

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

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

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-

The procedure followed for the determination of the feces and urine in the initial experiment was as follows: (a) Transfer a 100-ml sample into a 250-ml centrifuge bottle containing approximately 25 ml of acetonitrile and shake for 20 min. (b) Place the glass wool into a beaker of peat the extractant, trile, shake, centrifuge. (c) Flash evaporate the solvent removed, leaving the residue. Measure the weight of the residue.

TABLE	
PCB type	
1242	
1254	
1260	
1242, 1260	(50% r)
* Aroclor	

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

PCB types	Diets (%)				Feces (%)				Urine (%)			
	1	2	3	Av	1	2	3	Av	1	2	3	Av
1242	62	68	72	67	70	68	72	70	71	68	60	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

* Aroclor (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: ($\mu\text{g/day/subject}$) 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%.

DSW 031202

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.*

Sample types	Lindane		Kelthane		Chlorinated cyclodiene pesticides ^b		Arochlor (polychlor. biphenyl) ^c		DDT and metabolites ^d	
	($\mu\text{g/day/subject}$)	Max (%)	($\mu\text{g/day/subject}$)	Max (%)	($\mu\text{g/day/subject}$)	Max (%)	($\mu\text{g/day/subject}$)	Max (%)	($\mu\text{g/day/subject}$)	Max (%)
Diet	Tr-1.8		Tr-1.8		Tr-1.8		Tr-840.0		Tr-18.0	
Urine	Tr-0.00	3.12	Tr-0.00	3.12	Tr-0.10	6.24	Tr-5.8	0.89	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

* 32 subjects, 40 days (2 duplicate experiments); Tr = trace.

^b Aldrin, dieldrin, endrin, heptachlor, heptachlor epoxide.

^c Sum of PCB types I (1242), II (1854), 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 $\mu\text{g/day/subject}$. 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 $\mu\text{g/day}$ 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. Des Contam.
17. Gu (1970).
18. Ha
19. Hu
Technol
eds.), 2nd
New Yor

polychlorinated
hydrocarbons of Age.

DIT and
metabolites

g/day/ Max
subject (%)

Cr-18.0
Cr-2.1 11.80
Cr-3.7 20.74

Institute and
providing the
technicians Patri-
Beatty, who
analysis; and
stions in the

28) Bull. No.

Bull. No. 94,

Bull. No. 129,

Bull. No. 170,

Price, N. O., in
ed.), Chap.
6).

Miller, R. F., J.

Engel, R. W.,

over, Oct. 9, 9

nkous, W. N.,
Tech. Bull. 121

Toxicol. Appl.

O.A.C. 53, 761

(1966).
U.S. Dept.

News 49, 12

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.

DSW 031204