incubation media is an added proof of previous reports.

Contrary to a previous report for chicken liver preparations (Akhtar and Foster, 1977), addition of GSH (8.7×10^{-6} mol) to freshly prepared soluble fraction from sheep, cow, and pig livers produced only a small increase (approximately 10%) in the amount of I which was metabolized (Table III). This implies that the concentration of either GSH or *S*-transferase, or both, is higher in sheep, cow, and pig preparations than in that from chicken liver. This observation is in agreement with the report (Johnson, 1966) that dealkylation of iodomethane, under comparable conditions, by liver preparations from ox and pig was four to five times greater than by chicken liver.

Donninger et al. (1972) observed oxidative metabolism of I to desmethyl tetrachlorvinphos and formaldehyde by the microsomal preparation from dog liver and reported that 19 and 25% of I was metabolized in 30 min and 1 h, respectively. In the present study, when I was incubated with microsomal preparations from cow and pig liver, only 20–30 was converted in 4 h to a water-soluble metabolite that was identified as desmethyl tetrachlorvinphos. It appears that microsomal enzyme(s) has a less significant role in the metabolism of I. Therefore, this aspect was pursued no further.

The data presented show that all three species possess an effective GSH-dependent enzyme system(s), *S*-transferase(s), capable of degrading tetrachlorvinphos into a water-soluble metabolite. The soluble fraction (105000*g*) also contains enzyme systems such as hydrolase, dechlorinase, (probably sulfhydryl-dependent dechlorinase; Akhtar, 1978), and reductase. The data also indicate that in pig, cow, and sheep liver, it is the enzymes in the soluble fraction which are predominant in the metabolism of the insecticide, since microsomal enzyme metabolism proceeded much more slowly. Based on the details above, a metabolic pathway of tetrachlorvinphos by soluble fraction (105000*g*) from sheep, pig, and cow is shown in Figure 1. In vivo studies with the lactating cow are presently under investigation.

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A Metabolite of Polybrominated Biphenyls: Its Identification and Decomposition to a Brominated Dibenzofuran in the Gas Chromatograph–Mass Spectrometer

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The feces of dogs fed Firemaster BP-6, a mixture of polybrominated biphenyls, contained a metabolite identified as 6-hydroxy-2,4,5,2′,4′,5′-hexabromobiphenyl (hydroxy-HBB). Mass spectrometry (MS), thin-layer chromatography (TLC), and gas chromatography (GC) were used to compare the metabolite and synthetic hydroxy-HBB. The substitution pattern of the synthetic hydroxy-HBB was determined by comparing its nuclear magnetic resonance spectrum with that of 2,4,5,2′,4′,5′-HBB. MS analysis showed that, during GC on OV-101 columns at 230 to 260 °C, hydroxy-HBB unexpectedly decomposed into two pentabromodibenzofurans (PDBF). The hydroxy compound could be differentiated from the PDBF artifacts by TLC, infrared spectroscopy, formation of an acetate derivative, and MS analysis, using direct probe insertion into the source. Similar tests should be used to corroborate GC–MS data that indicate the presence of brominated dibenzofurans as contaminants or metabolites.

Polybrominated biphenyls (PBBs) are chemically similar to polychlorinated biphenyls (PCBs), a class of widespread environmental pollutants (Hutzinger et al., 1974). PBB has been found in milk, eggs, meat, and human blood in Michigan due to inadvertent feed contamination with Firemaster BP-6, a flame retardant (Hoeting, 1976). Firemaster BP-6 is a PBB mixture with 2,4,5,2′,4′,5′-hexabromobiphenyl (HBB) as its major component (Sundström et al., 1976a; Jacobs et al., 1976). (See Figure 1A for numbering of substituent positions in the biphenyl nucleus.)

