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REDISTRIBUTION AND ENHANCED URINARY EXCRETION OF 2,2′,4,4′,5,5′-HEXACHLOROBIPHENYL (HCB) IN RATS USING HCB-SPECIFIC IgG AND Fab FRAGMENTS

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Abstract—Drug-specific antibody fragments can enhance the elimination of some drugs by redistributing drug from tissues into serum and allowing renal excretion of the drug–antibody complex. This approach could potentially be used to enhance the elimination of compounds such as polychlorinated biphenyls that have very long elimination half-lives. As a first step in testing this hypothesis, the effects of 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCB)-specific antibodies and their corresponding Fab fragments on HCB disposition were studied in rats. Antibodies to HCB were produced in chickens, and the corresponding Fab fragments were produced by digestion with papain. To study antibody effects on HCB distribution, [^{14}C]HCB (0.1 mg) was administered i.v. to rats. Two weeks later, after distribution to tissues was complete, anti-HCB IgG or control IgG was administered i.v. The serum radiolabel concentration 2 hr after IgG administration increased 185 ± 64% in animals treated with specific antibody vs 51 ± 19% in control animals ($P < 0.001$). The increase in serum radiolabel concentration was apparent within 30 min and maximal at 2 hr. To study effects on HCB excretion, anti-HCB or control Fab fragment was administered 2 weeks after [^{14}C]HCB. Urinary HCB excretion over the next 24 hr, measured by gas chromatography, was 10-fold greater in the group treated with anti-HCB Fab ($P < 0.01$). These data demonstrate that anti-HCB IgG can redistribute HCB rapidly from tissues into serum and that anti-HCB Fab can enhance urinary HCB excretion. While the magnitude of these changes was small, the data suggest that increasing HCB excretion using drug-specific antibody fragments is feasible, and can serve as a model for enhancing the excretion of compounds that have very long elimination half-lives.

Key words: polychlorinated biphenyl; hexachlorobiphenyl; antibody; Fab fragment; immunotherapy

Environmental contaminants such as dioxins, PCBs‖ and DDT have very long elimination half-lives in animals and humans. Exposure to these compounds results in a body burden that may persist for months to years [1, 2]. Because they may be toxic [3–7], methods of enhancing their elimination would be of interest.

One approach that may be useful for such compounds is the administration of antibodies that can bind them with high affinity and specificity. Antibodies directed at drugs such as digitoxin [7] or the tricyclic antidepressant desipramine [8] can redistribute these drugs rapidly from tissues into serum and extracellular fluid. The resulting antibody–drug complex is only slowly eliminated, but renal excretion can be enhanced markedly by the use of smaller antibody fragments. Fab fragments, which are one-third the size of intact IgG, retain the ability to bind drug and redistribute it out of tissues. The elimination half-life of Fab, however, is much shorter than that of IgG (10 vs 61 hr in baboons), and 30–50% of Fab elimination is by renal excretion [9, 10]. Administration of drug-specific Fab can therefore be used to enhance urinary drug excretion [11–13]. For example, the renal excretion of digitoxin is increased 14-fold by the administration of digitoxin-specific Fab [11].

PCBs differ in their lipid solubility, tissue distribution and rate of elimination. HCB has an elimination half-life estimated at 1–4 months [14, 15]. This long half-life is due to both a very low clearance of 0.18 mL/min/kg (estimated from the data of Lutz *et al.* [16]) and a large volume of distribution. HCB therefore represents a suitable pharmacokinetic model of PCBs that accumulate in tissues. The current study is a preliminary investigation designed to test the hypothesis that HCB-specific antibody fragments can redistribute HCB from tissues into

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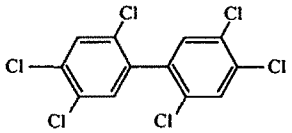
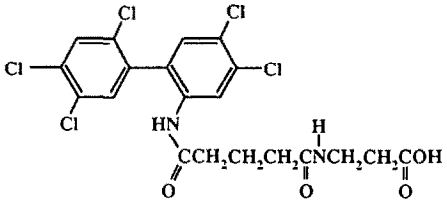
‖ Abbreviations: PCBs, polychlorinated biphenyls; DDT, dichlorodiphenyltrichloroethane; HCB, 2,2′,4,4′,5,5′-hexachlorobiphenyl; PCBG, *N*-(2′,4,4′,5,5′-pentachlorobiphen-2-yl)glutaramic acid; PCBGA, *N*-(2′,4,4′,5,5′-pentachlorobiphen-2-yl)glutaramyl-β-alanine; DMF, dimethylformamide; KLH, keyhole limpet hemocyanin; and TG, thyroglobulin.



