A vision for safer food contact materials: public health concerns as drivers for improved testing

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Abbreviations

AOP Adverse Outcome Pathway

BPA bisphenol A

CVD Cardiovascular Disease

FCC Food Contact Chemical

NCD Non-Communicable Disease

NIAS Non-Intentionally Added Substance

PFAS Per- and polyfluoroalkyl Substances

PFOA perfluorooctanoic acid

SCOD Six Clusters of Disease

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Food contact materials and articles are ubiquitous in today's globalized food system. Chemicals migrate from food contact materials into foodstuffs, but current regulatory requirements do not sufficiently protect public health from hazardous food contact chemicals (FCCs) because only individual substances used to make food contact materials are tested and mostly only for genotoxicity while endocrine disruption and other hazard properties are disregarded. Indeed, food contact materials are a known source of a wide range of hazardous chemicals, and they likely contribute to highly prevalent non-communicable diseases. Food contact materials can also include non-intentionally added substances (NIAS), which often are unknown and therefore not subject to risk assessment. To address these important shortcomings, we outline how the safety of food contact materials may be improved by (1) testing the overall migrate, including (unknown) NIAS, and (2) expanding toxicological testing beyond genotoxicity to multiple endpoints associated with non-communicable diseases relevant to human health. To identify mechanistic endpoints for testing, we group chronic health outcomes associated with chemical exposure into Six Clusters of Disease (SCOD) and we propose that finished food contact materials should be tested for their impacts on these SCOD. Future research should focus on development of robust, relevant and sensitive *in vitro* assays based on mechanistic information linked to the SCOD, e.g., through Adverse Outcome Pathways (AOPs) or Key Characteristics of Toxicants. Implementing this vision will improve prevention of chronic diseases that are associated with hazardous chemical exposures, including from food contact materials.

Keywords: food packaging; risk assessment; chronic disease; chemical safety

1. Introduction

In today's globalized food system, food contact materials and articles such as food packaging, tableware, and food processing equipment are ubiquitous, especially those made of plastic (1, 2). This increases exposures to food contact chemicals (FCCs) migrating from food contact materials (3-5). This widespread, continuous exposure to a wide range of synthetic chemicals requires a more stringent safety assessment of food contact materials than the current approaches used in both low- and high-income countries (6-10).

Food contact materials have been studied for over 50 years and are a known source of chemicals that migrate into foodstuffs (11-19). Numerous FCCs, either intentionally used in the manufacture of food contact materials or non-intentionally added substances (NIAS) that are present in the finished food contact material/article and that migrate into foodstuffs (5, 20, 21), are known to be hazardous and implicated with adverse human health impacts (22-29).

However, the current approach to chemical risk assessment for food contact materials is largely focused on assessing genotoxicity of single substances used to manufacture food contact materials and therefore fails to account for other highly relevant mechanisms of toxicity that are of equal concern as genotoxicity (10) and, what is more, the current approach does not assess NIAS that also migrate from food contact materials (Fig. 1) (17, 30). Addressing both issues is feasible in a cost-efficient way and necessary to protect public health.

Indeed, non-cancer non-communicable diseases (NCDs) of increasing prevalence in the global human population have been associated with several widely used FCCs, such as bisphenol A (BPA), bisphenol F, perchlorate, and di(2-ethyl hexyl) phthalate (DEHP), to name a few (Table 1). Given that humans are in daily contact with food contact materials, those materials are likely a relevant exposure source of hazardous chemicals that contribute to various NCDs globally.

In this article, we outline an improved assessment scheme for hazard identification of FCCs that captures all exposure-relevant chemicals including (unknown) NIAS, and we present a vision for assessing the safety of food contact materials that addresses biological effects linked to the most prevalent NCDs (31, 32). These include heart disease, stroke, cancer, diabetes, reproductive disorders, and several neurological conditions. We provide guidance on research and policy actions that should be developed to protect the public from avoidable chronic chemical exposures originating from food contact materials and articles.

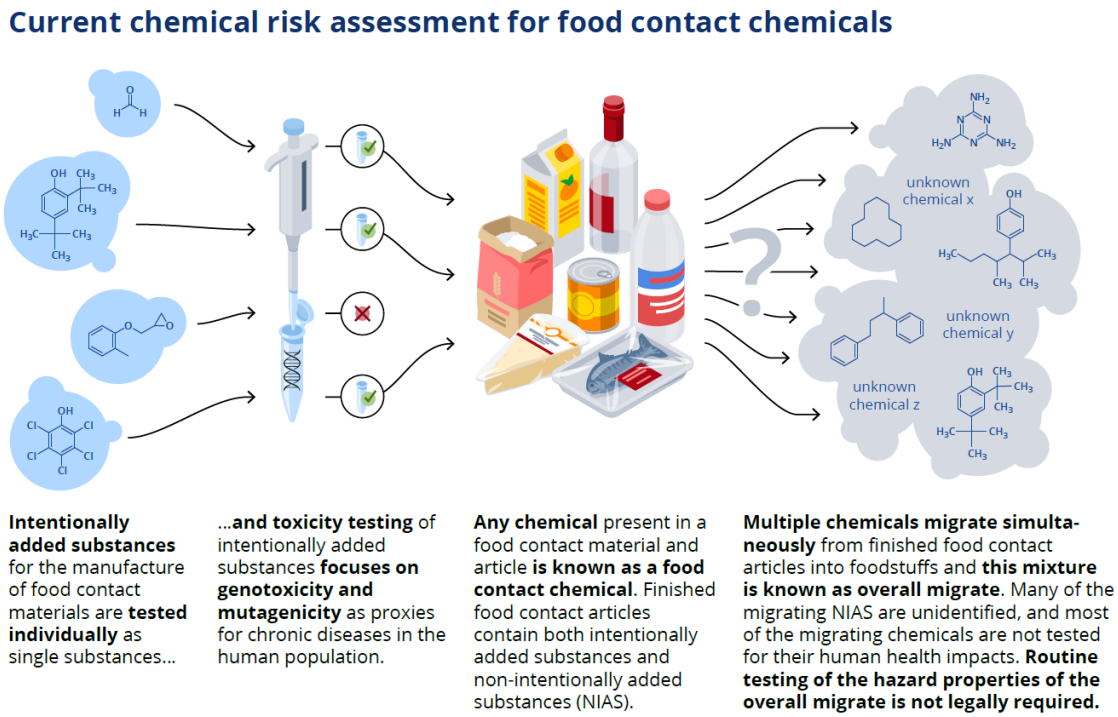

Figure 1. Chemical risk assessment for food contact chemicals (FCCs): current practice. The current approach for assessing the safety of FCCs focuses on testing single substances that are intentionally used to make food contact materials. The toxicological focus is on mutagenicity and genotoxicity, therefore only carcinogenicity is currently determined as a human health relevant endpoint for predicting chronic disease. However, many more chemicals can migrate simultaneously from the finished food contact material, including unidentified compounds that are non-intentionally added substances (NIAS). The migrating mixture is known as the overall migrate, and it can also exert adverse effects (mixture toxicity). Currently, the assessment of overall migrate mixture toxicity is not legally required. Illustrator: Michael Stünzi.

