AN ABSTRACT OF THE THESIS OF

G. Lalith Mahendra Aponso for the degree of Doctor of Philosophy in Toxicology presented on July 30, 2001. Title: Exposure and Health Risk Assessment for Farmers Occupationally Exposed to Chlorpyrifos in Sri Lanka; and Drinking Water and House Dust Analysis for Chlorpyrifos.

Abstract approved: _____
Ian J. Tinsley

This study was designed to assess chlorpyrifos exposure of a group of farmers by determining internal dose associated with a given application of this insecticide. This involved the monitoring of urinary levels of 3,5,6 trichloro-2-pyridinol (TCP), the major metabolite of chlorpyrifos. Incidental exposure was evaluated by determining the levels of chlorpyrifos and TCP in drinking water and house dust.

Nineteen full-time farmers from Kandy district, Sri Lanka, growing long-squash or bitter melon during the 2000 vegetable season (April-June) participated in the study. Information concerning their health history, agricultural practices, family background and pesticide-related issues were obtained using a questionnaire. All farmers used knapsack sprayers for applying a chlorpyrifos EC formulation. The amount of chemical applied, time required, and the safety precautions used were noted.

One urine sample was taken prior to application followed by three samples a day for 5 days post application from each farmer. Urine samples were extracted with hexane and analyzed for TCP using a gas chromatograph fitted with an electron capture detector. The limit of detection for TCP in urine was 6ng/mL.

TCP levels peaked within 24 hours post application and returned to the baseline after 5 days. Total TCP voided ranged from 71 to 299μg (average of 190.4ug) per 5g of creatinine, equivalent to a calculated internal dose of 0.0021-0.0084mg/kg (average 0.0055mg/kg) chlorpyrifos. It was assumed that 90% of the internal dose was voided in urine in 5 days. The dermal dose ranged from 4.8 to 19.6μg/cm^2 on exposed skin. The elimination half-life of the urinary TCP metabolite was 31.2 hours. The internal dose was correlated with the amount of active ingredient used ($p< 5 \times 10^{-7}$), the use of leaky tanks ($p<0.005$), and the use of protective clothing ($p<0.005$). Hazard quotient for cholinesterase inhibition based on the EPA reference dose for chlorpyrifos ranged from 0.8 to 2.7 and the margin of safety from 3.6 to 14.3 for the exposed farmers. None of the farmers were found to have symptoms of acute or sub-chronic poisoning in the medical examination carried out at the end of the season.

Drinking water was collected from three wells, and dust was collected as floor wipes from three houses located adjacent to treated areas. Chlorpyrifos was not detected in well water at levels that could be quantitated (minimum detection limit was 7ng/L). TCP was detected in well water 9 to 10ng/mL. Although some chromatograms suggest the presence of chlorpyrifos in some house dust samples (minimum detection limit 13ppb), a comparison of the responses on two different columns did not provide convincing evidence for the presence of chlorpyrifos. Failure to detect significant amount of chlorpyrifos in water and house dust was probably due to rapid break down due to high soil temperature and pH. Water and house dust did not add to the farmers' occupational exposure.

Exposure and Health Risk Assessment for Farmers Occupationally Exposed to Chlorpyrifos in Sri Lanka; and Drinking Water and House Dust Analysis for Chlorpyrifos

by

G. Lalith M. Aponso

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APPROVED

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Major Professor, representing Environmental and Molecular Toxicology

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Chair of Department of Environmental and Molecular Toxicology

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G. Lalith Mahendra Aponso, Author

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The various co-authors of the chapters are listed together with major contributions to the study.

Ian J. Tinsley	Technical support; critical review of the experimental design, data and manuscript; guidance; patience.
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DEDICATION

This thesis is dedicated to my loving son, Savinda Aponso, and daughter, Hiruni Marsha Aponso, with encouragement for their future studies.

EXPOSURE AND HEALTH RISK ASSESSMENT FOR FARMERS OCCUPATIONALLY EXPOSED TO CHLORPYRIFOS IN SRI LANKA; AND DRINKING WATER AND HOUSE DUST ANALYSIS FOR CHLORPYRIFOS

CHAPTER 1

INTRODUCTION

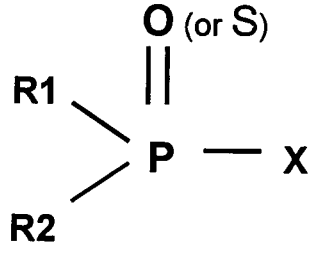
Pesticide use on agricultural crops is considered an efficient method for safeguarding against yield losses due to pests in a given ecosystem. Application of these substances poses a health risk to non-target species such as humans, domestic animals, and wildlife. Pesticide applicators, neighboring communities, and consumers of the produce can be at risk by oral, dermal, or inhalation exposure. The level of the risk depends on the inherent toxicity of the agent of interest and the magnitude of exposure.

PESTICIDES

A pesticide is defined as any substance or mixture of substances intended for destroying, repelling, or mitigating the activity of any pest. It is also described as any physical, chemical, or biological agent that will kill an undesirable plant or animal pest. Pesticides are mainly classified into different classes according to their usage such as insecticides, herbicides, fungicides, rodenticides, etc. Pesticides belong to different chemical classes such as organophosporus (OP), chlorinated hydrocarbons, bipiridyl, aminoacids, etc. Most of the OP pesticides are insecticides. Chemicals also can be assigned to one of five toxicity classes based on acute toxicity as indicated by the LD_{50} (oral, dermal) or LC_{50} (inhalation) values.

ORGANOPHOSPHORUS COMPOUNDS

OP compounds are widely used as pesticides throughout the world. These compounds are also used as plasticizers, lubricants, petroleum additives, and chemical warfare agents. OPs command the largest segment (more than 1/3) of the total $6.1 billion insecticide market worldwide. Over 89 million acres of the United States are sprayed annually with OP insecticides. Effectiveness as pesticides and rapid biodegradability favors the use of OP compounds. These compounds are normally esters, amides, or thiol derivatives of phosphoric or phosphonic acid. The general structure of an OP compound is given in Figure 2.1.



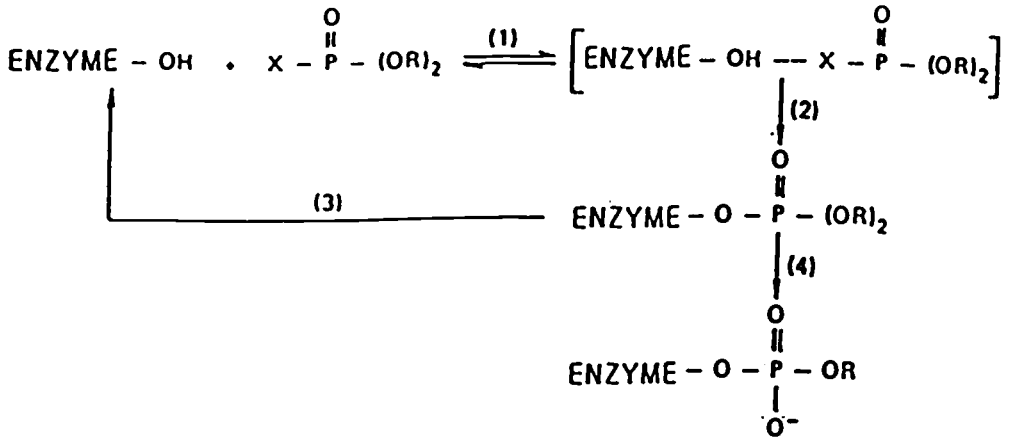
R1 and R2 are usually methyl or ethyl group, both of which may be bound directly to phosphorus (in phosphinates) or linked via -O- or -S- (in phosphates). R1 may be bound directly and R2 bonded via one of the above groups (phosphonates). In phosphoramidates, carbon is linked to phosphorus through a -NH group. X is called the leaving group, and it is usually bound via an -O- or -S- molecule. (WHO, 1986)

Figure 1.1: General structure of organophosphorus compound

TOXICITY OF ORGANOPHOSPHORUS COMPOUNDS

The toxicity of OP compounds is primarily due to the inhibition of acetylcholinesterase (AChE), an enzyme necessary for the normal function of the central and peripheral nervous system. AChE is a serine protease that hydrolyses the neuro-transmitter, acetylcholine (ACh). AChE (True cholinesterase) and pseudocholinesterase belong to an enzyme class called choline ester hydrolases (Ballantyne and Marrs, 1992). AChE is found postsynaptically in central and peripheral cholinergic synapses, including the preganglionic autonomic synapses and postaganglionic parasympathetic synapses (Palmer, 1980). It is also found at the motor end plate in the neuromuscular junction and is also associated with erythrocytes (Ballantyne and Marrs, 1992). Esterase enzymes such as AChE are inhibited by phosphorylation upon acute exposure to an OP compounds (Figure 1.2). Inhibition of AChE activity in nerve tissue leads to a range of effects resulting in dysfunction of central and peripheral nervous systems by over stimulating the target tissue and culminating in respiratory failure and death. Misra et al. (1985) reported that occupational exposure of applicators to the OP pesticide fenthion resulted in headache (59%), giddiness (50%), ocular symptoms (27%), and paresthesia (18%). A study on acute, chronic, and accidental exposure of OP pesticides to agricultural workers in California indicated that significant number of workers had signs of exposure (Brown et al., 1989).

AChE present on erythrocytes and cholinesterase (ChE) found in plasma does not have any known function in blood. Inhibition of erythrocyte AChE is proportional to the level of exposure and the affinity of the compound for the enzyme. In contrast, plasma ChE is more sensitive to inhibitors. Plasma ChE is inhibited to a greater degree by OP compounds such as chlorpyrifos, diazinon, dichlorvos, and malathion while the erythrocyte enzyme is more sensitive to dimefox, parathion, and parathion-methyl (Hays, 1982). Inhibition of blood ChE is not commonly considered as an adverse effect.



(1) Formation of Michaelis complex

(2) Phosphorylation of the enzyme

(3) Reactivation reaction

(4) Aging

Figure 1.2: Inhibition of an esterase enzyme by OP compounds

Blood AChE inhibition and the level of metabolites found in urine have been used as biomarkers for exposure (Knaak et al., 1979; Franklin et al., 1981) and biomarkers for adverse effects (Padilla et al., 1996) to OP or carbamate (another group of anti-cholinesterase pesticides) insecticides. Depression of blood AChE correlates with effects in the target tissue (generally, central or peripheral nervous system depending on the affinity), but the exact relationship depends on the time after exposure, the tissue, and the insecticide. The best correlation is achieved during maximal cholinesterase inhibition either after an acute dose or during repeated dosing. During maximal inhibition, the response in whole blood, plasma, and erythrocytes will exhibit good correlation with the target tissue, but this relation is not observed during initial exposure and recovery phases. In the recovery phase, erythrocyte and whole blood cholinesterase activity may lag behind recovery in the target tissue (Padilla et al., 1996). Further, they reported that some OP compounds show a linear relationship between blood cholinesterase inhibition and presence of clinical signs or change in behavior. As an example, chlorpyrifos-treated rats showed a linear relationship between blood ChE inhibition and motor activity impairment, but the ChE has to be depressed to at least 15% of control for a significant response. Animals fed with aldicarb (a carbamate pesticide) and paraxon (an OP pesticide) needed 50-60% inhibition of ChE to initiate a response. In a study using rats and beagle dogs, McCollister et al. (1974) reported plasma and erythrocyte ChE are depressed by smaller doses of chlorpyrifos than inhibit in brain ChE or produce signs of toxicity. Thus, changes in plasma and erythrocyte ChE have been used most frequently as a screen in evaluating an individual's exposure to chlorpyrifos (Nolan et al., 84). Gibson et al. (1998) suggested that plasma cholinesterase activity is the most sensitive indicator of exposure to chlorpyrifos.

Some OPs induce a delayed neuropathy, which develops weeks after a single exposure. Manifestation of OP induced delayed neuropathy differs among species with locomotor effects prominent in humans and hens for

example, but lacking in laboratory rats. Potential for the development of this progressive and irreversible neuropathy is determined by the capability of the OP to significantly and irreversibly inhibit neuropathic target esterases (NTE). Relative inhibition of NTE and AChE shortly after exposure may be used to distinguish the likelihood of causing delayed neuropathy or acute toxicity following exposure to OP compounds (Ehrich, 1996). A stable covalent bond at active sites of the enzymes causes the irreversible inhibition, and the process called aging further enhances stability of the bond when one of the alkyl groups of the diethylester is lost. Senanayake and Karalliedde (1987) described the acute neurotoxic effects during the cholinergic phase of OP insecticide poisoning and delayed neurotoxic effects that appeared 2-3 weeks later. In this study, they described patients appearing to have a distinct clinical entity (a so-called intermediate syndrome) that developed after the acute chlolinergic crisis and before the expected onset of the delayed neuropathy. OP pesticides reported to cause delayed neuropathy in man are mipafox (Bidstrup et al., 1953), leptophos (Xintaras et al., 1978), methamidophos (Senanayake and Johnson, 1982), trichlorphon (Shiraishi et al., 1977), trichlornat (Jedrzejowska et al., 1980), EPN (Xintaras and Burg, (1980) and chlorpyrifos (Lotti and Morretto, 1986).

The American Conference of Governmental Industrial Hygienist (ACGIH, 1995-1996) has established threshold limit values (TLV) to protect workers from exposure to solvents. TLV is the airborne concentration of a substance a worker could be exposed to daily without exhibiting adverse effects. There are three types of TLVs: (1) the time weighted average (TWA), which is a value for an 8-hr working day and for a 40-hr work week; (2) the short term exposure limits (STEL) is a value for a short period of time (usually 15 min); (3) the ceiling (TLV-C) is a value that should not be exceeded even briefly. The dermal exposure TLV and STEL for chlorpyrifos are 0.2 and 20 mg per cubic meter, respectively (USDHHS, 1997).

SRI LANKA

Sri Lanka is located in the Indian Ocean, 29 km off the southeastern coast of India. Its total area is 65,610 square kilometers and it is positioned between 5^0 and 10^0 north latitude. Sri Lanka has a warm climate moderated by ocean winds and considerable moisture. The mean temperature ranges from 15.8 °C in the central highlands to a high of 29 °C in the northeast coast, but some areas may reach 37 °C during July and August. Humidity is typically higher in the southwest and mountainous areas, and it varies with the seasonal patterns of rainfall. The country is divided climatically into a wet zone (southwestern quarter), a dry zone (north and eastern areas), and an intermediate zone (between wet and dry zone), based on annual precipitation. Average rainfalls are 250 cm, 120 cm, and 190 cm, respectively.

Over sixty percent of the 19 million population depends on agriculture or agricultural based industries. A majority of the vegetable farms are found in villages and most farmers own a land area of less than one acre. Crops grown depend on the rainfall and availability of irrigation water. In areas with no irrigation, rice is cultivated in the main rainy season and vegetables are grown during minor rains. Recently, a focus on high yield crops such as rice and other vegetables have resulted in an increased demand for fertilizers and pesticides. Currently, pesticides are used as the major method of pest control. Pesticide importation, formulation, distribution, storage and other related activities are monitored by the Control of Pesticide Act No. 33 of 1980 and it's amendment of 1994. Monitoring and controlling the use of pesticides in the field are lagging behind due to a lack of personnel in the pesticide control authority and in the agricultural extension service.

In Sri Lanka, insecticides are mainly used for pest control in agriculture and malaria vector control. According to the Registrar of Pesticides (the pesticide regulatory authority) of Sri Lanka, total technical grade insecticides (active ingredient) and formulated products imported to the country during

1998 were 393 and 3131 metric tons, respectively. Sixty one percent of total insecticides were OPs and 19% were carbamates (another anti-cholinesterase pesticides). Major contributions to OP's were from chlorpyrifos 40% emulsifiable concentrate (EC), dimethoate 40% EC, and diazinon 5% granules (G). Quantities of these formulated products used during 1999 were 198, 175 and 278 metric tons, respectively.

It has been estimated that 95% of fatal pesticide poisonings occur in developing countries, many of which are in the Asia-Pacific region. Agriculture-based economies, availability of pesticides, socioeconomic problems, lack of adequate protective clothing and limited treatment facilities are some of the factors contributing to the high intoxication and mortality (Fernando, 1995). In Sri Lanka, the number of hospital admissions due to OP pesticide poisoning in 1992 was 11,439, which was 73% of total pesticide poisonings (Fernando, 1995). Death records indicated that OPs are the major pesticides causing poisoning (Fernando, 1995; Senanayake and Peiris, 1995). In another study, Jayaratnam (1987) indicated that 5 out of 1000 agricultural workers in Sri Lanka were hospitalized yearly due to pesticide poisoning from occupational exposure. Many cases of intoxication due to occupational exposure may not require admission to a hospital and therefore go unreported.

Most of the farmers do not have adequate knowledge of the hazards of pesticides. Inappropriate activities such as using hands for mixing pesticides in knapsack sprayers, accidental spilling, leaking tanks, smelling pesticides, lack of protective clothing and many other factors may lead to increased dermal and inhalation exposure. Farmers of poor economic condition do not have the resources to replace or maintain spray equipment for optimal function. Knapsack sprayers are the primary equipment used for pesticide application. These sprayers are designed to be held with the nozzle in front of the operator and hence the area in front is sprayed, which causes the applicator to continually walk through the sprayed crop.

Due to the high cost of agricultural inputs and climatic uncertainties, farming is not a highly profitable business in Sri Lanka. Therefore, family labor and exchanged labor (with neighboring farm-families) is used for cultivation to minimize cost of production. Spray drift, could carry pesticide residue to drinking water sources and to nearby houses. In addition, pesticide contaminated clothes, dirty spray equipment, and improper storage conditions in houses may pose an exposure risk to children and other members of the house who are not involved in agricultural activities.

Rice cultivation is fed either by rain or channel irrigation depending on the monsoon season. Heavy monsoon rains cause runoff carrying soil and pesticide residues downstream which ends up in lakes and rivers. Exceeding recommended application rates especially on lowland crops such as rice, may accumulate the environmental impact. A study performed among vegetable farmers in three growing regions indicated that 63.5% of the farmers use more than the recommended dose of pesticides, 85.7% applied pesticides before the appearance of pests, and 8% sprayed pesticides prior to marketing (Chandrasekera et al., 1985).

In some agricultural areas purified tap water is not available and well water is used for drinking. Use of plastic pesticide containers for storing water and the use of river or lake-water for bathing and laundry may lead to a significant exposure to pesticides and other environmental contaminants.

CHLORPYRIFOS

Chlorpyrifos (O,O-diethyl-O-[3,5,6-trichloro-2-pyridyl] phosphorothioat) (CAS Register No. 2921-88-2) is a broad-spectrum OP insecticide widely used in agriculture and residential pest control. The structure of chlorpyrifos is given in Figure 1.3. According to the United States Environmental Protection Agency (US EPA), chlorpyrifos is registered for use on pests in fruits, nuts and

vegetables. Department of Agriculture Sri Lanka recommends chlorpyrifos 40EC formulation for pest control in rice and vegetables and as a treatment for soil termites.

As with the other OP compounds, the principle action of chlorpyrifos and its bio-activated product, chlorpyrifos-oxon, is inhibition of neural AChE (Namba et al., 1971). An oral LD_{50} of 152 mg/kg was reported for female mice and 169 mg/kg for female rats fed chlorpyrifos (Berteau and Deen, 1978). Oral LD_{50} values for male and female rats ranged from 118 to 245 mg/kg (Gaines, 1969). In a study of the pharmacokinetics of chlorpyrifos in human, no cholinergic signs were manifested at oral doses of 0.5 mg/kg, even though plasma cholinesterase activity was depressed to 15%. At this dose no toxicity signs were observed in any volunteers. Subchronic NOEL for human plasma cholinesterase activity depression was 0.03 mg/kg/day (Coulston et al., 1972).

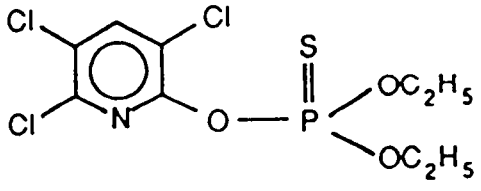


Figure 1.3: Structure of chlorpyrifos

DISTRIBUTION AND METABOLISM OF CHLORPYRIFOS

Distribution of orally administered ^{14}C-labeled chlorpyrifos has been investigated in male Wister rats (Smith et al., 1967) and Hereford crossbred heifers (Dishburger et al., 1977). Results of both studies indicated chlorpyrifos was distributed to all organs but liberation from fat was slower (half-life [$t_{1/2}$] is 67hr) than other tissues ($t_{1/2}$ is 10-16hr). Distribution of dermally exposed chlorpyrifos was investigated in goats (Cheng et al., 1989), mice (Shah et al., 1981) and bovine (Claborn et al., 1968; Ivery et al., 1972). The parent compound was reported to distribute throughout the body, but concentrations were comparatively higher in blood, liver and fat.

Microsomal cytochrome P-450 enzymes catalyze the oxidative desulfuration, bioactivation of chlorpyrifos to form chlorpyrifos-oxon (oxon) in rat and mouse liver (Sultatos and Murphy, 1983a; Ma and Chambers, 1994). In vitro studies showed that the oxon is 400 times more active than chlorpyrifos as an inhibitor of cholinesterase (Sultatos et al.,1982). Both chlorpyrifos and it's oxon are rapidly hydrolyzed to 3,5,6-trichloro-2-pyridinol (TCP) probably by A-esterase in humans (Sultatos and Murphy, (1983a, 1983b), rats and goats (Glas, 1981). Studies using liver perfusion have shown that both bioactivation and detoxification occurs rapidly hence only TCP can be detected in the hepatic effluent once steady-state conditions are reached (Sultatos and Murphy, 1983a, 1983b). Hydrolysis of oxon by A-esterase is probably the more common rout of detoxification, since TCP or a conjugate of TCP is the major metabolite of chlorpyrifos in humans (Nolen et al., 1984) and rodents (Bakke et al., 1976; Smith, 1967). The principle route of excretion in humans is through urine. This rapid conjugation and elimination reaction reduces occurrence of adverse health effects. Fate of chlorpyrifos in the human body is illustrated in Figure 2.4.

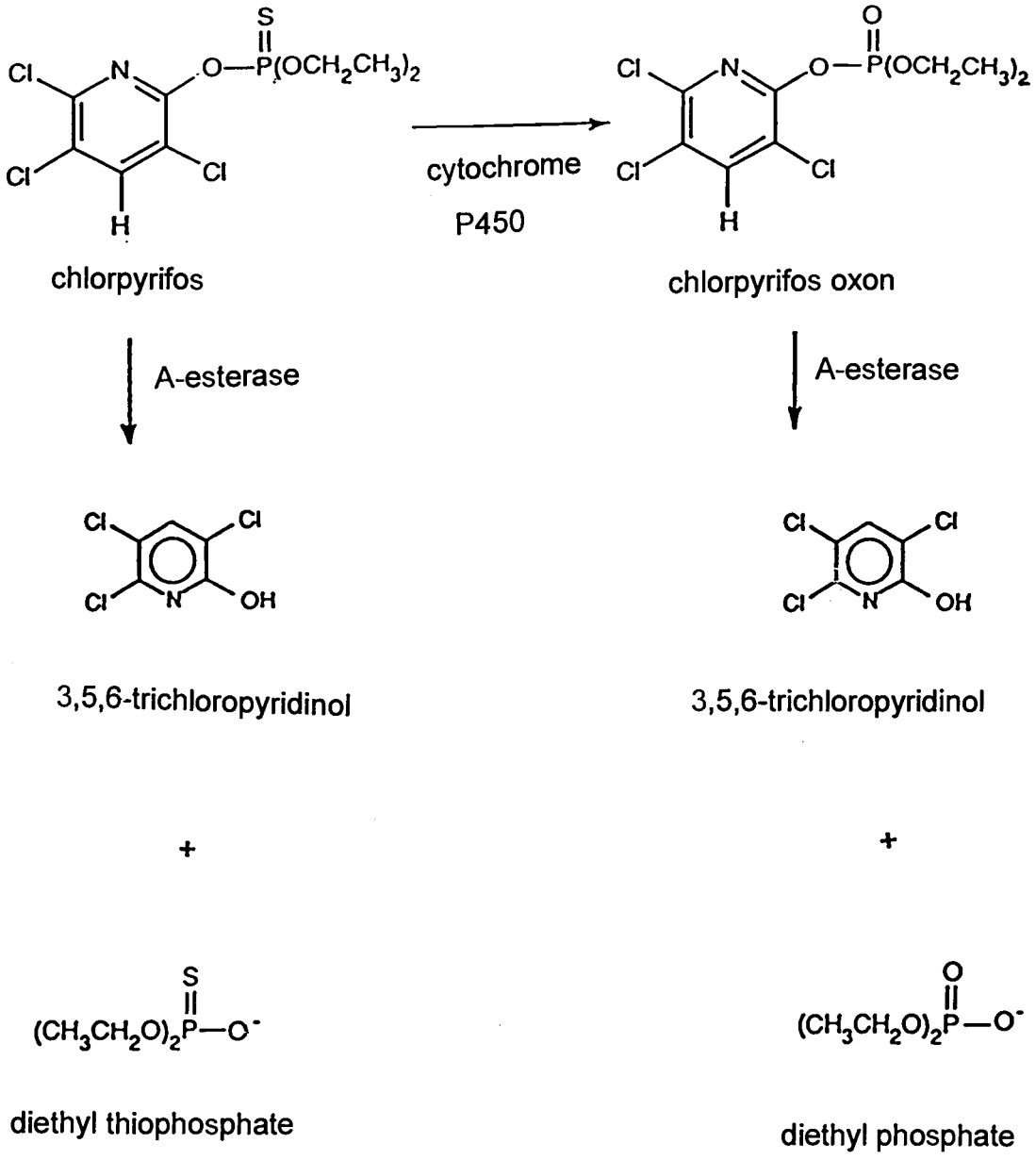


Figure 1.4: Fate of chlorpyrifos in human

In 1984, Nolan et al. studied the pharmacokinects of chlorpyrifos in human volunteers. They reported that chlorpyrifos is rapidly metabolized to TCP and excreted into urine in humans. Maximum blood concentration of this major metabolite was observed 6hr after oral exposure and 24hr after dermal exposure of chlorpyrifos. The mean half-life for the elimination of TCP from the blood was 26.9hr following both oral and dermal doses. The amount recovered as metabolites from urine was equivalent to 70% of the oral dose and 1.28% of the dermal dose (within 5 days).

Griffin et al. (1999) studied the oral and dermal absorption of chlorpyrifos using five human volunteers age range 26-45 years. All the subjects were given an oral dose of 1 mg of analytical grade chlorpyrifos. This dose is half that which would be absorbed if a subject were exposed to the Health and Safety Executive occupational exposure standard of 0.2 mg/m^3 over an 8hr period. Blood samples were taken over a 24hr period, and the total voided volume of urine was collected over 100hr. They reported that TCP, diethylphosphate, and diethylthiophosphate are the specific urinary metabolites of chlorpyrifos. In this study, the total diethylphosphate and diethylthiophosphate voided were determined for each volunteer as a biomarker of exposure. Ninety three percent of the oral dose was recovered in urine. Four weeks later, 28 mg of chlorpyrifos was administered dermally to the same volunteers over an 8hr period. Unabsorbed compound was washed off after this period. One percent of the dermal dose was recovered as metabolites in urine. This dermal dose was unable to depress plasma ChE, but detectable levels of metabolites were found in urine. Therefore, the authors concluded that urinary metabolites are the more sensitive biomarker of exposure. The US EPA reference dose (RfD) for oral exposure to chlorpyrifos is 0.003 mg/kg/day (US EPA, 1997). This RfD was obtained from a NOEL of 0.03 mg/kg (oral) for ChE inhibition (Coulston et al., 1972) and an uncertainty factor of 10 for human variability.

