HEALTH RISK ASSESSMENT AND BIOMARKERS OF CHLORPYRIFOS IN RICE FARMERS

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ABSTRACT: The purpose of this study was to assess health risk and cholinesterase levels due to chlorpyrifos exposure among rice farmers in Phatthalung Province. The 31 study subjects used chlorpyrifos insecticides. Air samples were collected in the breathing zone of the rice farmers using OSHA (Occupational Safety and Health Administration) versatile sampler (OVS-2) tubes, containing a glass fiber filter and two sections of XAD-2 adsorbent, following NIOSH method no. 5600. The accuracy, precision and detection limit of this method were also tested. Blood samples were collected and questionnaires were also administered by interviewers. Results revealed that the limit of detection of the method was 0.1 μg/tube. The percent recoveries of the method ranged from 99.20% to 102.83% with coefficients of variation of less than 7.00% for chlopyrifos concentrations of 1-3 μg/tube. The average occupational chlorpyifos exposure among rice farmers was 0.062 ± 0.092 mg/m^3. Thirty subjects (96.8%) had been exposed to chlorpyrifos concentrations less than the TLV-TWA of 0.1 mg/m^3 recommended by the American Conference of Governmental Industrial Hygienists (ACGIH). Many farmers had developed signs and symptoms, sweating (80.7%), chest tightness (32.3%), vomiting (25.8%) and blurred vision (35.5%). A high correlation coefficient was found between chlorpyrifos exposure and levels of cholinesterase in blood (r=0.872; p=0.01). The estimated daily intake of chlorpyrifos exposure through inhalation was 0.004 mg/ kg-day. The risk of exposure to chlorpyrifos was not acceptible (HQ ≥ 1).

Keywords: Chlorpyrifos, cholinesterase, rice farmers, health risk assessment

INTRODUCTION: Thailand is well known as an agricultural country. Rice is the main economic crop, which is consumed in the country and exported to the worldwide market. A survey of agriculturists in 2003 by the National Statistics Office[1)] found that there were 5.8 million agriculturists in Thailand. The Statistics Office of Agricultural Economics, Ministry of Agriculture and Cooperatives[2)] reported that the country's total area of rice farming was approximately 67 million rai, and overall rice production was approximately 29 million tons, in 2006. According to the statistics of Customs Department[3)], Thailand exported 7.4 million tons of milled rice to the worldwide market in 2006.

Most agriculturists use insecticide to kill insects. The statistics of imported agricultural dangerous material including insecticides, fungicides and herbicides has increased each year, and 86,905 tons were imported in the year 2004. Chlorpyrifos is an insecticide in the organophosphate group. These substances poison the nervous system by inhibition of cholinesterase enzyme. Health effects of chlorpyrifos are headache, nausea, dizziness, salivation, excess sweating, blurred vision, chest tightness, muscle weakness, abdominal cramps and diarrhea[4)]. Most agriculturists (66.6%) do not wear appropriate protective equipment while spraying insecticides[5)] There were 1,864 reported cases of pesticide poisoning and 9 pesticide-related deaths in Thailand in 2004.

Farmers in Phatthalung province do rice farming for the whole year (1-2 times a year). They have used several types of pesticide, such as malathion, parathion, glyphosate, chlorpyrifos and grammoxone. They used chlorpyrifis to kill insects. This research was aimed to assess health risk of occupational chlorpyrifos exposures through inhalation, cholinesterase levels and health symptoms among rice farmers after spraying chlorpyrifos.

MATERIALS AND METHODS: This study was a cross-sectional study to assess health risk of 31

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rice farmers exposed to chlorpyrifos. Personal air samples were collected from the breathing zone of farmers during spraying of chlorpyrifos insecticide for a full shift. Blood samples were also collected at the end of shift. The farmers were interviewed related to general characteristics, working factors and health symptoms at the end of shift.

Subjects

The subjects were 31 rice farmers who planted rice in Bangpraw Subdistrict, Papayom District, Phatthalung Province. Most of them have their own rice fields. They used chlorpyrifos insecticides.

Chemicals and reagents

Chlorpyrifos 99.5% (Standard grade) was purchased from Supelco, U.S.A. Triphennyl phosphate 98% (AR grade) was obtained from Unilab, Australia and other chemicals were of analytical grade.

Instrumentation

The Hitachi 263-80 gas chromatograph (Hitachi 263-80, HITACHI, Tokyo, Japan) with a OV-17 packed column (2 m x 3 mm I.D.), equipped with a flame photometric detector and an integrator, was used. The carrier gas was nitrogen at a flow-rate of 10 ml/min with a make-up gas of oxygen at 15 ml/min. The GC condition was isothermal; column, 220°C; injector, 240°C; detector, 300°C.

