Urinary Porphyrin Excretion in a Human Population Highly Exposed to Hexachlorobenzene

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Background: Data from an epidemic reported in Turkey (1955-1959) is the only information about the relationship between hexachlorobenzene (HCB) intake and porphyria cutanea tarda in humans. No information is available on the HCB threshold exposure level to induce porphyria cutanea tarda.

Objectives: To study HCB serum levels and urinary porphyrin excretion in the inhabitants of a village located near an organochlorine compound factory with high HCB concentrations in the air and to detect possible alterations in urinary porphyrin excretion and examine their relationship with HCB serum levels.

Design: Cross-sectional study.

Setting: Unit of Porphyrias of a tertiary care facility in Barcelona, Spain.

Participants: Six hundred four inhabitants of the village who were older than 14 years provided serum and urine samples (185 participants were factory workers).

Main Outcome Measures: Serum HCB was analyzed by gas chromatography coupled to electron capture detection. Quantification of urinary total porphyrins was performed by spectrofluorimetry. Porphyrin profile was determined by high-pressure liquid chromatography.

Results: Hexachlorobenzene was detected in all serum samples (mean, 39.8 ng/mL; range, 1.1-1616.0 ng/mL), and levels were higher in factory workers. Mean ± SD level urinary total porphyrin average concentration was 98 ± 69 nmol/L (range, 9-1009 nmol/L). Only the urine sample with the highest porphyrin concentration showed an increase of highly carboxylated porphyrins, with a typical profile of porphyria cutanea tarda. In the remaining 603 urine samples, coproporphyrin was the predominant fraction.

Conclusion: The airborne exposure to and increased body burden of HCB in the Flix village population are not enough to trigger a significant alteration of the heme biosynthesis pathway.

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PORPHYRIA CUTANEA tarda (PCT) is the most common human porphyria and results from a partial deficiency in the liver of an enzyme in heme biosynthesis, uroporphyrinogen decarboxylase, which causes an accumulation of acetylporphyrins (uporphyrins [UPs] and heptacarboxylporphyrins). The disease exists in clinically manifest and asymptomatic stages, which can be recognized on the basis of the amount and characteristic pattern of urinary porphyrin excretion.1

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A similar type of porphyria may be induced experimentally in some rodent strains by exposure to organochlorine compounds such as polychlorobiphenyls (PCBs), polychlorodioxins, and hexachlorobenzene (HCB). Some of these chemicals are widespread environmental pollutants that are occasionally involved in human poisoning episodes. The first evidence of a relationship between one of these chemicals, HCB, and porphyria in humans was reported in southeastern Turkey (1955-1959) when HCB-treated seed wheat was diverted for bread production. Porphyria cutanea tarda was diagnosed in 3000 to 4000 persons. The outbreak was attributed to HCB intoxication, but only experimental studies in rodents confirmed the porphyrinogenic potential of HCB. Unfortunately, no data regarding the concentrations of HCB in blood and tissue samples of the Turkish patients were available; consequently, the HCB threshold exposure level for PCT induction in humans is at present unknown, although HCB is a common organochlorine pollutant.
PARTICIPANTS, MATERIALS, AND METHODS

STUDY POPULATION

An epidemiological cross-sectional study was carried out on the 4178 inhabitants of Flix who were older than 14 years. A questionnaire about residence, occupation, lifestyle, and medical history was completed by 1800 inhabitants (43.1% of the total population). Biological samples were obtained from a random sample of the total population (n = 777), from which 324 inhabitants provided blood and 24-hour urine samples. We also included blood and 24-hour urine samples from 280 inhabitants who responded to the questionnaire but were not in the random sample. The total population studied was 604 (253 males and 351 females; mean ± SD age, 48.8 ± 17.6 years; age range, 14-91 years); 185 of these participants were employed in the chemical plant. Urine samples were collected in 2-L plastic flasks. Immediately after collection, sodium bicarbonate was added to obtain a solution of 5 g/L, and 5-mL aliquots were transferred to polypropylene tubes and stored at −20°C until analysis. Written consent for inclusion in the study was previously obtained from all participants.

ANALYSIS OF ORGANOCHLORINE COMPOUNDS IN SERUM

Hexachlorobenzene, PCBs, pp’-dichlorodiphenyldichloroethene (pp’-DDE), and β-hexachlorocyclohexane (β-HCH) in serum samples were analyzed by gas chromatography coupled to electron capture detection as previously described.12 Negative ion chemical ionization gas chromatography coupled to mass spectrometry was used to confirm the presence of these compounds in the samples.

