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Health risk assessment of dietary exposure to phthalates from plastic-coated paper among university students

Prasert MAKKAEW¹, Kanatpath CHALOEIJITKUL², Udomratana VATTANASIT^{1*} 💿

Abstract

Plastic-coated paper (PCP) is commonly used as a takeaway food container in Thailand. This study aimed to investigate the phthalate content in PCP and to evaluate the health risk of dietary exposure to phthalates. Determination of phthalates, namely, of benzyl butyl phthalate (BBP), dibutyl phthalate (DBP), and di(2-ethylhexyl) phthalate (DEHP), was carried out using a simple total immersion test followed by gas chromatography-mass spectrometry (GC-MS). Consumption data of 430 students in a university were collected by a questionnaire for health risk assessment. The results showed that DEHP was the most abundant compound detected in the PCP samples, followed by DBP and BBP, respectively. The hazard indexes (<1) showed that the health risk to all subjects was acceptable, but monitoring of phthalates in PCP on market shelves should be performed regularly to protect consumers' health.

Keywords: phthalates; food container; plastic-coated paper; health risk assessment.

Practical Application: Monitoring of phthalates from plastic-coated paper which is used as a take-away food container.

1 Introduction

Phthalates are among the most commonly found organic contaminants in food contact materials (FCM). They are used as plasticizers in many plastics applications, including packaging and other FCM (Barros et al., 2011; Giuliani et al., 2020). Moreover, inks, lacquers, adhesives, and recycled pulp are important sources of phthalates in paper and paperboard packaging (Aurela et al., 1999; Binderup et al., 2002; Bononi & Tateo, 2009; Mariani et al., 1999; Sturaro et al., 2006). In Thailand, several kinds of papers are used as food containers, including plastic-coated paper (PCP), which is commonly used as takeaway food containers (Figure 1). PCP is coated with a thin layer of polyethylene (PE) to prevent the penetration of oils and fats into the paper, protect against water and moisture vapor, and impart heat-sealing properties. Phthalates can migrate from the packaging to the food over time. Their migration under various conditions has been extensively documented in many studies (Alp & Yerlikaya, 2019; Bhunia et al., 2013; Fang et al., 2017; Galmán Graíño et al., 2018). The migration of plastic components into foodstuffs can lead to consumer exposure and possible health effects.

PCP was introduced as an alternative to plastic foam containers for containing food after the Department of Health launched the campaign "Say No to Foam" in 2015 (Department of Health, 2021) as food container/packaging trends paid more attention to environmental impact and chemical food safety. However, as described above, some components in PCP may present a source of phthalate contamination. Phthalate compounds can be categorized into two groups according to the length of the R and R' side chains, namely, low molecular weight (LMW) phthalates and high molecular weight (HMW) phthalates. The LMW phthalates contain 3-6 carbon atoms in their side chain. These phthalates include dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), and di(2-ethylhexyl) phthalate (DEHP), which have shown high endocrine-disrupting properties (Mirer, 2003). European Commission (EU) Regulation No. 10/2011 (European Commission, 2011) states that DBP, BBP, and DEHP are not allowed in the production of fat-containing food. Moreover, DEHP and BBP are present in the Registration, Evaluation, Authorization and restriction of CHemicals (REACH) Candidate List within the section "Substances of Very High Concern" (SVHC) (Wang et al., 2019).

Several human epidemiological studies, as well as in vitro and in vivo studies, have shown associations between phthalate exposure and endocrine and reproductive dysregulation, namely, early puberty, endometriosis, sex anomalies, infertility, and altered fetal development (Casals-Casas & Desvergne, 2011; Hatch et al., 2008). In addition, attention-deficit hyperactivity disorder, autism spectrum disorders, cardiotoxicity (Muscogiuri & Colao, 2017), hepatotoxicity, asthma, and allergy have been reported (Giuliani et al., 2020; Jaakkola & Knight, 2008). A study on contaminants in paper-based food packaging materials in the Czech Republic indicated that DBP, di-isobutyl phthalate (DIBP), and benzophenone were the most frequent contaminants of paper materials (Vápenka et al., 2016). Similarly, DEHP, DIBP, and DBP were detected in food paper packaging in China (Zhang et al., 2018). Human exposure to phthalates demonstrated by the study of Dong et al. (2017) in China showed a correlation between dietary consumption and phthalate excretion in the urine. Although the study found that certain types of food could be important sources of phthalate exposure, food contact materials are suspected and require more investigation.

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¹Department of Environmental Health and Technology, School of Public Health, Walailak University, Nakhon Si Thammarat, Thailand

² Department of Packaging and Materials Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand

^{*}Corresponding author: udomratana.va@mail.wu.ac.th



Figure 1. Common plastic-coated paper (PCP) used as a takeaway food container in Thailand.

In Thailand, there is no regulation relating to phthalate migration from PCP yet, even though ready-to-eat foods, sometimes oily and acidic, are generally contained in PCP at very high temperatures in food establishments. Up to the present, data on chemical food contamination from paperbased food packaging materials in Thailand has not been well established. More scientific data on the chemical safety of using this kind of packaging is still needed to form the basis for future recommendations and national legislation.