Ultrasonic bath. (Ultrasonic steri-cleaner, Coax group corporation Ltd, Thailand.)

SKC personal air sampling pump. (SKC Inc., Eighty Four, Pa, U.S.A.)

OVS-2 tube, 13-mm quartz, XAD-2, 270mg/140 mg. (SKC 226-58, SKC Inc., Eighty Four, Pa, U.S.A.).

Reactive paper (coated with 5, 5'-dithiobis-2-nitro benzoic acid and other chemicals) for cholinesterase enzyme in blood from Department of Health, Ministry of Public Health.

Preparation of solutions

A stock standard solution of chlorpyrifos (1 mg/ml) was prepared by dissolving 10 mg of chlorpyrifos in 10 ml of 90:10 toluene/acetone solution.

A stock internal standard solution of triphenyl phosphate was prepared by dissolving the 10 mg triphenyl phosphate in 10-ml of 90:10 toluene/acetone solution. The solution was kept at -20°C. The desorbing solvent was prepared by diluting 700 µl of the stock triphenyl phosphate (internal standard) with 90:10 toluene/acetone solution to 100 ml.

Calibration curve of chlorpyrifos

Chlorpyrifos solutions ranging 0.5-4.0 µg/ml was prepared and 2µl were injected into the GC. The calculated peak area ratio (the peak area of chlorpyrifos/the peak area of triphenyl phosphate internal standard), plotted against the concentration of chlorpyrifos, was used as a calibration curve.

Desorption efficiency of chlorpyrifos from OVS-2 tube

The known concentrations of 1,000 µg/ml chlorpyrifos were prepared in 90:10 toluene/acetone solution. Then, exactly 2, 4 and 6 µl were injected directly onto a quartz fiber filter in each tube for three replications. Finally, the tubes were capped and left overnight to assure complete adsorption of chlorpyrifos on the filters. The blank tubes were also handled in the same manner as the samples but no spiking the chlorpyrifos standard solution onto filter media. If the mass of chlorpyrifos is detected in the blank tube, it needs to be subtracted from the sample tube. The chlorpyrifos was extracted following NIOSH method number 5600[6]. After that exactly 2 µl of the chlorpyrifos extracted was injected and analyzed by GC.

Determination of atmospheric chlorpyrifos by gas chromatography

The chlorpyrifos was extracted following NIOSH method number 5600[6]. Exactly 2 µl of the chlorpyrifos extracts was injected and analyzed by GC. The peak area ratio of chlorpyrifos/triphenyl phosphate was measured and masses of chlorpyrifos were calculated from the respective standard curves.

Accuracy and precision

The known concentration of chlorpyrifos was prepared at 1,000 µg/ml. Then, exactly 2, 4 and 6 µl of known concentrations of chlorpyrifos was injected directly onto a quartz fiber filter in each tube Exactly 2 µl of the chlorpyrifos extracted was injected and analyzed by GC. The experiment was carried out for three days with three replications.

The accuracy and precision of three replications were presented as percent recovery and coefficients of variation.

Environmental monitoring of chlorpyrifos

Personal air samples were collected in the breathing zone of the farmers using OVS-2 tube, one sample per subject. The sampler was placed vertically with the large end down, which did not impede work performance. Air sample was collected at a known flow rate (1 L/min) for a total sample size of 12-240 L according to NIOSH method number 5600[6]. All samples were kept separately in capped plastic bags and stored at –20°C until analysis. The environmental conditions in the rice field were monitored during sampling such as air temperature, relative humidity and wind speed.

Biological monitoring of chlorpyrifos

Blood samples from rice farmers were collected using capillary tubes at the end of shift. The capillary tube was left at room temperature until there was separation of serum and red blood cells. The serum was transferred onto reactive paper and the whole area of the paper got soaked. The samples were left for 7 minutes and the result was read by comparing the developed color with the standard color to determine the levels of cholinesterase[7]. The scale of results is divided into 4 levels; when the reactive paper does not change the color, it indicates normal level of cholienesterase enzyme (≥100 units/ml). If the color of the paper has changed into yellow; it indicates safe level of cholinesterase enzyme (87.5 – 99.9 units/ml). If the color has changed into green, it indicates risky level of cholinesterase enzyme (75 – 87.4 units/ml). If the color has changed into green-blue, it indicates unsafe level of cholinesterase enzyme (<75 units/ml). The reactive paper is not specific to chlorpyrifos; it is designed for organophosphate pesticide.

