Plasma γ -hexachlorocyclohexane concentrations in forestry workers exposed to lindane

L DRUMMOND,¹ E M GILLANDERS,² H K WILSON¹

From the Occupational Medicine and Hygiene Laboratory,¹ Health and Safety Executive, London NW2 6LN, and Employment Medical Advisory Service,² Health and Safety Executive, Regent Centre, Newcastle-upon-Tyne, NE3 3JN, UK

ABSTRACT Plasma γ -hexachlorocyclohexane (γ -HCH) and three urinary trichlorophenols were measured in forestry workers who were engaged in planting seedlings treated with γ -HCH. These two procedures were assessed as potential biological monitoring methods and the data were compared with reported clinical symptoms. The measurement of plasma γ -HCH was considered to be a feasible and valid monitoring method for use in routine practice and is a useful indicator of γ -HCH absorption. The data were used to illustrate the need to be vigilant about personal hygiene and the efficacy of protective clothing. Plasma γ -HCH concentrations above 70 nmol/l were measured in two workers which coincided with persistent non-specific clinical symptoms. Trichlorophenols were identified in urine but the extensive and variable metabolism of γ -HCH makes this approach less suitable for biological monitoring.

γ -Hexachlorocyclohexane (γ -HCH), commonly known as lindane, has been used in veterinary, agricultural, and medical products. It has been used in applications ranging from the treatment of scabies to the control of agricultural pests. γ -HCH is the more toxic of the HCH isomers but accumulates in human fat to a lesser extent.¹ It primarily affects the central nervous system and signs of poisoning include non-specific symptoms such as headache, muscle pain, and nausea.

Occupational exposure to pesticides is recognised as a potential health risk and skin absorption is often an important route of entry into the body. Little is known about the mechanism of percutaneous absorption, however, and recent interest has been shown in combining the data from measurements in field studies and dermal absorption with risk assessment.²

In the United Kingdom the occupational exposure limit for γ -HCH is 0.5 mg/m³ with a short term limit of 1.5 mg/m³ and carries a skin notation.³ In 1982 the World Health Organisation recommended that a biological exposure limit of 20 μ g/l (70 nmol/l) in blood should be set for workers occupationally exposed to lindane.⁴ The central nervous system is viewed as the critical target organ and this level was to be regarded as an individual maximum and was

considered low enough to prevent a symptomatic adverse effect on the nervous system.

In 1985 we observed that some workers in the United Kingdom who were engaged in dipping conifer seedlings in γ -HCH complained of ill health. The symptoms were non-specific but it was subsequently established that these workers had plasma concentrations of γ -HCH considerably above that of the unexposed normal population (unpublished observations).

There are limited data available for plasma concentration profiles of γ -HCH measured throughout the season, and there is a need to establish a well validated routine monitoring procedure for workers exposed to this pesticide. In the present study plasma γ -HCH was evaluated as a suitable biological monitoring procedure for helping to control exposure to γ -HCH. Concurrently, full protective clothing and respirators were made available to help reduce potential exposure. In addition, we have explored the feasibility of measuring three major urinary metabolites (trichlorophenols). We report longitudinal data on two possible biological monitoring methods in a group of forestry workers exposed to γ -HCH.

Experimental

STUDY DESIGN

After consultation and discussion between the United Kingdom Forestry Commission, Civil Service

Occupational Health Service, Forestry workers, and the investigators, it was agreed that monitoring of plasma γ -HCH would be made available to all employees engaged in dipping, transporting, and planting operations throughout the Commission's operations in the United Kingdom. Pesticide Safety Precaution Scheme clearance was given to use Gamma-col* for planting 260 hectares. Gamma-col contains γ -HCH and was used as a 1.6% w/v γ -HCH suspension in water. A total of 45 men took part in the biological monitoring programme. Exposure resulted either from dipping conifer seedlings in the γ -HCH solution, transporting the dipped seedlings to the planting sites, or from planting the seedlings.

Protective clothing was supplied to all the workforce. The "dippers" wore a one piece neoprene boiler suit, rubber apron, boots, above elbow gloves, dust mask, and plastic face shield. "Planters" wore a waterproof suit, rubber boots, wrist length gloves, and a carbon cloth respirator.