ENVIRONMENTAL FATE OF CHLORPYRIFOS

Chlorpyrifos as granules is applied in significant quantities directly to soil or sprayed as liquid on plants, often at times when irrigation is employed to supplement natural rainfall. Both rainfall and irrigation can contribute significantly to chemical transport in runoff. Chlorpyrifos will degrade by both biotic and abiotic transformation processes in terrestrial and aquatic environments. In soil, water, plants and animals, the major pathway of abiotic and biotic degradation involves cleavage of the phosphorothioate ester bond (Racke, 1993) to form TCP (Figure 1.5). In the environment, TCP is degraded via photolysis with an aqueous half-life of about 4 min in surface water at 40^0N latitude (Dilling et al., 1984) and microbial degradation with an average half-life of 73 days at 25 ^{0}C (Bidlack, 1976). In terrestrial ecosystems, chlorpyrifos rapidly dissipates from plant foliage, with an observed half-life of 1 to 9 days (Racke, 1993). Chlorpyrifos dissipated at a moderate rate when incorporate into the soil profile with a half-lives of 33-56 days in Califonia, Michigan, and Illinois (Fontaine et al., 1987). However, dissipation from soil surfaces occurs rapidly compared to deep soil. Half-lives of 9-11 days have been noted for fallow soil surfaces and from 7-9 days from turf grass surfaces following spray application at sites in Indiana and Florida (Racke and Robb, 1993).

In aquatic ecosystems, chlorpyrifos is removed from the water column via hydrolysis, biodegradation, sorption to sediments, volatilization and photodegradation. Hydrolysis half-lives in sterile distilled water have been reported to range 16-72 days at pH 5-9, while laboratory photolysis half-lives of 30-52 days have been reported (Racke, 1993). Degradation half-lives in sediment water under aerobic and anaerobic conditions have been reported as 22-51 and 39-200 days, respectively in laboratory conditions (Racke, 1993).

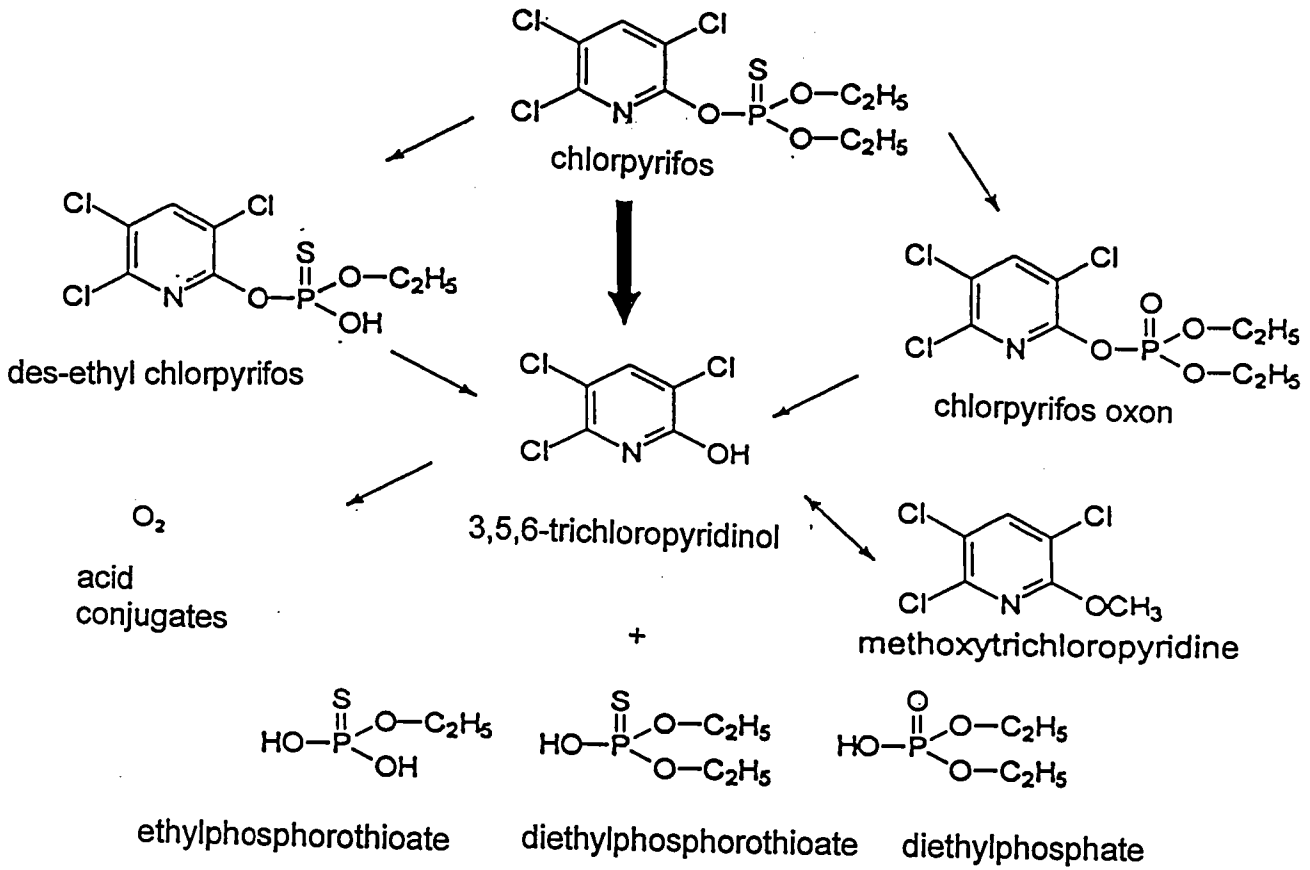


Figure 1.5: Environmental transformations of chlorpyrifos (adapted from Racke 1993)

RISK ASSESSMENT

Risk: Risk is a function of dose and the inherent toxicity of the compound. In general, risk is defined as the possibility of injury, harm, or other adverse and unwanted effects. Risks are commonplace in all of our lives. Risk assessments are conducted to estimate how much damage or injury can be expected from exposure to a given risk agent and to assist in judging whether these consequences are severe enough to warrant more intensive management or regulation. In the health, safety, and environmental fields, risk is usually identified as the likelihood that individuals (or population) will incur increased incidences of adverse effects such as disabling injury, disease, or death. Risk is frequently expressed in probability terms such as some number of additional deaths over a lifetime in a population of exposed people. Historically, a risk of less than 10^{-6} in magnitude has been considered acceptable in cancer incidence. The methods and sequence of steps involved in conducting a risk assessment vary with the kind of risk, i.e., threshold or non-threshold. In general, this process consists of four steps such as hazard identification, exposure assessment, dose-response assessment, and an integrative risk characterization.

a) Hazard Identification: This initial step of risk assessment seeks to identify the adverse health effect that can be caused by exposure to the chemical being studied. An adverse health effect can be temporary, permanent, or life threatening.

b) Exposure assessment: The objective of the exposure assessment is to estimate the route and magnitude of exposures to the chemicals of concern. Since risk is proportional to magnitude of exposure, this estimation is essential (and the more difficult parameter to assess) to calculate risk factors for individuals or to a population.

c) Dose response assessment: In this step, the extent of adverse effects resulting from a given level of exposure to a risk agent are evaluated, usually on experimental animals. This dose response relationship provides a toxicological reference that is used to estimate the likelihood or severity of adverse effect for the exposed individuals.

d) Risk characterization: This is the final step of risk assessment, which involves assembling prior analysis components to determine risk. In this step, the toxicity and exposure assessment are summarized and integrated into quantitative and qualitative expressions of risk. To characterize potential non-carcinogenic effects, comparisons are made between dose and toxicity values to provide a margin of safety. Risk quotient (or hazard quotient) is a function of dose (exposure level) and the inherent toxicity of the chemical.

OBJECTIVES

This study is focused on assessing the exposure and consequential risk for pesticide applicators by determining internal dose. Farmers in Sri Lanka take minimal safety precautions in handling pesticides. This may lead to high exposure levels via dermal, oral, and inhalation routes while handling concentrate, mixing, or applying pesticides in the field. Hence, farmers are at a high risk of pesticide exposure and poisoning. High temperature and humid conditions, which discourage the use of protective clothing, poor personal hygiene, and lack of knowledge of pesticide hazards of pesticides increases the potential for exposure. The main objective is to use urinary TCP levels (pre and post application) to calculate the internal dose. Since total urine collection is not practical under occupational conditions, TCP levels in urine are expressed as per gram of creatinine clearance.

Most residents in the farming community drink well water because purified tap water is unavailable. These wells and residence houses of farmers are located close to the farming land, where water and house floors could be contaminated by pesticide spray drift. The second objective of this study is to analyze drinking water and house dust for the parent compound and the major metabolite to assess potential secondary exposure.

Another objective of this study is to conduct a survey to understand personal details, cultivation practices, health status, and other relevant information about the participants which might influence exposure before the experiment. Finally, the adverse health effects caused by chlorpyrifos application by a medical examination after the experiment.

CHAPTER 2

EXPOSURE AND RISK ASSESSMENT FOR FARMERS OCCUPATIONALLY EXPOSED TO INSECTICIDE CHLORPYRIFOS IN SRI LANKA

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ABSTRACT

Urinary levels of 3,5,6 trichloro-2-pyridinol (TCP, CAS 6515-38-4), the major metabolite of chlorpyrifos, were measured in farmers occupationally exposed to the parent compound, chlorpyrifos. This study was designed to assess the internal dose experienced and the risk for farmers who applied chlorpyrifos on their crops during the major vegetable season (April-June) of the year 2000. Nineteen full time farmers from an agricultural community in Kandy district, Sri Lanka, participated in the study. A questionnaire was used to record health history, personal information, agricultural information, family background, pesticide-related issues, and health status. One urine sample was taken before application and sampling continued for 5 days (three samples per day) after application. TCP levels in urine peaked in the first day post application, returning to the baseline by the end of the fifth day. Cumulative TCP voided ranged from 71 to 299μg (average of 190.4μg) per 5g of creatinine and was equivalent to an internal dose of 0.0021-0.0084 mg/kg (average 0.0055 mg/kg) chlorpyrifos assuming 90% of the internal dose was voided in urine in five days. TCP levels were correlated with the amount of active ingredient used ($p< 5 \times 10^{-7}$) and the use of leaky tanks (p=0.005) and protective clothing (p=0.005). Calculated dermal dose ranged from 4.8 to 19.6 $\mu g/cm^2$ on exposed skin. The elimination half-life of the urinary TCP metabolite was 31.2hr. The calculated hazard quotient for cholinesterase inhibition using the EPA reference dose for chlorpyrifos ranged from 0.8 to 2.7, and margin of safety ranged from 3.6 to 14.3 for the farmers. Parent compound was not detected in any of the urine samples. None of the farmers were found to have acute or sub-chronic symptoms in the medical examination carried out at the end of the season.

Farmers do get higher doses than the reference dose by occupational exposure. Slow dermal uptake, rapid metabolism, and elimination of the parent compound seem to protect against an acute response. The short

application times and long intervals between application, may also be protective.

INTRODUCTION

Chlorpyrifos (*O,O*-diethyl-*O*-[3,5,6-trichloro-2-pyrdyl]) phosphorothioate (CAS Registry No. 2921-88-2) is a widely-used broad-spectrum insecticide recommended for use in many countries on various food crops and for the control of household insects. Chlorpyrifos is one of the most commonly used OP insecticides in agriculture with a high potential for inducing adverse health effects. Inhibition of AChE upon exposure and urinary 3,5,6-trichloro-2-pyrdynol (TCP) have been used as biological markers to assess chlorpyrifos exposure. Other agents such as carbamate compounds can inhibit AChE, but chlorpyrifos is one of only two insecticides that has TCP as a metabolite.

In a pharmacokinetics study, Nolan et al. (1980) reported on signs and symptoms of toxicity, changes in plasma and erythrocyte cholinesterase, and urinary TCP levels in six human volunteers administrated an oral dose (0.5 mg/kg) or dermal dose (5 mg/kg) of chlorpyrifos. In this study, the highest blood TCP concentrations of 0.93 μg/mL were reached 6h after an oral dose and 0.063 μg/mL 24h after a dermal dose. The average half-life ($t_{1/2}$) for TCP appearance in blood was 0.5h for oral and 22.5hr for dermal dose. Average TCP excreted in urine was 70±11% of the oral dose and 1.28±0.8% of the dermal. The mean $t_{1/2}$ of elimination of TCP from the blood was 26.9hr following both oral and dermal dose. Plasma cholinesterase was depressed 85% by the oral dose and 13% after the dermal dose. Erythrocyte ChE activity was essentially unchanged following the oral or dermal doses. Blood chlorpyrifos concentrations were less than 30 ng/mL, and no unchanged chlorpyrifos was found in the urine following either route of administration.

Griffin et al. (1999) determined the kinetics of elimination of urinary dialkylphosphate metabolites after oral and dermal exposure to human volunteers to doses of chlorpyrifos. Five volunteers ingested 1 mg (2852nmol) of chlorpyrifos, and 4 weeks later 28.59 mg (81567nmol) of chlorpyrifos was administrated to the skin of the same group by spreading 100μl of a

commercial preparation of chlorpyrifos (Durban 4, Dow Elanco), diluted in water on to an area of 78 cm^2 for 8hr. Total urine was collected over 100hr. It was observed that 93% of the oral dose and 1% of the dermal dose was recovered as urinary dialkylphosphate metabolites. Excretion after a dermal dose was delayed compared with the oral dose. The apparent elimination half-life of urinary dialkylphosphates after an oral dose was 15.5hr, and 30hr following the dermal dose. Plasma or erythrocyte cholinesterase activity was not depressed significantly by any of these doses. Urinary dialkylphosphate metabolites, which can be detected readily, are thus a more sensitive indicator of exposure.

Very limited studies are available for inhalation toxicology for chlorpyrifos. According to the United Stated Department of Health and Human Services (USDHHS) publication (1997) on toxicology profile for chlorpyrifos, no information is available on acute or sub-chronic inhalation exposure of chlorpyrifos to humans. In a chronic study, the prevalence of selected illnesses and symptoms in 175 employees involved in the production of chlorpyrifos were compared with 335 control matched subjects, but there was no significant difference found among groups (USDHHS, 1997). It was also reported that oral and inhalation exposure contributes to a greater internal dose than dermal absorption of chlorpyrifos. In mice, the inhalation LD50 of 94 mg/kg was determined after whole-body inhalation exposure to 6700-7900 mg/m^3 chlorpyrifos aerosol in xylene by varying the length of exposure from 27-50 min. Acute LD50 for virgin female Spague-Dawley rats similarly exposed to 5900-7500 mg/m^3 chlorpyrifos for varying length of time from 60 to 180 min was 78 mg/kg (Berteau and Deen, 1978).

OBJECTIVES

Chlorpyrifos is used widely to control agricultural pests in Sri Lanka. Farmers take minimal safety precautions in mixing and applying a pesticide. Protective clothing is rarely used because of the hot humid climate and, in most cases would not be available because of the farmers socioeconomic status. Farmers receive limited training and safety practices. The potential for exposure is obvious. This study designed to assess the exposure of farmers to chlorpyrifos by monitoring the urinary TCP levels after an application. Background information was completed to evaluate variables affecting exposure. The risk of a chlorpyrifos application to the farmer was determined using the internal dose.

MATERIAL AND METHOD

Field experiments: One of the main vegetable growing areas in Kandy district, Sri Lanka was selected for the study in 2000. Vegetables are grown during the minor rainy season (April to June) since no irrigation system is available. One of the cultivation sites selected is shown in Figure 2.1.

Farmers: Nineteen male farmers, 35-48 years of age participated in this study. Farmers were recommended by the Agricultural instructors and the head farmer (an on-site person employed by the Department of Agriculture) of the Kandy Provincial Department of Agriculture Sri Lanka. Selections were based primarily on the crops grown and the pesticides used to control insects. Farmers growing long squash (Figure 2.2) or bitter melon were chosen because the crop vines grow on canopies. Farmers completed questionnaire to provide background information on general health status, health history,

Figure 2.1: One of the areas selected for the study

method of pesticide application, area under crop of interest, cultural practices, level of education, and family background. Farmers were found to be in good health. None of the farmers were taking any medication for chronic health problems. They all agreed not to use chlorpyrifos for at least 10 days before the study and to collect urine samples as specified. The volume of chlorpyrifos concentrate used per tank mix, number of tanks sprayed, duration of application, protective measures used, method of cleaning spray tanks, personal hygiene and local weather conditions were recorded for each application. Average daytime temperature and relative humidity was 30-34 ^{0}C and 70-80%, respectively, during the study period.

Chemicals and Pesticides: Chlorpyrifos 40% EC; manufacturer & formulator Cheminova Agro A/S, Denmark, was a kind donation of BASF Finlay (Pvt.) Ltd., 186, Vauxhall Street, Colombo. TCP and chlorpyrifos standards were donated by Dow Agroscience, 9330 Zionsville Road, Indianapolis.

Pesticide application and sample collection: All farmers used their own or rented knapsack sprayers to apply pesticides. They applied pesticides on their crop using normal application techniques. Particular attention was given to collect the urine samples at the right time and bring them back to the laboratory. The head farmer of the area who had overall control of all the farmers was given the most responsible role in sample collection. This person provided the communication-bridge between the Department of Agriculture and the farmer. Two additional Agricultural Instructors from the Office of Registrar of Pesticides were also assigned to monitor applications and sample collection. Amber glass bottles (100 mL) were used to collect urine. All bottles were washed with hexane and methylene chloride before use. A volume of 100 mL of urine was collected from each farmer as the baseline (control) urine sample before application of chlorpyrifos. Since all the farmers apply pesticides in the morning, the first two samples were taken around 3pm and

Figure 2.2: Long squash plants and fruits

9pm on the same day of application, and the third sample required for the first day post application was collected from the first urination on the following day. The same cycle was repeated to collect 24hr samples for 5 days. Sample were returned to the laboratory daily and stored in the refrigerator until extracted.

Urine analysis for TCP: Conjugates of TCP were hydrolyzed by heating 10 mL of urine with 2 mL of concentrated H_2SO_4 acid for 1hr at 90 ^{0}C. TCP was extracted using 2 x 10 mL aliquots of hexane (Fisher Scientific, New Jersey, USA) and the two hexane extracts combined. Final volume was adjusted to 1 mL by evaporation under nitrogen gas (BOC group Inc. NJ). All samples and standards were derivatized with 5μL N,O-bis(trimethylsilyl) acetamide (BSA) prior to injection. Recovery was evaluated using four control urine samples spiked with different concentrations of TCP. Spiked concentrations and percent recoveries are listed in Table 2.2.

Table 2.1: Recovery of TCP from spiked urine

Amount spike (μg)	**Amount recovery (μg)**	**Percent recovery**
0.5	0.51	103
0.75	0.53	70
1.0	0.79	79
1.25	1.11	89

Average recovery 85.4%

GC analysis: A Varian 3600 gas chromatographic system with an electron capture detector was used for the analysis of TCP in urinary extracts. Two columns were fixed to the same injector port: a polar column, DB-XLB 30 m, 0.25 mm internal diameter, and 0.25μm film thickness and a non-polar column, DB-1 30 m, 0.25 mm internal diameter, and 0.25μm film thickness, manufactured by J&W scientific, USA. Data from both columns was used for confirmation. A 2μL split-less injection was used with a Varian auto sampler 8200. Temperatures at injector port and detector were 50^0 and 350 ^{0}C, respectively. The three-step column temperature program was 100^0, 190^0, and 300^0 for 6, 2.5, and 2.5 min, respectively. The rates of temperature increases were 100 to 190 at 20 ^{0}C/min and 190 to 300 at 25 ^{0}C/min, respectively.

Sets of 30 samples along with two sets of six standards were used for each run. One set of standards was injected before the samples were injected and the other at the end. Two standards from the first set were injected between every three-sample injections. Standard curves generated for TCP were polynomial, and this shape was reproduced over all GC runs (Figure 2.3). Polynomial standard curve was close to linear and the slope was higher over the concentration range of 0.25-1.25 μg/mL. Hence the samples with TCP levels higher than 1.25 μg/mL were diluted with hexane to work in the linear range.

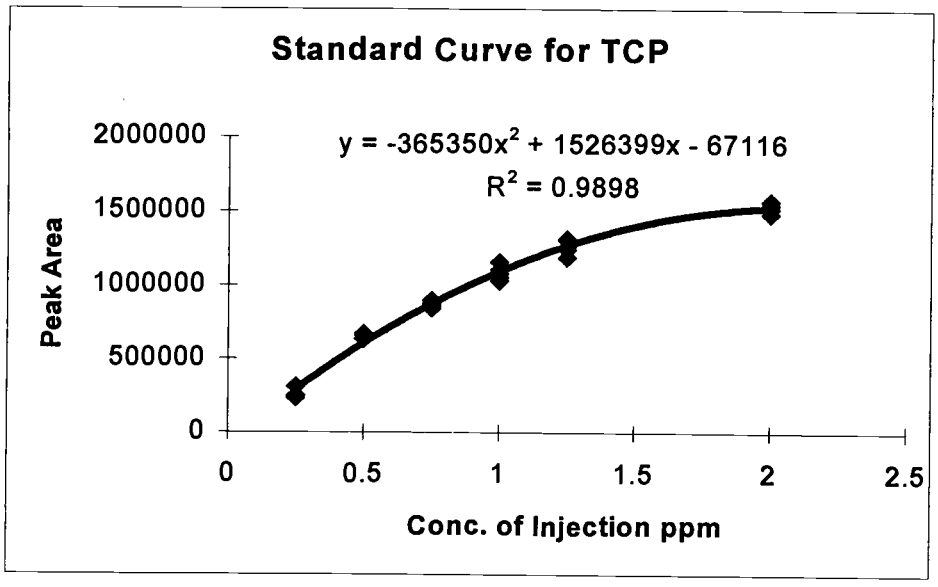

Figure 2.3: Standard curve generated for TCP

Instrument detection limits for urine analysis for TCP

Instrument noise = 0.5 mm (Figure 2.4)

Signal to Noise ratio = 3

Peak height of the 250 ng/mL standard = 113 mm

Calculation

Minimum measurable peak height = 0.5 mm x 3

= 1.5 mm

Minimum measurable concentration based on 1.5 mm peak

=(250 ng/mL/113 mm) x 1.5 mm

= 3.3 ng/mL

Instrument detection limit = 4 ng/mL

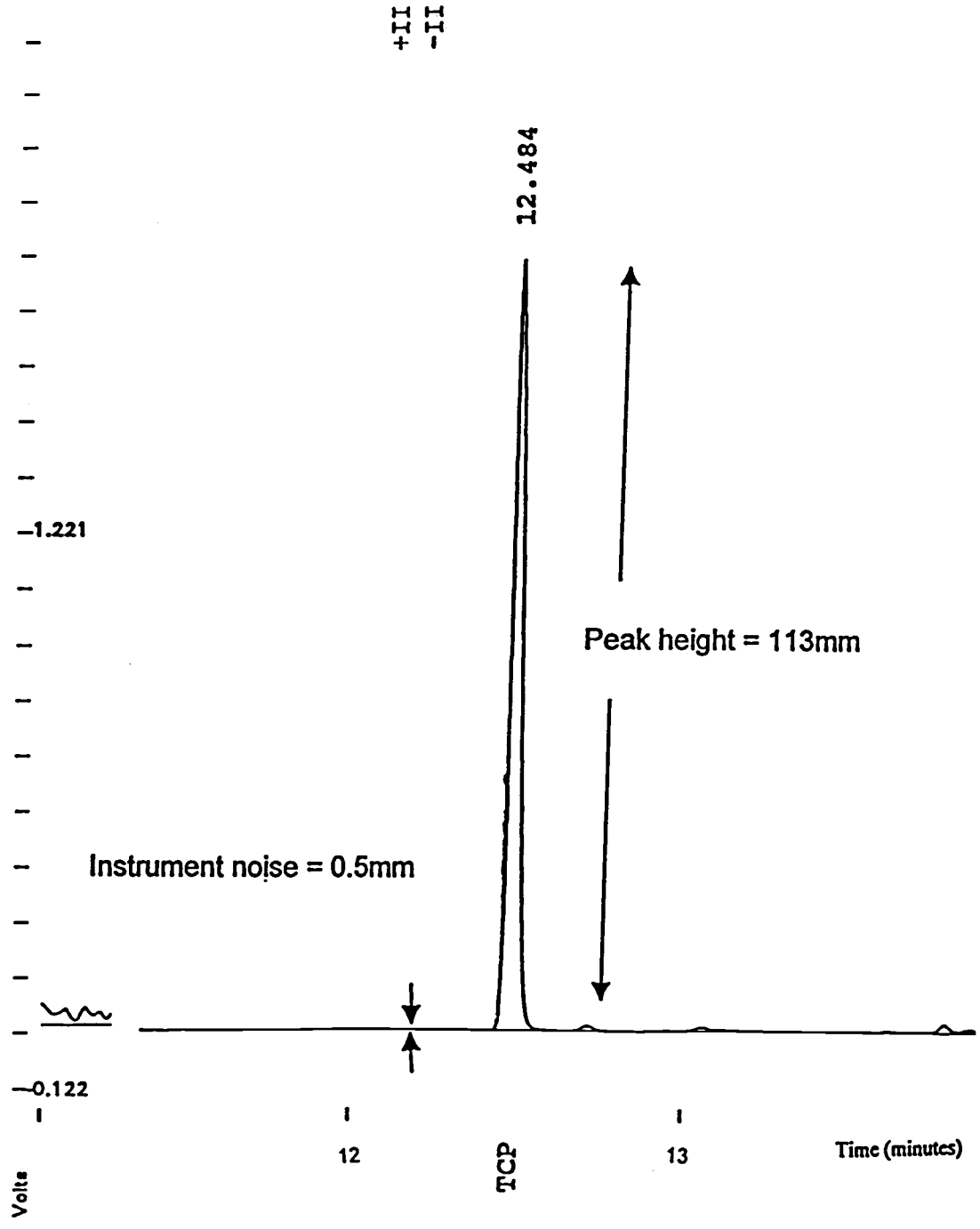

Figure 2.4: Chromatogram of 0.25 µg/mL TCP standard used to determine instrument detection limit in urine analysis for TCP (attenuation 1000)

Method detection limits for urine analysis for TCP

Method noise	= 8 mm (Figure 2.5)
Signal to noise ratio	= 3
Initial volume for the method	= 10 mL
Final volume for the method	= 1 mL
Peak height of the 250 ng/mL standard	= 113 mm

