Review

Exposure to endocrine disrupting compounds via the food chain: Is packaging a relevant source?

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ABSTRACT

Contamination of foodstuffs by environmental pollutants (e.g. dioxins, metals) receives much attention. Until recently, food packaging as a source of xenobiotics, especially those with endocrine disrupting properties, has received little awareness despite its ubiquitous use. This article reviews the regulations and use of endocrine disrupting compounds (EDCs) in food packaging and discusses their presence within the context of new toxicology paradigms.

I focused on substances known to be legally used in food packaging that have been shown to exhibit endocrine disruptive effects in biological systems. I compiled a list of 50 known or potential EDCs used in food contact materials and examined data of EDCs leaching from packaging into food, with a focus on nonylphenol. I included recent advances in toxicology: mixture effects, the developmental origins of adult disease hypothesis, low-dose effects, and epigenetics. I especially considered the case of bisphenol A. The core hypothesis of this review is that chemicals leaching from packaging into food contribute to human EDCs exposure and might lead to chronic disease in light of the current knowledge.

Food contact materials are a major source of food contaminants. Many migrating compounds, possibly with endocrine disruptive properties, remain unidentified. There is a need for information on identity/quantity of chemicals leaching into food, human exposure, and long-term impact on health. Especially EDCs in food packaging are of concern. Even at low concentrations, chronic exposure to EDCs is toxicologically relevant. Concerns increase when humans are exposed to mixtures of similar acting EDCs and/or during sensitive windows of development. In particular, non-intentionally added substances (NIAS) migrating from food contact materials need toxicological characterization; the overall migrate of the finished packaging could be evaluated for biological effects using bioassays. The widespread legal use of EDCs in food packaging requires dedicated assessment and should be updated according to contemporary scientific knowledge.

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Contents

1. Introduction

1.1. Food packaging market, economic value and importance

The packaging market is a highly important industrial sector, approximately equal in size to the pharmaceutical industry. In 2007, global market value amounted to around US $530 billion, with food and beverage packaging constituting more than half of all packaging uses (food 41%, industry and transport 21%, other 17%, beverages 14%, pharmaceuticals 4%, and cosmetics 3%) (Pira International, in: Schönrock 2008). When broken down by packaging material, the most important consumer packaging (by market value) is made of plastic (38%, both rigid and flexible plastics), followed by paper and cardboard (30%), metal (19%), glass (8%), and others (5%) (Pira International, in: Rexam, 2008). Around 70% of overall consumer packaging consumption is used for food and beverage packaging (Pira International, in: World Packaging Organization, 2008).

1.2. Packaging as a source of foodstuff contaminants

Food as a major xenobiotics and heavy metal exposure route to humans is studied intensively. Typical food contaminants, like pesticides, dioxins, PCBs, PBDEs, methylmercury, lead, arsenic, etc. are well characterized in food, with high public and regulatory awareness, as a recent debate on pesticides in food shows, spurred by an NGOs report (Schafer and Kegley, 2002). In contrast, the role of food and beverage packaging as an additional source of contaminants has received much less attention, even though food packaging contributes significantly to human xenobiotic exposure (Grob et al., 2006). This may now be changing. For example, a fierce public debate has unfolded during the past 5 years over the potential safety of bisphenol A (BPA), a plastic monomer that is one of the highest production–volume chemicals worldwide. BPA is extensively used in many different types of food packaging and a known endocrine disruptor (vom Saal et al., 2007). In fact, many intentionally-used substances in food packaging have been identified as endocrine disruptors in biological systems (Table 1). Therefore, it is important to consider food packaging as an important route of endocrine disrupting compounds (EDCs) exposure to humans by leaching from the packaging into the food and the environment by waste disposal.

1.3. Scope and purpose of this article

In this article I review the potential of common food and beverage packaging materials to act as food contaminant source. Several reviews have looked at leaching into foodstuff from packaging, but not with a focus on EDCs (Grob et al., 1999; Lau and Wong, 2000; Grob, 2002; Arvanitoyannis and Bosnea, 2004; Skjevrak et al., 2005; Garcia et al., 2006; Grob et al., 2006; de Fatima Pocas and Hogg, 2007; Marsh and Bugusu, 2007). The literature for leaching from food packaging is extensive, and this review will not do justice to all available information, but rather focus on selected EDCs that can leach from packaging into foodstuff. I pay particular attention to food packaging as source of those EDCs that have, either directly or indirectly, been implicated in epidemiological trends with potential links to endocrine disruption, and for which there are biomonitoring data documenting human exposure.

I also provide a brief overview of relevant regulations in the US and EU. Finally, I identify novel toxicological paradigms that should be integrated into the regulatory process of food contact material (FCM) authorization.

2. Food packaging types: chemistry and leaching into food

The purpose of food packaging, apart from marketing purposes, is to preserve food by protecting it from (i) air (and oxygen), (ii) loss of gas (e.g. for carbonated beverages), (iii) moisture loss/incorporation, (iv) light (and UV radiation), (v) foreign aroma compounds, (vi) microbial contamination, (vii) temperature instability, and (viii) mechanical influences. Different materials are used to package foodstuffs: plastics, paper, card board, metals, glass, regenerated cellulose, ceramics, rubbers and elastomers, waxes, wood, cork, and textiles. Most metal cans have polymeric coatings, and paper or carton packaging often is coated or laminated with plastic as the effective food contact material, essentially making plastics the main food contact material in today's packaging landscape (Castle, 2007). The main focus of this article is on plastic FCMs due to their abundance.

