Determination of 3,5,6-trichloro-2-pyridinol levels in the urine of termite control workers using chlorpyrifos.

Fumihiko Jitsunari*  Fumiyuki Asakawa†  Taichi Nakajima‡
Junko Shimada**  Masana Ogata††

*Kagawa Medical School,
†Kagawa Medical School,
‡Kagawa Medical School,
**Maeda Hospital,
††Okayama University,
Determination of 3,5,6-trichloro-2-pyridinol levels in the urine of termite control workers using chlorpyrifos.*

Fumihiko Jitsunari, Fumiyuki Asakawa, Taichi Nakajima, Junko Shimada, and Masana Ogata

Abstract

Chlorpyrifos, an organophosphorus insecticide, has been used to control termites since regulatory measures against the use of chlordanes were taken in September, 1986. We developed an improved gas chromatographic (GC) method for the assay of 3,5,6-trichloro-2-pyridinol (TCP) in the urine to use in the biological monitoring of exposure to chlorpyrifos. Urinary TCP was separated and determined accurately (C.V., 4%) with high sensitivity (detection limit, 10 ng/ml) and recovery (recovery greater than 90%) using a wide bore capillary column (WBC column). The accuracy and precision of the present GC method are satisfactory. The time course of urinary excretion of TCP was followed in workers. The urinary TCP level was low in the off-season and high in the busy season. Variation in the urinary TCP level corresponded to the termite control season and the length of the working period. The urinary TCP level showed a change reciprocal to the variations in the plasma cholinesterase activity. From these results, it is surmised that the urinary TCP level represents the extent of exposure to chlorpyrifos. The decrease in the level of cholinesterase activity is suggested to be due to exposure to chlorpyrifos. Determination of the urinary TCP level by GC using a WBC column is useful in the biological monitoring of chlorpyrifos in termite control workers and potentially has practical application to health care.

KEYWORDS: termite control worker, chlorpyrifos, urinary 3, 5, 6-trichloro-2-pyridinol, biological exposure index, biological monitoring

*PMID: 2481940 [PubMed - indexed for MEDLINE]
Copyright (C) OKAYAMA UNIVERSITY MEDICAL SCHOOL
Determination of 3,5,6-Trichloro-2-Pyridinol Levels in the Urine of Termite Control Workers Using Chlorpyrifos

Fumihiko Jitsunari*, Fumiyuki Asakawa, Taichi Nakajima, Junko Shimada and Masana Ogata

Department of Hygiene and Public Health, Kagawa Medical School, Kagawa 761-07, aMaeda Hospital, Kagawa 761, bDepartment of Public Health, Okayama University Medical School, Okayama 700, Japan

Chlorpyrifos, an organophosphorus insecticide, has been used to control termites since regulatory measures against the use of chlordanes were taken in September, 1986. We developed an improved gas chromatographic (GC) method for the assay of 3,5,6-trichloro-2-pyridinol (TCP) in the urine to use in the biological monitoring of exposure to chlorpyrifos. Urinary TCP was separated and determined accurately (C.V., 4%) with high sensitivity (detection limit, 10 ng/ml) and recovery (recovery > 90%) using a wide bore capillary column (WBC column). The accuracy and precision of the present GC method are satisfactory. The time course of urinary excretion of TCP was followed in workers. The urinary TCP level was low in the off-season and high in the busy season. Variation in the urinary TCP level corresponded to the termite control season and the length of the working period. The urinary TCP level showed a change reciprocal to the variations in the plasma cholinesterase activity. From these results, it is surmised that the urinary TCP level represents the extent of exposure to chlorpyrifos. The decrease in the level of cholinesterase activity is suggested to be due to exposure to chlorpyrifos. Determination of the urinary TCP level by GC using a WBC column is useful in the biological monitoring of chlorpyrifos in termite control workers and potentially has practical application to health care.

Key words: termite control worker, chlorpyrifos, urinary 3,5,6-trichloro-2-pyridinol, biological exposure index, biological monitoring

Chlorpyrifos, an organophosphorus insecticide, has been widely used to control termites since regulatory measures against the use of chlordanes were taken in September, 1986. With the arrival of the first termite control season (May, 1987) after the switch-over from chlordanes to chlorpyrifos, we found a rapid fall in the plasma cholinesterase activity in termite control workers (1, 2). We documented the status of work with a high risk of exposure to chlorpyrifos through the air or directly through the skin

*To whom correspondence should be addressed.
at high concentrations (3,4). The health hazard of organophosphorus poisoning among termite control workers frequently engaged in the spraying of chemicals in a small and closed environment has received attention in the field of public health. This problem is difficult to cope with in some respects due to conventional environmental control and work management, because the working place changes and the construction of houses is not uniform. Accordingly, biological monitoring based on exposure indices in the blood and urine is considered important from the viewpoint of health care.