Calculation

Minimum measurable peak height = 8 mm x 3

= 24 mm

Minimum measurable concentration based on 24 mm peak

= (250 ng/mL /113 mm) x 24 mm

= 53 ng/mL

Since final volume is 1 mL , final concentration = 53 ng/mL

Initial volume is 10 mL; therefore,

Minimum amount of TCP in 10 mL of urine = 53 ng

Minimum concentration in urine = 53/10 ng/mL

Method detection limit = 6 ng/mL in urine

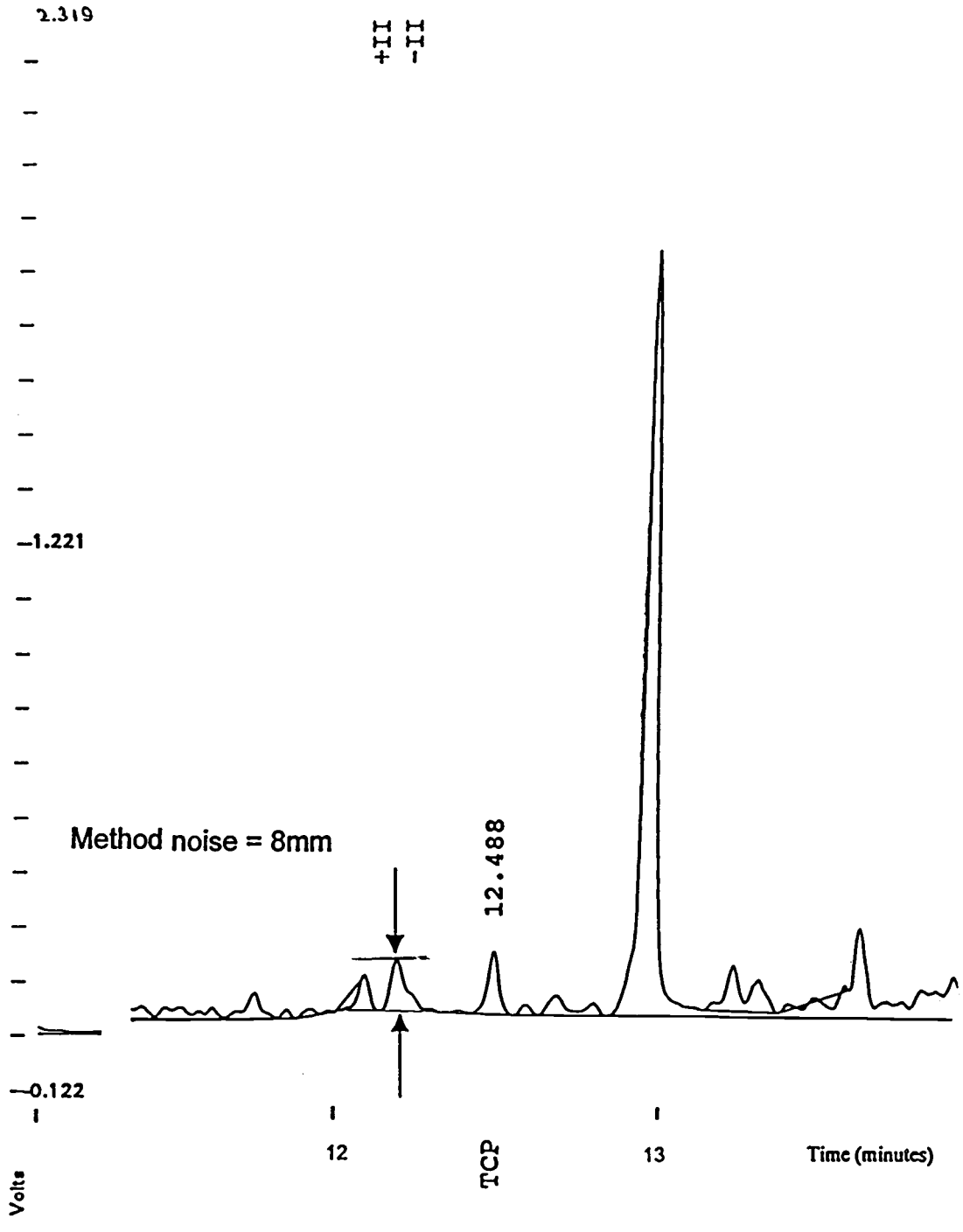

Figure 2.5: Chromatogram of blank urine extract used to determine method detection limit in urine analysis for TCP (attenuation 1000)

CREATININE ANALYSIS

Creatinine is a metabolic by-product of muscle, and an individual's muscle mass or lean body weight primarily determines its rate of production. It varies with age and gender, and for any given individual, the daily rate of creatinine production is assumed to be constant. Once creatinine is released from the muscle into plasma, it is eliminated almost exclusively by renal glomerular filtration. In the steady state, rate of creatinine production is equal to rate of elimination. In pharmacokinetic studies, urinary constituents are expressed as per gram of creatinine in urine.

Creatinine assay: Creatinine in alkaline solution reacts with picrate to form a colored complex, which increases the absorbance of the mixture. The change of absorbance over a specific time was measured in this assay (Henry, 1974).

Reagents:

1. Creatinine standard 2 mg/100ml (177μmol/L)
2. Picric acid 35 mmol/L
3. Sodium hydroxide 0.32 mmol/L

Procedure: Equal volumes of picric acid (35 mmol/L) and sodium hydroxide (0.32 mmol/L) were mixed as recommended (reagent mixture), and all reagents were stored in a refrigerator when not in use. Urine samples were diluted x50 with distilled water prior to the assay. Change in the absorbance at 492nm in first 2 min were recorded for the standard and samples. The instrument was set on kinetic mode for the assay. Standard solution or the samples were mixed with the reagent mixture in cuvettes as in Table 2.2 just before reading.

Table 2.2: Creatinine assay mixture

	Assay for standard	**Assay for samples**
Reagent mixture	2.0 mL	2.0 mL
Creatinine standard	0.2 mL	-
Sample	-	0.2 mL

Calculations:

A2-A1. Change in absorbance in standards($A_{standard}$)and samples ($A_{samples}$)

where A1= absorbance at 0 min

A2= absorbance at 2 min

$$\text{Urinary creatinine } \mu\text{mol/L} = \frac{A_{samples}}{A_{standard}} \times 177\ \mu\text{mol/L}$$

EXPOSED DERMAL AREA CALCULATION

Body surface area is a function of body weight (Wt) and height (Ht) of an individual. Mosteller's equation was used to calculate the total body surface area of the farmers (Mosteller, 1987). Areas of the different parts of the body of the farmers were calculated using the percentage values reported by Graber, 1997 (Figure 2.6, Table 2.3). Assumptions (a key) used to calculate uncovered skin area are given in Table 2.4.

Mosteller formula:

Body Surface Area (m^2)= (Height (cm) x Weight (kg)/3600) ^{½}

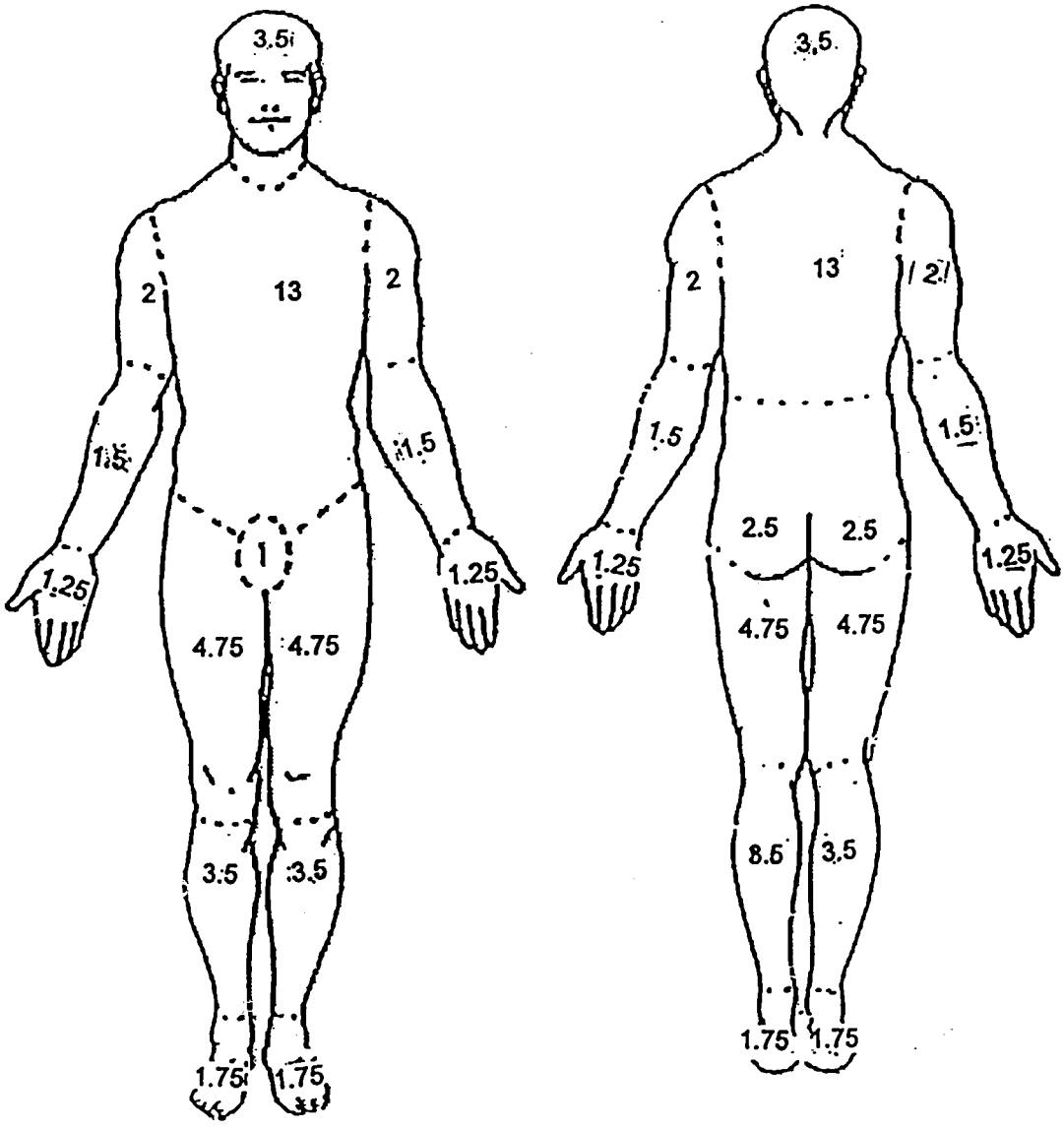

Figure 2.6: Area of different parts of the body

Table 2.3: Percent area of different parts of the body

Part of the Body	Percent of total area
Head	7
Neck	2
Anterior trunk	13
Posterior trunk	13
Right buttocks	2.5
Left buttocks	2.5
Genitalia	1
Right upper arm	4
Left upper arm	4
Right lower arm	3
Left lower arm	3
Right hand	2.5
Left hand	2.5
Right thigh	9.5
Left thigh	9.5
Right leg	7
Left leg	8
Right foot	3.5
Left foot	3.5

Table 2.4: A key used to calculate percent covered by clothing during application of chlorpyrifos

Protective clothing used	Percent covered
1	55
2	61
3	71
4	77
Hat	2.5
Gloves	5
Face cover	1.8

1= Short-sleeved shirt and covered up-to the knee

2= Long-sleeved shirt and covered up-to the knee

3= Short-sleeved shirt and long pans

4= Long-sleeved shirt and long pans

RESULTS

Questionnaire: Volume of pesticides applied on the crop varies for each farmer due to the fact that there were differences in area cultivated, density of the canopy (stage of the crop), mixing ratio and walking speed. Use of protective clothing and personal hygiene also varied. This variation among farmers led to different levels of individual exposure. The Department of Agriculture recommends using 28 mL of chlorpyrifos 40%EC formulated product per 16 liters of water, but about 30% of farmers used more than the recommended amount to achieve better pest control (Table 2.5). Many of the spray tanks were old and about 30% were leaking. Sprayers that are leaking result in an additional exposure through wet clothing. Half of the workers did not use a head cover. Personal information such as age, level of education body weight, size of family, alcohol consumption, and smoking habits are listed in Table 2.6. Only one farmer covered his face with a handkerchief. Many farmers bury left over pesticide in the bottle until it is used again. After pesticide application, there was about a 1hr delay before farmers washed in a stream.

Table 2.5: Agricultural details of the farmers

Farmer ID	Area cultivated (ac)	Chlorpyrifos 40% EC used (mL)	Number of tank loads	Spray mix (CPF* ml per 16L water)	Duration of application (hr)
F1	0.25	112	4	28	2.5
F2	0.25	168	4	42	3
F3	0.25	84	3	28	2.5
F4	0.3	112	4	42	3.5
F5	0.25	210	5	42	4
F6	0.3	210	5	42	4
F7	0.2	84	3	28	2.5
F8	0.3	140	5	28	4
F9	0.25	112	4	42	3
F10	0.25	210	5	42	4
F11	0.3	168	6	28	4.5
F12	0.25	112	4	28	3
F13	0.3	168	6	28	4
F14	0.25	112	4	28	3.5
F15	0.25	140	5	28	4.5
F16	0.2	84	3	28	2
F17	0.25	112	4	28	2.5
F18	0.25	140	5	28	4
F19	0.25	112	4	28	3

*CPF=chlorpyrifos

Table 2.6: Summery of personal details of the farmers

Farmer ID	Age	Level of education (grade)	Body weight (kg)	Members in the family	Alcohol consumption	Smoking
F1	35	8	65	3	Y	N
F2	38	10	70	4	Y	N
F3	41	8	75	3	Y	Y
F4	38	10	70	3	Y	Y
F5	47	8	73	3	Y	Y
F6	40	8	64	3	Y	Y
F7	41	8	65	4	Y	N
F8	35	10	68	3	N	N
F9	40	12	77	4	Y	Y
F10	42	6	81	3	Y	N
F11	41	6	73	3	Y	Y
F12	45	8	65	5	Y	N
F13	42	8	69	3	Y	Y
F14	39	6	73	4	Y	Y
F15	44	6	69	4	Y	Y
F16	39	10	70	2	Y	Y
F17	39	10	62	3	Y	Y
F18	42	8	63	3	N	Y
F19	46	8	65	4	Y	N

Pesticide application in the field: Chlorpyrifos was applied to the crop after each harvest. Farmers prepare pesticide spray mix by a stream which provides a convenient source of water. Most of them do not use gloves when handling concentrated pesticides. Figure 2.7 illustrates clothing used, preparation of spray mix for application, handing of concentrated pesticides, condition of some of the spray tanks, and the spray operation of some farmers. These conditions were similar for most of the farmers.

Figure 2.7: The process of applying pesticide on canopy

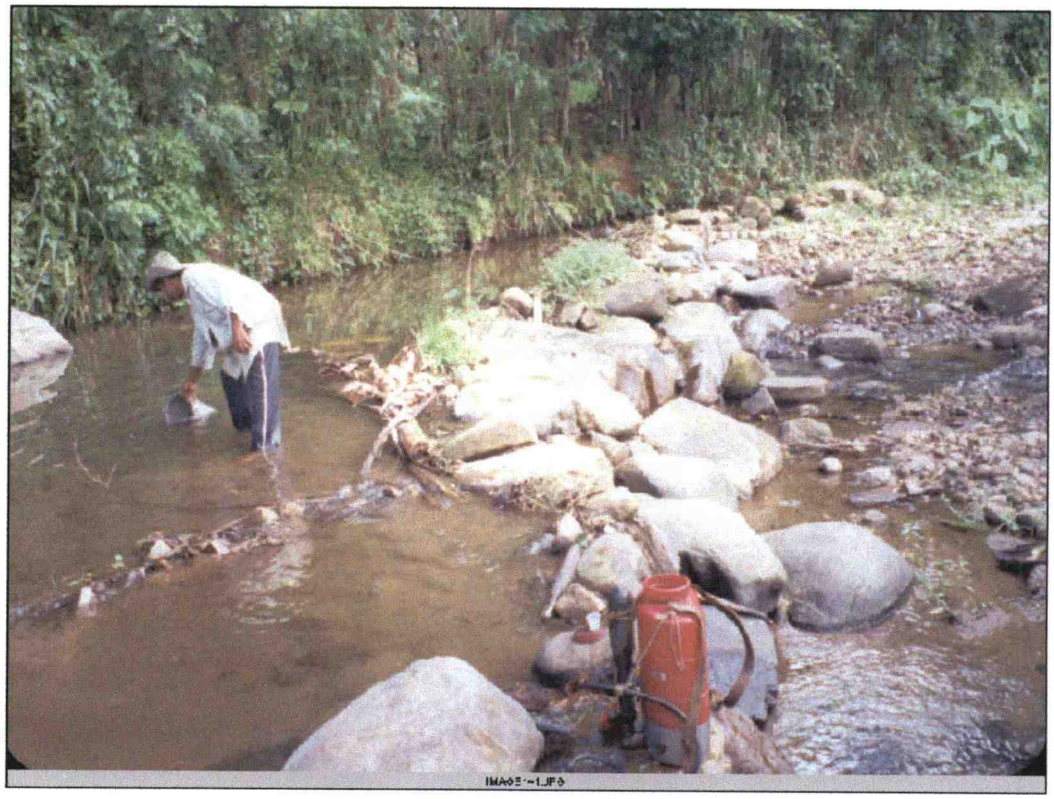

Figure 2.7a: Getting water from the stream for dilution

Figure 2.7b: Handling concentrate pesticide without gloves

Figure 2.7c: Applying pesticides with a leaking spray tank

Figure 2.7d: Spraying pesticides on an over-head canopy without a head protection, or facemask, and using minimal coverage.

Figure 2.7e: Ready to apply pesticides

Chromatograms of urine analysis for TCP: Chromatograms of pre and post application of chlorpyrifos of farmer number 4 is given in Figure 2.8(a-f) and a chromatogram of 0.25 µg/mL TCP standard is illustrated in Figures 2.9. Each sample run (method) was 25 min long and retention time of TCP-BSA was 12.47 min on a DB-1 column. A part of the chromatogram with interested peak is given as chromatograms in following figures.

Figure 2.8: Chromatograms for pre- and post-application urine extracts for farmer number three (a-f)

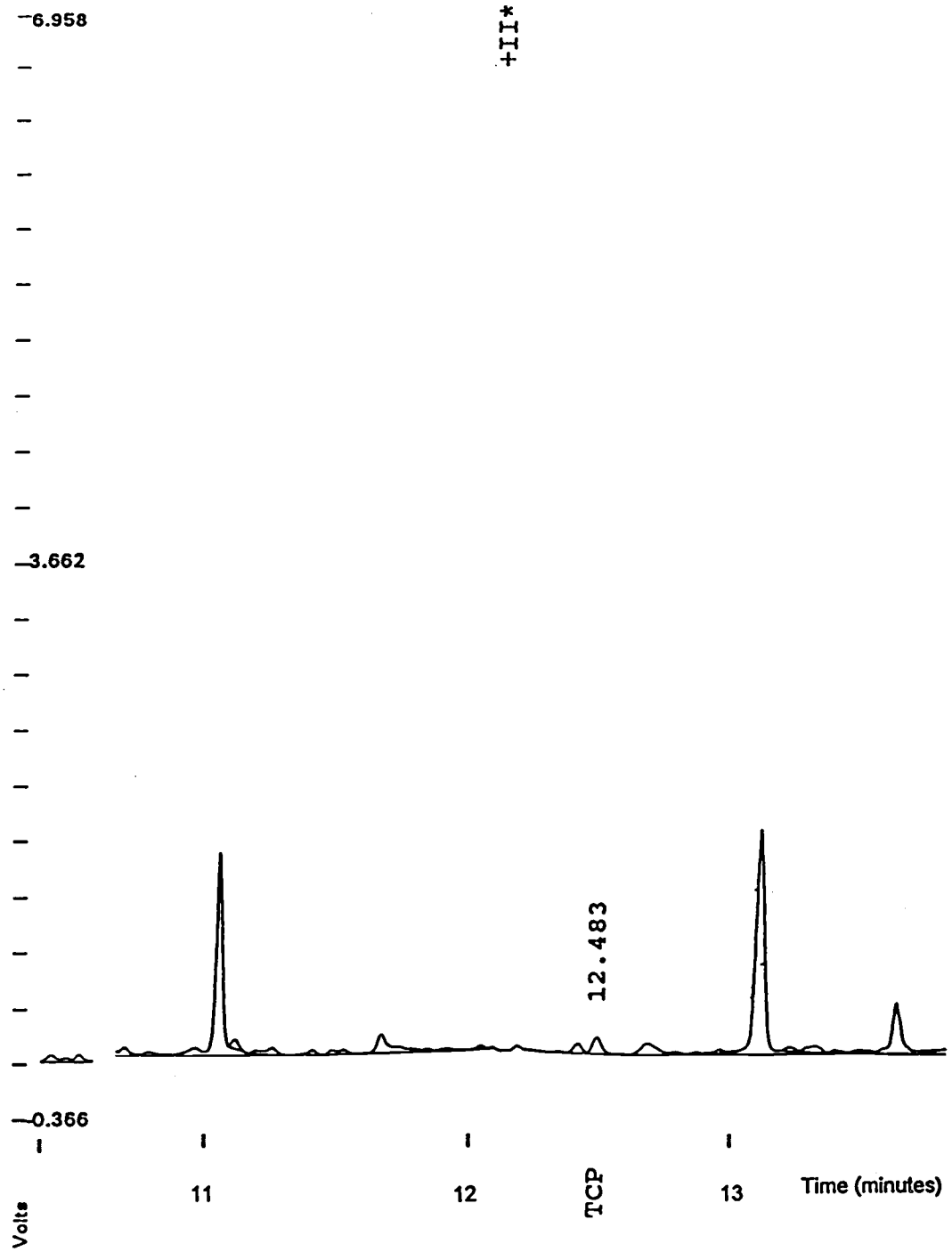

Figure 2.8-a: Chromatogram of pre-application urine extract of farmer number three (attenuation 3000)

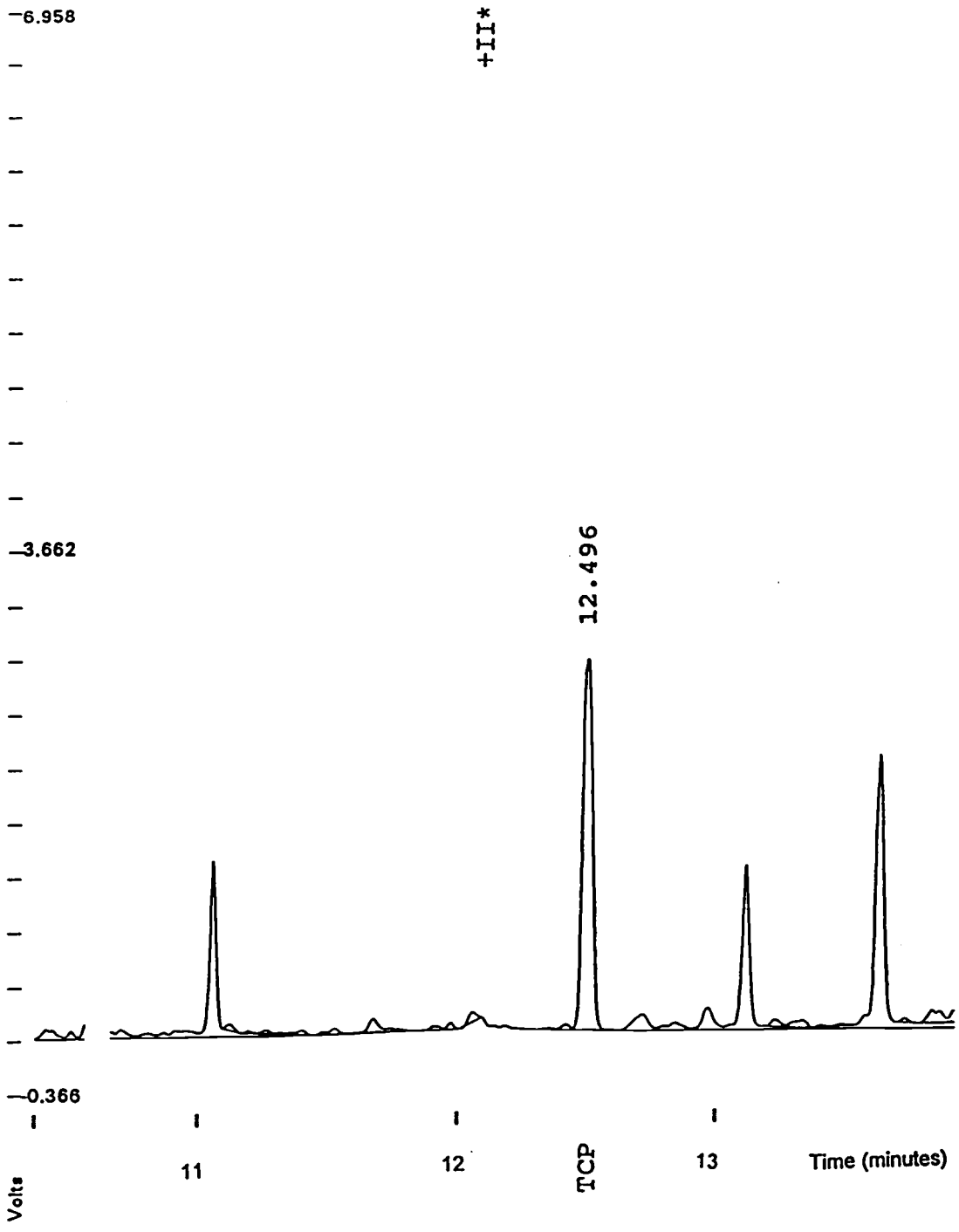

Figure 2.8-b: Chromatogram of 24 hr post-application urine extracts of farmer number three (attenuation 3000)

