Excretion of Hexachlorobenzene and Metabolites in Feces in a Highly Exposed Human Population

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A set of 53 individuals from a population highly exposed to airborne hexachlorobenzene (HCB) were selected to study the elimination kinetics of this chemical in humans. The volunteers provided blood, 24-hr urine, and feces samples for analysis of HCB and metabolites. The serum HCB concentrations ranged from 2.4 to 1,485 ng/ml (mean ± SD, 124 ± 278), confirming that this human population has the highest HCB blood levels ever reported. All analyzed feces samples contained unchanged HCB (range, 11–3,025 ng/g dry weight; mean ± SD, 395 ± 629). The HCB concentration in feces strongly correlated with HCB in serum (r = 0.85; p < 0.001), suggesting an equilibrium in feces/serum that is compatible with a main pulmonary entrance of the chemical and low intestinal excretion of nonabsorbed foodborne HCB. The equilibrium is also compatible with a nonbiliary passive transfer of the chemical to the intestinal lumen. Two HCB main metabolites, pentachlorophenol (PCP) and pentachlorobenzenoethyl (PCBT), were detected in 51% and 54% of feces samples, respectively. All urine samples contained PCP and PCBT, confirming the conclusions of a previous study [Environ Health Perspect 105:78–83 (1997)]. The comparison between feces and urine showed that whereas daily urinary elimination of metabolites may account for 3% of total HCB in blood, intestinal excretion of unchanged HCB may account for about 6%, thus showing the importance of metabolism in the overall elimination of HCB. The elimination of HCB and metabolites by both routes, however, appears to be very small (<0.05%/day) as compared to the estimated HCB adipose depot features. Features of HCB kinetics that we present in this study, i.e., non-saturated intestinal elimination of HCB and excretion in feces and urine of inert glutathione derivatives, may explain, in part, the absence of porphyrin cutanea in this human population heavily exposed to HCB. Key words: feces, hexachlorobenzene, humans, kinetics, urine. Environ Health Perspect 108:595–598 (2000). [Online 24 May 2000] 
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Hexachlorobenzene (HCB) is a chlorinated hydrocarbon with a high lipophility and a strong tendency to accumulate in food chains and lipid-rich tissues of animals and humans. It is ubiquitous in soil, water, air, and biological matrices. HCB was used as a fungicide for seed treatment, but most countries banned its use during the 1970s; currently, most HCB enters the environment by way of by-products and emissions from the chlorinated solvent industry. It was learned that HCB induces porphyrins in humans during an outbreak of porphyria cutanea tarda (PCT) in Turkey in the late 1950s, when several rural populations consumed HCB-contaminated bread (1). The effects of this chemical were most severe in breast-fed infants. Several decades of research have shown that HCB, in addition to inducing porphyrin, has a broad range of toxic effects in experimental animals, including immunotoxicity, endocrine effects, and cancer (1). However, because few data arose from the Turkish outbreak, there is little information on the threshold levels and dose-response relationship of HCB and on HCB-related adverse effects in humans.

Grimalt et al. (2) initiated a cross-sectional research project on the health effects of HCB in Flix, Spain (Tarragona Province), a rural village near a chlorinated solvent factory where high airborne HCB exposure regularly occurred during the last four decades. In the cohort studied in Flix, it was possible to quantify the HCB internal dose and assess the health status, including measurements of urinary porphyrin profiles (3–5). The authors, however, did not find an association between HCB serum concentrations and the appearance of PCT.

The Flix project was also intended to study HCB disposition in humans. The existence of a cohort of individuals with the highest HCB serum concentrations ever reported provided a unique opportunity to study HCB metabolism and elimination in humans. In a previous report (6) addressing the urinary elimination of HCB metabolites, we found a very strong correlation between HCB serum concentrations and pentachlorobenzenoethyl (PCBT) in urine. PCBT is a metabolite that arises from the conjugation of HCB with glutathione and the formation of an N-acetyl-l-cysteine derivative (6). This metabolite was found to be more concentrated in the urine than pentachlorophenol (PCP), a metabolite that arises from P450-mediated hydroxilation; this suggests the existence of an important detoxication pathway that leads to the formation of a mercapturic acid derivative.

