Binding of 2,2',4,4',5-Pentabromodiphenyl ether (BDE-99) and/or its metabolites to mammalian urinary and biliary carrier proteins

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INTRODUCTION

Polybrominated diphenyl ethers (PBDE) are used as flame retardants in the textile and electronics industries, and are globally produced in the range of 150,000 tons annually[1)]. They are lipophilic, as are other members of the polyhalogenated aromatic hydrocarbon family and, therefore, would also be expected to bioaccumulate in the environment and animal tissue. Recent reports have documented their presence in human plasma [2)], milk [3)], and adipose tissue [4)], and in aquatic species such as sperm whales, harbor seals, and whitebeaked dolphins [5)].

2,2',4,4',5-Pentabromodiphenyl ether (BDE-99) is the second most abundant member of the PBDE family in the environment, following 2,2',4,4'-tetrabromodiphenyl ether (BDE-47). Based on its lipophilicity, BDE-99 would be expected to require carrier proteins for mammalian *in vivo* transport. The purpose of the present study was to administer a single oral dose of BDE-99 to male rats, and investigate the binding with proteins present in the urine and bile.

EXPERIMENTAL

2,2',4,4',5-Pentabromo-[^{14}C]diphenyl ether (BDE-99), synthesized in-house by accepted methods [6)], was administered orally to six conventional male Sprague-Dawley rats, and ten bile-duct cannulated rats (2.2 mg/rat in peanut oil; 1.0 μCi). Urine and bile were collected every 24h for three days. The excreta were individually chromatographed by size exclusion on Sephadex G-75 (4.5 x 90cm) and Sephacryl S-200 (2.2 x 85cm), as described previously [7)]. The columns were eluted with 0.05 M phosphate buffer (pH 7.2). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 13% acrylamide) and immunoblot analysis (Western blot) were performed as described previously [8)]. TLC analysis was performed on aliquots of urine and bile after concentration by rotary evaporation. TLC plates (silica gel) were developed in 50:50 hexane:methylene chloride with a standard lane containing BDE-99.

RESULTS

Daily excretion of ^{14}C from BDE-99 into the urine or bile of male rats was minimal. Cumulative elimination into conventional or cannulated urine was less than 1% of the administered dose (Table 1). Cumulative biliary elimination was slightly higher (3.7%). TLC analysis of both urine and bile showed that metabolism was necessary for BDE-99 elimination in

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the urine or bile. These data suggest that metabolism of BDE-99 into water soluble metabolites by the xenobiotic metabolizing cytochromes was a stereochemically restricted process.

Due to low levels of radioactivity in the daily collections from conventional and cannulated rats, urine from all three days was pooled prior to G-75 chromatography (Figure 1A). The separation of 0-72h conventional rat urine revealed that 6.3% of the ^{14}C was protein bound (Table 2). The bound portion was associated exclusively with $\alpha 2_u$ globulin ($\alpha 2_u$). In the present study, none of the ^{14}C in urine from cannulated rats was bound to protein (Table 2). Presumably, BDE-99 metabolites formed in cannulated rat urine were sufficiently polar, and did not require a carrier system for excretion via the urine.

$\alpha 2_u$ is the chief protein component in male rat urine, and is hepatically synthesized under androgenic control [9]. The function of $\alpha 2_u$ has not been firmly established, however, it appears to be a pheromone carrier protein[10] and necessary to maintain spermatogenesis[11]. It belongs to a family of low molecular weight transport proteins, which includes human retinol binding protein (RBP) and bovine β-lactoglobulin (BL). $\alpha 2_u$, RBP, and BL have a similar eight-stranded β-barrel secondary structure, which serves as a hydrophobic ligand binding cavity. The cavity of each protein is lined with different hydrophobic amino acids which confers the specificity. A difference in specificity for xenobiotic binding apparently does exist with the proteins in rat urine, because data obtained from toxic[12] and non-toxic dioxins[13] demonstrated that the majority of urinary dioxins were preferentially bound to albumin, and minor amounts to $\alpha 2_u$.

