Endocrine disrupting chemicals in commercially available cling film brands in South Africa

Christiaan de Jager[a], Natalie Aneck-Hahn[a,b,*], Magdalena Van Zijl[a,b], Stefan Hayward[c], Pieter Swart[c], and Bettina Genthe[d]

[a]Environmental Chemical Pollution and Health Research Unit, School of Health Systems and Public Health, University of Pretoria, Arcadia, South Africa;

[b]Environmental Chemical Pollution and Health Research Unit, Department of Urology, University of Pretoria, Arcadia, South Africa;

[c]Department of Biochemistry, University of Stellenbosch, Matieland, South Africa;

[d]Council for Scientific and Industrial Research, Natural Resources and the Environment, Stellenbosch, South Africa

*Correspondence to: Natalie Aneck-Hahn, Department of Urology, University of Pretoria, Private Bag X323, Arcadia 0007, South Africa. Email: natalie.aneck-hahn@up.ac.za

ABSTRACT

Cling films comprise of plasticizers which are known endocrine disrupting chemicals (EDCs). Cling films are commonly used for food packaging and EDCs may leach into food, increasing exposure, leading to adverse health outcomes. We determined the levels of common selected EDCs expected in commercial cling film brands sold in South Africa. We tested for para-Nonylphenol (*p*-NP), Bisphenol A (BPA), bis(2-ethylhexyl) adipate (DEHA) and phthalates (di-2-ethyhexyl phthalate (DEHP) and dibutylphthalate (DBP)]. We selected eight brands of cling film and used standard chromatography methods to extract compounds. We found *p*-NP in one brand (2.06 µg/kg) and BPA in two brands (1.48 and 1.61 µg/kg). Five brands contained DEHP (2.1-2.59 mg/kg), DBP (0.62-1.21 mg/kg) and DEHA (165.89-674.07 mg/kg). Levels of DEHA and DEHP in the cling film are associated with potential human health risks. The maximum level of DEHA ranged from 1.6 to 16 times the safe level, with a calculated hazard

quotient of 1.4. The two cling film brands made from low density polyethylene (LDPE), both endorsed by the Cancer Association of South Africa (CANSA), had target chemicals below the detection limit.

Keywords: Health risk assessment; cling film; *p*-nonylphenol; phthalates; bis(2-ethylhexyl) adipate

INTRODUCTION

The awareness of endocrine disrupting chemicals (EDCs) has increased over the past decade. Endocrine disrupting chemicals include additives that have been found in materials and consumer goods such as pesticides, electronics, personal care products, pharmaceuticals and food packaging materials (Bergman et al. 2013). Close to 70% of consumer packaging is used for food and beverage packaging (Muncke 2009). Chemicals in food packaging can transfer into packaged food, aprocess known as migration. The migration of chemicals depends on the chemical properties of the packaging material, the food, the temperature during treatment and storage, exposure to ultraviolet light and storage time of the product (Muncke 2009; Muncke et al. 2014). Food contact materials are materials that are intended to be in contact with food and are assumed to be safe. Food contact materials may contain non-intentionally added substances (NIAS) like polymerisation by-products, additives, processing aids, impurities and breakdown compounds. Limited information is available on these NIASs due their presence at low levels and being difficult to analyse in complex mixtures (Muncke et al. 2014). Even though food packaging contributes significantly to human EDC exposure via environmental accumulation, the role of food and beverage packaging as an additional source of EDC exposure due to direct contact has not received much attention until recently.

Plastic food packaging films are commonly used to wrap foods and for reheating and cooking in microwave ovens (Inoue et al. 2001). Plastic film was first made from polyvinyl chloride (PVC). The PVC-based films were made softer and more flexible with the addition of plasticisers, most often bis (2-ethylhexyl) adipate (DEHA) and phthalates, including dibutyl phthalate (DBP) and bis (2-ethylhexyl) phthalate (DEHP) (ATSDR 2001, 2002; Latini et al. 2006). These plasticisers are not covalently bound to the plastic material and are consequently released into the environment with time and use (Latini et al. 2006). The migration of plasticisers from plastics into food has been studied, in particular some adipates and phthalates e.g, DEHA, which was found in foods wrapped in PVC film (Castle et al. 1987; Startin et al. 1987; Inoue et al. 2001). Lipophilic substances can migrate to foods, especially high fat foods such as cheeses, fatty fish and meat (Kozyrod and Ziaziaris 1989; Goulas et al. 2007; Fasano et al. 2012); Seiler et al. 2014), particularly when cooked or defrosted in microwave ovens (Moreira et al. 2014). Wrapped foodstuffs are therefore a source for human exposure (Fasano et al. 2012). Phthalate esters DEHP and DBP have been linked to reproductive abnormalities (Foster et al. 2001; Sharpe 2001; Latini et al. 2006); Martino-Andrade and Chahoud 2010; Manikkam et al. 2013; Chen et al. 2017).These compounds are now prohibited or regulated (EC 2002) in most countries due to their potential endocrine disrupting effects. Cling films can vary in thickness from 8-25μm with plasticisers making up 10-30% of the film (Fasano et al. 2012).