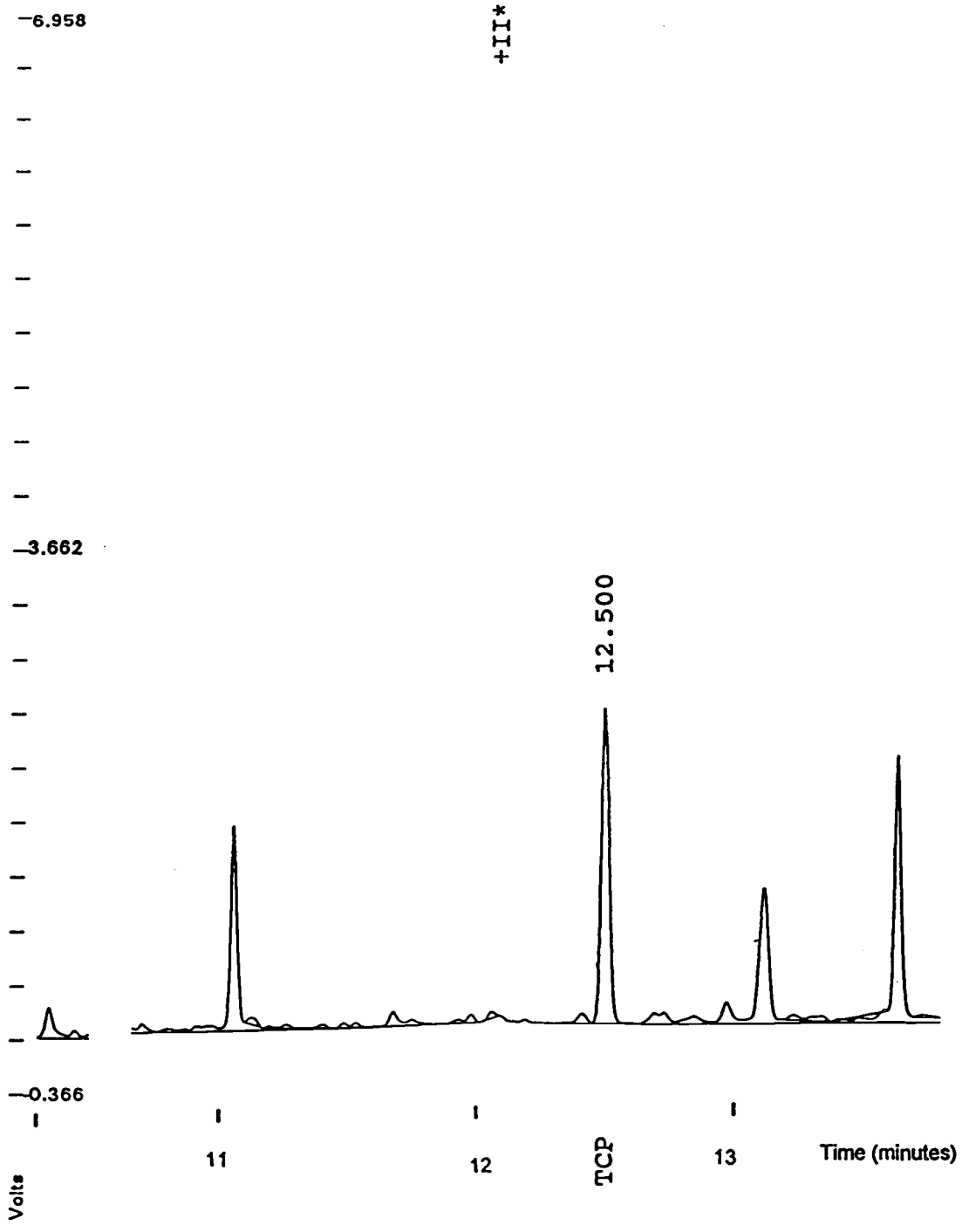

Figure 2.8-c: Chromatogram of 48 hr post application urine extract of farmer number three (attenuation 3000)

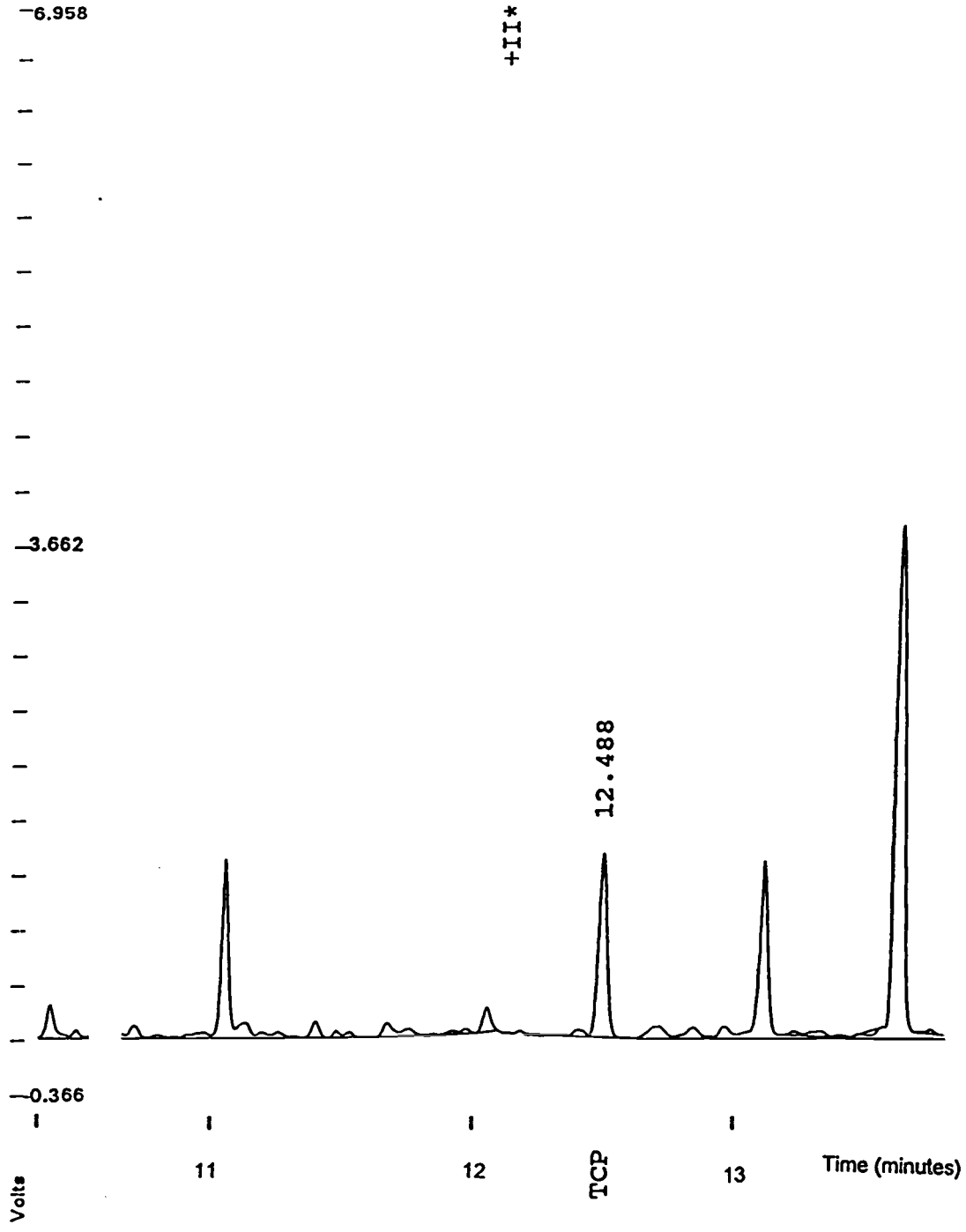

Figure 2.8-d: Chromatogram of 72hr post application urine extract of farmer number three (attenuation 3000)

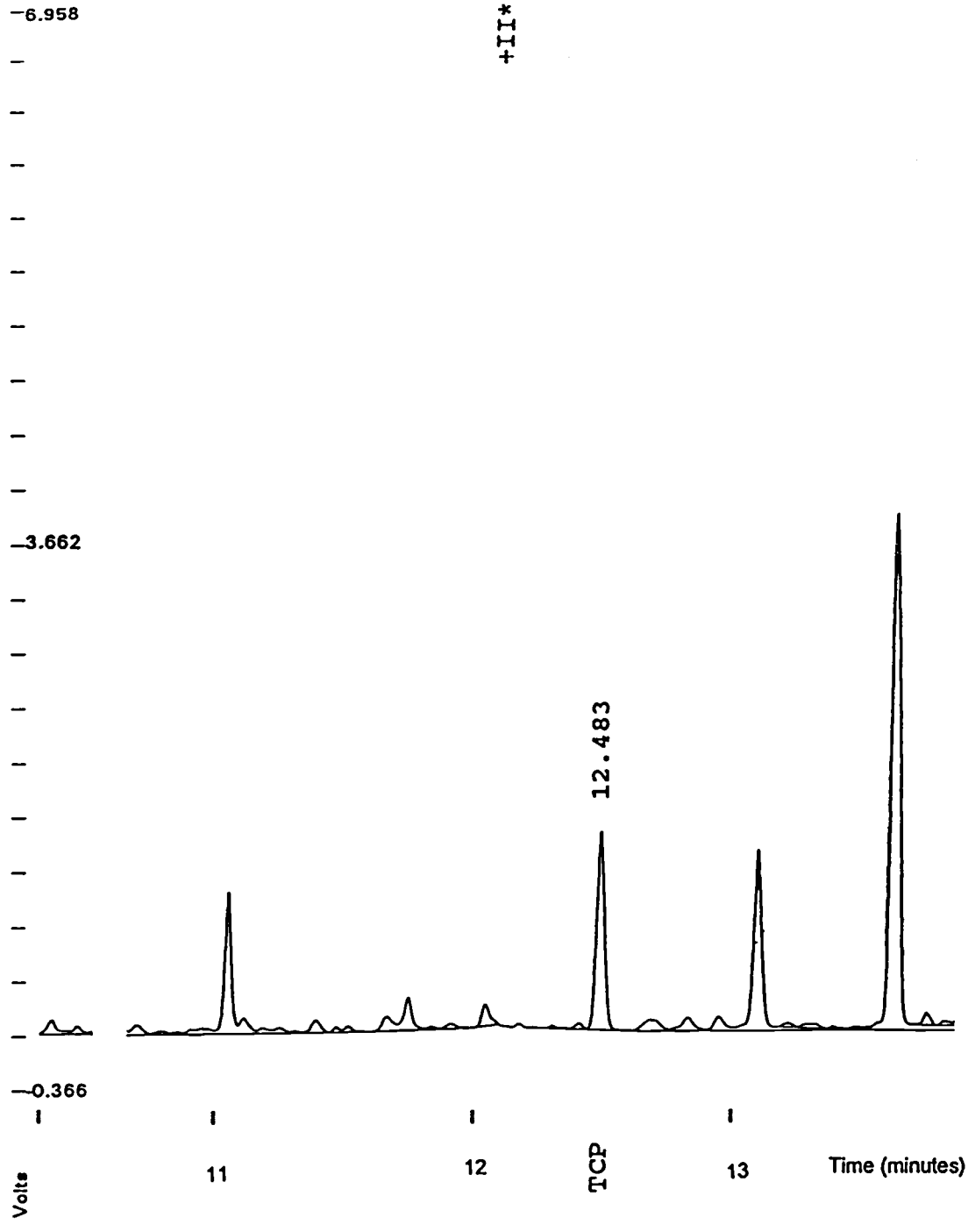

Figure 2.8-e: Chromatogram of 96 hr post application urine extract for farmer number three (attenuation 3000)

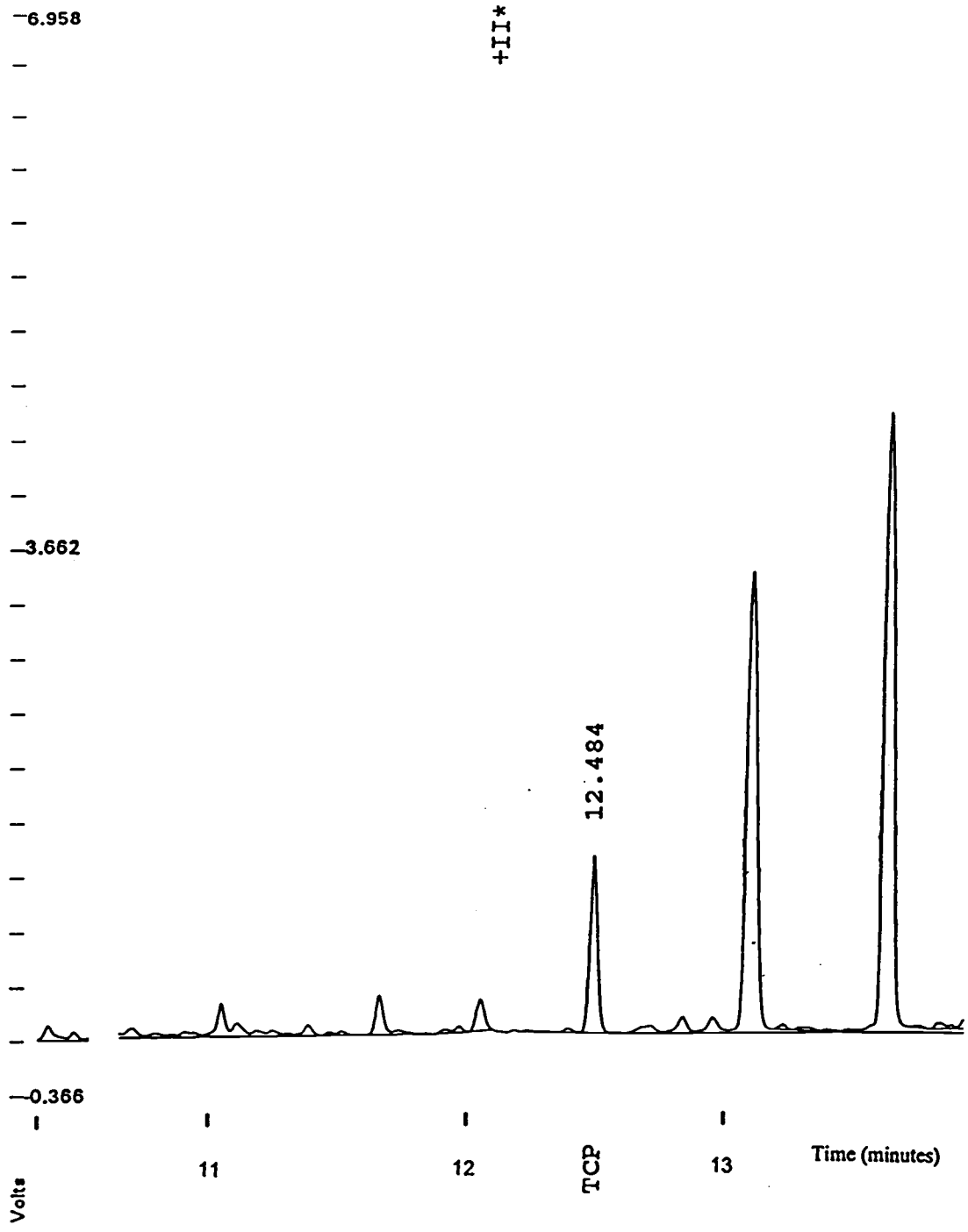

Figure 2.8-f: Chromatogram of 120 hr post application urine extract of farmer number three (attenuation 3000)

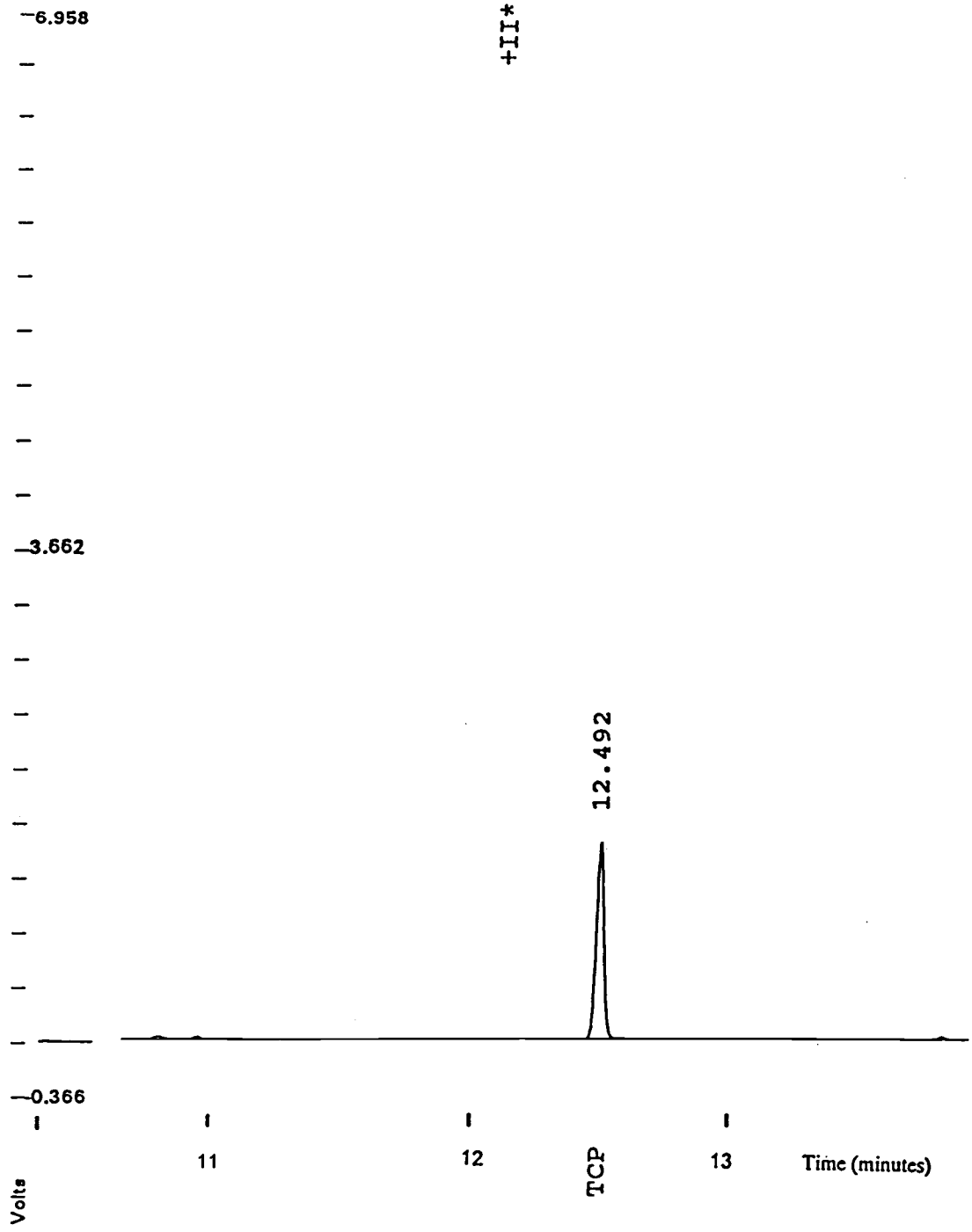

Figure 2.9: Chromatogram of 0.25µg/mL TCP standard (attenuation 3000)

Urinary TCP levels of individual farmers: Urinary TCP levels peaked 24hr after application and the levels dropped back to the baseline on the fifth day post application. The same pattern was observed for all farmers. Urinary TCP levels were expressed in μg/g of creatinine, assuming creatinine clearance of an adult is 1g per day. Average creatinine level was 0.95g/L for the farmers. Pre-(baseline) and post-application urinary TCP levels normalized for 1g of creatinine are given in Table 2.7. The values in Table 2.7 are derived from time verses TCP clearance curve for individual farmers, but not experimental values. Experimental values were obtained at 18, 42, 66, 90, and 114 hr post application and were extrapolated to 24 hr intervals using a polynomial curve and, as a result, some values obtained at 120 hr were negative. Total TCP excreted during the 5 day period ranged from 76.1-299.8 μg/5g of creatinine (mean 190.3 μg/5g of creatinine). All baseline samples except one had detectable levels of TCP, and baseline values were subtracted from all post application values for each farmer assuming this level was due to some continues exposure. Calculated cumulative TCP clearance values are given in Table 2.8. Since TCP elimination did not show exponential pattern, cumulative TCP clearance versus time graphs were used to calculate the time required to void half the amount of total TCP. Post-application urinary TCP levels with time for individual farmers and mean of all farmers with time are given in Figure 2.10(a-t) and 2.11, respectively.

Half time ($t_{1/2}$) : The time taken to eliminate 50% of the total TCP recovered in urine was considered as elimination $t_{1/2}$. The observed $t_{1/2}$ ranged from 24.8-35.1 days and the mean was 31.3 days.

Table 2.7: Pre- and post-application urinary TCP levels in μg/g of creatinine*

Farmer ID	Baseline	24 hour	48 hour	72 hour	96 hour	120 hour	Total (μg/5g of creatinine)
F1	0.02	83.6	53.3	30.5	7.7	-15.2	175.0
F2	43.57	92.9	60.4	36.3	12.2	-11.9	201.8
F3	5.04	35.1	24.8	16.5	8.2	-0.1	84.5
F4	3.94	60.0	39.8	28.5	17.2	5.9	151.4
F5	2.35	123.9	85.7	54.9	24.0	-6.9	288.6
F6	18.56	125.1	83.2	49.2	15.2	-18.8	272.7
F7	8.97	48.8	34.7	24.8	14.9	5.0	128.2
F8	5.90	116.3	86.7	54.5	22.4	-	279.9
F9	7.61	27.4	22.4	16.2	10.0	-	76.1
F10	2.84	115.4	83.4	58.5	33.6	8.8	299.8
F11	-6.04	89.3	64.8	47.0	29.3	11.5	241.9
F12	18.69	63.6	48.1	32.6	17.0	1.5	162.8
F13	8.52	96.8	75.7	48.8	22.0	-	243.3
F14	15.76	50.2	35.4	25.8	16.3	6.7	134.5
F15	14.56	119.4	82.2	51.3	20.4	-10.4	273.3
F16	9.59	58.9	41.3	24.2	7.2		131.6
F17	8.55	25.9	19.4	14.0	8.6	3.2	71.0
F18	12.16	90.8	66.6	47.0	27.4	7.8	239.7
F19	6.34	64.5	46.0	31.1	16.2	1.4	159.2
Mean	*9.84*	*78.31*	*55.46*	*36.40*	*17.35*	*-0.63*	*190.27*
Std.Devi	*10.28*	*33.02*	*23.05*	*14.46*	*7.66*	*8.62*	*75.92*
Std.Error	*2.36*	*7.57*	*5.28*	*3.32*	*1.76*	*2.03*	*17.41*

* These values are from the time versus TCP elimination curve (not experimental values)

Table 2.8: Post-application cumulative urinary TCP levels in μg/g of creatinine, calculated internal dose, and $t_{1/2}$

Farmer ID	24 hour	48 hour	72 hour	96 hour	120 hour	Internal dose mg/kg	$t_{1/2}$
F1	83.6	136.9	167.3	175.0	175	0.0053	24.8
F2	92.9	153.3	189.6	201.8	201	0.0057	26.3
F3	35.1	59.9	76.4	84.5	84.5	0.0023	30.4
F4	60.0	99.8	128.3	145.5	151.4	0.0044	33.5
F5	123.9	209.7	264.6	288.6	288.6	0.0080	29.3
F6	125.1	208.3	257.5	272.7	272.7	0.0084	26.1
F7	48.8	83.5	108.3	123.2	128.2	0.0040	36.0
F8	116.3	203.0	257.5	279.9	297.9	0.0082	29.2
F9	27.4	49.8	66.1	76.1	79.8	0.0021	34.0
F10	115.4	198.8	257.4	291.0	299.8	0.0075	33.2
F11	89.3	154.0	201.1	230.3	241.9	0.0067	35.1
F12	63.6	111.7	144.3	161.3	162.8	0.0051	32.3
F13	96.8	172.5	221.3	243.3	243.3	0.0070	30.6
F14	50.2	85.6	111.4	127.7	134.5	0.0037	34.9
F15	119.4	201.6	252.9	273.3	273.3	0.0080	28.4
F16	58.9	100.2	124.4	131.6	131.6	0.0038	27.4
F17	25.9	45.3	59.3	67.9	71.0	0.0023	37.6
F18	90.8	157.4	204.5	231.9	239.7	0.0077	33.8
F19	64.5	110.4	141.5	157.8	159.2	0.0050	31.8
Mean	*78.31*	*133.77*	*170.19*	*187.54*	*191.37*	*0.0055*	*31.29*
Std.Devi	*33.02*	*55.93*	*70.04*	*75.59*	*76.89*	*0.0022*	*3.65*
Std.Error	*7.57*	*12.83*	*16.07*	*17.34*	*17.64*	*0.00049*	*0.84*

Figure 2.10: Pre- and post-application urinary TCP levels of individual farmers (farmers number 1-19). X axis represent time in hr. Urinary TCP μg/g of creatinine is given in Y axis (Figures 2.10a-s)

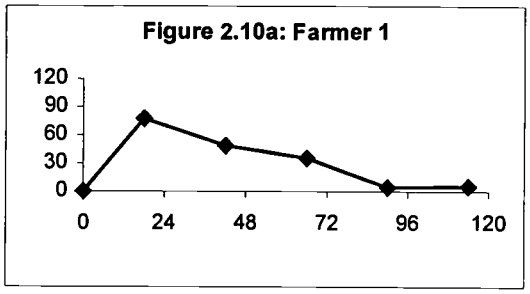
Figure 2.10a: Farmer 1
120
90
60
30
0
0
24
48
72
96
120

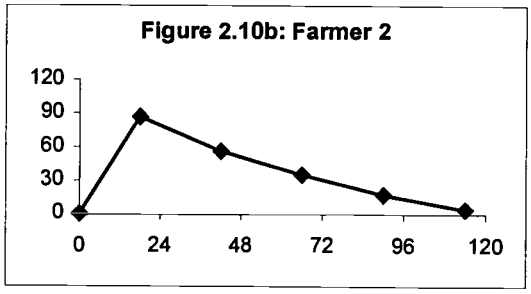
Figure 2.10b: Farmer 2
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90
60
30
0
0
24
48
72
96
120