HCB



PCBGA

Fig. 1. Structures of 2,2',4,4',5,5'-hexachlorobiphenyl (HCB) and *N*-(2',4,4',5,5'-pentachlorobiphen-2-yl)glutaramyl-β-alanine (PCBGA), which was conjugated to protein by carbodiimide coupling to produce immunogen.

serum and enhance HCB urinary excretion. Intact IgG was used to study HCB redistribution from tissues because IgG has a smaller volume of distribution than Fab and therefore provides a more sensitive assay of redistribution. Anti-HCB Fab fragment was used to study HCB urinary excretion.

MATERIALS AND METHODS

Synthesis of hapten. Synthesis of the hapten involved preparing the 2-glutaramyl derivative of 2',4,4',5,5'-pentachlorobiphenyl by extending the linker with β-alanine or [^{3}H]β-alanine to provide a radiolabeled marker, and then conjugating the resulting compound to carrier protein. Full details of the chemistry will be published elsewhere. In brief, 2-amino-2',4,4',5,5'-pentachlorobiphenyl was prepared by reduction of 2-nitro-2',4,4',5,5'-pentachlorobiphenyl with stannous chloride/hydrochloric acid essentially as previously described [17] and isolated by preparative thin-layer chromatography. Condensation of this product with glutaric anhydride gave PCBG. Finally, PCBGA was formed by the reaction of the *N*-hydroxysuccinimide ester of PCBG with *N*,*O*-bis(trimethylsilyl)-β-alanine (Fig. 1).

Conjugation of PCBGA to protein. A mixture of 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide HCl (18 mg, 0.79 mmol) and anhydrous DMF (0.5 mL) was warmed briefly to achieve solution, and this was added to a solution of PCBGA (3.0 mg) or the corresponding ^{3}H-labeled compound (1.0 mg) in anhydrous DMF (0.5 mL). After stirring for 30 min at room temperature, the reaction solution was added dropwise with stirring over 25 min to a solution of KLH (25 mg) in 0.05 M $NaHPO_4$, pH 7.75, buffer (15 mL). Additional DMF (0.25 mL) and then buffer (0.25 mL) were used to rinse the PCBGA-carbodiimide solution into the reaction solution. The reaction solution was stirred overnight at room temperature. The PCBGA–KLH conjugate was dialyzed extensively against $NaHPO_4$ buffer and separated from reagents using a Sephadex G-100 column. The extent of conjugation (molar substitution ratio) was calculated using radioactivity, and the protein concentration was measured by dye binding. PCBGA was conjugated to TG or BSA in an analogous manner.

Preparation of antibody. The PCBGA–BSA, PCBGA–KLH or PCBGA–TG conjugate (20 μg in 0.25 mL of 0.9% saline) was mixed 1 : 1 with complete Freund's adjuvant and injected i.m. at two sites (0.25 mL/site) into white leghorn chickens. The chickens were boosted at 1 month, and eggs were collected 1–2 weeks after the boost.

Intact egg yolks were washed with 10 mM Tris buffer, pH 7.5. Five yolks (approximate volume 80 mL) were processed at one time. One yolk volume of Tris, pH 7.5, was added, and the yolks were disrupted with a glass rod. One yolk volume of 10.5% polyethylene glycol (PEG 8000, Sigma Chemical Co., St. Louis, MO) was added; the mixture was stirred at 25° for 20 min and then centrifuged at 10,000 *g* for 20 min. The supernatant was decanted through gauze, 0.5 yolk volume of 42% PEG 8000 in Tris buffer was added, and the mixture was stirred for 30 min and then centrifuged at 10,000 *g* for 20 min. The supernatant was discarded, and 15 mL of Tris buffer was added. This was allowed to sit for at least 1 hr at 4°. The pellet was resuspended by gentle mixing and the volume was increased to 20 mL with Tris buffer. Twenty milliliters of 50% ethanol kept at −20° was added, and the suspended material was gently mixed, allowed to sit at −20° for 60 min, and centrifuged at 10,000 *g* for 30 min. The pellet was resuspended and dialyzed extensively against Tris buffer. Control antibody was similarly prepared from chickens immunized with an irrelevant antigen (porcine interleukin-6).