A human health risk assessment is the process of estimating the nature and probability of adverse health effects in humans who may be exposed to chemicals. Risk assessment is commonly represented by a hazard quotient (HQ) by comparing the estimated chemical intakes of an individual to an acceptable level of exposure. Since phthalate isomers have been demonstrated to exhibit similar toxicological actions, additive effects should be expected (Howdeshell et al., 2008). In this context, the hazard index (HI) was introduced in the assessment of the phthalate cumulative risk of exposure. The aims of this study were, therefore, 1) to determine LMW phthalates, namely BBP, DBP, and DEHP, in PCP, 2) to estimate average daily doses (ADDs), based on the average amount of phthalates and consumption data obtained from a questionnaire, 3) to assess the health risk (HQ) of oral exposure to phthalates, and 4) to assess the risk of cumulative exposure (HI) based on endocrine-disrupting endpoints of the phthalates.

2 Materials and methods

2.1 Sample collection

Three brands of PCP used in Walailak University canteens (our survey data) were purchased from local shops in Tha Sala district, Nakhon Si Thammarat Province, Thailand. They were only one size of 12 x 12 cm² and sold in a pack of 1 kg. The package labels specified that the plastic layer coated onto the paper was polyethylene (PE). The average whole thickness of the PCP (paper and PE) was 0.095 ± 0.003 mm. Two packs of each brand were purchased from local shops in Tha Sala district between May and July 2020. Two samples were randomly picked from each pack to be tested for LMW phthalates including BBP, DBP, and DEHP.

2.2 Determination of BBP, DBP, and DEHP

Determination of LMW phthalates, including BBP, DBP, and DEHP, was done based on a procedure described by Bradley and colleagues (Bradley et al., 2008) with minor modifications to the extraction procedure and the MS mode. As phthalates are widely used as a functional component of adhesives in food packaging materials and PE acts as a functional barrier, the coated plastic was detached from the PCP to extract all phthalates, representing the maximum level of phthalates in the PCP. The detached paper (dPCP) was cut into a small piece with an area of 1 cm². Three grams of the small piece of paper was weighed exactly by using a four decimal place balance (Sartorius LA310S, Germany). Mass per unit area of the paper (grammage) was determined by a standard method (International Organization for Standardization, 2019). The paper was placed in an amber bottle, and 30 mL of 99.8% ethanol (Merck, Germany) was then added into the bottle and left at room temperature for 24 h. A portion of the extract (10 mL) was dried under a stream of nitrogen gas, and the residue was redissolved and well-mixed with 1 mL of 99.8% ethanol. The extract of 1 mL was filtered and analyzed for the three phthalate compounds by gas chromatography-mass spectrometry (GC-MS; Agilent Technologies 6890N, USA).

The GC was operated with 99.999% He as the carrier gas at a constant flow rate of 1 mL/min. The injection (1 μ L) was splitless at 250 °C. Chromatography was performed on a (5%-phenyl)-methylpolysiloxane column (30 m x 0.25 mm ID x 0.25 μ m df: Agilent, USA). The column was held at 40 °C for 3 min and raised to 280 °C at a rate of 10 °C /min, where it was held for 5 min. The MS system was run using SIM mode at a different mass-to-charge (m/z) ratio as follows: m/z 91, 149, and 206.10 for BBP; m/z 149, 205, and 223.10 for DBP; and m/z 149, 167, and 207 for DEHP. The transfer line temperature was set at 280 °C, the MS source temperature was set at 230 °C, and the quadrupole temperature was set at 150 °C.

The linearity of the calibration curve was assessed by using the standard solutions at 0, 0.020, 0.040, 0.080, and 0.160 μ g/mL for BBP (Supelco, USA); 0, 100, 500, 1000, and 2000 μ g/mL for DBP (Fluka, Germany); and 0, 0.200, 0.400, 0.800, and 1.600 μ g/mL for DEHP (Fluka, Germany). Each data point on the calibration curves represents the average of seven replicate

injections. The correlation coefficients (R^2) were 0.9970 for BBP, 0.9994 for DBP, and 0.9986 for DEHP. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated as 3SD and 10SD, respectively, where SD is the standard deviation of the signal values based on seven replicated analyses of the standards at one-half of the lowest concentration. LOD were 0.0027, 0.0069, and 0.0066 µg/mL for BBP, DBP, and DEHP, respectively. LOQ for BBP, DBP, and DEHP were 0.0090, 0.0230, and 0.0220 µg/mL, respectively. The 99.8% ethanol that was used to extract the phthalates was also analyzed as a procedural blank for each experiment. The blank analysis was completed each day.

2.3 Study locations and subjects

The study was conducted at Walailak University, Nakhon Si Thammarat Province, Thailand, during May-July 2020. The study subjects were 1st-4th-year students. The questionnaire was distributed to students via Google Forms to obtain personal information (i.e., gender, study level, age, weight, height, and residence) and data on the consumption of food contained in PCP (i.e., consumption frequency and duration, and food types). Informed consent was obtained from all subjects involved in the study. This research was conducted following the Declaration of Helsinki. The study was approved by the Human Research Ethics Committee of Walailak University according to protocol number WU-EC-AH-2-132-63.

2.4 Exposure assessment

The exposure was calculated by using an equation proposed by Fátima de Poças & Hogg (2007) as follows (Equation 1):

Exposure
$$(mg / day) = Migration (mg / dm2) x$$

Packaging usage $(dm2 / day)$ (1)

The migration in the equation represents the amounts of BBP, DBP, and DEHP migrating to food. It depends on several variables, such as the packaging material itself, the chemical and physical properties of the food in contact, the initial concentration of the substance in the packaging material, time, and temperature, and it also depends on the ratio of the surface area of the packaging material to the amount of food product (Fátima de Poças & Hogg, 2007). However, this study intended to estimate under the worst-case scenarios. Accordingly, 100% migration was assumed using mean levels of each phthalate (mg/dm²) calculated from the concentration determined by GC-MS and grammage of the samples. The PCP usage was estimated based on the size of 12 x 12 cm² or 1.44 dm²/ sheet, an assumption of one sheet per meal, and the number of meals per day of an individual student, obtained from the questionnaire.

