Table I. Recovery of EDB from Fortified Citrus Samples[a]

substrate	fortification, ppb	recovery, % replicate A	B	C	mean ± SD
whole grapefruit	500	84	86	86	85 ± 1
	50	90	97	97	95 ± 4
	5	87	94	88	90 ± 4
grapefruit rind	500	82	84	86	84 ± 2
	50	94	94	100	96 ± 3
	5	74	73	81	76 ± 4
whole orange	500	88	96	90	91 ± 4
	50	90	86	86	87 ± 2
	5	92	84	83	86 ± 5
orange rind	500	98	88	90	92 ± 5
	50	83	89	86	86 ± 3
	5	81	78	75	78 ± 3
whole lemon	500	90	92	94	92 ± 2
	50	81	94	87	87 ± 7
	5	82	91	84	86 ± 5
lemon rind	500	88	96	84	89 ± 6
	50	86	94	86	89 ± 5
	5	79	78	76	78 ± 2

[a] Samples were processed by using benzene. Apparent EDB residues in control samples were too insignificant to be considered.

Table II. Recovery of EDB from Whole Citrus Fruits Fortified at 5 ppb[a]

substrate	recovery, % replicate A	B	C	mean ± SD
grapefruit	102	102	98	101 ± 2
orange	98	106	106	103 ± 5
lemon	102	98	102	101 ± 2

[a] Samples were processed by using hexane. Apparent EDB residues in control samples were too insignificant to be considered.

in a refrigerator, EDB residue levels changed erratically, and interfering background peaks appeared on the gas chromatograms.

Due to the adverse health effects ascribed to benzene, alternate solvents are sought by many laboratories. Use of ethyl acetate in place of benzene yielded a similar background profile as shown in Figure 1A. However, sulfuric acid cannot be placed safely in contact with ethyl acetate. The procedure of King et al. (1980) most likely designated benzene, rather than hexane, because samples, especially whole fruit, foam excessively when hexane is used. A dimethyl silicone defoaming agent was used with hexane with good results. Figure 3A shows a gas chromatogram prepared from 100 g of whole orange and by using a defoaming agent. Prior to use, the hexane needed to be passed through a column of activity grade I basic alumina to remove background interferences. Figure 3B shows the cleanup achieved by addition of acid-impregnated silica gel. Figure 3C shows that 5 ppb of EDB can be readily detected. Figure 3D shows that 1 ppb of EDB can also be detected.

Table II gives the percent recovery values obtained for samples of whole fruit fortified at 5 ppb and by using hexane with the steam distillation. The mean recovery value and standard deviation for all nine samples listed in Table II was 102 ± 3%. All samples were quantified on the same day that the samples were fortified and steam distilled.

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Registry No. EDB, 106-93-4; sulfuric acid, 7664-93-9.

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Lack of Gut Absorption of Solubilized Polystyrene by the Rat

[^{14}C]Polystyrene of a molecular weight range similar to that found in commercial expanded polystyrene containers was synthesized and dissolved in lemon oil. Two microcuries was administered intragastrically to male rats of the Long Evans strain. After 5 days all radiation was recovered from fecal samples. No radiation was detected in blood, urine, major organs, or tissue samples. Ninety-nine percent of ^{14}C label was excreted within 48 h after intubation.

Phillips (1979) indicated the possibility that polystyrene might be a food contaminant by reporting his observation of the deterioration of an expanded polystyrene container by lemon tea. Phillips, however, could not find polystyrene dissolved in tea from the damaged cups. Using a [^{14}C]-polystyrene we were able to explain the observations of Phillips (Monte, 1982) as well as quantitate the solubility of polystyrene in several essential oils and detect traces of the polymer solubilized in some cooking oils with which it made contact (Monte and Landau, 1982).

Oppenheimer et al. (1955) reported polystyrene film as a carcinogen, causing tumors when implanted in rats.

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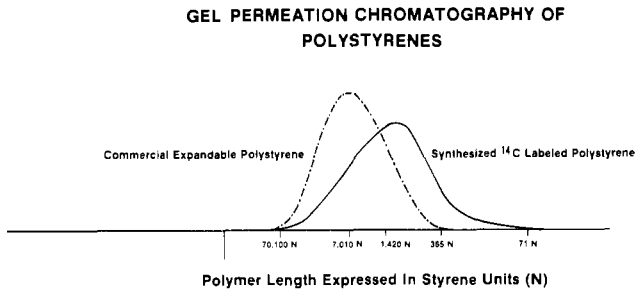

Figure 1. Molecular weight distribution of polystyrene synthesized for this study as compared to a sample of commercial expandable polystyrene cup material.