Antibody characterization. Antibody homogeneity was assessed by SDS–PAGE using a 5–15% acrylamide gradient. Antibody was screened for HCB binding activity by ELISA using rabbit anti-chicken IgG-peroxidase (Sigma). Wells were plated with PCBGA–BSA for assay of chickens immunized with PCBGA–TG or PCBGA–KLH. Specificity of binding was determined by competition with unlabeled HCB (Ultra Scientific, North Kingstown, RI). Chickens were selected to produce antibody for this study by comparison of their titers.

Fab preparation. IgG from a single chicken immunized with the KLH–PCBGA conjugate was digested to Fab by adding β-mercaptoethanol to a concentration of 10 mM and mercuripapain (Sigma) at a mecuripapain : IgG weight ratio of 1 : 50, and shaking at 37° for 4 hr. Digestion was terminated by adding iodoacetamide to a final concentration of 25 mM in Tris buffer, pH 8.0, for 1 hr in the dark. The product was dialyzed against Tris buffer, pH 7.5, and concentrated by ultrafiltration.

Drug. [^{14}C]HCB (sp. act. 17.6 mCi/mmol) was obtained from Sigma. Purity of drug was confirmed by gas chromatography. Because HCB is poorly

soluble in other diluents, [^{14}C]HCB was suspended in Emulphor/ethanol/water (1:1:8; GAF Corp., Linden, NJ) for administration to rats [15]. All doses were prepared immediately prior to use.

Animal preparation. Male Holtzman rats weighing 190–230 g were anesthetized with droperidol/fentanyl i.m., and a cannula was placed in one femoral vein. [^{14}C]HCB in 1 mL of Emulphor/ethanol/water was administered i.v. over 2 min, the cannula was removed, and the wound was stapled. Rats were placed individually in metabolic cages for the pharmacokinetics protocol or standard cages for the antibody administration protocol. The [^{14}C]HCB dose was 0.2 mg for the pharmacokinetics protocol and 0.1 mg (3.3×10^7 dpm) for the antibody administration protocols. A higher dose was used in the pharmacokinetics protocol to increase assay sensitivity at later time points.

Pharmacokinetics protocol. This protocol was performed to demonstrate the pharmacokinetics of [^{14}C]HCB in rats and to determine when [^{14}C]HCB distribution to tissues was complete. Six rats were kept in metabolic cages for 2 weeks after administration of [^{14}C]HCB (0.2 mg, i.v.) to allow collection of urine and feces. Blood was obtained periodically by tail vein bleeding under light droperidol/fentanyl anesthesia (see Fig. 2). Serum was immediately separated by centrifugation. For scintillation counting, 0.1 mL serum or urine was placed directly in scintillation fluid (Ecolite, ICN Biomedicals, Inc., Irvine, CA). A total of 3.3 mL of blood was removed over 50 days. Feces (1–2 g) was vortexed in 15 mL of water, allowed to stand for 24 hr, vortexed again and 0.1 mL placed in scintillation fluid. Control samples of serum, urine and feces were spiked with [^{14}C]HCB to determine quenching.

IgG administration protocol. On day 14 after [^{14}C]HCB administration, animals were anesthetized with droperidol/fentanyl, and a cannula was placed in the unused femoral vein. Anti-HCB or control IgG (100 mg in 2 mL of 10 mM Tris buffer, pH 7.5) was infused over 15 min with the order of treatments randomized. This large protein load was necessary because HCB-specific IgG was only a small fraction of total IgG. To compare different immunogens, three rats received antibody prepared from a single chicken immunized with HCB–TG and three rats received antibody prepared from a single chicken immunized with HCB–KLH. Six rats received control antibody. The cannula was flushed with 0.3 mL of 0.9% NaCl and was then used to obtain periodic blood samples of 0.3 mL. At 4 hr the femoral cannula was removed, the wound was stapled, and the animal returned to its cage. At 24 hr an additional blood sample was obtained by tail vein bleeding.