2.1. Migration from food packaging

Food packaging can interact with the packaged foodstuff by diffusion-controlled processes which mainly depend on chemical properties of the FCM and the foodstuff, temperatures at packaging, during heat treatment and storage, exposure to UV light, and storage time of the product (Arvanitoyannis and Bosnea, 2004). This interaction can lead to FCM compounds leaching from the packaging to the food, a process also known as "migration". Compounds that can leach from plastic FCMs are starting substances used for the initial polymerization step, like monomers or catalysts, and additives that are included during the manufacturing process to achieve special material properties (e.g. plasticizers for material softening, or fillers for hardening). Starting substances can leach either because of incomplete polymerization during the formation of the material, or because of material degradation over time. Furthermore, starting substances or additives can contain impurities, which again might leach from the packaging. These compounds are known as "non-intentionally added substances" (NIAS) and also include side-products from the complex polymerization reaction, like oligomers e.g. styrene trimer from polystyrene (Ohyama et al., 2007; Yanagiba et al., 2008) or the break-down product nonylphenol from the additive trisnonylphenyl phosphite (TNPP) (McNeal et al., 2000). The identity of NIAS is not always known (Grob, 2002; Bradley and Coulier, 2007).

Leaching also occurs from the other types of packaging materials; for example, glass bottles have been found to leach lead (Shotyk and Krachler, 2007), and metal closures of glass jars were a source of epoxidized soy bean oil (ESBO), di-iso-decylphthalate (DIDP) or di-iso-nonylphthalates (DINP) (Pedersen et al., 2008). Sea foods packaged in metal cans contained levels of Bisphenol A diglycidyl-ether (BADGE) and Bisphenol F diglycidyl-ether (BFDGE) that increased with storage time (Cabado et al., 2008). Paper food packaging was found to release perfluorinated compounds (Begley et al., 2005). Migration of benzophenone from beverage cartons into milk, fruit juices and wine has been demonstrated (Sagratini et al., 2008). Beverage cans were found to release the biocide ortho-phenylphenol (OPP) into beer (Coelhan et al.,

Table 1
List of 50 known or potential endocrine disrupters[a] with authorized use in food contact materials in the US and/or EU.

	CAS #	Compound Name	US CFSAN food additive EAFUS	US CFSAN indirect food additive	EU positive lists	EU specific migration limit SML [mg/kg]	EU priority substance[b]	References (selected)
1	59-50-7	4-chloro-3-methyl-phenol		x	–	No		Kruger et al. (2008)
2	74-31-7	Diphenyl-p-phenylenediamine	–	x	–			Yamasaki et al. (2002)
3	77-40-7	2,2-Bis(4-hydroxyphenyl)-n-butan (bisphenol B)		x	–		c	(Kitamura et al., 2005; Hashimoto et al., 2001)
4	80-05-7	4,4′-dihydroxy-2,2-diphenylpropane (Bisphenol A)		x	x	0.6	d	(vom Saal et al., 2007; Newbold et al., 2009; Leranth et al., 2008; Hugo et al., 2008)
5	80-46-6	p-(tert-pentyl)phenol	–	x	–			Yamasaki et al. (2002)
6	84-61-7	Dicyclohexyl phthalate		x	–			Kanayama et al. (2005)
7	84-66-2	Diethyl phthalate		x	–			Kanayama et al. (2005)
8	84-69-5	Diisobutyl phthalate (DiBP)		x	–		c	(Takeuchi et al., 2005; Boberg et al., 2008; Borch et al., 2006; Saillenfait et al., 2008)
9	84-74-2	Dibutyl phthalate (DBP)		x	x	0.3[e]	f	(Gray et al., 2000; Kanayama et al., 2005; Kruger et al., 2008)
10	84-75-3	Di-n-hexylphthalate (DnHP)		x	–		c	Yamasaki et al. (2004)
11	85-68-7	Butyl benzyl phthalate (BBP)		x	x	30[e]	d f	(Gray et al., 2000; Kanayama et al., 2005)
12	87-18-3	4-tert-Butylphenylsalicylate		x	x	12[g]		Ogawa et al. (2006)
13	88-24-4	2,2′-Methylenebis(4-ethyl-6-tert-butylphenol)		x	x	1.5[h]		Satoh et al. (2008)
14	88-99-3	Phthalic acid		x	–	No		(Gaitan, 1989; Masuyama et al., 2000)
15	90-43-7	2-Phenylphenol		x	–			(Kruger et al., 2008; Waring et al., 2008)
16	92-69-3	4-Phenylphenol		x			c	Ogawa et al. (2006)
17	92-88-6	4,4′-Biphenol	–	–	x	6	c	Yamasaki et al. (2004)
18	94-13-3	n-Propyl-p-hydroxybenzoate (Propylparaben)[i]		x	x	No	c	(Oishi, 2002; Kamiya et al., 2005)
19	96-69-5	4,4′-Thiobis(6-terc-butyl-3-methyl-phenol)		x	x	0.48		Satoh et al. (2008)
20	99-76-3	Methyl p-hydroxybenzoate (Methylparaben)[f]		x	x	No	c	(Routledge et al., 1998; Lemini et al., 2004)
21	99-96-7	p-Hydroxybenzoic acid	x	–	x	No	c	Lemini et al. (1997)
22	103-23-1	Diethylhexyl adipate		x	x	18		Kanayama et al. (2005)
23	104-40-5	4-Nonylphenol		x	–		c	Loyo-Rosales et al. (2004)
24	106-44-5	p-cresol		x	x	No	c	Letcher et al. (2005)
25	106-46-7	1,4-Dichlorobenzene		x[c]	x	12	d	Versonnen et al. (2003)
26	108-46-3	Resorcinol 1,3-dihydroxybenzene		x	x	2.4		Kruger et al. (2008)
27	117-81-7	Bis (2-ethylhexyl) phthalate (DEHP)		x	x	1.5[c]	c d f	(Gray et al., 2000; Kruger et al., 2008; Kanayama et al., 2005)
28	119-47-1	2,2′-Methylene bis(4-methyl-6-tert-butylphenol)		x	x	1.5[h]		Satoh et al. (2008)
29	119-61-9	Benzophenone		x	x	0.6		Kanayama et al. (2005)
30	120-47-8	Ethyl-4-hydroxy-benzoate (Ethylparaben)		x	x	No	c	(Lemini et al., 2004; Satoh et al., 2000)
31	121-79-9	Propyl gallate[f]		x	x	30		ter Veld et al. (2006)
32	121-91-5	Isophthalic acid		x	x	5		Gaitan (1989)
33	131-53-3	2,20-Dihydroxy-4-methoxybenzophenone		–	x	6[g,j]		Ogawa et al. (2006)
34	131-56-6	2,4-Dihydroxybenzophenone	–	–	x	6[j]	c	Yamasaki et al. (2004)
35	131-57-7	2-hydroxy-4-methoxybenzophenone (Oxybenzone)		x	x	6[j]	c	Ogawa et al. (2006)
36	301-02-0	9-octadecenamide (Oleamide)		x	x	No		McDonald et al. (2008)
37	599-64-4	p-Cumyl phenol		x	x	0.05		(Yamasaki et al., 2003; Terasaki et al., 2005; Hashimoto et al., 2001)
38	611-99-4	4,4′-Dihydroxybenzophenone	–	–	x	6[j]	c	Yamasaki et al. (2002)
39	620-92-8	Bis(4-hydroxyphenyl)methane (Bisphenol F)[k]		–	–			(Ogawa et al., 2006; Yamasaki et al., 2003)
40	683-18-1	Dibutyltin dichloride		x	–			Nakanishi et al. (2006)
41	1131-60-8	4-Cyclohexylphenol		x	–	No		(Yamasaki et al., 2002; Ogawa et al., 2006; Kamata et al., 2008)
42	1675-54-3	2,2-bis(4-hydroxyphenyl)propane bis (2,3-epoxypropyl) ether (BADGE)		x	–	9		(Wright et al., 2000; Letcher et al., 2005)
43	3380-34-5	2,4,4′-Trichloro-2′-hydroxydiphenyl ether (triclosan)			(x)	[5][l]		Kumar et al. (2009)
44	18964-53-9	2,4,6-triphenyl-1-hexene (Styrene trimer 1)		x	x[m]	–		(Yanagiba et al., 2008; Ohyama et al., 2001; Ohyama et al., 2007)
45	4809-35-2	2,2-bis[4-(3-chloro-2-hydroxypropoxy) phenyl]propane (BADGE.2HCl)		x	–		d	Satoh et al. (2004)
46	25013-16-5	tert.-Butylhydroxy-anisole (BHA)[f]		x	x	30	c d	ter Veld et al. (2006)
47	26027-38-3	Nonylphenol ethoxylate		x	–	No		Ogawa et al. (2006)