Health risk assessment

The chronic daily intake[8] of chlorpyrifos through an inhalation route was calculated as follows:

$$\text{Chronic daily intake (mg/kg-day)} = \frac{CA \times IR \times ET \times EF \times ED}{(BW \times AT)}$$

Where: CA = Chemical concentration in air (mg / m^3)
IR = Inhalation rate (m^3/hr)
ET = Exposure time (hr/day)
EF = Exposure frequency (days/year)
ED = Exposure duration (years)
BW = Average body weight (kg)
AT = Averaging time (period over which exposure is averaged in days)

Statistical analysis

The general characteristics of all data were analyzed in terms of descriptive statistics such as percentage, mean, standard deviation (SD), and coefficient of variation. The relationship between atmospheric chlorpyrifos concentrations and levels of cholinesterase concentrations, working factors was performed by spearman's rho correlation[9]. The relationship between the levels of chlorpyrifos concentrations and sign and symptom was performed by chi-square test.

RESULTS:

General characteristics of rice farmers

All thirty one rice farmers were male (100%). Their average age were 44.0±10.8 years ranging from 23 to 63 years (Table 1). The average exposure duration per day of the rice farmers was 2.71±0.86 hours/day. The average working duration of the farmers were 27.94±11.03 years ranging from 8 to 50 years. The possible factors that may affect the chlorpyrifos exposure as regards to number of rice field (Rai), personal behavior such as cigarette smoking, personal protective equipment used. It was founded that 54.8% (n=17) of rice farmers were smokers. They also used personal protective equipment (100%) when performing their working activities.

Calibration curve of chlorpyrifos

The standards of chlorpyrifos and triphenyl phosphate (internal standard) were analyzed by gas chromatography with a flame photometric detector (FPD). A linear relationship between the peak area ratio of chlorpyrifos and triphenyl phosphate and chlorpyrifos concentrations was found over the concentration range of 0.5 – 4.0 µg/ml chlorpyrifos. The equation of chlorpyrifos calibration curve was $Y = 0.2827X - 0.044$. The coefficient of determination (R^2) of the calibration

Table 1 General characteristic of rice farmers

Characteristics	Number (%)
Age (years)	
<35	5 (16.2)
35-49	14 (45.2)
≥ 50	12 (38.7)
Working duration (hours per day)	
≤ 2.0	15 (48.4)
2.1-3.0	12 (38.7)
3.1-4.0	2 (6.4)
4.1-5.0	2 (6.4)
Working experience (years)	
< 10	1 (3.2)
10-19	6 (19.4)
20-29	6 (19.4)
30-39	12 (38.7)
40-49	5 (16.1)
50-59	1 (3.2)
Area of rice field (Rai)	
<10	3 (9.7)
10-20	18 (58.1)
21-30	2 (6.4)
31-40	5 (16.1)
>40	3 (9.7)
Quantity of chlorpyrifos used (g/Rai)	
≤ 80	18 (58.1)
80.1-130.0	10 (32.3)
130.1-160.0	3 (9.7)
Spraying behavior	
Upward of air flow	24 (77.4)
Convenient	7 (22.5)
Hygienic behaviors	
Taking a bath immediately	13 (41.9)
Cleaning hand and face	9 (29.0)
No cleaning	9 (29.0)

graph for three replications was 0.9939. The detection limit of the method was 0.1 µg/ml at the coefficient of variation of 4.21% for three replications.

Desorption efficiency of chlorpyrifos

The average percent desorption efficiency of chlorpyrifos was 89%, 100% and 99.83% at the fortified mass of 1.0 µg, 2.0 µg and 3.0 µg, respectively. The coefficient of variations of chlorpyrifos desorption efficiency were 1.58, 7.07 and 4.01, respectively.

Accuracy and precision

The percent recoveries of the method for analysis of chlorpyrifos ranged from 99.20% to 102.83 % for between-day assay for three days in Table 2. The coefficient of variations was in the range of 1.51% to 6.81%.

Environmental monitoring of chlorpyrifos and environmental conditions in rice fields.

