Tissue Storage and Excretion in Milk of Polybrominated Biphenyls in Ruminants

In 1973–1974 many cattle and other farm animals became ill or died in Michigan from ingesting a flame retardant, consisting of a mixture of polybrominated biphenyl isomers (PBB) which had been inadvertently included in their rations. In the work reported, a lactating cow and a sheep were fed 50 ppm of PBB in their rations. High concentrations of PBB were excreted in milk and stored in the animal tissues. The disappearance rate ($t_{1/2}$) for PBB in milk following cessation of dosing was about 10.5 days. Post-mortem examination revealed marked glandular hyperplasia of the major intrahepatic bile ducts of the liver of the cow and the gall bladder of the sheep, an unusual condition whose only known cause in farm animals is ingestion of chlorinated naphthalenes.

FireMaster PB-6 is a flame retardant used in the textile industry consisting of a mixture of polybrominated biphenyl isomers (PBB) manufactured by the Michigan Chemical Corporation (St. Louis, Mich.). Its reported percentage isomeric composition of various brominated biphenyls is tetrabromo (2.0), pentabromo (10.6), hexabromo (about 63), and heptabromo (13.2), with the remainder unidentified. In 1973, hundreds of cattle among certain Michigan herds were reportedly ill or dying, the suspected cause being inadvertent ingestion of PBB. The material had somehow been confused with a magnesium oxide product of the above company, normally added to farm animal rations at a rate of about 0.4% to supplement those containing crop material low in magnesium. By early 1975, 11,000 cattle, 2000 hogs, 393 sheep, 1.5 million chickens, 4.6 million eggs, 753 tons of feed, and quantities of animal byproducts which were contaminated had to be disposed of. Also, PBB was detected in blood of 211 exposed farmers (*Chem. Eng. News*, 1975). PBB shows low oral (the LD_{50} for rats is 21.5 g/kg) and dermal (LD_{50} for rabbits is 2.15–10.0 g/kg) toxicity.

Several experimental cattle feeding studies (Platonow et al., 1971; Saschenbrecker et al., 1972; Fries et al., 1973) have been conducted with the related polychlorinated biphenyl isomeric mixture (PCB) illustrating their persistent secretion in milk and tissue storage. The only published work involving PBB in cattle is that of Jackson and Halbert (1974) on the toxic syndrome which resulted when the feed, first suspected of contamination in Michigan, was fed to cattle. In the work reported, PBB was fed to a cow and a sheep to study its elimination pattern in milk, tissue storage, and possible toxic effects.

EXPERIMENTAL SECTION

Feeding Experiments. A Holstein cow weighing 682 kg and with a daily average milk production of 7.4 kg was fed 1.13 g of PBB daily for 15 days. This corresponded to a concentration of 50 ppm (based on a daily ration of 22.7 kg). PBB in ethyl acetate was thoroughly mixed with the evening grain. The animal received 1.36 kg of grain in the morning and evening and hay ad libitum. The animal consumed the PBB-fortified feed completely during the first 2 days but would not thereafter. Crystalline PBB was therefore administered as one daily 1.13-g dose using a balling gun for the remaining 13 days. Morning and evening subsamples of the total mixed milk were taken 1 day prior to feeding (control sample), daily throughout the feeding period, and for 15 days thereafter.

The sheep was a wether weighing 63.6 kg. The animal was fed 50 mg of PBB per day in 1 kg of pelleted feed as a complete ration for 30 days. PBB in ethyl acetate was mixed with the feed. The animal consumed the feed completely throughout the experiment. At the end of the feeding period the cow and sheep were sacrificed by electrocution and tissues were examined pathologically and collected for analysis. Corresponding tissues were sampled from a lactating cow and a wether following slaughter which had received no PBB to serve as controls. Prior to chopping, mixing, and subsampling for analysis, meticulous care was taken to trim away all external fat from the various tissues so that the residues determined would truly represent those in the organ per se.