2. Problem set-up: Shortcomings of the current approach

2.1 Non-communicable diseases are increasingly prevalent and associated with chemical exposures

NCDs are a significant contributor to global mortality (33). However, the impact of NCDs is far greater than mortality alone, especially in low- and middle-income countries. Both mortality and morbidity of selected NCDs have increased substantially over the last 30 years. Premature deaths (<70 years) are primarily associated with cardiovascular disease (17.7 million deaths per year, accounting for 45% of all NCD deaths), cancer (8.8 million deaths per year, 22% of all NCD deaths), chronic respiratory disease (3.9 million deaths per year, 10% of all NCD deaths) and diabetes (1.6 million deaths per year, 4% of all NCD deaths) (33). Expressed in Disability-Adjusted Life Years, cardiovascular diseases have increased by a factor of 1.4 from 1990 to 2017, neoplasms by a factor of 1.5, and diabetes, urogenital, blood and endocrine diseases by a factor of 1.6 (from 1990 to 2016) (34) (Figure S1, Supplemental Material). Furthermore, among reproductive-age women and men, infertility is now the most prevalent chronic disease (35). Importantly, NCDs incur significant human suffering in addition to their estimated economic costs (36-40), which further stresses

the need for urgent action towards prevention of morbidities associated with NCDs.

Chemical exposures are an important contributor to NCDs. Several well-studied types of chemicals such as toxic metals, halogenated aromatics, and some pesticides (41, 42), as well as some members of the endocrine disrupting compounds (43-47) are associated with NCDs such as brain-related disorders, cancers, metabolic disorders and cardiovascular disease. Specific FCCs such as BPA and several members of the ortho-phthalates group are associated with NCDs such as heart disease, diabetes, and some forms of cancer (48, 49) (Table 1). Further, the effects of chemical exposures on risk of NCDs are complex and multifaceted, with some outcomes occurring across generations through transgenerational inheritance (47, 50, 51). It is also clear that these effects are not limited to laboratory animals, as mixtures of chemicals including FCCs have been associated with adverse health outcomes in prenatally exposed humans (46, 49, 52-55).

Table 1. Food contact chemicals (FCCs) associated with non-communicable diseases (NCDs) from each of the Six Clusters of Disease (SCOD) (non-exhaustive and non-systematic overview). Identification of FCCs was based on the Food Contact Chemicals database (FCCdb) (24) and the database on migrating and extractable food contact chemicals (FCCmigex) (17). This overview is not a complete list of FCCs that are associated with adverse health outcomes. Systematic reviews are indicated with*. Cancer agents are classified by cancer site (125).

Disease Cluster	Example disease	Associated FCC exposure	References
Cancers	Testicular cancer	PFOA	(229, 230)
	Kidney cancer	PFOA	(229, 231)
	Breast cancer	PFOA	(232)
		Ortho-phthalates	(232)
Cardiovascular diseases	Cardiovascular diseases: including myocardial infarction, arrhythmias, dilated cardiomyopathy, atherosclerosis, and hypertension	BPA	(233-236)
		Ortho-phthalates	(237)
Brain-related disorders	Hypothyroid	BPA	(238)
		Ortho-phthalates	(239)
		Perchlorate	(239)
		PFAS	(240)
	Abnormal neurodevelopment	Ortho-phthalates: DEHP, DBP, BBP and DEP	(241)
	Attention Deficit Hyperactivity Disorder/behavior	Lead, BPA, ortho-phthalates	(242-244)
	Lower Intelligence Quotient	Endocrine disrupting chemical (EDC) mixture (Ortho-phthalates)	(46, 245)
	Language delay	EDC mixture	(55)
Metabolic and endocrine diseases	Type-1 diabetes	BPA, Ortho-phthalates, PFAS	(246)

	Type-2 diabetes	BPA	(247-249)
		PFOA	(250)
	Pre-diabetes and diabetes	Ortho-phthalates	(241, 251, 252)
	Obesity (BMI, waist circumference)	BPA	(237, 253, 254)
		PFAS	(255, 256)
	Childhood Obesity	BPA	(257)
		Ortho-phthalates	(258)
	Gestational diabetes	Antimony	(259)
		Ortho-phthalates	(260)
	Non-alcoholic fatty liver disease	EDC mixture	(261)
		PFAS	(262)
Immunological disorders	Immunosuppression	PFAS: PFOS and PFOA	(263)
	Childhood asthma	Ortho-phthalates: DEHP and BBzP	(241)
	Kidney damage	Melamine	(264)
Reproductive disorders	Male infertility	BPA	(265)
		Dibutyl phthalate	(266)
	Semen quality	Ortho-phthalates: DBP, BBP, DEHP, and DINP	(241, 251, 267)
	Female infertility (reduced follicular count)	DEHP	(268)

NCDs that are increasingly prevalent in the human population and that are associated with hazardous chemical exposures can be grouped into disease clusters. On this basis, we developed the novel concept of Six Clusters of Disease (SCOD) (Figure 2). The six clusters are cancers, cardiovascular diseases, reproductive disorders, brain-related disorders, immunological disorders, and metabolic diseases. The SCOD concept provides a rationale for systematically assessing the safety of chemicals in food contact materials, with a focus on the prevention of chemical-associated, highly prevalent, and severe NCDs. As such, the SCOD concept expands current efforts for chemical risk assessment of FCCs.

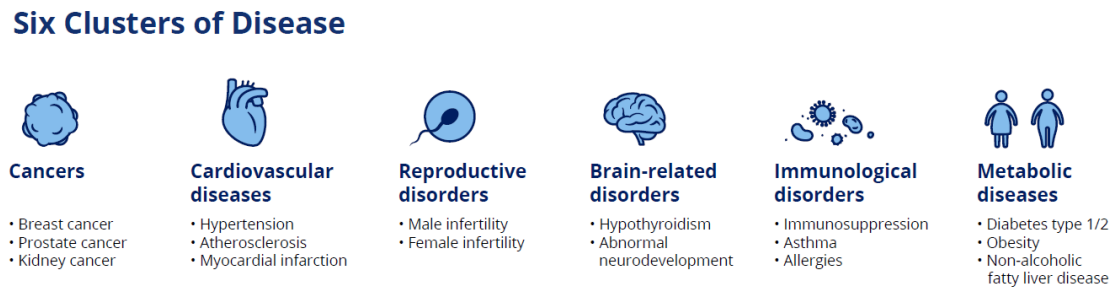

Figure 2. The novel Six Clusters of Disease (SCOD) concept comprises non-communicable diseases (NCDs) that are highly prevalent in the global human population, of increasing concern, and associated with hazardous chemical exposures that can be clustered by disease type. They include cancers, cardiovascular diseases, reproductive disorders, brain disorders, immunological disorders, and metabolic diseases. The SCOD are of major concern for public health and require novel approaches for prevention, namely the identification of chemical contributors. Chemical risk assessment of food contact chemicals (FCCs) should determine contributions to diseases of public health concern. Preventing exposure to chemicals in food contact materials that contribute to NCDs is critical for successful primary prevention strategies. Illustrator: Michael Stünzi.

2.2 Current risk assessment of food contact chemicals is not sufficiently protective of human health

The universe of known FCCs comprises at least 14,153 substances, and for at least 1,518 FCCs empirical evidence for migration from food contact articles and materials is publicly available (17). Evidence of human exposure exists for hundreds of these chemicals (4, 55-66). At least 388 FCCs in use today are known to be carcinogenic, mutagenic or toxic to reproduction, possess endocrine disrupting properties, or have other properties of concern such as persistence (22).

Currently, in the United States (US), Canada, the European Union (EU), China and other countries, chemical risk assessment is required for all migrating substances (Figure 1). In practice, however, it is predominantly the intentionally used substances that are assessed for their risk to human health. Humans are exposed to many more FCCs that are non-intentionally added to the finished food contact material or foodstuff. These NIAS include impurities of the starting substances, reaction by-products, or degradation products of starting substances (like additives) (5, 67-69). NIAS most often are unidentified, they are common in food contact materials with high chemical complexity, and they are likely to be biologically active (70). Under the current

chemicals risk assessment paradigm for food contact materials, where a chemical's identity must be known, unidentified FCCs cannot be assessed, although, for example, the EU plastic food contact regulation requires the risk assessment of NIAS (71), and also US FDA's Food Contact Notification has information requirements on impurities and reaction by-products (72).