A more comprehensive approach to HCB elimination kinetics in humans, however, required the quantification of HCB and its metabolites in feces and the comparison between both major elimination pathways. In this report we present this quantification based on a new subset of individuals from the Flix project who provided serum, urine, and feces samples for study.

Materials and Methods

Study population. An epidemiologic cross-sectional study was carried out on the 4,178 inhabitants of Flix who were >14 years of age. A questionnaire about residence, occupation, lifestyle, and medical history was completed by 1,800 inhabitants (43% of the total population). From these, we selected 777 individuals at random and asked them to donate biological samples for the study. A subset of 328 individuals responded positively. Other subjects (280) who responded to the questionnaire but had not been selected asked to be included. Thus, 608 individuals (328 randomly selected and 280 volunteers) provided biological samples.

Serum samples from all of these participants were analyzed to determine the HCB concentrations. Because HCB serum concentrations and sociodemographic characteristics did not differ by type of selection (3), we grouped all of the participants (randomly selected and volunteers).

We randomly selected a subset of 45 individuals for the present study. This resulted in a 10% overlapping by chance with the former urine study (6). In addition, 8 subjects with the highest blood HCB levels were included. Thus, the final subset was 53 individuals (25 men and 28 women; mean age of 47 and 42 years, respectively). These subjects...
were informed of the purpose of the study and provided 24-hr urine samples and feces samples for analysis of HCB and metabolites.

**Analysis of HCB and metabolites.** Feces and urine samples were analyzed at the Toxicology Unit (Hospital Clinic, University of Barcelona). Preanalytical management of samples (identification, chain of custody, storage, randomization of analysis order, blinding of technicians) was accomplished according to good laboratory practices and approved protocols of the hospital laboratory.

Feces samples (approximately 250 mg) were homogenized and dried at 65°C overnight. The drying process reduced the initial weight of the samples depending on the degree of hydration. The ratio of dried/fresh weight × 100 ranged from 14.2% to 55.65% (28.0 ± 9.02, arithmetic mean (AM) ± SD). Dried samples were weighed and digested under N₂ with 4 mL 2N H₂SO₄ for 4 hr at 70°C. Ascorbic acid and aldrin (as an internal standard) were added to the mix. This alkaline hydrolysis yields free PCP and PCBT. After cooling and acidification with concentrated HCl (pH 1), HCB and metabolites were extracted twice with 5 mL benzene; the solvent extracts were concentrated to 0.5 mL and treated with 0.5 mL diazothane in diethyl ether.

After derivatization (30 min in the dark), the excess diazothane was removed under an N₂ stream, the solvent extracts were concentrated to approximately 0.1 mL, and n-hexane (2 mL) was added. The resulting mixture was cleaned with H₂SO₄, and the organic phase was separated and concentrated to 25 μL. HCB and ethyl derivatives of PCP and PCBT were analyzed by gas chromatography (GC; Hewlett Packard 5890 II, Hewlett Packard, Palo Alto, CA) with 63Ni electron capture detection (ECD). Recovery of HCB, PCP, and PCBT was assayed with spiked wet feces and ranged between 88 and 100%. The limit of detection for HCB, PCP, and PCBT was 5 ng/g of dry feces. Metabolites of HCB in urine were analyzed as previously described (6). Briefly, aliquots of 24-hr urine were spiked with aldrin as an internal standard, hydrolyzed with NaOH, extracted with toluene, and derivatized with diazothane. The main urinary HCB metabolites ( conjugated PCP and pentachlorophenyl N-acetyl cysteine) were analyzed by GC-ECD as ethyl derivatives of free PCP and PCBT and confirmed by selective ion monitoring mass spectrometry (SIM-MS). Quantitation was calculated as micrograms of metabolites excreted in 24 hr. The detection limit was 0.5 μg/24 hr. The HCB in sera was analyzed as previously described (6). Briefly, serum aliquots were spiked with tetramobromobenzene as an internal standard and treated with n-hexane and sulfuric acid. HCB and other organochlorine compounds were analyzed by GC-ECD and confirmed by mass spectrometry. Quantitation was calculated as nanograms of HCB per milliliter of serum.

