Bull. Environm. Contam. Toxicol. 25, 345-352 (1980)

Phthalate Plasticizers: Accumulation and Effects on Weight and Food Consumption in Captive Starlings

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Phthalate plasticizers have recently been recognized to be widespread environmental pollutants (see, for example, GIAM et al. 1978). These compounds possess certain characteristics that are cause for concern: they are produced in massive quantities, they eventually reach the environment, and they have biomagnification factors at least as great as DDT within invertebrate food chains of model ecosystems (BOOTH et al. 1976, METCALF et al. 1973). Fortunately, however, in common laboratory mammals phthalates are rapidly metabolized, do not accumulate appreciably, and are of very low toxicity (for reviews on the toxicity and metabolism of phthalates see AUTIAN 1973, DANIEL 1978, FISHBEIN and ALBRO 1972, PEAKALL 1975, THOMAS et al. 1978).

In spite of their rapid metabolism and apparent lack of accumulation, we have nevertheless received reports of relatively high residues of phthalates in tissues of passerine birds, particularly starlings *(Sturnus vulgaris)*, from several regions of the United States. We suspected that these residues resulted from contamination of samples through contact with plastics, either in the field or in the laboratory. The possibility of accumulation of these pollutants in avian wildlife could have serious ramifications, however. Therefore, we designed an experiment to determine whether phthalate residues accumulate in starlings by exposing birds to dietary concentrations of di-2-ethylhexyl phthalate (DEHP) or di-n-hexyl phthalate (DHP) which were generally greater than those reported in various environmental samples (GIAM et al. 1978, HITES 1973, MAYER et al. 1972). We then examined the starlings for residue accumulation.

MATERIALS AND METHODS

Experimental Procedures. Wild starlings were captured during winter 1977-1978 and held in large outdoor enclosures (2.0 x 2.6 x 3.7 m) until the experiment was initiated. Birds were then banded, weighed, and randomly assigned to five smaller outdoor enclosures (1.8 x 1.8 x 3.7 m), 21 adult starlings of

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0007-4861/80/0025-0345 $01.60

mixed sexes per pen, on 13 October 1978. After a 2-week acclimation period, one of the following intended dietary dosages was assigned per cage: control, 25 ppm DEHP, 250 ppm DEHP, 25 ppm DHP, or 250 ppm DHP. Phthalates were dissolved in corn oil and mixed into a diet consisting of commercial turkey starter crumbles. The corn oil-phthalate mixture constituted 2 percent (%) of the diet by weight. An equal amount of corn oil was added to the control diet. Treated food was mixed at three 10-day intervals over the course of the 30-day exposure period, and one food sample was drawn within 72 hours after each of these three periods for analytical determination of phthalate concentrations. Results of these analyses appear in Table 1.

TABLE 1

Intended and recovered concentrations (in ppm) of phthalates in three feed samples drawn from each treatment group. ND = not detected.

Intended	Recovered	
	DEHP	DHP
25 DEHP	22, 24, 25	ND, ND, 0.25
250 DEHP	228, 254, 299	ND, ND, ND
25 DHP	0.80, 0.87, 4.6	24, 24, 27
250 DHP	0.32, 0.78, 1.3	254, 276, 299
Control	1.2, 2.3, 2.4	ND, ND, 0.24

Food and water were provided *ad libitum* at all phases of the study. Food consumption was determined at 24-hour intervals for 15 of the 30 days on treated diets and for 7 of the following 14 days on untreated feed. Daily food consumption was expressed as (g food provided - spillage)/number of birds per cage for each group.

At 30 days of exposure eight randomly chosen birds were removed from each pen, weighed, and sacrificed. The remaining birds were provided untreated food for the following 14 days *(cleanup period)*, at which time another randomly chosen set of eight individuals was weighed and sacrificed. Carcasses were prepared for residue analysis following methods employed in the starling subprogram of the National Pesticides Monitoring Program (MARTIN 1969), except that gastrointestinal tracts were removed in addition to beaks, feet, wings, and skin. Carcasses were then frozen before chemical analysis. Precautions were taken to avoid contact with plastics during all phases of the experiment.

Chemical Analysis. We homogenized samples with a Virtis homogenizer using stainless steel cups and blades and

teflon caps. Sample aliquots were mixed with anhydrous sodium sulfate, placed in glass thimbles, and extracted with hexane for 7 hours on a Soxhlet apparatus.

Lipids were removed by automated gel permeation chromatography (JOHNSON et al. 1976). Operating conditions were as follows:

Column: 600 mm x 25 mm i.d. packed with 80 g of 200-400 mesh BioBeads (SX-3)
Solvent system: (3+1) Ethyl Acetate/toluene
Flow rate: 5 ml/min
Dump cycle: 150 ml
Collect cycle: 125 ml

Phthalates were separated from organochlorine pesticides and polychlorinated biphenyls (PCBs) by Florisil column chromatography (BELISLE et al. 1975). Pesticides and PCBs were eluted with 200 ml 6% ethyl ether in hexane, followed by phthalates eluted with 275 ml 15% ethyl ether in hexane.