The volunteers agreed to provide a pre-exposure sample of blood and urine. Sampling of blood and urine and the clinical assessment was repeated at roughly fortnightly intervals for 20 weeks starting in April 1986.

SAMPLES

Blood samples (5 ml) were collected by venepuncture at appropriate field locations and placed in EDTA bottles. Urine samples were collected in polycarbonate bottles. All the samples were dispatched to the laboratory to arrive the next morning. On arrival, the blood samples were centrifuged and the plasma removed and stored at -20°C . The plasma samples were analysed for γ -HCH and for indicators of liver dysfunction. Urine samples were stored at -20°C and were analysed for trichlorophenol metabolites.

ANALYSIS OF γ -HCH IN PLASMA

γ -HCH was extracted from plasma (500 μl) with hexane (8 ml) and heptachlor was used as an internal standard. The hexane extract was evaporated to dryness and the residue was redissolved in hexane (500 μl). Initially, the analysis was performed using a temperature programme from 120°C to 270°C on a BP5 capillary column with electron capture detection (Carlo Erba Mega 5160 with AS 550 autoinjector). Using this procedure the chromatographic run time was 20 minutes a sample. This time was considerably reduced to eight minutes by using a "high oven temperature" cold on-column injector. This enabled the chromatography to be carried out isothermally at 210°C . In this system a secondary cooling coil

surrounds the pre-column (15 cm) which is maintained at 65°C using servo air. After injection the secondary cooling is switched off automatically and the pre-column reaches the oven temperature of 210°C within 45 seconds. The method has a coefficient of variation of 5% and a detection limit of 5 nmol/l.

The presence of γ -HCH was confirmed in six samples using capillary column gas chromatography mass spectrometry (VG 12-250) by comparison with a pure γ -HCH standard.

ANALYSIS OF α -, β -, AND γ -HCH ISOMERS

The method outlined above was modified to enable resolution of the isomers α , β , and γ -HCH by reducing the column temperature to 190°C .

ANALYSIS OF TRICHLOROPHENOL METABOLITES

Urine (1 ml) and concentrated hydrochloric acid (200 μl) were heated at 100°C for 30 minutes together with pentachlorophenol as an internal standard. Trichlorophenol (2,3,5-; 2,4,5-; and 2,4,6-) were extracted from urine with hexane (8 ml). The hexane extract was evaporated to dryness and the residue was derivatised with acetic anhydride (50 μl) and pyridine (50 μl). This solution was dissolved in hexane (500 μl) and then washed with 1M sodium hydroxide (500 μl). The hexane layer (1 μl) was injected (splitless) on to a BP5 capillary column fitted to a HP 5860 gas chromatograph and a VG12-250 mass spectrometer. Selective ion monitoring was used to quantify the trichlorophenol isomers (m/z 196) and internal standard (m/z 266). A temperature programme of 160°C for 0.5 minutes with a rate of $35^{\circ}\text{C}/\text{min}$ to 240°C for two minutes was used to separate the metabolites. The method has a coefficient of variation of 6% and a detection limit of 10 nmol/l.

Results were corrected for creatinine concentration (measured by the Jaffé reaction using a Cobas Bio autoanalyser).

LIVER FUNCTION TESTS

The standard clinical laboratory liver function tests (alkaline phosphatase, γ -GT, ASAT, ALAT) were determined by automated discrete and centrifugal analysers using commercially available kits.

QUALITY CONTROL SAMPLES

The day to day precision of the plasma γ -HCH analysis was monitored using quality control samples. Horse plasma was spiked with γ -HCH (nominal concentration 340 nmol/l) and dispensed (2 ml) into vials. The plasma was freeze dried and stored at -20°C . The sample was reconstituted with distilled water (2 ml) for analysis. The between batch coefficient of variation was 10.5%.

*Gamma-col ICI trade mark.

Fig 1 Mean plasma γ -HCH concentrations in a group of forestry workers from April to August (mean and SD; number in each group varied between 20 and 33).

Results

During our earlier investigations, only the γ -isomer was detected; we were unable to detect the presence of the other isomers, α and β -HCH, in the plasma samples.

