Analysis of Volatile Compounds in Wheat Germ Oil Responsible for an Aggregation Response in *Trogoderma glabrum* Larvae

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The volatile components of wheat germ oil were demonstrated by bioassay to be responsible for initiating aggregating activity of *Trogoderma glabrum* larvae. A strong aggregation response was induced by the neutral plus basic compound fraction of wheat germ oil but only a small one by the acidic fraction. Fractions collected from Carbowax 20M gas chromatography revealed highly active aggregation-inducing substances in the I_E regions of 7.8–8.1 and 14.1–14.6, but other fractions also showed activity. Principal compounds in the earlier I_E region were C_{13}–C_{16} saturated and unsaturated hydrocarbons, and a branched hexylbenzene. Major compounds in the latter region were octanoic acid, γ-nonalactone, an ethylnaphthalene, a methylethylnaphthalene, and a cyclic branched ketone. Synthetic octanoic acid was an active aggregating stimulant for *Trogoderma glabrum* larvae at some dilutions in mineral oil. Synthetic *cis*-3-hexenal, octanal, and γ-octalactone also induced aggregation when tested alone or in combinations. However, none of the synthetic or natural fractions yielded responses equivalent to that of intact wheat germ oil.

Integrated insect pest management techniques employing sex pheromones and food attractants for luring insects into traps or contact with pathogens or insecticides provide some interesting alternatives for control of stored-product insect infestations (Burkholder, 1977; Levinson and Levinson, 1977). It has been suggested that this approach could prove useful for controlling *Trogoderma* spp. which are universally distributed and are known for a wide variety of infestations of plant and animal materials. The sex pheromones of *Trogoderma glabrum* have been identified (Greenblatt et al., 1976), but their use is limited to control of sexually mature adults.

Even though adult *Trogoderma* feed minimally, they are attracted to potential food supplies for progeny. Attractant and arrestant responses to crude wheat germ fractions containing triglycerides have been observed for adult *Tribolium confusum* (Tamaki et al., 1971a). Subsequent studies (Tamaki et al., 1971b) have indicated that essentially nonvolatile triglycerides containing unsaturated fatty acids were attractive to *Tribolium confusum* adults. However, the potential role of volatile lipid oxidation products has not been investigated. *Tribolium confusum* adults have also been reported to be attracted to triglycerides isolated from the mycelium of *Nigrospora sphaerica*, a fungal contaminant of wheat (Starratt and Loschiavo, 1971). Substances attractive to adult *Sitophilus granarius* have also been isolated from wheat germ and appear to be associated with triglyceride fractions (Donat, 1970; Nawrot and Czaplicki, 1978). Similarly, extracts of grains have been shown to be attractive to *Sitophilus zeamais* adults (Ohsawa et al., 1970; Yamamoto and Yamamoto, 1970). Individual free fatty acids (C_5–C_{19}) have been shown to be attractive to adult *Tribolium castaneum* and *Trogoderma granarium* (Cohen et al., 1974).

For integrated management of whole populations, feeding attractants or aggregating compounds for the destructive larval stages are needed. Wheat bran was among attractive substances for *Trogoderma granarium* larvae in a study of a large number of synthetic and natural products by Bar-Zeev (1976). These larvae were also attracted to free fatty acids from lauric through linoleic. Recently, Nara (1979) investigated the aggregating behavior of *Trogoderma glabrum* larvae in the presence of a number of commercially available triglyceride oils and found that wheat germ oil stimulated aggregation.

A recent review of cereal grain volatiles by Maga (1978) indicates that only limited information is available for wheat, and these reports are generally limited to relatively simple aldehydes, ketones, alcohols, and esters (McWilliams and Mackey, 1969; Hougen et al., 1971; Lorenz and Maga, 1972; Shurpalekar and Rao, 1977; Buttery et al., 1978). Higher molecular weight hydrocarbons and fatty acids in wheat were found by Lorenz and Maga (1972), and Ivanova and Popov (1975) and Lercker et al. (1977) reported the identification of several fatty acids, sterols, and lipids in wheat germ oil.