A second problem is the lack of testing of substances present in the finished food contact material. Several approaches have been developed to approximate the health risks of unknown NIAS (73-79), but these approaches contain substantial uncertainties related to hazard estimation, chemical identification, and quantification (80, 81) because they are based on assumptions that cannot be entirely supported by empirical evidence. For example, generic thresholds for chronic exposures to nongenotoxic carcinogens were derived from testing chemicals at maximum tolerable doses (MTD) and at 1/2 MTD, but it depends on the exact mechanism by which a chemical exerts its toxicity whether a low-dose extrapolation from MTD dosing is appropriate or not (82, 83).

Finally, because some laws prohibit the use of chemicals that cause cancer in humans or animals, testing methods currently focus on genotoxicity as a proxy for predicting cancer risk (10, 84). This focus on genotoxic effects is at the expense of other hazards, including outcomes relevant to other chronic NCDs. Thus, there is a need for novel and more robust approaches to more fully evaluate all the relevant hazards to human health associated with FCCs.

3. Our vision: to make safer food contact materials

3.1 Assessing toxicological effects relevant to the Six Clusters of Disease

Chronic exposure to hazardous chemicals is a known modifiable risk factor for cancer and reducing exposure to hazardous or untested chemicals from consumer products, including food contact materials, is a recommended preventive measure (85). It is reasonable to assume that the same holds true for other NCDs that are associated with chemical exposures, especially for endocrine disrupting chemicals (Table 1). Indeed, exposure reductions can lower the incidence of disease (86), for example for neurodevelopmental disorders (87), obesity (88) or male reproductive disorders (89).

NCDs that are increasingly prevalent in the human population and that are associated with hazardous chemical exposures can be grouped into disease clusters. On this basis, we have developed the novel concept of SCOD (Fig. 2). The SCOD concept emerged from discussions with the Food Packaging Forum's Scientific Advisory Board (SAB) during several meetings between 2016 and 2022. The SCOD concept provides for the first time a rationale for systematically assessing the safety of chemicals in food contact materials, with a focus on the prevention of chemical-associated, highly prevalent and severe NCDs. As such, the SCOD concept expands current efforts for chemical risk assessment of FCCs beyond cancers induced via a genotoxic mechanism. For each disease cluster within the SCOD, many widely used FCCs have been associated with relevant diseases in both epidemiology and animal studies (Table 1). For some, mechanistic evidence strengthens these associations. It is also this mechanistic evidence that provides opportunities to use *in silico* and *in vitro* assays to better map toxicity profiles of individual FCCs in finished food contact materials,

before they are placed on the market, as well as mixtures, extracts and migrates from food contact materials and articles. The SCOD provides organizing principles for such an approach.

3.2 Assessing real-life chemical exposures: testing overall migrate from food contact materials

All FCCs that are relevant for human exposure should be tested, in other words, FCCs used in the manufacturing of food contact materials should be tested as single substances, and the real-life mixture of all migrating FCCs, the *overall migrate*, should also be tested. In addition, the overall migrate should be subjected to non-targeted chemical analyses that are aimed to identify its chemical composition, including NIAS (90). This combined testing and chemical identification approach could inform the development of safer food contact materials by selecting less hazardous ingredients and developing manufacturing processes that generate fewer and less biologically active NIAS. Such an approach would be aligned with the proposed Safe and sustainable by Design criteria included in the EU's Chemicals Strategy for Sustainability (91).

The already available as well as emerging *in vitro* assays provide an opportunity to identify hazardous properties of single substances and of the overall migrate. *In vitro* test systems are small-scale, often single-cell or small organism systems, for example human cancer cell lines, bacteria, and fungi (e.g. yeast). Other high-throughput screening assays utilize embryos and larvae from vertebrates such as zebrafish (*Danio rerio*) or African clawed frog (*Xenopus laevis*). These assays can be performed efficiently both in terms of time and cost and are usually based on mechanistic pathways (92, 93).

Test batteries, where several relevant assays are combined simultaneously, can also be operated as high-throughput screening methods such as those developed in Tox21 and ToxCast (94-96), which demonstrate the feasibility of this approach. In this way, diverse information about the interaction properties of a single chemical with different biological systems can be generated efficiently, and with lower cost, compared to whole-animal testing used in traditional toxicology.

These assessments should be guided by the SCOD concept. However, gaps exist in the current understanding of molecular pathways related to the SCOD, and these *in vitro* assays remain insufficient to identify the full panoply of potential hazards, especially those mediated by endocrine mechanisms. *In vitro* assays included in high-throughput test batteries need to be appropriate for predicting relevant human health outcomes; should be demonstrated to be reproducible, sufficiently specific and sensitive; and must be executed transparently (97, 98). Because of the limited *in vitro* assays for known pathways and mechanisms of action associated with endocrine disruption and other complex biological cascades, animal testing needs to continue, but at a reduced level than in the past. For example, no current *in vitro* approaches would have revealed what is now known to be a feature of some chemical exposures, e.g., transgenerational epigenetic inheritance (99). Acknowledging these and other gaps, the European Commission is funding EURION, a program to develop new testing and screening methods (including many *in vitro* approaches) for identifying endocrine disrupting chemicals (100).

3.3 Shifting from the status quo to a more comprehensive approach to testing

Within the SCOD, increasingly available mechanistic information enables an understanding of how chemicals contribute to highly prevalent NCDs. Two emerging frameworks are being implemented to describe how chemicals affect complex diseases and to provide a more uniform approach to evaluating mechanistic evidence: the key characteristics concept, and adverse outcome pathways (AOPs). Both offer opportunities to shift from the status quo, modernize hazard assessments, and develop suitable *in vitro* assays.

3.3.1 The Key Characteristics concept: modernizing chemical hazard assessments

The key characteristics concept makes use of information about the properties of hazardous chemicals that have empirical evidence linking them causally to relevant apical (disease) endpoints (101). The underlying premise is that chemicals that cause the same disease outcomes in whole organisms share molecular properties (i.e., key characteristics) that are relevant for their hazardous properties. The key characteristics for different disease outcomes are hence defined using empirical evidence for well-characterized chemicals, combined from epidemiological, *in vivo* and mechanistic studies. These disease-specific key characteristics can then be used to develop mechanistic *in vitro* assays to screen chemicals for their propensity to contribute to different disease clusters and thereby reduce the need for *in vivo* experiments while still decreasing scientific uncertainty normally associated with *in vitro* data.

The key characteristics were first developed for carcinogens, drawing from existing mechanistic information from thoroughly assessed chemicals that are known to be carcinogenic in humans (101-105). Additional key characteristics of other disease-causing chemicals have also been described, such as for hepatotoxicants (106), endocrine disrupting chemicals (107), female reproductive toxicants (108), male reproductive toxicants (109), cardiovascular toxicants (110), and immunotoxicants (111). For metabolic toxicants and neurotoxicants, work to describe key characteristics is ongoing. Taken together, the key characteristics approach provides an excellent starting point for the mechanistic understanding of how certain chemicals are associated with NCDs, such as those covered in the SCOD.