Fab administration protocol. Fab was administered in the same manner as intact IgG (described above) at a dose of 190 mg total protein in 2.1 mL of Tris buffer. As with IgG, this large protein load was necessary because HCB-specific Fab was only a small fraction of total Fab, and because products of digestion other than Fab were also present. Groups of six rats received either anti-HCB or control Fab in randomized order. Rats were kept in metabolic cages before and after Fab administration. Urine was collected in a beaker packed in dry ice for 24 hr prior to treatment with Fab, and for 48 hr after.

HCB assay. HCB serum concentrations in this study were estimated from radiolabel concentrations because this is inexpensive and because serum radiolabel concentrations correlate well with HCB concentrations measured by gas chromatography [14, 15, 18–20]. Estimation of HCB concentrations using radiolabel may not be as accurate for urine, owing to higher concentrations of metabolites. We therefore measured urinary HCB concentrations by gas chromatography on the 24-hr urine samples obtained just after Fab administration in order to confirm the results obtained by measuring radiolabel. The method used was similar to that reported by Mühlebach and Bickel [15]. In brief, internal standards (PCBs No. 14 and 166) were added to 1 mL of urine, and the sample was extracted three times with 3 mL hexane, washed through a sodium sulfate column, and concentrated to 1 mL. Samples were analyzed using a DB-5 column (J & W Scientific, Folsom, CA) and electron capture detection with injector temperature 250°, detector temperature 310°, and an oven program of 120° hold for 2 min, 1°/min ramp to 210°, 10°/min ramp to 250°, and hold at 250° for 10 min.

Data analysis. Estimates of pharmacokinetic parameters were obtained from mean data and the computer program PC NONLIN (version 4.2, Statistical Consultants Inc., Lexington KY) [21].

For the antibody administration protocol, the percent increase in serum radiolabel concentration compared with the pretreatment value (just prior to antibody administration) was calculated at each sampling time, and these values were compared by repeated measures ANOVA. For this analysis the three rats receiving antibody derived from the PCBGA–TG immunogen were combined with the three rats receiving antibody derived from the PCBGA–KLH immunogen, because results obtained with these immunogens were similar. Differences between groups at individual time points were compared using an unpaired, two-tailed *t*-test. For the Fab administration protocol, the percent change in urinary radiolabel excretion compared with the baseline value (the 24 hr prior to treatment) was calculated for the intervals 0–24 hr and 24–48 hr, and these values were compared by repeated measures ANOVA. Urine HCB concentration measured by gas chromatography was compared between groups using a one-tailed unpaired *t*-test.

Molecular weights used for calculation of urinary excretion were; HCB, 361 g/mol; Fab, 50,000 g/mol.

RESULTS

Immunogen. The PCBGA:protein molar substitution ratios for conjugated immunogen were: PCBGA–KLH, 371:1; PCBGA–TG, 39:1; and PCBGA–BSA, 29:1.

Antibody characteristics. IgG was >90% homogeneous by SDS–PAGE. All of seven chickens immunized with HCB–protein conjugates produced antibodies that bound HCB by ELISA. This binding

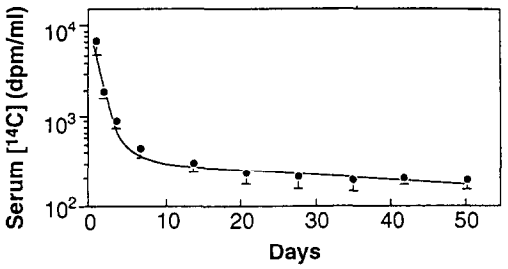


Fig. 2. Serum concentration of radiolabel after administration of 0.2 mg [^{14}C]HCB, i.v., at day 0. Lines represent a computer-generated best fit of the mean data. Values are means ± SD, N = 6.

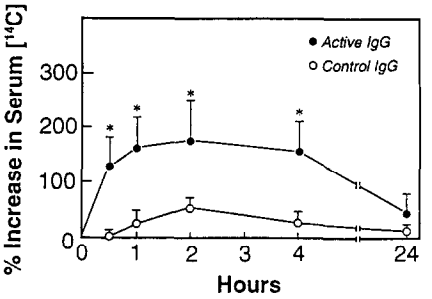


Fig. 3. Percent increase in the serum radiolabel concentration after administration of antibody. Antibody was administered at t = 0 (14 days after [^{14}C]HCB) as a 15 min i.v. infusion (N = 6 per group). Values are means ± SD. Key: (*) P < 0.05 compared with control IgG.