The existing literature inadequately describes the chemical basis for chemosensory responses of stored-product insects to wheat, wheat germ, or wheat germ oil. Therefore, this investigation was conducted to provide additional information on the components that induce an aggregating response by *Trogoderma glabrum* larvae to wheat germ oil.

MATERIALS AND METHODS

Trogoderma glabrum were reared for bioassays according to Hammack et al. (1973). Recently molted 4th instar larvae were employed in bioassays conducted in Petri dishes (10-cm diameter) filled to $^1/_3$ height with paraffin. The surface was covered with filter paper (9-cm diameter, Whatman No. 1) on which 2-cm circles were drawn at diametrically opposite locations with centers 5.5 cm apart (Nara, 1979). For assays a filter paper disk (12.7-mm diameter, Schleicher & Schuell, Keene, NH) was attached with a minuten pin to the surface of the arena in the center of each of the 2 cm diameter circles, and the substance to be assayed was applied to one of the disks. The chamber is a modification of one used successfully by Loschiavo et al. (1963) with a different insect species. Fifty *Trogoderma glabrum* larvae were released in the center of the Petri dish, and the cover was replaced. After 20 min the number of larvae found within each of the 2 cm diameter circles was counted. The percentage response was calculated as

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$(T-C)/50 \times 100$ where T was the number of larvae around the treated disk and C was the number around the untreated control disk.

Crude free fatty acid fractions were isolated from commercially available wheat germ oil (Viobin Corp., Monticello, IL) either by an alkaline arrestant column (Woo and Lindsay, 1980) or by extraction with saturated $NaHCO_3$ followed by acidification to pH 2 and extraction into ethyl ether. After removal of excess solvents, the crude fractions were assayed for *Trogoderma glabrum* larvae aggregating activity.

Wheat germ oil essentially devoid of volatile compounds was prepared by placing 5 mL of oil under reduced pressure (10 mmHg) in a 2-L round-bottom flask and heating intermittently at 150 °C for a total of 21 h during a 72-h period. Stripped oil was assayed immediately upon disassembly of the cooled apparatus to avoid additional volatile compound generation through autoxidation.

Volatile components from 7 L of wheat germ oil were collected for ~5 h by using an all-glass reduced-pressure (20–30 mmHg) steam distillation apparatus similar to that described by Withycombe et al. (1978). Ethyl ether concentrates were analyzed with a Varian 1740 gas chromatograph (Varian Associates, Palo Alto, CA) equipped with a flame ionization detector and a heated column effluent splitter located ahead of the detector.

Primary separations were carried out with a 305 cm × 31 mm o.d. stainless steel column packed with 10% Carbowax 20M on 60–80-mesh Chromosorb W A/W. Selected fractions were collected from Carbowax 20M separations on Tenax GC (Applied Science Laboratories, State College, PA) with the method described by Steinke (1978). Fractions collected on Tenax GC were either used for bioassays or rechromatographed with nonpolar stationary phases for bioassays or GC–MS analyses. The nonpolar columns were a 305 cm × 31 mm o.d. stainless steel column packed with 10% SE-30 on 100–120-mesh Chromosorb G A/W and a 180 cm × 2 mm i.d. glass column packed with 3% OV-101 on 100–120-mesh Varaport 30 which was used for GC–MS analysis.

Gas chromatography conditions were as follows: injector temperature, 260 °C; detector temperature, 260 °C; carrier gas flow, 24 mL/min; hydrogen and air flow to the detector, 24 and 240 mL/min, respectively. Column temperature program rates were 45–240 °C at 4 °C/min. The GC–MS system included a Varian Model 1740 gas chromatograph coupled with an all-glass interface to a Du Pont Model 21-491 mass spectrometer (E.I. Du Pont de Nemours & Co., Wilmington, DE) equipped with a single-stage glass jet separator. The ion source was maintained at 250 °C, and spectra were obtained at 70 eV for a mass range of 28–300 at a scanning rate of 4 s/decade. Spectra were recorded with a Du Pont 5-124 oscillograph, and chromatographic profiles were recorded from a total ionization monitor.

