Metabolism and Disposition of the Flame Retardant Tetrabromobisphenol A in Conventional Rats and Rats with Cannulated Bile Ducts

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Introduction

Tetrabromobisphenol A [4,4'-isopropylidenebis(2,6-dibromophenol)] (TBBPA) has been used as a flame retardant in printed circuit boards found in electronic equipment such as TV sets, computers and copying machines for many years. Recently, TBBPA has been detected in air sampled from office and computer rooms[1.] and in human serum at 15 ppb.[2.] In the late 1970's TBBPA was detected in stream sediments near two unidentified manufacturing sites in the USA and in stream water near one of the sites.[3.] In the early 1980's TBBPA was detected in river sediments in Japan in the concentration range of 22-140 ppb(dry wt.) and was reported to have undergone microbial methylation.[4.] To our knowledge this later study represents the only metabolic information available on TBBPA. In this study we investigated the metabolism and disposition of TBBPA in the rat.

Material and Methods

Bis-[^{14}C]phenol A was prepared from [^{14}C]phenol (2.0 mCi, 25 mCi/mmol) and acetone.[5.] The product isolated was brominated by 4.2 equivalents of bromine in methanol:water (1:1). The specific activity of the 3,3',5,5'-tetrabromo-bis-[^{14}C]phenol-A isolated was 48.4 mCi/mmol as determined by gas chromatography and radioactivity measurements.

Male Sprague-Dawley rats (300-350g) were each given a single oral dose of ^{14}C-ring labelled TBBPA (2 mg/kg body wt.). Eight rats (275-325g) were bile duct cannulated as previously described[6.] and dosed as described above. Bile, urine and faeces were collected every 24 hours for three days. The animals were anesthetized with halothane, a ventral midline incision made, and killed by exsanguination from the dorsal aorta. Blood, heart, lungs, kidneys, thymus, fat, testes, small and large intestine, and carcass were collected. Urine and bile samples were assayed for ^{14}C by pipetting an aliquot into scintillation cocktail and counting the sample

in a liquid scintillation counter (LSC). Lyophilized faeces, blood, and homogenized tissues were combusted in a sample oxidizer and $^{14}CO_2$ was analyzed by LSC. Faecal samples were freeze-dried and extracted successively with ethyl acetate and methanol. Bile samples collected from 0-24h, 24-48h and 48-72h were each applied to a Porapak Q column.[6] The column was eluted first with water followed by methanol. The methanol fraction was taken to dryness, solubilized in water and applied to a Sephadex LH20 (LH-20) column eluted with water.[6] Radioactive fractions eluting from the LH-20 column were each subjected to reverse-phase HPLC chromatography with a water/acetonitrile mobile phase, beginning with a 95% water a six step gradient to 100% acetonitrile. The isolated metabolites were treated with β-glucuronidase (Type VII-A, E. coli) or arylsulfatase as described later.

Freeze-dried 0-24h, 24-48h and 48-72h faeces were each extracted with ethyl acetate followed by methanol. Each fraction was subjected to chromatography on HPLC as described above.

Isolated bile and faecal metabolites were subjected to negative ion fast atom bombardment mass spectrometry (-FAB/MS), negative ion electrospray mass spectrometry, or were derivatized [methoxy (diazomethane), or TMS (trimethylsilyl, Regisil)] and analyzed by GC/MS.

A VG Auto Spec with a cesium gun was used for FAB/MS. GC/MS was performed on a VG Auto Spec (70 eV) with a HP5890GC using a 15 m DB5MS column (70°C to 310°C @ 10°C/min). Electrospray mass spectrometry was conducted on a HP 5988 mass spectrometer retrofitted with an atmospheric pressure ionization source (source voltages: Vcyl = 3065 volts, Vend = 3618 volts and Vcap = 4052) from Branford, Inc. (Branford, CT) using a Harvard syringe pump to pump H_2O:MeOH (1:1) at 50 µl/min.

Results and Discussion

Most of the radioactivity was excreted in the bile and faeces (71% and 92%, respectively, Table 1) 72 hrs after dosing. Biliary excretion levels peaked in the first 24 hrs (48%) while faeces excretion levels peaked 24h later (24-48h, 66%). These excretion data indicate that biliary radioactivity undergoes enterohepatic circulation. Because limited amounts of radioactivity were excreted in urine (0.3% of the dose) in 72 h, metabolites were not pursued. About 2.1% of the dose remained in the tissues. Tissues with the highest levels of radioactivity were lung, carcass, and small and large intestinal tracts (0.2%, 0.2%, 0.7%, and 1.0% of dose, respectively; Table 1).