The indirect approach was employed for the exposure assessment. The estimated daily exposure from the calculation above and the data on consumption frequency, duration, and body weight obtained from the questionnaire were applied to calculate the average daily dose (ADD; mg/kg-day) as follows (Equation 2):

$$ADD = \frac{x52 \text{ weeks / year x Exposure duration (year)}}{Body \text{ weight (kg) x Exposure duration (year)}}$$
(2)

2.5 Health risk assessment

In general, the human health risk that a packaging material represents depends on two main factors, namely, the extent and intrinsic toxicity of the substances of concern. The calculated ADD was compared with an acceptable daily intake (ADI) from international authorities, namely the United States Environmental Protection Agency (USEPA) and the European Food Safety Authority (EFSA), to estimate the human health risk of phthalates for non-carcinogenic effects from ingestion. USEPA proposed the oral reference doses (oral R_tD) for BBP (0.2 mg/kg bw/day) (U.S. Environmental Protection Agency, 2020a), DBP (0.1 mg/kg bw/day) (U.S. Environmental Protection Agency, 2020b), and DEHP (0.02 mg/kg bw/day) (U.S. Environmental Protection Agency, 2020c). The health risk was represented by a hazard quotient (HQ), which is the ratio between the exposure (ADD) and the R₄D. EFSA also established the tolerable daily intake (TDI) for BBP (0.5 mg/kg bw/day) (European Food Safety Authority, 2005a), DBP (0.01 mg/kg bw/day) (European Food Safety Authority, 2005b), and DEHP (0.05 mg/kg bw/day) (European Food Safety Authority, 2005c). HQ was calculated by the general Equation 3 as follows:

$$Hazard \ Quotient \ (HQ) = ADD \ (mg / kg \ bw / day) \ / \ ADI \ (mg / kg \ bw / day)$$
(3)

BBP, DBP, and DEHP are phthalates with endocrine-disrupting properties. The cumulative risk was estimated using a hazard index (HI) under the implicit assumption that components are toxic in the same target. HI is the sum of individual HQ, as shown in the following Equation 4:

$$Hazard index (HI) = HQ_{BBP} + HQ_{DBP} + HQ_{DEHP}$$
(4)

2.6 Statistical analysis

Descriptive statistics were used to examine the mean and median of numeric data and the frequency of observations as percentages for nominal data. A Student t-test or a one-way ANOVA were employed for parametric tests. Furthermore, a Mann-Whitney U-test or a Kruskal-Wallis test were employed to test group medians of non-parametric data. A *P*-value of less than 0.05 was considered to represent statistical significance.

3 Results

3.1 Phthalates in PCP

The levels of three LMW phthalates found in dPCP are presented in Table 1. The levels of BBP, DBP, and DEHP were in the range of 0.0001-0.0003, 0.0004-0.0032, and 0.0007-0.0052 mg/dm², respectively. Mean levels of the three phthalate compounds in dPCP (n = 12) were significantly different (P = 0.000). Among

Brand	n	Phthalates in mg/dm ² (Mean \pm SD)		
		BBP	DBP	DEHP
А	4	0.0002 ± 0.0001	0.0018 ± 0.0014	0.0046 ± 0.0005
В	4	0.0002 ± 0.0000	0.0004 ± 0.0001	0.0020 ± 0.0014
С	4	0.0003 ± 0.0001	0.0012 ± 0.0001	0.0028 ± 0.0017
SUM	12	0.0002 ± 0.0001	0.0011 ± 0.0009	0.0031 ± 0.0016

Table 1. The amount of BBP, DBP, and DEHP in dPCP.

SUM represents the average concentration of BBP, DBP, or DEHP in all samples (Brand A, B, and C; n = 12). SD represents the standard deviation.

the three compounds, DEHP was the predominant phthalate found in the tested dPCP. The mean levels of DEHP were 2.82 and 15.50 times higher than those of DBP and BBP, respectively. Among the three brands, brand B showed the lowest level of DBP and was significantly different from brands A and C at P = 0.037 and P = 0.020, respectively.

3.2 Subject characteristics and consumption behavior

A total of 430 students (mean age 21; range 18-27 years) were recruited for the study. Most of the subjects were female (83.26%). The proportion of subjects was highest in the 2nd-year students (30.93%), followed by the 4th-year (23.49%), 1st-year (23.02%), and 3rd-year students (22.56%). The body mass index (BMI) of approximately one-half of the subjects (48.84%) was normal (18.5-22.9) according to the Asia-Pacific guidelines (Lim et al., 2017; Pan & Yeh, 2008). The remaining subjects were underweight (BMI < 18.5; 23.02%), overweight (BMI 23-24.9; 12.33%), or obese (BMI \geq 25; 15.81%). Most of the subjects lived in on-campus dormitories (67.21%). The remaining subjects lived in off-campus dormitories (28.37) or their houses (4.42%). Thirty students (6.98%) reported that they did not consume any food contained in PCP. Data on consumption behavior were, therefore, collected from the remaining 400 subjects, as shown in Table 2. Consumption frequency in most of the subjects was one meal/day (42.50%) and 3-4 days/week (37.00%). Most of the subjects (35.50%) reported consumption of food contained in PCP for more than 10 years, whereas 24.25% and 26.75% of the subjects reported consumption for 1-3 years and 4-6 years, respectively. There were several types of food for which the use of a PCP container was reported. The three most common food types contained in PCP were à la carte (89.75%), sticky rice with grilled chicken/fried chicken or pork (78.50%), and curry and rice (63.50%).