Other chemicals of interest include *para*-nonylphenol (*p*-NP) and bisphenol A (BPA). *para*-Nonylphenol (*p*-NP) is used in the manufacturing of surfactants and plastics (Inoue et al. 2001; Votavová et al. 2009). It is a well-studied EDC with estrogenic properties (Soto et al. 1991; Votavová et al. 2009)Puy-Azurmendi et al. 2014; Kim et al. 2015; Noorimotlagh et al. 2017), it is found in food (Guenther et al. 2002; Muncke 2009), food packaging (Fasano et al. 2012) and also in environmental water samples (Inoue et al. 2001); Careghini and Mastorgio

2015). *para*-Nonylphenol is known to leach from plastic cling film into food (Bornman et al. 1997; Inoue et al. 2001) and occurs in domestic and commercial cling films at levels ranging from < 500 -3300 µg/g (Inoue et al. 2001). Aside from plastic contamination, *p*-NP can accumulate in foods via different pathways, including disinfectancts and pesticides used to manufacture food products (Guenther et al. 2002) and, as a result, they are found in almost all foods. A study investigating the migration of NP from high density polyethylene (HDPE) milk containers found that temperature had an effect on NP levels found in the milk surrogate after 15 days at 40 °C, 580 ± 25 ng/L this was was higher than that in water, 230 ± 12 ng/L (Loyo-Rozales et al. 2004). Bisphenol A (BPA) is extensively used in many different types of food packaging (Muncke 2009); von Goetz et al. 2010; Fasano et al. 2012). Bisphenols and their derivatives are known endocrine disruptors in humans and may also be potentially carcinogenic (Keri et al. 2007; Pocas et al. 2008; Soto et al. 2013) especially at chronic low dose exposure (Welshons et al. 2006; Muncke 2009).

In South Africa, cling film is commonly used to cover meat, vegetables, cheese and commercially available ready cooked meals. Contact with plastic films usually extends from the marketplace to the home. To date, limited studies have examined the presence of EDCs in plastic cling films in South Africa (Bornman et al. 1997). We investigate the presence, concentration and potential health risks of *p*-NP, BPA, DEHA and selected phthalates in common cling films used for domestic and commercial use in South Africa.

MATERIALS AND METHODS

Sample Selection

We conducted a survey in 2011 to identify the most common cling film brands stocked in South African supermarkets and grocery stores. We identified two national house brands, two brands endorsed by the Cancer Association of South Africa (CANSA) and one additional brand that

Table 1. Domestic and commercial cling film brands available in South Africa and tested for phthalates, BPA's and *p*-NP

Sample code	Sample description	Use
A[a]	Inert; non-toxic polyethylene; Width 330 mm; Length 30 m; CANSA approved	Domestic
B[b]	VL30; Light; 10 micron; Width 300mm; Length 1400m	Commercial
C[b]	VL38-10; Light; 10 micron; Width 380mm; Length 1000m	Commercial
D	Environmentally friendly; food grade; plastic code 3 - Poly vinyl chloride; Width 350 mm; Length 30 m; For professional quality catering	Domestic/ commercial
E	Length 30 m	Domestic
F[a]	Low density polyethylene; no plasticisers added; free from any carcinogens (CANSA approved); photodegradable; Fully recyclable; Width 300 mm; Length	Domestic
G	Polyethylene; Width 330 mm; Length 50m	Domestic
H[b]	FDA approved	Commercial

[a] CANSA endorsed

[b] Commercial use

is available nationally. Additionally, we identified three commercial brands at two wholesale outlets in Pretoria, South Africa. These brands are usually used at food outlets, take away stores and in the meat and dairy sections in supermarkets for food packaging. We selected these eight

brands (A-H) because they represent products bought by consumers and poducts used for commercial food packaging (Table 1).

Chemicals and Reagents

We prepared reagents using deionised water that was passed through an EDS-Pak® Polisher (Merck, Darmstadt, Germany), containing activated carbon and designed to remove endocrine disruptors such as BPA, diethyl phthalate, DBP and *p*-NP from ultrapure water. We first washed all glassware with chromatography grade ethanol and methanol (Sigma-Aldrich, SA) and then rinsed the glassware with water that was passed through the EDS-Pak® Polisher. We prepared stock solutions of the following standards (Sigma-Aldrich [St. Louis, MO, USA]) in dichloromethane (DCM), *p*-NP, BPA, DEHA, DEHP and DBP. We used 5α-cholestane as an internal standard for gas chromatography-mass spectrometry (GC-MS) analysis and methanol as the solvent blank.

Extraction Procedure

We cut each cling film sample (1.5 g (equivalent to 100 cm^2)) into smaller pieces and extracted under DCM (150 mL) reflux by Soxhlet extraction for 18 hours (de Castro et al. 2013). The resulting extract was evaporated to dryness under vacuum at 40°C. The dried extract was subsequently resuspended in 1 mL DCM and split into two samples of 500 µL each. The solvent of one aliquot was evaporated under a thin stream of nitrogen at 40°C and prepared for target chemical analysis.

