for *Trogoderma glabrum* larvae aggregating activity, and selected combinations of these compounds were assayed in mineral oil and nearly deodorized wheat germ oil (Table VII). The deodorized wheat germ oil had been exposed to air for ~2 h before tests were conducted and served primarily as a fixative for volatile compounds. However, the slight activity for this sample may have been due to autoxidation products or nonvolatile arrestants. Comparison of the bioassay results for compounds tested singly or in combinations indicated that synergistic effects were nonexistent, and combinations frequently gave lower aggregation responses than would be expected from simple dilution effects.

In summary, it appears that the aggregation of *Trogoderma glabrum* larvae induced by wheat germ oil is due to the combined effects of several volatile compounds. Further work should yield information which will allow preparation of synthetic mixtures with equal or greater attractancy than that obtained for native wheat germ oil. Future attention should be directed at confirmation of the identity of active compounds, identification of additional active compounds, and investigations on concentration/synergistic/antagonistic effects of active compounds.

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Occurrence of Dibutyl and Di(2-ethylhexyl) Phthalate in Chicken Eggs

Mitsuharu Ishida, Kyozo Suyama, and Susumu Adachi*

Low levels of dibutyl phthalate (DBP) and di(2-ethylhexyl) phthalate (DEHP) have been shown to be present in chicken eggs collected from retail stores in Japan in 1977. The concentration of DBP and DEHP in the egg white were in the range of trace to 0.15 ppm and 0.05 to 0.40 ppm, respectively, but no phthalates were detected in the egg yolk. After administration of DEHP to laying hens, it was observed that the eggs were contaminated with the DEHP.

Phthalates are widely used in the industrial production of plastics as plasticizers. Phthlates migrate easily from plastic products because they do not bind chemically with the plastics (Marx, 1972) so phthalate contamination is widespread. For example, the contamination was found in animal tissues (Nazir et al., 1971; Tarosky, 1967), milk (Cerbulis and Ard, 1967), fish (Mayer et al., 1972; Williams, 1973), and also human blood (Jaegar and Rubin, 1972; Rubin and Nair, 1973), air (Marx, 1972), and water (Marx, 1972; Mayer et al., 1972). Although little attention has been given to the contamination of avian eggs by phthalates, Suyama et al. (1977) confirmed the presence of DEHP in the shell and shell membrane.

We report here the presence of dibutyl phthalate (DBP) and di(2-ethylhexyl) phthalate (DEHP) in the edible structures of the eggs obtained from retail stores in Japan

Laboratory of Animal Products Technology, College of Agriculture, Tohoku University, Sendai 980, Japan.

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and from laying hens administered with DEHP.

MATERIALS AND METHODS

Reagents. All organic solvents and water were distilled in an all-glass apparatus. The 200-fold (100–0.5 mL) concentrated solvents contained no detectable phthalates as verified by gas–liquid chromatography (GLC). All glassware was washed, heated in an oven at 120 °C for 3–4 h, and/or rinsed with ethyl ether before use. Anhydrous sodium sulfate was washed with ethyl ether, dried, and heated at 150 °C for 2–3 h. The silicic acid (Mallinckrodt AR, 100 mesh) was washed also with ethyl ether, dried, and activated at 150 °C for 3–4 h.

DEHP Administration. Two kinds of White Leghorn laying hens were assigned at random to individual laying steel cages with individual steel feed cups. The hens were fed commercial laying mash (Parl mash, Taiyo Feed Co. Japan) and adjusted to the laboratory environment for a 10-day conditioning period. All eggs were used for the analysis of DEHP were gathered on the same day of laying. Administrations were carried out as follows. (A) Nine 12-month-old Babcock hens were divided into three groups of three hens each. Group 1 was kept as a control, and group 2 was given DEHP at the level of 1.0 g/kg of body weight directly into the stomach by the use of silicon tubing every morning. Group 3 was given 3.0 g/kg DEHP in the same way. The administrations were continued for 33 days. (B) Four 10-month-old Iwaya hens were divided into two groups of two hens each. One group was fed mash containing DEHP (1.0 g/100 g of mash) ad libitum and the other was the control. The administration was continued for 45 days.

Egg Samples. Eggs produced in seven different areas of Japan were obtained from the local markets in Feb 1977. The eggs were maintained in a refrigerator until analyzed, and 10 eggs of each lot were analyzed in the form of mixtures of the separated egg whites and yolks.

The eggs from the hens used in the administration experiment were collected from the A-group hens in lots at 3–4-day intervals and from the B-group hens in lots at 5-day intervals.