**Statistical analysis.** Because HCB concentrations in feces and blood were skewed to the right, we used the natural logarithmic transformation (ln) in the analysis.

We performed multiple linear regression models using SSPSSPC (SPSS Inc., Chicago, IL) to assess the association between HCB concentrations in blood and feces (and between blood HCB and PCBT/PCP in feces and urine) while adjusting for other possibly confounding variables such as sex, age, body mass index, and current smoking status.

**Results**

All feces samples analyzed contained unchanged HCB, with values ranging from 11 to 3,025 ng/g (calculated on a dry weight basis). The AM, SD, geometric mean (GM), and range of values are shown in Table 1, and the frequency distribution is shown in Figure 1.

The HCB concentration in feces was strongly associated with HCB in serum (r = 0.85; p < 0.0001; Figure 2, Table 2), suggesting that only a small amount of the chemical found in feces is nonabsorbed intestinal HCB. Among the remaining variables, only sex was associated with HCB concentrations in feces (Table 2). Because men had significantly higher blood HCB concentrations than women, this association between sex and HCB in feces disappeared after adjusting for blood HCB concentrations in a multiple regression analysis. The association between HCB in blood and feces was stronger in males (slope = 0.89; SE = 0.05) than in females (0.32; SE = 0.29), and this difference was statistically significant (p = 0.046). The association between HCB concentrations in blood and those in feces was not confounded by the variables included in Table 2. Also, there were no differences in the association of HCB concentrations in blood and feces by age or body mass index.

PCP was detectable in only 51% of the feces samples (range 5–70 ng/g; Table 1) and PCBT was detectable in 54% (range 5–139 ng/g). We were unable to detect other known metabolites of HCB in rodents, such as tetrachlorohydroquinone and tetrachloro-1,4-benzenediol.

As expected from the lipophilicity of the compound, HCB could not be detected in any of the urine samples (detection limit 0.5 ng/mL), whereas PCP and PCBT were detected in 100% (Table 1). PCBT in urine strongly correlated with HCB in serum (r = 0.80), thus confirming the findings of the previous study using a different subset of individuals (6).

The concentration of HCB in feces does not allow a precise calculation of the amount of HCB that is eliminated daily by the intestines. This would require a (difficult) collection of 24-hr feces from all of the participants. Thus, an accurate comparison between the amount of HCB eliminated through feces and urine is not possible. Based on the data in Table 1, however, we estimated the excretion of HCB in feces; these estimates are presented in Table 3. Supposing a standard volume of 5.4 L of blood in a healthy individual who weighs 70 kg, 13–8,019 μg HCB (GM 163 μg) may be in circulation. The 24-hr urinary output in the form of metabolites (PCP and PCBT) ranges

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**Figure 1.** Frequency distribution of HCB concentration in feces.