Chemical Analysis

We prepared all standard stock solutions and dilutions in DCM. We used two separate chromatographic methods to analyse the samples. The BPA and NP were analysed using ultra-

performance liquid chromatography (UPLC-MS), and DEHA and phthalates were analysed using gas chromatography (GC-MS) for DEHA (Cao 2010; Russo et al. 2015). We could not use liquid chromatography to analyse phthalates since the solvents used for liquid chromatography contain high concentrations of phthalates (Griffiths et al. 1985; Cao 2010).

During chromatography, we determined the limit of detection (LOD) and limit of quantification (LOQ) for each analyte. The LOD is the concentration that corresponds to the sum of the mean of blanks and three times the standard deviation while the LOQ is the sum of the mean of blanks at the analyte retention time and 10 times the standard deviation (Armbruster and Pry 2008; Quanson et al. 2016). We determined the LOD and LOQ for each analyte by injecting five blank replicates of deionized water.

GC-MS analysis

GC-MS analysis was done according to de Jager et al. (2013). The dried extract was resuspended in 1 mL DCM yielding a two-fold dilution. Each sample was analysed using an Agilent 6890N GC (Agilent Technologies Wilmington, USA) coupled to an Agilent 5975 MS detector. We separated samples using a Zebron (ZB 7HG-G010-11) 30m x 0.25 mm (inner diameter) x 0.25 µm (film thickness) ZB-5MS Guardian capillary column. We injected 5 µl of each sample using a CTC PAL auto sampler in split-less injection mode. The injection port temperature was set at 250°C and used high purity Helium (99.999%) as a carrier gas, at a flow rate of 1.2 mL/min. We used the following GC oven temperature program: the initial oven temperature was set at 40°C and increased 260°C at a rate of 10°C per min and maintained at 260°C for 3 min. The oven temperature was subsequently increased to 280°C at a rate of 25°C and held at 280°C for 7 min. Finally, the oven temperature was increased to 330°C at a rate of 50°C per min and maintained at this temperature for 6.2 min after which the method was ended. Full scan ms ($^{m}/_{z}$ 40 – 800) and single ion monitoring (SIM) in positive mode was used to

detect analytes. SIM was used to quantify DEHP ($^{m}/_{z}$ 149, 167), DEHA ($^{m}/_{z}$ 129,147) and DBP ($^{m}/_{z}$ 104, 149), at the retention times of 18.763, 22.670, and 24.219 min respectively. Data were analysed using Agilent ChemStation software.

UPLC-MS/ MS analysis

Prior to the UPLC-MS/MS procedure, all samples and standards were derivatized with dansyl chloride prior to injection using an adapted method described by Zang et al. (2012)). Prepared samples were analysed using a Waters Acquity UPLC coupled to a Waters Xevo TQ MS detector. We injected and separated 5µL of each sample on a Waters BEH 2.1 x 100 mm, 1.7 µm C18 UPLC column eluted with a gradient of acetonitrile: methanol: isopropanol (49:49:2) in 7.5% formic acid at 50°C. We used multiple reaction monitoring (MRM) to detect and quantify each analyte. MRM transitions of $^{m}/_{z}$ 454.2 → 171, $^{m}/_{z}$ 504 → 171, $^{m}/_{z}$ 506 → 171, $^{m}/_{z}$ 530 → 171, $^{m}/_{z}$ 695.4 → 170 was used for derivatized *p*-NP and BPA. The ms capillary voltage was set at 2.8 kV with a source temperature of 120°C. Samples were desolvated at 400°C with desolvation gas flow rate of 600 L/h. In order to prevent carry-over from the standards to the samples, the standards were run subsequent to the samples. Prior to injection of the lowest standard concentration duplicate injections of a solvent blank (methanol) was injected. Solvent blanks were regularly injected throughout analysis to further limit possible carry-over.

Health Risk Assessment

We conducted a human health risk assessment according to the guidelines described by the US Environmental Protection Agency (USEPA 1987, 1992, 2011) and the World Health Organization (WHO 2010). The health risk assessment comprised of four compounds namely;

(a) hazard identification, (b) dose-response assessment, (c) exposure assessment and (d) risk characterisation.

Exposure parameters used in the human health risk calculations are shown in Table 3. For the non-cancer toxic effects of chemicals, we calculated a hazard quotient (HQ). The HQ compares the expected exposure to safe exposure levels. Expected exposures were calculated as the average daily dose (ADD) (see equation 1), which were determined from the highest concentrations of chemicals measured in the cling film samples in order to establish a worst case scenario. The safe exposure levels are given as the reference dose (RfD) (Table 2). We assumed that food was in contact with a piece of cling film of the size of a standard dinner plate (10 cm × 10 cm). Using the concentrations of chemicals extracted from the cling film, we assumed that either 10% or 100% of plasticizers leached. Ten percent is based on US Food and Drug Administration (US-FDA) recommendations of food contact materials in simulants (Bhunia *et al.* 2013) and 100% represents the maximum possible exposure. Any HQ (see equation 2) less than 1 is considered safe for a lifetime exposure..