3,5,6-Trichloro-2-pyridinol (TCP) is a urinary metabolite (5) of chlorpyrifos. We determined the time course of urinary TCP levels in workers after improving the assay method of this compound. Then, we discussed the relationship among urinary TCP levels, variations in plasma cholinesterase activity and working hours.

Materials and Methods

Reagents. 3,5,6-Trichloro-2-pyridinol, supplied by Dow Chemical Company, Midland, MI, USA, was dissolved in benzene to prepare a 1000 ppm standard solution. The solution was diluted in benzene before use when needed. N,O-Bis(trimethylsilyl)acetamide (BSA) from Wako Pure Chemical Co., Osaka, Japan, was used as the silylating agent. Organic solvents and other reagents for analysis of residual agricultural chemicals or their equivalents were used.

Gas chromatography (GC). We used the wide bore capillary column (WBC column) to improve method for the determination of urinary TCP. The recently developed WBC column has the merit of both a convenience of the packed column (P column) and a high separation ability of the capillary column (6). The P column was used according to the conditions described by Nolan et al. (7) with some modifications.

A GC-7A gas chromatograph (Shimadzu Works, Kyoto, Japan) with electron capture detector was used in connection with a data processor, chroma-
work above or under the floor. Preparations used are a 40-fold diluted emulsion of 40% chlorpyrifos and an oil preparation containing 1% chlorpyrifos. Chlorpyrifos was dissolved in emulsifier or kerosene. Chemical stabilizers were not added, so other hazardous compounds were not present. Preparations are sprayed or injected with power sprayers. The workers wore a hood, overalls, rubber gloves, rubber boots and a mask provided with a direct connection type small-sized absorption can for organic gasses. In the busy termite control season, the control work was done on about 2 houses a day per team. The termite control season generally spanned a period from May to September. The period from October or November to March or April of the following year was referred to as the non-busy season.

The detailed relationship between the working situation and exposure to chlorpyrifos has been reported elsewhere (3).

Results

Gas chromatograms of TCP through the P and WBC columns are illustrated in Fig. 1. When 1 μl of the standard TCP solution (0.1 μg/ml) was injected, a sharp peak was obtained with the WBC column, while tailing occurred with the P column. The attenuation in GC was raised to 2 for the P column. Analysis could be made at attenuation 8 in the case of the WBC column. Accordingly, the analytical sensitivity of the WBC column is four times that of the P column. The theoretical plate of the P column was 422 ± 56 (mean ± SD, n = 3), and that of the WBC column was 10,517 ± 408 (mean ± SD, n = 3). Thus, with the WBC column, we were able to determine TCP levels at a higher sensitivity and separation ability than with

![Gas chromatograms](image)
the P column. The number of samples analyzed per unit time was almost equal. TCP in the urine of the workers was detected as a clearly separated peak in gas chromatograms obtained with the WBC column (Fig. 1).

The TCP standard solution was added to urine to give a final concentration of 0.1 μg/ml, and determination was carried out according to the standard analytical procedure. The recovery rate was 91.5 ± 1.3% (mean ± SD, n = 4), and the detection limit was 10 ng/ml in terms of the urinary TCP level assuming that 1 μl is injected into the GC. In this experiment, the detection limit was taken as amount represented by a peak height three times the background height. A silylated derivative of TCP was formed immediately after addition of BSA, but it decomposed with time, most markedly in the urine samples (stored in a cool, dark place after silylation). Accordingly, determination should be done soon after silylation, at least within the same day. Urinary TCP was determined quantitatively at a high level of sensitivity with the WBC column. This

![Diagram](image-url)