Table 1 (*continued*)

	CAS #	Compound Name	US CFSAN food additive EAFUS	US CFSAN indirect food additive	EU positive lists	EU specific migration limit SML [mg/kg]	EU priority substance[b]	References (selected)
48	26523-78-4	Tris(nonylphenyl)phosphate (TNPP)		x	–	No	[c] [d]	Ogawa et al. (2006)
49	26761-40-0	Diisodecyl phthalate (DiDP)		x	x	9[e]		Kruger et al. (2008)
50	–	1e-phenyl-4a-(1′-phenylethyl)tetralin (Styrene trimer 4)		x	x[m]	–		(Ohyama et al., 2001; Ohyama et al., 2007)

[a] Listed substances have been shown to be EDC *in vitro* or *in vivo*.
[b] Substance listed by EU institution (s. footnotes c, d, and f).
[c] Community Strategies for Endocrine Disrupters List http://ec.europa.eu/environment/endocrine/documents/sec_2007_1635_en.htm.
[d] ORATS EU Joint Research Center (JRC) priority list http://ecb.jrc.it/esis/index.php?PGM=ora.
[e] Specific migration limit (SML) with restrictions.
[f] European Chemical Agency (ECHA) Candidate List of substances of very high concern for REACH Annex XV http://echa.europa.eu/chem_data/candidate_list_table_en.asp.
[g] Metabolite is estrogenic.
[h] SML(T): Specific migration limit as total of all substances listed, for 88-24-4 and 119-47-1.
[i] GRAS status (US): substance is classified as generally recognized as safe.
[j] SML(T) for 131-53-3, 131-56-6, 131-57-7 and 611-99-4.
[k] Banned in EU since 2006; stocks are allowed to be used up.
[l] EFSA provisional positive list of additives, to be implemented 01.01.2010 http://ec.europa.eu/food/food/chemicalsafety/foodcontact/2008_2004_2010_provisional_list_additives_used_plastics.pdf.
[m] Styrene monomer: EU positive list starting substances; US polystyrene approved (21 CFR 177.1640).

2006). Dry foods are also affected by packaging leachates: Migration of triclosan from packaging into flour and rice has been demonstrated (Silva et al., 2008). Recycled board leached diisobutyl phthalate (DiBP), dibutyl phthalate (DBP) and benzophenone into dry foods (Brauer and Funke, 2008), and migration of xenoestrogens used in printing inks has been demonstrated for recycled cardboard used for food contact (Lopez-Espinosa et al., 2007).

When assessing food contamination from packaging it is not sufficient to only sample retail products and analyze them for certain contaminants. While this will give a good indication of actual food pollutant levels, their presence in food cannot be clearly attributed to leaching from packaging because other contaminant sources, like processing prior to packaging, are not taken into account. To determine actual leaching from food packaging contaminant levels need to be assessed over time. Such experiments are often carried out using food simulants, e.g. water, 3% acetic acid, 10% ethanol, oils etc. instead of actual foods. However, the use of food simulants might lead to an underestimation of actual migration into food (Grob, 2008). For example, this is the case for perfluorinated compounds used in grease-proof paper packaging (Begley et al., 2008).