In the present study no plasma γ -HCH was detected in any of the pre-exposure samples. The results from the samples collected during the exposure period are plotted against time in fig 1. The data are presented as the γ -HCH plasma concentration (mean and SD) for all those workers with detectable levels of γ -HCH. A

Fig 2 Percentage of workers with no detectable plasma γ -HCH (less than 5 nmol/l) from April to August.

wide range of interindividual plasma concentrations was observed. The percentage of workers with no detectable γ -HCH is illustrated in fig 2.

For the first eight weeks of the study (April–June) all the workers had plasma γ -HCH concentrations less than the detection limit (5 nmol/l) and it was only in the eighth week that five workers reported sporadic symptoms. During this period (April–June) the mean maximum temperatures were 13–17°C.

From June onwards, there was an upward trend in the number of workers with raised γ -HCH concentrations in plasma; by the end of July all workers had detectable levels of γ -HCH. The group mean increased from zero at the beginning of June to a maximum of 40 nmol/l in mid-July. During this period there was a pronounced change in the daily temperatures which ranged from 15° to 19°C in early June to 20° to 26°C in late June. Temperatures declined in July (17–20°C) but the humidity increased and there was an exceptionally high population of midges.

On 9 July the highest plasma γ -HCH concentration was 59 nmol/l. On 16 July two workers had values of 123 and 75 nmol/l respectively. These two workers complained of feeling unwell with flu like illness, tiredness, sore throat, and nausea. The symptoms in these two workers lasted for about 14 days, at which time the γ -HCH level for each man had fallen to 26 and 23 nmol/l respectively. It had been previously agreed that if levels above 70 nmol/l were found then exposed workers would be removed from further exposure. As this occurred concurrently with a deterioration in the condition of the young saplings in the cold store, however, all planting operations were suspended and the dipping shed was cleaned out.

When exposure ceased at the end of July, those employees who showed raised γ -HCH in plasma were monitored until August. At this time 80% of the workers had no detectable γ -HCH in their plasma. For those workers who had raised plasma γ -HCH, the group mean was 16 nmol/l. No further symptoms were reported. Subsequent resampling in early September indicated that levels had now returned to pre-exposure values—that is, below the detection limit.

Three trichlorophenols (2,3,5-; 2,4,5-; 2,4,6-) were identified in the urine of some workers exposed to γ -HCH. The presence of these metabolites was confirmed by comparison with authentic standards using gas chromatography mass spectrometry based on relative retention times (120, 123, and 110 seconds respectively) and a characteristic ion m/z 196 ($M-\text{COCH}_3$).

All three trichlorophenols were quantified using single ion monitoring and the data have been compared with plasma γ -HCH concentrations. No statistical relation was found between individual trichlorophenol metabolites and γ -HCH; however, when total

Fig 3 Relation between plasma γ -HCH and urinary trichlorophenols.

trichlorophenol excretion was related to plasma γ -HCH (fig 3) there appears to be a weak relation between these two parameters (correlation coefficient 0.46).

During the period April to June when no γ -HCH was found in any of the plasma samples, small quantities of trichlorophenols were found occasionally in some urine samples (between 1 and 10 nmol/mmol creatinine). It is worth noting that similar concentrations were found in some of the pre-exposure samples. These metabolites may have originated from exposure to γ -HCH but they are not specific indicators of γ -HCH uptake.

There was no evidence of hepatotoxicity as all the liver function test results were in the normal range.

Discussion

Numerous papers and reviews have been published on the effects of γ -HCH and its accumulation and metabolism in man.⁵⁻⁷ It is generally agreed that skin

absorption is an important route of entry into the body. The process of percutaneous absorption is a complex multifunctional process and simply monitoring external exposure may have little relevance in vivo.⁸ It is in this area that biological monitoring can play an important part. After exposure to γ -HCH, the uptake may be monitored by measuring the blood or plasma concentration. These techniques may be used to encourage good hygienic practice and to determine the effectiveness of protective clothing.

After an earlier observation that workers exposed to γ -HCH in the course of dipping and planting young trees had raised plasma concentrations of γ -HCH, and in some cases reported non-specific symptoms, we arranged to collect longitudinal data over a 20 week period. The workers were provided with respiratory masks and protective clothing. The γ -HCH plasma concentrations were determined at fortnightly intervals together with a record of reported clinical symptoms.

