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Uptake of Di-(2-ethylhexyl) Phthalate of Vegetables from Plastic Film Greenhouses

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ABSTRACT: Uptake of di-(2-ethylhexyl) phthalate (DEHP) of nine vegetables including potherb mustard, bok choy, celery, spinach, cabbage, leaf of tube, lettuce, garlic, and edible amaranth in plastic film greenhouses with different plastic films, film thickness, greenhouse age, and greenhouse height was studied. The results showed that the higher the DEHP content of film, the thicker the film, the lower the height of the greenhouse, and the younger the age of the greenhouse were, the higher the DEHP concentration of vegetables was. The results afford significant information for production of safe vegetables with low level DEHP contamination.

KEYWORDS: Vegetables, DEHP uptake, greenhouse, cover plastic film

INTRODUCTION

Di-(2-ethylhexyl) phthalate (DEHP) is one of the most widespread phthalate plasticizers, used in numerous consumer products, commodities, medical devices, food packaging, and building materials.[1−4] However, DEHP is not chemically bonded to the polymer and can be easily released into the environment from the products made of such polymers.[5] Therefore, consumer products containing DEHP can result in human exposure through direct contact and use, indirectly through leaching into other products, or general environmental contamination.[6−9] As to health effects from human exposure to DEHP, DEHP is teratogenic, mutagenic, and carcinogenic, and the U.S. Environmental Protection Agency has classified them as priority pollutants.[10,11] Because of the large and widespread applications, DEHP has been widely detected in food, air, water, soil, and sediments.[12,13] Recently, Du et al.[14,15] have reported that the application of plastic mulch film for the cultivation of vegetables lead to DEHP being taken up by plants and entering the human food chain, with a potential risk for human health. Few studies are available concerning the pollution of DEHP from the greenhouse plastic film in the vegetable plants.[16,17] This paper presents the results from field studies on the DEHP uptake of vegetables cultivated in greenhouses covered by plastic films. The factors included in the raw materials of plastic film, film thickness, height, and the age of the greenhouse, which related to the DEHP uptake of vegetable, were investigated.

MATERIALS AND METHODS

Solvents and Reagents. All solvents and reagents used for extraction were of analytical grade and purchased from Huadong Chemicals Co. y (Hangzhou, China). DEHP and chromatographically pure chloroform for dissolving DEHP standard were purchased from Sigma Shanghai Division (Shanghai, China).

Film Material. The technology of the plastic film greenhouse was applied in cultivating vegetables, which can greatly relieve the problem of vegetable supplies in the off-season. The film was manufactured by Liaochen Plastic Film (Shangdong, China) and was used for the field plant experiments.

Field Plant Experiments. *Plants Used for Experiments.* Nine vegetables cultivated in the plastic film greenhouse, including potherb mustard (*Brassica juncea*), bok choy (*Brassica chinensis*), celery (*Apium graveolens*), spinach (*Spinacia oleracea*), cabbage (*Brassica oleracea*), leaf of tube (*Allium tuberosum*), lettuce (*Lactuca sativa*), garlic (*Allium saticum*), and edible amaranth (*Amaranthus mangostanus*), were selected for testing DEHP uptake.

Treatment. Nine vegetables were planted in each greenhouse with 3 m length and 1 m width, respectively.

Sampling. The time of sampling was at 9:00 a.m. for the same group experiment. The edible parts of each vegetable were collected for DEHP determination. For each DEHP determination, three replications were carried out.

Determination of DEHP. *Extraction of DEHP.* Vegetable samples were freeze-dried, ground, and sieved to less than 0.2 mm. For each sample, 5−10 g was extracted in a Soxhlet extractor for 24 h with 200 mL of acetone/dichloromethane (1:1,v/v) in a water bath at 75 °C. The extracts were reduced to 5.0 mL using a rotary evaporator in a water bath at 50 °C. The concentrated sample was subjected to cleanup.

Plastic film samples were cleaned with distilled water, dried, and cut to chips with 2 mm × 2 mm. For each sample, 2−5 g was extracted in a Soxhlet extractor for 24 h with 200 mL of acetone/dichloromethane (1:1,v/v) in a water bath at 55 °C. The extracts were reduced to 5.0 mL using a rotary evaporator in a water bath at 50 °C. The concentrated sample was subjected to cleanup.