Salts were removed from the bile in the Porapak Q column water wash and ^{14}C-TBBPA metabolites were eluted in the methanol fraction. Three 0-24h [^{14}C]-peaks were separated on LH20 from the biliary Porapak Q methanol fraction. Negative ion electrospray mass spectrometry of the first [^{14}C]-peak purified by HPLC gave a mass spectrum consistent with the diglucuronide conjugate of TBBPA (891, $[M-H]^-$; 913, $[M-2H+Na]^-$; 715, [M-glucuronide (GluUA)]$^-$; and 539, $[M-2GluUA+H]^-$). All four of these masses contained a four bromine isotope cluster. This fraction represents 34% of the radioactivity excreted in 0-24h bile. The second [^{14}C]-peak was purified on HPLC and gave a -FAB/MS consistent with the sulfate ester-glucuronide diconjugate of TBBPA (795, $[M-H]^-$; 817, $[M-2H+Na]^-$; 737, $[M-2H-SO_3+Na]^-$; and 537, $[M-2H-SO_3-GluUA]^-$). This metabolite represented 21% of the radioactivity excreted in 0-24h bile. The third [^{14}C]-peak gave a negative ion FAB/MS mass spectrum consistent with the monoglucuronide of TBBPA (715, $[M-H]^-$; 737, $[M-2H+Na]^-$ and 539, $[M-GluUA]^-$).

Additionally, this third peak was treated with β-glucuronidase and subsequently derivatized with diazomethane. The GC/MS of the methylated aglycone gave a mass spectrum identical to authentic methylated TBBPA (568, $[M]^{+}$). This monoglucuronide represented 45% of the radioactivity excreted in the 0-24h bile.

The major metabolite detected in the ethyl acetate and methanol extracts of faeces collected over the three day period was TBBPA. The TMS derivative of the sample purified by HPLC gave a mass spectrum identical to the TMS derivative of authentic TBBPA (diTMS; 684, $[M]^{+}$ and 669, $[M-15]^{+}$). In 0-24h, 24-48h and 48-72h faeces 96%, 97% and 92%, respectively, of the faecal radioactivity was found to be TBBPA. It should be noted from these results that the gut flora deconjugated the glucuronide and sulfate ester conjugates excreted in the bile.

Summary

Most (71%) of a single oral dose of ^{14}C-labelled TBBPA was excreted in bile in 72h with 48% excreted in the first 24h. The characterized biliary metabolites were a diglucuronide, a monoglucuronide and a glucuronide-sulfate ester conjugate of TBBPA. The major biliary metabolite was the monoglucuronide. In the conventional rat, 95% of the ^{14}C-TBBPA was excreted in the faeces as TBBPA. Most (66%) of the faecal excretion occurred in the 24-48h period. This delay in fecal excretion of TBBPA resulted from enterohepatic circulation of TBBPA because the biliary TBBPA conjugates were deconjugated and reabsorbed in the lower gut, reconjugated and re-excreted in the bile. About 2.0% of the dose remained in the rat 72h after dosing with the highest levels in the large (1.0%) and small (0.6%) intestine, lung (0.2%) and carcass (0.2%).

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Table 1. Recovery of ^{14}C from conventional and bile-duct cannulated rats dosed orally with [^{14}C]-TBBPA.

		Conventional (n=9)		Bile-duct Cannulated (n=7)	
Urine	0-24	0.11 ± 0.07		0.38 ± 0.40	
	24-48	0.19 ± 0.07		0.32 ± 0.40	
	48-72	0.02 ± 0.02		0.03 ± 0.04	
Subtotal for urine			0.32 ± 0.07		0.73 ± 0.84
Bile	0-24			48.4 ± 15.6	
	24-48			21.0 ± 13.1	
	48-72			1.87 ± 1.97	
Subtotal for bile					71.3 ± 7.2
Faeces	0-24	6.64 ± 7.13		6.03 ± 6.02	
	24-48	65.6 ± 22.7		15.3 ± 5.61	
	48-72	19.5 ± 8.78		4.99 ± 5.34	
Subtotal for faeces			91.7 ± 17.4		26.3 ± 6.33
Spleen		0		0	
Heart		0		0	
Liver		0.06 ± 0.04		0.05 ± 0.05	
Thymus		0		0	
Lung		0.23 ± 0.26		0.07 ± 0.12	
Fat		0		0	
Kidney		0.003 ± 0.001		0.001 ± 0.001	
Carcass		0.15 ± 0.12		0.33 ± 0.50	
Small intestine		0.68 ± 0.61		0.16 ± 0.18	
Large intestine		0.96 ± 0.66		0.37 ± 0.33	
Testes		0		0	
Subtotal for tissues			2.08		0.98
Total recovered			94.1%		99.3%