal standard should be used that is, for example, the same, or structurally very similar (isotope labelled) compared to the NIAS under investigation. This ensures that the detector response for the internal standard and NIAS is the same or very similar. Migrates may be obtained using migration conditions simulating the intended use of the FCM. One or more internal standards should be added at a level in the range of the expected migration of the NIAS. It may also be considered whether worst-case migration calculations, i.e. calculations that use compositional data or extraction data and assume 100 % transfer to the foodstuff, can be used.

5.2.2 Non-targeted analysis/screening for unpredicted NIAS

Screening analysis can be conducted for unpredicted NIAS, but also detects predicted NIAS as well as IAS. In this approach, a FCM or a starting substance(s) is extracted with one or more simulant/extraction solvents followed by analysis using several analytical methods to provide maximum coverage for all substances possible, e.g. headspace/solid phase microextraction (SPME), gas chromatography flame ionisation detection (GC-FID) or gas chromatography mass spectrometry (GC-MS) to detect volatile substances; GC-FID or GC-MS for semi-volatile substances; liquid chromatography ultraviolet detection (LC-UV), LC-evaporative light scattering detection (ELSD) or LC high resolution MS for non-volatile and polar compounds; inductively coupled plasma (ICP)-MS for trace elements; and nuclear magnetic resonance (NMR) for general screening. High resolution GC-MS may also be useful for the analysis of NIAS. NIAS with a molecular weight up to 1000 Da need to be considered. NIAS with a molecular weight exceeding 1000 Da are generally not considered of relevance for risk assessment (EFSA, 2008) and are therefore not taken into account.

The screening approaches are described in the next paragraphs (sections 5.2.2.1 to 5.2.2.4). The composition of the FCM should be assessed to judge whether the analytical techniques used are sufficient to cover all classes of substances to be predicted. In case a material may contain (classes of) substances which cannot be detected with the above mentioned techniques, and which can be regarded as toxicologically relevant, additional analytical methods should be used, e.g. screening for ionic substances. The migration or extraction experiments can be performed according to the guidance given in, for example, Annex V of the Regulation EU 10/2011 on plastic materials and articles intended to come into contact with food (EU, 2011), and EUR 23814[13] which refers to several European Committee for Standardisation (CEN) methods relevant for different FCMs.

A commonly used approach is to initiate the NIAS screening using a solvent extraction to gain high concentrations of the substances (and NIAS) to be analysed. This results in a relatively high concentration of potential NIAS, which may make identification easier. Also, an estimated quantitative analysis of the NIAS concentration may be determined (see section 5.2.2.4) using internal standards to allow worst-case migration calculations to estimate worst-case migration of the detected NIAS to be carried out. If potential NIAS are detected that may exceed the LOI, migration experiments under realistic migration conditions may be performed to determine whether these NIAS have migrated under these circumstances and at what concentration.

The identities of each of the peaks present in the GC or LC chromatograms for substances that exceed or may exceed the LOI should then be determined by comparison with library spectra or with databases of accurate masses for known FCM substances as well as databases of potential NIAS proposed after consideration of the starting materials used to make the FCM. If no database is available or match quality is not sufficient, predictions can be made based on fragmentation patterns and calculated formulas.

Confirmation of the identities and quantitative determination of concentration in the solvent extracts/migrate can then be achieved through the analysis of purchased or synthesised analytical standards alongside the simulant/extraction solvent.

The screening analysis will not always lead to full identification of the NIAS. Many examples can be found in the literature where the screening analyses allow observing the presence of substances without the possibility to give a full identification (Nerin *et al.*, 2013). Note that full identification is not necessary, since only substances that exceed the LOI need to be identified. Beside identification of NIAS, chromatographic or NMR fingerprints of starting substances/materials may be useful to decide what signals were generated during the actual processing and what signals are parts of the raw materials used.

Provided that the LOI has been exceeded, full identification needs to be the goal of the NIAS assessment but may not always be possible and necessary since the partially identified substances may help to classify the unidentifiable NIAS into a specific chemical category and may give an indication of the toxicological potential of this unidentified NIAS.

5.2.2.1. Volatile substances

Homogeneous FCMs can be cut into pieces and transferred to headspace vials. Depending on the type of FCM, thermic desorption takes place at a temperature below the decomposition temperature of the material. Alternatively or in addition, solid phase microextraction (SPME) can be used to analyse volatile substances.

13. EUR 23814 EN – Joint Research Centre – Institute for Health and Consumer Protection, Guidelines on testing conditions for articles in contact with foodstuffs (with a focus on kitchenware) – A CRL-NRL-FCM Publication, 1st edition, 2009.

5.2.2.2 Semi-volatile substances

The extracts/migrates are obtained and an internal standard is added to the extract at the LOI. Analysis takes place using a gas chromatograph (GC) with possibly dual columns with diverting polarity (polar and apolar column) in order to detect both polar and apolar semi-volatile substances. Screening with the polar column may not be necessary if liquid chromatography, which also analyses polar substances, is also used as a screening technique.

5.2.2.3. Non-volatile and polar substances

The extracts/migrates are obtained and an internal standard is added at the LOI. Analysis takes place using LC typically in reversed phase or hydrophilic interaction liquid chromatography (HILIC) gradient mode and the use of, for example, an evaporative light scattering detector or a corona charge aerosol detector and mass spectrometer. Derivatisation techniques for GC-MS to make non-volatile molecules more volatile is an approach which is also frequently used.

5.2.2.4. Estimated quantitative analysis of unknown NIAS

Analysis and determination of known substances in an extract/migration media are normally carried out using a series of calibration standards containing known amounts of the substance of interest. This is not possible when the identities of the migrating substances have not been confirmed as is often the case for the NIAS. Instead, the concentration of a given substance is estimated by comparison with the response of an internal standard. Given the wide range of NIAS that may be present, it is important to choose a detector which gives a relatively comparable response for a large spectrum of analytes; the so-called universal detection. Thus, the response of the internal standard used for estimated quantitative analysis purposes should be similar to that of the NIAS being estimated. In this way, a reasonable estimation of concentration is obtained for each NIAS. If needed, upon identification of the NIAS, specific standard(s) may be used to prepare a calibration range to determine the accurate concentration of the NIAS in the extract/migration media and the resulting exposure. Note that, below the LOI, identification of the unidentified NIAS is not necessary.

For GC, the flame ionisation detector (FID) usually gives a universal response. Electron ionisation mass spectrometry (EI-MS) and chemical ionisation mass spectrometry (CI-MS) allow easy substance identification through a library search but response may vary more between substances than with FID. The state-of-the-art of estimated quantitative analysis is to use GC-MS for identification and GC-FID to estimate the quantity with a series of internal standards selected according to the chemistry of the FCM. For LC, aerosol detectors (e.g. evaporative light scattering detection (ELSD); charged aerosol detection (CAD); and nano quantity analyte detection (NQAD)) are available which give a reasonably universal response for semi- and non-volatile substances but may vary depending on the functional groups a molecule contains. Note that a variation in the response for different substances will always exist except when the internal standard used is structurally very similar or the same as the NIAS to be determined. For the detectors mentioned above, the difference in detector response is normally less than one order of magnitude for the majority of non-volatile substances. It may also be appropriate to include more than one internal standard in the analysis. However, volatile substances cannot be sensitively detected by aerosol detectors and reliable estimated quantitative analysis by internal standards is impossible. These substances should be detected in a headspace or SPME analysis, see section 5.2.2.1. Identification with light scattering techniques is not possible.

LC-high resolution MS (mainly electrospray ionisation) is another technique widely used for the analysis of non-volatile substances. Electrospray ionisation (ESI) is mainly used, but alternative ionisation techniques such as atmospheric pressure chemical ionisation (APCI) and atmospheric pressure photo-ionisation (APPI) are also used. High-resolution mass spectrometry may give information on the elemental composition of the molecules detected. Fragmentation techniques (MSn) can be used to fragment the molecules detected to generate specific fragmentation profiles of these molecules that contain structural information. The detection sensitivity of the technique is unsurpassed. The main drawback of LC-high resolution MS is that the detector response for different molecules present in a matrix may be very different making the estimated quantitative analysis much more difficult than for other LC detectors.

One should realize that with LC-high resolution MS, it is difficult to perform an estimated quantitative analysis since the same or structurally similar standards would be necessary. When LC-high resolution MS is used for the screening of NIAS, most likely all substances detected will need to be identified, quantified and risk assessed as far as possible in a pragmatic way.

As the identity and concentration of substances found in the FCM migrate are unknown, the recovery of analytes cannot be determined, see section 5.2.4. The recovery should therefore be estimated based on one or more substances (covering a range of chemical properties, e.g. polarity and volatility) which are spiked to the simulant after the migration experimental phase. Furthermore, care should be taken to ensure that the analytical detection limit is appropriate to determine the substances at a relevant concentration.

Besides the chromatographic methods, NMR has recently been demonstrated to be a powerful tool for quantifying or at least estimating the concentration of structural units even without knowing the complete structure of a migrating molecule. Practical examples are the determination of migrating polysiloxanes, phthalate plasticisers, aromatic moieties being released from rubber, unsaturated moieties in polyolefin oligomeric saturated hydrocarbons (POSH), and many more (Helling *et al.*, 2012).

5.2.3 Limitations of non-targeted screening

Estimated quantitative analysis and uncertainty

Quantification of analytes in analytical chemistry is performed using an internal standard that has a very similar or known detector response compared to the substance that needs to be quantified (here the NIAS). In the case of unpredicted NIAS, a non-target screening is required to detect these. It is not known which substances will be detected and therefore the choice of an internal standard that has a very similar detector response compared to the NIAS present is impossible. The best practice today is to use detectors that give a response fairly independent of the analyte's physical and chemical properties such as GC-FID/EI-MS and/or aerosol detectors as discussed in section 5.2.2.4. The choice of the internal standard should be such that the detector response of this internal standard versus other classes of substances can be related. Preferably, the response of the internal standard should be relatively low compared to other substances to ensure that the measured level of the unpredicted NIAS in the migrate is worst case.

For example, the response of the internal standard difluorobiphenyl in GC-MS was compared to a mixture of 23 substances containing different functional groups (Koster *et al.,* 2014). These substances were:

- Alkanes: undecane, dodecane, tetradecane, pentadecane, eicosane, triacontane.
- Alkenes: cyclooctene, octadecene, squalene.
- Alcohols: phenol, 1-octanol.
- Aldehydes/ketones: benzaldehyde, decanal, caprolactam.
- Ether: diphenylether.
- Carboxylic acids: octanoic acid, decanoic acid.
- Chlorinated substances: dichlorobenzene, dichlorophenol.
- Aromatics: anisol, naphthalene, xylene.
- Amines: aniline, butylbenzylamine.

The response of the detector (peak area/ng substance) for most substances was higher than the detector response of an equal amount of difluorobiphenyl. This demonstrates that when difluorobiphenyl is used as internal standard, the levels measured for the majority of substances in a migrate will be overestimated. This makes the estimated quantitative analysis of the screening conservative (Koster *et al.,* 2014). It may be decided to use a number of internal standards distributed over the entire retention time window to improve the estimated quantitative analysis.

In the case of targeted analysis, the chemical compound to analyse is known and the method can be calibrated with reference substances. Regarding the trueness or bias, related to the efficiency of extraction and other sample preparation related issues, reference materials containing the known chemical compound can be used. However, for unidentified NIAS this cannot be performed and remains an uncertainty.

Identification

Though electron impact (EI)-MS is amongst the best detectors in analytical chemistry for the identification of unknown substances, it is possible that a mass spectrum is recorded that is not present in the mass spectral databases. Experienced mass spectrometrists may be able to manually interpret the mass spectra and to derive the structure of the molecule it concerns (or at least give an indication). Confirmation of the tentative identification should be performed using a commercially available reference standard of the proposed substance or a reference substance that is equal to or structurally similar to the NIAS that has been tentatively identified. The retention time (or retention index) may help in this confirmation. Additional experiments with chemical ionisation (CI) may be used to confirm the molecular weight of the NIAS detected.