It is often desirable to study the animal metabolism of industrial chemicals found as food contaminants because the metabolites may be more toxic than the parent materials. While examining fecal extracts for possible toxic metabolites of PBB by gas chromatography–mass spectrometry (GC–MS), we observed a substance having a mass spectrum of a brominated dibenzofuran (DBF). (See Figure 1B for dibenzofuran structure.) Certain chlorinated DBFs are known and are very toxic compounds (Kimbrough, 1972; McKinney et al., 1976). They have been found as contaminants of PCB (Bowes et al., 1975) and there is evidence that they were excreted in the urine of

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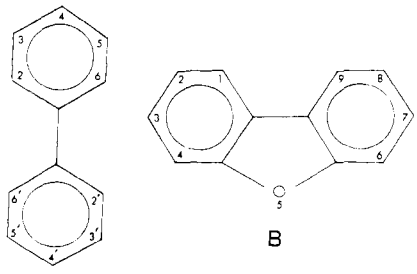

Figure 1. Numbering of the biphenyl (A) and dibenzofuran (B) nucleus.

rats fed PCB (Curley et al., 1975).

This report describes the subsequent identification of the PBB metabolite found in the feces of dogs fed PBB as a hydroxy-PBB. The conversion of 2-hydroxy-PBBs to DBFs at GC temperatures is also discussed.

EXPERIMENTAL SECTION

Reagents. Organic solvents used for chromatography, synthesis, and extraction were distilled in glass (Burdick and Jackson Laboratories, Inc.).

Standards and Derivatives. Purified 2,4,5,2′,4′,5′-HBB and Firemaster BP-6 (bag code 3124) were obtained from the reference materials collection of the Division of Chemical Technology, Food and Drug Administration, Washington, D.C. Synthetic 6-hydroxy-2,4,5,2′,4′,5′-HBB was prepared by mixing 250 mg of anhydrous aluminum chloride into a solution of 200 μL of bromine, 50 mg of 2-hydroxybiphenyl (Aldrich, gold label), and 0.5 mL of methylene chloride in a test tube at room temperature. The solution was repeatedly evaporated to dryness and dissolved in methylene chloride until the color due to bromine disappeared. One milliliter of distilled water was added to decompose the aluminum chloride. 6-Hydroxy-2,4,5,2′,4′,5′-HBB was then separated from the other brominated hydroxybiphenyl products in the methylene chloride layer by TLC, using 50% benzene–hexane as the developing solvent. The R_f of the main product was 0.62. The acetate of 6-hydroxy-2,4,5,2′,4′,5′-HBB was prepared by reacting 1 mL of acetic anhydride with 0.5 mg of 6-hydroxy-HBB in a test tube for 10 min. Unreacted acetic anhydride was evaporated with a jet of nitrogen. Synthetic 6-hydroxy-2,4,5,2′,4′,5′-HBB was thermally decomposed to DBFs by heating 1 to 5 μg in a glass test tube capped with aluminum foil for 20 min in an oven at temperatures ranging from 230 to 260 °C. (Caution: Small amounts of halogenated DBF may be toxic. Samples should be handled the same as other very toxic materials in the laboratory.) Solutions of 1–5 μg of DBF in 0.1–5 mL of hexane or ethyl acetate were used for chromatographic analyses. The solvent was evaporated with nitrogen for sampling DBF for infrared (IR) analysis.

Instruments. IR spectra were obtained on a Perkin-Elmer Model 180, using the micro-KBr disc technique (Chen, 1965). A Finnigan Model 1015 S/L instrument equipped with a Finnigan 6000 Data System was used for mass spectrometric (MS) analysis. Proton nuclear magnetic resonance (NMR) spectra were determined in deuterated chloroform solutions on a Varian HA-100 instrument.

Chromatography. Gas chromatographic (GC) columns were 4 mm (i.d.) × 1.81 m coiled glass columns packed with OV-101 on 80/100 mesh Chromosorb WHP. Loadings of 1, 3, and 10% OV-101 were used in conjunction with a mass spectrometer, a flame ionization detector, and a tritium foil electron-capture detector, respectively. Isothermal column temperatures were 235, 230, and 260 °C for the respective detectors. The nitrogen carrier gas flow was 100 to 120 mL/min; the helium carrier gas flow was 20 mL/min in the gas chromatograph used with the mass spectrometer. Thin-layer chromatography (TLC) was performed on 0.5-mm thick silica gel GF (type 60) plates (E. Merck), using hexane or 50% benzene–hexane as the developing solvent. Plates were prewashed by development in a mixture of equal amounts of acetone, ethyl acetate, and acetonitrile. TLC was used to clean up the biological extracts and to separate and characterize the metabolite and synthetic products.