The average chlorpyrifos exposures of 31 rice farmers were 0.062 ± 0.092 mg/m^3 ranging from 0.022 to 0.550 mg/m^3. Almost rice farmers 96.8%, (n=30) were exposed to the concentration of chlorpyrifos below 0.1 mg/m^3, the airborne exposure limit recommended by the American Conference of Governmental Industrial Hygienist (ACGIH). Table 3 shows the levels of chlorpyrifos exposure in rice field classified by the levels of exposure. Only one worker exposed to chlorpyrifos concentrations greater than TLV-TWA of 0.1 mg/m^3. Regarding environmental conditions in the rice field during air sample collection, the average temperature was 30.08±0.58 °C with a range from 29.30 to 31.12 °C. The humidity ranged from 59.03% to 75.46% with mean of 70.92±3.64%. The average wind speed was 2.45±0.46 m/s ranging from 1.51 to 3.45 m/s.

Biological monitoring of chlorpyrifos in rice farmers

The result showed that more than half of studied rice farmers (58.1%) had the cholinesterase levels < 75 units/ml in an unsafe condition and 32.3% in a risky condition (Table 4).

Sign and symptom of rice farmers exposed to chlorpyrifos

Most farmers developed signs and symptoms, sweating (80.7%), chest tightness (32.3%), vomiting (25.8%) and blurred vision (35.5%) (Table 5).

The relationship between atmospheric chlorpyrifos exposure with working factors of farmers in the rice field.

The relationship between chlorpyrifos exposure and the working factors was calculated by the Spearman's rho correlation. The correlation coefficients between atmospheric chlorpyrifos exposure and quantity of chlorpyrifos used and number of rice fields were 0.415 (p= 0.02) and 0.438 (p= 0.014), respectively.

The relationship between chlorpyrifos exposure and the levels of cholinesterase among rice farmers

The relationship between chlorpyrifos exposure and the levels of cholinesterase enzyme in this study was calculated by the Spearman's rho

Table 2 The between-day assay for the determination of accuracy and precision of chlorpyrifos

Concentration (μg)		% Srt	% Recovery
Added	**Found***		
1.00	1.01 ± 0.015	1.51	100.66
2.00	2.06 ± 0.140	6.81	102.83
3.00	2.98 ± 0.090	3.04	99.20

*(Mean ± SD) (n=3)

Table 3 Atmospheric chlorpyrifos exposures among rice farmers

Chlorpyrifos concentration (mg/m^3)	Number (%)
< 0.05	17 (54.8)
0.05 – 0.1	13 (41.9)
> 0.1	1 (3.2)

Table 4 The levels of cholinesterase in serum of rice farmers

Interpretation	Cholinesterase levels (units /ml.)*	Number (%)
Normal	≥ 100	0 (0)
Safe	87.5-99.9	3 (9.7)
Risky	75-87.4	10 (32.3)
Unsafe	<75	08 (58.3)

* Following the color of the paper[7)]

Table 5 Sign and symptoms developed from rice farmers

Sign and symptoms	Number (%)
Chest tightness	10 (32.5)
Vomiting	8 (25.8)
Sweating	25 (80.7)
Blurred vision	11 (35.5)
Urinary incontinence	5 (16.1)
Muscular spasm	5 (16.3)
Headache	7 (22.6)
Total	31 (100.0)

correlation. The correlation coefficient of chlorpyrifos concentration (mg/m^3) and the levels of cholinesterase were 0.872 at the p-value of 0.01. The chlorpyrifos exposure had high correlation with the levels of cholinesterase enzyme.

The relationship between chlorpyrifos exposure and the sign and symptom

The relationship between chlorpyrifos exposure and sign and symptoms was calculated by the Chi-square test. The relationship of chlorpyrifos concentration and muscular spasm was statistically significant (p=0.027); the relationship of chlorpyrifos concentration and other symptoms was not significant.

Health risk assessment of farmers to chlorpyrifos by inhalation route

The occupational exposures to chlorpyrifos of rice farmers was used for estimation of chronic daily intake to chlorpyrifos, as shown below:

$$\text{Chronic daily intake (mg/kg-day)} = \frac{CA \times IR \times ET \times EF \times ED}{(BW \times AT)}$$

$$= \frac{0.062\ mg/m3 \times 30\ m3/24\ hr \times 2.71\ hr/day \times 3\ days/week \times 6\ weeks/year \times 27.94\ years}{70\ kg \times 27.94\ years \times 365\ days\ /\ year}$$

$$= 0.004\ mg/\ kg\text{-}day$$

$$\text{Hazard quotient (HQ)} = \frac{\text{Chronic daily intake (CDI)}}{RfD}$$

$$= \frac{0.004}{0.003}$$

$$= 1.33$$

The estimated acceptable daily intake for humans was 0.004 mg/ kg-day for chlorpyrifos. The reference dose of chlorpyrifos exposure through inhalation route for general population was 0.003 mg/ kg-day[10)]. The hazard quotient (HQ) presented was 1.33. Therefore, the health risk of chlorpyrifos exposures through inhalation route was not acceptable (HQ > 1).