3.3.2 Using other mechanistic information to develop suitable in vitro assays

In addition to the key characteristics, further important mechanistic understanding is becoming available and can be useful to inform development of dedicated *in vitro* screening assays for hazard assessments of FCCs. Chemicals exert toxic effects by combinations of many different molecular-level events. These mechanistic events leading to apical endpoints of toxicity can be organized in an AOP (112). Several AOPs relevant to NCDs in the SCOD have been proposed, such as estrogen receptor activation leading to breast cancer (113) and the upregulation of thyroid hormone catabolism (via activation of hepatic nuclear receptors) leading to subsequent adverse neurodevelopmental outcomes in mammals, specifically the loss of cochlear function (114).Thus, AOPs are an emerging approach to organize mechanistic information so that molecular or cellular-level targets can be identified for developing *in vitro* assays that are relevant to the SCOD.

3.3.3 The novel approach: A vision for safer food contact materials

Based on the presumption that mechanistic *in vitro* testing of chemicals supports the prevention of NCDs within the SCOD, we propose a novel approach for testing FCCs that

(1) covers individual FCCs as well as real-life mixtures, migrating (or extractable) from finished food contact materials, including all known and unknown NIAS,
(2) assesses the health impacts of FCCs and real-life mixtures with respect to the most prevalent NCDs in the human population, and
(3) evaluates effects that are upstream from the disease, relying on mechanistic information and *in vitro* screening approaches (wherever possible) to accurately predict health effects induced by FCCs and migrates.

This shift from current practice to the proposed approach is summarized in Fig. 3, and a detailed overview is provided in Fig. 4. Our approach overcomes the most challenging shortcomings of the current testing paradigm of chemical hazard assessment of food contact materials, fully recognizing that to assess all adverse effects of chemicals on biological systems, adequate *in vivo* testing is required, where additional aspects would be addressed such as metabolic activation, unknown modes of action leading to apical endpoints, and transgenerational effects. However, we also realize that such extensive, multigeneration *in vivo* testing may not always be feasible for various reasons, including ethical and practical ones. Therefore, we propose this vision to improve FCC testing from the currently too limited scope towards a much more comprehensive yet feasible approach that holds promise for better protection of public health.

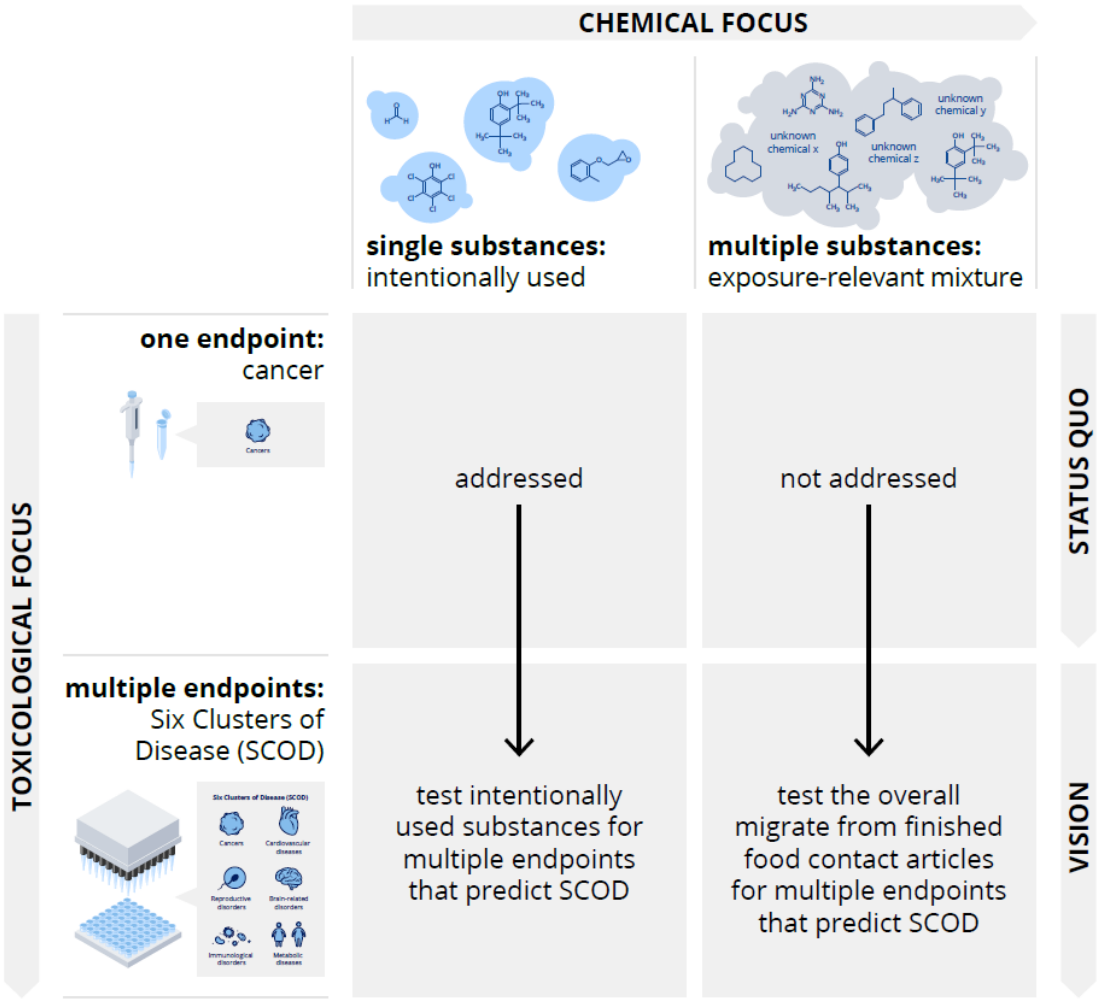

Figure 3. Schematic overview of the current vs. proposed approach to food contact chemical (FCC) testing. Currently, single substances intentionally used to make food contact materials are tested for genotoxicity using *in vitro* assays. The proposed new approach focuses on testing the overall migrate (i.e., the human exposure-relevant mixture of all migrating FCCs) for its potential to contribute to the Six Clusters of Disease (SCOD). Notably, single substances used to make food contact materials would also be tested individually for the SCOD-relevant endpoints and, if found to have biological activity, excluded from use in the manufacturing of food contact materials. Illustrator: Michael Stünzi.