LC-high resolution MS is an excellent detector to analyse the mass of non-polar molecules and the mass of fragments generated during MS/MS. Extensive databases of substances related to these methods are however not available, which makes it frequently more difficult to identify detected substances compared to EI-MS. Some LC-high resolution MS systems such as time of flight mass spectrometry (TOF-MS), Orbitrap and Fourier transform mass spectrometry (FT-MS) record the accurate mass of a NIAS to 4 or 5 decimal places. This enables the elemental composition of the NIAS detected to be determined.

It may remain the case that even though a lot of time has been spent trying to identify the unknown NIAS, it is not possible to perform the identification. If the estimated exposure of this unidentifiable NIAS exceeds the LOI, other analytical techniques should be considered such as NMR. If these techniques do not result in the identification of the NIAS, it may be considered appropriate to use the TTC concept applied to unknowns as described in section 5.5.3.

Recovery

Another problem related to the estimated quantitative analysis of NIAS present in migrates is that it is not possible in non-targeted screening to estimate the analytical recovery of the respective NIAS. Since the analyte and thereby its physico-chemical properties are unknown, extraction/migration conditions may be unsuitable (e.g. extraction of perfluoro octanoic acid from FCM (Begley *et al.,* 2005)). Additionally, after extraction/migration, sample pre-treatment and analysis, analytes may be unstable or may be lost due to irreversible adsorption, precipitation, evaporation, etc. As long as the identity of the unpredicted NIAS remains unknown, it is not possible to correct for the recovery.

A pragmatic but limited approach to this problem may be to add one or more internal standards before the exposure phase of the migration test in order to be able to see whether recovery of these analytes is acceptable. It should be noted that the recovery of an internal standard can not necessarily be compared to the recovery of a NIAS since its physical/chemical properties may be very different. If migration of a NIAS has exceeded the LOI, the NIAS should be identified and a specific migration performed for the NIAS concerned using a suitable internal standard.

Homologous compounds

Non-targeted screening experiments result in the detection of single substances whereas some NIAS may consist of a number of structurally similar substances that may all need to be summed to take possible cumulative effects into account. Examples are mineral oils that contain a distribution of structurally similar substances that may be chromatographically separated. If only a screening analysis is performed, structurally similar substances that are chromatographically separated may be missed. Techniques capable of detecting groups of substances such as NMR may be a solution to this.

Non-detected NIAS

The non-target screening techniques are developed such that a wide coverage of NIAS with varying physical-chemical properties are detected. It is however possible that an unpredicted NIAS is present with physical-chemical properties that fall outside of the range of the non-target screening techniques used. The chance that this happens should be minimised by proper choice of extraction/migration procedures, analytical techniques and prior knowledge/evaluation of the predicted NIAS. Note that information on the starting materials used may provide valuable information on the analytical methods to be used for the NIAS evaluation, which is considered to include expert judgment.

Derivatisation followed by GC-MS may help to detect NIAS that are difficult to detect with other techniques. Co-elution may cause some NIAS with low abundance to remain non-detected.

5.3. Hazard identification and hazard characterisation of NIAS

Hazard identification and hazard characterisation are important steps in the risk assessment of a substance. The goal of hazard identification is to identify the potential adverse health effects in humans associated with an exposure to a chemical. Hazard identification requires an adequate and

documented review of relevant scientific information obtained from appropriate databases, peer-reviewed literature and/or study reports, if available. This approach places emphasis on studies in the following order: human (epidemiological/safety) studies, animal toxicological studies, *in-vitro* bioassays, and lastly, read-across and (quantitative) structure–activity relationships ((Q)SAR).

Hazard characterisation deals with dose–response analysis for the key toxicological effects identified. Classically, it is a process leading either to the development of safety level of exposure (e.g. of acceptable daily intake) or to an exposure level associated with a predetermined level of risk.

Before approving a substance intended to be used in a FCM in the EU, a toxicological evaluation of migrating substances from the FCM should be conducted. Toxicological studies could also be an option at stages of development of substances, when the nature of the NIAS is known. In that case, the set of toxicity data required is the same as for NIAS and is dependent on the anticipated level of migration:

1. migration below 0.05 mg/kg of food/food simulant: three negative mutagenicity tests covering different end points. Discussion are ongoing at EFSA to change this to two mutagenicity tests; in the case of suspected genotoxicity of NIAS which cannot be avoided by change of technological parameters or replacement of materials, a Margin of Exposure (MoE) of at least 10 000 should be reached (EFSA, 2012c) or should not exceed the lowest TTC threshold of 0.0025 µg/kg bw/day;

2. migration between 0.05 and 5 mg/kg of food/food simulant: in addition to (1.), an additional 90-day oral toxicity study and data to demonstrate the absence of potential for accumulation in man;

3. migration above 5 mg/kg of food/food simulant: in addition to (2.), a study on absorption, distribution, metabolism and excretion, studies on reproduction (in one species), and developmental toxicity (normally in two species), and studies on long-term toxicity/carcinogenicity (normally in two species).

In the absence of toxicological data, the Threshold of Toxicological Concern (TTC) approach (Kroes *et al.*, 2004) can be applied. Based on the chemical identity of the substance, the chemical classification can be determined using the TTC decision tree which includes the classification as published by Cramer et al. (1978). However, data on exposure is needed. TTC was not developed to apply to mixtures containing unknown substances. The decision tree is automated in the open source application Toxtree (free download via: http://toxtree.sourceforge.net/). It should be noted that in Toxtree, both the original Cramer classification as well as a Cramer classification adjusted to the latest knowledge can be used.

5.3.1. Use of in-vitro *bioassays*

It is frequently the case that limited or no toxicity data are available for the detected and identified NIAS, making hazard identification and characterisation very challenging. Moreover, for unidentified NIAS which can represent more than half of the compounds present in the migrate (Grob *et al.*, 2006), substance specific toxicity data cannot be searched for. It is increasingly acknowledged that a traditional approach based on the identification/quantification of all substances together with their full toxicological characterisation is neither practical (highly resource intensive) nor desirable; many NIAS are likely to be at such low exposure levels, that these are not likely to be inducing effects in in vivo test systems. However, toxicological interactions in such complex mixtures are still under discussion.

Instead, efforts are increasingly focused on non-targeted screening using analytical methods, *in-vitro* bioassays and *in-silico* toxicological evaluation. In such a case, the use of *in-vitro* cell culture-based assays (*in-vitro* bioassays) could be a very helpful tool in hazard identification to screen for

toxicological end points such as cytotoxicity, genotoxicity, and potential endocrine activity. Short-term *in-vitro* bioassays are widely recognised to play an increasing role in toxicological hazard identification, increase speed, and reduce cost and animal use. *In-vitro* bioassays have historically been used to assess the hazard of a complex mixture of chemicals present at very low levels in the environment (Depledge and Fossi, 1994; Walker *et al.,* 1996; Calow, 1998). From an agreed scientific point of view, they have to be used in conjunction with chemical analyses. Their main advantage is that *in-vitro* bioassays investigate the contamination as a whole and may detect a hazard originating from unidentified, not quantified and/or not detected chemicals. However, batteries of *in-vitro* bioassays have to be used to assess hazards.

Today, the use of a battery of *in-vitro* bioassays is a regulatory requirement in the field of the environment (i.e. wastes classification regarding their hazards (Directive 2008/98/CE) (EC, 2008); assessing the surface water quality (EC Water Framework Directive 2000/60/EC) (EC, 2000)). Regarding synthetic polymers, the use of *in-vitro* bioassays is also regulatory. Their application depends on the nature of the contact of the device with the human body (DIN, 2003).

Regarding food contact materials and NIAS, *in-vitro* bioassays may be used to acquire additional hazard information on the biological activity of migrates/extracts especially when considering that these are complex mixtures. This is because eventual interaction, which may be increasing as well as decreasing a certain effect, between NIAS and/or IAS in the FCM extract, may also occur between components. These effects may not be easily picked up with other tools and currently an interaction threshold is not available (even cumulative effects are recommended for substances having the same mode of action as a pragmatic approach).

Specific *in-vitro* biological assays permit to acquire more insight into the biological activity of migrating substances in the case of concerns that a specific hazard (e.g. endocrine active substance) may be present (EFSA, 2012). *In-vitro* bioassays can provide comprehensive information on hazard assessment of mixtures because they may be used to indicate possible interactions between the different components present. If present at very low dose levels, the health relevance of possible cumulative effects is considered to be so low that a need for a correction factor to cover possible cumulative effects is very low to absent (Leeman *et al.,* 2013). For some known contaminants such as endocrine disruptors (Welshons *et al.,* 2003; Kortenkamp *et al.,* 2011) and genotoxic-carcinogens, a combination effect at low dose may need to be considered.

Today's state-of-the-art in *in-vitro* bioassays, however, is not destined to replace the current chemical analysis (which is a detection tool for the presence of a substance), or for complete risk/compliance assessment, but should be used in addition (especially in the case of unpredicted NIAS) to obtain an indication of the mechanism of action of the adverse effect and then assist with further prioritisation, which is in line with the opinion of EFSA (2012c).

There are a plethora of tests available. *In-vitro* bioassays can be semi-quantitative, give binary (Yes or No) responses and inform on a toxicological mode of action such as genotoxicity, cytotoxicity or potential endocrine activity. The need and choice of a specific *in-vitro* bioassay or a battery of *in-vitro* bioassays to be conducted may be dependent on the toxicological targets to be assessed and on the FCM under evaluation (e.g. whether it is expected that the *in-vitro* bioassay might give additional valuable information for the toxicological safety evaluation) and is therefore dependent on expert judgment. Table 6 depicts some *in-vitro* bioassays currently under investigation, with a focus on the mechanisms/mode of action thought to act at low doses (genotoxicity and receptor mediated potential endocrine activity).

Table 6: In-vitro *bioassays currently used in NIAS research.*

Potential endocrine activity	Cytotoxicity*	Genotoxicity/ potentialcarcinogenicity
Oestrogen receptor (**ER**) redistribution **Androgen** receptor (**AR**) redistribution	**Cell Organelle Health (COH)**; end points: DNA content, cytochrome C, mitochondrial membrane potential, RNA synthesis kinetic inhibition.	**Indicator assays for genotoxicity** (PARP, GADD45, …), Comet-FPG assay
Transcriptional activation assay **Oestrogen** receptor (**ER**) (anti) **androgen** receptor (**AR**) **Glucocorticoid** receptor (**GR**) **Progesterone** receptor (**PR**) **Thyroid** receptor (**TR**) Peroxisome Proliferator Activated Receptor (**PPARγ**)	**Cell Proliferation and Cell Death (CPD)**; end points: apoptosis – caspase3, p53; DNA content, DNA proliferation - BrdU	**Mutagenicity test** (Ames test, mammalian cell gene mutation tests, micronucleus (MN) test) **Potential carcinogenicity** Cell Transformation Assay (detection of both geno- and non-genotoxic carcinogens)
H295R Steroidogenesis assay (changes in hormone production)		

* Primarily for monitoring purposes.

The following in vitro bioassays are currently in use for the assessment and/ or monitoring of FCM extracts:

- Genotoxicity assays to assess purified NIAS migrating from plastic FCM using the required set of genotoxicity tests (EFSA, 2008). Three genotoxicity tests are needed for acceptance by EU legislation.
- High throughput genotoxicity assays (Hughes *et al.*, 2012) to assess NIAS in carton FCM migrates/ extracts (Koster *et al.*, 2014).
- A specific cytotoxicity assay using human cells for the detection of unknown pollutants and NIAS in water for human consumption in contact with distribution channels (AFNOR, 1996).
- Ah receptor-based *in-vitro* bioassays for the quantification of toxic equivalents of dioxins and dioxin-like substances (EC 589/2014) (EC, 2014). The receptor-based *in-vitro* bioassays are acceptable as a screening tool in this legislation.

