Pesticide Residues and Polychlorinated Biphenyl Levels in Diets, Urine, and Fecal Matter of Preadolescent Girls (36347)

NELSON O. PRICE, R. W. YOUNG, AND JEAN K. DICKINSON
(Introduced by G. E. Bunce)

Department of Biochemistry and Nutrition, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

Description of six earlier Southern Regional Studies with preadolescent girls 7 to 9 years of age have been published (1–3). The 1970 study, including overall objectives, subject management, exact dietary compositions, and methodology of sample collection, was reported (4). Also included in some of the past work in these metabolic studies are the determinations of certain trace minerals in the diets, urine, and feces (5–7).

This work summarizes the screening of weekly composite samples of diets, urine, and fecal matter for pesticide residues. This is the first work reported on polychlorinated biphenyl (PCB) residues from a human metabolic study where controlled conditions of the diets and sample collection were established. The residue levels, including PCB, may hopefully be of great value as a basis for further investigation in this field with human subjects.

The chlorinated aromatic compounds have been used extensively in industry since 1929 (8). In 1952, a bovine hyperkeratosis disease appeared in a number of cattle herds in Virginia. Highly chlorinated naphthalene was established as the causative agent (9) for this disease. This compound was found in certain petroleum products (lubricants) essential for various types of farm machinery (10). The petroleum industry eliminated the highly chlorinated naphthalene from their products and the hyperkeratosis disease of cattle disappeared in VA. Recently, Vos and Beems (11) have shown that the application of PCB to the skin of rabbits produced hyperplasia and hyperkeratosis which resembled the changes observed in animals exposed to the chlorinated naphthalenes. Polychlorinated biphenyls are structural analogs of the chlorinated naphthalenes and are used as lubricants (11). It would be reasonable to suggest a more vigorous effort to identify the occurrence of PCB in petroleum products.

Procedure for sample collection and diet composition. Two replicate nutrition studies, each with sixteen 7- to 9-year-old girls, were conducted at Blacksburg, VA, during June, July and Aug., 1970. In each study, the first 8 days (two 4-day periods) were used for adjustment to the basal diet. The diet employed was considered typical of the foods consumed by lower socioeconomic groups in the southeastern United States. Each diet provided about 2000 kcal of energy, 24 g of protein, and was marginal in vitamin A, thiamine, riboflavin, and niacin equivalents. After the adjustment period, the subjects were randomly assigned within weight groups to four modifications of the basal diet and were maintained on them for two periods, each consisting of 6 consecutive days. The modifications consisted of variations in calcium (300–1300 g/day) or nitrogen (L-lysine·HCl, 1.139 g; L-threonine, 0.63 g; L-methionine, 0.189 g; or ammonium citrate to provide an equivalent amount of nitrogen), but the data from each treatment have been pooled for this report since there were no patterns evident as a function of dietary variation.

Composites of food, feces, and urine were made as follows: The complete diet for each day, per subject, was weighed and adjusted to 2000 g with ion-free water. From this daily composite, portions were transferred into a liter plastic container, capped, and frozen. At the end of each period, the daily aliquots were thawed, blended thoroughly, and sam-

TABLE I. Recovery (%) of Polychlorinated Biphenyls (PCB)[a] in Diets, Feces, and Urine.

	Diets (%)				Feces (%)				Urine (%)			
PCB types	1	2	3	Av	1	2	3	Av	1	2	3	Av
1242	62	68	72	67	70	68	72	70	71	68	66	68
1254	66	68	72	68	65	69	70	68	66	68	62	65
1260	62	68	65	65	68	61	64	64	66	69	70	68
1242, 1254 (50% mixture)	68	67	69	68	70	66	67	67	65	62	68	65

[a] Arochlor (PCB) types, 1242, 1254, 1260 supplied by Monsanto Chemical Co., St. Louis, MO.

pled. The 24-hr urine collections were measured for each subject and the total volume made to 2000 ml with ion-free water, from which a composite sample was taken. The feces for each subject were collected and weighed; ion-free water was added to bring the net weight of the composite to a total of 1000 g for the two 4-day periods and 15000 g for the two 6-day periods. All composite samples for the residue analyses were stored in Nalgene plastic bottles. Ten grams of the diets, 10 g of the fecal matter, and 50 ml of the urine were used for the residue extractions and determinations.