Migration usually is assessed using chemical analysis of known single substances. Such studies however do not cover all possible migrants (Fig. 1). A first study using an invertebrate organism bioassay to determine overall migrating compounds from PET bottles found estrogenic substances originating from the packaging, however their chemical identity so far is unknown (Wagner and Oehlmann, 2009). Also genotoxicity has been found for overall migrants from recycled and virgin paper FCM using *in vitro* bioassays (Ozaki et al., 2004); only some of the migrating substances were identified, but their concentration did not fully explain the observed genotoxic effect of the total migrate.
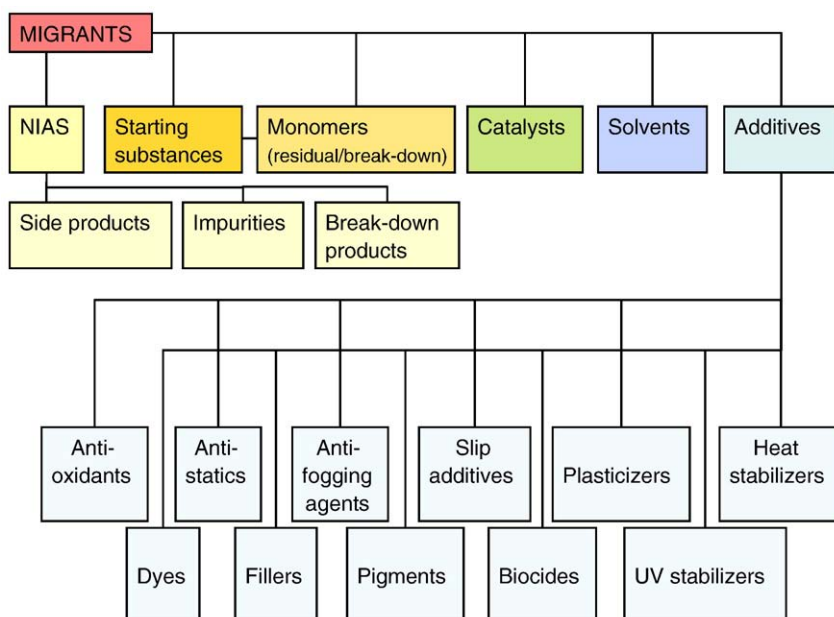

Fig. 1. Classification of possible migrants from food packaging. An overview of which compounds are used as additives in plastics is given in Bolgar et al., 2008 and Akovali, 2007. Detailed information can be found in Ash and Ash, 2008.

Table 2
Nonylphenol migration from different types of food packaging into various food simulants/foods.

Packaging type	Food type	Concentration	Time	Temp. [°C]	Reference
Glass bottle (closure)	Apple juice	22.5 ng/mL	10 days	40	McNeal et al. (2000)
Glass jar (closure)	Infant formula	81.0 ng/g	10 days	40	McNeal et al. (2000)
PET bottle	Dist. water	n.d. quantification limit 8 ng/L	240 days	40	Loyo-Rosales et al. (2004)
Food can	Apple juice	n.d. detection limit 0.2 ng/mL	10 days	40	McNeal et al. (2000)
Beverage carton	Apple juice	7.0 ng/mL	10 days	40	McNeal et al. (2000)
HDPE bottle	Dist. water	230 ng/L	120 h	40	Loyo-Rosales et al. (2004)
	10% ethanol (milk surrogate)	580 ± 25 ng/L	15 days	40	Loyo-Rosales et al. (2004)
		180 ± 53 ng/L	50 h	20	
PVC bottle	Dist. water	140 ng/L	120 h	40	Loyo-Rosales et al. (2004)
	10% ethanol (milk surrogate)	580 ng/L	15 days	40	Loyo-Rosales et al. (2004)
PVC films	Dist. water	9.7 ng/cm^2[a]	30 min	60	Inoue et al. (2001)
	4% acetic acid	7.2 ng/cm^2[b]	30 min	60	
	n-heptane	1.6 µg/cm^2[c]	60 min	25	
	Cooked rice	Max. 35 ng/g	30 min	20	
	Cooked rice	Max. 172 ng/g	1 min	Microwave 500 W, 2450 MHz	

Note: When assessing the contribution of food packaging to food contamination, actual migration needs to be assessed, not just the presence of the contaminant in the food as this could have other sources than the packaging. However, when retail samples are used this means that migration is underestimated, as original food is discarded and previously leached contaminant is not taken into account.
When analyzing data on migration from food packaging in the literature, different units are used, either mg/kg or mg/dm^2. The latter refers to surface of the packaging in contact with the food stuff. In the EU and US different conventions are used for packaging surface/food volume ratios (see Table 3).
n.d. not detected.
[a] Corresponds to 194 ng/mL (for 200 cm^2 surface area).
[b] Corresponds to 144 ng/mL (for 200 cm^2 surface area).
[c] Corresponds to 30,000 ng/mL (for 200 cm^2 surface area).

2.2. Example 1: nonylphenol in foods and food packaging and migration from packaging into food

Nonylphenol is a well-studied model EDC with estrogenic properties (Soto et al., 1991) that is widely present in foods (Guenther et al., 2002). In food packaging, nonylphenol originates from oxidation of the antioxidant additive trisnonylphenyl phosphite (TNPP) (McNeal et al., 2000). Levels of nonylphenol in different packaging materials were recently assessed and were found to range from below 0.03 µg/g in a PET water bottle to 287 µg/g in PVC cling film (Fernandes et al., 2008). Nonylphenol was also detected in different types of retail-purchased foods: up to 78 ng/L were found in PET-bottled mineral water (Toyo'Oka and Oshige, 2000), up to 40 µg/kg in beverage cartons of UHT-whole milk and up to 32.3 µg/kg in HDPE-bottled milk (in bottle sterilization) (Casajuana and Lacorte, 2004). To determine the contribution of food packaging to food levels, appropriate study designs are necessary. Several such studies have been carried out for this specific EDC and are summarized in Table 2). It has been shown that initial concentrations of nonylphenol in the packaging material correlate with migration (Inoue et al., 2001).