A number of *in-vitro* bioassays (e.g. cytotoxicity, genotoxicity, endocrine activity) have been developed and (pre-)validated. Their applicability in complex matrices of food-contact paper and board (with many unknown substances and/or contaminants (recycled fibres)) has been assessed in a European project (BIOSAFEPAPER), giving rise to normalised cytotoxicity assays for water extract (industry guideline for the compliance of paper and board materials and articles for food contact) (EN 15845, EN 16 418). The feasibility of *in-vitro* bioassays applied to FCMs was checked further in the European project (MIGRESIVES) related to adhesives (Migresives 2010). Other assays have been used in the pre-screening/pre-selection of suspicious samples or new formulations enabling replacement of undesirable substances, routine biomonitoring, and quality management, and were tested for different materials such as paperboards, polymers, polyethylene terephthalate (PET), polyvinyl chloride (PVC), polystyrene (PS) and coatings or to biobased plastics (EU Ecopack project, www.ecopack-label.eu).

For FCM with no specific EU regulation such as paper board, hazard identification using the migrate of the finished material can be applied (ILSI Europe Report (Ottenio *et al.,* 2004), industry GMP paperboard FCM guidance). This approach was recommended by AFSSA (formerly ANSES), and the Confederation of European Paper Industries (CEPI) in complement to analytical methods using standardised methods (AFSSA, 2006).

Several papers were published in which the relevance of *in-vitro* bioassays is indicated. It was found that *in-vitro* bioassays allowed distinguishing non-food grade samples from food grade samples (Bradley *et al.,* 2008, 2010). Moreover, the chemical analysis could not always explain the toxicity observed (Honkalampi-Hämäläinen *et al.,* 2010; Ozaki *et al.,* 2004,) suggesting that testing the whole migrate therefore offers an opportunity to reduce uncertainty (Muncke, 2011).

Limitations of in-vitro *assays for hazard identification and characterisation*

The feasibility of *in-vitro* bioassays in vitro, for example, the choice of adequate models and pertinent toxicological end points measured is a challenge as it determines the quality and reliability of the results. The sensitivity of *in-vitro* bioassays should be sufficient to detect biological effects at a relevant concentration and should not result in false positives/negatives. The current generations of genotoxicity assays have not been demonstrated to detect genotoxic substances at low levels, for example, in the ppb range.

5.3.2. Use of in-silico *tools*

In the absence of specific toxicological data, *in-silico* strategies may have some advantages. To be applicable, knowledge of the structure of the studied chemicals is necessary. Such models will provide not only qualitative information on the potential hazardous properties of chemicals (e.g. an alert for genotoxicity), but also quantitative information (e.g. Lowest Observed Adverse Effect Levels (LOAELs), or carcinogenic potency such as the median toxic dose (TD50)). It could allow direct comparisons with exposure estimates (Schilter *et al.,* 2014; Lo Piparo *et al.,* 2011).

Such an approach has already been extensively used in various research and development fields (Benfenati *et al.,* 2009) and has been identified to be of key importance for the food sector (JRC, 2010). Several *in-silico* models have been identified as potentially usable but there is still effort required to obtain full regulatory acceptance.

Computational methods

Computational toxicology is based on the assumption that the toxicity of a chemical can be predicted from its molecular structure. Recently, a number of models and methods have been identified as suitable for practical use (Schilter *et al.,* 2014; JRC, 2010). Suitable model means fit for purpose and properly validated according to internationally recognised guidelines. Many models have focused on the identification of hazards or mechanisms of action (e.g. hepatotoxicity, genotoxicity). These include Structure–Activity Relationships (SARs) which are based on expert knowledge or statistical correlation that links a particular toxicological effect with fragments or sub-structures in a molecule. Mechanistic end points such as nuclear receptor binding (e.g. oestrogen receptor) can be predicted through docking, for example, using commercial software (e.g. Vedani *et al.,* 2012). Quantitative Structure–Activity Relationship (QSAR) models are increasingly available, providing quantitative predictions of toxicological end points relevant for risk assessment such as chronic toxicity (Mazzatorta *et al.,* 2008; Venkatapathy *et al.,* 2004) and carcinogenic potency (Lo Piparo *et al.,* 2014; Contrera, 2011). In addition to QSAR methodologies, grouping/read-across has also

been increasingly developed and promoted to address the absence of toxicological data. Read-across is aimed at predicting toxicological properties/end points of an uncharacterised chemical based on existing information on other, toxicologically well tested chemical analogues (Schilter *et al.,* 2014; Wu *et al.,* 2010).

Application of computational toxicology for establishing the level of interest

In the frame of an ILSI Europe expert group, a decision tree (DT) reflecting the risk assessment paradigm has been developed to integrate exposure information with predicted toxicological values (Schilter *et al.,* 2014). In this DT, the size of the Margin of Exposure (MoE), established as the ratio between predicted toxicological values and estimated exposure, determines the level of safety concern and can be interpreted using the standard default uncertainty factors applied in hazard characterisation (e.g. for inter-species and inter-individual differences). Importantly, the DT makes full use of all available data, including information on mechanism/mode of action (MoA) and ensures an adequate degree of conservatism.

Limitations of in-silico *tools*

Any risk assessment is inherently associated with a degree of uncertainty. In a specific risk assessment, uncertainty is an important element to be communicated to decision-makers. For traditional risk assessment, many sources of uncertainties have been identified on both exposure and hazard characterisation (COT, 2007; EFSA, 2006). Because of its design, the application of the DT as described above was considered to bring uncertainties similar to the ones identified in classical risk assessment (Schilter *et al.,* 2014). Although there are currently no generally accepted *in-silico* tools to quantitatively predict chronic toxicity and carcinogenic potency (Lapenna, 2010), promising models are now available (Schilter *et al.,* 2014). An analysis of the average errors of QSAR models for chronic toxicity (Mazzatorta *et al.,* 2008) and carcinogenic potency (Lo Piparo *et al.,* 2014) was actually close to the biological variability of the experimental data, meaning that the use of predicted toxicological values may not bring a significant additional uncertainty. This is particularly true when several validated models and approaches are used in an integrated way. Overall, the uncertainty analysis and other considerations, such as case studies, indicated that if integrated properly as proposed in the decision tree, *in-silico* tools can be used to establish level of safety concern (Schilter *et al.,* 2014). In this context, it has to be kept in mind that applying *in-silico* methodologies requires knowledge of the chemical structure and significant inter-disciplinary expertise.

5.4. Exposure assessment

Exposure assessment is a key part of the risk assessment and is defined by the Codex Alimentarius Commission (CAC, 2003) as 'the qualitative and/or quantitative evaluation of the likely intake of biological, chemical and physical agents via food as well as exposure from other sources if relevant'. ILSI Europe published a guidance for exposure assessment of substances migrating from food packaging materials which was reviewed by a workshop held in March 2007 (Brands *et al.,* 2007). According to this guidance, exposure can be assessed using the following information:

- Which substances are occurring in the FCM (discussed in Chapter 5.2)?
- Is the exposure assessment necessary only for one application or does it concern the application of a substance in different materials?
- With which foodstuff is the material intended to come into contact?

- How much of the packaged food is consumed?
- What is the availability, relevance and suitability of different food consumption surveys for use in assessing exposure?

In section 5.4.1, a brief description is given of what information is needed for this. Sections 5.4.2 and 5.4.3 give a description of some of the information needed that has undergone developments since 2007, the year of publication of the guidance document.

5.4.1. Conducting an assessment of exposure

The ILSI Europe guidance on exposure assessment of substances migrating from food packaging materials (Brands *et al.*, 2007) gives an overview of data that are required to assess exposure. The following elements are needed: the amount consumed in the daily diet, the concentration and presence or absence of the chemical in question in each and every foodstuff consumed.

More specifically, the following list of questions/information requests was proposed in the ILSI Europe guidance to perform a risk assessment for migrants in general and is also applicable to NIAS. More information on specific subjects and how to perform an exposure assessment can be found in the ILSI Europe guidance.

Consumption

1. Obtain estimates of consumption for the foods and population groups relevant to the assessment.
2. If further assessment is necessary, develop refined estimates of consumption by the following steps:
   a. Firstly collect and compare all sources of consumption data that are considered relevant.
   b. Review suitability of consumption data and its relationship to its packaging. Is a packaging description included in the description of the food item consumed or purchased, etc.?
   c. If only food consumption data are available, then it is necessary to obtain information about its packaging by a different route.
   d. Identify the packaging containing the substance(s) of interest.
   e. Identify and list foodstuffs that could possibly be packaged in any of that packaging.
   f. Allocate market shares for consumption of foodstuffs in the packaging of interest. It could be assumed that all or only some of the food items consumed were packaged in the material of interest.
   g. Calculate estimates of exposure for each relevant type of packaged foodstuff and sum them to give an estimate of total exposure.

Concentration and occurrence

3. Obtain estimates of concentrations of the substance(s) of interest in relevant foodstuffs. For a first tier assessment, measurements in standard food simulants may be sufficient.

4. If further assessment is necessary, develop refined estimates of concentrations by the following steps:
   a. Allocate concentration data to any foodstuff in the packaging of interest. This may be simulant or foodstuff data or a combination of both. Modelling may also be used.
   b. With lack of concentration data, it may be necessary to be conservative and apply concentration values at the top end of a range, e.g. the SML.
   c. Decide how to treat limit of detection (LOD) and limit of quantification (LOQ) values. There are various treatments available.

Exposure assessment

5. Decide if a value can be set below which the exposure can be declared as safe or not of concern to human health.
6. Use a screening conservative approach first. Is this value exceeded? If not, there is no need for further assessment.
7. If it is exceeded then use a stepwise-tiered approach to estimate exposure. This involves deterministic, refined deterministic as well as probabilistic approaches.
8. Whichever method is used, link the foodstuffs consumed with their packaging and their concentration data to estimate exposure for each type of packaged foodstuff, and sum these to give an estimate of total exposure. Include other sources of exposure if relevant.
9. Compare each stepwise estimate of exposure against an appropriate end point, allowing for uncertainties. If the end point indicates that there is no problem, paying due regard to any impact of packaging loyalty, socio-economic or ethnic groups, regional variation and vulnerable groups, then there is no need for a more refined approach.

5.4.2. Migration assessment

As discussed in Chapter 4, each step in the supply chain should take responsibility over the safety of the product they manufacture and commercialise. This will be part of the declaration of compliance (DoC) and includes information on both IAS and NIAS. Three different scenarios were introduced in Chapter 4 to define how responsibilities are transferred throughout the supply chain.

The type of information and how this information is obtained that each partner in the supply chain will need to support depends on the type of material they manufacture/process. For instance, a manufacturer of a monomer will not be able to perform a migration study with their product since it does not come close to an article that will come into contact with food. The manufacturers may generate peak patterns for their products to check the reproducibility of, for example, processing, see also section 5.2.4, step 4. The identity and quantity of NIAS can be determined in these chromatographic peak patterns. Another example is the manufacturing of final articles that will be in contact with food. Here migration or extraction experiments may be performed to obtain insight into the IAS and NIAS that migrate, see section 5.2.1.

Table 7 shows an illustrative supply chain for the manufacturing of final articles made of plastic. Similar tables can be constructed for other types of FCM, but for some FCM, it will be more difficult to prepare reliable predictions (e.g. paper/board). A suggestion is made on what type of experiment can be used at the several levels in the supply chain to perform the estimation of migration of IAS and NIAS.

Table 7: Available approaches for the estimation of migration of NIAS from plastic materials.