**Fig. 2** Monthly variations in urinary 3,5,6-trichloro-2-pyridinol (TCP) concentration of workers in 1988. The busy season was from May to September. Workers: A, ×; B, △; C, ■; D, ◻; E, ●; F, ○; G, ◻; H, ×.
method is accurate and precise. Urine samples were collected on the first Friday of every month upon returning to the company after work. Venous blood for the determination of cholinesterase activity in the plasma was taken from a cubital vein at 8 a.m. on the first day of every month. Urinary TCP concentrations of workers are illustrated in Fig. 2. Generally, the urinary TCP level was lower than 0.10 mg/g creatinine in February (off-season). However, it increased markedly in the beginning of the termite control season, and reached a peak of 0.07-4.18 mg/g creatinine in August (busy season). The highest level of TCP was 40 times that in the off-season. Thereafter, the urinary TCP level decreased sharply, and fell to below 0.20 mg/g creatinine in December. Workers G and H, who were engaged in sales showed lower levels throughout the season than workers exclusively engaged in the control work. In the busy season, however, they sometimes helped with the termite control work, and on such occasions, an increase in the urinary TCP level was noted. Of the termite control workers, B showed the highest level throughout the season, followed by A, C, E, F and D in that order. TCP was not detected in urine which was collected by the same method at the same time from persons not exposed to chlorpyrifos.

The relationships of the urinary TCP level of worker C, as a typical example, to the variation in the plasma cholinesterase activity and to the duration of his work are illustrated in Fig. 3. The working hours started to increase gradually from January, showed a marked increase in May, remained at about the same level up to July, and began decreasing from August to the off-season. The duration of work corresponded to the urinary TCP level. The plasma cholinesterase activity showed a reciprocal relationship. When the urinary TCP was at the peak, the plasma cholinesterase activity was the lowest (the beginning of August), and the number of hours worked had passed the peak. Changes in working hours, the TCP level and cholinesterase activity in worker C were similar to those in two other workers (E and G) (Fig. 3). Among all eight workers, there was a significant positive correlation between the working hours and the urinary TCP level \(r = 0.641, n = 72, p < 0.05\), a significant negative correlation between the working hours and plasma cholinesterase activity \(r = -0.593, n = 82, p < 0.05\) and a significant negative correlation between the urinary TCP level and plasma cholinesterase activity \(r = -0.767, n = 65, p < 0.05\).

Discussion

Chlorpyrifos, an organophosphorus insecticide, is frequently used as a termite control agent. Accordingly, the health hazard of chlorpyrifos to termite control workers needs to be considered. Since the work pattern of termite control workers is irregular, biological monitoring based on exposure indices in blood and urine is important from the viewpoint of health care. TCP is a urinary metabolite of chlorpyrifos which may be used as an exposure index. Sultatos et al. (5) have reported that chlorpyrifos was excreted in the urine as TCP and as dialkylphosphates such as diethylthiophosphate and diethylphosphate. Generally, dialkylphosphates are analyzed as exposure indices of organophosphorus insecticides (11-13). However, phosphate compounds lack specificity since they are common to all organophosphorus insecticides. On the other hand, TCP is mostly excreted into urine (7,14) and is highly specific for chlorpyrifos, given the unique chemical structure and metabolism (5) of chlorpyrifos.
Fig. 3-1

Fig. 3-2
TCP was separated and determined accurately (C.V., 4%) with high sensitivity (detection limit, 10 ng/ml) by using the WBC column instead of the P column. The WBC column is a fused silica capillary column with high column efficiency, heat stability and solvent tolerance. This column can easily be used with existing apparatus by simply connecting an attachment. It is as convenient as the P column and has the high separation ability of the capillary column. Thus, GC using the WBC column is useful for biological monitoring of urinary metabolites.

We determined the urinary TCP levels in termite control workers and confirmed that there was a definite difference between workers with a high risk of being exposed to chlorpyrifos and those with a low risk or no risk. The urinary TCP level varied with the termite control season and the number of working hours. These results suggest that the detection of urinary TCP indicates exposure to chlorpyrifos and that the variation in the urinary TCP level reflects the amount of chlorpyrifos to which the workers have been exposed.

On the basis of observations made since October, 1986, we have reported that the variation in the plasma cholinesterase activity level in workers reflects exposure to chlorpyrifos (1, 2). In the present cases, it was confirmed that plasma cholinesterase activity decreased with an increase in the working hours. The plasma cholinesterase activity shows a change reciprocal to the urinary TCP level. The plasma cholinesterase activity decreased in the order of workers B, A, C, E, D, F, G and H in August, 1988 (15). This result was consistent with the present result obtained in the same month, August, in which the urinary TCP
level became high in the order of workers B, A, C, E, F, D, G and H. Therefore, the plasma cholinesterase activity correlates well to the urinary TCP level among the workers. It is suggested that the decrease in plasma cholinesterase activity mainly occurs due to chlorpyrifos exposure.

From these results, urinary TCP appears to be a biological index of exposure to chlorpyrifos. Determination of TCP by GC with the WBC column is useful in carrying out health care for termite control workers using chlorpyrifos.

References


Received April 21, 1989; accepted June 6, 1989