Methods and sample preparation. A Microtek 220 GLC was used for the residues and PCB determinations. The polychlorinated biphenyls (PCB) were separated from DDT and its analogs using the method of Armour and Burke (12). The average recovery of PCB from spiked samples of diet, urine, and fecal matter was 67.0% (Table I). Sensitivity for all residue determinations was 0.001 ppm.

The procedure of Samuel (13) was followed for the determination of the residues in the feces and diet, with some modifications in the initial extractions and preparations: (a) Transfer a 10-g sample of fecal matter into a 250-ml centrifuge bottle. Add approximately 25 ml of ion-free water and 150 ml of acetonitrile and shake for 1 min. (b) Centrifuge for 20 min at 1500 rpm. Decant through glass wool into 500-ml Erlenmeyer flask. Repeat the extraction with 100 ml of acetonitrile, shake, centrifuge, and decant as before. (c) Flash evaporate until the acetonitrile is removed, leaving only the water layer. (d) Measure the water layer, transfer to a 500-ml separatory funnel, and add an equal amount of methylene chloride. Shake well and allow to settle for 15 min. Transfer the methylene chloride layer through an anhydrous Na_2SO_4 column into a 500-ml Erlenmeyer flask. Extract with three more portions of methylene chloride, add to the original extract, evaporate to dryness on a flash evaporator. (e) Add 50 ml of ethyl acetate to the sample and pour onto a column which is prepared by making 15 g of 1 part acid-washed attapulgus clay and 1 part Celite 545 (John Manville) topped with anhydrous Na_2SO_4. Attapulgus clay is substituted in place of charcoal (Norit A) (14) in cleanup section.

The procedure of Teasley and Cox (14) was followed for the determination of residues in urine. The PCB's were separated from DDT and its analogs as previously stated. Results of the residue analysis are shown in Table II and are expressed: (μg/day/subject) and (maximum percentage of intake excreted).

Summary and Conclusions. The pesticide residue levels in the diets, urine, and fecal matter were not affected by supplementation of the diets with amino acids, ammonium citrate, or calcium lactate. Pesticide residues were found in all samples of the diets, urine, and feces. With the exception of the chlorinated cyclodienes, excretion of all residues was greater in the feces, suggesting the feces as the primary route of excretion. Excretion of the PCB's was predominately through the feces with 6.2% of the total excretion appearing in the urine. With the exception of DDT and its metabolites, excretion of residues was 9 to 12%. Maximum excretion of DDT and its metabolites was approximately 32%.

TABLE II. Range and Maximum Percentage of Intake of Pesticide Residues and Polychlorinated Biphenyl (PCB) Levels in Diets, Urine, and Fecal Matter of Preadolescent Girls, 7 to 9 Years of Age.[a]

Sample types	Lindane		Kelthane		Chlorinated cyclodiene pesticides[b]		Arochlor (polychlor. biphenyl)[c]		DDT and metabolites[d]	
	(μg/day/ subject)	Max (%)	(μg/day/ subject)	Max (%)	(μg/day/ subject)	Max (%)	(μg/day/ subject)	Max (%)	(μg/day/ subject)	Max (%)
Diet	Tr[e]–1.8		Tr–1.8		Tr–1.8		Tr–840.0		Tr–18.0	
Urine	Tr–0.06	3.12	Tr–0.06	3.12	Tr–0.10	6.24	Tr–5.8	0.69	Tr–2.1	11.80
Feces	Tr–0.12	6.21	Tr–0.12	6.21	Tr–0.12	6.21	Tr–87.0	10.36	Tr–3.7	20.74

[a] 32 subjects, 40 days (2 duplicate experiments); Tr = trace.
[b] Aldrin, dieldrin, endrin, heptachlor, heptachlor epoxide.
[c] Sum of PCB types I (1242), II (1354), III (1260).
[d] DDT and metabolites, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDD, *o,p'*-DDT.
[e] Trace (<.001 ppm).