Equation 1: $ADD = (C \times IR \times EF)/BW$

Where ADD = average daily dose (mg/kg/day), C = contaminant concentration in cling film (mg/kg), IR = intake rate (kg/dag), EF = exposure frequency, BW = body weight (kg)=70 kg

Equation 2: $HQ = ADD/RfD$

HQ = hazard quotient and RfD = reference dose (Table 2).

Table 2. Guideline values used in human health risk calculations

Contaminant	RfD mg/kg /day	Critical effect	Human carcinogen	Slope factor per mg/kg/day
Dibutyl phthalate (DBP)	[a] 0.1 (USEPA 2000b)	Mortality Feto-toxic Embryo-toxic	not classified	-
Di(2-ethylhexyl) phthalate (DEHP)	0.02 (USEPA 2000a)	Liver toxicity and tumours	probable human carcinogen	0.014 (USEPA 1991)
Bisphenol A (BPA)	0.0125 (EC 2002)	Anti-androgenic effects	not classified	-
Di(2-ethylhexyl) adipate (DEHA)	0.6 (USEPA 1992)	Reproductive and developmental effects	possible human carcinogen	0.0012 (USEPA 1992)
para-Nonylphenol (*p*-NP)	0.1 mg/kg/d (No IRIS RfD exists)[a] (USFR 2010)	Reproductive effects	not classified	-

[a]US Federal Register Vol 75, No.94 / Monday, CFR May 17, 2010/ Rules and Regulations (https://www.gpo.gov/fdsys/pkg/FR-2010-05-17/pdf/2010-11687.pdf)

TDI, tolerable daily intake; RfD, reference dose; NOAEL, no observed adverse effect level; LOAEL, lowest observed adverse effect level

Table 3. Exposure parameters used in the human health risk calculations (default values specified by USEPA (2011))

Exposure parameters used in dose calculations	
number of times used per year	350
body weight	70 kg
life time	70 years
dose	Chemical concentrations based on concentrations from 10% and 100% migration (leaching) from 10 cm X 10 cm cling film in order to represent a range of daily exposures[a]
chronic exposure duration	30 years

[a] 10% based on FDA recommendations of food contact materials in stimulants, described in (Bhunia et al. 2013). 100% represents the maximum exposure possible.

For the risk of carcinogens for exposures that last less than a lifetime average daily dose (LADD), exposure is calculated as (see equation 3):

Equation 3: $LADD = (ADD \times ED)/Lft$

Equation 4: $Risk = LADD \times \beta$

Where: ED = exposure duration (years), Lft = lifetime (years), β = oral slope factor (USEPA 2011).

The risk estimates (see equation 4) represent the theoretical excess cancer risk. This is the risk of developing cancer in addition to the background cancer incidence. For example, if

the cancer risk is found to be 1 x10^{-5} = 0.00001 = 1/ 100 000, then it can be said that there is an additional risk of developing cancer of 1 in a hundred thousand. The WHO suggests an acceptable risk level of 10^{-5} (WHO 2001, WHO 2017).

We performed a Monte Carlo simulation to model the probabilistic outcome of the risks associated with exposure to cling film. This was carried out using the Excel add-in, @Risk (Palisade). The HQs and cancer risks were assumed to have an exponential distribution and 10,000 iterations were run.

RESULTS

GC-MS Analysis

The LOD and LOQ were, DBP (LOD: 5.7 ng/l; LOQ: 19.0 ng/l), DEHA (LOD: 8.7 ng/l; LOQ: 29.0 ng/l) and DEHP (LOD: 4.4 ng/l; LOQ: 15 ng/l), respectively. DEHP and DBP were detected in five and four of the eight cling film brands respectively. DEHP values were more consistent ranging between 2.10-2.59 mg/kg. DBP values ranged between 0.62-1.21 mg/kg. DEHA was present in four of the eight brands with concentrations ranging between 165.89-674.07 mg/kg. Pthalates were detected in samples B, C, D and H (Table 4).

UP /MS Analysis

The LOD for BPA and *p*-NP was 13 and 3.2 ng/l respectively with a LOQ of 42 ng/l for BPA and 13 ng/l for *p*-NP. We found low levels of NP and BPA in cling film brands C, D and G (Table 4).

Table 4. *p*-NP, BPA, DEHA and phthalates (DEHP and DBP) concentrations in cling film samples

Sample code	*p*-NP (mg/kg)	BPA (mg/kg)	DEHA (mg/kg)	DEHP (mg/kg)	DBP (mg/kg)
A	Nd	Nd	Nd	Nd	Nd
B[a]	Nd	Nd	674.07	2.10	1.21
C[a]	0.00206	Nd	395.09	2.15	1.13
D	Nd	0.00148	275.25	2.15	0.62
E	Nd	Nd	Nd	2.27	Nd
F	Nd	Nd	Nd	Nd	Nd
G	Nd	0.00161	Nd	Nd	Nd
H[a]	Nd	Nd	165.89	2.59	0.81

[a] Commercial cling film brands

Nd, not detected.