Quantification of phthalate residues was done on a gas-liquid chromatograph (GLC) equipped with a N_i^{63} electron-capture detector. Operating conditions were as follows:

Column: 1.83 m x 4 mm i.d. glass packed with 1.5/1.95% SP2250/SP2401 on 80-100 mesh Supelcoport
Column temp: 210°C
Injection port temp: 250°C
Detector temp: 300°C
Carrier gas: (95+5) Argon/Methane at 60 ml/min

Recovery studies were done by fortifying clean starling tissue with 1.0 ppm DEHP and DHP. Recovery of DEHP and DHP averaged 100% and 111%, respectively. The lower limit of quantification was 1.0 ppm.

Special precautions were taken to reduce laboratory contamination of samples during analysis. All solvents were tested for DHP and DEHP contamination by GLC before use. Anhydrous sodium sulfate was heated at 675°C for 2 1/2 hours to reduce background contamination. We made volume reductions using a rotary evaporator and tube heater. All glassware was prerinsed with 15% ethyl ether in hexane. With these precautions, background levels of DEHP in procedural blanks averaged 0.87 µg. Residue values reported were corrected for this background. DHP was not detected in procedural blanks.

Statistical Analysis. Statistical procedures generally follow SOKAL and ROHLF (1969). F-max tests for homogeneity of variance were employed before parametric tests of hypotheses. Analysis of variance (ANOVA) on food consumption was computed on a randomized block design (dates x treatment), with data from exposure and cleanup periods analyzed separately. Changes in

body weight were positive in all individuals and were expressed as percentage increase over weight at the initiation of the experiment. Percentages were subjected to an arcsine-squareroot transformation before hypothesis testing. Differences in mean percentage increases in weight due to treatment were tested by one-way ANOVA and Duncan's multiple range test at 30 days exposure and again after 14 days on untreated feed. Differences in mean weight increases at 30 days exposure versus the cleanup period were analyzed by t-tests for each treatment group. Percentage lipid in carcass homogenates was subjected to transformation as above and tested with one-way ANOVA and Duncan's method of mean separation, and simple linear regression was performed on data for percentage carcass lipids and percentage increase in body weight. In all instances an alpha probability of 0.05 or less was considered significant. Summary statistics are presented as means and standard deviations or as retransformed means and 95% confidence intervals (CI).

RESULTS

Residue Accumulation. Phthalates were not detected in carcasses of control birds or birds exposed to either dietary concentration of DHP. One of eight birds fed 25 ppm DEHP had detectable residues (1.6 ppm) after 30 days exposure. Five of eight birds fed 250 ppm DEHP contained an average of 1.8 ± 0.31 ppm of this plasticizer. Five of eight starlings exposed to 250 ppm DEHP for 30 days and then fed an uncontaminated diet for 14 days also contained detectable concentrations, averaging 1.3 ± 0.25 ppm. The decline in DEHP residue means among birds with residues is statistically significant ($P<0.05$; $t=2.89$). No chemical analyses were performed on starlings from the other groups following the cleanup period because residues were essentially not evident at 30 days exposure.

Body Weights. All birds gained weight over the course of the experiment. After 30 days on treated feed both groups of starlings fed DEHP had significantly greater mean percentage weight increases than controls or DHP-fed groups ($P<0.01$). No other treatment-related differences were detected at 30 days exposure. Significant differences attributable to treatment were not evident from ANOVA on weight increases in birds sacrificed at the cleanup period. For all groups except controls, however, the mean percentage increases seen at the cleanup period were significantly less than the increases that were evident at the 30-day exposure period ($P<0.01$; Table 2).

Percentage lipid in carcass homogenates was determined for all birds sacrificed at the 30-day exposure period; all phthalate-dosed groups had significantly greater lipid means than controls but did not differ among themselves (Table 3). The percentage increase in weight (combined data for the above birds and the 250 ppm DEHP-dosed birds at the cleanup period) was significantly correlated with percentage lipid in carcass homogenates ($P<0.01$; $r=0.66$; $Y=0.018 + 1.61X$).

TABLE 2

Percentage increase in body weight for starlings sacrificed after 30 days exposure or after a 14-day cleanup period.