3.3 Estimated average daily dose (ADD) and health risk assessment (HQ and HI)

Data on consumption of food contained in PCP of 324 subjects (81.00%) out of 400 students were used for the health risk assessment of exposure to BBP, DBP, and DEHP. Data of 76 subjects (19.00%) were excluded because some of their data required for calculating ADD were missing. Levels of exposure by ingestion to BBP, DBP, and DEHP were estimated and are presented in Table 3. The median daily exposure to DEHP was highest (6.97 x 10^{-5} mg/kg bw/day), followed by DBP (2.47 x 10^{-5} mg/kg bw/day) and BBP (4.50 x 10^{-6} mg/kg bw/day). Based on these data, the HQ (daily exposure level divided by oral

Consumption data	Number of subjects (n = 400)	Percent (%)
Meals/day		
1	170	42.50
2	137	34.25
3	42	10.50
> 3	51	12.75
Days/week		
1-2	77	19.25
3-4	148	37.00
5-6	102	25.50
Every day	73	18.25
Years		
1-3	97	24.25
4-6	107	26.75
7-9	27	6.75
≥ 10	142	35.50
Missing data	27	6.75
Food types		
À la carte	359	89.75
Sticky rice with grilled chicken/fried chicken or pork	314	78.50
Noodles	163	40.75
Yum (spicy and sour salad)	62	15.50
Curry and rice	254	63.50
Sushi	32	8.00

 Table 2 Consumption behavior of food contained in PCP

 $R_{f}D$ or TDI) of individual phthalates was calculated. The median hazard index (HI) based on cumulative exposure to the three phthalates was 3.78 x 10⁻³ and 3.88 x 10⁻³, when $R_{f}D$ and TDI were applied, respectively. The HI of all subjects was less than 1.

Hazard indices classified by subject characteristics are presented in Table 4. The median HI of females (3.88×10^{-3}) was significantly higher than that of males (3.17×10^{-3}) (P = 0.042). The average consumption of females and males was 1.64 ± 0.70 and 1.59 ± 0.61 meals/day and 7.72 ± 5.41 and 7.02 ± 5.03 years, respectively. In addition, the median HI of 1st-year students (2.37×10^{-3}) was significantly lower than 2nd-year (4.73 $\times 10^{-3})$ and 3rd-year students (4.33×10^{-3}) at P = 0.012 and P = 0.010, respectively. The average consumption of 1st-, 2nd-, and 3rd-year students was 1.50 ± 0.68 , 1.75 ± 0.68 , and 1.69 ± 0.68 meals/day and 7.65 ± 5.67 , 7.60 ± 5.66 , and 8.06 ± 5.19 years, respectively. Moreover, the median HI of underweight students (4.44×10^{-3}) was significantly higher than that of obese students (2.39×10^{-3})

(3.31 x 10⁻⁴ - 1.59 x 10⁻²)

3.78 x 10⁻³

(3.57 x 10⁻⁴ - 1.71 x 10⁻²)

 $(1.32 \times 10^{-4} - 6.36 \times 10^{-3})$

3.88 x 10⁻³

 $(3.68 \times 10^{-4} - 1.77 \times 10^{-2})$

Phthalates		Median (Min-Max)			
Phinalates	ADD (mg/kg-day)	HQ_{RfD} (ADD/R _f D)	HQ _{tdi} (ADD/TDI)		
BBP	4.50 x 10 ⁻⁶	2.25 x 10 ⁻⁵	8.99 x 10 ⁻⁶		
	$(4.27 \text{ x } 10^{-7} - 2.05 \text{ x } 10^{-5})$	(2.14 x 10 ⁻⁶ – 1.03 x 10 ⁻⁴)	(8.55 x 10 ⁻⁷ – 4.10 x 10 ⁻⁵)		
DBP	2.47 x 10 ⁻⁵	2.47 x 10 ⁻⁴	2.47 x 10 ⁻³		
	(2.35 x 10 ⁻⁶ – 1.13 x 10 ⁻⁴)	(2.35 x 10 ⁻⁵ – 1.13 x 10 ⁻³)	(2.35 x 10 ⁻⁴ – 1.13 x 10 ⁻²)		
DEHP	6.97 x 10 ⁻⁵	3.49 x 10 ⁻³	1.39 x 10 ⁻³		

 $(6.62 \times 10^{-6} - 3.18 \times 10^{-4})$

HI

Table 3. Health risk assessment of phthalates (n = 324).

Table 4. HI classified by subject characteristics.