For the first eight weeks of the study there was no indication of γ -HCH absorption, which coincided with few reported symptoms. In the eighth week non-specific symptoms, such as flu like illness, were reported by five workers and the number of workers with detectable γ -HCH began to increase. By mid-July the symptoms were pronounced in two workers who had plasma concentrations of 123 and 75 nmol/l respectively. These symptoms were reported to the medical officer at the time of blood sampling when the subjects were completely unaware of the plasma concentration of γ -HCH.

The use of γ -HCH ceased in July and a reduced plasma concentration in all workers was immediately evident. After about two weeks all symptoms had disappeared from all the workers. The non-specific symptoms reported in this study have been noted previously by other workers.⁹ Kashyap reported that 90% of formulators and 75% of workers engaged in γ -HCH manufacture showed toxic reactions—for instance, headache, paraesthesia of face, malaise, and vomiting.¹⁰ These groups of subjects had mean serum γ -HCH concentrations of 196 and 55 nmol/l respectively. Nevertheless, a neurological examination of chronically exposed workers with a mean blood value of 86 nmol/l, reported by Baumann *et al*, found no evidence for impairment of the central nervous system or peripheral motor nerves.¹¹ Czegledi-Janko *et al* examined 37 lindane production workers and observed that the number of men with clinical symptoms and EEG changes increased when the blood concentration exceeded 70 nmol/l.¹² After reviewing published reports the WHO concluded that a blood concentration of 70 nmol/l should be considered an individual maximum and is a level below which no clinical symptoms are expected.⁴ Many of these results

cannot be compared directly since some investigators have used blood measurements and others have used serum or plasma which would be expected to be about twice the blood concentration.

It is not clear whether the uptake of γ -HCH resulted because the protective clothing lost its efficacy or because there was increased accidental contamination of the skin. The increase in the ambient temperature and high humidity may have resulted in mopping of the skin or removal of some clothing. The results are consistent with the explanation that either occupational hygiene was poor and the operators had deviated from the recommended operating procedures or the protective equipment was effective in preventing exposure for the first eight weeks only.

The mean value for the half life of γ -HCH in plasma was calculated to be about eight days. There appears, however, to be evidence for at least a two compartment model, the half life being about five hours and eight days.¹³ The rapid uptake of γ -HCH through the skin is well established. Peak concentrations have been found in serum two to five hours after dermal application.^{13,14}

γ -HCH undergoes extensive metabolism and over 20 metabolites have been identified in human urine. The trichlorophenols (2,4,6-; 2,3,5-; 2,4,5-) have been shown to be the major metabolites of γ -HCH in man.¹⁵ These metabolites have been identified in this study and have been considered as a possible non-invasive approach to biological monitoring. As might be expected, the correlation between γ -HCH in plasma and three trichlorophenols in urine is not high, although in cases where γ -HCH uptake is highest there is a corresponding upward trend in urinary metabolite excretion. During the first eight weeks when no γ -HCH was detected in plasma, nine workers occasionally had low concentrations of trichlorophenols in urine (less than 10 nmol/mmol creatinine). This pattern was observed in the pre-exposure urine samples. Owing to the extensive metabolism of γ -HCH and the non-specificity of these metabolites, the measurement of trichlorophenols appears to have limited value in biological monitoring. Chlorophenols are metabolites of all HCH isomers and some chlorobenzenes and are not specific for γ -HCH.¹⁵ It has been suggested that the chlorophenol metabolites of γ -HCH are responsible for chronic hepatotoxicity.¹⁵ We did not observe any signs of hepatotoxicity in this study.

We suggest that for routine monitoring measuring γ -HCH in plasma is more appropriate. The analytical method is precise, sensitive, and specific, and it is thought that it is γ -HCH per se that is responsible for acute toxicity of the central nervous system.¹⁶

The present study illustrates the value of biological monitoring in assessing uptake of γ -HCH. Regular monitoring has identified when absorption of the

pesticide has occurred and illustrates the need to be vigilant concerning personal hygiene and operator protection. We have also followed up the time taken for the workforce to return to normal concentrations after cessation of exposure. We found no reliable evidence for a causal relation between plasma γ -HCH and clinical symptoms. The limited findings in this investigation, however, suggest that a lower action level than that proposed by the World Health Organisation may be appropriate.

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