Clean-up. The concentrated samples were loaded on a combined column of silica gel and alumina. The glass chromatography column (25 cm long, 1 cm i.d.), was packed with 3 cm alumina plus 10 cm silica, followed by 2 cm anhydrous sodium sulfate. Dichloromethane (20 mL) was used for elution. The collected fraction was reduced to 0.5 mL under a gentle stream of nitrogen.

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Table 1. DEHP Content in Edible Parts of Nine Vegetable Plants from the Three Greenhouses with Different Material Films[a]

	μg g^{-1}, dry weight		
vegetables	PVC	PE	PVC + PE
potherb mustard	33.96 ± 2.46 a	8.63 ± 1.90 a	27.34 ± 0.70 a
bok choy	20.29 ± 2.67 de	ND	19.59 ± 0.56 c
celery	22.04 ± 3.41 cd	ND	18.79 ± 0.58 c
spinach	18.19 ± 0.87 ef	ND	16.62 ± 0.42 d
cabbage	15.80 ± 0.31 f	ND	13.02 ± 0.84 e
leaf of tube	15.76 ± 2.06 f	ND	16.03 ± 0.84 d
lettuce	24.98 ± 2.78 bc	6.27 ± 1.89 b	20.06 ± 0.23 bc
garlic	15.75 ± 0.87 f	ND	11.21 ± 1.67 f
edible amaranth	28.21 ± 1.40 b	9.38 ± 2.02 a	21.32 ± 1.37 b

[a] Note that a difference at $p < 0.05$ between two vegetables is indicated with different letters in the same column.

Gas Chromatography−Mass Spectrometry (GC-MS). The cleaned samples were analyzed by GC-MS[18,19] using a Hewlett-Packard 5890/5971 GC-MSD (Agilent Technologies, Palo Alto, CA) equipped with an HP-5 trace analysis column (30 m, 0.32 mm i.d., 0.25 mm film thickness). The GC oven temperature was held at 150 °C for 3 min and programmed to increase at 20 °C min^{-1} to 300 °C, and finally held at 300 °C for 3 min.

The temperature of the injector was 250 °C. Helium was the carrier gas at a linear flow rate of 20.7 cm s^{-1}. Full scan electron ionization data were obtained as follows: solvent delay, 5 min; electron ionization energy, 70 eV; source temperature, 200 °C; emission current, 150 μA; scan rate, 4 scan s^{-1}; and detector voltage, 350 V. The DEHP level in the sample was taken as the average of three injections. The amounts of DEHP were calculated from a calibration curve: $y = 0.0014 \times -0.0005$ (concentration range, 0.001−0.05 mg mL^{-1}; $r^2 = 0.9925$) and $y = 0.0017 \times -0.0142$ (concentration range, 0.05−0.5 mg mL^{-1}; $r^2 = 0.9972$). The final contents were expressed as μg g^{-1} based on the amounts of the dried samples.

Statistical Analysis. Statistical analysis was performed using analysis of variance Duncan's multiple range test to assess the relationship between DEHP levels in vegetables and greenhouse factors. All statistical analyses were performed using SAS 8.0 (SAS Inc., 2000). A probabilistic $p < 0.05$ value was considered significant. The DEHP content results were expressed as the mean ± standard deviation of three replicates.

RESULTS AND DISCUSSION

DEHP Uptake of Vegetables in Different Greenhouses Covered by Plastic Films with Different Raw Materials. Three types of plastic films with a thickness of 0.04 mm, made of polyvinyl chloride (PVC), polyethylene (PE), and a PVC−PE compsite, respectively, were selected as the covers of three greenhouses with the same capacity and shape. In each greenhouse, the same nine vegetables were cultivated, respectively, for DEHP uptake experiments. Table 1 showed the DEHP contents in edible parts of nine vegetables from the three greenhouses with PVC film, PE film, and PVC−PE composite film. DEHP contents from the PVC, PE, and PVC−PE compsite films were 22.4%, nearly zero, and 10.5%, respectively. The DEHP contents of nine vegetables were 15.75 ± 0.87−33.96 ± 2.46 μg g^{-1} (dry weight) from the greenhouse with the PVC film and 11.21 ± 1.67−27.34 ± 0.70 μg g^{-1} (dry weight) from the greenhouse with the PVC−PE composite film. However, for vegetables from the greenhouse with the PE film, there were only three vegetables in which DEHP was detected. There were significant differences between the three greenhouses ($p < 0.01$). The higher the DEHP content was in the film, the higher the DEHP content was in the vegetables. This result indicated that DEHP from the two films (PVC and PVC−PE) was emitted into the atmosphere, diffused in the greenhouses, and then was absorbed by the vegetables. In addition, the DEHP concentrations of four vegetables (*B. juncea*, *A. graveolens*, *L. sativa*, and *A. mangostanus*) were higher than the other five vegetables, and it might be associated with the different cultivated locations; the four vegetables were cultivated near the plastic film of the greenhouse.