3. Regulation of food contact materials and compounds

An important difference distinguishes the European Union's approach to food packaging regulation compared to that of the United States: the EU regulation focuses on substance migration from the packaging into food simulants, while the US regulation is based on estimated consumer exposure.

Table 3
Conventions for migration, exposure and risk assessment of food contact material migrants in US and EU.

	US	EU
Food/packaging ratio[a]	1 kg food/6.45 dm^2 (10 g/in.^{2})	1 kg food/6 dm^2 (11 g/in.^{2})
Weight per person	60 kg (132 lb)	60 kg
Food consumption per day	3 kg all foods (solid + liquid)	1 kg of any given food
Risk management tool	ADI based on CEDI	SML based on TDI

[a] Mass of food stuff in contact with packaging surface, for all types of packaging.

3.1. US food contact regulation

Food contact material regulation in the US originates from the Federal Food, Drug and Cosmetic Act of 1958, Section on Food Additives (21USC348). Substances used as food additives require authorization, unless they were used in food packaging prior to September 6, 1958.

New substances authorization is centered on consumer exposure levels. The cumulative estimated daily intake, or CEDI, is an approximation of consumer exposure to packaging compounds, derived from leaching into food surrogates, or "food simulants". This migration is determined experimentally or by modeling. Compounds added to food packaging need to be authorized by the Food and Drug Administration (FDA), and different requirements apply depending on the CEDI (Table 4). This CEDI is determined by estimating which type of packaging material is in contact with which food, and consequently how much of a migrant would be consumed on average per person and day. The FDA sets the consumption factors for all materials and food-type distribution factors for all foods based on own market data estimates.

If a manufacturer wants to obtain authorization for a novel additive, the extent of data requirement essentially will depend on the CEDI. For a CEDI of 1.5 µg/person/day or below, the "Threshold of Regulation" (TOR) applies, a concept introduced in 1995: Authorization is granted if the substance has no structural similarity to known carcinogens and there are no data indicating carcinogenicity (Begley, 1997). In this case, experimental toxicological data are not required. If the CEDI is above 1.5 µg/person/day but below 150 µg/person/day, *in vitro* genotoxic data must be submitted, while for a CEDI above 150 µg/person/day but below 3 mg/person/day subchronic toxicity studies *in vivo* are required (in rodent and non-rodent species) and possibly also chronic studies on reproductive/developmental toxicity. For substances with a CEDI below 3 mg/person/day a Food Contact Notification (FCN) can be filed, an authorization pathway that is much faster (6 months) than the Indirect Food Additive Petition that would be required for a CEDI of or above 3 mg/person/day.

Inventories of TOR, FCN and Indirect Food Additive substances are available online (Table 4). Both TOR and FCN were introduced with the aim to speed up the authorization process for food contact substances and, in the case of FCN, for economic advantage as the FCN is valid only for the applying company. Another pathway for food contact substances authorization is the generally recognized as safe, or GRAS,

Table 4
Toxicological testing requirements for food contact substances authorization in the US and EU.

	US				EU		
Authorization based on	Cumulative estimated daily intake (CEDI) [µg/person/day] of food contact substance				Migration (M) [µg/kg food] of food contact substance		
Authorization threshold	CEDI ≤ 1.5[a]	1.5 < CEDI ≤ 150	150 < CEDI < 3000	CEDI ≥ 3000 (for biocides: 600)	M < 50	M < 50–5000	M > 5000 (max. 60,000)
Concentration in food	≤ 0.5 ppb	0.5 ppb–50 ppb	50 ppb–1 ppm	> 1 ppm	< 50 ppb	50 ppb–5 ppm	> 5 ppm
Specific applicable regulation	21CFR170.39 Threshold of Regulation since 1995	21CFR170.101 Food Contact Notification since 1997	21CFR170.101 Food Contact Notification since 1997	21CFR171.1 Indirect Food Additive Petition since 1958	2002/72/EC	2002/72/EC	2002/72/EC
Toxicological testing[b]		• Gene mutations (bacteria) • Mammalian *in vitro* cytogenicity assay or tk+ assay	• Gene mutations (bacteria) • Mammalian *in vitro* cytogenicity assay or tk+ assay • Chromosomal damage in rodent hematopoietic cells *in vivo* • 2 subchronic oral toxicity tests *in vivo* (rodent and non-rodent species) (90 days) • Further testing (chronic exposure) with further endpoints can be recommended (metabolism studies, teratogenicity, reproductive toxicity, neurotoxicity, Immunotoxicity studies)	• Gene mutations (bacteria) • Mammalian *in vitro* cytogenicity assay or tk+ assay • Chromosomal damage in rodent hematopoietic cells *in vivo* • 2 subchronic oral toxicity tests *in vivo* (rodent and non-rodent species) (90 days) • Chronic toxicity and carcinogenicity in two rodent species (2 years), one study incl. *in utero* phase • Two generation reproductive toxicity study (in rats) • Further testing with further endpoints can be recommended	• Gene mutations (bacteria) • Gene mutations in mammalian cells *in vitro* (tk+ assay) • Chromosomal aberrations in mammalian cells *in vitro*	• Gene mutations (bacteria) • Gene mutations in mammalian cells *in vitro* (tk+ assay) • Chromosomal aberrations in mammalian cells *in vitro* • 2 subchronic oral toxicity tests *in vivo* (rodent and non-rodent species) (90 days)	• Gene mutations (bacteria) • Gene mutations in mammalian cells *in vitro* (tk+ assay) • Chromosomal aberrations in mammalian cells *in vitro* • 2 subchronic oral toxicity tests *in vivo* (rodent and non-rodent species) (90 days) • ADME study (absorption, distribution, metabolism and excretion) *in vivo* • Reproduction study (one species), developmental toxicity (in two species) • Chronic toxicity and carcinogenicity in two species (2 years)
Further information requirements	• Structure analysis (carcinogen) • Chemical and physical properties • Migration into foods (as basis for setting the EDI) • Literature review; risk assessment if a constituent is carcinogenic	• Structure analysis (carcinogen) • Chemical and physical properties • Migration into foods (as basis for setting the EDI) • Literature review	• Structure analysis (carcinogen) • Chemical and physical properties • Migration into foods (as basis for setting the EDI) • Literature review	• Structure analysis (carcinogen) • Chemical and physical properties • Migration into foods (as basis for setting the EDI) • Literature review	• Chemical, physical and microbial properties • Migration, residual levels in food contact materials • Literature review	• log KOW data; if >3 ADME study might be required • Microbial properties • Migration, residual levels in food contact materials • Literature review	• Microbial properties • Migration, residual levels in food contact materials • Literature review
Inventory	http://www.cfsan.fda.gov/~dms/opa-torx.html CEDI/ADI database http://www.cfsan.fda.gov/~dms/opa-edi.html	http://www.cfsan.fda.gov/~dms/opa-fcn.html	http://www.cfsan.fda.gov/~dms/opa-fcn.html	http://www.cfsan.fda.gov/~dms/opa-indt.html	http://ec.europa.eu/food/food/chemicalsafety/foodcontact/eu_substances_en.pdf http://ec.europa.eu/food/food/chemicalsafety/foodcontact/docs/2008_2004_2010_provisional_list_additives_used_plastics.pdf		
Guidance	CFSAN Guidance and Reference Documents for Petitions and Notifications http://www.cfsan.fda.gov/~dms/opa-guid.html				Guidelines of the Scientific Committee on Food for the presentation of an application for safety assessment of a substance to be used in food contact materials prior to its authorization http://ec.europa.eu/food/fs/sc/scf/out82_en.pdf		
Notes	• for CEDI<150 ppb (dietary concentration in food <50 ppb) no ADI calculated if substance is not of toxicological concern) • "Information on many polymeric FCSs and constituents, such as monomers, are presently not available." • If no migration data is available for a substance a default CEDI of 7 ppb is assumed. • Generally recognized as safe.				SML is based on TDI (tolerable daily intake) value if available		