Retention indices (I_E) of gas chromatographic fractions and peaks were determined in relation to those of ethyl esters of n-alkyl fatty acids according to the procedure of Van den Dool and Kratz (1963).

RESULTS AND DISCUSSION

Aggregation responses of *Trogoderma glabrum* larvae to crude acidic and neutral plus basic compound fractions for alkaline-arrestant column isolates are shown in Table I. Sodium bicarbonate extracts gave similar results. The acidic fractions contained only limited aggregation-inducing activity. While the bulk of the acidic fraction would be expected to be aliphatic free fatty acids, aromatic carboxylic acids, multifunctional group compounds containing carboxyl groups, and possibly very polar phenolic compounds would also be present. It is likely that the observed responses were overall net effects, and both attractive and repellent compounds were probably present in the mixtures. Increasing the amount of the acidic fraction did not influence the results of the bioassay. However, increasing the amount of the neutral plus basic compound fraction substantially increased the aggregating response. This result supports the hypothesis that the free fatty acids alone are not highly active attractive substances for *Trogoderma glabrum* larvae.

Table I. Aggregation of *Trogoderma glabrum* Larvae to Crude Acidic and Neutral Plus Basic Compound Fractions from Wheat Germ Oil Separated by an Alkaline Arrestant Column

test substances, mL	% response[a]
crude acidic fraction	
0.005	14.0
0.010	12.0
crude neutral plus basic fraction	
0.010	66.0
0.020	85.0

[a] Average of two replicates.

Table II. Aggregation Response of *Trogoderma glabrum* Larvae to Wheat Germ Oil Fractions

test substances	% response[a]
wheat germ oil	
0.01 mL of native	90.0
0.01 mL of deodorized	2.5
wheat germ oil steam distillate extract	
0.01 μL of distillate extract plus 0.01 mL of deodorized wheat germ oil	84.5
0.01 μL of distillate extract plus 0.01 mL of mineral oil	68.0

[a] Means of four replicates.

Extended vacuum deodorization of wheat germ oil at an elevated temperature resulted in nearly complete removal of attractancy of the oil for *Trogoderma glabrum* larvae (Table II). The deodorized wheat germ oil reconstituted with the volatile distillate extract fraction elicited a response approaching that of the native oil which indicates that the heat treatment did not induce the formation of repellent compounds. The activity of the distillate extract dissolved in mineral oil was less than that for the extract dissolved in deodorized wheat germ oil. This may reflect differences in release rates of volatile compounds from triglycerides in the wheat germ oil and hydrocarbons in mineral oil. On the other hand, it could indicate that wheat germ oil contains essentially nonvolatile feeding stimulants which induce wandering larvae to remain with the oil in the bioassay arena. Earlier experiments had shown that mineral oil alone did not induce aggregation of *Trogoderma glabrum* larvae. Thus, these data proved conclusively that the volatile fraction of wheat germ oil was responsible for the aggregation of *Trogoderma glabrum* larvae.

Aggregation bioassays of volatile extracts were difficult to perform with the enclosed, equilibrium system employed. Aggregation responses to unfixed, concentrated extracts were dependent upon the amount of substances placed in the test system (Table III). The progressive decrease in aggregation responses with increasing sample size demonstrates the confounding effects of either repellency, chemosensory fatigue, or disorientation due to saturation of the test environment with volatile compounds. Qualitative fixing of volatile extracts in either deodorized wheat germ oil or mineral oil greatly suppressed