Level in supply chain	Worst case migration	Mathematical modelling	Actual migration
Substance Responsibility: *chemical industry*	Worst case calculation for impurities from list of substances and certificate of analysis	Not appropriate	Migration not possible, peak pattern analysis (see section 5.1.4, step 4)
Polymer Responsibility: *polymer manufacturer*	Worst case calculation for oligomers or predicted IAS/NIAS	Estimate migration of known NIAS or oligomers	Migration not appropriate, peak pattern analysis (see section 5.1.4, step 4)
Plastic intermediate Responsibility: *converters, film/pellet manufacturer*	Worst case calculation from information received	Estimate migration of known NIAS	Evaluation of migration by using appropriate simulant(s) according to food products
Plastic article Responsibility: *film, article manufacturer*	Worst case calculation from information received	Estimate migration of known substances in inks, adhesives, coatings	Evaluation of migration by using appropriate simulant(s) according to food products
Plastic article in contact with food Responsibility: *food producer*	Worst case calculation from information received	Modelling with information received or composition analysis (screening of FCM)	Evaluation of migration in food

5.4.2.1. Migration testing

How migration testing should be performed is referred to in section 5.2.1. Results from this migration testing should be used. A FCM migrate is made using the simulants and time/temperature conditions as required by Regulation EU 10/2011 on plastics or other relevant legislation for other types of FCM (EU, 2011). For Forest-of-Peak (FOP) screening, the use of oils from plant origin is not recommended as the oil itself already contains a large amount of substances which may interfere with the FOP screening. Instead, 95 % ethanol and/or iso-octane might be considered for use. The migrate should be prepared in such a way that it can be analysed, e.g. a 3 % acetic acid migrate may be extracted into an organic solvent before analysis with GC-MS.

5.4.2.2. Worst case calculation

For the predicted NIAS, the assessment of the migration of NIAS, at an early stage of the packaging material's development/manufacture can be realized theoretically by using worst case calculation (the total mass transfer) or by simulation with mathematical models.

An example of worst case calculation has been developed by Plastics Europe in their document entitled 'Risk Assessment Of Non-Listed Substances (NLS) And Not-Intentionally Added Substances (NIAS) Under Article 19'[14].

Also for non-identified substances, based on residual levels determined by estimated quantitative analysis, worst case calculation can be used to determine whether the levels of NIAS are below or above the level of interest, taking into account exposure data. Polymer and plastic producers are using the results of the exposure matrix project for that purpose, see section 5.4.4.

All of the manufacturers of packaging elements, such as polymers, cardboards, glass, inks, adhesives and coatings should conduct a worst case migration assessment (i.e. a calculation that assumes 100% transfer from the material or article to the foodstuff) when they manufacture a substrate (e.g. polymer, cardboard, etc.) or formulate a mixture (e.g. inks, adhesives and coatings). Several European associations of packaging material manufacturers, such as the European Printing Ink Association (EuPIA) for inks, the Association of the European Adhesive & Sealant Industry (FEICA) for adhesives, or CEPI for cardboard, propose decision trees for the selection of raw additives. The worst case migration calculation could be applied to NIAS present in raw additives in the same way as for the additives themselves.

5.4.2.3. Mathematical modelling of migration

For the predicted NIAS, mathematical modelling of migration can be applied. The European Union introduced the option to use generally recognised migration models as a novel compliance and quality assurance tool with Directive 2001/61/EC (EC, 2001). In 2010, the Joint Research Centre at ISPRA, the official laboratory of EU Commission, published a Technical Report in which the input parameters for mathematical modelling of migration are reported for plastic materials. It is assumed that the migration of organic substances from polymeric materials such as PP, PE or PET is governed by Fick's 2nd equation of diffusion. To obtain quantitative results, the diffusion coefficient D of the organic substance in the polymer needs to be known. The approach to estimate D is to correlate this coefficient with the relative molecular mass, M, of the migrant with a polymer specific parameter, Ap, and a polymer specific "activation energy", τ.

It is well established that, in most cases, the mass transfer (migration) of substances from polymeric materials to contact media obeys Fick's laws of diffusion (Franz and Brandsch, 2013). Knowledge about the diffusion properties of polymeric materials enables the simulation of their migration behaviour under real conditions of use, that is, calculation of migration levels for a given migrant from a polymer of any thickness, area to volume ratio, and at any time and temperature conditions of interest. For the usual high volume polymers such as polyolefins, PET, polyamides, PS and PVC, such a pragmatic migration model has been established which is today scientifically recognised and widely used for food law compliance evaluation purposes and to substantiate technical dossiers for petitioning of new polymer additives to authorities such as the EFSA or the US FDA.

Open software tools

Several companies/institutes offer software for migration modelling such as: INRA Safe Food Packaging Portal version 3[15], MIGRATEST software[16] and AKTS-SML Software.[17]

14. http://www.plasticseurope.org/plasticssustainability/consumer-protection.aspx
15. http://modmol.agroparistech.fr/SFPP3/SFPP3download.html
16. http://www.fabes-online.de/software.php?lang=en&mode=migratest
17. http://www.akts.com

5.4.3. Food consumption assessment

For plastic FCMs, it has generally been conventionally assumed in the past that a surface to volume ratio of 6 dm^2 packaging material is used to package 1 kg of food. However, in many applications, the actual packaging to mass of food ratio varies considerably from this value. Examples include foods individually packaged in cartons or plastics instead of bulk packaging, and pizza boxes which typically have a surface area to volume ratio higher than 6 dm^2/kg food and small parts of, for example, coffee machines that will have a surface to volume ratio lower than 6 dm^2/kg food. Also, infant food requires a different approach since children have a low body weight. The surface to volume ratio of 6 dm^2/kg food should therefore be considered carefully when assessing exposure.

A tool that may be used to refine exposure to NIAS was developed in the FACET project. The FACET exposure tool (Flavours Additives and Food Contact Materials Exposure Tool) is the product of an EU FP-7 funded project that ran from 2008 to 2012. The FCMs covered are the packaging of retail foods, these being plastics (both flexible and rigid), metal containers, light metal packaging, paper and board, as well as the adhesives and inks used on them. The FACET tool is available at the JRC website[18] and it is maintained and developed further by the FACET User Group (Oldring *et al.*, 2014a, 2014b).

It should be considered that FACET is only a tool for packaging material exposure and cannot be used for a general exposure assessment of all food contact materials. For example, exposure to cyclic polyamide oligomers may be related to the use of polyamide films in food packaging as well as to the common use of nylon kitchen utensils. FACET would only cover the packaging part.

The tool makes estimating exposure of European consumers to packaging substances both easier and more accurate. It contains actual food consumption data for eight EU countries for different age groups with 15 surveys in total each covering typically one to two thousand consumers. It also has databases on packaging composition (e.g. layer-by-layer descriptions), packaging usage (which materials are used in contact with which foods) along with details of the substances used to make these packaging materials. Each of these information sources has market share details to represent the EU market. These data in FACET are combined to produce concentration distributions for substances in foods, using a mathematical migration model. In the case of coatings on light metal packaging, where migration can be exhaustive and migration modelling is not so useful, migration or extraction data (on a mg/dm^2 basis) are used as direct inputs thus bypassing the migration module. The concentration distributions are then linked probabilistically to the amounts of each food item consumed, as recorded in the National food consumption survey diaries, in order to estimate exposure to packaging migrants. Estimates of exposure are at the level of the individual consumer and thus can be expressed for various percentiles of different populations and sub-populations covered by the national dietary surveys.

When considering exposure, packaging loyalty has to be taken into account. Unlike brand loyalty, where a consumer may always drink, for example, one brand of cola irrespective of its packaging, a packaging loyal consumer will always drink a can or bottle of cola irrespective of its brand. The user of the FACET software has the option to run the program with and without loyalty. This will give a range of exposures. Loyalty can be selected for any food item at any food tier. As a generalisation, running an exposure assessment with loyalty should stretch-out the exposure distributions and give the highest exposure and so can be considered to be conservative. It should be borne in mind that, for some foodstuffs which have a high market share in a particular type of package, there may be only small differences in setting the loyalty flag off or on.

18. http://ihcp.jrc.ec.europa.eu/our_activities/food-cons-prod/chemicals_in_food/FACET

FACET has an extensive database of the substances used to make FCMs but no database on the occurrence and concentrations of NIAS. Estimates of exposure to NIAS are addressed in one of three ways using the FACET tool for a NIAS associated with (i) an existing substance; (ii) a packaging material; (iii) a type or group of foodstuffs. The input data are different for light metal packaging and other FCMs as the migration model is not used for the former.

1. For a NIAS that is associated with a known substance

This would be the case if the NIAS was an impurity or a transformation product of an existing substance. The user has the option to pick an existing substance, say Substance A, and associate a NIAS to it. For the NIAS, the user enters a molecular weight and the log Pow (needed to run the migration module) as well as a percentage value which indicates the concentration of the NIAS in Substance A, whenever Substance A appears in a material. For light metal packaging, migration or extraction data are used. The software then draws upon the extensive databases to link the NIAS with all known uses of Substance A and thereby estimate exposure.

2. For a NIAS that is associated with a particular material or process

The NIAS can be associated with a material or materials by selecting the materials that the NIAS has been/could be found in. The user needs to enter the concentration (either for migration modelling or measured) of the NIAS in each material (inputs can be point estimates or statistical distributions) along with the market share the NIAS has in each material (as a percentage).

3. For a NIAS that is associated with one or more different food groups

This would be the case, for example, if a NIAS was discovered to affect a certain type of group of foodstuffs. The concentration(s) of the NIAS in each food group is uploaded along with estimates of the probability of occurrence – what fraction of foods may be affected. The tool then simply uses these concentration data with the food consumption statistics to estimate exposure.

5.4.4. Migration level relevant in the safety evaluation of NIAS

Two options can be considered to set a migration level above which a safety assessment of NIAS is needed.

1. detection above the detection limit of the analytical method or,
2. detection above a level corresponding to a safe exposure threshold, the so-called exposure-based approach.

In the first option, the figure of 10 µg/kg food is the European conventional limit of detection. This value has no relation to a health-derived threshold but was introduced in the Regulation EU 10/2011 as a typical detection limit of the analytical techniques used (EU, 2011). As no exposure is anticipated at the detection level, this threshold is accepted as a no risk level for the consumer. This level is quoted in Annexes I and II of Regulation EU10/2011 and used in the definition of functional barrier for the IAS as far as they are not classified as CMR and are not in the nano-form.

This 10 µg/kg threshold, although widely used for the assessment of NIAS and other non-listed substances migrating (also from materials that do not have a functional barrier), could represent a challenge with some FCM as they may release many NIAS exceeding this limit that are sometimes difficult or not possible to identify.

In the second option, a level of migration corresponding to a safe exposure threshold (based on substance specific data or *in-silico* tools) could be derived. This level, also called the Level Of Interest (LOI), may be derived as introduced in the Matrix project[19]. There, the actual consumption of the packaged food is needed to calculate the surface area of the packaging used to package the daily consumed foods. Together with the measured migration from the packaging, the exposure can be calculated:

$$\text{Exposure [µg/person/day]} = \text{migration [µg/dm}^2\text{]} \times \text{surface area [dm}^2\text{/person/day]}$$

The Matrix database was jointly initiated, financed and supported by Cefic-FCA, European Plastics Converters (EuPC), Flexible Packaging Europe (FPE) and PlasticsEurope and is publicly available. A free web-based tool allows one to calculate exposure based on the Matrix consumption tables (https://matrixcalculation.eu/). The Matrix Project derived country data sets for Germany, France, Italy, Spain and United Kingdom with the respective packaging surface to which consumers are exposed per plastic material group and per consumed food and the respective calculation of LOIs.

As with FACET, MATRIX is also a tool for packaging material exposure and cannot be used for a general exposure assessment of all food contact materials.

In the context of the revision of its guidelines for evaluation of FCM for authorisation purposes, the EFSA is also considering different exposure scenarios. Three age groups are under discussion (infants, toddlers, and adults)[20] together with different food categories. The Guidelines are not yet available.

This approach has been used in the US for many years. The US FDA has generated consumption data for packaged food which are used in risk assessments for regulatory purposes. The food consumption data for different packaging materials (Consumption Factors and Food-Type Distribution Factors) are described in the document 'Guidance for Industry: preparation of premarket submissions for Food Contact substances: chemistry recommendations' and can be found on the internet.[21] Tables I and II in annex IV of this FDA document give the overview of the figures that can be used in the determination of the estimated daily intakes. Additionally, the guidance document contains detailed instructions on how to calculate consumer exposure to substances migrating from packaging materials.