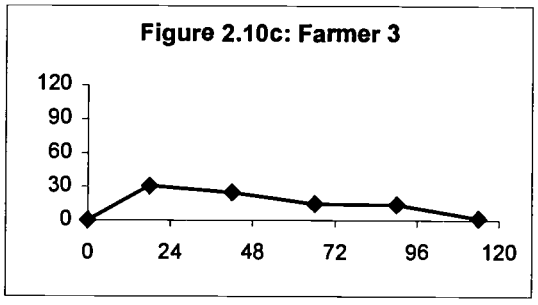


Figure 2.10c: Farmer 3
120
90
60
30
0
0
24
48
72
96
120

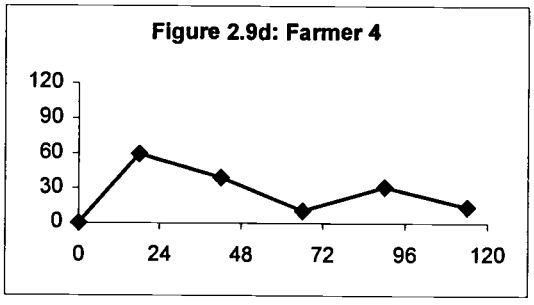


Figure 2.9d: Farmer 4
120
90
60
30
0
0
24
48
72
96
120

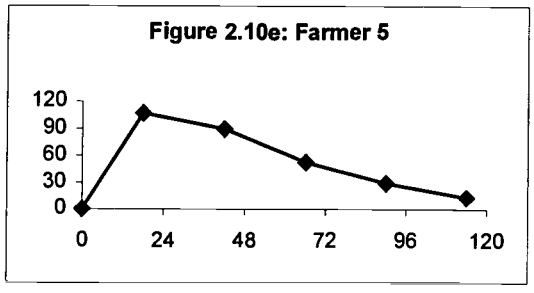


Figure 2.10e: Farmer 5
120
90
60
30
0
0
24
48
72
96
120

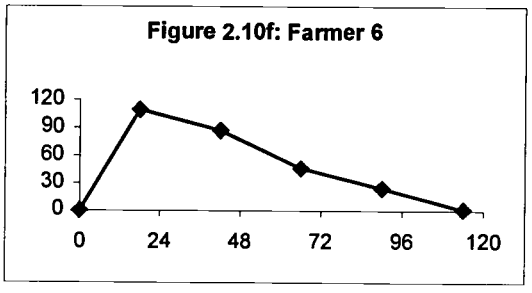


Figure 2.10f: Farmer 6
120
90
60
30
0
0
24
48
72
96
120

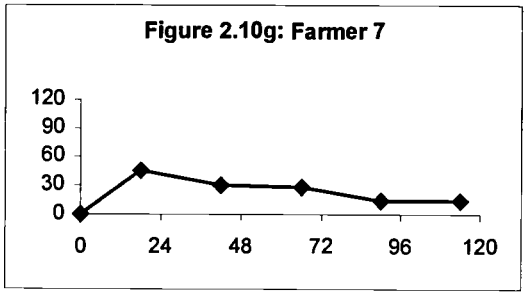


Figure 2.10g: Farmer 7
120
90
60
30
0
0
24
48
72
96
120

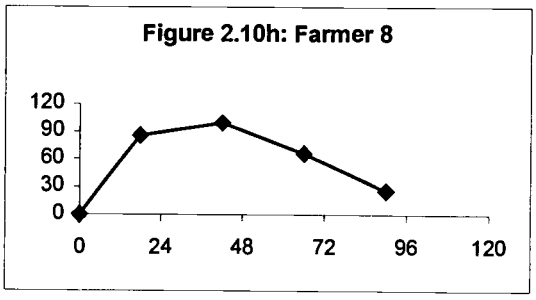


Figure 2.10h: Farmer 8
120
90
60
30
0
0
24
48
72
96
120

Figure 2.10i: Farmer 9

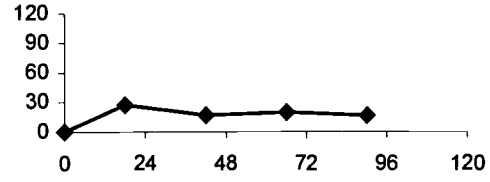


Figure 2.10j: Farmer 10

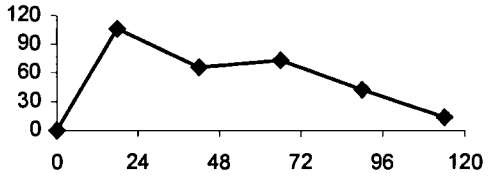


Figure 2.10k: Farmer 11

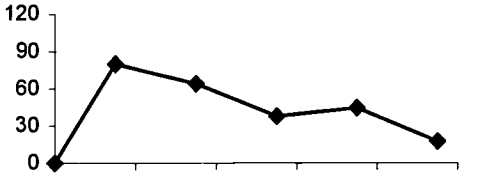


Figure 2.10l: Farmer 12

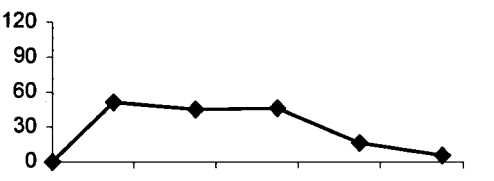


Figure 2.10m: Farmer 13

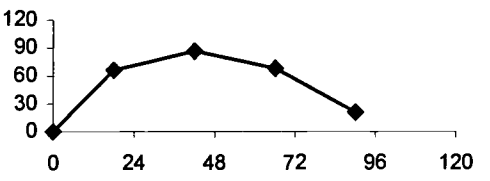


Figure 2.10n: Farmer 14

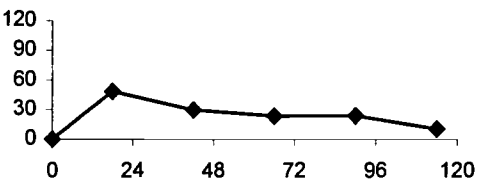


Figure 2.10o: Farmer 15

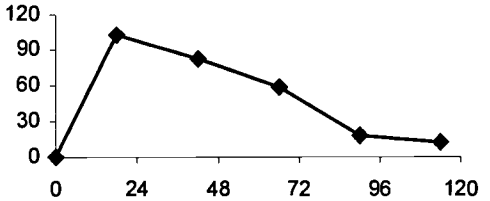
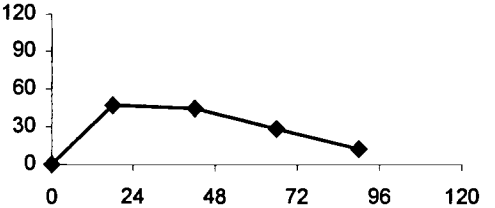


Figure 2.10p: Farmer 16



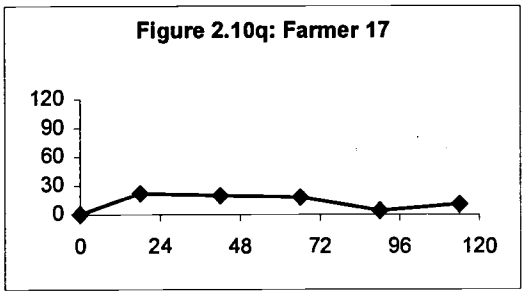


Figure 2.10q: Farmer 17
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90
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72
96
120

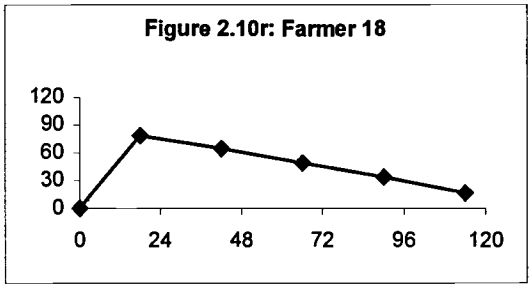
Figure 2.10r: Farmer 18
120
90
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0
0
24
48
72
96
120

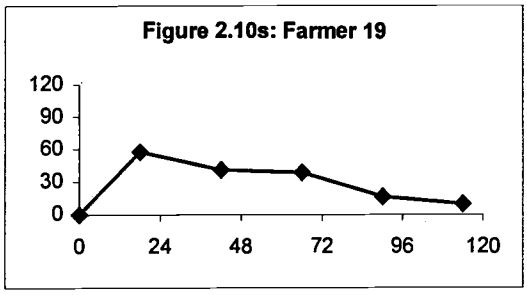
Figure 2.10s: Farmer 19
120
90
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72
96
120

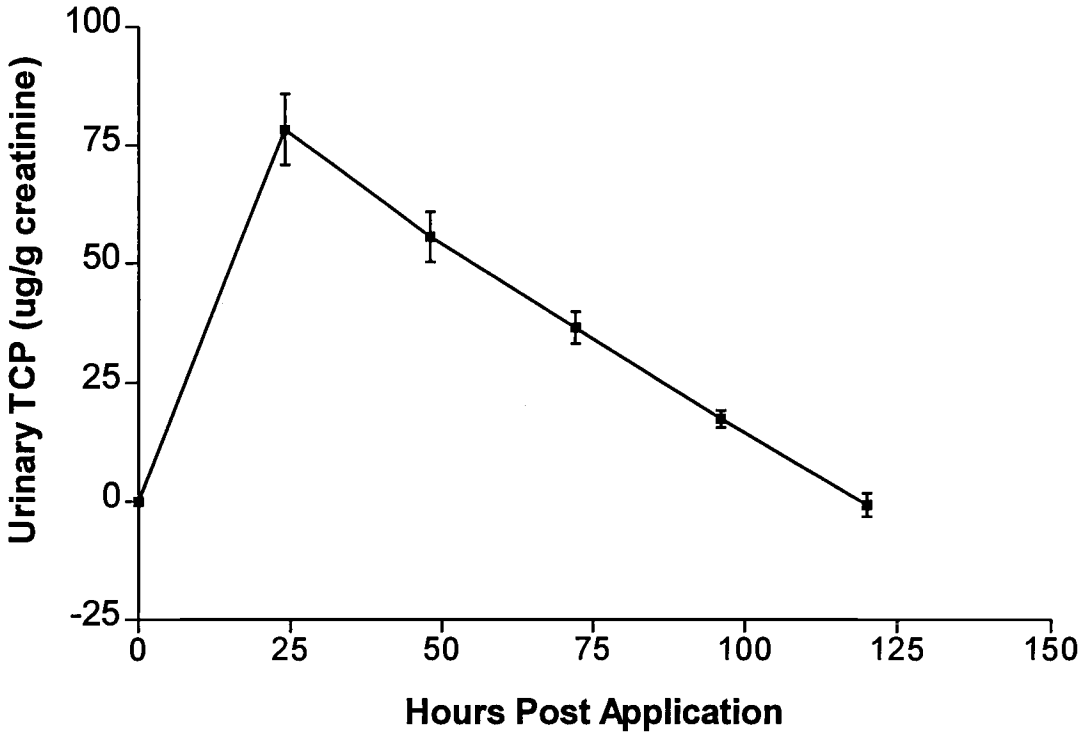

Figure 2.11: Mean urinary TCP levels of all farmers

Risk calculation: Based on Nolen et al. (1984) and Griffin et al. (1999), we assume 90% of the TCP was voided in urine over 5 days and one mole of chlorpyrifos generates one mole of TCP in the body.

Calculated total open skin surface areas for farmers are given in Table 2.9. Mosteller formula was used to calculate body surface area for each farmer using their body weight and height.

Amount of active ingredient used, body weight, calculated open skin area based on protective clothing used, dosage on skin, calculated internal dose, hazard quotient (HQ), and margin of safety (MOS) are given in Table 2.10. Griffin et al. (1999) reported no signs or symptoms of toxicity or change of erythrocyte or plasma cholinesterase levels with a dose of 28.59 mg over an area of 78 cm^2 on human skin. It was assumed that total internal dose was due to dermal exposure. Griffin et al. (1999) reported that 1% of the total dose was recovered after an 8hr exposure from the water based chlorpyrifos formulation applied on the skin. Therefore, proportional dermal dose was calculated for all farmers based on the duration of exposure and urinary TCP level. Duration of exposure was calculated from time started applying pesticide through the body-wash after application. Calculated internal dose values, HQ and MOS values ranged from 0.0021-0.0084 mg/kg, 0.8-2.7, and 3.6-14.3, respectively. EPA oral sub chronic NOEL and reference dose (RfD) used for the calculations was 0.03 and 0.003 mg/kg, respectively. An uncertainty factor of 10 was used for human variability to obtain the RfD (EPA, 1997). Equations used to calculate MOS and HQ are given below.

$$\text{Margin of Safety} = \frac{\text{NOEL (mg/kg body weight)}}{\text{Exposure Dose (mg/kg body weight)}}$$

$$\text{Hazard Quotient} = \frac{\text{Exposure Dose (mg/kg body weight)}}{\text{Reference Dose (mg/kg body weight)}}$$

All except three farmers showed an HQ higher than 1 (average 1.8), which indicates a risk to the applicator. The MOS values were greater than 1 in all cases. The farmers received an occupational dose higher than RfD of chlorpyrifos, but it was below the NOEL.

Medical Examination: A standard physical examination was conducted to assess possible adverse neurological effects of the farmers participating in this study. This examination evaluates the function of cranial nerves, muscle power, reflexes, co-ordination and sensations. No significant abnormalities were found in any of the farmers.

Table 2.9: Calculation of total body surface area and exposed area for farmers

Farmer ID	Total body surface area (m^2)	Percent exposed	Area exposed cm^2
F1	1.70	42.5	7225
F2	1.80	45.0	8100
F3	1.87	45.0	8415
F4	1.80	34.7	6246
F5	1.84	29.0	5336
F6	1.73	45.0	7785
F7	1.74	45.0	7830
F8	1.78	26.5	4717
F9	1.89	36.5	6899
F10	1.94	20.5	3977
F11	1.84	42.8	7875
F12	1.74	42.5	7395
F13	1.79	45.0	8055
F14	1.84	23.0	4232
F15	1.79	23.0	4117
F16	1.80	26.5	4770
F17	1.70	26.5	4505
F18	1.72	45.0	7740
F19	1.74	45.0	7830
Mean	1.790	36.26	6476.26
Std. Error	0.015	2.16	372.67
Std Dev.	0.066	9.42	1624.45

Calculation based on the Mosteller formula

Body Surface Area (m^2)= (Height (cm) x Weight (kg)/3600) ^{½}

Table 2.10: Calculated risk values for individual farmer

Farmer ID	Active Ingredient used (g)	Internal Dose of Chlorpyrifos mg/kg	Open skin area (cm^2)	Dose on Skin* mg/kg	$\mu g/cm^2$	HQ	MOS
F1	48.3	0.0053	4505	1.1	15.8	1.8	5.7
F2	72.5	0.0057	3690	1.0	19.6	1.9	5.3
F3	36.3	0.0023	7293	0.5	4.8	0.8	13.0
F4	48.3	0.0044	7326	0.7	6.8	1.5	6.8
F5	90.6	0.0080	5336	1.2	15.8	2.7	3.8
F6	90.6	0.0084	5017	1.2	15.5	2.8	3.6
F7	36.3	0.0040	7830	0.8	6.9	1.3	7.5
F8	60.4	0.0082	7565	1.2	10.6	2.7	3.7
F9	48.3	0.0021	3875	0.4	7.5	0.7	14.3
F10	90.6	0.0075	7081	1.1	12.3	2.5	4.0
F11	72.5	0.0067	6771	0.9	9.5	2.2	4.5
F12	48.3	0.0051	7395	0.9	8.0	1.7	5.9
F13	72.5	0.0070	5191	1.0	13.4	2.3	4.3
F14	48.3	0.0037	5336	0.6	8.1	1.2	8.1
F15	60.4	0.0080	8055	1.0	8.9	2.7	3.8
F16	36.3	0.0038	6570	0.9	9.7	1.3	7.9
F17	48.3	0.0023	3485	0.5	8.6	0.8	13.0
F18	60.4	0.0077	4988	1.1	14.0	2.6	3.9
F19	48.3	0.0050	7830	0.9	7.5	1.7	6.0

Assumption: 1) * Calculated assuming 1% of the dermal dose recovered in 8 hour period
2) Internal dose was used as the exposure and subchronic RfD for oral is 0.003 mg/kg

MOS= NOEL mg/kg/ Exposure mg/kg NOEL is 0.03mg/kg

Hazard Quotient = Exposed dose / RfD

STATISTICAL ANALYSIS

A statistical analysis was conducted to determine which of several application variables were associated with the internal dose of chlorpyrifos (μg/kg bodyweight). The variables included the amount of active ingredient (g) applied by the farmer and protective measures used which include condition of the spray tank (good, average and leaky) the protective clothing used, (short/long pants and/or short/long-sleeved shirt - see Table 2.11), and whether a hat and/or gloves were used. The duration of application was not considered since it was likely to be associated with the amount of pesticide applied, i.e., the more pesticide that is sprayed by a particular farmer (g), the longer the application period (hr). Also, since only one farmer wore a mask, an analysis of the significance of the use of a mask with dose was not possible.

The internal dose of chlorpyrifos for the 19 farmers participating in the study was computed from the total TCP (μg TCP/g creatinine per day) excreted in 5 days following exposure using the following formula assuming 90% of the internal dose is excreted in urine. Calculated internal doses are listed in table in Table 2.8.

Internal dose (μg/kg body weight) = 1111 x TCP(μg/g of creatinine)/Body weight (kg)

With a limited number of observations, the analysis was divided into two steps. The first analysis screened for existing interactions between: 1) The amount of active ingredient applied by the farmer, spray tank rating (good, average leaky), and total exposed skin surface area (cm^2). 2) The amount of active ingredient applied by the farmer (g chlorpyrifos) and the spray tank condition. The reason for substituting total exposed skin area in place of indicator variables for shirt, pants, gloves, and hat during the first analysis was to conserve degrees of freedom in the analysis for interaction. A weakness of the model, which will be overlooked for the time being, is that it assumes each

Table 2.11: Safety measures used while application of chlorpyrifos in the field

Farmer ID	Protective clothing used	Gloves		Tank		Hat		Total exposure points
		Yes/No	Exposure points	Condition	Exposure points	Yes/No	Exposure points	
F1	3		0	Leaking	1	H	1	5
F2	4		0	Good	5	F(cloth),H	2	11
F3	4		0	Good	5	-	0	9
F4	1	Used	2	Good	5	-	0	6
F5	3		0	Average	3	-	0	6
F6	3		0	Average	3	-	0	6
F7	1		0	Average	3	-	0	4
F8	1	Damaged	1	Leaking	1	H	1	4
F9	4	Used	2	Good	5	H	1	12
F10	2		0	Leaking	1	H	1	4
F11	2		0	Good	5	H	1	8
F12	1		0	Average	3	H	1	5
F13	3		0	Leaking	1	-	0	4
F14	3		0	Average	3	-	0	6
F15	1		0	Leaking	1	-	0	2
F16	2	Used	2	Average	3	H	1	6
F17	4		0	Good	5	H	1	10
F18	3		0	Leaking	1	-	0	4
F19	1		0	Average	3	-	0	4

1= Short sleeved shirt and short pans
2= Long sleeved shirt and short pans
3= Short sleeved shirt and long pans
4= Long sleeved shirt and long pans
H= A hat was used F= Face cover was used

area unit of exposed skin, regardless of location, will have a uniform degree of association with internal dose.

The second analysis, which would be conducted if no interactions were observed between spray tank or total exposed skin area and the amount of active ingredient, would be to explore an additive model computing internal dose associated with particular clothing worn by farmers (long-sleeved or short-sleeved shirt, long pants or short pants, hat, and gloves) after accounting for the amount active ingredient used and tank condition (assuming the latter two are significant factors).

Table 2.12: Analysis of Variance (ANOVA) results for interaction model: *Internal dose ~ a.i. + TANK + skin + a.i.:skin + a.i.:TANK*

Parameter	Degrees of Freedom	Sum of Squares	Mean Square	F Value	2-sided p-value (F) for terms added sequentially
a.i.	1	49.8659	49.8560	66.4731	0.0000047
TANK	2	16.5651	8.2825	11.3731	0.00210
Skin	1	4.1353	4.1353	5.6783	0.0363
a.i.:TANK	2	3.6300	1.8150	2.4923	0.128 (0.231)*
a.i.: skin	1	0.6171	0.6171	0.8474	0.377 (0.144)*
Residuals	13	8.0108	0.7283		

*p-value when a.i.:skin is added before a.i.:tank

RESULTS OF ANOVA:

The results in Table 2.12 indicate there is no evidence of interaction between the amount of active ingredient applied by the farmer and the condition of the spray tank, nor is interaction observed between exposed skin area and the amount of active ingredient applied. The results of a linear regression of the significant terms in the above model are listed in Table 2.13.

Table 2.13: Linear regression of model: *Internal dose ~ a.i. + TANK* + skin,* R^2=0.852

Internal Dose – (μg Chlorpyrifos/kg body weight) = β_0 + β_1·*a.i. (g)* +β_2·LEAKY_TANK +β_3·AVERAGE_TANK + β_4·*Skin*) d.f. = 14 (degrees of freedom), qt (0.975, 14) = 2.145

Parameter	**Coefficient value (β)**	**Std. Error (β)**	**95% u.c.l. (β)**	**95% l.c.l. (β)**	**p value (β) (2-sided)**
Intercept	-1.923	1.380	1.037	-4.883	0.185
a.i.	0.091	0.013	0.119	0.063	5.7×10^{-6}
AVERAGE_TANK	0.439	0.288	1.061	-0.183	0.150
LEAKY_TANK	0.502	0.151	0.828	0.176	0.005
.Skin	3.4×10^{-4}	1.6×10^{-4}	6.7×10^{-4}	2.1×10^{-6}	0.047

u.c.l.-upper confidence limit
l.c.l.-lower confidence limit

Internal Dose - (μg Chlorpyrifos/kg body weight) - computed from measured TCP levels in urine (μg TCP /g creatinine in urine)

a.i. - (g) Mass of active ingredient applied by farmer

LEAKY_TANK - indicator for leaking spray tank (1= leaky, 0=good or average)

AVERAGE_TANK - indicator for a spray tank rated as average (1=average, 0=good or leaky)

Skin (cm^2) - exposed skin area of the farmer during pesticide application.

*The categorical variable, *TANK*, consists of the indicator variables *LEAKY_TANK* and *AVERAGE_TANK*.

qt - t multiplier for 95 % confidence interval

SUMMARY OF STATISTICAL FINDINGS

Internal dose vs. active ingredient applied: There is overwhelming statistical evidence that the internal dose of chlorpyrifos increase with amount of active ingredient (a.i.) applied ($p< 6 \times 10^{-6}$; d.f.=14). An increase of 91 ng for the mean internal dose is associated with each additional gram of chlorpyrifos applied by the farmer (95% confidence interval is 63 ng/kg to 119 ng/kg increase per gram of active ingredient applied). The scope of inference of this model includes the 19 farmers studied and an application amount of chlorpyrifos between 36.3g and 90.6g.

Internal dose vs. spray tank condition: There is strong evidence (p=0.005) that an increase in internal dose is associated with the use of a leaky spray tank. A 502 ng chlorpyrifos/kg body weight increase in the mean dose is associated with farmers who used a leaky spray tank over farmers who used a spray tank in good condition (95% confidence interval is 151 ng/kg to 828 ng/kg). The model does not indicate a difference in internal dose between farmers who used tanks in either good or average condition (p=0.150). Farmers who used spray tanks rated as "average" were likely to have a mean increase in internal dose of 439 ng/kg body weight over farmers who used spray tanks rated in good condition; 95% confidence range 288 ng/kg *increase* to 183 ng/kg *decrease* in internal dose.

Internal dose vs. exposed skin area: An increase in internal dose is associated with increased exposed skin surface area (p=0.047). An internal dose increase of 0.339ng chlorpyrifos/kg body weight is associated with each additional square centimeter of exposed skin surface area (95% confidence interval 0.156 to 0.677ng chlorpyrifos/kg body weight). According to Table 2.10, the farmers had exposed skin areas ranging from 3485 cm^2 to 8055 cm^2 with an average of 6060 cm^2. This corresponds to an increase in internal dose

between 1.18 to 2.73 μg chlorpyrifos/kg body weight with an average of 2.05 μg/kg associated with exposed skin area.

Since no interaction was observed between any variables, a second analysis (an additive model) was conducted to explore the degree to which internal dose could be associated with the protective garments worn by the farmer (see Figure 2.4 and Table 2.8). A full model was constructed using categorical variables for tank condition (good, average, and leaking), whether the farmer wore a short-sleeved shirt or long-sleeved shirt; short pants or long pants. Numerical variables in the model included the amount of active ingredient, a.i. (g), applied by the farmer and the number of gloves worn by the farmer.

The full model was then subject to a stepwise regression to determine which parameters were significant. Table 2.14 lists the Analysis of Variance (ANOVA) results of the selected model: *Internal Dose ~ a.i. + LONG_SHIRT + LONG_PANTS + TANK.*

Table 2.14: ANOVA results for model selected from step-wise regression procedure

Parameter	d.f.	Sum of squares	Mean square	F Value	2-sided p-value (F) for terms added sequentially
a.i.	1	49.9865	49.9865	90.6307	3.17×10^{-7}
TANK*	2	16.5651	8.2825	15.0534	0.000413
SHIRT	1	5.8497	5.8497	10.6317	0.006200
PANTS	1	3.3908	3.3908	6.1627	0.027482
Residuals	13	7.1527	0.5502		

d.f.- degrees of freedom

*The categorical variable, *TANK,* consists of the indicator variables *LEAKY_TANK* and *AVERAGE_TANK.*

Full Model: (Upper case variables correspond to factor, i.e. 1 or 0; lower case are numerical)
Internal Dose ~ a.i. + LONG_SHIRT + LONG_PANTS + LEAKY_TANK + AVERAGE_TANK + hat + gloves

Table: 2.15: Results of a linear regression of the stepwise regression from the full model: Internal Dose (μg chlorpyrifos/kg body weight) ~ a.i. + LEAKY_TANK + AVERAGE_TANK + LONG_SHIRT + LONG_PANTS,
$R^2 = 0.914$

Internal Dose – (μg chlorpyrifos/kg body weight) = β_0 + β_1·*a.i. (g)* +β_2·LEAKY_TANK +β_3·AVERAGE_TANK + β_4·LONG_SHIRT + β_5· LONG_PANTS , n = 19 (number of farmers) d.f. = 13 (degrees of freedom), qt (0.975, 13) = 2.160

Parameter	**Coefficient value (β)**	**Std. error (β)**	**95% u.c.l. (β)**	**95% l.c.l. (β)**	**p value (β) (2-sided)**
Intercept	-0.094	0.6232	1.2521	-1.4401	0.8823
a.i.	0.092	0.0101	0.1138	0.0702	5.47×10^{-7}
LEAKY_TANK	0.420	0.1226	0.6848	0.1552	0.00452
AVERAGE_ TANK	-0.173	0.3054	-0.4866	-0.8327	0.58145
LONG_SHIRT	-0.857	0.2485	-0.3202	-1.3938	0.00453
LONG_PANTS	0.442	0.1784	0.8273	0.0567	0.02748

u.c.l. - upper confidence limit

l.c.l. - lower confidence limit

Internal Dose - (μg Chlorpyrifos/kg body weight) - computed from measured TCP levels in urine (μg TCP /g creatinine in urine)

a.i.- (g) Mass of active ingredient applied by farmer

LONG_SHIRT - indicator for whether farmer wore a long or short-sleeved shirt (1= long-sleeved, 0= short-sleeved)

LONG_PANTS - indicator for whether farmer wore long or short pants (1=long pants, 0= short pants)

LEAKY_TANK - indicator for leaking spray tank (1= leaky, 0=good or average)

AVERAGE_TANK - indicator for a spray tank rated as average (1=average, 0=good or leaky)

hat - numerical variable for the degree of facial protection used (0=no hat used, 1=hat used, 2=hat + mask used)

gloves - numerical variable for the number of gloves worn by the farmer during application, 0=no gloves, 1=one glove, and 2= two gloves worn.

SUMMARY OF STATISTICAL FINDINGS

Internal dose vs. active ingredient applied: There is overwhelming statistical evidence that computed internal dose levels of chlorpyrifos from measured levels of TCP in urine for the nineteen farmers are associated with amount of active ingredient (a.i.) applied ($p< 5 \times 10^{-7}$;d.f.=13). Similar results were found in the previous linear regression model. The mean internal dose level increases by 92ng Chlorpyrifos/kg body weight per gram of chlorpyrifos applied by the farmer (95% confidence interval is 70 μg/kg to 114 μg/kg increase per gram of active ingredient applied). These results are in agreement with the previous analysis in Table 2.13.

Internal dose vs. spray tank condition: Both full model and liner regression model had strong evidence (p=0.005) that the use of a leaky spray tank corresponds to an internal dose increase of 420ng Chlorpyrifos/kg body weight, over farmers who used a spray tank rated in good condition (95% confidence interval is 155 ng/kg to 685 ng/kg). The model does not indicate a difference in internal dose between farmers who used spray tanks evaluated in either good or average condition (p=0.581). The *decrease* in dose associated with the use of a spray tank evaluated in average condition is 173 ng/kg body weight over farmers who used a spray tank in good condition; 95% confidence

range 581 ng/kg *increase* to 832 ng/kg *decrease* in internal dose. These results are also in agreement with the previous analysis in Table 2.13.

Internal dose vs. clothing: There is strong evidence that a decrease of internal dose is associated with the use of a long-sleeved shirt instead of a short-sleeved shirt (p=0.004). A mean *decrease* in internal dose of 857 ng Chlorpyrifos per kilogram bodyweight was observed for the farmers who wore a long-sleeved shirt over those who wore short-sleeved shirts; 95% confidence interval, 320ng/kg to 1,39 ng/kg decrease.

Since the parameters for GLOVES and HAT were eliminated in the stepwise regression, it is concluded that there is no statistical evidence that a change in additive internal dose for the farmers is associated with the use of gloves or a hat.