	Number of subjects (%) n = 324	HI		
Characteristics		Median	min - max	
Gender				
Male	51 (15.74)	3.17 x 10 ⁻³	5.53 x 10 ⁻⁴ - 1.20 x 10 ⁻²	
Female	273 (84.26)	3.88 x 10 ⁻³	3.57 x 10 ⁻⁴ - 1.71 x 10 ⁻²	
Study level				
1 st year	78 (24.07)	2.37 x 10 ⁻³	5.91 x 10 ⁻⁴ - 1.43 x 10 ⁻²	
2 nd year	99 (30.56)	4.73 x 10 ⁻³	3.57 x 10 ⁻⁴ - 1.50 x 10 ⁻²	
3 rd year	72 (22.22)	4.33 x 10 ⁻³	5.53 x 10 ⁻⁴ - 1.71 x 10 ⁻²	
4 th year	75 (23.15)	2.95 x 10 ⁻³	6.85 x 10 ⁻⁴ - 1.60 x 10 ⁻²	
BMI (Asia-Pacific guidelines)				
< 18.5 (Underweight)	77 (23.76)	4.44 x 10 ⁻³	6.85 x 10 ⁻⁴ - 1.64 x 10 ⁻²	
18.5-22.9 (Normal)	158 (48.77)	3.89 x 10 ⁻³	5.52 x 10 ⁻⁴ - 1.71 x 10 ⁻²	
23-24.9 (Overweight)	42 (12.96)	3.95 x 10 ⁻³	5.71 x 10 ⁻⁴ - 1.36 x 10 ⁻²	
≥ 25 (Obese)	47 (14.51)	2.39 x 10 ⁻³	3.57 x 10 ⁻⁴ - 1.16 x 10 ⁻²	
Residence				
On-campus dormitory 215 (66.36)		4.57 x 10 ⁻³	3.57 x 10 ⁻⁴ - 1.71 x 10 ⁻²	
Off campus	109 (33.64)	2.34 x 10 ⁻³	5.53 x 10 ⁻⁴ - 1.41 x 10 ⁻²	

(*P* = 0.006). The average consumption of underweight students and obese students was 1.57 ± 0.66 and 1.64 ± 0.67 meals/day and 7.77 ± 5.57 and 8.29 ± 5.62 years, respectively. Furthermore, the median HI of students who lived in on-campus dormitories (4.57×10^{-3}) was significantly higher than that of students who lived off-campus (2.34×10^{-3}) (*P* = 0.000). The average consumption of students who lived on-campus and off-campus was 1.73 ± 0.71 and 1.44 ± 0.60 meals/day and 7.31 ± 5.29 and 8.21 ± 5.44 years, respectively.

4 Discussion

The quality of paper raw materials and adhesives can contribute to the contaminant levels (Song et al., 2000; Vápenka et al., 2016). Therefore, good manufacturing practice (GMP) should be properly enforced by all PCP manufacturers to ensure that the product is consistently produced and controlled according to quality standards. In addition, GMP labeling can provide basic criteria of PCP selection for food sellers to prevent the migration of PCP into foodstuffs, leading to consumer exposure. We observed that the manufacture and expiration dates, as well as lot numbers, were not present on any PCP packaging labels. A lot number can provide the identity of products by breaking identical products into batches at various production times. Lot traceability is essential when a product defect is reported.

The present study showed that DBP, BBP, and DEHP were detected in all dPCPs. Among the three compounds, DEHP was the predominant phthalate (0.0031 mg/dm²) detected in the food simulant, followed by DBP (0.0011 mg/dm²) and BBP (0.0002 mg/dm²). These results were consistent with a study on time-dependent migration of phthalates in packaged food samples, which reported that DEHP was the most abundant phthalate (Alp & Yerlikaya, 2019). Many studies have shown that the migration of phthalates is associated with the characteristics of foods. Phthalates are soluble in oil; hence, they are generally found in fatty foods (Giuliani et al., 2020). Moreover, it has also been found that foods with strong acidity increase phthalate migration (Giuliani et al., 2020). It has been reported that the lower the pH values, the higher the level of migration (Jasna et al.,

2007). Hydrolysis is suggested as one of the main factors that affect phthalate migration in acidic and basic solutions. It has also been shown that DEP and DBP are degraded by hydrolysis under acidic conditions (Keresztes et al., 2013). A study by Lau et al. (2005) revealed that DEP and DBP degradation were higher at a pH range of ≥ 10 and ≤ 3 (Lau et al., 2005). A study on the migration of DEHP and DBP from polypropylene food containers in China also showed that the highest migration was found under strong acidity (pH = 3) (Fang et al., 2017). These foods contained in PCP should also be avoided to prevent exposure to the possible migrants coming from PE instead.

As described above, many studies have shown that the migration of phthalates is associated with the characteristics of foods. Data obtained from the questionnaire revealed that there was a variety of food types that the subjects reported the use of PCP containers. The three most common food types contained in PCP were: 1) à la carte (89.75%), 2) sticky rice with grilled/ fried chicken or pork (78.50%), and 3) curry and rice (63.50%). Some of the food reported could increase phthalate migration into foods. For example, à la carte refers to various Thai dishes cooked to order. Food is boiled, stir-fried, deep-fried, or crispfried at high temperature, such as stir-fried crispy pork with holy basil and sunny-side up, and Tom Yum Koong (spicy and sour prawn soup). They are served immediately with plain rice. Yum (spicy and sour salad) contains raw or cooked meat, seafood, or mushrooms mixed with vegetables and seasoned with lime juice (or citric acid), fish sauce, and chopped Thai chilies or dried chili flakes. Its specific flavor is extremely sour. Curry and rice is a Thai local dish of plain rice topped with prepared foods. A customer selects different types of daily cooked food that are displayed in heated pots or trays at a storefront. The most common foods are coconut milk-based (red, yellow, and green) curry, spicy curry, sour curry, bean curd soup, and pan-fried salty meat or fish fillet. The certain characteristics of such foods described above can enhance phthalate migration, therefore accounting for a greater health risk.