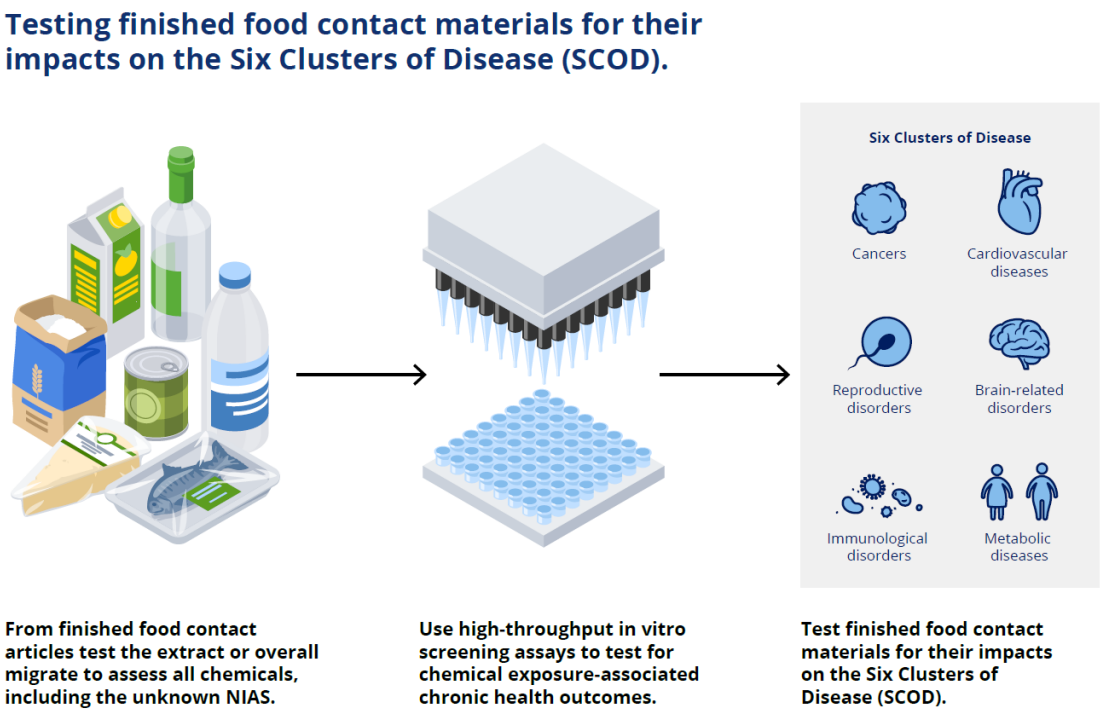

Figure 4. The vision for a novel approach to safety assessment of food contact materials and articles. Finished food contact materials and articles are tested for their real-life mixture of all migrating chemicals (the overall migrate), using *in vitro* screening assays as well as non-targeted chemical analyses. The screening assays are mechanism-based and identify the key characteristics, key initiating events, or other mechanisms of action of the overall migrate. Screening assays are selected around the Six Clusters of Disease (SCOD) concept. In addition, intentionally added substances used for the manufacture of food contact materials are also tested as individual substances prior to their authorization, and the overall migrate is chemically characterized using non-targeted approaches. Illustrator: Michael Stünzi.

4. Implementing the vision: assessing impacts of FCCs and relevant mixtures on human health outcomes in the SCOD using mechanistic approaches

Here we review the mechanistic basis for each of the disease clusters included in the SCOD, and selectively highlight available *in vitro* testing methods. Importantly, some available assays cover key characteristics that are relevant for several disease clusters.

This vision for expanded hazard assessment of food contact materials is based on the finding that for each of the disease clusters included in the SCOD, some mechanistic understanding is available for the way that chemicals cause disease (Table 2).

4.1 Cancer

As defined by Willis,

A neoplasm is an abnormal mass of tissue, the growth of which exceeds and is

uncoordinated with that of the normal tissues and persists in the same excessive manner after cessation of the stimulus which evoked the change (115).

Regarding cancer causation, the somatic mutation theory posits that cancer is a cellular disease caused by mutations of genes that disrupt the control of cell proliferation. Yet, substantive contradictions exist between this theory and empirical evidence (116), which inspired competing theories consider cancer as a problem of tissue organization akin to organogenesis (117-119). Importantly, not all carcinogens are mutagens (120) and, thus, carcinogenicity cannot be equated with genotoxicity. Yet, because legal requirements restrict the use of cancer-causing agents in food contact materials, testing of FCCs has focused on genotoxicity as a proxy to identify carcinogenic substances.

Both carcinogens and mutagens are found in food contact materials including 1) formaldehyde, a known human carcinogen (121), which migrates from various plastics including melamine-formaldehyde plastics used as tableware for children, and polyethylene terephthalate plastic (PET) (122, 123); 2) antimony trioxide, which "is reasonably anticipated to be a human carcinogen" (124) and "probably carcinogenic to humans" (125), and it is used in the manufacture of PET, where antimony is found to migrate into soft drinks (123, 126); and 3) per- and polyfluoroalkyl substances (PFAS) are widely used in the manufacture of food contact materials as processing aids in plastic and paper food contact material production (127, 128), and perfluorooctanoic acid has limited evidence for testicular and kidney cancers in humans (129).

The key characteristics for carcinogens reveal that these chemicals can be mutagens, but that there are numerous other common features for these agents as well (101-105). Guyton and Schubauer-Berigan (2021) recommended the use of *in vitro* assays based on the key characteristics to identify carcinogens in high-throughput screening (105). Further, Rider et al. (2021) proposed methods to use the key characteristics to test chemical mixtures and their propensity to affect cancer development including in mixtures of chemicals with different key characteristics of carcinogens (130). Approaches such as these will provide important information for testing mixtures such as the overall migrate from finished food contact materials.

Methods for evaluating genotoxicity are readily available, validated, and trusted. Chemicals are considered genotoxic if they damage the structure, information content, or segregation of DNA, with mutagenicity (i.e. changes to the nucleotide sequence) being a sub-type of genotoxicity (131).

These methods include:

- **Mutagenicity:** The Ames test, based on bacterial reverse mutagenicity, is the most employed test for mutagenicity (Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 471). A mammalian cell (mouse lymphoma) gene mutation test (OECD TG 490) is also available (132)
- **Chromosomal aberration:** Cultured mammalian cells are assessed for the presence of chromatid-type and chromosome-type aberrations during metaphase (OECD TG 473)
- **Micronucleus:** Micronuclei represent chromosomal damage (chromosome fragments or whole chromosomes) that have been transmitted to daughter cells. Micronuclei can be assessed *in vitro* by using mammalian cells (OECD TG 487)

or *in vivo* with erythrocytes collected from bone marrow or peripheral blood (OECD TG 874)

These methods are recommended or required for assessing intentionally used FCCs (72, 133). Several other *in vitro* assays for assessing the genotoxic potential of FCCs are also available (134). However, these strategies have not kept pace with discoveries in cancer biology (135). Currently, no *in vitro* assays are available that capture features of carcinogenicity beyond genotoxicity, but research is underway to address this technical gap (136). On the other hand, the causal role of the microenvironment in carcinogenicity, as put forward by tissue-based theories on carcinogenicity (118), is not captured by such *in vitro* assays, because the reciprocal interactions between stroma and parenchyma during development, regeneration, and remodeling are not being considered (137). Although *in vivo* assays involving mammals are available, traditional 2-year rodent carcinogenicity studies (OECD TG 451), either alone or in combination with chronic toxicity studies, are rarely performed for FCCs.

4.2 Cardiovascular diseases

Cardiovascular diseases (CVDs) are a group of disorders arising due to disfunction of the heart and blood vessels. The most recognized forms of CVD, coronary heart disease and cerebrovascular disease, result in damage to tissues caused by limited or complete loss of blood supply (138).

FCCs including several phthalates and bisphenols contribute to the causation of CVDs, independent of obesity and diabetes (110). Bisphenols can disrupt calcium signalling in myocardium and vasculature; and phthalates and bisphenols are oxidant stressors that accelerate coronary and other arterial inflammation (110). In the US alone, 100,000 premature deaths from CVD among 55–64-year-olds each year are attributed to exposure to one phthalate, DEHP (139). Other FCCs, such as antimony, may also impair cardiovascular function and accelerate CVDs (140).

Lind et al. (2021) compiled the key characteristics of cardiovascular toxicants and provided a comprehensive overview of robust and sensitive *in vitro*, *ex vivo* and *in vivo* assays that are available for measuring dysregulation of Ca^{2+} ion homeostasis and resulting arrhythmogenic activities of chemicals. For example, the increased risk for CVDs associated with higher exposures to BPA is mechanistically associated with Ca^{2+} release and reuptake resulting in proarrhythmic delays after depolarizations in isolated cardiomyocytes. BPA promotes Ca^{2+}-mediated arrhythmias *ex vivo* in the whole heart of rats and mice (141). However, this is only one of many possible mechanisms for inducing CVDs, and further assay development is required.