ANALYTICAL PROCEDURE

The isolation of PBB was performed using the published method (Pesticide Analytical Manual, 1971) involving fat extraction, isolation using acetonitrile partitioning, and column chromatography on Florisil. Final determination was made by electron affinity gas chromatography. The instrument was a Barber-Colman Model 10 with a battery-operated No. A-4071, 6-cm^3 electron affinity detector containing 56 μCi of radium-226. The recorder was a Leeds and Northrup, 0–10 mV equipped with 18-cm chart paper running 38 cm/hr. The electrometer gain setting was 3000. The column was U-shaped, made of borosilicate glass, 6 mm i.d., 61 cm long, and containing 5% OV-17 on 80–100 mesh acid-washed Chromosorb W. The operating temperatures for the column, flash heater, and detector were 240, 280, and 270°, respectively, and nitrogen (80 cm^3/min) was the carrier gas. PBB eluted as five discernible peaks with retention times of 7.4, 12.3, 18.0, 24.0, and 28.2 min. Quantitation was made by measuring the height of the third and largest peak eluting at 18.0 min which possibly represented the hexabromo isomer. The recoveries of PBB added to control samples are listed in Table I.

RESULTS AND DISCUSSION

The pattern of PBB excretion in milk is illustrated in Figure 1. The cow excreted a total of 376.7 mg of PBB in the milk during the 31-day period or 2.22% of the total dose (16.95 g). Daily milk production did not decrease appreciably during the 30-day period although daily consumption of hay diminished. Jackson and Halbert (1974) similarly noted anorexia in cattle whose forage was suspected to contain PBB.

Figure 2 shows a semilogarithmic plot of the disappearance of PBB in milk during the 15-day period (day 16 through day 30) following cessation of PBB dosing. In Figure 2 the y intercept = 10.74 and the slope = −0.066. The linear correlation coefficient (r) was 0.95. The half-life ($t_{1/2}$) for PBB in milk is therefore 10.47 days. Based on this value for $t_{1/2}$, after 10 half-lives, 105 days, total daily excretion of PBB in milk would diminish to less than 0.01 mg/day.

Residues of PBB found in the tissues of the animals are listed in Table II. With the exception of thyroid, residues of PBB are consistently up to several fold higher in the sheep tissues. This may be due in part to more efficient intestinal absorption by the sheep which consumed its feed containing PBB as compared to the cow which had to be force-fed using capsules. Jackson and Halbert (1974) similarly found high concentrations of PBB in milk and fat deposits of cows foraging on PBB contaminated food.

Figure 3 shows gas chromatograms of PBB in heart tis-

Table I. Recovery of PBB Added to Control Samples

Sample	Added, ppm (fresh wt)	Recovery, % Cow	Recovery, % Sheep
Milk	0.01	75	
	0.02	79, 64	
	1	83	
	2	80	
Adrenal	5	69	81
Brain	1	71	100
Fat (brisket)	5	78	100
(omental	5	75	84
(renal)	5	63	88
Heart	1	70	100
Kidney	1	85	90
Liver	1	75	103
Mammary	2	78	
Muscle (chuck)	1	73	80
(loin)	1	78	78
Spleen	1	83	90
Thyroid	5	73	79

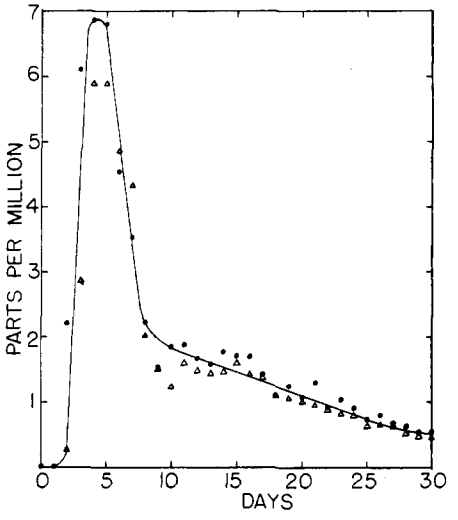

Figure 1. The pattern of excretion of PBB in milk. Feeding of BP-6 began on the first day and ended on the 15th day: a.m. milk (△); p.m. milk (●).