ANALYSIS OF URINARY PorphyrINS

Quantitative assessment of urinary total porphyrin was made by spectrofluorimetry11 (model F-200; Hitachi, Barcelona, Spain) in all 604 urine samples. The reference value for this method was less than 300 nmol/L. Mole fractions for UP and coproporphyrin (CP) were estimated at their respective maximum intensity wavelengths.12 Normal urine samples showed CP/UP ratios of 80:20 or more. Urinary porphyrin patterns were analyzed in 185 factory workers by high-pressure liquid chromatography according to Lim and Peters.13 Briefly, 1 mL of sample urine was acidified with 50 µL of concentrated hydrochloric acid,14 and 400 µL of this solution was injected into a chromatograph (Waters Corp, Milford, Mass) that was equipped with a fluorescence detector (model 474; Waters Corp) (excitation and emission wavelengths set at 405 and 618 nm, respectively), 2 pumps (model 510, Waters Corp), an automatic controlled gradient, an automatic injector (model 717-plus; Waters Corp) fitted with a 2000-µL sample loop, and an electronic integrator (model CR-6A; Shimadzu Corp, Tokyo, Japan).

Porphyrin separation was achieved using an analytical column (250 × 4.6 mm, 5-µm particle size) (BDS-Hypersil; Shandon HPLC, Cheshire, England). The solvents for the gradient elution were 10% (volume per volume) acetonitrile in ammonium acetate, 1 mol/L, pH 3.16 (solvent A), and 10% (volume per volume) acetonitrile in methanol (solvent B). The column was equilibrated with 100% solvent A before the sample was injected. Porphyrins were separated with a 30-minute linear gradient elution from 100% solvent A to 35% solvent A, followed by isocratic elution for another 10 minutes. The flow rate was 1 mL/min.

STATISTICAL ANALYSIS

Because the distribution for data points is skewed to the right, the variables were analyzed in logarithmic scale. The association of urinary porphyrin levels and HCB exposure was assessed with the standard linear regression method, producing normally distributed residuals. Adjustments for age, sex, and alcohol intake were made with a multiple regression model. Similarly, associations of porphyrins with age, sex, alcohol intake, and occupation were performed by linear regression.

RESULTS

Hexachlorobenzene was detected in all serum samples, with a mean value of 39.8 ng/mL (range, 1.1-1616.0 ng/mL). Hexachlorobenzene levels were higher in males (mean, 72.8 ng/mL; range, 1.1-1616.0 ng/mL) than in females (mean, 17.7 ng/mL; range, 1.7-180.0 ng/mL). Hexachlorobenzene levels were higher in the 185 factory workers, most of whom were male (Table 1). Polychlorobiphenyls, pp’-DDE, and β-HCH were also detected but in lower concentrations than HCB and not different from those found in other populations.15

None of the participants answering the questionnaire admitted to having manifestations of overt porphyria. Mean ± SD urinary total porphyrin concentration was 98 ± 69 nmol/L (range, 9-1009 nmol/L). The mean was higher in males than in females (Table 2). Total porphyrin amount was inversely related to age in males and females. Although the total porphyrin level increased with alcohol intake, the association was seen only in males and was not statistically significant (P = .49). Porphyrin levels did not differ by liver or kidney diseases or by occupation in the factory. Six participants had elevated porphyrin levels. Only the urine sample with a total porphyrin level of 1009 nmol/L showed a CP/UP ratio of 20:80 (Table 3). The CP/UP ratios were 80:20 or greater in the 603 other urine samples.

High-pressure liquid chromatographic profiles of urinary porphyrins were studied in the 185 factory work-
ers. In 184 of them, CP III was the major porphyrin excreted, according to the normal pattern (Table 4). Only the worker with porphyrin excretion of 1009 nmol/L had an increase of UPs and heptacarboxyporphyrins. This participant showed the following profile: UP I, 492.7 nmol/L; UP III, 186.74 nmol/L; heptacarboxyporphyrin I, 24.08 nmol/L; heptacarboxyporphyrin III, 261.0 nmol/L; CP I, 18.34 nmol/L; and CP III, 16.81 nmol/L (Figure). All these features are consistent with the biochemical diagnosis of PCT. The participant was a 33-year-old male who consumed alcohol (>80 g/d). He did not have a past or present history of skin or hepatic disease or antibodies against hepatitis C virus, and his serum HCB level was 256 ng/mL. Serum levels of PCBs, β-HCH, and pp′-DDE were 2.9, 0.4, and 0.7 ng/mL, respectively. This participant died in an accident after the samples were taken, preventing any further study. Subsequent determination of the erythrocyte uroporphyrinogen decarboxylase activity in his family (both parents and a sister) showed normal values.

After correlating the measured HBC concentrations in serum with porphyrin levels for the total population of 604 participants, no statistically significant association was found. Profiles of workers with the highest HCB serum levels are presented in Table 5. No association was found with other organochlorine compounds (β-HCH, pp′-DDE, and PCBs).