was inhibited in a concentration-dependent manner by HCB. Because the percent of specific IgG was not known, the molar doses of IgG or Fab administered to rats could not be calculated precisely. However, they may be estimated as follows. (1) Of the unpurified total protein administered as "Fab" (190 mg), only 2% was actually Fab as estimated from SDS–PAGE. The actual Fab dose was, therefore, 3.8 mg. Of this amount, only a fraction is specific for HCB. In a previous study, 3.2% of chicken IgG recovered from egg yolk was specific for antigen [22]. If we assume the same percent of specific Fab in the current study, then the actual dose of HCB-specific Fab administered was 3.8 mg·3.2% = 0.12 mg or 2.5 nmol (molecular weight of Fab = 50 kDa). Using the same estimates, the actual dose of anti-HCB IgG administered was 3.2 mg.

The HCB dose administered was 0.1 mg or 277 nmol (molecular weight of HCB = 361). The dose of HCB-specific Fab administered, based upon these estimates, therefore represented about 2.5 nmol/277 nmol = 0.9% of the HCB dose.

Pharmacokinetics protocol. All rats tolerated [^{14}C]HCB infusion without adverse effects. Two rats died at days 23 and 28. Cause of death was not apparent at necropsy. Weight gain had been normal. Data from these rats up to the time of death were included in the pharmacokinetic analysis. All other rats appeared healthy and gained weight normally (4.5 ± 0.8 g/day) during the 50-day study period. The serum radiolabel disappearance curve showed biexponential decay (Fig. 2) with a terminal half-life of 118 days. Fecal excretion over 1 week was 4.0 ± 0.9% of the HCB dose and urinary excretion was 0.20 ± 0.04%. These values were similar if calculated excluding the two animals that died.

IgG administration. Antibody was administered at 14 days because the pharmacokinetic study showed that radiolabel distribution was complete by this time. Serum radiolabel concentrations in the treated and control groups was comparable prior to IgG administration (650 ± 230 vs 800 ± 410 dpm/mL, P > 0.5). Anti-HCB IgG produced a prompt increase in the serum radiolabel concentration that was apparent by 30 min and maximal at 2 hr (Fig. 3). This increase was significantly greater than the

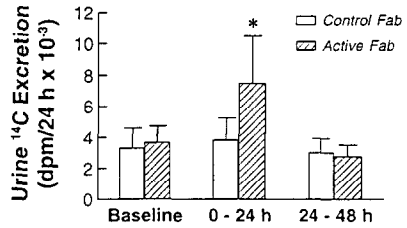


Fig. 4. Urine radiolabel excretion over the 24-hr period prior to Fab administration (baseline) and for the intervals 0–24 hr and 24–48 hr after Fab administration. Values are means ± SD, N = 6 per group. Key: (*) P = 0.02 compared with control Fab. Note that urinary HCB excretion, *measured by gas chromatography rather than by radiolabel*, increased 10-fold in the group treated with anti-HCB Fab. These data are not shown here because HCB concentrations were measured by gas chromatography only for urine collected for the 24 hr after Fab administration, whereas radiolabel concentrations were measured at additional times and serve to show the time course of the anti-HCB Fab effect.

increase produced by control antibody (P < 0.001). Differences between groups were significant (P < 0.05) at all time points except 24 hr (P = 0.08).

Fab administration. Urine output was not affected by anesthesia or Fab administration. The 24-hr urine volumes before and after Fab administration were 16.3 ± 2.8 and 18.5 ± 4.8 mL for the control Fab group and 16.6 ± 4.6 and 18.5 ± 4.4 mL for the anti-HCB Fab group. Urinary radiolabel excretion in the treated and control groups was comparable in the 24 hr prior to Fab administration (3.3 ± 1.3 × 10^3 vs 3.6 ± 0.8 × 10^3 dpm/24 hr, P > 0.5). Active Fab produced a doubling of the urinary radiolabel excretion over the period 0–24 hr, whereas no increase was observed with control Fab (P = 0.02, Fig. 4). Active Fab also produced a small increase in the serum radiolabel concentration compared with control Fab (66 ± 23 vs 31 ± 16%, P = 0.01) at 15 min after Fab administration. There was no

difference in fecal radiolabel excretion between groups (data not shown).