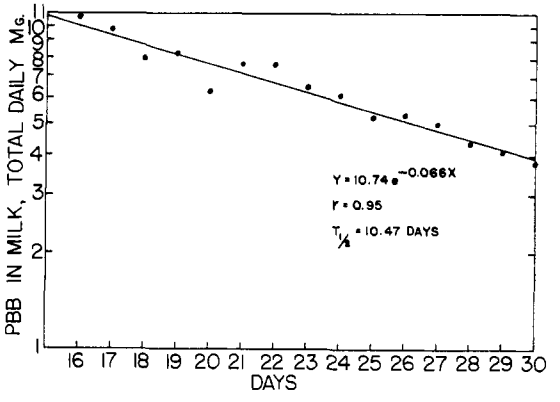

Figure 2. Semilogarithmic plot of disappearance of PBB in milk during the period from day 16 through day 30 following cessation of PBB dosing on day 15.

Table II. Parts per Million (Fresh Weight) of PBB in Animal Tissues Uncorrected for Percent Recovery

Tissue	Cow Control	Cow Fed BP-6	Sheep Control	Sheep Fed BP-6
Adrenal	<0.3	0.4	<0.5	1.8
Brain	<0.1	0.5	<0.1	1.9
Fat (brisket)	<0.4	3.8	<0.6	17.2
(omental)	<0.6	9.2	<1.0	25.0
(renal)	<0.6	10.0	<1.0	41.6
Heart	<0.1	1.0	<0.1	4.3
Kidney	<0.1	0.5	<0.1	0.9
Liver	<0.1	3.1	<0.1	12.0
Mammary	<0.2	1.3		
Muscle (chuck)	<0.1	1.3	<0.1	8.0
(loin)	<0.1	1.2	<0.1	6.0
Spleen	<0.1	0.4	<0.1	0.9
Thyroid	<0.4	3.1	<0.5	2.2

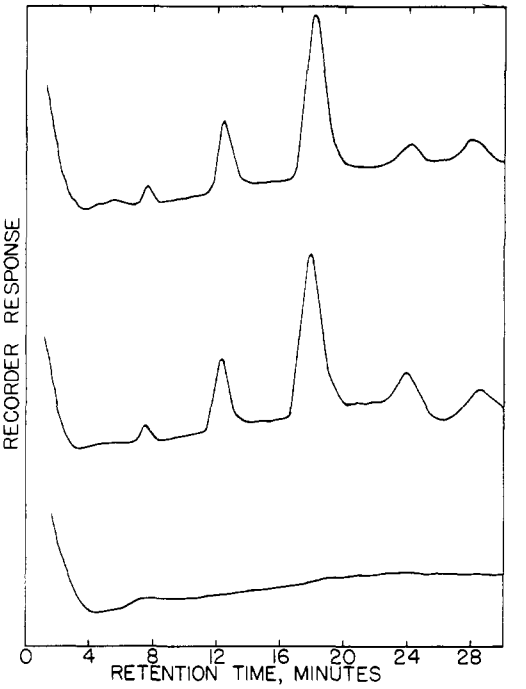

Figure 3. Gas chromatograms showing: (top) PBB in the heart tissue of the sheep fed the mixture for 30 days, (middle) recovery of 1 ppm of BP-6 added to heart tissue of control sheep, and (bottom) heart tissue of control sheep.

sue of the dosed sheep as well as those of the recovery of 1 ppm of PBB added to control heart tissue and of the control heart tissue itself. It was observed that the peak eluting at 12.3 min (which is possibly the pentabromo isomer of PBB) was relatively smaller in height than those of the other PBB isomers in the chromatograms of the milk samples as compared to its relative height in the chromatograms of standards or recoveries. This may have been due to more rapid in vivo metabolism of this isomer. The route of metabolism of PBB isomers is possibly ring hydroxylation and urinary and fecal excretion as conjugates but this possibility was not investigated. Excretion of intact PBB in the animal feces was probably also considerable based on the concentrations found in feces of Japanese quail fed PBB (Babish et al., 1975).

