Elimination of hexachlorobenzene and metabolites by feces and urine in a highly exposed human population.

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Introduction

Hexachlorobenzene (HCB) is a widespread chlorinated hydrocarbon with a strong tendency to accumulate in food chains and lipid-rich tissues of animals and man. Nowadays its main emission sources are impurities present in other chlorinated pesticides and by-products of several industrial emissions, notably in the chlorinated solvent industry. HCB may induce porphyria in humans and has a broad range of toxic effects in experimental animals, including inmunotoxicity, reproductive toxicity, endocrine adverse effects and cancer (1).

A cross sectional research project on the health effects of HCB is being carried out in Flix (Tarragona,Catalonia,Spain), a rural village located near an a chlorinated solvent factory where high airborne HCB exposure has regularly occurred during the last four decades (2). Since very few studies have addressed HCB kinetics in humans, this project has also been used to get more insight into HCB metabolism and disposition. In a previous study (3) we have reported an association between serum HCB levels and the elimination by urine of a sulfur derivative that after hydrolysis yielded pentachlorobenzenethiol (PCBT; figure 1).

In the present study we report the analysis of HCB and metabolites in feces in the same population in order to obtain a more complete approach of HCB excretion pattern in humans.

Material & Methods

Feces of 40 subjects, whose serum and urine had been previously analyzed (3) was collected for analysis of HCB and metabolites. This subset were 25 males and 15 females of a mean age of 47 and 42.6 years respectively. Feces were homogenised and samples (aprox 250 mg) were dried at 65ºC overnight. Dried samples were weighed and digested under N_2 with 4 mls of 2N NaOH for 4h at 70ºC . Ascorbic acid and aldrin (as internal standard) were added to the mix. This alkaline hydrolysis yields free PCP and PCBT. After cooling and acidification with conc HCL (pH=1), HCB and metabolites were extracted twice with 5 ml of benzene; the solvent extracts were concentrated to 0.5 mls and treated with 0.5 ml of diazoethane in diethyl ether.

After derivatization (30 min in the dark), the excess diazoethane was removed under a N_2 stream, the solvent extracts were concentrated to approximately 0.1 ml and n-hexane (2 ml) was added. The resulting mixture was cleaned-up with H_2SO_4, the organic phase was separated, concentrated to 25 µl. HCB and ethyl derivatives of PCP and PCBT were analyzed by GLC (Hewlet Packard 5890 II) with ^{63}Ni electron capture detection. Recovery of HCB, PCP and PCBT was assayed with spiked wet feces and ranged between 88-109%. Urine was analyzed similarly and as previously described (3). HCB in sera was analyzed in the Department of Environmental Chemistry (CID-CSIC) by GLC/ECD after treatment of sera with sulfuric acid (3).

Results

All feces analyzed contained unchanged HCB with values ranging from 10 to 2300 ng/g (concentrations calculated on a dry weight basis); X ± s.d.: 458 ± 620. Differently, none of the urines previously analyzed contained unchanged HCB at detectable limits (> 0.5 ng/ml). HCB concentration in feces strongly correlated with HCB in serum (R^2 =0,87; p<0.0001; fig 2). Pentachlorophenol (PCP) was detectable in only 40 % of the samples (range: 3-22 ng/g; X ± s.d.: 5.95±5.5) and pentachlorobenzenethiol (PCBT) in only 34 % (range: 3-200 ng/g; X ± s.d.:24 ± 48 ng/g. PCP and PCBT had been detected in 100 % the urines previously analyzed. Other known metabolites of HCB in rodents (tetrachlorohydroquinone, tetrachloro-1,4-benzenedithiol) could not be detected in feces or urine.