Although several FCCs have been associated with CVDs, cardiovascular toxicity is generally not evaluated for FCCs, whether they are intentionally used to make food contact materials or NIAS present in finished food contact materials. This is in part due to a reliance on *in vivo* guideline testing of general toxicity for chemicals migrating at very high levels and limited to assessment of neoplastic and non-neoplastic cardiac lesions in rodent models, which can be confounded by a high incidence of background pathology in many of the rodent strains used for toxicity testing (142). However, these are insensitive apical endpoints that only identify highly cardiotoxic chemicals that result in robust pathology but miss subtle molecular effects (143, 144).

We recommend that comprehensive testing for all new chemicals include *in vitro* and *in silico* testing harmonized with the Comprehensive *in vitro* Proarrhythmia Assay approach (145, 146).

4.3 Brain-based disorders

Disrupted neurodevelopment can have numerous consequences including a lower intelligence quotient, delayed language acquisition, ADHD, and autism (53, 55, 147). Because the role of thyroid hormone in brain development is well established, hypothyroidism, especially during early development, is also a condition of concern upstream of neurodevelopmental disorders. Neurotoxicity can also result from impaired neuronal function due to a variety of factors, such as neuronal misplacement during development, altered synapses, hypomyelin, or degeneration. Other neurodegenerative conditions that typically arise later in life include Parkinson's disease, Alzheimer's disease, and other forms of dementia.

The role of FCCs in the causation of many brain-based disorders is well established, with substantial contribution to the burden of disease for both neurodevelopmental and neurodegenerative disorders (37). For example, FCCs that interfere with thyroid hormone systems or sex steroids (e.g., phthalates and perchlorate) can affect brain development as well as cognitive function in adults (87, 148). The vulnerability of the developing brain and the lack of systematic assessment of neurodevelopmental toxicity for FCCs raises serious concerns (149). At present, the key characteristics of neurotoxicants remain undescribed, but relevant work is ongoing.

In addition to assays covering interference with the thyroid and sex steroid axes, *in vitro* testing of neurotoxicants requires sophisticated and reliable models due to the complexity of the brain. Neuronal cell lines, primary central nervous system cells, transformed neuronal precursors and stem cell derived progenitor cells are used in neurotoxicity assays (150) to evaluate endpoints including migration, synapsis formation, network activity and differentiation. Although single-cell cultures are informative, multi-cell type and three-dimensional models utilizing microfluidics more adequately represent the diversity and spatial properties of the brain (151-154), but high throughput versions of these methods are not yet available, and thus their use in evaluating FCCs has been limited. Additional *in vitro* assays for chemical screening of neurotoxicants are under development in EU-funded research programs (155) and research is ongoing to develop further *in vitro* assays targeting the thyroid system (156). Recently, the establishment of a human cell-based *in vitro* battery has been reported; it combines 10 assays selected to cover major key events in the relevant AOPs (157) and was shown to provide 82% sensitivity in that it was able to identify 24 out of 28 known neurotoxicants (158).

New low- and medium-throughput screening assays have been developed. For example, the nematode is a promising model for evaluating known neurodevelopmental toxicants and could be expanded to profiling chemicals with unknown neurotoxicity (159, 160). Spontaneous movements (161), number and location of neurons (162), and behavioral effects (163) are some of the neurological endpoints measured in zebrafish. Validated high-throughput screening assays using African clawed frog tadpoles are also available (OECD TG 248).

In vivo testing in rodents can be used to assess different functional aspects of neurotoxicity including impacts on cognition, learning and memory; and anxiety-like, depressive-like and reproductive behaviors. OECD developmental neurotoxicity (OECD TG 426) and extended one-generation reproductive toxicity assays (OECD TG 443) include optional measurements of learning and memory, motor and sensory function, motor activity, and auditory startle. Neurodegeneration is not covered because animals are not kept until the end of their lifetime (164).

4.4 Obesity and Metabolic diseases

Metabolic diseases, including obesity, involve the many tissues that comprise the metabolic system (165). These include adipose tissue, skeletal muscle, pancreas, liver, gastrointestinal tract, bone, and brain. Type-2 diabetes, an important metabolic disease with increasing prevalence in human populations, occurs due to systemic insulin resistance, often with an increasing production of insulin by the pancreas. Type-1 diabetes occurs due to a progressive loss of β-cell insulin secretion. Non-alcoholic fatty liver disease is another metabolic disease with increasing prevalence in human populations.

While poor diet and insufficient physical activity are considered the chief drivers of the obesity and diabetes twin pandemics, chemical exposures (for example, to phthalates, bisphenols, parabens, PFAS, etc.) can disrupt the balance between energy expenditure and energy intake (166). A large comprehensive review of metabolic disrupting chemicals, including those that can induce obesity (obesogens), provides strong evidence that numerous FCCs are associated with type-2 diabetes, obesity, and fatty liver disease (167). The key characteristics of metabolic disruptors and obesogens are being compiled. Rusyn et al. (2021) have described the key characteristics of acute and chronic human hepatotoxicants and note that only one of 12 key characteristics are specific to liver tissue (KC9: causing cholestasis) (106).

The simplest assays to identify an obesity hazard are those that measure the effect of chemical exposures on the development of adipocytes (168-170). Primary preadipocyte cultures, or mesenchymal stem cell assays, use animal or human cells to assess proliferation and differentiation into adipocytes (169, 171-176), and a recent study found that around one third of tested food contact articles contained metabolic disrupting chemicals (177). Recently, spheroid adipocyte models have been developed that improve the efficiency and speed of differentiation (178) and can be used for a more comprehensive understanding of adipocyte physiology than monolayer cultures. Other non-adipocyte cell lines, when well characterized, are also useful for mechanistic studies (97, 179). In addition to adipocyte differentiation, several other mechanisms are implicated with metabolic disease causations, for example the disruption of energy homeostasis at the level of the hypothalamus and brain. Therefore, *in vitro* assays that examine effects on hypothalamic neurons are useful (180, 181).

No assays have been developed to identify metabolic disruptors acting as diabetogens. Ongoing projects are developing assays to measure β-cell function and survival (182-184) using rodent β-cell lines (INS-1E and MIN-6) and a human β-cell line (ENDOC-βH1). Assays of insulin function on the human liver cell line HepaRG, the skeletal muscle cell line C2C12, and adipocytes are also under investigation (183). One well established system of assays employing both *in vitro* and *in vivo* methods has

been used to explore the relationship between BPA and type-2 diabetes (185).

The most used assay to screen chemicals for effects on the liver uses the HepaRG cell line. This cell line can be customized with different expression levels of various drug metabolizing enzymes (186). Other 2D and 3D *in vitro* approaches use primary hepatocytes (187) and other liver models (188) to screen for effects on liver outcomes.

4.5 Immunological disorders

The immune system is an intricate network of many different, highly specialized cells interacting with each other and with the nervous and endocrine systems (189). Disorders of the immune system include autoimmune disorders such as multiple sclerosis, Graves' and Hashimoto's diseases, lupus, Celiac's, Addison's, and rheumatoid arthritis, among others. Other diseases including type-1 diabetes and asthma have an important immune component. Therefore, assays for immunotoxicity need to capture a multitude of potential effects, including immunosuppression, immunostimulation, hypersensitivity reactions, mechanisms of autoimmunity, and developmental immunotoxicity, e.g., delayed immunotoxic responses to toxic influences (190).

The human immune system is highly effective, but also sensitive to synthetic chemical insults during development and adult life. Effects of chemicals on the immune system are less well understood in humans than other disease endpoints, but emerging evidence implicates PFAS exposure in reducing immune response to vaccines and increasing susceptibility to infections in early life (191). Other FCCs including bisphenols and phthalates increase the risk of atopy and asthma (192-194), and infections in early life (195).