Although, to the best of the authors' knowledge, there are at present no publically available tables handling the food consumption data for different packaging materials at the EU level, we can envisage that in the near future, similar tables will be published. Those tables might serve as a guidance to determine the level of interest for those types of materials based on estimated daily intake.

5.4.5. Limitations of exposure assessment

A few tools are available that may be used to estimate exposure to NIAS, for example, by listing for which applications and food types a specific NIAS containing material is used. FACET could be helpful for this purpose. However, the source of exposure to NIAS may not only be from packaging but also from other types of FCM such as kitchen utensils, from food or other sources. Except for packaging, these sources are not covered by FACET. An example is the waxing of fruit to avoid water loss and mineral oils from cocoa beans. Furthermore, food consumption and food packaging types for certain foods can vary markedly from one EU country to another. Since food consumption data from 'only' eight EU member states have been included in FACET, this is also a source of uncertainty of exposure assessment.

19. http://www.plast.dk/billeder/fakta/PlasticsEuropeexposurematrixprojectOct2009.pdf
http://www.ilsi.org/Europe/Documents/117.pdf

20. Guidance of selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured date, EFSA Journal 2012; 10(3): 2579.

21. http://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/ingredientsadditivesgraspackaging/ucm081818.htm

However, with today's tools in exposure assessment, it is not possible to estimate exposure to unpredicted unknown NIAS coming from different sources. Even for many known predicted NIAS, sufficient information is lacking to perform a full exposure assessment. An exception to this is, for example, bisphenol-A (EFSA, 2014).

5.5. Risk assessment of NIAS

The data retrieved from Chapters 5.1-5.4 will be combined in a substance specific risk assessment resulting in a conclusion on the safety of the NIAS.

5.5.1 Threshold of Toxicological Concern (TTC)

The threshold of toxicological concern (TTC) is a pragmatic risk assessment tool that is based on the principle of establishing a human exposure threshold value for all chemicals, below which there is a very low probability of an appreciable risk to human health (Kroes *et al.,* 2004). If the chemical structure of a substance is known, its likely health risk can be evaluated on the basis of generic human thresholds of exposure for chemicals (these are called 'TTC values'). TTC values have been established for substances of similar chemical structure and likelihood of toxicity, based on extensive published toxicological data. The TTC approach is not meant to be a replacement for the risk assessment of regulated substances such as plastic monomers and additives, where there is a legislative requirement for the submission of toxicological data (EFSA, 2012b).

The 'TTC values' (LOIs, see section 5.4.4.) are:

- <0.15 µg/person per day (or 0.0025 µg/kg bw/day) for substances with a structural alert for genotoxicity,
- <18 µg/person per day (or 0.3 µg/kg bw/day) for organophosphate and carbamate substances with anti-cholinesterase activity,
- <90 µg/person per day (or 1.5 µg/kg bw/day) for Cramer Class III and Cramer Class II substances, and
- <1800 µg/person per day (or 30 µg/kg bw/day) for Cramer Class I substances.

However, some substance groups are excluded from the TTC approach (EFSA, 2012c; Koster, 2011) since they are either not safe at the <0.15 µg/person/day value ('Cohort of concern', CoC; Kroes *et al.,* 2004) or not sufficient information has been gained within the TTC approach. Therefore, these groups require an independent hazard assessment as described above (e.g. toxicological data from the literature, read-across). The following list summarises the TTC exclusion groups with special respect to FCMs:

- High potency carcinogens for which even the 0.15 µg/person per day level is too high

  o N-Nitroso and N-nitrosatable substances[22]

  Rubber materials in particular are known to contain N-nitrosoamines and N-nitrosatable substances, respectively, and are regulated in Commission Directive 93/11/EEC (EEC, 1993). Since an endogenous nitrosation of N-nitrosatable substances can take place in humans, the latter are limited as well and should be analysed along with N-nitrosamines if there are indications for the presence of the latter.

22. Cohort of concern according to Kroes et al. (2004): three high potency genotoxic carcinogens (aflatoxin-like compounds, N-nitroso-compounds and azoxy-compounds), and two non-genotoxic carcinogens (steroids and polyhalogenated dibenzo-p-dioxins and-dibenzofurans).

o Aflatoxin-like substances[22]

o Azoxy-compounds[22]

o Benzidines

Benzidine and other primary aromatic amines (PAAs) can be NIAS (impurities, breakdown products) in certain pigments used in colourants/printing inks for plastics and paper and board, respectively.

o Hydrazines

Semicarbazide (a hydrazine derivative) has been identified as a NIAS formed during the degradation of the blowing agent azodicarbonamide in PVC compounds of twist-off caps. However, in recent toxicological studies, semicarbazide has not been identified as a 'high potency carcinogen.'

- Substances that are known or predicted to bioaccumulate

o Polyhalogenated dibenzo-p-dioxins, dibenzofurans and dioxine-like PCBs[22]

These substances are found in products of biological origin as result of accumulation in the environment. Materials such as paper, cork and wood should at least be evaluated for the presence of these substances. Dioxins are also known as impurities of pigments for printing inks due to the use of halogenated solvents for finishing of the pigments.

o Perfluorooctanoic acid (PFOA)

PFOA has been used as a surfactant in the emulsion polymerisation of fluoropolymers which are used for coatings of kitchen utensils such as frying pans and grease release coatings for paper and board, respectively. PFOA is persistent in the environment and bioaccumulates in blood, liver and kidneys.

o Mineral oil

Mineral oil saturated hydrocarbons (MOSH, C16–C35) which are found as contaminants, for example, in recycled paper and board can accumulate in human tissues (investigations in progress)

- Metals and organometallics
- Steroids[22]
- Nanomaterials
- Radioactive substances
- Proteins

o Allergens

Latex proteins (e.g. in gloves, cold seals) can trigger contact allergies

Application of the TTC approach to NIAS from Food Contact Materials

In case the calculated exposure exceeds the Limit of Interest (LOI, for instance derived from the TTC level of the specific substance or structure), the production process of the FCM or starting substance should be adjusted to eliminate or reduce the presence of the NIAS in the FCM below the relevant LOI.

Generally, a food contact manufacturer would like to know as much as possible on the identities of the substances detected above the LOI (see section 5.4.4) from their FCM or starting substances (exposure and risk). A substance specific risk assessment will then typically be performed. There are however situations where not all migrants can be identified above this migration level. Examples are from coatings where hundreds of molecules may be formed that cannot all be identified (see also previous chapters). Therefore, the risk assessment approach to be used for NIAS depends on the possibility to identify all peaks in a chromatogram and also depends on the type of material and the exposure to the migrating substances. The following NIAS risk assessment approaches can be distinguished:

1. Substance specific risk assessment for the predicted NIAS
2. Substance specific risk assessment for identified unpredicted NIAS
3. Risk assessment for detected but unidentifiable unpredicted NIAS

These approaches are elaborated in sections 5.5.2–5.5.4. In the risk assessment strategies described in these sections, the outcome of *in-vitro* bioassays may be used as supporting information, see section 5.3.1.

5.5.2. Substance specific risk assessment for the predicted NIAS and identified NIAS

For many of the predicted NIAS, toxicological evaluations have frequently been reported in scientific opinions, for example, by EFSA or other national/European authorities which establish health-based guidance values such as the TDI. These can be used to establish the level of concern or LOI. If no toxicological evaluations from national/European authorities are available, substance specific toxicological data can be retrieved from the literature and used through a MoE approach. The level of concern or LOI will depend on the size of the MoE and can be assessed according to scientific principles (Schilter *et al.,* 2014). If no toxicological data are available, TTC in combination with computational toxicology may be applied to arrive at a MoE estimate.

In cases where the predicted NIAS exceeds the TDI, the production process of the FCM or starting substance should be adjusted to eliminate or reduce the presence of the NIAS in the FCM below the relevant TDI. In cases where no safe threshold can be determined or where the MoE is considered insufficient, the production process of the FCM or starting substance should be adjusted to reduce or eliminate the presence of the NIAS in the FCM. If it is not possible to reduce the NIAS below its LOI, the normal scheme for toxicity testing needs to be followed for this NIAS, see section 5.4 (EFSA, 2008).

5.5.3. Substance specific risk assessment for the identified unpredicted NIAS

For unpredicted NIAS that were identified, a substance specific risk assessment should be performed if exposure exceeds the LOI. In cases where not all peaks detected in a chromatogram can be identified, more attempts should be performed to identify them using, for example, different analytical techniques. The first step in the substance specific risk assessment is a similar process to what was described in section 5.5.2 for predicted and identified NIAS.

5.5.4. Substance specific risk assessment for detected but unidentifiable unpredicted NIAS

Unpredicted NIAS that could not be identified and could not be eliminated or reduced below the LOI (see section 5.5.5) by adjusting the production process for manufacturing the FCM or starting substance, should undergo additional analytical chemistry testing to identify the NIAS.

If the use of several analytical techniques does not help to identify the structure of the NIAS, partial characterisation may help to classify the unidentifiable NIAS into a specific chemical category (e.g. saturated branched alkanes, etc.).

If partial identification does not help the risk assessment process, the risk assessment approach for unknowns (Rennen *et al.*, 2011; Koster *et al.*, 2014) could be applied. This approach is a stepwise analytical approach based on exclusion of specific groups of compounds following the TTC decision tree of Kroes et al. (2004) with modifications as proposed by Munro et al. (2008). The LOI (Cramer class III threshold) is only applicable in cases where it can be demonstrated that the unidentified NIAS is:

- not genotoxic,
- not an organophosphate/carbamate,
- does not belong to one of the special categories for which use of TTC is excluded (such as non-essential metals), see below.

In the case of partly identified substances with similar chemical structure and thus anticipated mode of action (MoA), the cumulative exposure can be taken into account. Note that the value for Cramer Class III substances (1.5 µg/kg bw/day or 90 µg per person per day for an adult of 60 kg) may be subject to changes in the future (higher or lower value) if new toxicological insights become available (e.g. Leeman *et al.*, 2014; EFSA, 2015).

If the NIAS exceeds the LOI, it has either to be reduced during the manufacturing process or the EFSA scheme for toxicity testing as outlined in the 'Note for Guidance' needs to be followed for this NIAS, see Appendix 2 (EFSA, 2008).

The derived LOI depends on the possible presence of genotoxic substances. Although there are several efforts being made to improve (sensitivity of) genotoxicity assays, it is difficult to exclude the possibility that the unidentified unpredicted NIAS is genotoxic since the substance might be present at a low concentration in the migrate/extract. Therefore, information on the samples under investigation in section 5.1, steps 1–5 may serve as supporting information to demonstrate that genotoxic substances are unlikely to be present. The use of *in-vitro* bioassays may help in the risk assessment for unidentifiable unpredicted NIAS as described in section 5.3.1.

5.5.5. Interpreting and managing the data of in-vitro bioassays

The *in-vitro* bioassays that have been selected intend to cover mechanisms of action known to act at low doses and possibly without threshold, i.e. genotoxicity. They are intended to detect a certain adverse activity in the sample at a detection level that has to be determined. This is especially of interest when full chemical identification is impossible. However, this approach is hazard-based as it does not per se inform on the potential health risk for the consumer.

The results of the *in-vitro* bioassays may theoretically result in two main situations:

- No activity is observed in both genotoxicity/cytotoxicity and endocrine activity bioassays, or
- Some activity is observed in one or both of the *in-vitro* bioassay, genotoxicity or endocrine activity.

In the first case, no concern is anticipated at this stage as no hazard linked to genotoxicity/cytotoxicity and endocrine activity has been observed. The risk assessment made in parallel with the detectable NIAS (both identified and unidentified) of which the quantity was estimated, will drive the final decision. Note that, until now, *in-vitro* genotoxicity bioassays cannot reach down to the (sub) ppb level for genotoxic substances to exclude their presence. The same is the case for non-targeted analytical chemistry screening to exclude the presence of molecules containing a structural alert for genotoxicity. These limitations should be considered when performing a risk assessment or when making a risk management decision.