Group		Exposure	Cleanup	t
Control	$\bar{X}$	13.4	12.2	0.42
	CI	9.4-17.9	7.6-17.8	
25 ppm DEHP	$\bar{X}$	26.3	6.5	7.17
	CI	21.4-31.6	3.4-10.5	
250 ppm DEHP	$\bar{X}$	25.1	7.4	6.01
	CI	19.8-30.8	4.0-11.8	
25 ppm DHP	$\bar{X}$	14.1	5.5	3.29
	CI	10.0-18.8	2.3-10.0	
250 ppm DHP	$\bar{X}$	18.7	5.8	5.64
	CI	13.7-24.3	3.7-8.5	

TABLE 3

Percentage lipid in carcass homogenates of birds sacrificed at the end of the 30-day exposure period.

		DEHP		DHP	
	Control	25 ppm	250 ppm	25 ppm	250 ppm
$\bar{X}$	8.0	12.7	12.2	10.9	12.2
CI	5.3-11.1	10.1-15.5	9.4-15.3	9.4-12.6	10.3-14.2
n	7	8	8	8	8

Food Consumption. Significant effects on food consumption due to date of measurement (blocks) occurred during both the exposure and cleanup periods ($P<0.01$). These can be attributed to climatic variables which act to change energetic or nutritional demands on birds held in outdoor enclosures. Some significant effects due to treatment were also detected. Although during the exposure period birds in all treatment groups appeared to consume less food than controls (Table 4), significant differences existed only for the groups fed 25 ppm DEHP or 250 ppm DHP. These two groups

did not differ from each other but consumed significantly less food than the other groups. During the cleanup period the only significant difference occurred between controls and birds previously fed 250 ppm DHP.

TABLE 4

Food consumption (g/bird/day) in captive starlings exposed to different dietary concentrations of phthalates.

Group	Exposure Period	Cleanup Period
Control	22.3 ± 4.2	17.4 ± 3.0
25 ppm DEHP	20.5 ± 4.6	16.5 ± 2.6
250 ppm DEHP	21.6 ± 3.9	16.5 ± 2.3
25 ppm DHP	21.5 ± 3.7	17.3 ± 2.7
250 ppm DHP	20.6 ± 4.8	15.7 ± 2.9

DISCUSSION

Our residue analyses demonstrate that DHP does not accumulate in starlings. The analyses also show that DEHP was not detectable in all individuals exposed, and in those in which it was found it occurred only at low concentrations. This was true even after a 30-day period of exposure to amounts that were very high relative to levels that are known to occur in the environment (see, for example, GIAM et al. 1978, HITES 1973, MAYER et al. 1972). These results are in accord with other studies which also show a rapid metabolism and lack of accumulation of DEHP and other phthalates in tissues of warm-blooded vertebrates (BELISLE et al. 1975, BOOTH et al. 1976, IKEDA et al. 1978, SCHULZ and RUBIN 1973, TANAKA et al. 1978, WILLIAMS and BLANCHFIELD 1974). Captive starlings fed 250 ppm DEHP for 30 days (about 5,000 µg per day) had total amounts averaging 9.1µg in the prepared carcasses of the five individuals with detectable residues. This indicates that 99.8% of the phthalate ingested during the previous 24 hours was either excreted, or discarded with the gastrointestinal tracts. The low quantities present continued to decline; mean concentrations in carcasses among birds with residues decreased by 28% over the 14-day cleanup period. Such findings suggest to us that reports of high phthalate concentrations in tissues of avian wildlife should be met with skepticism unless it can be thoroughly demonstrated that both laboratory and field contamination of samples did not occur, or that the phthalates in question are unusual compounds with atypical properties.

Our findings also indicate that DEHP-dosed starlings gained more weight than birds in the DHP or control groups, and that birds fed both DEHP and DHP showed an increase in lipid deposition. The increases in body weights and carcass lipids

were not accompanied by increases in food consumption. Individuals placed on clean food following exposure showed significantly smaller weight gains than those sacrificed immediately after exposure, but this was not true of controls. We are unable to explain the physiological basis for these changes. Similar findings have been reported by STEIN et al. (1973), however, who noted that rats showed an increase in fat deposition and gained weight when fed 1,000 ppm DEHP in a diet which included fats. A related group of compounds, the terephthalates, are used commercially on a large scale to enhance the physical properties of animal feeds and the bioavailability of other additives (AUTIAN 1973). Perhaps phthalates acted similarly and simply increased assimilation efficiency in our captive starlings. However, phthalates are also known to cause biochemical alterations in lipid metabolism (see, for example, BELL et al. 1979, 1978a, b; BELL and NAZIR 1976) and, it may be pertinent that the effects noted in our study occurred during the hormonally-mediated autumnal fat deposition phase of the annual physiological cycle. Thus without additional data the effects on weight and carcass lipids could also be attributed to some undetermined toxicological property of these compounds.

ACKNOWLEDGEMENTS

Cheryl L. Bratsch provided invaluable assistance during most phases of this experiment. We also thank other members of the Patuxent staff who helped in various ways. Eugene H. Dustman reviewed the manuscript.

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