Discussion

The whole set of results suggest a pattern of HCB elimination in humans with a major elimination of the unmetabolized compound by feces. These findings in humans agree with studies in rhesus monkeys feed with [^{14}C] HCB were aprox 90% of the radioactivity found in feces was found to be the parent compound.(4). The correlation found between HCB in feces and HCB in sera suggest an equilibrium kinetics with few variations due to non-absorbed food-borne HCB. This is in agreement with other observations which indicate that in the population under study, HCB exposure is due, mainly, to inhalation of contaminated air (2). Some authors have suggested that fecal elimination of HCB is produced mainly by direct transfer of the chemical to the large intestines via the lymphatic system and not by biliary secretion (5). This could easily induce an equilibrium between fat depots and fecal excretion. Our results, showing relatively high levels of HCB in feces and a strong correlation serum/feces would tend to reinforce this point of view. As expected from the lipophilicity of the compound, unmetabolized HCB do not appear to be eliminated by urine. The metabolism, although showing a whole poor efficacy, results in the elimination by urine of conjugated PCP and a cysteine derivative (probably S-pentachlorophenyl-N-acetyl-L-cysteine) that after hydrolysis yields PCBT (6). Urine appears to be the main route of elimination of these metabolites since they could not be detected in all the feces analyzed and sometimes they were found at only trace amounts. An approximate estimation of HCB depots in adipose tissue can be calculated from the serum levels and the known adipose/serum partition coefficients.

This shows that the whole metabolite out-put is very small compared to HCB internal dose. This is particularly evident for PCP which present low levels in feces and urine, suggesting a relatively poor efficiency of the HCB oxidative pathway. Differently, the glutathione conjugation and formation of the mercapturate appears to be a major metabolic pathway in those cases with higher HCB internal dose, clearly surpassing the formation of hydroxylated derivatives. This is one of the first opportunities to study the kinetics of HCB in a highly exposed human population. Other reports will present the health status of this population and the possible HCB adverse effects.

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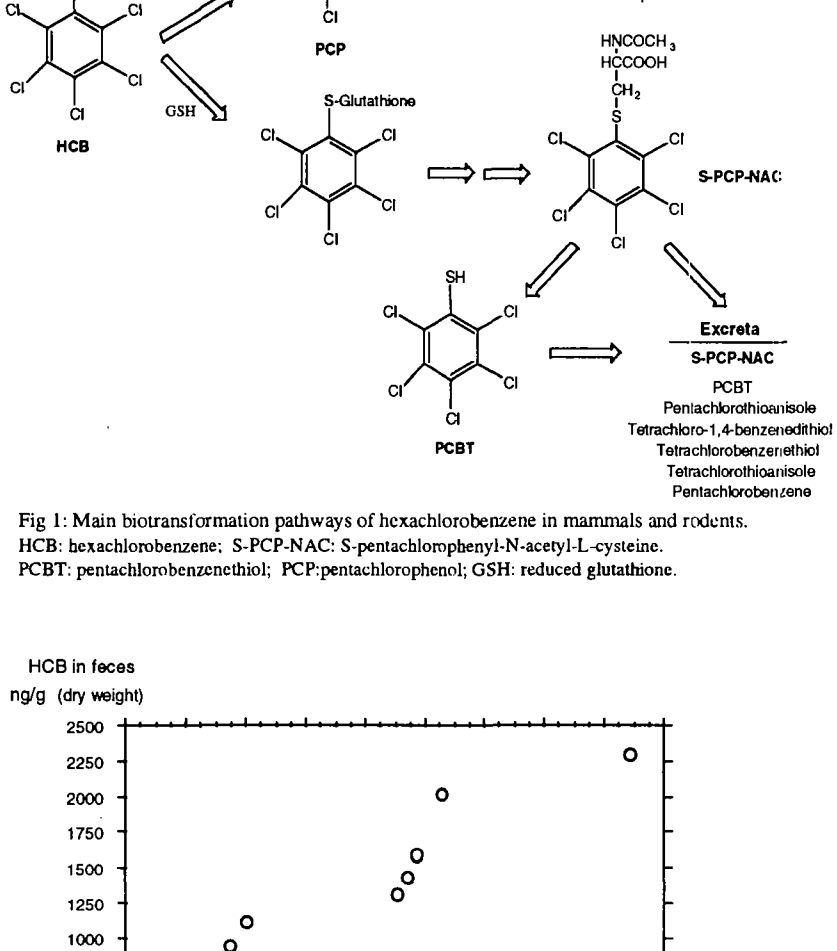

Fig 1: Main biotransformation pathways of hexachlorobenzene in mammals and rodents.
HCB: hexachlorobenzene; S-PCP-NAC: S-pentachlorophenyl-N-acetyl-L-cysteine.
PCBT: pentachlorobenzenethiol; PCP:pentachlorophenol; GSH: reduced glutathione.

Figure 2: Correlation between HCB levels in serum and HCB in feces