Health Risk Assessment

All risk parameters, calculations and resulting risks are shown in Table 5. Cling film brand B, had the highest concentration of DEHA (Table 4), at this level the HQ = 16.07, which may result in adverse health effects, in addition to carcinogenic risks ranging from 5 in 1,000 to 5 in 10,000, depending on the dose used.

Table 5. Human health risks based on exposure to BPA, *p*-NP, DEHA and phthalates (DEHP, DBP) in cling film

Plasticiser	**?A**	**DEHP**	**DEHA**	**DBP**	***p*-NP**
mg/L leached from 100 cm^2 based on daily ingestion of food exposed to cling film (equivalent to 1.5 g)	0.002	2	675	1.2	0.002
ADD[a] based on ingestion of phthalates leached from 100% 10 cm X 10 cm cling film	2.86E-05	2.86E-02	9.64E+00	1.71E-02	2.86E-05
ADD based on ingestion of phthalates leached from 10% of 10 cm X 10 cm cling film	2.86E-06	2.86E-03	9.64E-01	1.71E-03	2.86E-06
RfD mg/kg/d #	0.0125	0.02	0.6	0.1	0.1
Hazard Quotient based on ingestion of phthalates leached from 100% 10 cm X 10 cm cling film	0.00	**1.43**	**16.07**	0.17	0.00
Hazard Quotient based on ingestion of phthalates leached from 10% of 10 cm X 10 cm cling film	0.00	0.14	**1.61**	0.02	0.00
Cancer Slope factor per mg/kg/d	-	0.014	0.0012	-	-

Risk based on ingestion of phthalates leached from 100% of 10cm X 10 cm cling film	-	**2E-04**	**5E-03**	-
Risk based on ingestion of phthalates leached from 10% of 10 cm X 10 cm cling film	-	2E-05	**5E-04**	-

[a] ADD, Average Daily Dose mg/kg/d; # RfD, reference dose mg/kg/d.

Our uncertainty analysis, using Monte Carlo probabilistic determinations (supplementary material), show that expected DEHA values range between 0.8 and 48 times the value considered safe for life-time exposure, 90% of the time. Cancer risks resulting from exposure to DEHA would range from the highest at 1 in 100, to 2 in 10,000 with 90% certainty, with a mean cancer risk of 5 in 1,000. This is 50 times higher than the 'acceptable' cancer risk.

Exposure to DEHP results in risks an order of magnitude less than to DEHA even though the concentrations were 2-3 orders of magnitude lower in the cling film extracts.

DISCUSSION

Only two of the cling film brands tested in this study (brands A and F) were below the detection limit for all target chemicals, both brands are made from low density polyethylene (LDPE). Brands A and F are also the only two brands in this study with the CANSA smart choice seal. (http://www.cansa.org.za/cansa-smart-choice-seal- cling-wrap). We measured DEHP, DBP and DEHA concentrations that were above the US-FDA threshold of 0.5 ng/ml for single substances present in the diet from food packaging material (Muncke 2011). This may pose a potential health risk as the overall migrate from the packaging material may act synergistically or additively (Muncke 2011). A similar study by Bonini et al. (2008) in Italy could not measure

plasticisers in PVC-free cling films, but found DEHA, BBP and DEHP in other cling films and plastic bags used for food freezing (Inoue et al. 2001).

We detected *p*-NP in one of the commercial brands at a concentration of 0.002 µg/g. This level is much lower than the levels of *p*-NP detected in cling films by Inoue et al. (2001), who recorded concentrations of up to 3300 µg/g. PVC cling films used in German supermarkets also had high levels of *p*-NP, ranging from 0.44-1.72 mg/g (Votavová et al. 2009). In another South African study, Bornman et al. (1997) found higher levels of *p*-NP in nine cling film brands ranging between 1-440 mg/kg. We detected low levels of BPA in the two brands of cling film (D = 0.0015 mg/kg and G = 0.0016 mg/kg), these levels were much lower than those found in PVC-based films, where four of the five PVC films had BPA levels ranging from 40-500 mg/kg (Lopez-Cervantes and Paseiro-Losada 2003). Since 2006, BPA and *p*-NP in cling film have been recognised as NIAS, as neither are used to manufacture LDPE. The low levels of BPA and *p*-NP found in samples (this study) confirm these changes in manufacturing. These levels pose a negligible health risk (Table 5) according to US EPA guidelines. These NIASs may originate from antioxidants in laboratory equipment and materials (vessels, tubes, detergents, etc.), or pollution during the washing steps when the packaging was manufactured (Poças and Hogg 2007). The fact that considerably lower levels of *p*-NP were detected in cling film samples in this study compared to the study by Bornman et al. (1997) is encouraging, indicating that the manufacturers of cling film are using safer alternatives compared to the materials previously used.