Post-mortem examination revealed marked glandular hyperplasia of the major intrahepatic bile ducts of the liver in the cow and the gall bladder of the sheep. This unusual condition is characteristic of hyperkeratosis, the only known cause of which in farm animals is ingestion of chlorinated naphthalenes (Clarke and Clarke, 1970). Symptoms of hyperkeratosis in cattle foraging on PBB contaminated feed were also observed by Jackson and Halbert (1974).

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A Specific Gas–Liquid Chromatographic Method for Analysis of Some Amine Salts of 2,4-Dichlorophenoxyacetic Acid

The methyl-, dimethyl-, *n*-butyl-, and *n*-dodecylamine salts of 2,4-dichlorophenoxyacetic acid (2,4-D) were analyzed by conversion to the corresponding amide and subsequent gas–liquid chromatographic analysis. As the amide function retains the identity of the original acid and amine, the original amine salt can be identified.

The herbicidal halophenoxyacetic acids are usually formulated as a mixture of esters, or of amine salts. It is often desirable to know which derivative was originally present.

The amine salts of 2,4-dichlorophenoxyacetic acid (2,4-D) are too involatile for direct gas–liquid chromatography. Thus, these compounds need to be derivatized to more volatile forms to take advantage of the sensitivity of this method of analysis. Various derivatives of 2,4-D can be quantitated individually by such methods as alkaline hydrolysis with subsequent free acid isolation and then esterification (Que Hee et al., 1975; Henshaw et al., 1975), or by total acid equivalent methods (Horwitz, 1970). Unfortunately, the quantitative nature of these methods is at the expense of the specificity of the derivative originally present, although the type of halophenoxyacetic acid present can be found (Henshaw et al., 1975). In the case of the amine salts, previous work (Que Hee and Sutherland, 1974) has shown that the corresponding amides are produced after pyrolysis. These pyrolysis products retain the identity of both acid and amine, and so the original amine salt can be identified. This paper presents a specific analytical technique for some amine salts of 2,4-dichlorophenoxyacetic acid (2,4-D) based upon this fact.

EXPERIMENTAL SECTION

Reagents. Commercial *n*-dodecyl- and *n*-butylamines (Aldrich) were purified by vacuum distillation. Commercial 2,4-D (Aldrich) was recrystallized from benzene until a constant melting point of (140–143 ± 0.5°) was attained. The purity of these chemicals was confirmed by NMR and mass spectroscopy. Reagent grade dimethylamine (Eastman) and a 20% aqueous solution of methylamine were used without further purification.

Salts (1:1) of 2,4-D were made by adding stoichiometric amounts of the amines dissolved in benzene–acetone solutions to solid 2,4-D at 10°. The solutions were shaken until all the 2,4-D had disappeared. The solvent was then removed under vacuum at room temperature. The resultant salts were recrystallized from ether–acetone–hexane (1:1:1) by volume to constant melting points.

Pyrolysis Experiments. Known masses of solid salts (ca. 200 mg) were pyrolyzed directly in sealed Pyrex tubes covered with aluminum foil for 1 hr at 160 and 190°. The tubes were then cooled. Experiments were done in triplicate.

Quantitation and Analysis. The pyrolyzed salts and equivalent amounts of corresponding nonpyrolyzed salts were dissolved in known volumes of methanol and also of acetonitrile. In the case of the methylamine salt, enough methanol was added to the acetonitrile solution to dissolve the salt.

Known aliquots (ca. 25 μl) of pyrolyzed salts were injected onto a 6-ft long × 3.5 mm i.d. copper column packed with 10% SE-30 on 60–80 mesh Chromosorb W (DMCS-AW). The injector, column, and thermal conductivity detector were maintained at temperatures of 200, 170, and 210°, respectively. The flow of helium carrier was 25 ± 1 ml/min. The filament current was 200 mA. The separated amides were collected manually in tared glass tubing, cooled externally. The weight of collected amide was compared with the amount of amide expected if pyrolysis was 100% efficient, and the purified amides then used to quantitate the amide produced in the original pyrolysis. This was done by injecting known masses of purified amide and constructing calibration curves using a 6 ft × 3.5 mm i.d. Pyrex U-tube column packed with 10% SE-30 impregnated