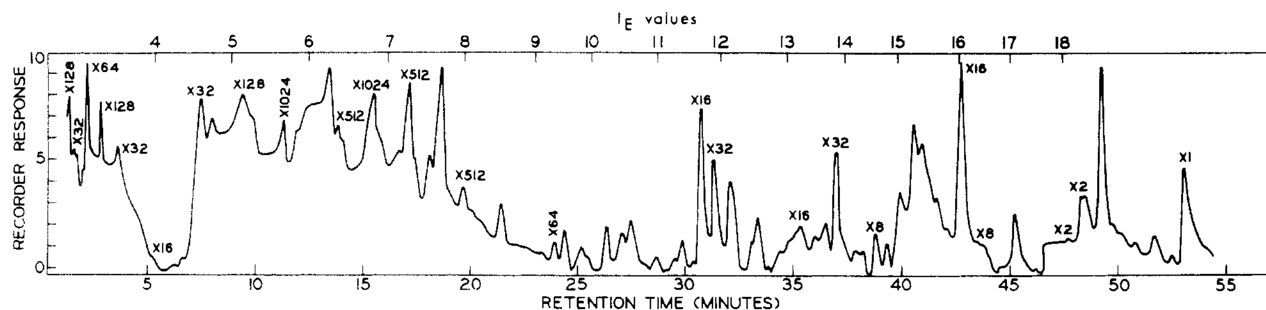

Figure 1. Gas chromatogram of the wheat germ oil steam distillate extract separated on a 10% Carbowax 20M packed column.

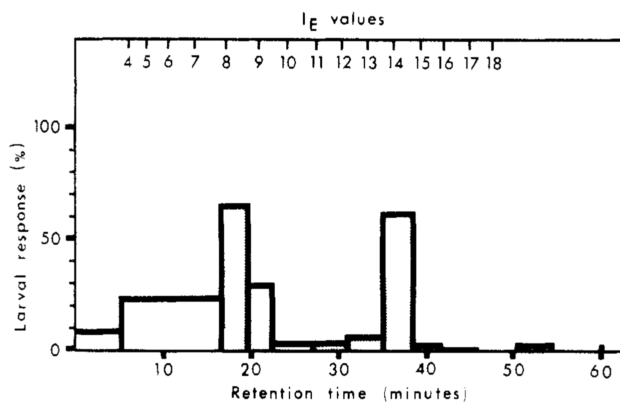

Figure 2. Aggregation responses of *Trogoderma glabrum* larvae to fractions of the wheat germ oil steam distillate extract separated on Carbowax 20M and collected on Tenax GC.

Table III. Aggregation Response of *Trogoderma glabrum* Larvae to Varying Amounts of Unfixed Wheat Germ Oil Distillate Extract

amount of extract, μL	% response[a]
4.0	22.0
2.0	20.0
1.0	48.0
0.5	48.0
0.2	76.0

[a] Results of one bioassay for each amount of extract.

these effects, but caution must always be exercised in interpreting data where concentrations of compounds are unknown.

A typical gas chromatogram of the steam distillate extract of wheat germ oil separated on Carbowax 20M is shown in Figure 1. Bioassay of gas chromatographic fractions collected on Tenax GC showed that many of the fractions contained active substances (Figure 2). The low resolution capabilities of the column and the effects of peak tailing probably affected the bioassays, but high activity was observed for compounds eluting in the I_E regions of 8 and 14. Bioassay of more narrow subfractions from these regions showed the greatest aggregating activities in the I_E spans of 7.8–8.1 and 14.1–14.6, and these fractions were analyzed further.

Rechromatographing of the fraction in the Carbowax 20M I_E region of 7.8–8.1 on a packed SE-30 column yielded 10 peaks which were again collected on Tenax GC for either bioassay or mass spectral analysis. Based on I_E values for similar compounds and published mass spectral data ("Index of Mass Spectral Data", 1969; Cornu and Massot, 1966; Steinke, 1978), the tentative identifications shown in Table IV were assigned. However, authentic compounds were not available for direct comparisons. All of the compounds tentatively identified in this fraction were hydrocarbons and would be included in the nonsaponifiable fraction (7.2%) of wheat germ oil reported by