[a] The Threshold of Regulation (TOR) is not based on the EDI but applicable for substances at or less than 0.5 ppb in the diet. The underlying assumption for setting the EDI is that 3 kg solid and liquid food is consumed per person per day which would set the EDI at 1.5 µg/person/day or below in the case of TOR substances.
[b] Recommended (US) and required (EU).

regulation (21CFR186.1; more information and inventory http://www.cfsan.fda.gov/~dms/opa-noti.html). Not all compounds that are marketed as GRAS necessarily are listed (Twaroski et al., 2007).

Polymer resin NIAS are assessed for toxicity when authorization for the polymer is given; however, this can be problematic and costly (Twaroski et al., 2007). The FDA currently is reviewing specific oligomers for further toxicity testing requirements (Twaroski et al., 2007).

3.2. Food contact regulation in the EU

Food contact regulation in the EU is complex, as in the US. The underlying Framework Regulation 1935/2004 for all types of food packaging is directly applicable and legally binding to all member states of the EU. Its *Article 3* sets the general requirements for all food contact materials, stating that materials and articles intended to contact food shall be manufactured "so that under normal or foreseeable conditions of use, they do not transfer their constituents to food in quantities which could endanger human health […]".

Further detailed provisions for all-plastic food packaging are made in the "Plastics FCM Directive" (2002/72/EC and amendments: 2004/1/EC, 2004/19/EC, 2005/79/EC, 2007/19/EC, 2008/39/EC). It does not apply to multi-material packaging like beverage cartons or food cans, where the FCM is plastic.

Authorization of food contact materials in the EU is based on migration into food. The Plastics FCM Directive sets a maximum level for non-specific leaching from packaging into food at 60 mg/kg foodstuff, or 10 mg/dm^2 of the packaging surface, determined as overall, non-specific migration from the polymer into food simulants (Table 4). For individual substances authorized for food contact use, specific migration limits (SML) may be issued. The authorized starting substances (monomers or compounds that initially react to form the monomer) are inventoried in the "positive list" (Annex II, Section A, 2002/72/EC), and SMLs are given, if applicable. An SML is derived from the compound-specific Tolerable Daily Intake (TDI) which is not available for all listed substances; hence not all compounds have an SML value.

Only those chemicals listed are permitted for use in plastic-only food packaging. For additives, an analogous approach is being taken (Annex II, Section B, 2002/72/EC). The additives positive list will be legally binding after January 2010, applicable to all-plastics packaging materials.

Like in the US, authorization in the EU follows a tiered toxicological testing approach (Table 4). The NIAS do not require specific authorization, and there is scarce publicly available information on their occurrence and toxicity.

A novel concept in the EU is the "functional barrier": Compounds used or present in packaging behind a functional barrier do not require authorization, as long as their migration to the foodstuff does not exceed 10 ppb and they are not carcinogenic, mutagenic or toxic to reproduction (European Union, 2007).

Another unique feature of EU regulation is the Fat (Consumption) Reduction Factor (FRF): For foods that contain more than 20% fat the measured migration of a compound into food simulants can be divided by the FRF, to establish the legal migration. The FRF takes actual fat consumption into account, which is estimated to be less than 200 g fat per day on average. When food has a higher fat content than 20%, migration of lipophilic compounds is higher but consumption of this particular food is assumed to be low (Grob et al., 2007). The FRF is the ratio of food fat content divided by 20. For example, for a lipophilic compound maximal 5 times reduction of the actual migration is permitted, to derive the legally relevant migration value, by using the FRF concept.