Sample Preparation. The lipids of shell associated with shell membrane (50 g) and yolk (200 g) were extracted with chloroform–methanol (2:1 v/v; 200 mL) (Folch et al., 1957) 4 times. The lipid of egg white (400 g) was extracted with chloroform–methanol (1:1 v/v; 300 mL) at first and then with chloroform–methanol (2:1 v/v; 250 mL) 3 times. All extracts were separately washed twice with water (100 mL) in a separatory funnel. The chloroform layer obtained was dried with anhydrous sodium sulfate (20 g) and subsequently concentrated to dryness under reduced pressure at 35 °C.

The extracted lipids of shell associated with shell membrane (0.01 g) and egg white (0.05 g) were dissolved with 2 mL of ethyl ether, and an aliquot was injected into the GLC. The lipid of yolk (1.0 g) was dissolved with exactly 100 mL of chloroform and treated with silicic acid (10 g) to remove polar lipid. After the mixture was stirred for 3 min, exactly 10 mL of the chloroform layer was taken and concentrated to dryness. The dried residue was dissolved with 1.0 mL of ethyl ether, and an aliquot was then injected into the GLC.

Various known quantities of DBP and DEHP (Table I) dissolved in ethyl ether were added to the egg white and yolk in order to determine the percent recovery. After being mixed completely with the phthalates, the egg edible structures were analyzed.

Gas–Liquid Chromatography. A Hitachi gas–liquid chromatograph, Model 063, equipped with a flame ionization detector was used for all analyses. A 2 m long × 3 mm i.d. stainless steel column packed with 1.5% SE-52 on 60–80-mesh Chromosorb W provided adequate resolution for all quantitative analyses operated isothermally at 230 °C for DEHP and linearly programmed at the rate of 5 °C/min from 150 to 240 °C for DBP. The temperatures at the detector and the injection part were 300 °C, and the carrier gas was nitrogen at a flow rate of 30 mL/min. Diethyl phthalate (DEP) and di-*n*-octyl phthalate (DOP) were used as internal standards for the determination of DBP and DEHP, respectively. Under these conditions, DEP, DBP, DEHP, and DOP had retention times of 3.9, 9.5, 9.5, and 16.8 min, respectively. For reconfirmation of the presence of the phthalates, a 2 m long × 3 mm i.d. stainless steel column packed with 8.0% diethylene glycol succinate polyester (DEGS) on 80–100-mesh Diasolid L was used. The operating conditons were as follows: column, 200 °C isothermally; detector and injection part, 270 °C; nitrogen flow rate, 30 mL/min. The retention times of DBP and DEHP were 16.0 and 47.3 min, respectively.

The chromatograph failed to remove all of the phthalates from the reagents and the equipment used. Therefore, the phthalates were detected as background in the egg samples. A blank test was performed on the same scale as that of the sample throughout each extraction, and the cleanup procedure for the accurate analysis of phthalates was described above. The background levels of phthalates in each 100-g sample were about 14 ppb for DBP and 71 ppb for DEHP in shell associated with shell membrane, 5 ppb for DBP and 21 ppb for DEHP in egg white, and 23 ppb for DBP and 105 ppb for DEHP in yolk. The values of phthalates in the egg samples were corrected for

Table I. Recovery of Dibutyl and Di(2-ethylhexyl) Phthalate Added in Egg Edible Structures[a]

	added, ppm	found, ppm[b]	recovery, %
DBP			
egg white	0	0.028 ± 0.004	
	0.1	0.067 ± 0.007	39.0
	0.3	0.203 ± 0.027	58.3
yolk	0	ND[c]	
	5	3.58 ± 0.27	71.6
	10	9.28 ± 0.50	92.8
DEHP			
egg white	0	0.060 ± 0.002	
	0.5	0.351 ± 0.027	58.2
	1.0	0.894 ± 0.073	83.4
yolk	0	ND	
	10	8.34 ± 1.19	83.4
	20	17.94 ± 2.01	89.7

[a] Detection limitation: 0.01 ppm in egg white and 1.0 ppm in yolk. [b] Average ± standard deviation. [c] Not detectable.