SCOPE OF INFERENCE

The scope of inference includes the 19 farmers having characteristics, using application methods and following safety measures listed in Tables 2.8 - 2.11. Table 2.16 summarizes the contribution of each factor towards the internal dose of a farmer representative of the average of the body weight, computed background internal dose, and amount of active ingredient applied. The average contribution of each factor is computed from the coefficient values in Table 2.15 multiplied by the average value of the factor.

Table 2.16: Additive contributions of each significant factor to computed Internal Dose (μg/kg) of the average farmer studied, using results in Table 2.15

Model: *Internal Dose~ Background + a.i. + Leaking Tank + Short Shirt + Long Pants*

Factor	Avg. contribution of factor to internal dose (ug/kg)	Cumulative internal dose (ug/kg)	% Total internal dose
Active ingredient applied (Avg. = 58.82 g)	5.41	5.67	76%
Leaking Spray tank	0.42	6.09	6%
Short-sleeved shirt	0.86	6.95	12%
Long Pants	0.44	7.39	6%
Total Internal Dose (μg/kg)	7.13		
Total Dose (μg) for Avg. body wt. (69.3 kg)	512.46		

Avg. =Average

DISCUSSION

Consistent TCP excretion patterns were observed in all 19 farmers. The average TCP excretion half-life of 31.3 days was consistent with a 30 day half-life reported, by Griffin for dermal exposure of alkylphosphates. With a 1:1 relation with diethyl thiophosphate and diethyl phosphate one might expect a comparable half life from the two metabolites. Griffin also reported a 15hr half-life from oral exposure to chlorpyrifos. One can conclude that the farmers exposure to chlorpyrifos is dermal rather than respiratory. Backpack sprayers would find to provide larger droplets rather than the fine aerosols that could enhance respiratory uptake. Structural applicators working in confined areas show only 26% of their exposure coming through the respiratory route.

The use of the EPA reference dose of 0.003 mg/kg/day is not as appropriate toxicological reference for this exposure scenario. The RfD is based on a sub-chronic study in human were exposed to 19 days (0.03 mg/kg/day) with plasma acetyl cholinesterase monitored as the response. While the later enzyme is sensitive to chlorpyrifos it is not a reliable indicator of adverse effect. Aslo farmers are experiencing a one time exposure. A better reference is the toxico-kinetic study by Griffin who did not observed a cholinesterase inhibition with dermal dose of 28.59 mg. The maximal exposure received by the farmer was 29.9 mg, which is almost same as dermal exposure study. On this basis, the farmers experience a minimal risk despite taking limited precautions.

There has been concern that farmers in tropical regions are particularly vulnerable because of the reluctance to use protective clothing under the hot and humid climatic conditions that are common in these regions. This study demonstrates that the farmers risk can be minimize by limiting the amount applied and the frequency of applications. It should also be noted that the

study deals with a worst-case scenario where the farmer is spray an overhead canopy.

With observations on 19 farmers and variation in the internal dose experienced a statistical analysis provided perspective on the effects of different variables influencing exposure. It is clear that the amount of compound applied is the over siding factor. However, the use of sound equipment and long-sleeved shirt can reduce exposure by 6-10%. The observation that wearing long pants actually increased exposure was surprising and would need to be confirmed. However, the farmer may not wash his legs after application when wearing long pants and it he were to continue to wear these pants his exposure could be prolonged.

CONCLUSION

Farmers applying chlorpyrifos showed a consistent excretion pattern of the metabolite, TCP, characteristic for this organophosphate. The excretion half-life ranged from 24.8 to 37.6hr with an average value 31.3hr. The cumulative TCP excreted over 120hr was used to calculate the internal dose of chlorpyrifos, which ranged from 0.0021 to 0.0084 mg/kg. It was assumed that major exposure route was skin, and a dose of 0.4 to 1.2 mg/kg was estimated, based on 1% dermal uptake. This dose was considered to give a marginal risk with hazard quotients range from 0.7 to 2.7 and margin of safety from 4 to 14. Statistical analysis established that the internal dose was determined, in large part, by the amount of chemical applied. In addition, it was demonstrated that faulty spray equipment and the amount of skin exposed also was associated with an increase in the internal dose. Analysis also indicated that wearing long pants could increase the internal dose, although the reason for this unexpected response is not clear. This study provides quantitative information for that program, which can be used to train farmers in the use of safer application practices.

CHAPTER 3

ANALYSIS OF DRINKING WATER AND HOUSE DUST FOR CHLORPYRIFOS AND 3,5,6-TRICHLORO-2-PYRIDINOL, COLLECTED FROM A FARMING COMMUNITY IN SRI LANKA

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ABSTRACT

Drinking water was collected from three wells and dust was collected from three houses located in a major vegetable growing area in Kandy district. Wells and houses were located adjacent to the cultivated land, some of which had been treated with chlorpyrifos. Water samples were analyzed for chlorpyrifos and the major metabolite, 3,5,6-trichloro-2-pyridinol (TCP). Floor wipes were analyzed for possible contamination by the parent compound. Chlorpyrifos in drinking water was below quantifiable level, but 9, 10, and 0.6 ng/mL of TCP were detected in well water samples. In the dust analysis, quantifiable peaks were found in the same window as chlorpyrifos, but the results were not confirmed on a second column. Recoveries of 94% and 86.8% of chlorpyrifos and TCP from water were achieved with detection limit of 13 ng/L, and 18ng/mL, respectively. Recovery of the parent compound from spiked dust was 72% with a detection limit of 167 ng/L.

Prevailing climatic conditions favor dissipation of chlorpyrifos from water and soil, limiting the risk of chlorpyrifos exposure from these sources.

INTRODUCTION

Chlorpyrifos is used widely on soil and crops to control insect pests on farm animals, to control ticks, and in houses to control cockroaches, fleas, and termites. The manufacturer voluntarily withdrew chlorpyrifos from most indoor and pet uses in 1997 (United Stated Department of Health and Human Services, 1997). Chlorpyrifos neither bio-accumulates nor persists in the environment for extended periods.

Soil level of chlorpyrifos depends mainly on the amount applied and the disposal of waste containers in soil. Much of the compound applied to foliage eventually reaches soil, either as parent compound or metabolite (Racke, 1993). Re-deposition of atmospheric chlorpyrifos (Racke, 1992) and spills during storage, transportation, mixing, or cleaning of spray equipment could also contribute to soil levels of chlorpyrifos. Environmental factors such as moisture, pH, and organic carbon can greatly influence the fate of chlorpyrifos in soil (Harmaker et al., 1972; Getzin, 1981a,b,; Chapman and Chapman, 1986). Chlorpyrifos undergoes hydrolysis and microbial degradation in soil. The rate of hydrolysis is pH and temperature dependant (Miller and Zepp, 1983). The half-life was shorter in natural soils than in sterile soils, which illustrates the role of microbes. Under laboratory conditions, chlorpyrifos degradation half-life varies from less than 10 days to greater than 120 days in different soils (Meikle and Hedlund, 1973; Davis and Kuhr, 1976). The primary hydrolysis product, 3,5,6-trichloro-2-pyridonil (TCP), and secondary metabolite, 3,5,6-trichloro-2-methoxy pyridine will mineralize to CO_2 (Bidlack, 1979; Chapman and Harris, 1980; Getzin, 1981a; Racke et al., 1988). The fate of chlorpyrifos in the environment is illustrated in Figure 1.5.

Racke et al. (1990) evaluated the potential for enhanced microbial degradation of chlorpyrifos in different soils under laboratory conditions. Repeated chlorpyrifos applications to soils did not alter the rate of degradation or product distribution. The reported half-life of chlorpyrifos was 4-9 days in

soils with a pH greater than 8 and repeated applications of insecticides failed to control target pests. They concluded that chlorpyrifos is not susceptible to enhanced microbial degradation and repeated applications did not have any increased effect on the efficacy or persistence due to higher rate of metabolism. This was explained by the fact that the hydrolysis step was not due to microbial activity. Accumulation or mineralization of TCP was unrelated to the rate of chlorpyrifos hydrolysis, which was a function of microbial activity.

Chlorpyrifos has an average sorption coefficient (Koc) of 8500 mL/g (Recke, 1993) and will tend to sorb in soil; hence, there is less potential to leach from soil in solution. While chlorpyrifos has been considered immobile in soil (Racke et al., 1993), TCP is moderately mobile due to its greater water solubility. Chlorpyrifos may degrade by photo-induced reactions on the soil surface. Laboratory studies using UV light (254nm from mercury lamp) demonstrated that photochemical processes such as hydrolysis, dechlorination, and oxidation take place simultaneously (Walia et al., 1988). Dehalogenated and oxidized products undergo further photolysis to form chloropyridinol and O,O-deethyl phosphorothioic acid. In the same study, the levels of these metabolites was also decreased with time, suggesting mineralization taking place under UV-photo-irradiation conditions.

In water, partition into colloids, evaporation, hydrolysis, and photosensitized oxidation are likely to be the major pathways of dissipation. Distilled water with pH 1 or 12.9 had a half-life of 89 or 0.01 days, respectively, at 25 ^{0}C (Macalady and Wolfe, 1983). In a similar study, Freed et al. (1979) reported a half-life of 120 and 53 day at pH of 6.1 and 7.4 at 20 ^{0}C. The activation energy for the hydrolysis of chlorpyrifos at pH 7.4 is 14 kcal/mol, indicating its sensitivity to temperature change. Hydrolysis can be catalyzed by copper ions (Blanchet and St. George, 1982). Henry's law constant (H) for chlorpyrifos is 6.6×10^{-6} atm-m3/mol (Downey, 1987), and the vapor pressure is 1.9×10^{-5} mmHg at 25 ^{0}C (Racke, 1993). Compounds with H of less than 10^{-5}

atm-m3/mol may volatilize slowly from water (Lyman et al., 1990), but it will also partition into available airborne particulate (Eisenreich et al., 1981).

In an exposure assessment study performed for residential environments in Arizona, Sydney et al. (1999) reported that chlorpyrifos level in indoor air was 3.3μg/m^3. The range of chlorpyrifos levels found in floor wipes and windowsill wipes was 0.004-48.5 and 0.07-16100 ng/m^2, respectively.

A farm worker might be exposed to chlorpyrifos during mixing and application or by consuming contaminated foods or water. Little children walking or crawling on contaminated house floors are also susceptible to exposure. US Environmental Protection Agency (US EPA) recommends a 24hr waiting period prior to reentering a chlorpyrifos-treated field. Chlorpyrifos has been found in at least seven current and former EPA National Priority List (NPL) hazardous waste sites (HazDat, 1996) and, thus, the potential for chlorpyrifos exposure is significant.

OBJECTIVES

A prior study evaluated occupational exposure to chlorpyrifos by analyzing urinary metabolites. The overall analysis of risk should consider other possible routes of exposure. Since wells used for drinking water were located in/or adjacent to treated areas and dust could be blown or tracked into homes. Therefore, the objective of this study is to analyze drinking water and house dust for the parent compound and the major metabolite to assess potential background exposure to chlorpyrifos. The study was carried out at the same site (Kandy district of Sri Lanka) as the prior experiment (Figure 3.1).

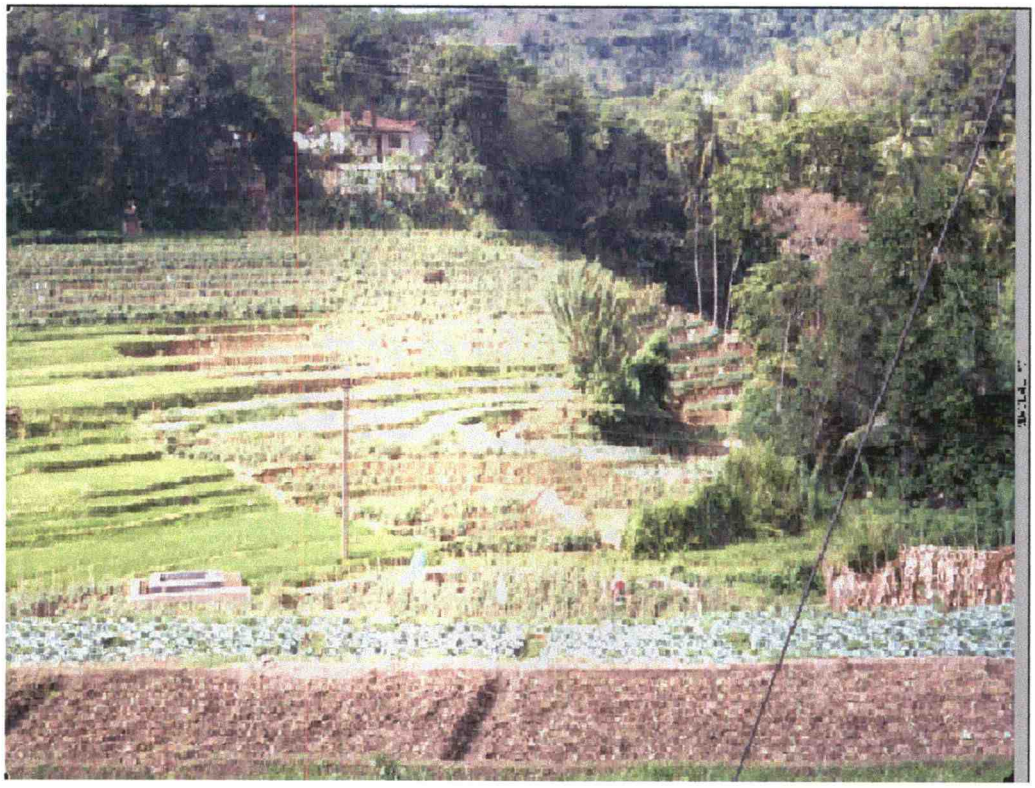

Figure 3.1: Location of drinking water wells and houses in the selected agricultural site

METHOD

Solvents and standards: Acetone, methylene chloride, ethyl acetate, hexane, and methanol were from Fisher Scientific, New Jersey. Chlorpyrifos and 3,5,6-trichloro-2-pyridinol standards are kind donations from Dow Agroscience, Indianapolis. All glassware was baked for 10hr at 350 ^{0}C before use.

Sample collection: Water samples were collected (about 3 weeks after the season) from three drinking water wells located in the selected farming community in Kandy district, Sri Lanka. This area uses contour landscaping, and crops are grown on contour plots. Houses are located at higher elevations around the field, and water wells are in the center, close to the lowest point, where the water level is near the ground water table (Figure 3.2). Samples were collected from three drinking water wells (three 1L samples from each well) at the end of the season. Bottles made with polyethylene terepthalate manufactured by CISCO Specialty Packaging (Pvt.) Ltd., Pannipitiya, Sri Lanka were used. Water pH was adjusted to 2 as specified in the EPA method 525.2 (USEPA, 1994) to minimize both hydrolysis and microbial activity. Samples were refrigerated until used.

Dust was collected from three houses located in the same farming community. Two out of three houses were facing the cultivated field and the other house was about 100m away. Dust was collected using cotton balls from an area of 2ft^2 from 3 locations of the house, i.e., front porch, living room and kitchen (two replicates from each locations). Samples were stored in five mL glass vials and kept refrigerated until analyzed. Both water and dust were brought to the Food Safety and Environmental Stewardship Program Laboratory at Oregon State University for extraction and analysis.

Figure 3.2: Location of a drinking water well and sampling

ANALYSIS OF DRINKING WATER FOR CHLORPYRIFOS

A sub-sample of 200 mL was extracted with hexane (2 x 10 mL aliquots) in separatory funnels. Extracts were combined and the volume adjusted to 20 mL using weight and density of hexane at room temperature. An aliquot of 10 mL from the total volume was concentrated to 1 mL prior to analysis. A varian gas chromatograph system fitted with an electron capture detector was used and 2 μL injections were made using the auto-sampler.

Recovery of chlorpyrifos from spiked water: Deionized water (4 L) was spiked using a chlorpyrifos standard in acetone. Fortified water was diluted to give different final concentrations using deionized water. Spiked levels and percent recovery is given in Table 3.1. pH of distilled water was adjusted to 2 before the experiment as it is done for sample water.

Table 3-1: Recovery of chlorpyrifos from spiked water

Concentration of chlorpyrifos in water (ppb)	Chlorpyrifos in 200 mL water (ng)	Chlorpyrifos detected (ng)	Percent recovery
0.37	74	71.8	99.7
1.49	298	269.4	90.4
2.97	594	470.4	79.2
7.43	1486	1610.8	108.4

Average recovery is 94.4%

Instrument detection limits for water analysis for chlorpyrifos

Instrument noise for solvent = 1 mm (Figure 3.3)
Signal to Noise ratio = 5
Final volume for the method = 1 mL
Peak height of the 12.5 ng/mL standard = 99 mm

Calculation

Minimum measurable peak height = 1 mm x 5
= 5 mm

Minimum measurable concentration based on 5 mm peak
= (12.5/99) x 5 ng/mL
= 0.631 ng/mL

Instrument detection limit = 0.7 ng/mL

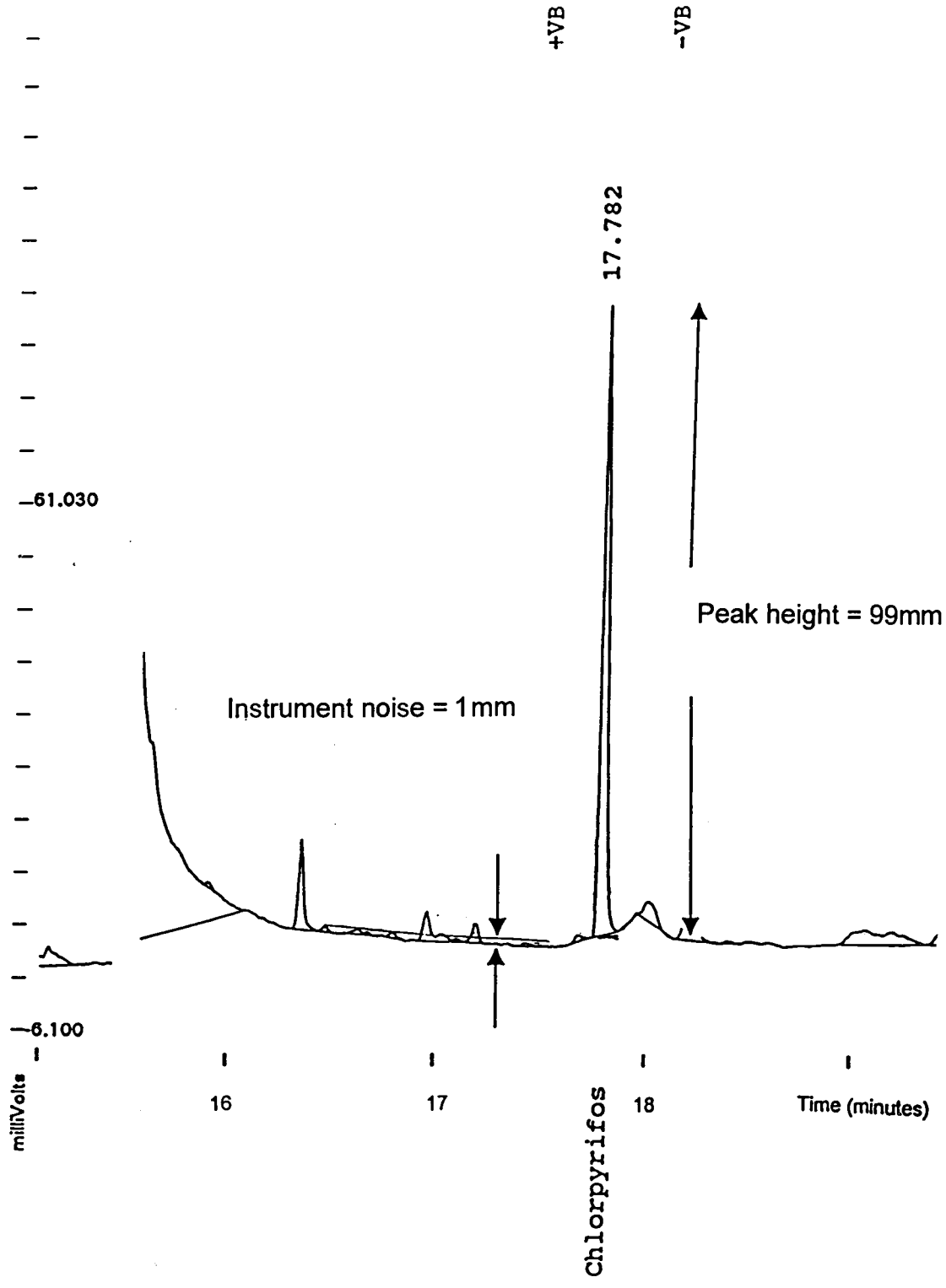

Figure 3.3: Chromatogram of 12.5ng/mL chlorpyrifos standard used to determine instrument detection limit (attenuation 50)

Method detection limits for water analysis for chlorpyrifos

Method noise = 2 mm (Figure 3.4)
Signal to Noise ratio = 5
Initial volume for the method = 200 mL
Final volume for the method = 1 mL
Peak height of the 12.5 ng/mL standard = 99 mm

Calculation

Minimum measurable peak height = 2 mm x 5
= 10 mm

Minimum measurable concentration based on 10 mm peak
= (12.5 ng/mL /99 mm) x10 mm
= 1.262 ng/mL

Since final volume is 1 mL , final concentration = 1.262 ng/mL

Initial volume is 200 mL, therefore,

Minimum amount of chlorpyrifos in 200 mL water = 1.262 ng

Minimum concentration in water = 1.262/200 ng/mL
= 6.13 ng/L in water

Method detection limit = 7 ng/L in water

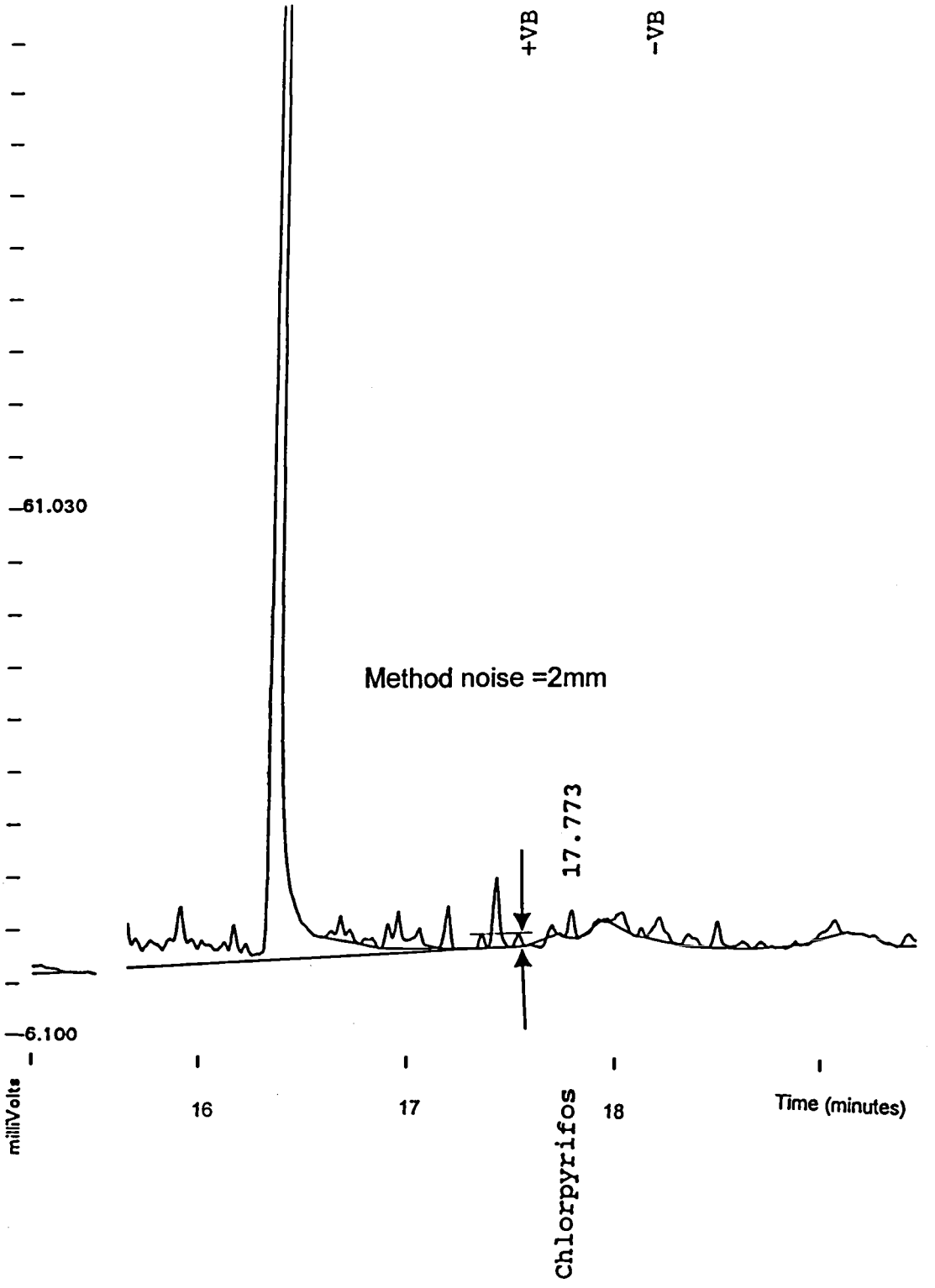

Figure 3.4: Chromatogram of blank water analysis used to determine method detection limit (attenuation 50)

HOUSE DUST ANALYSIS FOR CHLORPYRIFOS

The method used was a modified version of Sydney et al. (1999) and EPA method 525.2. Cotton balls were extracted with 2 x 5 mL aliquots of acetone and the extracts combined. Cotton balls were placed in tubes with caps (diameter of ~1 cm), 5 mL of acetone was added (acetone level is above cotton) and sonicated for 30 min (Figure 3.5-A). Sonicated tubes were inverted into larger tubes (diameter of ~2 cm) and centrifuged for 5 min at 10,000rpm (Figure 3.5-B) allowing only acetone to drain into the large tube. Cotton was pressed down in the small tube using a spatula to avoid moving down during centrifuge. A small glass stopper was placed on the bottom of the large tube to make enough space for acetone to drain. Acetone extracts were transferred to volumetric tubes using disposable pipettes (Figure 3.5-C and D). Small tubes were re-centrifuged if necessary to recover at least 4 mL from each extract. About 9 mL of acetone was recovered from cotton from both extracts. The acetone extracts (8 mL) were diluted with 200 mL of water (pH adjusted to 2 using 6N H_2SO_4) keeping the same ratio (of 4 mL of acetone diluted in 100 mL of water) described by Sydney et al. (1999). 1 mL (5% of total volume of water) of methanol was also added to each acetone water mixture.