The health risk assessment of individual LMW phthalates, namely BBP, DBP, and DEHP, was done by calculating a hazard quotient (HQ), i.e., the ratio between the exposure (average daily dose; ADD) and an acceptable daily intake (ADI). The results from the indirect exposure assessment method showed that the median daily exposure to DEHP was the highest, followed by DBP and BBP. This trend relied on the levels of the three phthalates used for the calculation. We found that the HQ value of each phthalate calculated by comparing the daily exposure with an oral reference dose (RfD) and tolerable daily intake (TDI) was different. Nevertheless, the cumulative risks of exposure represented by the hazard indices (HIs) were in the same range $(HI_{RD} 3.57 \times 10^{-4}$ -1.71 x 10^{-2} and $HI_{TDI} 3.68 \times 10^{-4}$ -1.77 x 10^{-2}). Furthermore, the HI_{RD} was classified according to subject characteristics, namely gender, study level, BMI, and residence. We found that the HI of females was significantly higher than that of males (P < 0.05), whereas the average consumption was slightly different. In addition, we observed that the HI of the 1st-year students was significantly lower than that of 2nd-year and 3^{rd} -year students (P < 0.05). This was based on consumption frequency and duration, which was lower for 1st-year students compared to 2nd-year and 3rd -year students. Moreover, the HI of underweight students was significantly higher than that of obese students (P < 0.01). However, the average consumption of underweight students was less than that of obese students. This finding might result from the inverse proportion of body weight in the ADD calculation.

The students were also categorized into two groups according to their residences, namely on campus and off campus. The HI of students who lived on campus was significantly higher than that of students who lived off campus (P < 0.001). The average consumption of the students who lived on campus was higher. PCP was promoted for use by food sellers after the "Say No to Foam" campaign was launched by the university. Accordingly, PCP is mainly used in food containers in most of the food shops in the university, depending on the type of food. However, there were many other types of takeaway food containers used in food shops outside the university. The present study showed that the health risk of exposure to phthalates from PCP among the students was acceptable. Nevertheless, the students were exposed not only to phthalates from PCP but also to other types of plastic food containers. They might also be exposed to phthalates from contaminated environmental media and various products in everyday life, for example, pharmaceuticals and personal care products (Giuliani et al., 2020). Therefore, total exposure levels from several sources may be greater and require further investigation. Moreover, FCM such as starch films from tapioca (Othman et al., 2019), cassava, jackfruit seed, and mango kernel (Rodrigues et al., 2021) that is more environmental friendly and less toxic to human health should be explored and investigated to be used as an alternative FCM.

5 Conclusion

DBP, BBP, and DEHP were detected in all dPCPs. Among the three phthalates, DEHP was the dominant compound with the highest amount of 0.0052 mg/dm². The cumulative risk for 324 students evaluated from their estimated dietary exposure under the worst-case scenarios to all three phthalates was acceptable (HI < 1). Additionally, PCP is coated with PE, which is used as a functional barrier, so the results in this study suggested that PCP is safe for use as a food container. However, the use of PCP to contain oily and acidic foods should be done with caution. Phthalates and other chemicals of health concern should be regularly monitored, especially in takeaway food shops where sellers use PCP from manufacturers with inadequate quality control, to protect consumers' health.

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References

- Alp, A. H., & Yerlikaya, P. (2019). Phthalate ester migration into food: effect of packaging material and time. *European Food Research and Technology*, 246(3), 425-435. http://dx.doi.org/10.1007/s00217-019-03412-y.
- Aurela, B., Kulmala, H., & Soderhjelm, L. (1999). Phthalates in paper and board packaging and their migration into Tenax and sugar.

Food Additives and Contaminants, 16(12), 571-577. http://dx.doi. org/10.1080/026520399283713. PMid:10789379.