Collection, Extraction, and Cleanup of Feces and Liver. Dog feces and livers were obtained from T. Farber (Food and Drug Administration), who was conducting a PBB toxicity study. In that experiment two female beagles about 1-year-old were fed Firemaster BP-6 in a corn oil capsule at 1 mg kg^{-1} day^{-1} for 6 weeks. On the last day of dosing, these dogs and two female control dogs were placed in individual metabolism cages to collect the feces and isolate it from the urine. After the dogs were sacrificed the livers were removed and frozen immediately. Collected samples were kept below 0 °C until analyzed.

Ten to forty-gram portions of feces were mixed with twice as much anhydrous sodium sulfate and blended at high speed for 5 min with 200 mL of ethyl ether. (Caution: A blender equipped with an explosion-proof motor and switch such as manufactured by Waring should be used.) The mixture was then filtered in a Büchner funnel under vacuum. The filter cake was reextracted twice by blending with 200-mL portions of ethyl ether. The pooled filtrates were concentrated under a jet of nitrogen to 5 mL for TLC cleanup. TLC cleanup was necessary before GC or GC–MS analysis. Extracts were streaked on TLC plates and developed with hexane. The materials at R_f from 0.05 to 0.20 (hydroxy-HBB) or 0.55 to 0.65 (DBF) were removed from the adsorbent as described by Gardner et al. (1976).

Twenty grams of dog liver tissue was homogenized with 50 mL of hexane in a Model 45 Virtis homogenizer. The mixture was filtered in a Büchner funnel under vacuum and the solid residue was blended again in the homogenizer with 50 mL of ethyl ether. This mixture was filtered and the solid residue was mixed with 50 mL of distilled water and 12 mL of concentrated hydrochloric acid in order to release conjugated metabolites. After 20 h at room temperature the mixture was shaken in a 500-mL separatory funnel containing 120 mL of ethyl ether. This ether phase and the hexane and ether phases from the previous extractions were combined and concentrated to 5 mL for TLC cleanup of hydroxy-HBB as described above.

RESULTS AND DISCUSSION

Chromatographic characteristics and the initial MS data for the PBB metabolite extracted from dog feces are given in Table I. The GC–MS data indicating the presence of a pentabromodibenzofuran (PDBF) were unexpected since most metabolites of the chemically related PCBs have been identified as hydroxylated PCBs (Sundström et al., 1976b). GC analyses of Firemaster BP-6 cleaned up by TLC showed the absence of the metabolite in Firemaster BP-6.

2-Hydroxybiphenyl was brominated in order to obtain analytical data on the 6-hydroxy-PBBs. The main product showed a mass spectrum of a hydroxy-HBB when the sample was introduced into the mass spectrometer using the probe insertion technique. The molecular ion was at 638 amu, the first of a cluster of halogen isotope ions that were indicative of six bromine atoms. The NMR data (Table II) identified the synthetic product as 6-hydroxy-2,4,5,2′,4′,5′-HBB. Chemical shifts (δ) of ring

Table I. Chromatographic Properties and GC-MS Data for Hydroxy-PBBs and Their Thermal Decomposition Products

compound[a]	GLC[b]	GLC of acetate[b]	TLC R_f (in hexane)
PBB metabolite	1.67[c] 2.08	1.52	0.10
synthetic 6-OH-2,4,5,2′,4′,5′-HBB[d]	1.67[c] 2.10[c]	1.52	0.10
product of 6-OH-2,4,5,2′,4′,5′-HBB and heat (brominated DBFs)[e]	1.62 2.05[c]		0.59

[a] All the compounds have the following GC-MS spectra, mass fragments (amu): M = 558 (5 Br), M − COBr = 451, and M − $COBr_3$ = 293. [b] Retention time relative to 2,4,5,2′,4′,5′-HBB on a 10% OV-101 column at 260 °C. [c] GC-MS mass spectrum for this peak. [d] 6-Hydroxy-2,4,5,2′,4′,5′-HBB identified by NMR and IR. [e] Heated in a glass tube at 230 to 260 °C for 20 min.