Sample cleanup was performed using 1g of octadecyl (^{18}C) in solid phase extraction (SPE) columns (manufactured by Baker Bond). SPE columns were conditioned by eluting each cartridge with a 5 mL aliquot of ethyl acetate followed by a 5 mL aliquot of methylene chloride. The cartridge was allowed to drain dry after each flush. Then each cartridge was eluted with a 10 mL aliquot of methanol, not allowing the methanol to elute below the top of the cartridge packing. Ten mL aliquots of de-ionized water were added to the cartridge, but before the water level droped below the top edge of the packing, sample water was added to the reservoir (EPA 525.2). Columns were dried under vacuum for 40 min (Sydney et al., 1999) to make sure no more water

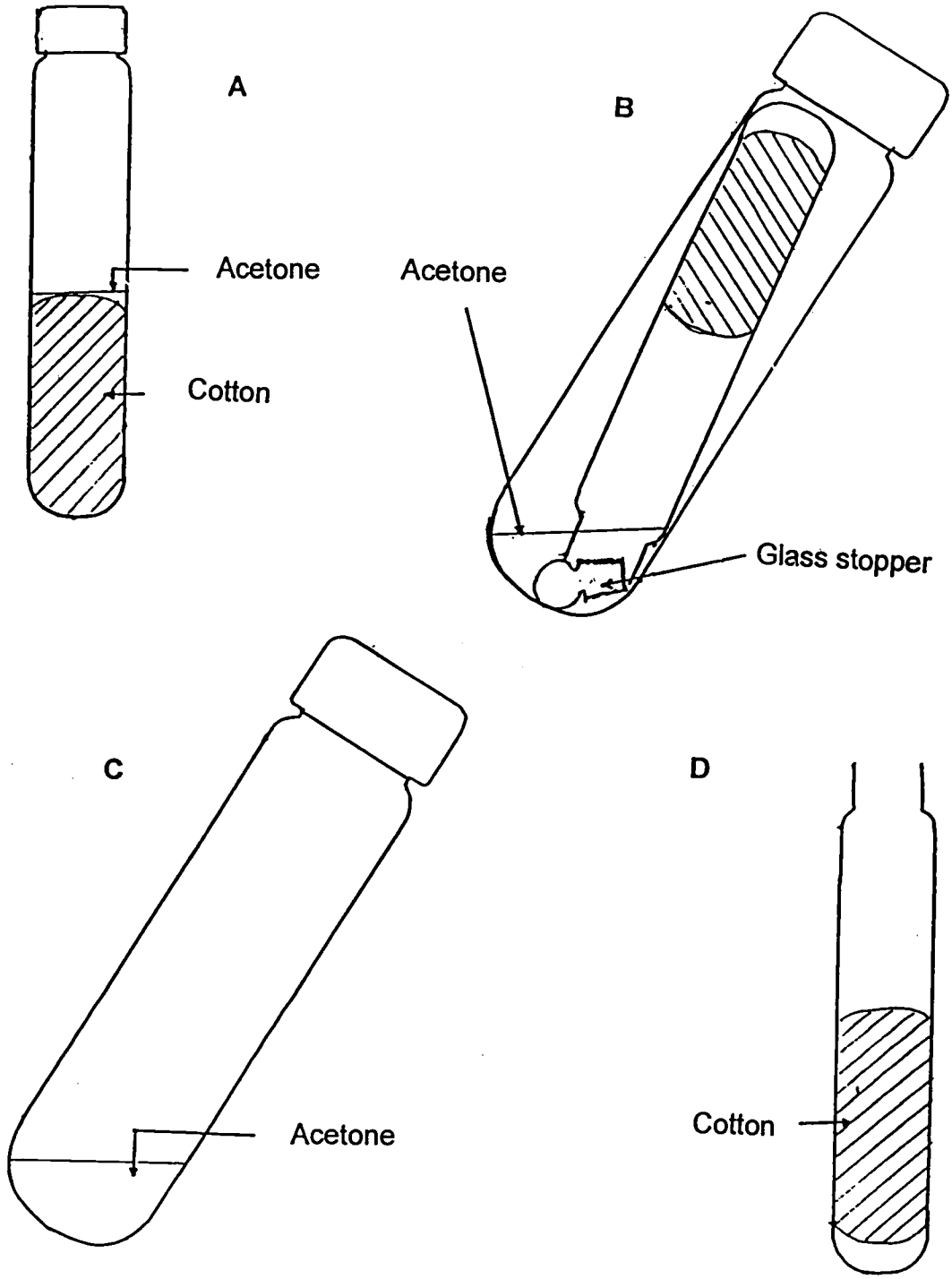

Figure 3.5: Extraction of cotton with acetone

was present. The elution apparatus (15 mL glass tube) was set under the column to collect eluants. Five mL aliquots of ethyl acetate was transferred after making sure container was free of water and rinsed inside. The solvent was used to elute columns. The same steps were followed with a 5 mL aliquot of methylene chloride. As a final rinse, 2 mL of methylene chloride was passed through the columns. Eluants were collected, combined and concentrated to 1 mL before analysis.

Fortified dust in cotton: Cotton balls were used to collect dust from the front porch of none agricultural area for the recovery studies. Four levels of chlorpyrifos-spiked dust (in cotton) were extracted with samples to validate the method. Spike levels and percent recovery are listed in Table 3.2.

Table 3.2: Recovery of chlorpyrifos from spiked cotton balls (with and without dust)

Chlorpyrifos in cotton ball (ng)	Chlorpyrifos in 200 mL water (ng)	Chlorpyrifos recovered (ng)	Percent recovery
250.0	181.8	134.5	74
100.0	72.7	51.6	71
25.0	18.2	12.9	71
0	0	0	-

Average recovery 72%

* only cotton (no dust)

Instrument detection limits for dust analysis for chlorpyrifos

Instrument noise for solvent = 2 mm (Figure 3.6)
Signal to noise ratio = 5
Final volume for the method = 1 mL
Peak height of the 25 ng/mL standard = 104 mm

Calculation

Minimum measurable peak height = 2 mm x 5
= 10 mm

Minimum measurable concentration based on 10 mm peak
= (25 ng/mL/ 104 mm) x 10 mm

Instrument detection limit = 3 ng/mL

Method detection limits for dust analysis for chlorpyrifos

Method noise = 10 mm (Figure 3.7)
Signal to Noise ratio = 5
Final volume for the method = 1 mL
Peak height of the 25 ng/mL standard = 104 mm

Calculation

Minimum measurable peak height = 10 mm x 5
= 50 mm

Minimum measurable concentration based on 50 mm peak
= (25 ng/mL /104 mm) x 50 mm

Since final volume is 1 mL, final concentration = 12.019 ng/mL

Initial floor area is $2ft^2$, minimum amount in 2 ft^2 area is = 12.019 ng

Method detection limit = 2 ng/ft^2

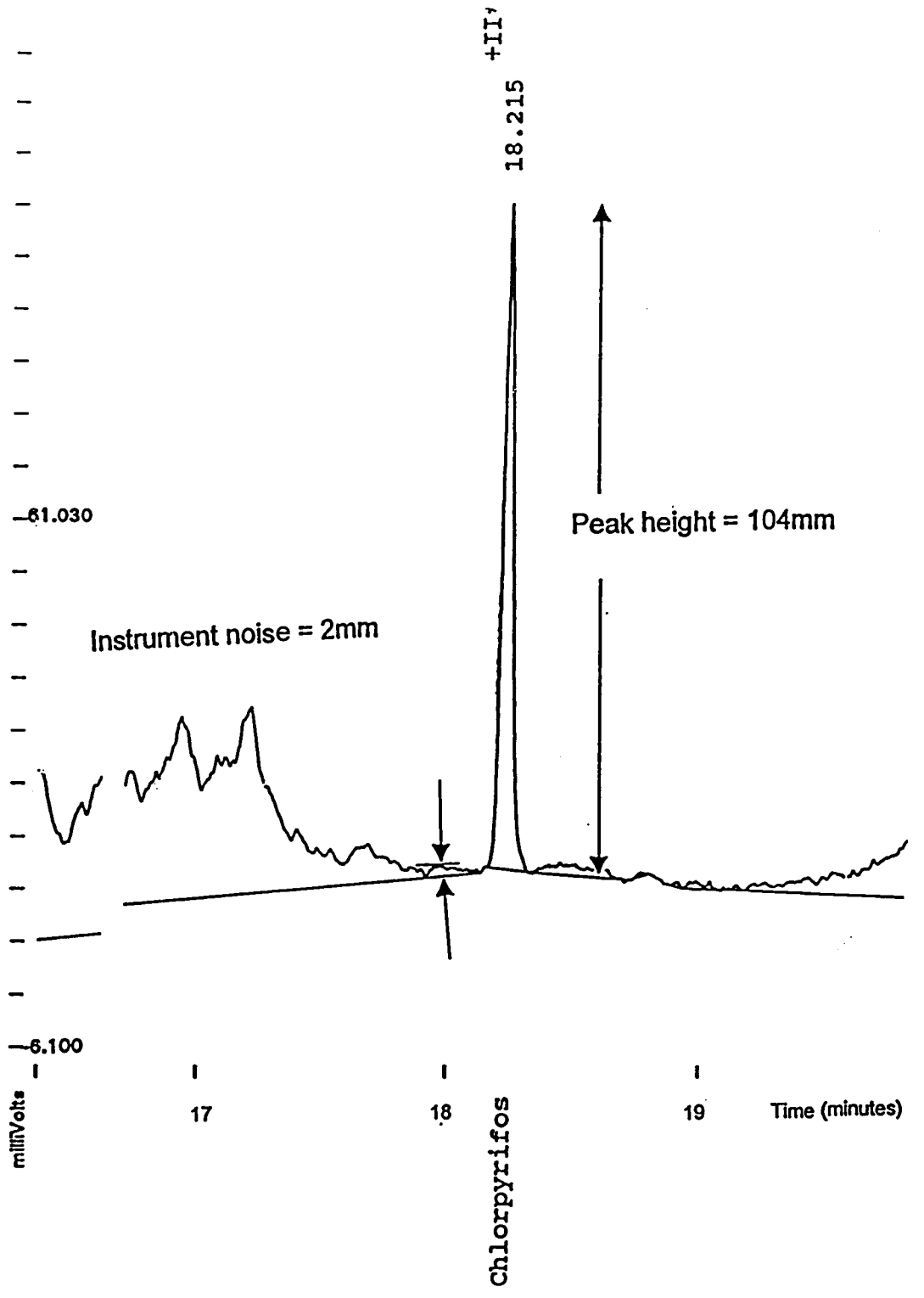

Figure 3.6: Chromatogram of 25 ng/mL chlorpyrifos standard used to determine instrument detection limit in dust analysis for chlorpyrifos (attenuation 50)

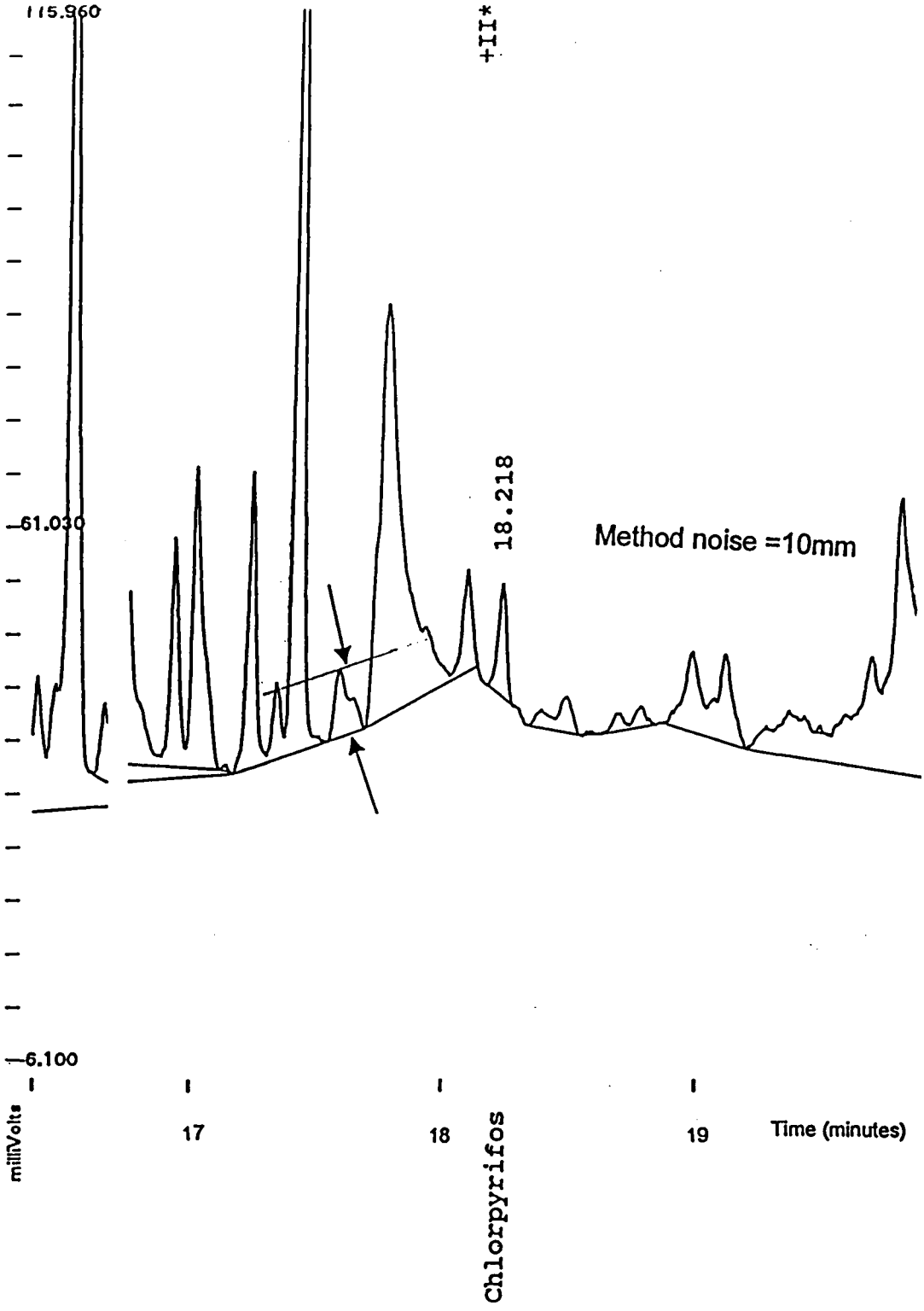

Figure 3.7: Chromatogram of blank dust extract used to determine method detection limit in dust analysis for chlorpyrifos (attenuation 50)

Gas chromatography conditions: A varian workstation 3600 gas chromatographic system with two electron capture detectors was used for the analysis of chlorpyrifos in water and dust. Two columns DB1 (non-polar) and DB-XLB (polar) were attached to the same injection port, and the responses on both columns were compared to confirm the presence of chlorpyrifos. Both columns were 30 m in length, 0.25 mm (internal diameter), and 0.25μm film thickness manufactured by J&W scientific, USA. Ultra-pure helium and 99.9% pure nitrogen gas were used as carrier and makeup, respectively. Two micro liter samples were injected using a split-less injection system of the Varian auto sampler 8200. Temperature at injector port, and detector were 50 and 350 ^{0}C, respectively. The 3-step column temperature program was 100, 190, and 250^{0} C for 6, 2.5, and 3 min, respectively. The rates of temperature increases were 100 to 190 at 20 ^{0}C/min and 190 to 250 at 20 ^{0}C/min.

Standard curve: Standard curve (Figure 3.8) was generated using six different concentrations and repeated with each sample set.

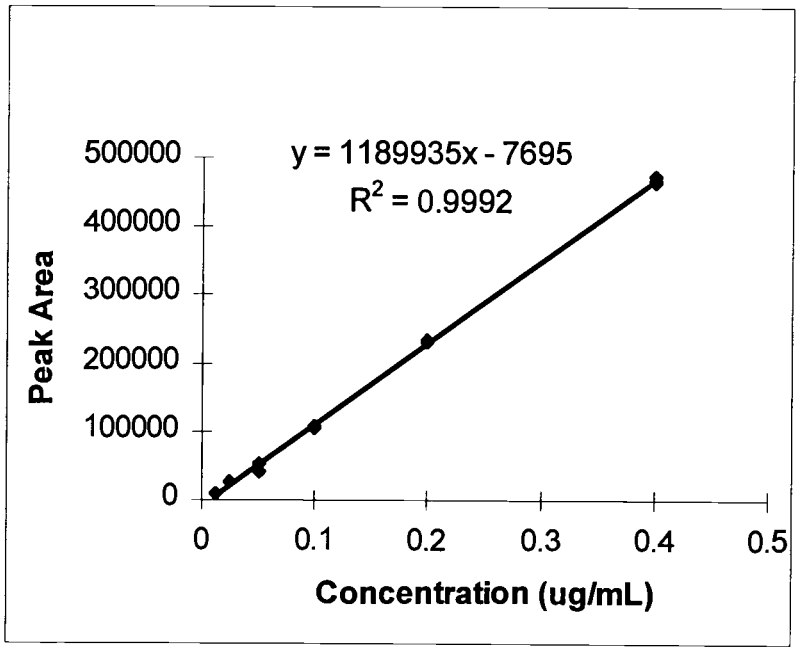

Figure 3.8: A standard curve generated for chlorpyrifos

ANALYSIS OF TCP FROM WATER

Water (15 mL) was transferred to glass tubes (25 mL) and acidified with two drops of 6 M sulfuric acid. Then, 0.4 g of sodium chloride was added to each tube before extracting twice with 5 mL of benzene. Benzene layers were removed using disposable pipettes, and the extracts combined, and concentrated to 1 mL. 5 μL of N,O-bis(trimethylsilyl)acetamide (BSA) was added just before injecting 2 μL in to the varian gas chromatograph system.

Recovery study: A volume of 4 L of water was fortified with a TCP standard in acetone, and different dilutions with deionized water were used for recovery studies. Final concentrations of TCP in spiked water and percent recoveries are given in Table 3.3.

Table 3.3: Recovery of TCP from spiked water

Concentration of TCP in water (ppb)	TCP in 15mL water (ng)	TCP recovered (ng)	Percent recovery
16.4	246.1	184.3	75
9.8	147.7	131.6	89
6.6	98.4	93.6	95
3.3	49.2	55.8	88

Average recovery 86.8%

Instrument detection limits for water analysis for TCP

Instrument noise for solvent = 3 mm (Figure 3.9)
Signal to noise ratio = 5

Final volume for the method = 1 mL
Peak height of the 10 ng/mL standard = 100 mm

Calculation

Minimum measurable peak height = 3 mm x 5
= 15 mm

Minimum measurable concentration based on 15 mm peak
= (10 ng/mL/100 mm) x 15 mm
= 1.5 ng/mL

Instrument detection limit = 2 ng/mL

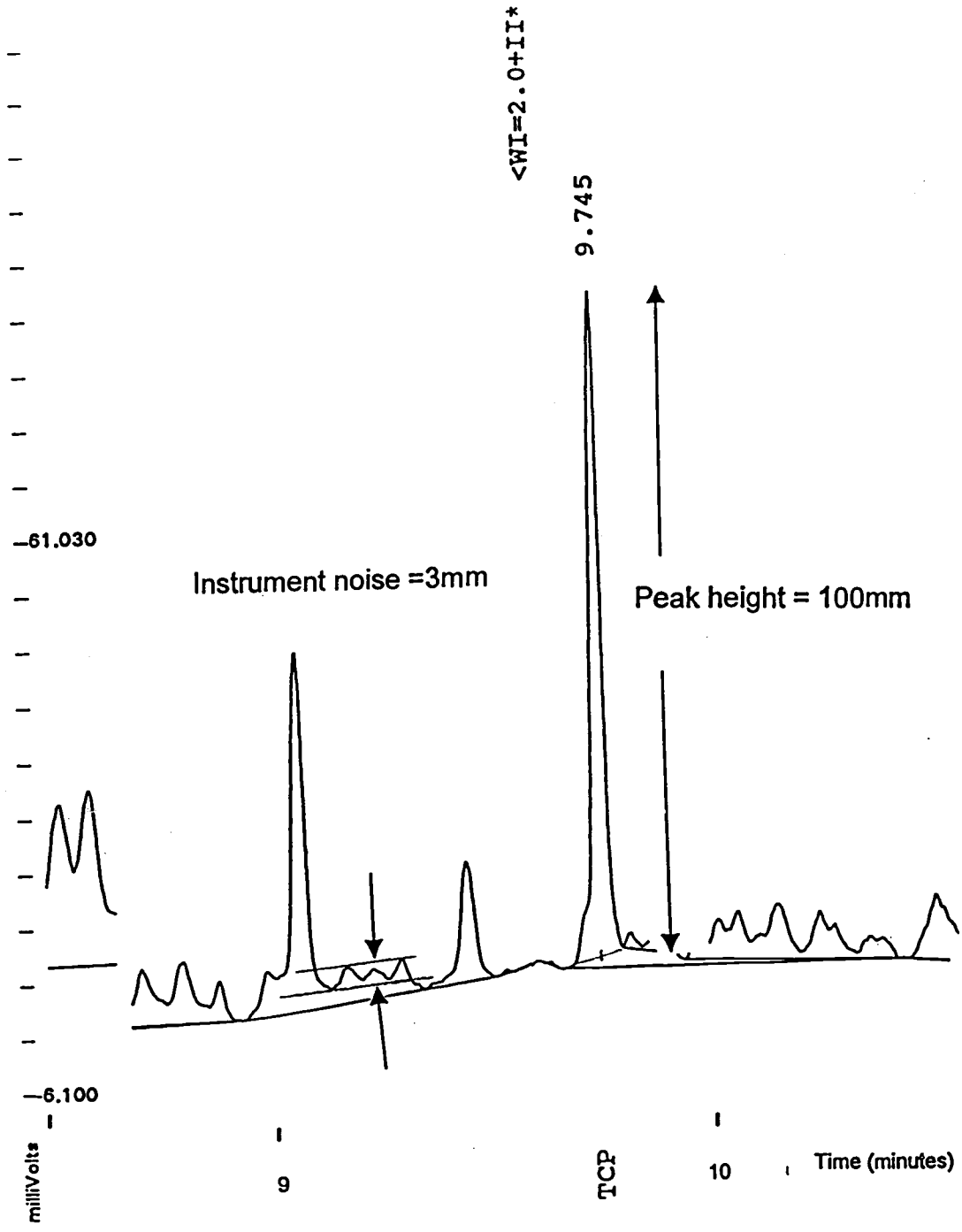

Figure 3.9: Chromatogram of 10ng/mL TCP standard used to determine instrument detection limit in water analysis for TCP (attenuation 50)

Method detection limits for water analysis for TCP

Method noise	= 5 mm (Figure 3.10)
Signal to noise ration	= 5
Initial volume for the method	= 15 mL
Final volume for the method	= 1 mL
Peak height of the 10 ng/mL standard	= 100 mm

Calculation

Minimum measurable peak height = 5 mm x 5

= 25 mm

Minimum measurable concentration based on 25 mm peak

= (10 ng/mL /100 mm) x 25 mm

= 2.5 ng/mL

Since final volume is 1 mL , final concentration = 2.5 ng/mL

Initial volume is 15 mL; therefore,

Minimum amount of TCP in 15 mL water = 2.5 ng

Minimum concentration in water = 2.5/15 ng/mL

Method detection limit = 167 ng/L in water

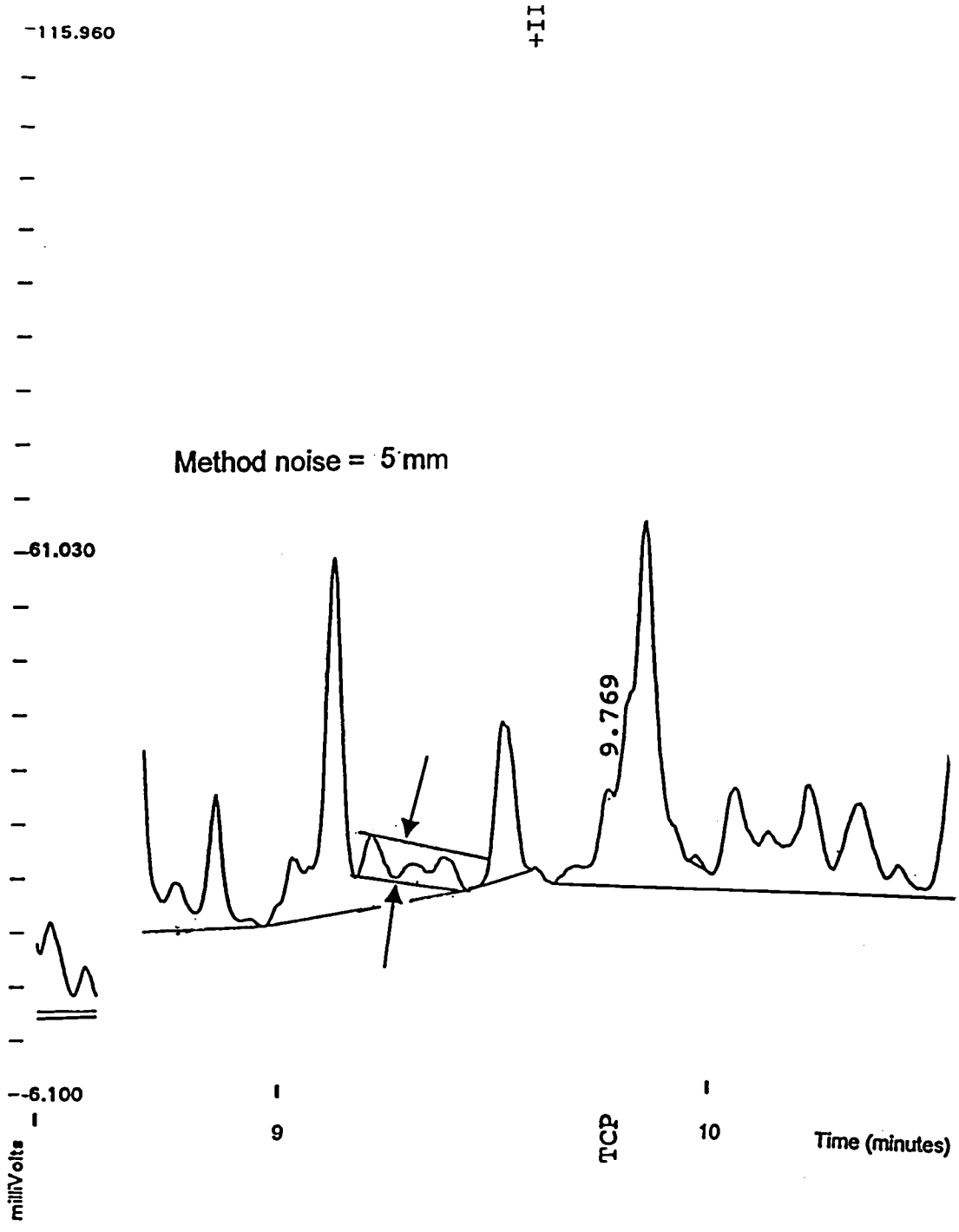

Figure 3.10: Chromatogram of blank water extract used to determine method detection limit in water analysis for TCP (attenuation 50)

Gas chromatography analysis: The same Varian work station 3600 gas chromatographic system was used. Temperature at injector port and detector were 50 and 350 ^{0}C, respectively. The 3-step column temperature program was 100, 190, and 300 ^{0}C for 6, 2.5, and 2 min, respectively. The rates of temperature increases were 100 to 190 at 20 ^{0}C/min and 190 to 300 at 25 ^{0}C/min.

Standard curve: Standard curve was generated using four points and the curve was reproduced with each set of samples. A standard curve used for calculations is given in Figure 3. 11.