- Barros, H. D., Zamith, H. P. S., Bazílio, F. S., Carvalho, L. J., & Abrantes, S. M. P. (2011). Identification of fatty foods with contamination possibilities by plasticizers when stored in PVC film packaging. *Food Science and Technology*, 31(2), 547-552. http://dx.doi.org/10.1590/ S0101-20612011000200041.
- Bhunia, K., Sablani, S. S., Tang, J., & Rasco, B. (2013). Migration of chemical compounds from packaging polymers during microwave, conventional heat treatment, and storage. *Comprehensive Reviews in Food Science and Food Safety*, 12(5), 523-545. http://dx.doi. org/10.1111/1541-4337.12028. PMid:33412668.
- Binderup, M. L., Pedersen, G. A., Vinggaard, A. M., Rasmussen, E. S., Rosenquist, H., & Cederberg, T. (2002). Toxicity testing and chemical analysis of recycled fibre-based paper for food contact. *Food Additives and Contaminants*, 19(Suppl. 1), 13-28. http://dx.doi. org/10.1080/02652030110089878. PMid:11962701.
- Bononi, M., & Tateo, F. (2009). Identification of diisobutyl phthalate (DIBP) suspected as possible contaminant in recycled cellulose for take-away pizza boxes. *Packaging Technology & Science*, 22(1), 53-58. http://dx.doi.org/10.1002/pts.805.
- Bradley, E. L., Honkalampi-Hämäläinen, U., Weber, A., Andersson, M. A., Bertaud, F., Castle, L., Dahlman, O., Hakulinen, P., Hoornstra, D., Lhuguenot, J.-C., Mäki-Paakkanen, J., Salkinoja-Salonen, M., Speck, D. R., Severin, I., Stammati, A., Turco, L., Zucco, F., & von Wright, A. (2008). The BIOSAFEPAPER project for in vitro toxicity assessments: Preparation, detailed chemical characterisation and testing of extracts from paper and board samples. *Food and Chemical Toxicology*, 46(7), 2498-2509. http://dx.doi.org/10.1016/j. fct.2008.04.017. PMid:18508176.
- Casals-Casas, C., & Desvergne, B. (2011). Endocrine disruptors: from endocrine to metabolic disruption. *Annual Review of Physiology*, 73(1), 135-162. http://dx.doi.org/10.1146/annurev-physiol-012110-142200. PMid:21054169.
- Department of Health. (2021). Summary of campaign results to reduce and stop using foam food containers for the good health of Thai people during 2014-2017. Retrieved from https://foodsan.anamai.moph. go.th/ewt_dl_link.php?nid=1393&filename=NoFoam
- Dong, R., Zhou, T., Zhao, S., Zhang, H., Zhang, M., Chen, J., Wang, M., Wu, M., Li, S., & Chen, B. (2017). Food consumption survey of Shanghai adults in 2012 and its associations with phthalate metabolites in urine. *Environment International*, 101, 80-88. http:// dx.doi.org/10.1016/j.envint.2017.01.008. PMid:28117142.
- European Commission. (2011, January 15). Commission Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food. *Official Journal of the European Union*. Retrieved from https://eur-lex.europa.eu/legal-content/EN/ ALL/?uri=CELEX%3A32011R0010
- European Food Safety Authority EFSA. (2005a). Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to butylbenzylphthalate (BBP) for use in food contact materials. *EFSA Journal*, 3(9), 241. http://dx.doi.org/10.2903/j.efsa.2005.241.
- European Food Safety Authority EFSA. (2005b). Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to di-butylphthalate (DBP) for use in food contact materials. *EFSA Journal*, 3(9), 242. http://dx.doi.org/10.2903/j.efsa.2005.242.
- European Food Safety Authority EFSA. (2005c). Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to di(2-ethylhexyl)

phthalate (DEHP) for use in food contact materials. *EFSA Journal*, 3(9), 243. http://dx.doi.org/10.2903/j.efsa.2005.243.

- Fang, H., Wang, J., & Lynch, R. (2017). Migration of di(2-ethylhexyl) phthalate (DEHP) and di-n-butylphthalate (DBP) from polypropylene food containers. *Food Control*, 73, 1298-1302. http://dx.doi.org/10.1016/j.foodcont.2016.10.050.
- Fátima de Poças, M., & Hogg, T. (2007). Exposure assessment of chemicals from packaging materials in foods: a review. *Trends in Food Science & Technology*, 18(4), 219-230. http://dx.doi.org/10.1016/j. tifs.2006.12.008.
- Galmán Graíño, S., Sendón, R., López Hernández, J., & Rodríguez-Bernaldo de Quirós, A. (2018). GC-MS screening analysis for the identification of potential migrants in plastic and paper-based candy wrappers. *Polymers*, 10(7), 802. http://dx.doi.org/10.3390/ polym10070802. PMid:30960727.
- Giuliani, A., Zuccarini, M., Cichelli, A., Khan, H., & Reale, M. (2020). Critical review on the presence of phthalates in food and evidence of their biological impact. *International Journal of Environmental Research and Public Health*, 17(16), 5655. http://dx.doi.org/10.3390/ ijerph17165655. PMid:32764471.
- Hatch, E. E., Nelson, J. W., Qureshi, M. M., Weinberg, J., Moore, L. L., Singer, M., & Webster, T. F. (2008). Association of urinary phthalate metabolite concentrations with body mass index and waist circumference: a cross-sectional study of NHANES data, 1999-2002. *Environmental Health*, 7(1), 27. http://dx.doi.org/10.1186/1476-069X-7-27.
- Howdeshell, K. L., Wilson, V. S., Furr, J., Lambright, C. R., Rider, C. V., Blystone, C. R., Hotchkiss, A. K., & Gray, L. E. Jr. (2008). A mixture of five phthalate esters inhibits fetal testicular testosterone production in the Sprague-Dawley rat in a cumulative, dose-additive manner. *Toxicological Sciences*, 105(1), 153-165. http://dx.doi.org/10.1093/ toxsci/kfn077. PMid:18411233.
- International Organization for Standardization ISO. (2019). *ISO 536:2019* (*EN*): paper and board: determination of grammage. Geneva: ISO. Retrieved from https://www.iso.org/obp/ui/#iso:std:iso:536:ed-4:v1:en
- Jaakkola, J. J. K., & Knight, T. L. (2008). The role of exposure to phthalates from polyvinyl chloride products in the development of asthma and allergies: a systematic review and meta-analysis. *Environmental Health Perspectives*, 116(7), 845-853. http://dx.doi. org/10.1289/ehp.10846. PMid:18629304.
- Jasna, B., Puntarić, D., Galić, A., Škes, I., Dijanić, T., Klarić, M., Matijana, G., Mario, Č., & Zdenko, Š. (2007). Migration of phthalates from plastic containers into soft drinks and mineral water. *Food Technology* and Biotechnology, 45(1), 91-95.
- Keresztes, S., Tatár, E., Czégény, Z., Záray, G., & Mihucz, V. G. (2013). Study on the leaching of phthalates from polyethylene terephthalate bottles into mineral water. *The Science of the Total Environment*, 458-460, 451-458. http://dx.doi.org/10.1016/j.scitotenv.2013.04.056. PMid:23688967.
- Lau, T. K., Chu, W., & Graham, N. (2005). The degradation of endocrine disruptor di-n-butyl phthalate by UV irradiation: a photolysis and product study. *Chemosphere*, 60(8), 1045-1053. http://dx.doi. org/10.1016/j.chemosphere.2005.01.022. PMid:15993151.
- Lim, J. U., Lee, J. H., Kim, J. S., Hwang, Y. I., Kim, T.-H., Lim, S. Y., Yoo, K. H., Jung, K.-S., Kim, Y. K., & Rhee, C. K. (2017). Comparison of World Health Organization and Asia-Pacific body mass index classifications in COPD patients. *International Journal of Chronic Obstructive Pulmonary Disease*, 12, 2465-2475. http://dx.doi. org/10.2147/COPD.S141295. PMid:28860741.
- Mariani, M. B., Chiacchierini, E., & Gesumundo, C. (1999). Potential migration of diisopropyl naphthalenes from recycled paperboard