**Figure 2.** Correlation plot of HCB concentration in feces versus HCB concentration in serum.

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Table 1. Concentration of HCB and its metabolites in serum, feces, and urine (n = 53).

| Sample Type | Percent Detected | AM (ng/mL) | SD (ng/mL) | GM (ng/mL) | Minimum | Maximum |
|-------------|-----------------|------------|------------|------------|---------|---------|
| HCB serum  | 100             | 124.2      | 278.2      | 30.2       | 2.4     | 1485.0  |
| HCB feces  | 100             | 395.4      | 629.9      | 149.1      | 11.0    | 3025.0  |
| PCP feces  | 51              | 12.3       | 16.8       | 6.0        | 5.0     | 70.0    |
| PCBT feces | 55              | 32.2       | 42.0       | 7.6        | 5.0     | 139.0   |
| PCP urine | 100             | 3.8        | 4.0        | 2.5        | 0.6     | 18.0    |
| PCBT urine| 100             | 8.8        | 17.0       | 2.6        | 0.5     | 88.9    |
between 0.9 and 105 µg (GM 5.1 µg), which represents 3.1% of the calculated GM in circulation. Calculation of the total amount of HCB excreted in feces is far more tentative. Assuming the standard 24-hr production of feces to be 150–250 g (7), we used the maximum value of 250 g and an average weight reduction of 28% after the drying process (see “Materials and Methods”) to calculate the amount of dry feces per day: 250 g × 28% = 70 g dry feces/day. According to the concentrations presented in Table 1, the total daily unchanged HCB eliminated by feces would range between 0.8 and 211.7 µg (GM 10.4 µg; 6.4% of total HCB in circulation; Table 3). Approximately 0.9 µg 24-hr metabolites (sum of the GM of the PCP concentration in feces × 70 g/24 hr dry feces = 0.42 µg plus the GM of the PCBT concentration × 70 g = 0.53 µg; Table 1) could also be excreted in feces (still only detected in 50% of the samples).

Discussion
This is the first study of humans in which the HCB elimination pattern can be studied in a general population highly exposed to HCB and with the highest HCB serum concentrations ever reported (5). The quantitative results shown in Table 1 clearly suggest that in humans there is a major elimination of unmetabolized HCB in feces. These findings agree with studies performed in rhesus monkeys fed [14C]HCB, which found that a significant amount of the radioactivity detected in feces corresponded to the unchanged parent compound (8). In mammals, the elimination patterns of other organochlorines, such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins, and polychlorinated dibenzofurans, usually show a shift from urine to feces. This shift seems to be based on the size and number of halogen substitutions in the compound; thus, the most lipophilic congeners within each family are eliminated primarily by feces (9). In some cases, virtually unmetabolizable PCB congeners (2,4,5,2’,4’,5’-hexachlorobiphenyl) are eliminated only in feces (10). According to our results for HCB, there is not such a clear shift in humans. We found a large amount of unchanged HCB excreted in feces, but the polar derivatives PCP and PCBT still make up a relatively high proportion of the total output.

HCB found in feces may be, in part, the nonabsorbed and recently ingested compound. However, the strong correlation between HCB content in feces and sera (Figure 2) suggests an equilibrium kinetics with very few variations because of nonabsorbed foodborne HCB. Previous reports have shown that the population under study is exposed to HCB primarily through inhalation of contaminated air (2); the appearance of a correlation between feces and blood is compatible with a main pulmonary entrance and incompatible with irregular amounts of HCB that were recently ingested, not absorbed, and currently circulate in the gastrointestinal tract.

Some authors have suggested that fecal elimination of some lipophilic chemicals is produced mainly by direct passive transfer to the intestinal lumen and not by biliary secretion (11). This could be the case for HCB in humans because mineral oil has been shown to stimulate the intestinal excretion of HCB in rhesus monkeys (12). Our results do not directly confirm this possibility, but the relatively high concentrations of unchanged HCB found in feces and the strong correlation of serum and feces (which in turn suggests a correlation between adipose tissue, serum, and feces) seem compatible with the hypothesis of a nonbiliary passive transfer that is probably mediated by the lymphatics independent of the liver.