The key characteristics of immunotoxicants have been described (111). This offers a starting point for development of suitable *in vitro* assays for testing FCCs for immunotoxicity. Due to the complexity of the immune system components and responses, a comprehensive battery of *in vitro* assays covering all relevant aspects of immunotoxicity has not been established. However, several *in vitro* assays, dealing for example with direct immunosuppression, allergic hypersensitivity, or autoimmunity, are being developed to detect a range of immunotoxicants (196-199) and these assays could be used to screen FCCs (200).

4.6 Reproductive disorders

In industrialized countries, male reproductive health has declined over the past decades, including a 50-60% decrease in sperm counts since 1973 (201, 202) and an increase in testicular cancer (203). Female fertility is also affected, as are maternal health and pregnancy outcomes, and conditions such as polycystic ovary syndrome (PCOS), endometriosis, and premature ovarian failure (204).

The sperm count decrease is associated with chemical exposures (to, e.g. phthalates), especially during fetal development (205). Strong evidence from animal experiments support this interpretation (43, 206-208). FCC exposures are also associated with PCOS (209), and other aspects of reproductive toxicity (210, 211). These adverse outcomes have even been found for FCCs promoted as safer alternatives

to hazardous chemicals such as the plasticizer 1,2-cyclohexane dicarboxylic acid diisononyl ester (tradename Hexamoll DINCH) (212), which is used as a replacement for DEHP and other phthalates. Several FCCs such as BPA have been studied for mechanistic-level impacts on female fertility, including oogenesis, folliculogenesis, and altered expression of gonadotropin and gonadotropin hormone-releasing hormone receptors (213). The key characteristics of male (109) and female reproductive toxicants (108) have been described. Development and function of the reproductive system is fundamentally dependent on sex hormone action. Thus, the key characteristics of endocrine disrupting chemicals (114) are also relevant to the study of chemicals that affect reproductive outcomes. However, a systematic overview of available *in vitro* assays for hazard identification of endocrine disrupting chemicals that affect male and female fertility is unavailable.

In vitro assays that identify chemical interference with sex hormone production and signalling have been validated (OECD TG 493, 455, 458, 456). These include assays based on nuclear receptor activation and steroid hormone synthesis. The bovine oocyte maturation assay (ECVAM TM 2010-05) is also a reproduction-relevant *in vitro* assay. A good correlation between *in vitro* results and *in vivo* observations has been established for female fertility endpoints (214, 215). Validated *in vivo* assays exist to evaluate reproductive toxicity for impacts on both male and female fertility (OECD TG 443), but these may not be sufficiently sensitive or comprehensive.

Table 2. Examples of food contact chemicals (FCCs) that are associated with diseases from the Six Clusters of Disease (SCOD) by mechanisms from *in vitro* and/or *in vivo* studies. Not a complete list: Select references only.

Disease Cluster	Food Contact Chemical	Reference
Cancers	**Melamine** (CAS 108-78-1)	(269)
	Formaldehyde (CAS 50-00-0)	(121)
	Benzidine (CAS 92-87-5)	(270)
	4,4′-Diamino-3,3′ - Dichlorodiphenylmethane (MOCA) (CAS 101-14-4)	(121)
	Antimony trioxide (CAS 1309-64-4)	(271)
	Perfluorooctanoic acid (PFOA) (CAS 335-67-1)	(272-274)
	Di (2-ethylhexyl) phthalate (DEHP) (CAS 117-81-7)	(275, 276)
	Bisphenol A (BPA) (CAS 80-05-7)	(277-280)
Cardiovascular diseases	**Bisphenol A (BPA)** (CAS 80-05-7)	(143, 281-285)
	Triclosan (CAS 3380-34-5)	(284)
	Tributyltin chloride (CAS 1461-22-9)	(284)
	Diethanolamine (CAS 111-42-2)	(286)
	DEHP	(287)
Brain-related	**Perchlorate** (CAS 14797-73-0)	(288)

disorders	**Ortho-phthalates**	(289)
	BPA	(290, 291)
	Bisphenol S (BPS) (CAS 80-09-1)	(290, 292)
Metabolic diseases	**BPA**	(293-297)
	Bisphenol A diglycidyl ether (BADGE) (CAS 1675-54-3)	(298)
	Organotins	(299)
	Perchlorate	(300)
	Perfluorooctanesulfonic acid (PFOS) (CAS 1763-23-1)	(301, 302)
	Bisphenol F (BPF) (CAS 620-92-8)	(303)
	BPS	(303)
	2,4,7,9-tetramethyl-5-decyne-4,7-diol (TMDD; Surfynol) (CAS 126-86-3)	(304-306)
	DEHP	(297, 307)
Immunological disorders	**Melamine**	(269)
	BPA	(290)
	BPF	(290)
	BPS	(290, 308)
	2,4-di-tert-butylphenol (CAS 96-76-4)	(309)
	DEHP	(308, 310)
Reproductive disorders	**BPA**	(311-314)
	BADGE	(298, 306)
	BPS	(315)
	DEHP	(287, 312)

5. What is needed to implement the vision for safer food contact materials?

To achieve our vision, we propose a multi-pronged approach that is grounded in the SCOD concept, which includes many of the most prevalent NCDs of high relevance to human health. We identified three components needed to realize this vision: analytical methods and testing strategies, data integration and interpretation, and science to inform decision making.

5.1 Analytical methods and testing strategies

In Section 4 we list several available and emerging assays used in the identification of hazard for each of the SCOD. However much more is needed, especially high-throughput non-animal and low-medium throughput assays with non-mammalian models. These assays would overcome challenges with cost, time, and scientific relevance as the selection of suitable *in vitro* assays would be based on robust mechanistic evidence from key characteristics and AOPs. Identification of the key characteristics for brain disorders and metabolic diseases will form the basis for identification and/or development of relevant *in vitro* assays to identify hazardous chemicals related to these clusters. For *in vitro* testing based on mechanistic pathways to succeed, additional dedicated expertise and financial support are needed to identify assays that would address relevant key characteristics. This work is ongoing and the website keycharacteristics.org collates all available information and publications in this area (216).

Another important aspect of testing is the development and validation of methods that reflect real-world chemical exposures from food contact materials. Migration testing protocols exist but ongoing research efforts need to be expanded and validated to ensure minimal loss of potentially hazardous chemicals during sample preparation (e.g. by using polar and apolar food simulants and by capturing not only non-volatile compounds, but also those that are semi-volatile and volatile).

Lastly, a battery of screening assays addressing the SCOD needs to be defined and validated. This step will need the contribution of experts in each field to ensure that the selected endpoints are reliable and result in high confidence.

5.2 Data interpretation and integration

Methods must be developed to interpret and corroborate *in vitro* test results. Individual assays should be integrated into an overall high-level / aggregated scheme (e.g. using visualization approaches such as ToxPi (217, 218)). Also, non-targeted chemical analysis needs advancing to allow for better identification of currently unknown compounds, especially when present at low concentrations. One way to improve the latter is to create comprehensive and open mass spectrometry libraries of FCCs, including NIAS. Ideally, an open-access repository of information about food contact material manufacturing processes and the major FCCs associated with specific materials should be generated. Confidential business information poses a critical obstacle, as the full disclosure of the chemical composition of food contact materials is commonly not available. Accordingly, a mechanism needs to be developed that enables such an FCC library without infringing on intellectual property rights.

