Cytotoxicity of Migrates from Plastic Packaging

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Abstract

Migrates (95% EtOH, 4 h, 60°C) of a food packaging film have been characterised analytically and additionally been tested in the neutral red assay [1] for their cytotoxicity using human cell lines (Hep-G2 and HT-29). Migrates contain several plasticizers (part of the printing inks and lacquer), slip additives and antioxidants (part of PE food contact layer). 33 mg/l (50 mg/l) of the dissolved migration residue (food contact side of the packaging film) caused a 50% decrease (IC_{50}) of Hep-G2 (HT-29) viability. Yet, we could not fully identify the causative agents for the cytotoxic effect of the packaging film migrate.

Composition of the laminate

The food contact surface of the packaging film consists of a LDPE/LLDPE-blend (fig. 1). The external printed surface is covered by a clear varnish. Printing inks and varnish contain a cellulose nitrate resin (CN).

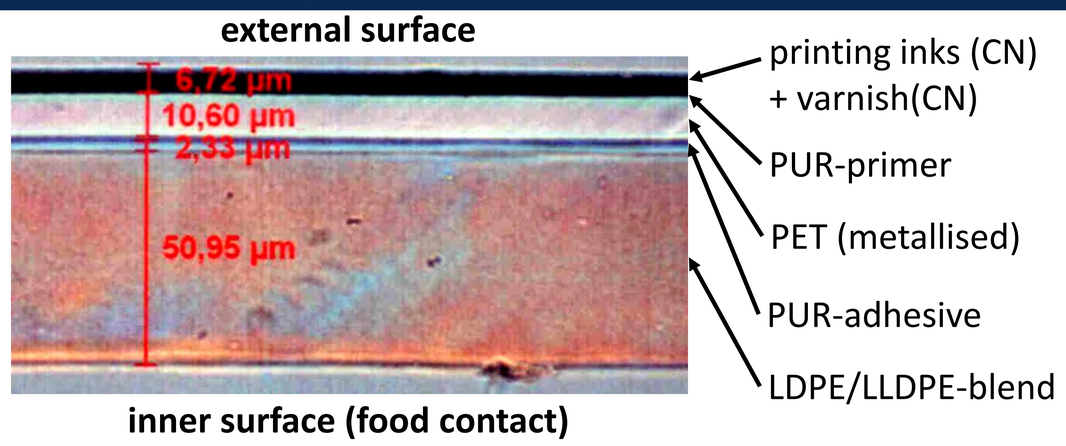

Fig. 1: Microtome cut of the packaging film (photo: WIPAK Walsrode, Germany).
PUR: polyurethane,
PET: polyethylene terephthalate,
(L)LDPE: (linear) low-density polyethylene

Characterisation of the inner and external surface migrates

Where do the migrating substances come from?

	plasticisers							slip additives		antioxidants		
	ATBC	NETSA	DEHA	DBS	Triacetin	EHDPP	DBP	Ole-amide	Eruc-amide	Irganox 1010	Irganox 1076	Irgafos 168
migrate from external surface[a] [mg/dm²]	2.33	0.05	0.39	0.50	0.05	0.11	0.23	0.39	0.05	<5·10⁻⁵ [b]	<8·10⁻⁵ [b]	<7·10⁻⁵ [b]
clear varnish	+	-	+	+	+	+	+	+	-	-	-	-
printing inks: gold	+	+	+	-	+	+	-	-	+	-	-	-
printing inks: yellow/ black/green	+	+	+	-	+	+	-	-	-	-	-	-
printing inks: red	+	+	+	-	+	+	-	-	-	-	-	-
printing inks: white	+	-	-	-	+	+	-	-	-	-	-	-
PUR-primer												
PET-film (metallised)	-	-	-	-	-	-	-	-	-	-	-	-
PUR-adhesive												
PE-film	-	-	-	-	-	-	-	-	+	+	+	+
migrate from inner surface[a] [mg/dm²]	0.56	0.02	0.52	0.47	0.005	0.04	0.001	0.11	0.12	0.005	0.22	0.22
SML [mg/dm²][c]	OML	n.l.	3	OML	OML	0.4	0.05	OML	OML	OML	1	OML

set-off or migration | migration

Fig. 2: Analysis of the single packaging film components and comparison with the migrates (95% EtOH, 4 h, 60°C) from the inner and external surface of the packaging. **+**: contained, **-**: not detectable. n.l.: not listed. OML: limited by overall migration limit of 10 mg/dm². ATBC: acetyl tributyl citrate, NETSA: N-ethyl-2/4-toluenesulfonamide, DEHA: diethylhexyl adipate, DBS: dibutyl sebacate, EHDPP: 2-ethylhexyl diphenyl phosphate, DBP: dibutyl phthalate. [a] Overall migrate (gravimetry): inner surface 3.0 mg/dm², external surface 11.4 mg/dm². [b] limit of detection (RP-HPLC-UV 220 nm). [c] according to Directive 2002/72/EC [4]