Table IV. Tentative Identifications and Aggregation Responses of *Trogoderma glabrum* Larvae to Wheat Germ Oil Volatile Fractions Separated by an SE-30 Column after Original Collection from a Carbowax 20M Column between I_E = 7.8 and I_E = 8.1

peak no.	tentative identification	estimated I_E on SE-30	*T. glabrum* larval response, %[a]
1	unknown	8.0	7.0
2	a branched hexylbenzene (162)[b]	8.4	8.0
3	unknown	8.6	21.0
4	unknown	9.3	8.0
5	a tridecadiene (180)	9.5	8.0
6	a tetradecadiene (194)	9.7	9.0
7	unknown	10.0	4.0
8	a branched hexadecadiene (222)	10.3	15.0
9	a branched hexadecene (224)	10.7	12.0
10	5-propyltridecane (226)	10.8	6.0
wheat germ oil			97.0

[a] Mean of two replicates. [b] Molecular weight is shown in parentheses.

Table V. Identification of Compounds in Volatile Fractions from Wheat Germ Oil after Original Collection from a Carbowax 20M Column between I_E = 14.1 and I_E = 14.6 and Rechromatography on an OV-101 Column

peak no.	compd identification[a]	I_E on OV-101
1	octanoic acid (144)[b]	8.3
2	unknown	8.7
3	a dibasic ester (188)[b]	9.2
4	γ-nonalactone (156)[b]	9.5
5	unknown	9.8
6	unknown	10.2
7	an ethyl naphthalene (156)[b]	10.4
8	unknown	10.7
9	cyclic, C_{16} branched-chain ketone (238)[b]	10.8
10	a methylethylnaphthalene (170)[b]	11.0
11	unknown	11.2

[a] Number in parentheses is the molecular weight. [b] Tentative identification.

Lercker et al. (1977). Each of the fractions collected from the SE-30 separation showed some aggregating activity (Table IV) even though only a few micrograms of each fraction were represented in each assay. In the absence of authentic reference compounds, it was not possible to verify the attractiveness of the indicated compounds.

Similar gas chromatographic and mass spectral analysis of the fraction collected from Carbowax 20M separations in the I_E region of 14.1–14.6 allowed the separation and partial identification of the compounds listed in Table V. Bioassay of octanoic acid in mineral oil showed that the presence of 250–1000 μg of the compound in 0.01-mL assay

Table VI. Percentage Response of *Trogoderma glabrum* Larvae to γ-Octalactone at Different Concentrations in Mineral Oil

test 1 (very high levels)			test 2 (high levels)			test 3 (moderate levels)		
concn, ppm	dose on disk, μg[a]	response, %[b]	concn, ppm	dose on disk, μg[a]	response, %[b]	concn, ppm	dose on disk, μg[a]	response, %[b]
800 000	8000	−7.6	40 000	400	−4.0	1250.0	12.5	46.0
400 000	4000	−5.6	20 000	200	−2.0	625.0	6.3	44.4
200 000	2000	−2.8	10 000	100	2.8	312.5	3.1	28.4
100 000	1000	−6.0	5 000	50	19.6	156.3	1.6	23.6
50 000	500	−4.4	2 500	25	24.4	78.1	0.8	27.6
						39.0	0.4	34.0
wheat germ oil		94.6	wheat germ oil		94.6	wheat germ oil		94.0

[a] Calculated from the concentration assuming a specific gravity of 1.0. [b] Mean of five replicates.

Table VII. Aggregation Responses of *Trogoderma glabrum* Larvae to Selected Combinations of Compounds Diluted with either Mineral Oil or Deodorized Wheat Germ Oil