5.3 Science for decision making

The results of testing single chemical or overall migrate from a food contact material using a battery of assays for each of the SCOD would need to be interpreted and integrated with available evidence to reach a conclusion within a regulatory context. A framework, similar to that available for read-across (219, 220), should be developed to effectively utilize results and support conclusions that are actionable for policy makers and regulatory enforcement. The experience gained from development of effect-based trigger values for water quality assessment in Europe could be highly informative (221, 222). Here, effect-based trigger values have been developed as a means to interpret the results of *in vitro* assays through linking the existing water quality guideline values to observed levels of bioactivity elicited by a reference chemical. Then, if a test chemical or mixture causes an activity above the trigger value set for a specific assay, it is highlighted for a follow-up assessment, such as calculation of concentration factors and *in vitro* to *in vivo* extrapolation (223-225). In theory, effect-based trigger values for food contact materials could be developed following the same principle, e.g. by matching effect concentrations in relevant bioassays with existing specific migration limits for FCCs of concern, and possibly factoring in additional exposure-related parameters. This approach appears highly promising, since it has been demonstrated that derivation of effect-based trigger values greatly facilitates regulatory and practical uptake of *in vitro* methods into specific assessment pipelines (222).

6. Conclusion

The novel approach we present here is in line with the goals laid out in the EU's Chemicals Strategy for Sustainability (91), the EU Farm to Fork Strategy (226), and the European Parliament's report on food contact materials (227), which emphasize the need for revising food contact material regulation in Europe to adequately reflect recent scientific understanding and improve compliance. Further, this work adds to previous publications on policies and methods related to the risk assessment of food contact chemicals and materials (10, 22, 30).

We think that our vision to create safer food contact materials by linking hazard identification more directly to human health has the potential to spur innovation in assay development and testing, and ultimately, for safer materials as such. Additionally, new findings on the key characteristics for the NCDs included in the SCOD, as well as mechanistic understanding derived from AOP research, will support the development of new assays.

Awareness of adverse health effects of synthetic chemicals is increasing globally, and the need is obvious for significant and urgent improvements in the ways that risks are assessed and managed for FCCs (228).

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Declarations

Competing Interests

The authors have no competing interests to declare. For the sake of transparency, the authors list their relationships with various research funders and not-for-profit organizations in the following. As researchers employed by the Food Packaging Forum Foundation (FPF) (JMB, BG, JM, LZ) or working pro bono as members of the Foundation's board (TB, JPM, MS) and its Scientific Advisory Board (SAB) (AMA, TJC, KJG, JJH, MVM, OVM, AN, CN, AMS, LT, MW, RTZ), we are reporting that the FPF receives donations from diverse companies that may be affected by the research reported in the enclosed paper. FPF funders have no influence on any of the work at FPF and were not involved in any way in the preparation of this manuscript. TB declares that he serves as the board member of the International Panel on Chemical Pollution (IPCP), the Swedish Toxicological Council and the EU Commission's Committee on Health, Environmental and Emerging Risks (SCHEER). All those activities are pro bono and have no bearing on the content of the manuscript. None of the aforementioned organizations have had any impact on the content of the manuscript. TJC declares that he is the creator-founder of Sudoc, LLC, which deploys TAML catalysts for many applications and has potential for remediating FCCs in water. JL reports that she receives funding for another research project (ZonMw/Health-Holland Microplastics and Health project MOMENTUM 458001101) of which some partners may be affected by the research reported here. MVM is a paid consultant to the FPF. OVM is one of the representatives of the European Parliament on the European Chemical Agency's Management Board. JPM is co-founder and board member of Sudoc and he declares to have given all his shares to an irrevocable grantor trust so that he will not benefit financially if the company is successful. AN declares to have received travel reimbursement from universities, NGOs and scientific societies, to speak about endocrine-disrupting chemicals. LNV has received travel reimbursements from universities, governments, NGOs, and industry. She has received research funding from the US National Institutes of Health, the University of Massachusetts Amherst, and NGOs including the Cornell Douglas Foundation, the Allen Family Foundation, and the Great Neck Breast Cancer Coalition. She is a scientific advisor to Sudoc LLC. The FPF foundation board, whose members have no connection with any of the FPF's funders and receive no remuneration for their work, is legally obliged to guarantee that the work of the FPF is in no way influenced by the interests or views of the funders.

Authors' contributions

This manuscript was initiated by the FPF's SAB and guests participating in SAB meetings in 2017, 2018, 2019, 2020, 2021; AMA, MVM, JM, JPM, RTZ and MS were responsible for preparing an outline and a first version; JM, LVM, MVM and MS edited the final draft, and all authors contributed to the various intermediate versions, wrote separate sections of the manuscript, and approved the final version.

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Supplemental Material

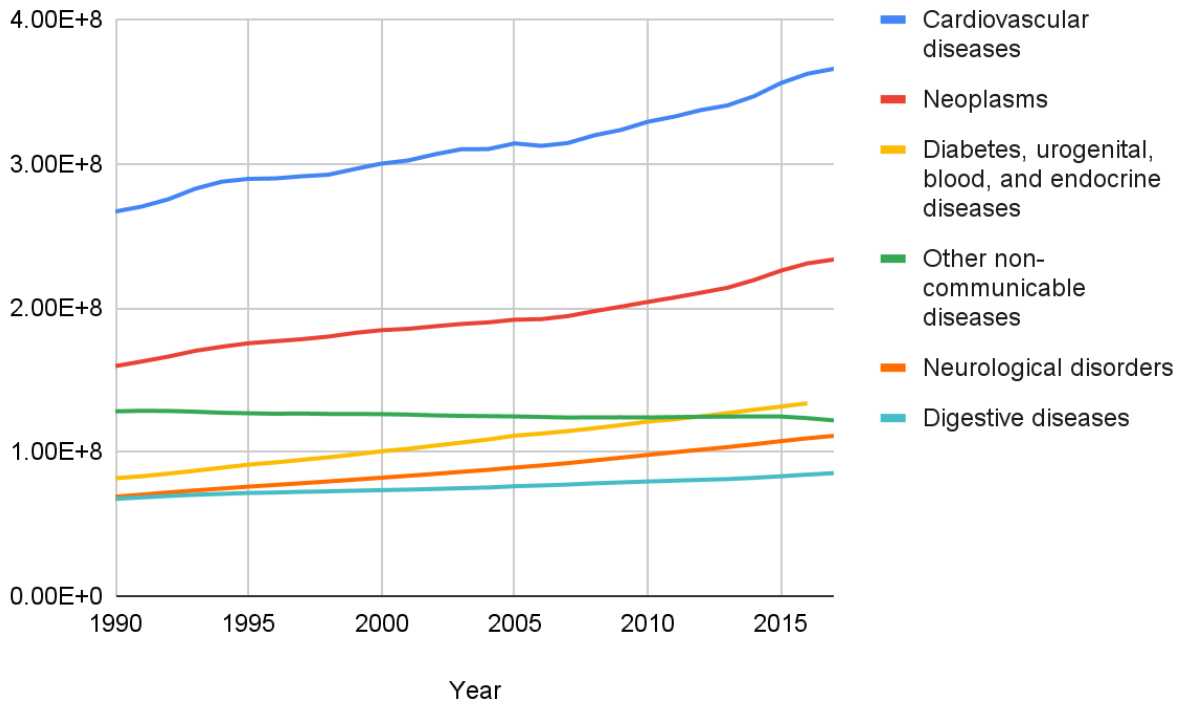

Figure S1: Disability-Adjusted Life Years (DALYs) of worldwide selected non-communicable diseases in both sexes and all age groups, 1990 - 2017 (Diabetes, urogenital, blood, and endocrine diseases: data 1990-2016). Data: Global Burden of Disease 2021.