4. Food packaging and human health issues

4.1. Increasing human diseases and use of industrial chemicals

Certain human diseases that have been attributed to environmental factors, amongst others, are increasing in parallel to global synthetic chemical production and use (Baillie-Hamilton, 2002). Examples are hormonally mediated cancers of the breast (Brody and Rudel, 2003; Bray et al., 2004), prostate (Moller, 2001) and testis (Skakkebaek et al., 2001), which are frequent cancer types worldwide (Parkin et al., 2005); insulin resistance or metabolic syndrome (reviewed in (Biddinger and Kahn, 2006)), associated with type 2 diabetes (Zimmet et al., 2001) and obesity (Grun and Blumberg, 2006; Newbold et al., 2007; James, 2008); allergies and autoimmune diseases (Inadera, 2006); infertility (Skakkebaek et al., 2006) and malformations of newborn male genitalia (Steinhardt, 2004); and neurodevelopmental diseases including autism (Colborn, 2004; Grandjean and Landrigan, 2006; Roman, 2007; Hertz-Picciotto and Delwiche, 2009). While these correlations cannot be used to infer causality, they do identify a series of hypotheses about potential contributions to these trends that warrant serious study.

4.2. Do endocrine disrupting compounds affect human health?

Considering the vast amount of different chemicals that are used in food packaging and other consumer goods, humans are constantly exposed to a mixture of many different chemicals, most of them at low concentrations. Human exposure to synthetic chemicals and heavy metals has been shown using advanced targeted chemical analysis (CDC, 2005), and biomonitoring is an important tool for public health management (Angerer et al., 2007). Food packaging-associated compounds have been detected in humans, like bisphenol A (Calafat et al., 2007; Vandenberg et al., 2007), nonylphenol (Calafat et al., 2005), perfluorinated compounds (Midasch et al., 2006; Lau et al., 2007), and certain phthalates (Wittassek et al., 2007).

Whether exposure to EDCs influences disease development in humans is not causally proven. However, there are reasons that lead to the assumption that this might be the case:

(i) developmental exposure to hormonally active chemicals has been linked to adverse effects in animals (McLachlan et al., 1998), and in humans (Newbold and McLachlan, 1996; Newbold et al., 2007; Swan, 2008);

(ii) mixtures of EDCs have been shown to induce effects where the individual substance concentrations in the mixture had no statistically significant effect *in vitro* and *in vivo* (Silva et al., 2002; Brian et al., 2005; Hass et al., 2007);

(iii) at low-dose, but not at high-dose, certain adverse effects are seen with EDCs (Welshons et al., 2006; Newbold et al., 2007);

(iv) disruption of fetal development can lead to adult disease (Barker, 2004), and some EDCs have been shown to cross the placental barrier (Main et al., 2007; Midasch et al., 2007; Chen et al., 2008), potentially affecting the human fetus by hormonal disruption at sensitive stages of development which could lead to adult diseases (Fenton, 2006); an example is the tragic intentional exposure of fetuses to diethylstilbestrol (DES), a synthetic estrogen given to millions of pregnant mothers from 1939 to the 1960s, leading to an increased risk for breast cancer in women that were exposed *in utero* (Palmer et al., 2006);

(v) some EDCs disturb epigenetic imprinting, making exposed animals susceptible to certain disease phenotypes and also affecting subsequent generations as a consequence of effects on the germ line (Anway et al., 2005).

4.3. Low-doses of EDCs in food packaging are a public health issue

For all these reasons, the extensive legal use of EDCs in food packaging needs reconsideration. The US' Threshold of Regulation concept, as well as the EU's Functional Barrier concept, is based on the hypothesis that most chemicals below a defined concentration are not harmful to human health (Begley, 1997). However, these thresholds are based on decades-old toxicological data, meaning that high-dose

single substance experiments were extrapolated to lower doses for risk assessment purposes, and some of the studies were neither peer-reviewed nor is the data publicly accessible. Furthermore, the most sensitive life stages, embryonic, fetal and neonatal, were not included in these assessments. For example, the phthalates, additives in plastics, adhesives, pigments and inks, are of most concern during prenatal exposure (Gray et al., 2000; Parks et al., 2000), but toxicity studies in primates so far have not included this development stage and thus are of limited relevance for risk assessment (Tomonari et al., 2006). In addition, it is important to realize that exposure to many chemicals, even if they are present at or below their individual NOAELs, can lead to adverse effects because NOAELs are not zero effect levels (Kortenkamp et al., 2007). Hence, the more chemicals are present in a mixture the more concern is indicated, also for dissimilar acting substances.

When unidentified substances, i.e. the NIAS, are migrating, there are two major problems with these threshold concepts: (i) precise quantification will not be possible due to the lack of an internal standard, hence there is no way of controlling that the threshold is not being exceeded, and (ii) toxicological properties cannot be determined in the absence of the chemical's identity, hence it is unclear if migrating substances are carcinogenic, mutagenic or toxic to reproduction. This means that new approaches need to be put in place to characterize whole packaging toxicity, including printing inks, adhesives and secondary packaging, to understand what consumers are actually being exposed to and whether there is a safety issue or not. With the current approach there seems to be insufficient safety for sensitive population groups in the mixture reality of human exposure (Kortenkamp, 2007).

4.4. Example 2: low-dose toxicity of bisphenol A

In the case of bisphenol A (BPA) it has been shown that at low-doses adverse effects occur, while at high doses these effects are not seen (Welshons et al., 2003). On the other hand, scientific studies on BPA low-doses that were used by risk assessors in the EU and the US were designed, funded and co-authored by the BPA producing industry, and found no such effects (Tyl et al., 2002; Tyl et al., 2008). Several aspects of these studies' methodologies have been criticized as scientifically flawed and thus inappropriate for risk assessment (Myers et al., 2009).

In the US, people of all ages are widely exposed to BPA, with an average urinary concentration of 2.7 ppb that is thought to reflect body burdens (Calafat et al., 2007).