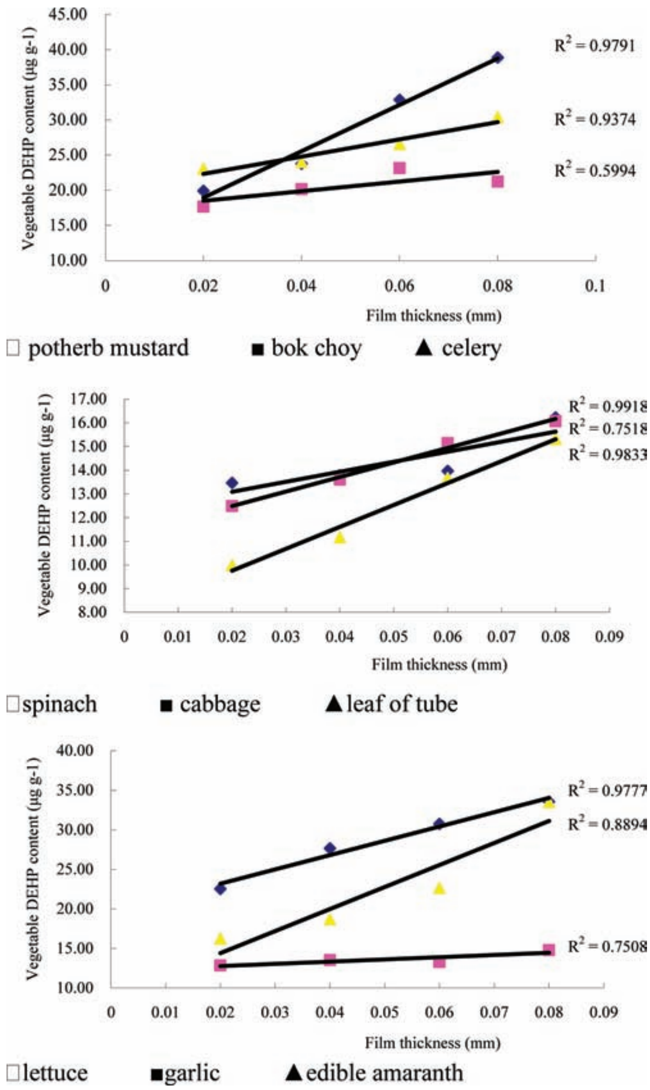

Figure 1. Correlation analysis between DEHP contents of nine vegetables and film thickness of the greenhouses.

Relationship between DEHP Uptake of Vegetables and the Thickness of Greenhouse Plastic Films. Four PVC films with thicknesses of 0.02, 0.04, 0.06, and 0.08 mm were selected as the covers of four greenhouses with the same capacity and shape. In each greenhouse, the same nine vegetables were cultivated for DEHP uptake experiments. The relationship between the DEHP content of the nine vegetable plants and the film thickness of the greenhouses was analyzed by a correlation analysis (Figure 1).

Table 2. DEHP Content in Edible Parts of Nine Vegetable Plants from the Different Height Greenhouses[a]

	μg g^{-1}, dry weight		
vegetables	0.5 m	1.0 m	1.5 m
potherb mustard	34.25 ± 2.43 c	26.05 ± 0.11 c	21.09 ± 3.74 c
bok choy	22.05 ± 1.94 d	16.80 ± 0.62 e	14.57 ± 1.10 cd
celery	20.76 ± 0.59 e	18.50 ± 0.73 f	11.78 ± 1.06 d
spinach	15.75 ± 0.63 c	12.48 ± 0.37 b	12.46 ± 1.96 cd
cabbage	12.45 ± 0.88 a	10.31 ± 0.89 a	10.86 ± 0.79 ab
leaf of tube	17.92 ± 1.44 b	15.22 ± 0.15 a	11.09 ± 0.64 b
lettuce	27.11 ± 0.24 d	25.54 ± 1.64 d	18.81 ± 2.34 cd
garlic	16.47 ± 0.53 d	12.20 ± 1.24 e	10.14 ± 0.57 d
edible amaranth	32.55 ± 2.36 a	26.74 ± 0.80 a	23.15 ± 2.52 a

[a] Note that a difference at $p < 0.05$ between two vegetables is indicated with different letters in the same column.