test 1		test 2		test 3	
sample tested (in mineral oil)	response, %[a]	sample tested (in mineral oil)	response, %[a]	sample tested (in deodorized wheat germ oil)	response, %[a]
250 μg of octanoic acid plus 1000 μg of *cis*-3-hexenal	62.8	1000 μg of *cis*-3-hexenal plus 12.5 μg of γ-octalactone	6.4	250 μg of octanoic acid plus 1000 μg of *cis*-3-hexenal	60.4
250 μg of octanoic acid plus 2000 μg of octanal	62.0	250 μg of octanoic acid plus 1000 μg of *cis*-3-hexenal plus 2000 μg of octanal	32.4	250 μg of octanoic acid plus 2000 μg of octanal	27.6
250 μg of octanoic acid plus 12.5 μg of γ-octalactone	63.6	250 μg of octanoic acid plus 2000 μg of octanal plus 12.5 μg of γ-octalactone	8.8	250 μg of octanoic acid plus 12.5 μg of γ-octalactone	76.0
1000 μg of *cis*-3-hexenal plus 2000 μg of octanal	38.0	250 μg of octanoic acid plus 1000 μg of *cis*-3-hexenal plus 12.5 μg of γ-octalactone	16.4	10 μL of deodorized wheat germ oil	10.0
2000 μg of octanal plus 12.5 μg of γ-octalactone	25.2	1000 μg of *cis*-3-hexenal plus 2000 μg of octanal plus 12.5 μg of γ-octalactone	40.4	10 μL of wheat germ oil	85.2
10 μL of wheat germ oil	85.0	250 μg of octanoic acid plus 1000 μg of *cis*-3-hexenal plus 2000 μg of octanal plus 12.5 μg of γ-octalactone	23.6		
		10 μL of wheat germ oil	76.0		

[a] Mean of five replicates; a 0.01-mL sample was used in each assay.

samples caused aggregation responses of 31–38% whereas higher and lower amounts gave much lower responses. However, similar levels of γ-nonalactone did not elicit any aggregating response, indicating that octanoic acid contributed much of the aggregating activity of this fraction. The presence of naphthalene and alkylnaphthalenes in wheat flour has been reported by Buttery et al. (1978), but the lack of positive identifications of individual isomers precluded assays in the current study.

The fraction eluting in the Carbowax 20M I_E region of 5.8–8.0 showed considerable aggregating activity (Figure 2). While this fraction was not subjected to mass spectral analysis, I_E values of some authentic compounds for this elution region were found compatible with observed aromas, predictable lipid oxidation products (Hoffmann, 1962), and previously reported wheat-related compounds. Bioassay of octanal (Carbowax 20M I_E 6.6; McWilliams and Mackey, 1969; Mulders, 1974) gave up to 49% *Trogoderma glabrum* larvae aggregation responses while *trans*-2-octenal (Carbowax 20M I_E 8.0; Buttery et al., 1978) did not appear to be attractive. Similarly, *trans*-2-hexenal (Carbowax 20M I_E 5.8; Hoffmann, 1962) gave little or no aggregation activity. On the other hand, *cis*-3-hexenal caused up to 56% *Trogoderma glabrum* larval response, and *cis*-3-hexenol gave up to 19% aggregation response. Aggregating responses to some aldehydes suggest that lipid peroxidation through autoxidative and lipoxidase enzymic processes play a key role in the attractancy of certain insects to grains and grain products. Since many vegetable oils exhibit aggregating activity for *Trogoderma glabrum* larvae (Nara, 1979), it can be hypothesized that this general attractancy is due to common oxidation products developed in the oils. Both primary aldehyde products (i.e., octanal) and secondary oxidation products (i.e., octanoic acid; Lillard and Day, 1964) appear to have a potential role in attracting *Trogoderma glabrum* larvae to vegetable oils.

Synthetic γ-octalactone was studied by accident because it was misidentified in early trials. However, it was found attractive to *Trogoderma glabrum* larvae, and it bears a common structural relationship with octanal and octanoic acid which were also quite attractive. The data in Table VI demonstrate important influences which affect the results of the assay procedure. In these assays γ-octalactone was found to elicit moderate aggregation at concentrations of 40–5000 ppm. At high concentrations (>10 000 ppm), γ-octalactone was either repellent or caused chemosensory fatigue or disorientation similar to that observed previously for the concentrated wheat germ oil distillate extract. The assay method was not especially effective for testing repellency, however, because repelled larvae would not necessarily aggregate around the control disk.

Octanoic acid, *cis*-3-hexenal, octanal, and γ-octalactone were the most attractive synthetic compounds evaluated

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

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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

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