HCB renal excretion was measured by gas chromatography only in the urine samples collected for the first 24 hr after Fab administration. HCB excretion was 1.3 ± 0.6 ng/24 hr in controls and 12.2 ± 8.5 ng/24 hr in the group treated with anti-HCB Fab ($P < 0.01$). The excess HCB excretion attributable to anti-HCB Fab was, therefore, 10.9 ng/24 hr (0.03 nmol/24 hr).

DISCUSSION

We chose to study HCB for several reasons: (1) HCB pharmacokinetics has been well characterized in rats, (2) HCB has a very large volume of distribution and a very long elimination half-life, and therefore serves as a model for compounds that are highly persistent in tissues, and (3) HCB undergoes very limited biotransformation, so that radiolabel concentrations provide a good estimate of parent drug concentration [14, 15, 18–20]. For example, Mühlebach and Bickel [15] found that only 17% of an HCB dose administered to rats was excreted over 40 weeks (16% in feces and 1% in urine), and that virtually all of the dose remaining at that time was in the form of parent compound. These authors estimated that <3% of the HCB dose was excreted as metabolites. Likewise, Wyss *et al.* [23] recovered 89% of an HCB dose from rat tissues as parent compound 56 days after dosing. It is not clear whether the disposition of HCB is dose dependent. However, the 0.1 and 0.2 mg HCB doses used in the current study are similar to the HCB doses used in the above-mentioned pharmacokinetic studies, and produce serum HCB concentrations similar to reported values for the total serum PCB concentration in PCB-exposed humans [2, 24].

The pharmacokinetic parameters estimated in this study were comparable to previously reported values for both radiolabeled HCB, as well as for HCB measured using methods that distinguish parent compound from metabolites. The terminal elimination half-life in serum of 118 days compares with reported values of 37.2 days [14] and 16.9 days [19], and with a fecal excretion half-life of 100 days [15]. This variability in estimated half-life is not unexpected, in that sampling times differed and in some cases (including the current study) were relatively short compared to the elimination half-life. Thus, the terminal [^{14}C]HCB half-life estimated in the current study should not be taken as a definitive pharmacokinetic value, but rather as confirmation that HCB pharmacokinetic parameters in this model are similar to those described in other studies of HCB in rats. Fecal and urinary excretion of radiolabel over the first 2 weeks after [^{14}C]HCB dosing in the current study (4.0 and 0.2%, respectively, of the HCB dose) were also similar to previously reported values of 5.5% for fecal excretion [20] and <1% for urinary excretion [15, 23]. The distribution phase of radiolabel in the current study was largely complete by 10 days (Fig. 2). We chose 14 days as the time to administer antibody so that any effect of antibody on radiolabel concentration would clearly represent redistribution of radiolabel out of tissues. While the i.v. dosing route used in the current study differs from the route of exposure of humans to PCBs, the tissue distribution of HCB in rats has been shown to be quite similar after either i.v. or repeated oral dosing [15].

Anti-HCB IgG promptly increased the serum radiolabel concentration compared with control antibody, demonstrating redistribution of HCB from tissues into serum. This effect was achieved rapidly, being apparent at the first sampling time (30 min) and maximal at 2 hr after IgG administration. It is interesting that control antibody also increased the serum radiolabel concentration, although to a much lesser extent than the specific antibody. It is possible that HCB is nonspecifically bound by control antibody. However, we have observed a small increase in the serum radiolabel concentration when saline, rather than IgG, was used as the control treatment (unpublished data). We are unaware of any previous studies addressing the binding of HCB, or of other PCBs, to IgG.

One previous study evaluated the effect of antibodies on PCB distribution. Colburn and White [25] administered 4-monochlorobiphenyl or 2,2′,3,3′-tetrachlorobiphenyl to mice i.p. and 15 min later administered a specific rabbit antiserum i.v. The antiserum resulted in a modest redistribution of these compounds into serum as measured by the tissue/serum ratios. Based upon their observations, these authors suggested that smaller drug-specific antibody fragments might be used to enhance PCB elimination. These results generally support the concept of using antibodies to alter PCB disposition, but differ from the current study in several ways. First, the compound used by Colburn and White [25] has a smaller volume of distribution and undergoes much more rapid elimination than HCB. Second, the antiserum was administered very soon after the PCB, and it is unlikely that PCB distribution to tissues was complete. The current study, therefore, provides a more suitable pharmacokinetic model of PCBs (or other compounds) that are highly persistent in tissues.