**COMMENT**

Hexachlorobenzene is considered a public and occupational health concern when high concentrations are found in the environment. Hexachlorobenzene can induce porphyria in rodents by decreasing uroporphyrinogen decarboxylase activity. A diagnostic indicator of this is the increased levels of highly carboxylated porphyrins (UP and heptacarboxyporphyrin) in urine. Results of the present study confirm that a significant percentage of the Flix population has the highest HCB serum concentration ever reported (Table 6). These levels were particularly elevated in workers from the chemical plant. However, the...
levels of other major organochlorine compounds—PCBs, pp’-DDE, and β-HCH—were not particularly high compared with previous reports in the literature. Thus, the study of porphyrin excretion in this population with long-term exposure to high levels of HCB (≈4 decades) may provide a reference guideline for the feasibility of HCB to induce PCT at current exposure values.

Results of this study show that an increase in UP I and heptacarboxylyporphyrin III levels, according to the PCT profile, was detected in only 1 participant. In the remaining 603 individuals, CP was the major porphyrin excreted, even in the 5 participants in whom an elevated urinary porphyrin level was found. High-pressure liquid chromatography, performed in the group of factory workers, demonstrated that CP was a type III isomer. Marked excretion of CP alone is not an indicator of uroporphyrinogen decarboxylase inhibition. Several different diseases, especially hepatobiliary disorders and lead intoxication, are associated with mild or moderate increases in urinary CP concentration.

There was no relationship between urinary total porphyrin excretion and HCB serum levels or with the other organochlorine compounds. The only participant with high HCB levels; however, individuals with higher levels did not show any alteration in urinary porphyrin excretion (Table 5).

In the Turkish outbreak, almost 90% of the victims were younger than 16 years. In our study, we could not investigate people younger than 14 years. However, physicians and pediatricians who were responsible for the health care of the Flix population informed us that there was no clinical evidence of symptomatic porphyria in persons younger than 45 years. Furthermore, 259 of the individuals analyzed were younger than 45 years, which means they grew up with this severe HCB exposure. Porphyrin cutanea tarda was detected only in the above-reported single participant within this age range (14-45 years).

Prevalence of PCT in the population studied (1.64 per 1000 population, 95% confidence interval, 0.63-2.65) is similar to that found in Madrid, Spain (1.24 per 1000 population), both prevalences being based on the discovery of asymptomatic forms of the disease. The lack of relationship between serum HCB levels and urinary porphyrin excretion, and the absence of other participants with uroporphyrinuria, points away from the possibility that the only case of PCT detected was induced by HCB exposure as the main factor. Serum HCB levels of this population, although much higher than those found in all other human populations studied to date, are probably not as high as those (never studied) of the Turkish victims, who had an estimated oral HCB intake of 50 to 200 mg/d for an undetermined but extended period.

Also, the HCB liver input might have been higher in the Turkish patients than in the Flix population, who had high HCB airborne exposure but relatively low food HCB contamination.

Our results suggest that the airborne exposure to and increased body burden of HCB in the Flix population are not high enough to trigger a significant alteration of the heme biosynthesis pathway. Thus, the HCB exposure limit values and serum threshold levels for a trigger of PCT in humans remain unknown. Our data show that values as high as 1616.0 ng/mL in serum may not induce clinical or subclinical alterations in porphyrin metabolism and excretion. Because not all rodent strains are equally susceptible to HCB-induced porphyria, the question is whether genetic factors may modulate the response and susceptibility to HCB and other porphyrinogenic chemi-

### Table 5. Characteristics of the 15 Factory Workers With the Highest HCB Serum Levels

<table>
<thead>
<tr>
<th>Participant No./ Age, y</th>
<th>HCB, ng/mL</th>
<th>Total Porphyrin, nmol/L</th>
<th>UP I, mmol/L</th>
<th>UP III, nmol/L</th>
<th>Hepta I, nmol/L</th>
<th>Hepta III, nmol/L</th>
<th>CP I, nmol/L</th>
<th>CP III, nmol/L</th>
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<td>85.62</td>
</tr>
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</table>

*HCB indicates hexachlorobenzene; UP, uroporphyrin; Hepta, heptacarboxylyporphyrin; CP, coproporphyrin; and ellipsis, data not detected.

### Table 6. Comparison of Serum Levels of Hexachlorobenzene (HCB) Among Different Populations

<table>
<thead>
<tr>
<th>Subjects, No.</th>
<th>Country</th>
<th>HCB Level, Mean (Range), ng/mL</th>
<th>Study</th>
</tr>
</thead>
<tbody>
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<td>604</td>
<td>Spain (Flix)</td>
<td>39.80 (1.10-1616.00)</td>
<td>Present study</td>
</tr>
<tr>
<td>100</td>
<td>Spain (Barcelona)</td>
<td>4.13 (0.70-19.70)</td>
<td>To-Figuras et al</td>
</tr>
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<td>370</td>
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<td>0.19 (0.05-3.21)</td>
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</tr>
<tr>
<td>15</td>
<td>Croatia</td>
<td>1.00 (0.50-4.00)</td>
<td>Krauthacker</td>
</tr>
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<td>6</td>
<td>Germany</td>
<td>1.23 (0.33-2.66)</td>
<td>Van der Ven et al</td>
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</table>
tals. This could also apply to the human population and remains an area of further research.

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