Described in this report are the results of a feeding study done to determine if polystyrene, when solubilized in a food oil, can be absorbed by the digestive system of the rat.

MATERIALS AND METHODS

Synthesis and Preparation of [^{14}C]Polystyrene. A glass ampule containing styrene (ring-^{14}C labeled) (New England Nuclear) with an activity of 0.246 mCi/mmol was submerged in liquid nitrogen and evacuated several times to remove oxygen (which was replaced with nitrogen) and sealed. The ampule was heated in an oil bath, once at 110 °C for 144 h and then at 120 °C for 10 h. Purification and removal of all residual styrene and much of the very low molecular weight polymers not found in commercial grades of expanded polystyrene were accomplished by solution in methyl ethyl ketone and precipitation of polymer with addition of ethanol (Boundy and Boyer, 1952). Samples of this synthesized polystyrene and commercial polystyrene were subjected to gel permeation chromatography (Figure 1). A subjective comparison of the molecular weight distributions as determined by integration of the chromatograms allowed us to treat them as equivalent for the purpose of this absorption study.

Seventy milligrams of the [^{14}C]polystyrene was dissolved in 10 mL of lemon oil (Sunkist Growers No. 3428) to produce an activity of 20 μCi/mL of solution (solubility of polystyrene is greater than 200 mg/mL of lemon oil).

Animal Study. Twenty male rats of the Long Evans strain (130–160 g) received 2 μCi of [^{14}C]polystyrene dissolved in 100 μL of lemon oil as above. The oil was administered intragastrically via a 5 French size polyethylene feeding tube (Pharmaseal Laboratories) passed perorally. The tube was rinsed with several aliquots of distilled water. Immediately afterward the rats were housed in individual metabolic cages. They were allowed free access to Wayne Lab-Blox (Allied Mills) and water. Urine and feces were collected at 8-h intervals. Animals were weighed before entering the test, and during the experiment records were kept of body weights, food eaten, appearance, and any signs of illness. Animals were killed 120 h after intubation and tissues prepared immediately for examination. Appropriate samples of the following tissues were subjected to digestion in Protosol (New England Nuclear) and detection of activity in Econofluor (New England Nuclear): blood, skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes (neck), heart, liver, pancreas, stomach, large and small intestines, kidney, urinary bladder, testes, and brain. The stomach and large and small intestines were washed in saline and scraped carefully with a glass slide to remove epithelium and gut contents to avoid possible contamination from this source. Aliquots of the urine were dissolved in Aquasol (New England Nuclear) before scintillation. Fecal material was first extracted exhaustively with methyl ethyl ketone. Both the extract (after evaporation of the solvent) and the residue were subjected to scintillation determination of ^{14}C activity.

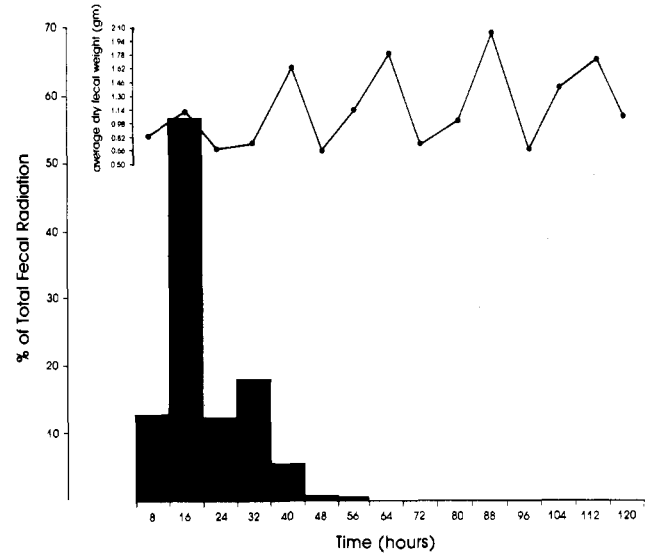

Figure 2. Percent of total administered [^{14}C]polystyrene recovered from fecal samples of 20 rats over a period of 120 h as compared to average dry fecal weight at each collection.

RESULTS

The rats intubated with [^{14}C]polystyrene appeared normal and gained weight at the same rate as the controls. None of the tissue samples showed any activity above background. Two of the urine samples showed slight activity (<0.001% of administered ^{14}C) but both had been subjected to inadvertent fecal contamination prior to collection. Within the bounds of experimental error, all ^{14}C was found in the fecal samples (Figure 2) and 99% was excreted within 48 h after intubation. This study indicates that polystyrene of this molecular weight distribution solubilized in an absorbable solvent does not pass through the intestinal barrier of the rat.

Registry No. Polystyrene, 9003-53-6.

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