Migrates of the inner and external packaging surface have been investigated concerning migrating substances. Migration experiments (95% EtOH, 4 h, 60°C) simulated sterilization of fatty foods (121°C, 30-60 min) in the packaging as a worst case scenario [2]. 74% and 33% of the migration residue of the inner and external surface, respectively, could be clarified by RP-HPLC-DAD/CLND/ELSD/MS, GC-MS/FID and ^{1}H-NMR (fig. 2).
The identified plasticisers are part of the printing inks and the CN-varnish (fig. 2). They improve flexibility of the dried inks and varnish. They get from the printed external surface to the food contact side via set-off or migration through the film. The antioxidants and the slip agent erucamide, found in the inside migrate, originate from the PE-layer. No additives have been found in an ethanolic extract of the PET-layer (95% EtOH, 4 h, 60°C). Despite worst case conditions for migration SMLs of the identified substances have not been exceeded.

In vitro cytotoxic effect of the identified additives

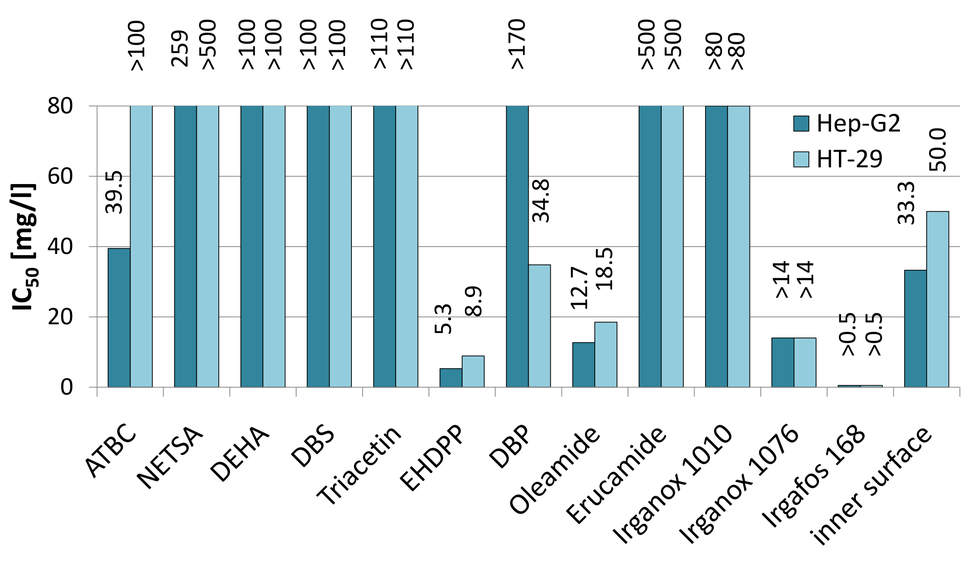

Fig. 3: Results of the neutral red assay of the identified additives and the inner surface migrate of the packaging film. Tested concentrations vary due to solubility. IC_{50}: 50% decrease of the cell viability.

Migrates of the inner surface of the packaging film and standard substances of the identified migrants were tested in the neutral red assay [1] on human cell lines Hep-G2 (hepato blastoma) and HT-29 (colon carcinoma). Oleamide, EHDPP, DBP and ATBC, in high concentrations also NETSA, showed a cytotoxic effect (fig. 3). So, about 28% (14%) of the cytotoxic effect of the inner surface migrate on Hep-G2 (HT-29) cells could be clarified so far, according to the concept of concentration addition [3]. This suggests that not all causative substance(s) have been identified in the migration residue so far.

Literature: [1] Borenfreund E, Puerner JA (1985). Toxicol. Lett. **24**, 11-124 [2] Directive 97/48/EC from 29th of july 1997 [3] Berenbaum MC (1985). J. theor. Biol. **114**, 413
[4] Directive 2002/72/EC from 6th of august 2002