In the current study we estimated urinary HCB excretion from measurement of radiolabel concentrations as well as by direct measurement of HCB by gas chromatography. Radiolabel excretion was doubled by the administration of anti-HCB Fab. This increase was apparent only for the first 24 hr, consistent with previous data showing that most Fab excretion occurs during this time [26]. Measurement of HCB concentrations by gas chromatography showed a much larger increase, with ten times more HCB excreted in the anti-HCB group than in the control group. This larger increase was likely due to some excretion of radiolabel as HCB metabolites, which served to elevate the background radiolabel concentration in urine. Nevertheless, these two measures gave similar values for the increase in HCB excretion after anti-HCB Fab: 10 ng/24 hr as estimated from radiolabel excretion and 11 ng/24 hr as measured by gas chromatography.

The absolute increase in HCB excretion after anti-HCB Fab treatment (10.9 ng/24 hr) was small, representing only 0.011% of the administered HCB dose. The small magnitude of this increase may have

been due to a number of factors. First, the amount of HCB-specific Fab actually administered (2.5 nmol) represented only an estimated 0.9% of the HCB dose on a molar basis (see Results; antibody characteristics for this calculation). Second, only 30% of administered Fab is expected to be excreted in urine [26]. Thus, the maximum amount of HCB that could have been excreted in urine, if all Fab had HCB bound to it when excreted, was 0.75 nmol HCB (2.5 nmol·0.3). The actual HCB excretion (10.9 ng, 0.03 nmol) was only 4% of the predicted amount (0.03 nmol/0.75 nmol). This lower figure could be due, in part, to overestimation of the Fab dose administered, although this magnitude of overestimation is unlikely, or to the anti-HCB Fab having a relatively low affinity for HCB. Further study with affinity-purified Fab to allow precise measurement of the Fab dose and the K_a would address these questions. It is more likely that the amount of HCB excreted was low because the rate of redistribution of HCB from tissues into serum was slow compared to the rate of Fab elimination. This possibility is also testable, for example, by administering the Fab as a slow continuous infusion rather than as a bolus injection, to allow more time for HCB redistribution to take place. In support of this possibility, it has been shown that the highly lipid soluble PCBs accumulate in milk, and lactation can serve as a route of elimination. In one study, mice eliminated only 2.7% of their HCB body burden during 20 days of pregnancy, but eliminated over 95% of their HCB body burden during the subsequent 20 days while lactating [3]. Thus, slower administration of Fab may be more efficient in enhancing HCB elimination. The rapid rise in the serum radiolabel concentration observed after IgG administration (Fig. 3) does not necessarily contradict this hypothesis; the HCB that appeared rapidly in serum may have come from a shallow compartment, while most HCB remains in a deeper compartment that redistributes into serum more slowly. Further study of these issues is clearly needed. We emphasize that the current study was a preliminary effort, intended primarily to demonstrate the feasibility and potential utility of using anti-HCB Fab to manipulate HCB disposition.

Fecal radiolabel excretion was not increased by anti-HCB Fab. This could be anticipated, as there is no evidence that Fab is excreted in feces. It is also possible that Fab would decrease HCB excretion in feces, by binding HCB in serum and reducing its excretion into bile. This possibility would have been difficult to detect, as the amount of HCB excreted in feces over 24 hr is very low. Tissue radiolabel concentrations were not measured in this study because the magnitude of change with this dose of antibody was anticipated to be small. The question of which tissues the redistributed HCB comes from is important but will require further study. It is unlikely, however, that the redistributed HCB simply came from red blood cells, as <20% of HCB in blood is contained in red blood cells [15].

In summary, the administration of HCB-specific IgG to rats 14 days after administration of [^{14}C]-HCB resulted in a prompt redistribution of radiolabel from tissues into serum. Administration of HCB-specific Fab substantially increased urinary HCB excretion. While the absolute amount of radiolabel excreted was small, these data suggest that further study may provide insight into the pharmacokinetics of HCB as well as potential strategies for enhancing HCB elimination. To the extent that other environmental contaminants share the pharmacokinetic characteristics of HCB, this approach may also be of interest for other compounds that have very long elimination half-lives.

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