DISCUSSION: In agriculture, pesticides are applied with vehicle-mounted equipment, such as a tractor, or hand held equipment, e.g. a knapsack sprayer. The spray drift can be directed downwards, upwards or outwards, depending on the height and properties of the crop to be protected. The work involving pesticides can be divided into two phases: the mixing and loading of pesticide; and the application of pesticide. During mixing and loading, concentrated pesticide formulations are diluted and/or loaded into an applicator, such as knapsack sprayer. During application, pesticides are spread on the area or the target. Additionally, pesticide work includes the maintenance, repair and cleaning of the application equipment. The environmental conditions may have an effect on the pesticide exposure of the sprayer. Environmental factors

including high temperature, low relative humidity, and air movement tend to increase pesticide volatilization[11]. It leads to high exposure of sprayer. The humidity during the sample collection is rather high (59.03-75.46%), the moisture may reduce the dispersion of pesticide and dissolve pesticide particulates; as a consequence, it reduces exposure of sprayers.

The average chlorpyrifos exposure of 31 rice farmers in the rice field was 0.062 ± 0.092 mg/m^3 ranging from 0.0216 to 0.5500 mg/m^3. Only one of them was exposed to the concentration of chlorpyrifos over 0.1 mg/m^3 because he used high concentration of chlorpyrifos. The chlorpyrifos exposures among rice farmers in the rice field in this study were slightly less than in previous studies[12,13]. Concentrations of chlorpyrifos and methyl parathion were measured in farmers; they exposed to organophosphate pesticide ranging from 0.004 to 0.61 mg/m^3 in vegetable fields and four of them exposed to the pesticide over the recommended TLV-TWA of ACGIH[12]. The two mixer and loader workers exposed to chlorpyrifos averaged of 0.37 mg/m^3 but both of them wore respirators[13].

The factors that may affect chlorpyrifos exposure include working hours, personal behavior such as cigarette smoking, personal protective equipment and concentration of chlorpyrifos used. Approximately 58.1% of workers in this study mixed insecticide and water at the ratio of 50 ml of pesticide and 20 L of water. Data from the questionnaires showed that 54.8% (n=17) of all rice farmers were smokers. They also used personal protective equipment when performing their working activities but their PPE used were cotton mask and cotton clothing. This could not protect workers from exposure to chlorpyrifos. Most farmers developed sign and/or symptoms associated with exposure to chlorpyrifos.

The chlorpyrifos exposure has relationship with quantity of chlorpyrifos used and area of rice fields; probably because these workers have their own rice fields. The workers having large area of rice fields have to spend more time spraying chlorpyrifos; they have high exposure. The workers use high concentration of chlorpyrifos should have higher exposure to chlorpyrifos.

The relationship between chlorpyrifos exposure and the levels of cholinesterase was calculated by the spearman's rho correlation. The correlation coefficient of chlorpyrifos concentration (mg/m^3) and the levels of cholinesterase enzyme were 0.872 at the p-value of 0.01 indicating the chlorpyrifos exposure had linear correlation with the levels of cholinesterase enzyme. The results from this study found that the concentration of chlorpyrifos exposure affected the inhibition of acetylcholine breakdown in the central and peripheral nervous system due to the reduction of enzyme acetylcholine esterase in blood of all subjects.

The health risk of chlorpyrifos exposure through inhalation route is not acceptable due to hazard quotient of 1.33. The reference dose of chlorpyrifos exposure (inhalation) is 0.003 mg/kg/day. The reference dose is for general population. The information about how long chlorpyrifos has been used in Thailand is not known. From the interview, the average duration of pesticide used was 27.94 years; the subjects may have used other chemicals in the old time before chlorpyrifos has been imported into Thailand. The health risk calculated may be the maximum risk. Actually, these workers may also expose to chlorpyrifos through dermal absorption, contamination of chlorpyrifos in the ground water and soil in the rice fields.

The prevention, minimization and elimination of chlorpyrifos exposure in rice farmers can be done by giving them the knowledge about the health effect of insecticide exposure. The positive approach about reducing chlorpyrifos exposure may be effective by good hygiene practice of the rice farmers such as keeping their hands and clothes cleaned from insecticide. In addition to the hygienic practice between working hour, they should never smoking while on duty.

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