In the second case, further work to link the biological activity to specific substance(s) could be needed. Then different options are possible.

- Identify the triggering substance or substance group, and perform additional studies (i.e. *in-silico* toxicology or *in-vivo* toxicology studies) to conduct a risk assessment of this substance(s). Note that the chemical structure needs to be known when performing *in-silico* toxicology.
- Check to use a different suitable material without activity in *in-vitro* bioassays and without other drawbacks.
- Test measures on the material that eliminate or reduce the *in-vitro* bioassay activity (may be supported by pattern analysis of detectable NIAS).

5.5.6. Limitations of the risk assessment

The risk assessment of FCMs strongly depends on a proper exposure assessment. This is probably the most critical piece of information as the source to which exposure to NIAS occurs is an uncertain step in the risk assessment process. It should be noted, however, that this is an uncertainty which is not specific for FCM. Accurate exposure information is difficult to obtain for most risk assessments due to a lack of data on the full production process and the different non-FCM sources of the NIAS.

A risk assessment is typically only performed on NIAS that exceed 10 µg/kg food. This is a regulatory defined LOD which is not based on analytical or toxicological facts. It finds limited use for the risk assessment of NIAS since many NIAS exceed this threshold making risk assessment difficult if not impossible and, on the other hand, a lower concentration of NIAS does not guarantee the absence of concern. A better and more realistic approach is to move to exposure driven risk assessment.

Combination toxicity where two or more substances are present below the LOI but which may have the same target organ or mechanism or mode of action, is in principle, not covered by the NIAS screening approaches. According to two literature studies (EU-SCENIHR, 2012; Boobis *et al.,* 2011) and a publication by Leeman et al. (2013), the health relevance of possible cumulative effects at low (TTC) dose levels is considered to be so low that a need for a correction factor to cover possible cumulative effects is very low to absent. However, for endocrine active substances and genotoxic substances, possible cumulative effects at low dose cannot be excluded (Leeman *et al.,* 2013). The latter can be covered by performing *in-vitro* bioassays on these end points.

Risk assessment of NIAS should ascertain that these do not contain a potential for genotoxicity. For non-identified NIAS, it is not possible to determine whether these have a structural alert for genotoxicity. The alternative approach is to perform a genotoxicity *in-vitro* assay. However, the sensitivity of the applied assay has to be validated towards known FCM related genotoxic substances (e.g. certain primary aromatic amines) and be related to the FCM under investigation. Some promising *in-vitro* bioassays are in development that will inform about the mechanism of action of substances (EFSA, 2011). Therefore, genotoxicity testing according to the current acceptable state-of-the-art is being advised.

Although some limitations are described, it should be noted that the risk assessment strategy for unidentified NIAS as described in this report is currently the only approach available for unknown chemicals. The approach should therefore be considered to be state-of-the-art until new and/or more sensitive methods become available. However, since no alternative is available to evaluate these unidentifiable substances, the best one can do today is that described in this guidance.

FCM	NIAS	Authors	Origin/source
Plastic	Cyclic polyamide oligomers	Heimrich *et al.* (2012)	Cyclic oligomers in polyamide for food contact material: quantification by HPLC CLND and single substance calibration
	Styrene oligomers	Kawamura *et al.* (1998)	Migration from polystyrene containers into food
	Nonylphenol	Toyo'Oka and Oshige (2000)	PET bottled mineral water
	Multiple NIAS	Aznar *et al.* (2011)	Migration of NIAS originating from adhesive in multilayer materials
	Multiple NIAS	Skjevrak *et al.* (2005)	Migration of NIAS such as breakdown products/impurities from bottles and multi-layer materials
	Multiple NIAS	Simoneau *et al.* (2012)	Identification and quantification of the migration of chemicals from plastic baby bottles used as substitutes for polycarbonate
	Polyolefin oligomers	Biedermann-Brem *et al.* (2012)	Migration of POSH into food
	Odour active substances in PP and irradiated PP	Tyapkova *et al.* (2009)	Characterisation of flavour compounds formed by --irradiation of polypropylene
	NIAS	Bach *et al.* (2012)	Migrants from PET bottles for drinking water
	Oligomers	Nelson *et al.* (2011)	Assessing toxicity of low molecular weight oligomers that may migrate from several types of FCM
Paper/carton	Diisopropylnaphthalene isomers	Honkalampi-Hämäläinen *et al.* (2010)	Migration from board FCM
		Bradley *et al.* (2010)	
	Phthalates	Gartner *et al.* (2009)	Migration to infant food packaged in recycled paperboard
	Mineral oil	Droz and Grob (1997)	Contamination by mineral oil from printed cardboard
	Phthalates	Brauer and Funke (2008)	Contamination by phthalates from recycled fibres
	Benzophenone	Brauer and Funke (2008)	Photo-initiator in UV cured printing inks
	UV initiators; benzophenone, ITX, etc	Sagratini *et al.* (2008)	Migration of photo-initiators to packaged beverages

FCM	NIAS	Authors	Origin/source
Paper/carton	Perfluorinated substances	Begley *et al.* (2005)	Migration of perfluorinated substances to simulants
	Genotoxic effect for overall migrants	Ozaki *et al.* (2004)	Genotoxic effect detected for overall migrants from recycled and virgin paper FCM
Coatings	BADGE	Cabado *et al.* (2008)	Migration of BADGE and BFDGE to seafoods
	BADGE	Petersen *et al.* (2008)	BADGE migrating from packaging material 'disappears' in food: reaction with food components
	BADGE derivatives	Schaefer and Simat (2004)	Migration from can coatings: Part 3. Synthesis, identification and quantification of migrating epoxy-based substances below 1000 Da
	Polyester oligomers	Schaefer *et al.* (2004)	Migration from can coatings: Part 2. Identification and quantification of migrating cyclic oligoesters below 1000 Da
Adhesives	Reaction products	Félix *et al.* (2012)	Polyurethane adhesive in multilayer packaging materials
	Biocides	Canellas *et al.* (2010, 2012)	Biocides suspected to be allergenic/cytotoxic in acrylic adhesives
Kitchen utensils		Skjevrak *et al.* (2005)	Migration of NIAS such as breakdown products/impurities from kitchen utensils
	Cyclic dimethylsiloxanes	Helling *et al.* (2009)	Determination of the overall migration from silicone baking moulds into simulants and food using 1H-NMR techniques

BADGE, Bisphenol-A diglycidyl ether; BFDGE, bisphenol-F diglycidyl ether; FCM, food contact materials and articles; HPLC-CLND, high-performance liquid chromatography with chemiluminescent nitrogen detection; ITX, ; NMR, nuclear magnetic resonance; PET, polyethylene terephthalate; POSH, polyolefin oligomeric saturated hydrocarbons; PP, polypropylene.

GLOSSARY

AAS	Atomic absorption spectroscopy
AP	Aids to polymerisation
APCI	Atmospheric pressure chemical ionisation
APPI	Atmospheric pressure photoionisation
BADGE	Bisphenol-A diglycidyl ether
BPA	Bisphenol-A
CAD	Charged aerosol detection
CCP	Critical control point
CEN	European Committee for Standardisation
CEPI	Confederation of European Paper Industries
CI	Chemical ionisation
CMR	Carcinogenic/mutagenic/reprotoxic
CoC	Cohort of concern
DoC	Declaration of Compliance
DT	Decision tree
EFSA	European Food Safety Authority
EI-MS	electron ionisation mass spectroscopy
ELSD	Evaporative light scattering detection
FACET	Flavours additives and food contact materials exposure tool
FCM	Food contact materials and articles
FCN	Food contact notification
FCS	Food contact substance
FDA	Food and Drug Administration
FEICA	Association of the European Adhesive & Sealant Industry
FID	Flame ionisation detection
FOP	Forest-of-peaks
FT-MS	Fourier transform mass spectrometry
GC-MS/FID	Gas chromatography mass spectrometry/Flame ionisation detector
GMP	Good manufacturing practice
HILIC	Hydrophilic interaction liquid chromatography
HPLC-CLND	High-performance liquid chromatography with chemiluminescent nitrogen detection
IAS	Intentionally added substance
ICP-MS/OES	Inductively coupled plasma mass spectrometry/optical emission spectrometry
ITX	Isopropylthioxanthone
LC-MS	Liquid chromatography mass spectrometry

LC-UV	Liquid chromatography ultraviolet detection
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
LOI	Level of interest
LOQ	Limit of quantification
MoA	Mechanism/mode of action
MOAH	Mineral oil aromatic hydrocarbon
MoE	Margin of exposure
MOH	Mineral oil hydrocarbon
MOSH	Mineral oil saturated hydrocarbon
MS	Mass spectrometry
MSDS	Materials safety data sheet
MW	Molecular weight
NIAS	Non-intentionally added substance
NLS	Non-listed substance
NMR	Nuclear magnetic resonance
NQAD	Nano quantity analyte detection
PAA	Primary aromatic amine
PET	Polyethylene terephthalate
PFOA	Perfluorooctanoic acid
POSH	Polyolefin oligomeric saturated hydrocarbons
PP	Polypropylene
PPA	Polymer production aid
PS	Polystyrene
PVC	Polyvinyl chloride
QSAR	Quantitative structure activity relationship
SARs	Structure–Activity Relationships
SML	Specific migration limit
SPME	Solid phase microextraction
TD50	Median toxic dose (carcinogenic potency)
TDI	Tolerable daily intake
TOF-MS	Time of flight mass spectrometry
TTC	Threshold of toxicological concern
UVCB	Substances of unknown or variable composition, complex reaction products or biological materials

REFERENCES

AFSSA (2006). Etat des connaissances sur une approche globale de l'appréciation de l'innocuité appliquée à des migrats issus de matériaux au contact des denrées alimentaires. AFSSA, 2006.

AFNOR XP (1996). Effet des matériaux sur la qualité des eaux destinées à la consommation courante. Matériaux organiques. Partie 3 : Méthode de mesure de cytotoxicité. p 41-250-3.

Aznar M, Vera P, Canellas E, Nerín C, Mercea P, Störmer A (2011). J. Mater. Chem. 21, 4358-4370.

Baan R, et al. (2008). Carcinogenicity of some aromatic amines, organic dyes, and related exposures. *Lancet Oncol.* 9, 322-332.

Bach C, Dauchy X, Chagnon M-C, Etienne S (2012). Chemical compounds and toxicological assessments of drinking water stored in polyethylene terephthalate (PET) bottles: a source of controversy reviewed. *Water Res.* 3, 571-583.

Begley TH, White K, Honigfort P, Twaroski ML, Neches R, Walker RA (2005). Perfluorochemicals: potential sources of and migration from food packaging. *Food Addit. Contam.* 22, 1023-1031.

Belgian Royal Decree (Koninklijk besluit/Arrêté royal). May 11th 1992.

Benfenati E, Benigni R, Marini D, Helma C, Kirkland D, Martin TM, Mazzatorta P, Meunier J-R, Ouédraogo-Arras G, Richard A, Schilter B, Schoonen WGEJ, Snyder R, Yang C, Youne DM (2009). Predictive models for carcinogenicity and mutagenicity. Frameworks, state-of-the-art and perspectives. J. Environ. Sci. Health, Part C 27, 57-90.

Bentayeb K, Batlle R, Romero J, Nerin C. (2007). UPLC-MS as a powerful technique for screening the nonvolatile contaminants in recycled PET. *Anal. Bioanal. Chem*. 388, 1031-1038.

Biedermann-Brem S, Kasprick N, Simat T, Grob K (2012). Migration of polyolefin oligomeric saturated hydrocarbons (POSH) into food. Food Addit. Contam. *Part A Chem. Anal. Control Expo. Risk Assess.* 29, 449-460.

Boobis AR, Budinsky R, Collie S, Crofton K, Embry M, Felter S, Hertzberg R, Kopp D, Mihlan G, Mumtaz M, Price P, Solomon K, Teuschler L, Yang R, Zalesky R (2011). Critical analysis of literature on low-dose synergy for use in screening chemical mixtures for risk assessment. Crit. *Rev. Toxicol.* 41(5), 369-383.