A significant portion of the bile ^{14}C was protein bound following G-75 (Figure 1B). After further chromatography on S-200, SDS-PAGE, and linear regression MW analysis with standards, it was determined that the ^{14}C was bound to a protein of 79 kDa. The amount bound increased with time during the experiment, and ranged from 28-47% (Table 2). Previous work with dioxins[13] had also shown that this novel 79 kDa protein in rat bile binds polyhalogenated aromatics or their metabolites. The identity or role of this protein is not known, but our studies indicate it is an N-terminally blocked, monomeric protein with an isoelectric point of 5.7. The native role of this protein, as well as $\alpha 2_u$, may be affected by binding to BDE-99 metabolites.

Table 1: Recovery of ^{14}C in the urine and bile of male rats dosed orally with 2,2',4,4',5-pentabromo-[^{14}C]diphenyl ether (BDE-99).

	Percent of Dose	
Excreta	Conventional (n=6)	Bile-duct Cannulated (n=10)
Urine		
0-24 h	0.4 ± 0.06	0.1 ± 0.08
24-48 h	0.3 ± 0.1	0.2 ± 0.2
48-72 h	0.2 ± 0.01	0.05 ± 0.03
Bile		
0-24 h	----	0.6 ± 0.8
24-48 h	----	1.7 ± 1.1
48-72 h	----	1.4 ± 1.0

Table 2: Protein binding of 2,2',4,4',5-pentabromo-[^{14}C]diphenyl ether (BDE-99) metabolites in rat urine and bile.

Excreta	% Unbound	% Bound			
			Albumin	$\alpha 2_u$	79 kDa
Conventional					
0-72 h Urine	88.0	6.3	---	6.3	---
Bile-duct cannulated					
0-72 h Urine	100.0	---	---	---	---
0-24 h Bile	61.0	28.4	---	---	23.9
24-48 h Bile	58.0	34.4	---	---	29.9
48-72 h Bile	43.2	46.9	---	---	44.6

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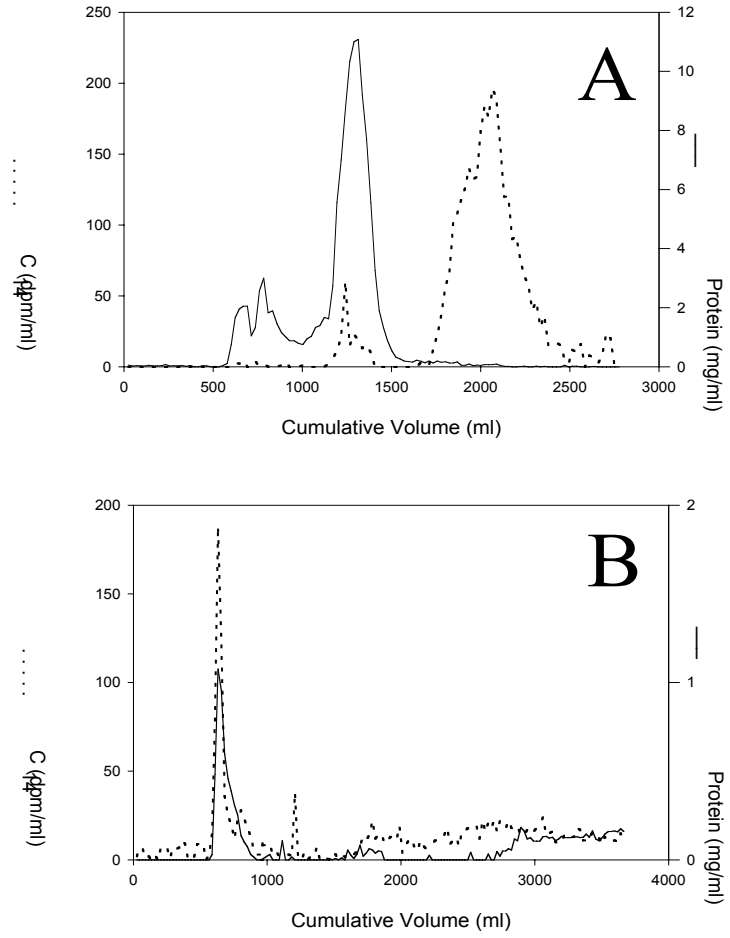

Figure 1. Elution pattern from Sephadex G-75 of 0-72h conventional rat urine (A) and 0-24h bile (B) following a single oral dose of 2,2',4,4',5-pentabromo-[^{14}C]diphenyl ether (BDE-99).
Fractions were assayed for protein (—) and radioactivity (- - -).