Table II. Proton NMR of HBB and Synthetic 6-Hydroxy-HBB

compound	integral	chemical shift, ppm[a] proton 3	3′	6	6′	6-OH
2,4,5,2′,4′,5′-HBB[b]	1:1	7.47	7.47	7.93	7.93	
2,4,5,2′,4′,5′-HBB[c]	1:1	7.46	7.46	7.93	7.93	
synthetic 6-OH-2,4,5,2′,4′,5′-HBB[c]	1:1:1:1	7.40	7.48		7.90	5.75

[a] Relative to tetramethylsilane. [b] Data from Sundström et al. (1976a) obtained on the Varian HR-220 instrument in deuterated chloroform. [c] Data obtained by authors on the Varian HA-100 instrument in deuterated chloroform.

protons were very close to those of protons of recrystallized 2,4,5,2′,4′,5′-HBB reported by Sundström et al. (1976a) and found by us. Increased shielding of the proton at the 3 position in the hydroxy-PBB due to the *p*-hydroxyl group at position 6 would cause the slight upfield shift from 7.46 ppm, observed in HBB, to 7.40 ppm (Jansson and Sundström, 1974). A hydroxyl O–H stretching band at 3482 cm^{-1} in the IR confirmed the presence of the hydroxyl group in the synthetic compound.

When the synthetic 6-hydroxy-HBB was heated at 230 to 260 °C, two products were isolated after TLC with hexane development which had GC retention times matching those of the metabolite and the synthetic hydroxy-HBB, as shown in Table I. The GC–MS mass spectrum of the major product was identical with those of the metabolite and the synthetic hydroxy-HBB (Table I). The two products were unresolved by TLC. Their TLC R_f in hexane, however, was higher than that of hydroxy-HBB or the metabolite. The mass spectrum of the major decomposition product obtained by using the probe insertion technique was typical of halogenated DBF (Roach and Pomerantz, 1974), displaying a molecular ion at 558 amu and fragmentary losses of COBr and $COBr_3$. The IR spectrum of the products supported the DBF structure showing a C–O–C stretching band at 1197 cm^{-1} and the absence of an O–H stretching band in the 3500 cm^{-1} region.

A tentative thermal decomposition scheme for 6-hydroxy-2,4,5,2′,4′,5′-HBB is shown in Figure 2. 6-Hydroxy-2,4,5,2′,4′,5′-HBB (I) probably decomposes at 230

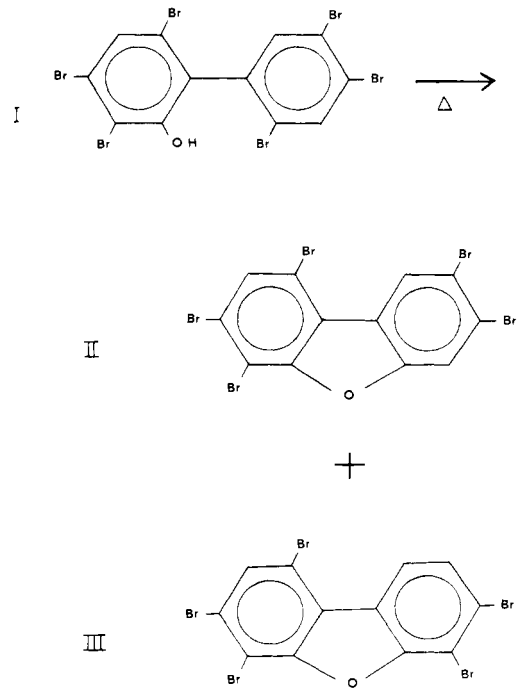

Figure 2. Tentative thermal decomposition scheme for 6-hydroxy-HBB.

to 260 °C to 1,3,4,7,8-PDBF (II). It is known that certain 2-methoxy-PCBs readily yield fragments isomeric with DBF ions on electron impact in the mass spectrometer (Jensen and Sundström, 1974; Sundström and Wachtmeister, 1975); but the thermal decomposition of hydroxy- or methoxy-PCB to DBF has not been previously reported. The thermal loss of HBr demonstrated with 6-hydroxy-HBB is probably facilitated by the lower strength of the C–Br bond relative to the C–Cl bond. Since two well-separated DBF chromatographic peaks were usually detected by GC–MS analysis, it is possible that both the 2′ and the 6′ positions in the molecule (see Figure 1A) are attacked by the reactive oxygen atom after thermal loss of HBr, producing two isomeric DBFs. DBF III (Figure 2) may therefore be deduced by postulating the migration of the 6′ hydrogen to the 2′ position after oxygen attack at the 6′ carbon. The ratio of the two peak areas varied with the amount of sample injected when a flame ionization detector was used for monitoring. Formation of the peak eluting first at a retention time relative to HBB of 1.67 (Figure 2, III) was favored when smaller amounts of the sample were injected. DBF structural assignments in Figure 2 were based on GC data obtained on OV-101 columns, which indicated that halogen substitution in the 4 and 6 positions (see Figure 1B) decreases retention times of isomeric DBFs (Phillipson, 1977).