We found high levels of DEHA and DEHP in commercial bands (B, C, and H), which are PVC based cling films and may pose a significant health risk (Table 1). We based our health risk assessment on the highest concentration of DEHA and DEHP observed in all samples. Exposure to DEHP resulted in carcinogenic risks slightly greater than the acceptable risk level, ranging from 2 in 10,000 to 2 in 100,000 risk. These risks represent a worst-case scenario, and

are based on using cling film daily over a 30 year period. The risk associated with DEHA exposure was higher, with possible toxic (non-carcinogenic) effects possibly over 15 times safe levels. Brands B, C and H are used by many caterers and in the meat industry for food packaging, resulting in increased exposure to the general public. *In-utero* and early-life nutritional factors and exposures to environmental toxicants ranging from heavy metals to EDCs like those found in cling film can result in the development of non-communicable diseases and can affect metabolism, immune system function, neurodevelopment, and reproductive function, possibly having an effect at epigenetic level in adults (Balbus et al. 2013; Baroukas et al. 2012). This highlights the need for caution and more detailed research to establish additional representative exposures.

Our study identifies brands of cling films containing chemicals that are known endocrine disruptors with potential carcinogenic effects (USEPA 1991, 1992). Some of these chemicals might be a NIAS from the raw material contaminants, or formed during the production process or migrate from other sources like printing inks on the packaging etc (Nerin et al. 2013). We found that cling films, used both domestically and commercially, pose exposure risks to South African consumers. It must be kept in mind that unknown and unidentified migrating substances might be present whichare not included in a risk assessment (Muncke et al. 2017) and therefore the actual risks might be underestimated in this study. In addition, sensitive windows of exposure as described in Balbus et al. (2013) where effects may occur if exposure takes place during highly sensitive life stages such as foetal development and early childhood are not considered in the health risk assessment. A migration study, exploring the volatility of plasticizers under different conditions is recommended but was beyond the scope of this study. Countries such as Canada, the European Union (EU) and the United States are looking for safer alternatives to replace low molecular weight phthalates, which have recently been classified as Category 1B Reproductive agents. However, sometimes the

products that show promise as safer alternatives may later prove to have endocrine disruptive activity (Boisvert et al. 2016). Three of the phthalates, DBP, BBP and DEHP are subject to restrictions for their use in the production of plastics in the EU, as they have been classified as carcinogenic, mutagenic or toxic to reproduction (Geuke et al. 2014). Both consumers and commercial suppliers should be alerted to the differing quality of plastic packaging materials and their potential health effects.

REFERENCES

Armbruster D, Pry T. 2008. Limit of blank, limit of detection and limit of quantitation. Clin Biochem Rev 29**:**S49-S52.

ATSDR (Agency for Toxic Substances and Disease Registry). 2001. Toxicological profile for di-n-butyl phthalate. Available at https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=859&tid=167

ATSDR (Agency for Toxic Substances and Disease Registry). 2002. Toxicological profile for di(2-ethylhexyl)phthalate (DEHP). Available at https://www.atsdr.cdc.gov/toxprofiles/TP.asp?id=684&tid=65

Balbus JM, Barouki R, Birnbaum LS, *et al.* . 2013. Early-life prevention of non-communicable diseases. The Lancet 381(9860):3 - 4.

Barouki R, Gluckman PD, Grandjean P,*et al.* 2012. Developmental origins of noncommunicable diseases: implications for research and public health. Environ Health 11:42.

Bergman A, Heindel JJ, Jobling S*, et al.* 2013. State of the science of endocrine disrupting chemicals 2012. United Nations Environment Programme and the World Health Organization, Geneva, Switzerland.

Bhunia K, Sablani SS, Tang J, *et al.* 2013. Migration of chemical compounds from packaging polymers during microwave, conventional heat treatment, and storage. Compr Rev Food Sci Food Saf 12**:**523-545.

Boisvert A, Jones S, Issop L, *et al*. 2016. In vitro functional screening as a means to identify new plasticizers devoid of reproductive toxicity. Environ Res 150:496-512.

Bonini M, Errani E, Zerbinati G, *et al.* 2008. Extraction and gas chromatographic evaluation of plasticizers content in food packaging films. Microchem J 90**:**31-36.

Bornman MS, De Jager C, Vorster H, *et al.* p-Nonylphenol in food packaging material: An absorption study. 10th Biennial meeting of SASRSS, 22-25 August 1997.

Cao XL. 2010. Phthalate esters in foods: sources, occurrence, and analytical methods. Compr Rev Food Sci Food Saf 9**:**21-43.

Careghini A, Mastorgio AF. 2015. Bisphenol A, nonylphenols, benzophenones, and benzotriazoles in soils, groundwater, surface water, sediments, and food: a review. Environ Sci Pollut Res 22:5711-5741.

Castle L, Mercer AJ, Startin JR, *et al.* 1987. Migration from plasticized films into foods. 2. Migration of di-(2-ethylhexyl)adipate from PVC films used for retail food packaging. Food Addit Contam 4**:**399-406.