The higher concentration of HCB in feces from men as compared to women is dependent on the higher serum HCB found in men in this population subset. A similar difference has also been found in the overall serum study (5). The most probable explanation for these sex differences is that more men that women were employed in an electrochemical factory, which was the main determinant of the variation in HCB body burden (5). The small numbers used in this study and the wide range of HCB body burdens do not allow us to confirm possible sex- and/or age-related variations regarding HCB metabolism and excretion, which would require a further investigation with a larger number of participants.

The amount of HCB that accumulated in adipose tissue in this population can be estimated based on the HCB adipose:serum concentration ratio of 246:1 (lipid weight vs. whole weight) reported by Needham et al. (13). According to this, the population under study may have HCB concentrations in adipose tissue ranging from 0.59 to 365 µg/g (calculated on lipid basis; AM 30 µg/g; GM 7.4 µg/g). Thus, a standard individual who weighs 70 kg and who has 10 kg of adipose tissue (14) could accumulate an adipose HCB burden ranging from 5.9 to 3,653 mg (AM 300 mg; GM 74 mg). This should be corrected for the percentage of extractable lipids (approximately 75%) (15) yielding a range of 4.4–2,737 mg (AM 225 mg; GM 55.5 mg).

Calculations using the previous assumptions on excretion of HCB and metabolites in feces and urine yields a total amount of 16.3 µg excreted in 24 hr (GM 10.3 µg unchanged HCB, 5.1 µg metabolites in urine, and 0.9 µg metabolites in feces; Table 3). These tentative calculations show that the total daily elimination of HCB and metabolites by both routes (urine and feces) may be very small as compared to the adipose depot (0.029%/day; relative to previously estimated GM 55.5 mg of HCB adipose burden) and even as compared to the total HCB in blood (10%/day, relative to GM 163 µg; Table 3). Assuming a first order kinetics, this excretion rate would suppose an average estimated whole-body HCB half-life of 6 years, which is in accordance with the upper limit of calculations made in monkeys (16).

Previous studies have addressed the health status of this HCB-exposed human population (2,3). An increase in soft-tissue sarcoma and thyroid cancer in men was observed, but prevalence of self-reported common chronic diseases, thyroid pathology,  

| Sex | HCB in feces (ng/g) | No. | Mean (SD) | GM |
|-----|---------------------|-----|-----------|----|
| Men | 25                  | 708 (813) | 245* |
| Age | < 45                | 19   | 401 (547) | 138 |
| > 45| 24                  | 517 (778) | 211 |
| Body mass index (kg/m²) | 10 | 92 (56) | 75 |
| Current smoking | No | 37 | 313 (481) | 144 |
| Yes | 13 | 711 (832) | 233 |
| Sample | Random | 17 | 397 (491) | 181 |
| Volunteers | 36 | 394 (692) | 130 |

* p < 0.05.

| Range | HCB in blood (µg/5.4 L) | Urinary excretion (µg PCP + PCBT/24 hr) | Fecal excretion (µg HCB/24 hr)* |
|-------|------------------------|----------------------------------------|---------------------------------|
| 1–10  | 57 (44)                | 0.92–105                               | 0.8–211.7                       |
| 10–16 | 83 (38)                | 5.1 (3.1%)*                            | 10.4 (8.4%)*                    |
| 16–25 | 125 (79)               | 12.5 (8.7%)*                           | 27.6 (4.4%)*                    |

*Estimated based on the assumption of an average excretion of 70 g dry feces/24 hr. Relative to the calculated geometric mean of HCB in blood. Relative to the calculated arithmetic mean of HCB in blood.
Parkinson’s disease, all cancers, and reproductive outcomes did not differ from other populations. Most striking was the absence of porphyria cutanea, a disease known to be associated with HCB exposure in humans (4). However, several peculiarities of HCB kinetics that emerge from this study (adipose sequestration, nonsaturated elimination of unchanged compound by feces, nonsaturated formation of inert glutathione derivatives) could explain why HCB, even present in this human population with the highest blood concentrations ever reported, may not be high enough to trigger hepatic toxicity or other effects typically associated with organochlorines.

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