Data in Table I show that the fecal metabolite has more of the properties of synthetic hydroxy-HBB than of the DBF formed by thermal decomposition of the hydroxy-HBB. Identification of the metabolite as 6-hydroxy-2,4,5,2′,4′,5′-HBB was confirmed by the preparation of acetate derivatives from the metabolite and synthetic 6-hydroxy-HBB; these derivatives had identical GC retention times of 1.52 relative to HBB.

Using the extraction and cleanup given above, no hydroxy-HBB was found in the liver of a dog fed PBB, although PBB was present. Recovery of hydroxy-HBB added to liver was 75%. Hydroxylation of highly chlorinated or brominated biphenyls at the 2 or 6 positions is rare in animals (Sundström et al., 1976b; Hutzinger et al., 1974). We suspect that the metabolite found in the feces

was formed by microbial metabolism of PBB in the dogs' gut and that it was then excreted in the feces. PBB levels in the feces were about 7 ppm. The hydroxy metabolite levels in the feces were about an order of magnitude less. DBF metabolites were not found in the feces.

This work has demonstrated that the high temperatures in the gas chromatograph may cause 6-hydroxy-2,4,-5,2′,4′,5′-HBB to decompose to DBFs, which are then detected by MS. GC–MS is extensively used as the sole screening method for metabolites and contaminants occurring in foods and in the environment (Alford, 1977). Results obtained by this technique which indicate the presence of brominated DBFs should be confirmed by other analytical techniques in order to show that the brominated DBFs have not been formed from hydroxy-PBBs during analysis.

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Worker Environment Research: Methidathion Applied to Orange Trees

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Methidathion (Supracide) was applied to orange trees, and residue levels of methidathion and its oxygen analogue on substrates pertinent to field worker safety were determined. Primary emphasis was placed on dislodgable foliar residues, but dislodgable fruit residues, grove dust residues, and pesticide concentration in air were also determined. Prebloom, postbloom, and early and late summer treatments were made to encompass the normal seasonal application periods; climatic data were collected. Three different geographical areas in California were used. Applications included two dilute spray rates and one low-volume rate. Posttreatment residue levels depended on initial pesticide deposition. High atmospheric oxidant levels did not correlate with enhanced oxon formation due to the many variables operative in the tests conducted.

Methidathion (Supracide, GS-13005) is currently recommended for use on citrus trees in California for the chemical control of the California red scale, *Aonidiella aurantii* (Mask.) (Ciba-Geigy Corp., 1977; University of California, 1976). It has an assigned tolerance for residues of 2 ppm in or on grapefruits, lemons, and oranges (*Federal Register*, 1975). To insure below-tolerance fruit, no more than two applications per growing season can be made and at least 45 days must elapse between applications; it cannot be applied within 14 days of harvest (Ciba-Geigy Corp., 1977). Methidathion is highly toxic to honey bees and severe losses may be expected if used when bees are present at treatment time or within a day thereafter (University of California, 1975). Thus, it is used as a pre- or postbloom and/or summer spray. In California, the probable application period for this scalicide is from late February to late March and from mid-May to late October. Methidathion is used extensively in California; of the organophosphorus pesticides, its usage in 1976 ranked third after dimethoate and parathion (California Department of Food and Agriculture, 1976). The Department of Food and Agriculture temporarily set 30 days as the reentry interval or time that must elapse between pesticide application and legal entry into the treated field by workers to engage in any activity requiring substantial contact with treated foliage such as pruning or harvesting (California Administrative Code, 1976). The 30-day interval was considered temporary until a more definitive reentry study could be conducted under California conditions and guidelines.

The California Department of Food and Agriculture conducted limited studies on foliar residues following application of methidathion to orange trees (Maddy, 1975). Reported here are more extensive residue data for methidathion and its oxygen analogue (GS-13007) in the orchard environment to assist in setting a worker reentry

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