Chen Q, Yang H, Zhou N, *et al.* 2017. Phthalate exposure, even below US EPA reference doses, was associated with semen quality and reproductive hormones: Prospective MARHCS study in general population. Environ Int 104:58-68.

de Castro LMD, Jiménez RJ, Ayuso GLE 2013. Soxhlet extraction. Ref Modul Chem Mol Sci Chem Eng 2000: 2701–2709.

de Jager C, Aneck-Hahn NH, Swart P, et al. 2013. Estrogenic activity and endocrine disrupting chemical (EDC) status in water obtained from selected distribution points

in Pretoria and Cape Town. Water Research Commission, South Africa. WRC Report No KV 317/13.

EC (European Commission). 2002. European Commission Directive 2002/72/EC of 6 August 2002, relating to plastic materials and articles intended to come into contact with foodstuffs. Official Journal of the European Union L39**:**1-42.

Fasano E, Bono-Blay F, Cirillo T, *et al.* 2012. Migration of phthalates, alkylphenols, bisphenol A and di(2-ethylhexyl)adipate from food packaging. Food Control 27**:**132-138.

Foster PM, Mylchreest E, Gaido KW, *et al.* 2001. Effects of phthalate esters on the developing reproductive tract of male rats. Hum Reprod Update 7**:**231-5.Geueke B, Wagner CC, Muncke J. 2014. Food contact substances and chemicals of concern: a comparison of inventories, Food Addit Contam: Part A, 31:1438-1450, DOI: 10.1080/19440049.2014.931600

Goulas AE, Zygoura P, Karatapanis A, *et al.* 2007. Migration of di(2-ethylhexyl) adipate and acetyltributyl citrate plasticizers from food-grade PVC film into sweetened sesame paste (halawa tehineh): kinetic and penetration study. Food Chem Toxicol 45**:**585-91.

Griffiths WC, Camara P, Lerner KS. 1985. Bis-(2-ethylhexyl) phthalate, an ubiquitous environmental contaminant. Ann Clin Lab Sci 15**:**140-151.

Guenther K, Heinke V, Thiele B, *et al.* 2002. Endocrine disrupting nonylphenols are ubiquitous in food. Environ Sci Technol 36**:**1676-80.

Inoue K, Kondo S, Yoshie Y, *et al.* 2001. Migration of 4-nonylphenol from polyvinyl chloride food packaging films into food simulants and foods. Food Addit Contam 18**:**157-64.

Keri RA, Ho SM, Hunt PA, *et al.* 2007. An evaluation of evidence for the carcinogenic activity of bisphenol A. Reprod Toxicol 24**:**240-52.

Kim Y-S, Hwang K-A, Hyun S-H, *et al*. 2015. Bisphenol A and nonylphenol have the potential to stimulate the migration of ovarian cancer cells by inducing epithelial−mesenchymal transition via an estrogen receptor dependent pathway. Chem Res Toxicol 28:662-671.

Kozyrod RP, Ziaziaris J. 1989. A survey of plasticizer migration into foods. J Food Prot 52**:**578-580.

Latini G, Del Vecchio A, Massaro M, *et al.* 2006. Phthalate exposure and male infertility. Toxicology 226**:**90-8.

Lopez-Cervantes J, Paseiro-Losada P. 2003. Determination of bisphenol A in, and its migration from, PVC stretch film used for food packaging. Food Addit Contam 20**:**596-606.

Loyo-Rosales JE, Rosales-Rivera GC, Lynch AM, *et al.* 2004 Migration of Nonylphenol from Plastic Containers to Water and a Milk Surrogate. J Agric Food Chem 52:2016−2020

Manikkam M, Tracey R, Guerrero-Bosagna C, *et al*. 2013. Plastic derived endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. PLoS One 8:e55387. doi:10.1371/journal.pone.0055387.

Martino-Andrade AJ, Chahoud I. 2010. Reproductive toxicity of phthalate esters. Mol Nutr Food Res 54:148-157.

Moreira MA, Andre LC,Cardeal ZL 2014. Analysis of phthalate migration to food simulants in plastic containers during microwave operations. Int J Environ Res Public Health 11**:**507-26.

Muncke J. 2009. Exposure to endocrine disrupting compounds via the food chain: Is packaging a relevant source? Sci Total Environ 407**:**4549-4559.

Muncke J. 2011. Endocrine disrupting chemicals and other substances of concern in food contact materials: an updated review of exposure, effect and risk assessment. J Steroid Biochem Mol Biol 127**:**118-27.

Muncke J, Myers JP, Scheringer M, *et al.* 2014. Food packaging and migration of food contact materials: will epidemiologists rise to the neotoxic challenge? J Epidemiol Community Health 68**:**592-594.

Muncke J, Backhaus T, Geueke B, *et al.* 2017. Scientific challenges in the risk assessment of food contact materials. Environ Health Perspect 125. doi.org/10.1289/EHP644

Nerin C, Alfaro P, Aznar M, *et al.* The challenge of identifying non-intentionally added substances from food packaging materials: A review. Anal Chim Acta 775:14-24.