Table 3. DEHP Content in Edible Parts of Nine Vegetable Plants from the Different Age Greenhouses[a]

	μg g^{-1}, dry weight		
vegetables	0 month	after 3 months	after 6 months
potherb mustard	35.39 ± 0.54 a	26.32 ± 2.91 b	23.12 ± 2.82 a
bok choy	23.65 ± 0.65 c	16.50 ± 0.25 d	13.85 ± 0.88 b
celery	31.29 ± 0.43 b	26.95 ± 1.75 ab	21.17 ± 1.73 a
spinach	21.24 ± 0.61 cd	15.68 ± 0.27 d	12.66 ± 2.08 b
cabbage	23.70 ± 1.81 c	21.23 ± 2.90 c	15.44 ± 2.39 b
leaf of tube	17.17 ± 1.32 e	17.27 ± 0.85 d	13.14 ± 0.41 b
lettuce	36.16 ± 3.13 a	29.82 ± 0.58 a	23.73 ± 0.18 a
garlic	20.08 ± 0.63 d	17.64 ± 1.31 d	14.43 ± 0.40 b
edible amaranth	31.06 ± 1.08 b	27.04 ± 2.50 ab	24.30 ± 2.05 a

[a] Note that a difference at $p < 0.05$ between two vegetables is indicated with different letters in the same column.

The linear correlation coefficients of nine vegetables (*B. juncea, B. chinensis, A. graveolens, S. oleracea, B. oleracea, A. tuberosum, L. sativa, A. saticum*, and *A. mangostanus*) were 0.9791, 0.5994, 0.9374, 0.7518, 0.9918, 0.9833, 0.9777, 0.7508, and 0.8894, respectively. The linear trends showed that the DEHP concentration in the vegetables was going up with an increase in the film thickness. The results showed that the thicker film emited more DEHP into the atmosphere of the greenhouse since it contained more DEHP.

DEHP Uptake of Vegetables in the Greenhouse with Different Heights. The PVC film with a 0.04 mm thickness was selected for the greenhouse covers. Three greenhouses with heights of 0.5, 1, and 1.5 m, respectively, were built. In each greenhouse, the same nine vegetables were cultivated for DEHP uptake experiments. The DEHP contents in the edible parts of the nine vegetables from the three greenhouses with the different heights are shown in Table 2. The DEHP contents of the nine vegetables were 12.45 ± 0.88−34.25 ± 2.43 μg g^{-1} (dry weight) at the greenhouse with a height of 0.5 m, while they were 10.14 ± 0.57−23.15 ± 2.52 μg g^{-1} (dry weight) at that of the 1.5 m greenhouse. The difference in the DEHP content of vegetables among the different heights of greenhouses was significant ($p < 0.05$). The lower the height of the greenhouse is, the higher the DEHP content of the vegetables is. The results indicate that the nearer the air to the plastic film, the higher the atmospheric DEHP content, and the vegetable in low greenhouse is easier to take DEHP from the plastic film.

DEHP Uptake of Vegetables in the Greenhouses with Different Ages. Three PVC greenhouses with the same capacity and shape, one new, one 3 months old, and one 6 months old, were applied for the cultivation of the nine vegetables for DEHP uptake experiments. Table 3 shows the DEHP contents in edible parts of nine vegetables from the three greenhouses with the different ages. There were significant differences between the 0 month and after 6 months ($p < 0.01$). The DEHP contents of the nine vegetables in the 0 month old greenhouse were 17.17 ± 1.32−36.16 ± 3.13 μg g^{-1} (dry weight), while they were 12.66 ± 2.08−24.30 ± 2.05 when in the 6 month old greenhouse. The results showed that the uptake of DEHP by vegetables was obviously decreased with the age of the greenhouse increasing. It revealed that the DEHP of the plastic film was emitted in the atmosphere after a short exposure to the environment, and so, the DEHP content in the greenhouse was highest at the beginning and then decreased gradually.

A previous study[16] found that the PAE (including DEHP and DBP) concentrations in the air of the greenhouse were increased sharply at the early days (5 days). Zheng et al.[20] reported that the PAE contents of the greenhouse air were decreased after 6 months. Our results are in agreement with their observations.

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