Table II. Concentrations of Dibutyl and Di(2-ethylhexyl) Phthalate in the Egg White of Commercial Eggs from Different Regions in Japan

producing region	DBP, ppm	DEHP, ppm
Fukuoka	0.08	0.09
Aichi	0.13	0.05
Ibaraki	trace	0.08
Ibaraki[a]	0.15	0.40
Saitama	0.12	0.23
Miyagi	0.06	0.19
Aomori	0.05	0.27
average[b]	0.098 ± 0.041	0.182 ± 0.125

[a] Brown-shell eggs. The other eggs have white shells. [b] Average ± standard deviation. Neither DBP nor DEHP was detected in the yolks.

Table III. Di(2-ethylhexyl) Phthalate Concentration in the Eggs from Treated Hens

	administered DEHP level[d]	no. of samples	av concn, ppm egg white	yolk	shell[a]
A:	control	69	0.21 (0.01-0.41)[b]	ND[c]	1.73 (0.61-2.76)
	1 g kg^{-1} day^{-1}	65	0.57 (0.06-1.82)	43.64 (11.71-74.54)	3.34 (0.85-5.13)
	3 g kg^{-1} day^{-1}	6	1.96	26.85	0.92
B:	control	86	0.15 (0.02-0.38)	ND	2.07 (0.90-3.66)
	1 g/100 g of mash	72	1.25 (0.72-2.28)	148.35 (9.44-254.88)	4.39 (1.05-9.21)

[a] Shell associated with shell membrane. [b] The value of the range is in parentheses. [c] Not detectable. [d] (A) Administered directly into stomach; (B) administration of ad libitum feeding of mash containing DEHP.

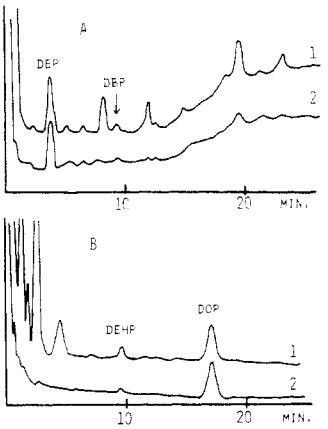

Figure 1. Representative gas chromatograms of DBP and DEHP in egg white and that of the blank. Chromatograms: (1) egg white; (2) blank. Conditions: (A) for DBP, column SE-52 and column temperature 150–240 °C (5 °C/min); (B) for DEHP, column SE-52 and column temperature 230 °C. The egg sample from Miyagi is used and the blank is the background from the reagents and equipment used. The levels of background are approximately 6 ppb for DBP and 20 ppb for DEHP.

background contamination but not for recovery.

RESULTS AND DISCUSSION

Recoveries. The results of the recovery tests are presented in Table I. The recoveries varied from 39.0 to 92.8% for DBP and 58.2 to 89.7% for DEHP. In the case of the analysis of yolk, because it contains a much higher level of lipid concentration than egg white, the noise of the gas chromatogram obtained was higher than that of egg white. The relative sensitivity for the determination of phthalates in the yolk appears to be at a considerably lower level than that of egg white. When the original yolk samples contained phthalates below 1.0 ppm, the phthalates were not detected.

Phthalate Levels in the Eggs on the Markets in Japan. The phthalate levels in the edible structures of the eggs are given in Table II. The representative gas chromatograms of the phthalates in egg white are shown in Figure 1. The concentraion was in the range of trace to 0.15 ppm for DBP and 0.05 to 0.40 ppm for DEHP in the egg whites. Neither DBP nor DEHP was detected in the yolks. However, the possibility of the presence of phthalates at levels below limitation (1.0 ppm) of detection remains for some of the yolks.

DEHP Levels of Eggs Obtained from Hens Administered with DEHP. The levels of DEHP in the structures of the eggs obtained from the administration experiments are given in Table III. The hens taking 3 g/kg dosage stopped laying eggs in the first 4 days and developed diarrhea and anorexia 10 days after the administration was started. Only six eggs from the three hens were obtained during the experimental period. The results of the DEHP administration indicated that DEHP entered into the body of the hens was transferred partially to the eggs and was accmulated at the highest level in the yolk.

Each hen consumed about 100 g of mash daily, and the daily dosage for each hen in the treated mash experiment was evaluated to be about 1 g of DEHP. The DEHP concentration of the eggs obtained from hens fed the treated mash was higher than that from the hens given directly the higher DEHP dosage (1.6–2.0 g/day). It seems most reasonable to conclude that the continuous ingestion of DEHP in the treated mash led to the increase absorption of DEHP in the digestive tract and caused a higher accumulation of the phthalate residue in the egg structures. Thus, oral administration of phthalate is probably responsible for the occurrence of phthalate in chicken eggs. Another possible route of contamination may be through the lungs or skin.

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