Continuous exposure of rats to 2.5 ppb during fetal development lead to a significant increase in preneoplastic lesions in mammary tissue, while at higher concentrations this effect decreased, displaying a non-monotonic dose-response (Murray et al., 2007). Mice exposed as fetus' to 0.1 ppb BPA were found to have statistically significantly more preneoplastic and neoplastic lesions in the ovary and reproductive tract compared to the control and higher concentration treatments (Newbold et al., 2009). Rats exposed to 3 single doses of 10 ppb BPA during neonatal development had a significantly increased risk of developing adult cancer, if subsequently exposed to steroid hormones (Ho et al., 2006).

But not only specifically sensitive population groups might be at risk. In a recent landmark epidemiological study, Lang et al. (2008) found statistically significant correlations between cardiovascular diseases, as well as diabetes, and increased BPA body burdens. Molecular markers for these diseases were shown to be affected by low-doses of BPA both *in vivo* at 10 ppb (Alonso-Magdalena et al., 2006) and *in vitro* from 0.023 ppb (Hugo et al., 2008) using human tissue explants. Interestingly, BPA showed the same potency as estradiol in inducing the effect at lowest concentrations.

In another recent study using an adult primate model, ovariectomized females were exposed to doses of 50 μg BPA/kg bw/day, the

current (April 2009) tolerable daily intake (TDI) adopted by the EFSA and the FDA's reference dose (RfD), via implanted osmotic pump (Leranth et al., 2008). After 4 weeks exposure to BPA a statistically significant inhibition of estradiol-mediated synaptic regeneration was observed. Hence, BPA exposure at previously believed safe doses results in reduced synapse connections in primates, a condition that is also observed in early stages of Alzheimer's Disease (Selkoe, 2002) and in schizophrenia (Crayton and Meltzer, 1976) in humans.

Risk management warrants a safety factor of 100 or 1000 for extrapolation of the TDI from animal toxicological data, indicating that the TDI for BPA should be at least reduced to 30 μg/day (adults) and 2 μg/day (infants) — for all exposure routes, based on recent scientific findings. The FDA has so far not adjusted the RfD accordingly (vom Saal, 2009). In the EU the TDI for BPA was recently increased from 10 μg/kg bw/day to the US' RfD of 50 μg/kg bw/day (EFSA, 2006), however leaving the corresponding former migration limit for BPA of 0.6 mg/kg so far unchanged.

Levels found in some foods are high, for example with canned foods on the Japanese market containing up to 842 μg/kg BPA (Sajiki et al., 2007). Leaching from polycarbonate baby bottles due to polymer degradation has been shown to increase with higher alkalinity; under certain conditions levels exceeding 100 ppb can be reached (Biedermann-Brem and Grob, 2009). In a recent Canadian study BPA was detected in almost all canned soft drinks samples at levels ranging from 0.032 μg/L to 4.5 μg/L (Cao et al., 2009). Increasingly, other exposure sources of BPA than food packaging are being taken into consideration (Stahlhut et al., 2009).

5. Discussion and conclusions

Current EU regulation has been criticized for permitting too high contamination levels from food packaging migrants (Grob et al., 1999). In particular, coatings are of concern for their often high leaching, but also the trend towards smaller convenience packaging contributes to increasing food contamination, because of larger surface/volume ratios (Grob et al., 2007). Also, for foods with >20% fat content the use of the FRF in the EU can lead to high levels of food contaminants which are legally compliant (Grob et al., 2007). Food contact materials thus are a major source of food contaminants, and many of these compounds, possibly with endocrine disruptive properties, remain unidentified. Hence, in addition to the 50 known or potential EDCs legally used in FCM (Table 1) there might be even more such substances present in food packaging and potentially leaching into food.

There is sufficient evidence that EDCs pose a risk to human and environmental health. Even at low concentrations, chronic exposure to EDCs is of toxicological concern and this concern increases when humans are exposed to mixtures of similar acting EDCs and/or during sensitive windows of development. The widespread use of chemicals with endocrine disrupting properties in food packaging thus might present a risk, and it requires dedicated assessment. Only few EDCs have been researched so far with respect to migration, presence in food, human body burdens, and their impact on human health (Waring and Harris, 2005). Furthermore, not all substances migrating from food packaging have been characterized for their endocrine disruptive potential, which is sometimes discovered only by chance long after they are widely in use (Hunt, 2008; McDonald et al., 2008; Soto et al., 1991). The current situation implies chronic exposure to EDCs with essentially unknown effects and presents a health risk that also might affect next generations. Such practice is not in accordance with the principles of sustainable development (The World Commission on Environment and Development, 1987).

Taken together, the knowledge summarized in this review indicates that policies governing the use of EDCs in food contact material should be revised to reflect contemporary scientific understanding. Substances that have been in use for several decades should be

reassessed for their endocrine disruptive properties by modern toxicology principles. Assessments of health risks used for establishing packaging standards must also include the NIAS, especially oligomers of plastic packaging that in some cases have already been shown to be EDCs, like for polystyrene (Ohyama et al., 2001; Yanagiba et al., 2008). With today's toxicological knowledge threshold concepts for unidentified food packaging migrants require thorough reconsideration and validation according to latest scientific developments, including non-monotonic dose-responses.

Furthermore, an additional biological effect evaluation might prove useful for exposure and risk assessment; it could determine the whole migrants from the finished packaging into foodstuffs, for existing and new authorizations. This requires establishment of the relevance of such bioassays for human and environmental health.

Strategies how to minimize EDCs exposure need to address food packaging as a potentially large source, given the ubiquitous presence of packaged foodstuffs. Detailed information on human exposure to EDCs from food packaging is required from academia, as well as substantiating or discarding the risk that chronic exposure to EDCs poses for human health. In terms of fetal exposure to EDCs and chronic health effects later in life, longitudinal studies are underway but results will take some time to emerge. These studies could also assess the contribution of food packaging to EDCs exposure. All these efforts would certainly benefit from a larger awareness within the scientific community for food packaging as a major food contaminant source.

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