packaging into dry foods. *Food Additives and Contaminants*, 16(5), 207-213. http://dx.doi.org/10.1080/026520399284073. PMid:10560574.

- Mirer, F. E. (2003). Comment from the union participant in the IARC Working Group that downgraded DEHP. *International Journal of Occupational and Environmental Health*, 9(1), 85-87. http://dx.doi. org/10.1179/oeh.2003.9.1.85. PMid:12749640.
- Muscogiuri, G., & Colao, A. (2017). Phthalates: new cardiovascular health disruptors? *Archives of Toxicology*, 91(3), 1513-1517. http://dx.doi.org/10.1007/s00204-016-1780-1. PMid:27358237.
- Othman, S. H., Majid, N. A., Tawakkal, I. S. M. A., Basha, R. K., Nordin, N., & Shapi'i, R. A. (2019). Tapioca starch films reinforced with microcrystalline cellulose for potential food packaging application. *Food Science and Technology*, 39(3), 605-612. http:// dx.doi.org/10.1590/fst.36017.
- Pan, W.-H., & Yeh, W.-T. (2008). How to define obesity? Evidencebased multiple action points for public awareness, screening, and treatment: an extension of Asian-Pacific recommendations. *Asia Pacific Journal of Clinical Nutrition*, 17(3), 370-374. PMid:18818155.
- Rodrigues, A. A. M., Costa, R. R., Santos, L. F., Silva, S. M., Britto, D., & Lima, M. A. C. (2021). Properties and characterization of biodegradable films obtained from different starch sources. *Food Science and Technology*, 41(2, Suppl. 2), 476-482. http://dx.doi.org/10.1590/fst.28520.
- Song, Y. S., Park, H. J., & Komolprasert, V. (2000). Analytical procedure for quantifying five compounds suspected as possible contaminants in recycled paper/paper board for food packaging. *Journal of Agricultural and Food Chemistry*, 48(12), 5856-5859. http://dx.doi. org/10.1021/jf000512x. PMid:11141258.
- Sturaro, A., Rella, R., Parvoli, G., Ferrara, D., & Tisato, F. (2006). Contamination of dry foods with trimethyldiphenylmethanes

by migration from recycled paper and board packaging. *Food Additives and Contaminants*, 23(4), 431-436. http://dx.doi. org/10.1080/02652030500526052. PMid:16546890.

- U.S. Environmental Protection Agency USEPA. (2020a). *Chemical* assessment summary: Butyl benzyl phthalate CASRN 85-68-7. Washington. Retrieved from https://cfpub.epa.gov/ncea/iris/ iris_documents/documents/subst/0293_summary.pdf
- U.S. Environmental Protection Agency USEPA. (2020b). *Chemical assessment summary: Dibutyl phthalate (DBP) CASRN 84-74-2.* Washington. Retrieved from https://cfpub.epa.gov/ncea/iris/ iris_documents/documents/subst/0038_summary.pdf
- U.S. Environmental Protection Agency USEPA. (2020c). *Chemical assessment summary: Di(2-ethylhexyl)phthalate (DEHP) CASRN 117-81-7*. Washington. Retrieved from https://cfpub.epa.gov/ncea/ iris/iris_documents/documents/subst/0014_summary.pdf
- Vápenka, L., Vavrouš, A., Votavová, L., Kejlova, K., Dobiáš, J., & Sosnovcová, J. (2016). Contaminants in the paper-based food packaging materials used in the Czech Republic. *Journal of Food* and Nutrition Research, 55, 361-373.
- Wang, Y., Zhu, H., & Kannan, K. (2019). A review of biomonitoring of phthalate exposures. *Toxics*, 7(2), 21. http://dx.doi.org/10.3390/ toxics7020021. PMid:30959800.
- Zhang, Y., Qi, Y., & Wei, Q. (2018). Study on migration model of phthalic acid esters plasticizer in different kinds of plastic bottle packaging materials. In P. Zhao, Y. Ouyang, M. Xu, L. Yang & Y. Ren (Eds.), *Applied sciences in graphic communication and packaging* (pp. 525-531). Singapore: Springer. http://dx.doi.org/10.1007/978-981-10-7629-9_65.