Bradley E, Coulier L (2007). An investigation into the reaction and breakdown products from starting substances used to produce food contact plastics. FD 07/01. *London: Central Science Laboratory.*

Bradley EL, Honkalampi-Hämäläinen U, Weber A, Andersson MA, Bertaud F, Castle L, Dahlman O, Hakulinen P, Hoornstra D, Lhuguenot JC, Mäki-Paakkanen J, Salkinoja-Salonen M, Speck DR, Severin I, Stammati A, Turco L, Zucco F, von Wright A (2008). The BIOSAFEPAPER project for in vitro toxicity assessments: Preparation, detailed chemical characterisation and testing of extracts from paper and board samples. *Food Chem. Toxicol.* 46(7), 2498-2509.

Bradley EL, et al. (2010). Test procedures for obtaining representative extracts suitable for reliable in vitro toxicity assessment of paper and board intended for food contact. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* 27(2), 262-271.

Brands B, Castle L, Duffy E, Franz R, Garia L, Gibney M, Hart A, Oldring P (2007). Guidance for exposure assessment of substances migrating from food packaging materials. ILSI Europe Workshop Proceedings. (http://www.ilsi.org/Europe/Publications/O2007Gui_Exp.pdf)

Brauer B, Funke T (2008). Bestimmung von Kontaminanten – Papier aus recycelten Fasern und verpackten Lebensmittel. *Dtsch. Lebensm.-Rundsch.* 104(7), 330-335.

Cabado AG, Aldea S, Porro C, Ojea G, Lago J, Sobrado D (2008). Migration of BADGE (Bisphenol-A diglycidyl-ether) and BFDGE (bisphenol F diglycidyl-ether) in canned seafood. *Food Chem. Toxicol.* 46, 1674-1680.

CAC (2003). Codex Alimentarius Commission. Working principles for risk analysis. In: *CAC Procedural Manual*, 13th edition. FAO, Rome, pp. 42-48.

Calow P (1998). General principles and overview. In: *Handbook of Ecotoxicology*, P. Calow editor. Blackwell Science, Oxford, pp. 1-6.

Canellas E, Nerín C, Moore R, Silcock P (2010). New UPLC coupled to mass spectrometry approaches for screening of non-volatile compounds as potential migrants from adhesives used in food packaging materials. *Anal. Chim. Acta* 666, 62-69.

Canellas E, Vera P, Domeño C, Alfaro P, Nerín C (2012). Atmospheric pressure gas chromatography coupled to quadrupole-time of flight mass spectrometry as a powerful tool for identification of non-intentionally added substances in acrylic adhesives used in food packaging materials. *J. Chromatogr.* A 1235, 141-148.

Chung KT (2000). Mutagenicity and carcinogenicity of aromatic amines metabolically produced from Azo Dyes. J. Environ. Sci. Health Part C Environ. *Carcinogen. Ecotoxicol. Rev.* 18(1).

Contrera JF (2011). Improved in silico prediction of carcinogenic potency (TD50) and the risk specific dose (RSD) adjusted Threshold of Toxicological Concern (TTC) for genotoxic chemicals and pharmaceutical impurities. *Regul. Toxicol. Pharmacol.* 59(1), 133-141.

COT (Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment) (2007). Variability and uncertainty in toxicology of chemicals in food, consumer products and the environment. (http://cot.food.gov.uk/pdfs/vutreportmarch2007.pdf)

Cramer GM, et al. (1978). Estimation of toxic hazard – a decision tree approach. *Food Cosmet. Toxicol.* 16, 255-276.

Depledge MH, Fossi MC (1994). The role of biomarker in environmental assessment (2). *Ecotoxicology* 3, 161-172.

DIN (2003). DIN EN ISO 10993-1, Biological evaluation of medical devices – Part 1: Evaluation and testing.

Droz C, Grob K (1997). Determination of food contamination by mineral oil material from printed cardboard using on-line coupled LC-GC-FID. Z. *Lebensm. Unters. Forsch.* 205(3), 239-241.

EC (2000). Directive 2000/60/EC of 23 October 2000 establishing a framework for Community action in the field of water policy. Off. J. Eur. Comm. L327/1.

EC (2001). Commission Directive 2001/61/EC of 8 August 2001 on the use of certain epoxy derivatives in materials and articles intended to come into contact with foodstuffs. Off. J. Eur. Comm. L215/26.

EC (2004). Regulation EU 1935/2004 of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. Off. J. Eur. Union. L338/4.

EC (2005). Regulation EU 1895/2005 of November 2005 on the restriction of use of certain epoxy derivatives in materials and articles intended to come into contact with food. Off. J. Eur. Union. L302/28.

EC (2006). Regulation EU 2023/2006 of 22 December 2006 on good manufacturing practice for materials and articles intended to come into contact with food. Off. J. Eur. Union L384/75.

EC (2007). Commission Directive 2007/42/EC of 29 June 2007 relating to materials and articles made of regenerated cellulose film intended to come into contact with foodstuffs. Off. J. Eur. Union. L172/71.

EC (2008). Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives. EFSA practical guide. Food contact materials, practical guide (available at EFSA website).

EC (2014). EC 589/2014. Regulation EU 589/2014 of 2 June 2014 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation EU 252/2012.

ECHA (European Chemicals Agency) (2012). Guidance for identification and naming of substances under REACH and CLP. ECHA-11-G-10.1-EN.
(http://echa.europa.eu/documents/10162/13643/substance_id_en.pdf)

EEC (1993). Commission Directive 93/11/EEC of 15 March 1993 concerning the release of the N-nitrosamines and N-nitrosatable substances from elastomer or rubber teats and soothers. Off. J. Eur. Comm. L93/37.

EFSA (European Food Safety Authority (2003). Practical Guide. SANCO D3/LR D (04.2003).

EFSA (European Food Safety Authority) (2006). Guidance of the scientific committee on a request from EFSA related to uncertainties in dietary exposure assessment. *EFSA J.* 438, 1-54.

EFSA (European Food Safety Authority) (2008). Food contact materials: Note for guidance for petitioners presenting an application for the safety assessment of a substance to be used in food contact materials prior to its authorisation. *EFSA J. Issue 7*, 1-125.
(http://www.efsa.europa.eu/en/efsajournal/doc/21r.pdf).

EFSA (European Food Safety Authority) (2011). Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. *EFSA J.* 9, 2379.

EFSA (European Food Safety Authority) (2012a). Report of ESCO WG on non-plastic FCMs. Supporting Publications 2012, 139.

EFSA (European Food Safety Authority) (2012b). FAQ on the Threshold of Toxicological Concern. (http://www.efsa.europa.eu/de/faqs/faqttc.htm)

EFSA (European Food Safety Authority) (2012c). Scientific opinion on exploring options for providing advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC). EFSA J. 10, 2750-2853. (http://www.efsa.europa.eu/de/efsajournal/doc/2750.pdf)

EFSA (European Food Safety Authority) (2014). Draft Scientific Opinion on the risks to public health related to the presence of Bisphenol-A (BPA) in foodstuffs.

EFSA (European Food Safety Authority) (2015). Threshold of toxicological concern approach: conclusions and recommendations of the EFSA/WHO expert workshop.
(http://www.efsa.europa.eu/en/consultations/call/150212.pdf)

EN15845 BS EN 15845:2010 Paper and board. Determination of the cytotoxicity of aqueous extracts. 31 March 2010.

EN16418 BS EN 16418:2014 Paper and board. Determination of the cytotoxicity of aqueous extracts using a metabolically competent hepatoma cell line (HepG2). 30 April 2014.

EPA (United States Environmental Protection Agency) Chemical substances of unknown or variable composition, complex reaction products and biological materials: UVCB substances. (available at http://www.epa.gov/opptintr/newchems/pubs/uvcb.txt).

EU (2011). Regulation EU 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. *Off. J. Eur. Union.* L12/1.

EU-SCENIHR (European Commission – Scientific Committee on Emerging and Newly Identified Health Risks) (2012). Toxicity and Assessment of Chemical Mixtures; European Commission; Scientific Committee on consumer Safety (SCCS), Scientific Committee on Health and Environmental Risks (SCHER), Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). (http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_155.pdf)

Félix JS, Isella F, Bosetti O, Nerín C (2012). Analytical tools for identification of non-intentionally added substances (NIAS) coming from polyurethane adhesives in multilayer packaging materials and their migration into food simulants. *Anal. Bioanal. Chem.* 403(10), 2869-2882.

Franz R, Brandsch R (2013). Migration of acrylic monomers from methacrylate polymers – establishing parameters for migration modeling. *Packaging Technol. Sci.* 26(8), 435-451.

Gartner S, Balski M, Koch M, Nehls I (2009). J. *Agric. Food Chem.* 57(22), 10675-10681.

Grob K, Biedermann M, Scherbaum E, Roth M, Rieger K (2006). Crit. Rev. Food Sci. Nutr. 46, 529-535.

Heimrich M, Bönsch M, Nickl H, Simat TJ (2012). Cyclic oligomers in polyamide for food contact material: quantification by HPLC-CLND and single-substance calibration. *Food Addit. Contam.* 29, 846-860.

Helling R, Mieth A, Altmann S, Simat TJ (2009). Determination of the overall migration from silicone baking moulds into simulants and food using 1H-NMR techniques. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* 26, 395-407.

Helling R, et al. (2012). Characterisation and migration properties of silicone materials during typical long-term commercial and household use applications: a combined case study. *Food Addit. Contam. Part A* 29(9), 1489-1500.

Honkalampi-Hämäläinen U, Bradley EL, Castle L, Severin I, Dahbi L, Dahlman O, Lhuguenot JC, Andersson M, Hakulinen P, Hoornstra D, Mäki-Paakkanen J, Salkinoja-Salonen M, Turco L, Stammati A, Zucco F, Weber A, von Wright A (2010). Safety evaluation of food contact paper and board using chemical tests and in vitro bioassays: role of known and unknown substances. *Food Addit. Contam.* 27(3), 406-415.

Hughes C, Rabionowitz A, Tate M, Birrell L, Allsup JM, Billington N, Walmsley R (2012). Development of a high-throughput gaussia luciferase reporter assay for the activation of the GADD45a gene by mutagens, promutagens, clastogens, and aneugens. *J. Biomol. Screen.* 17, 1302-1315.

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 99. Some Aromatic Amines, Organic Dyes, and Related Exposures (http://monographs.iarc.fr/ENG/Monographs/vol99/mono99.pdf). Also refer to Volumes 1, 4, 29, 57, or 77, or to Supplement 7.

JRC (Joint Research Centre) (2010). Applicability of QSAR analysis to the evaluation of the toxicological relevance of metabolites and degradates of pesticide active substances for dietary risk assessment. Report from the European Commission's Joint Research Centre (JRC) to the European Food Safety Authority (EFSA). (http://www.efsa.europa.eu/en/scdocs/scdoc/50e.htm.)

Kawamura Y, Nishi K, Maehara T, Yamada T (1998). Migration of styrene dimers and trimers from polystyrene containers into instant foods. *Shokuhin Eiseigaku Zasshi (J. Food Hyg. Soc. Japan)* 39(6), 390-398.

Kortenkamp A, Martin O, Faust M, Evans R, McKinlay R, Orton F, Rosivatz E (2011). State of the art assessment of endocrine disrupters.
(http://ec.europa.eu/environment/chemicals/endocrine/pdf/sota_edc_final_report.pdf)

Koster S, et al. (2011). Application of the TTC concept to unknown substances found in analysis of foods. *Food Chem. Toxicol.* 49, 1643-1660.

Koster S, Rennen M, Leeman W, Houben G, Muilwijk B, van Acker F, Krul L (2014). A novel safety assessment strategy for non-intentionally added substances (NIAS) in carton food contact materials. *Food Addit. Contam. Part A* 31(3), 422-443.

Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F, Vos JG, Würtzen G (2004). Structure-based thresholds of toxicological concern (TTC): Guidance for application to substances present at low levels in the diet. *Food Chem. Toxicol.* 42, 65-83.

Lapenna S, Fuart Gatnik M, Worth A (2010). Review of QSAR models and software tools for predicting acute and chronic systemic toxicity. Publications Office of the European Union. JRC61930. EUR24639 EN.

Leeman WR, Krul L, Houben GF (2013). Complex mixtures: relevance of combined exposure to substances at low dose levels. *Food Chem. Toxicol.* 58, 141-148.

Leeman WR, Krul L, Houben GF (2014). Reevaluation of the Munro dataset to derive more specific TTC thresholds. *Regul. Toxicol. Pharmacol.* 69, 273-278.

Lo Piparo E, Worth A, Manibusan M, Yang C, Schilter B, Mazzatorta P, Jacobs MN, Steinkelner H, Mohimont L (2011). Use of computational tools in the field of food safety. *Regul. Toxicol. Pharmacol.* 60(3), 354-362.

Lo Piparo E, Maunz A, Helma C, Vorgrimmler D, Schilter B (2014). Automated and reproducible read-across like models for predicting carcinogenic potency. *Regul. Toxicol. Pharmacol.* 70, 370-378.

Mazzatorta P, Estevez MD, Coulet M, Schilter B (2008). Modeling oral rat chronic toxicity. *J. Chem. Inf. Model.* 48(10), 1949-1954.

Migresives (2010). Research program on migration from adhesives in food packaging materials in support of European legislation and standardization. COLL-CT-2006-030309.

Muncke J (2001). Endocrine disrupting chemicals and other substances of concern in food contact materials: An updated review of exposure effect and risk assessment. *J. Steroid Biochem. Mol. Biol.* 127(1-2), 118-127.

Munro IC, Renwick AG, Danielewska-Nikiel B (2008). The threshold of toxicological concern (TTC) in risk assessment. *Toxicol Lett.* 180, 151-156.

Nelson CP, Patton GW, Arvidson K, Lee H, Twaroski ML (2011). Assessing the toxicity of polymeric food-contact substances. *Food Chem. Toxicol.* 49, 1877-1897.

Ohsawa K, et al. (2000) Genotoxicity of o-aminoazotoluene (AAT) determined by the Ames test, the in vitro chromosomal aberration test, and the transgenic mouse gene mutation assay. *Mutat. Res.* 20, 113-126.

Oldring PKT, O'Mahony C, Dixon J, Vints M, Megehan J, Dequatre C, Castle L (2014a). Development of the FACET exposure tool to assess migrants from food packaging. *Food Addit. Contam.* Part A 31(3), 444-465.

Oldring PKT, Castle L, O'Mahony C, Dixon J (2014b). Estimates of dietary exposure to Bisphenol-A (BPA) from light metal packaging using food consumption and packaging usage data: a refined deterministic approach and a fully probabilistic (FACET) approach. *Food Addit. Contam.* Part A 31(3), 466-489.

Ottenio D, Escabasse J-Y, Podd B (2004). Packaging materials 6. Paper and board for food packaging applications. *ILSI Europe Report Series.* 1-24.

Ozaki A, Yamaguchi Y, Fuijita T, Kuroda K, Endo G (2004). Chemical analysis and genotoxicological safety assessment of paper and paperboard used for food packaging. *Food Chem. Toxicol.* 42, 1323-1337.

Petersen H, Biereichel A, Burseg K, Simat TJ, Steinhart H (2008). Bisphenol-A diglycidyl ether (BADGE) migrating from packaging material 'disappears' in food: reaction with food components. *Food Addit. Contam.* Part A 25(7), 911-920.

Rennen M, Koster S, Krul CA, Houben GF (2011). Application of the threshold of toxicological concern (TTC) concept to the safety assessment of chemically complex food matrices. *Food Chem. Toxicol.* 49, 933-940.

Sagratini G, Caprioli G, Cristalli G, Giardina D, Ricciutelli M, Volpini R (2008). Determination of ink photoinitiators in packaged beverages by gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry. *J. Chromatogr. A* 1194(2), 213-220.

Schaefer A, Simat TJ (2004). Migration from can coatings: part 3. Synthesis, identification and quantification of migrating epoxy-based substances below 1000 Da. *Food Addit. Contam.* 21(4), 390-405.

Schaefer A, Ohm VA, Simat TJ (2004). Migration from can coatings: part 2. Identification and quantification of migrating cyclic oligoesters below 1000 Da. *Food Addit. Contam.* 21(4), 377-389.

Schilter B, Benigni R, Boobis A, Chiodini A, Cockburn A, Cronin MTD, Lo Piparo E, Modi S, Thiel A, Worth A (2014). Establishing the level of safety concern for chemicals in food without the need for toxicity testing. *Regul. Toxicol. Pharmacol.* 68, 275-296.

Simoneau C, Van den Eede L, Valzacchi S (2012). Identification and quantification of the migration of chemicals from plastic baby bottles used as substitutes for polycarbonate. *Food Addit. Contam.* 29(3), 469-480.

Sinsheimer JE, et al. (1992). The in vivo and in vitro genotoxicity of aromatic amines in relationship to the genotoxicity of benzidine. *Mutat. Res.* 268, 255-264.

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

Toyo'Oka T, Oshige Y (2000). Determination of alkylphenols in mineral water contained in PET bottles by liquid chromatography with coulometric detection. *Anal. Sci.* 16(10), 1071-1076.

Tyapkova O, Czerny M, Büttner A (2009). Characterisation of flavour compounds formed by γ-irradiation of polypropylene. *Polym. Degrad. Stabil.* 94, 757-769.

Vedani A, Dobler M, Smiesko M (2012). VirtualToxLab – A platform for estimating the toxic potential of drugs, chemicals and natural products. *Toxicol. Appl. Pharmacol.* 261, 142-153.

Venkatapathy R, Moudgal CJ, Bruce RM (2004). Assessment of the oral rat chronic lowest observed adverse effect level model in TOPKAT, a QSAR software package for toxicity prediction. *J. Chem. Inf. Comput.* Sci. 44, 1623-1629.

Welshons WV, Thayer KA, Judy BM, Taylor JA, Curran EM, vom Saal FS (2003). Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. *Environ. Health Perspect.* 111(8), 994-1006.

Walker C, Hopkin SP, Sibley RM, Peakall DB (1996). *Principles of Ecotoxicology.* London: Taylor & Francis.

Wu S, Blackburn K, Amburgey J, Jaworska J, Federle T (2010). A framework for using structural, reactivity, metabolic and physicochemical similarity to evaluate the suitability of analogs for SAR-based toxicological assessments. Regul. *Toxicol. Pharmacol.* 56, 67-81.

ILSI Europe Reports

- Addition of Nutrients to Food: Nutritional and Safety Considerations
- An Evaluation of the Budget Method for Screening Food Additive Intake
- Animal-Borne Viruses of Relevance to the Food Industry
- Antioxidants: Scientific Basis, Regulatory Aspects and Industry Perspectives
- Applicability of the ADI to Infants and Children
- Application of the Margin of Exposure Approach to Compounds in Food which are both Genotoxic and Carcinogenic
- Approach to the Control of Entero-haemorrhagic *Escherichia coli* (EHEC)
- Assessing and Controlling Industrial Impacts on the Aquatic Environment with Reference to Food processing
- Assessing Health Risks from Environmental Exposure to Chemicals: The Example of Drinking Water
- Beyond PASSCLAIM – Guidance to Substantiate Health Claims on Foods
- Campylobacters as Zoonotic Pathogens: A Food Production Perspective
- Considering Water Quality for Use in the Food Industry
- Consumer Understanding of Health Claims
- Detection Methods for Novel Foods Derived from Genetically Modified Organisms
- Emerging Technologies for Efficacy Demonstration
- Evaluation of Agronomic Practices for Mitigation of Natural Toxins
- Evaluation of the Risks Posed in Europe by Unintended Mixing of Food Crops and Food Crops Developed for Non-Food Uses
- Exposure from Food Contact Materials
- Foodborne Protozoan Parasites
- Foodborne Viruses: An Emerging Problem
- Food Consumption and Packaging Usage Factors
- Food Safety Management Tools
- Food Safety Objectives – Role in Microbiological Food Safety Management
- Frontiers in Food Allergen Risk Assessment
- Functional Foods in Europe – International Developments in Science and Health Claims
- Functional Foods – Scientific and Global Perspectives
- Guidance for the Safety Assessment of Botanicals and Botanical Preparations for Use in Food and Food Supplements
- Impact of Microbial Distributions on Food Safety
- Markers of Oxidative Damage and Antioxidant Protection: Current status and relevance to disease
- 3-MCPD Esters in Food Products
- MCPD and Glycidyl Esters in Food Products
- Method Development in Relation to Regulatory Requirements for the Detection of GMOs in the Food Chain
- Micronutrient Landscape of Europe: Comparison of Intakes and Methodologies with Particular Regard to Higher Consumption
- *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and the Food Chain
- Nutrition in Children and Adolescents in Europe: What is the Scientific Basis?
- Overview of the Health Issues Related to Alcohol Consumption
- Overweight and Obesity in European Children and Adolescents: Causes and consequences – prevention and treatment
- Packaging Materials: 1. Polyethylene Terephthalate (PET) for Food Packaging Applications
- Packaging Materials: 2. Polystyrene for Food Packaging Applications
- Packaging Materials: 3. Polypropylene as a Packaging Material for Foods and Beverages
- Packaging Materials: 4. Polyethylene for Food Packaging Applications
- Packaging Materials: 5. Polyvinyl Chloride (PVC) for Food Packaging Applications
- Packaging Materials: 6. Paper and Board for Food Packaging Applications
- Packaging Materials: 7. Metal Packaging for Foodstuffs
- Packaging Materials: 8. Printing Inks for Food Packaging Composition and Properties of Printing Inks
- Packaging Materials: 9. Multilayer Packaging for Food and Beverages
- Practical Guidance for the Safety Assessment of Nanomaterials in Food
- Recontamination as a Source of Pathogens in Processed Foods – A Literature Review
- Recycling of Plastics for Food Contact Use
- Risk Assessment Approaches to Setting Thermal Processes in Food Manufacture
- Safety Assessment of Viable Genetically Modified Micro-organisms Used in Food
- Safety Considerations of DNA in Foods
- Safety Implications of the Presence of Nucleic Acids of Inactivated Microorganisms in Foods'
- *Salmonella* Typhimurium definitive type (DT) 104: A multi-resistant *Salmonella*
- Significance of Excursions of Intake above the Acceptable Daily Intake (ADI)
- The Safety Assessment of Novel Foods
- The Safety Assessment of Novel Foods and Concepts to Determine their Safety in use
- Threshold of Toxicological Concern for Chemical Substances Present in the Diet
- Tools for Microbiological Risk Assessment
- Transmissible Spongiform Encephalopathy as a Zoonotic Disease
- Trichothecenes with a Special Focus on DON
- Using Microbiological Risk Assessment (MRA) in Food Safety Management
- Validation and Verification of HACCP
- Water Use of Oil Crops: Current Water Use and Future Outlooks

ILSI Europe Reports can be downloaded from:
http://www.ilsi.org/Europe/Pages/ReportSeries.aspx

ILSI Europe publishes also Concise Monographs in its Concise Monograph Series. They can be downloaded from:
http://www.ilsi.org/Europe/Pages/ConciseMonographSeries.aspx

Predominantly, ILSI Europe publishes articles and proceedings in peer-reviewed journals. Most of them can be downloaded from:
www.ilsi.org/Europe/Pages/JournalArticles.aspx

To order copies
ILSI Europe a.i.s.b.l.
Avenue E. Mounier 83, Box 6
B-1200 Brussels, Belgium
Phone (+32) 2 771 00 14
E-mail: publications@ilsieurope.be
www.ilsi.eu

ISSN: 2294-5504
ISBN 9789078637424