Noorimotlagh Z, Haghighi NJ, Ahmadimoghadam M, *et al*. 2017. An updated systematic review on the possible effect of nonylphenol on male fertility. Environ Sci Pollut Res 24:3298-3314.

Poças MF,Hogg T. 2007. Exposure assessment of chemicals from packaging materials in foods: a review. Trends Food Sci Technol 18**:**219-230.

Pocas MF, Oliveira JC, Oliveira FA, *et al.* 2008. A critical survey of predictive mathematical models for migration from packaging. Crit Rev Food Sci Nutr 48**:**913-28.

Puy-Azurmendi E, Olivares A, Vallejo A, *et al*. 2014. Estrogenic effects of nonylphenol and octylphenol isomers in vitro by recombinant yeast assay (RYA) and in vivo with early life stages of zebrafish. Sci Total Environ 466-467:1-10.Quanson JL, Stander MA, Pretoriusa E, *et al.* 2016. High-throughput analysis of 19 endogenous androgenic steroids by ultra-performance convergence chromatography tandem massspectrometry. J Chromatogr B 1031**:**131-138.

Russo MV, Avino P, Peruginia L, *et al.* 2015. Extraction and GC-MS analysis of phthalate esters in food matrices: a review. RSC Adv 5**:**37023–37043.

Seiler A, Bach A, Driffield M, *et al*. 2014. Correlation of foodstuffs with ethanol–water mixtures with regard to the solubility of migrants from food contact materials

. Food Addit Contam Part A 31:498-511.

Sharpe RM. 2001. Hormones and testis development and the possible adverse effects of environmental chemicals. Toxicol Lett 120**:**221-32.

Soto AM, Brisken C, Schaeberle C, *et al.* 2013. Does cancer start in the womb? Altered mammary gland development and predisposition to breast cancer due to in utero exposure to endocrine disruptors. J Mammary Gland Biol Neoplasia 18**:**199-208.

Soto AM, Justicia H, Wray JW, *et al.* 1991. P-nonylphenol, an estrogenic xenobiotic released from 'modified' polystyrene. Environ Health Perspect 92**:**167-173.

Startin JR, Sharman M, Rose MD, *et al.* 1987. Migration from plasticized films into foods. 1. Migration of di-(2-ethylhexyl)adipate from PVC films during home-use and microwave cooking. Food Addit Contam 4**:**385-98.

USEPA (United States Environmental Protection Agency). 1987. Risk assessment guidelines of 1986. EPA/600/8-87/045. Office of Health and Environmental Assessment, Washington, DC, USA.

USEPA (United States Environmental Protection Agency). 1991. Integrated Risk Information System (IRIS). National Center for Environmental Assessment, Washington, DC, USA.

USEPA (United States Environmental Protection Agency). 1992. Integrated Risk Information System (IRIS). National Center for Environmental Assessment, Washington, DC, USA.

USEPA (United States Environmental Protection Agency). 2000a. Bis(2-ethylhexyl) phthalate (DEHP) Fact Sheet, CAS 117-81-7. United States Environmental Protection Agency, Washington, DC, USA .

USEPA (United States Environmental Protection Agency). 2000b. Dibutyl Phthalate Fact Sheet, CAS 84-74-2. United States Environmental Protection Agency, Washington, DC, USA .

USEPA (United States Environmental Protection Agency). 2011. Exposure Factors Handbook: 2011 Edition. National Centre for Environmental Assessement, Washington, DC, USA.

USFR (United States Federal Register). 2010. α-(p-Nonylphenol)-whydroxypoly(oxyethylene) sulfate and phosphate esters; Time-limited exemption from the requirement of a tolerance *In:* Agency, UEP (ed.) Vol. 75, No. 94 ed.: US Federal Register. Rules and Regulations.

Von Goetz N, Wormuth M, Scheringer, *et al.* 2010. Bisphenol A: How the most relevant exposure sources contribute to total consumer exposure. Risk Anal 30:473-487.

Votavová L, Dobiáš J, Voldřich M, *et al.* 2009. Migration of nonylphenols from polymer packaging materials into food simulants. Czech J Food Sci 27**:**293-299.

Welshons WV, Nagel SC,Vom Saal FS. 2006. Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. Endocrinology 147**:**S56-69.

WHO (World Health Organization). 2001.Water Quality: Guidelines, Standards and Health. orld Health Organization, Geneva, Switzerland.WHO (World Health Organization). 2010. Human health risk assessment toolkit. World Health Organization, Geneva, Switzerland.

WHO (World Health Organisation). 2017. Guidelines for drinking-water quality, fourth edition incorporating first addendum. ISBN 978-92-4-154995-0. Geneva: World Health Organization.

Zhang S, You J, Sun Z, *et al.* 2012. A sensitive method for extraction and determination of endocrine-disrupting compounds from wastewater using 10-ethyl-acridone-2-sulfonyl chloride as pre-column labeling reagent by high-performance liquid chromatography with fluorescence detection. Microchem J 103**:**90-96.