PCB's were found in all eight diets analyzed. Four contained traces and four contained 200, 420, 580 and 840 μg/day/subject. The FDA has not yet established tolerance levels for PCB. Albert Kolbye, deputy director for the FDA's Bureau of Foods has, however, recently stated that PCB is regarded as moderately toxic but certainly less toxic than DDT. He suggested that 150–300 μg/day would be within the safe range as far as temporary dietary intake is concerned (15). Eighty-eight percent of the ingested PCB's were not excreted. Presumably these have been retained in the body. The comparable percentage for DDT and its metabolites was 67.5%.

It is difficult to explain the presence of high levels of PCB found in these diets though PCB has been identified in such sources as sediments, river water, sewage outfalls, ink, newsprint, plastic bags, plastic products, poultry and animal tissues (16–23). The plastic containers used for storage may be a possible source of contamination, though this possibility has not been tested. The diets did consist of 20% fat which is considered normal for low income diets of the southeastern United States. Fats in the diets of the subjects before the study were primarily animal, whereas fats in the prepared experimental diets were chiefly vegetable.

The authors gratefully acknowledge the Southern Regional Nutritional Project (S-64) and its Technical Committee; the Department of Human Nutrition and Foods at Virginia Polytechnic Institute and State University, Blacksburg, VA for providing the samples and other technical data; technicians Patricia L. Marshall and Sylvie R. Beatty, who prepared and extracted the samples for analysis; and Dr. R. E. Webb for his suggestions in the preparation of the data for this article.

1. S. Regional Nutr. Res. Proj. (S-28) Bull. No. 64, Blacksburg, VA (1959).
2. S. Regional Nutr. Proj. (S-28) Bull. No. 94, Blacksburg, VA (1962).
3. S. Regional Nutr. Proj. (S-64) Bull. No. 129, Blacksburg, VA (1967).
4. S. Regional Nutr. Proj. (S-64) Bull. No. 170, Blacksburg, VA (1970).
5. Engel, R. W., Miller, R. F., and Price, N. O., *in* "Zinc Metabolism" (A. S. Prasad, ed.), Chap. 18, p. 326. Thomas, Springfield, IL (1966).
6. Engel, R. W., Price, N. O., and Miller, R. F., J. Nutr. **92**, 197 (1967).
7. Price, N. O., Bunce, G. E., and Engel, R. W., Amer. J. Clin. Nutr. **23**, 258 (1970).
8. Henninger, D., The National Observer, **Oct. 9**, 9 (1971).
9. Bell, W. B., Va. J. Sci. **3**, 169 (1952).
10. Engel, R. W., Price, N. O., Linkous, W. N., and Bell, W. B., Va. Agr. Exp. Sta. Tech. Bull. **121** (1953).
11. Vos, J. G., and Beems, R. B., Toxicol. Appl. Pharmacol. **19**, 617 (1971).
12. Armour, J., and Burke, J., J.A.O.A.C. **53**, 761 (1971).
13. Samuel, B. L., J.A.O.A.C. **49**, 346 (1966).
14. Teasley, J. I., and Cox, W. S., U.S. Dept. H.E.W., **1**, 11C, 4b (1965).
15. Kolbye, A. C., Chem. Eng. News **49**, 12 (1971).

16. Devos, R. H., and Peet, E. W., Bull. Environ. Contam. Toxicol. **6**, 1648 (1971).

17. Gutafson, C. G., Environ. Sci. Technol. **4**, 814 (1970).

18. Hayes, J. W., Jr., Dale, W. E., and Pirkle, C. I., Arch. Environ. Health **22**, 119 (1971).

19. Hubbard, H. L., *in* "Encyclopedia of Chemical Technology" (R. E. Kirk and D. F. Othmar, eds.), 2nd ed., Vol. 5, p. 289. Wiley (Interscience), New York (1964).

20. McCune, E. L., Savage, J. E., and O'Dell, B. L., Poultry Sci. **41**, 295 (1962).

21. Peakall, D. B., and Lincer, J. L., Bioscience **20**, 958 (1970).

22. Reynolds, L. M., Bull. Environ. Contam. Toxicol. **4**, 128 (1969).

23. Risebrough, R. W., Rieche, P., Herman, S. G., Peakall, D. B., and Kirren, M. N., Nature (London) **220**, 1098 (1968).

Received Nov. 5, 1971. P.S.E.B.M., 1972, Vol. 139.