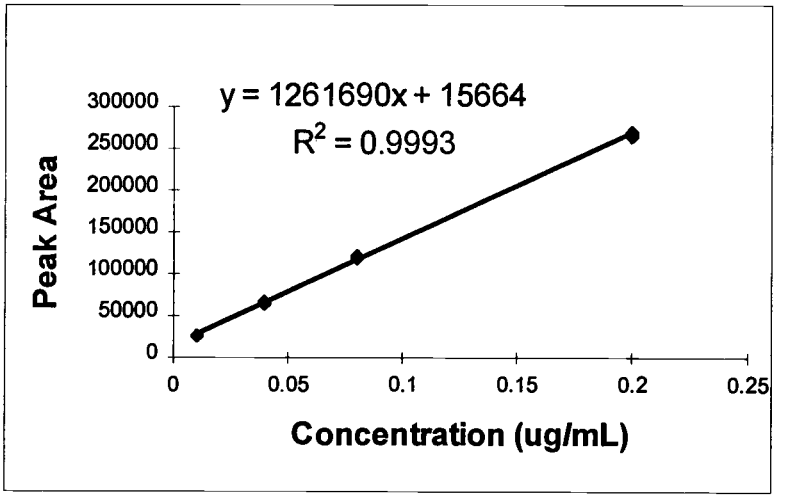

Figure 3.11: Standard curve for TCP

RESULTS

Drinking water analysis for chlorpyrifos: The method detection limit for chlorpyrifos in water was 7 ng/L and the mean recovery of chlorpyrifos from spiked water was 94.4% (Table 3.1). Retention time for chlorpyrifos on DB-1 column was 17.782 min for the method. Spiked water samples were analyzed along with drinking water samples. Chromatograms from the DB-1 column for sample water, blank water analysis, and chlorpyrifos standard are given in Figure 3.12(a-e). None of the drinking water samples contained quantifiable amounts of chlorpyrifos.

Figure 3.12: Chromatograms for drinking water analysis for chlorpyrifos

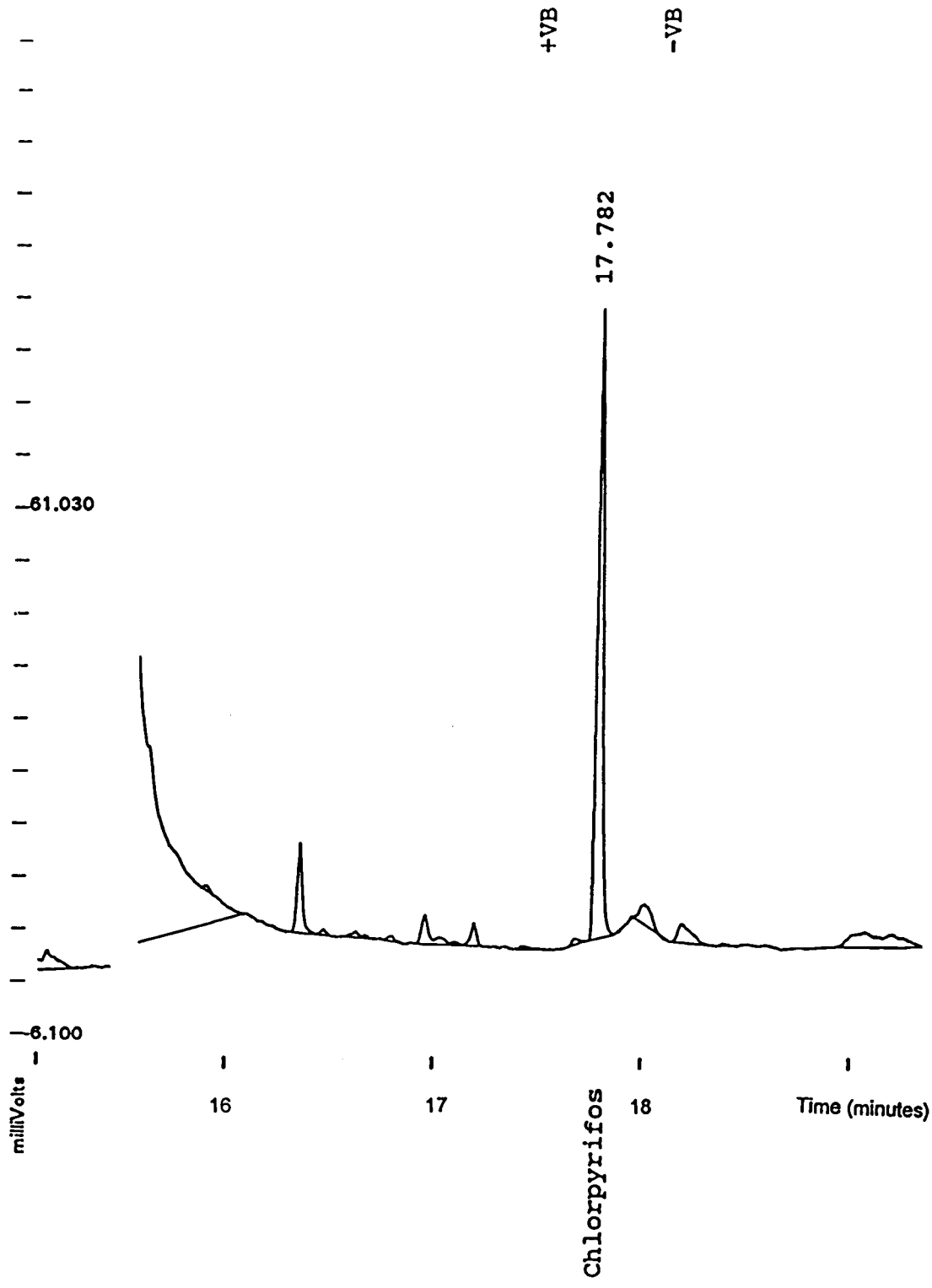

Figure 3.12a: Chromatogram of 12.5ng/mL chlorpyrifos standard (attenuation 50)

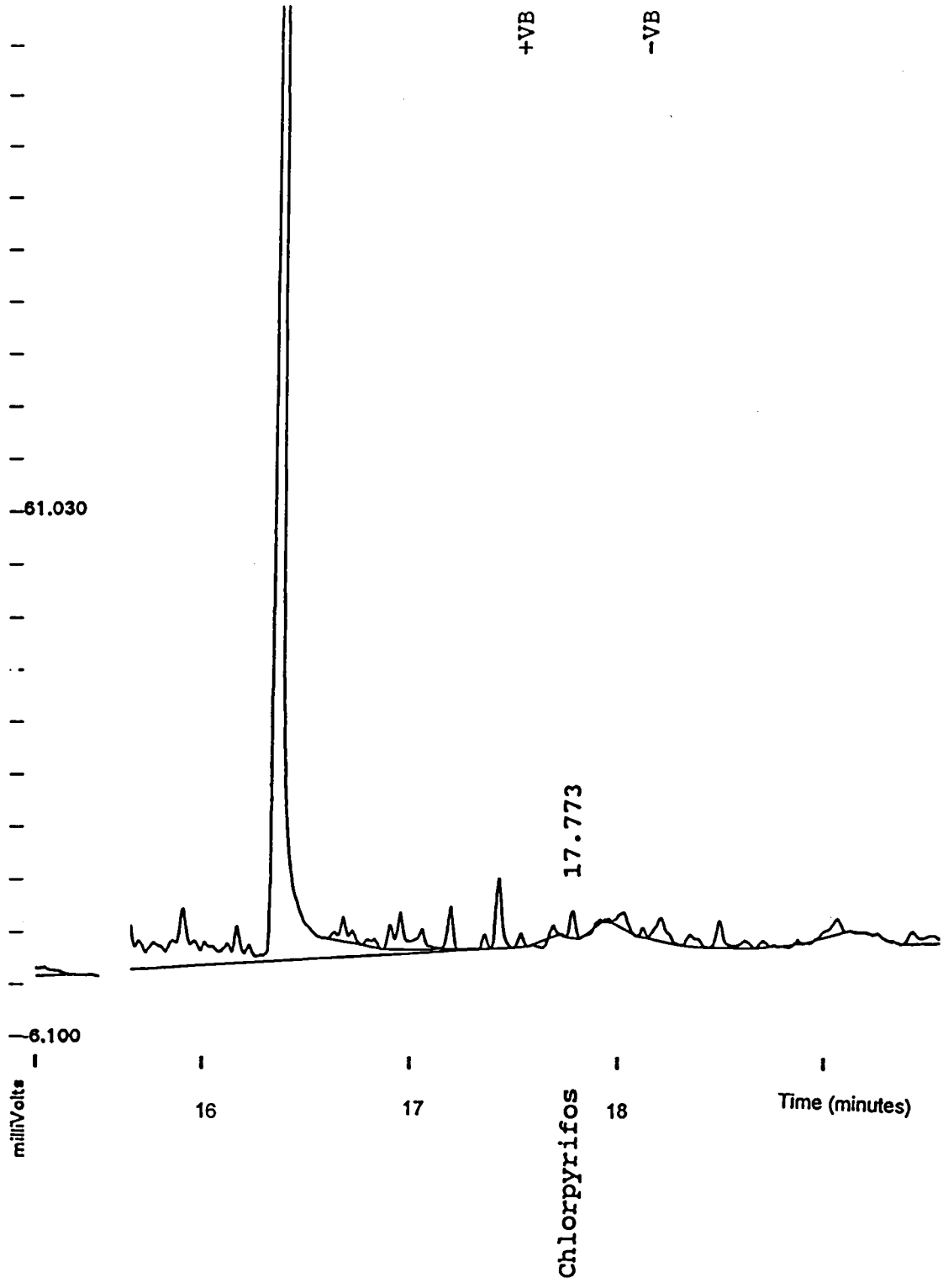

Figure 3.12b: Chromatogram of blank water analysis for background level of chlorpyrifos (attenuation 50)

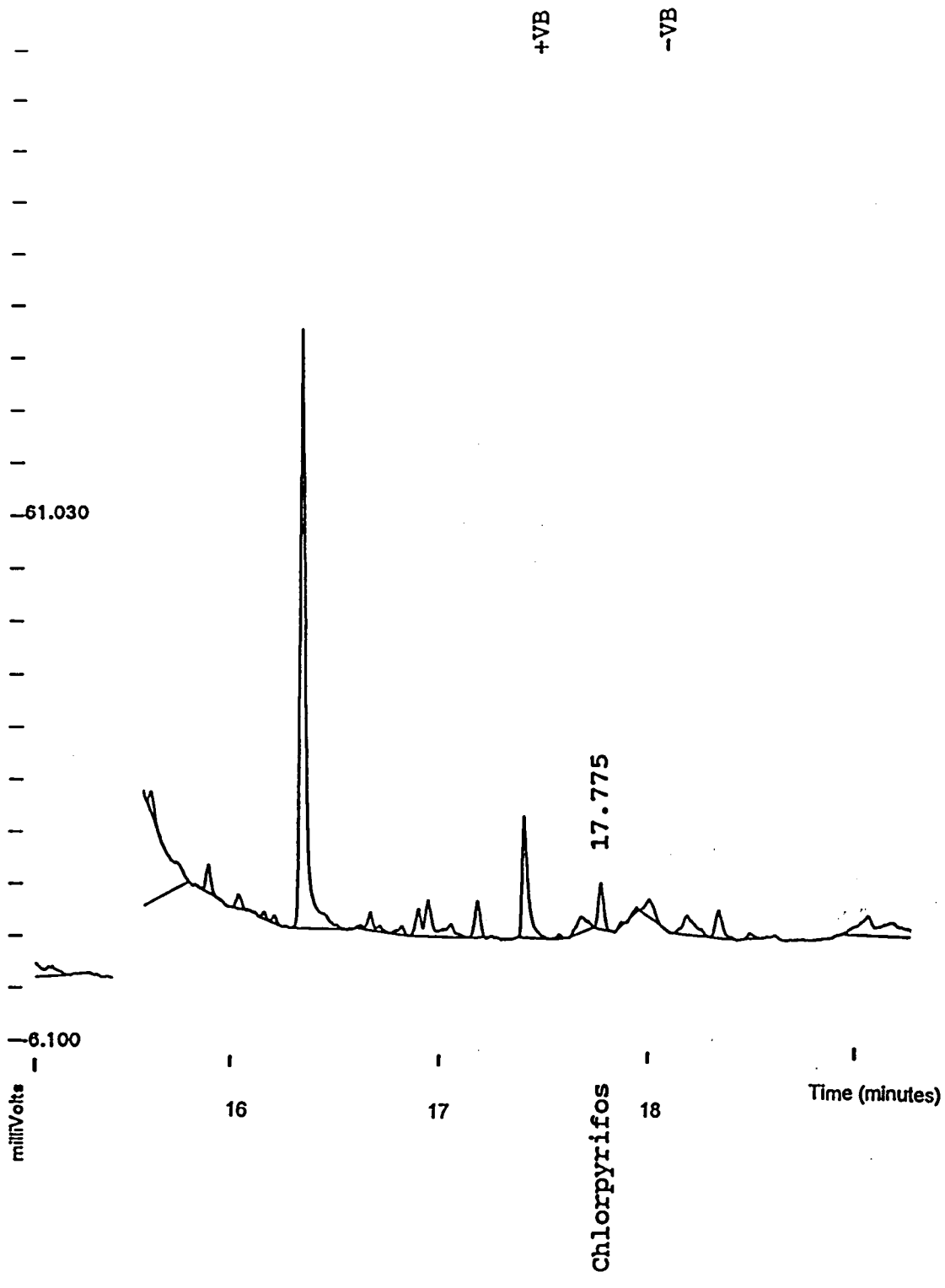

Figure 3.12c: Chromatogram of water (from well 1) analysis for chlorpyrifos (attenuation 50)

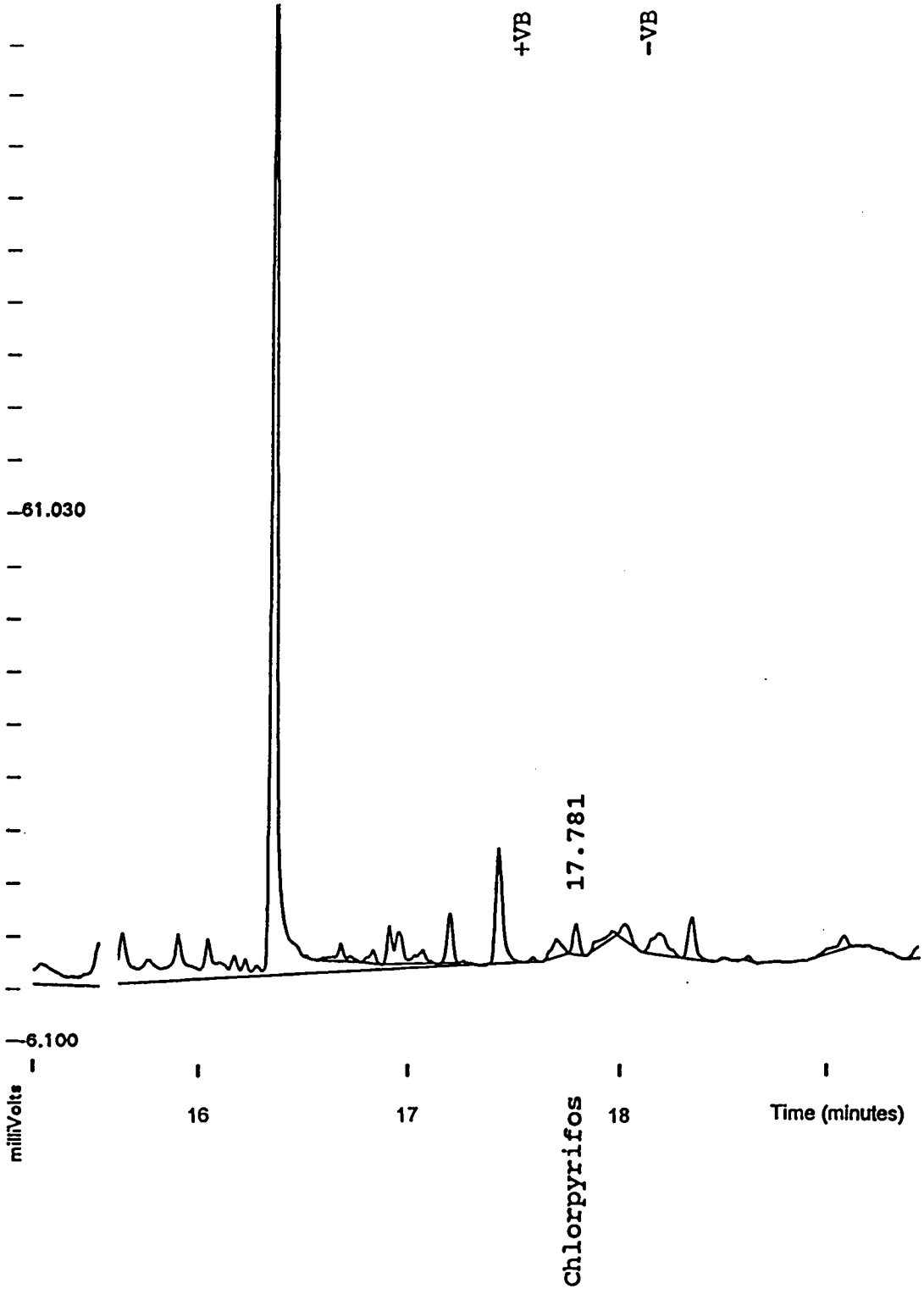

Figure 3.12d: Chromatogram of water (from well 2) analysis for chlorpyrifos (attenuation 50)

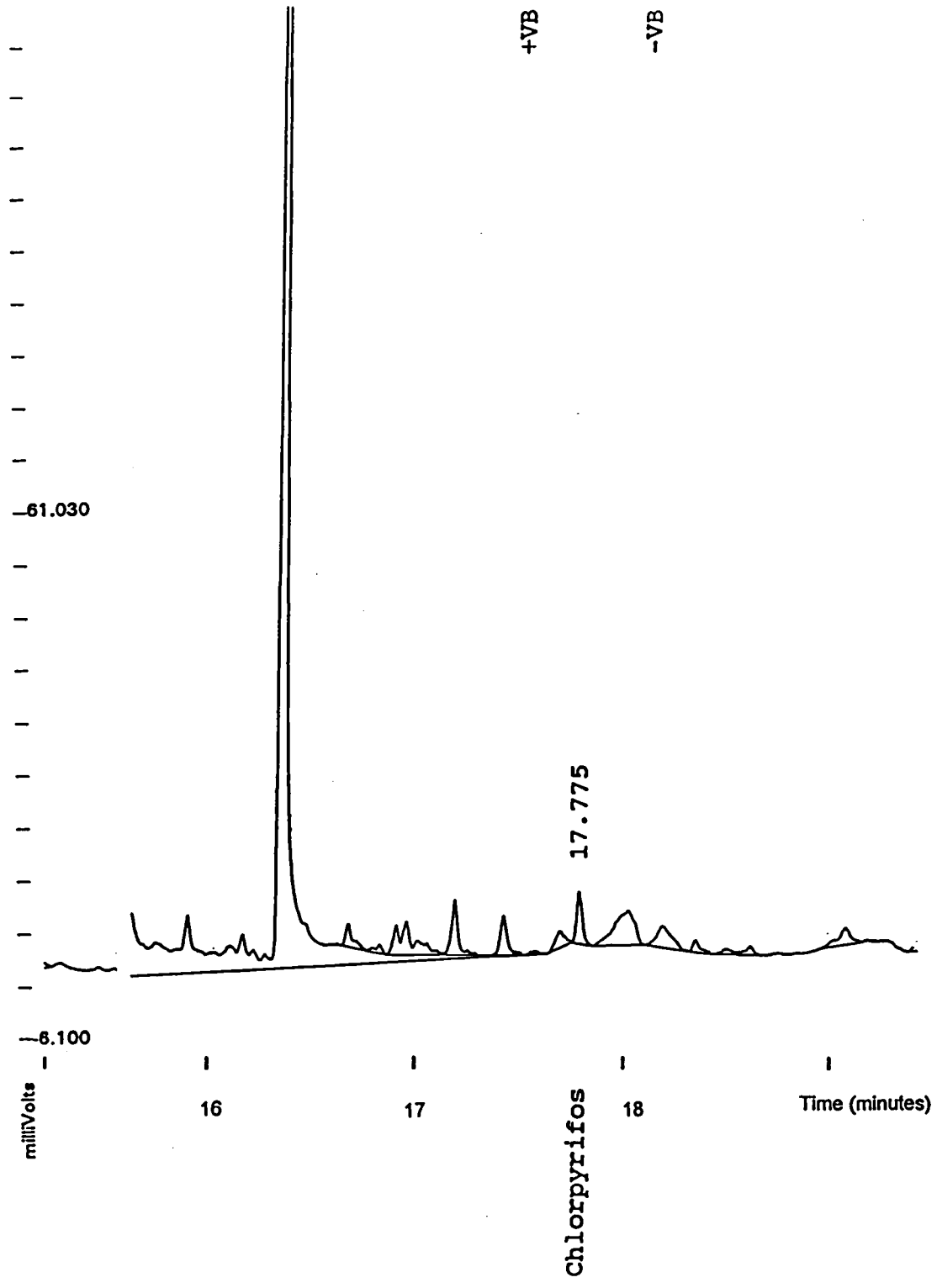

Figure 3.12e: Chromatogram of water (from well 3) analysis for chlorpyrifos (attenuation 50)

Dust analysis for chlorpyrifos:. In the dust analysis, method detection limit was 2 ng/ft^2, and the mean recovery from spiked cotton was 72%. Retention time for chlorpyrifos was 18.215 min on a DB-1 column, and 15.227 min for a DB-XLB column. The electron capture detector (ECD) is a very sensitive but has limited specificity. This detector responds to any electrophilic compound and peaks were found in all dust samples. Dust samples gave peaks with retention time of chlorpyrifos on a DB-XLB column in samples from all locations of each house. The same samples (same injection) did not show comparable peaks on the DB-1 column with respect to magnitude and retention time. In Figure 3.13b (DB-XLB column), the peak at 15.227 min (dust for front porch) is lower than the 0.05 μg/mL standard. From the same injection on the DB-1 column (Figure 3.13a) the peak is higher than the 0.05 μg/mL standard and shows a retention time different from chlorpyrifos. When Figures 3.15a and 13.15b are considered, front porch samples do not have the same retention time as chlorpyrifos in DB-1 column, but on DB-XLB the same sample had a peak similar to chlorpyrifos. The magnitudes of those two peaks were also different. Double peaks were found (one peak having retention time close to chlorpyrifos) on DB-1 column for dust sample from living room (Figure 3.13a). The same sample gave a peak identical with chlorpyrifos standard in DB-XLB (Figure 3.13b) suggesting the possibility of a small amount of chlorpyrifos in that sample. Similarly, in Figure 3.15a a sample from the kitchen (DB-1 column) had a double peak (a little peak with retention time close to chlorpyrifos), but the same injection gave a larger peak identical to chlorpyrifos in BD-XLB column (Figure 3.15b). There was no consistent response among sampling sites in the same house. A comparison among houses indicated that samples from kitchens (K1,K2 and K3) had suspect peaks in both columns. Analytical results do not provide any convincing evidence for the presence of chlorpyrifos in house dust. It was not possible to use more selective but less sensitive detector to establish whether the response was due to the phosphorus containing compounds.

Figure 3.13: Comparison of dust analysis results from house 1

1F - Dust from front porch
1L - Dust from living room
1K - Dust from kitchen

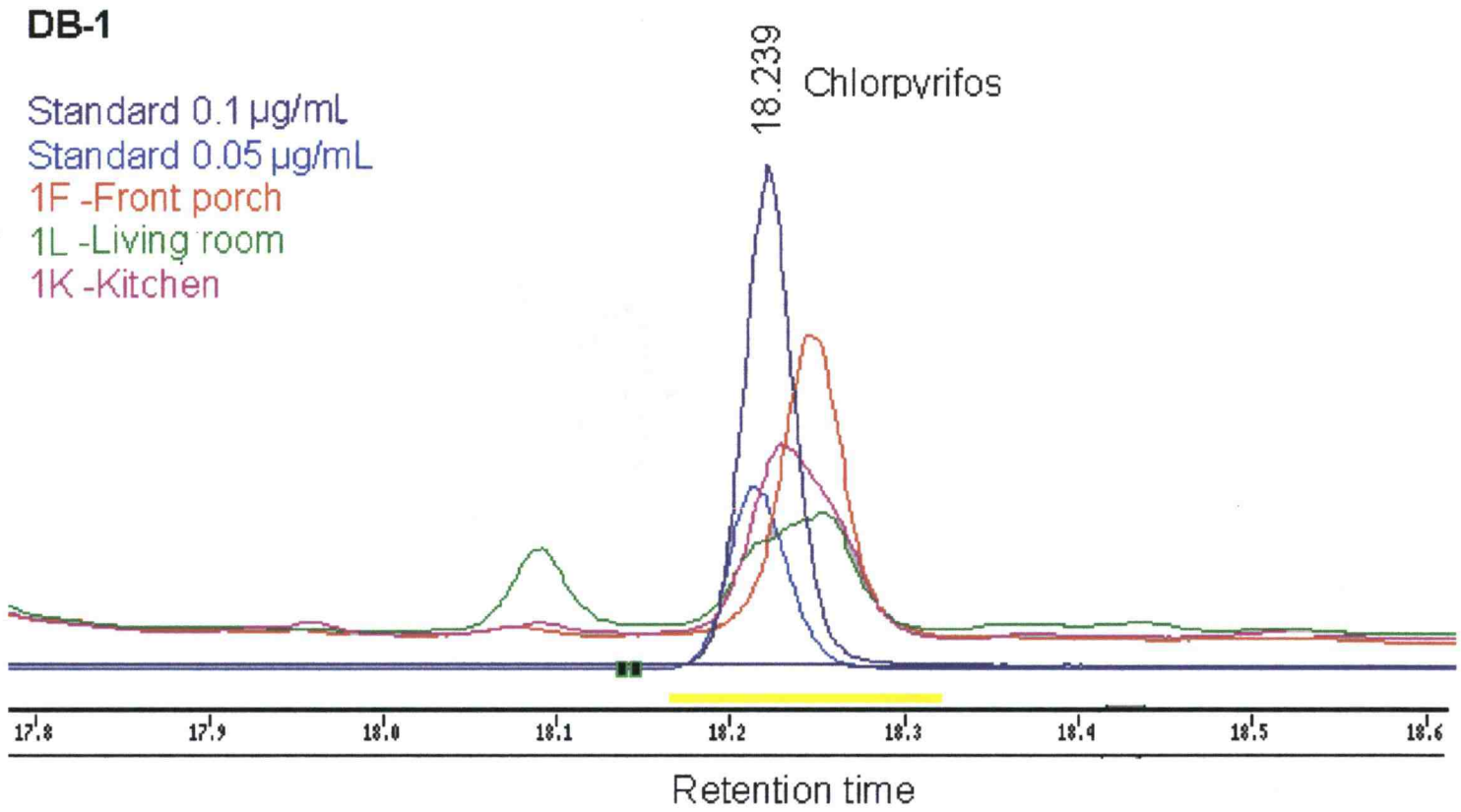

Figure 3.13a: Comparison of dust analysis results from house 1 on DB-1 column

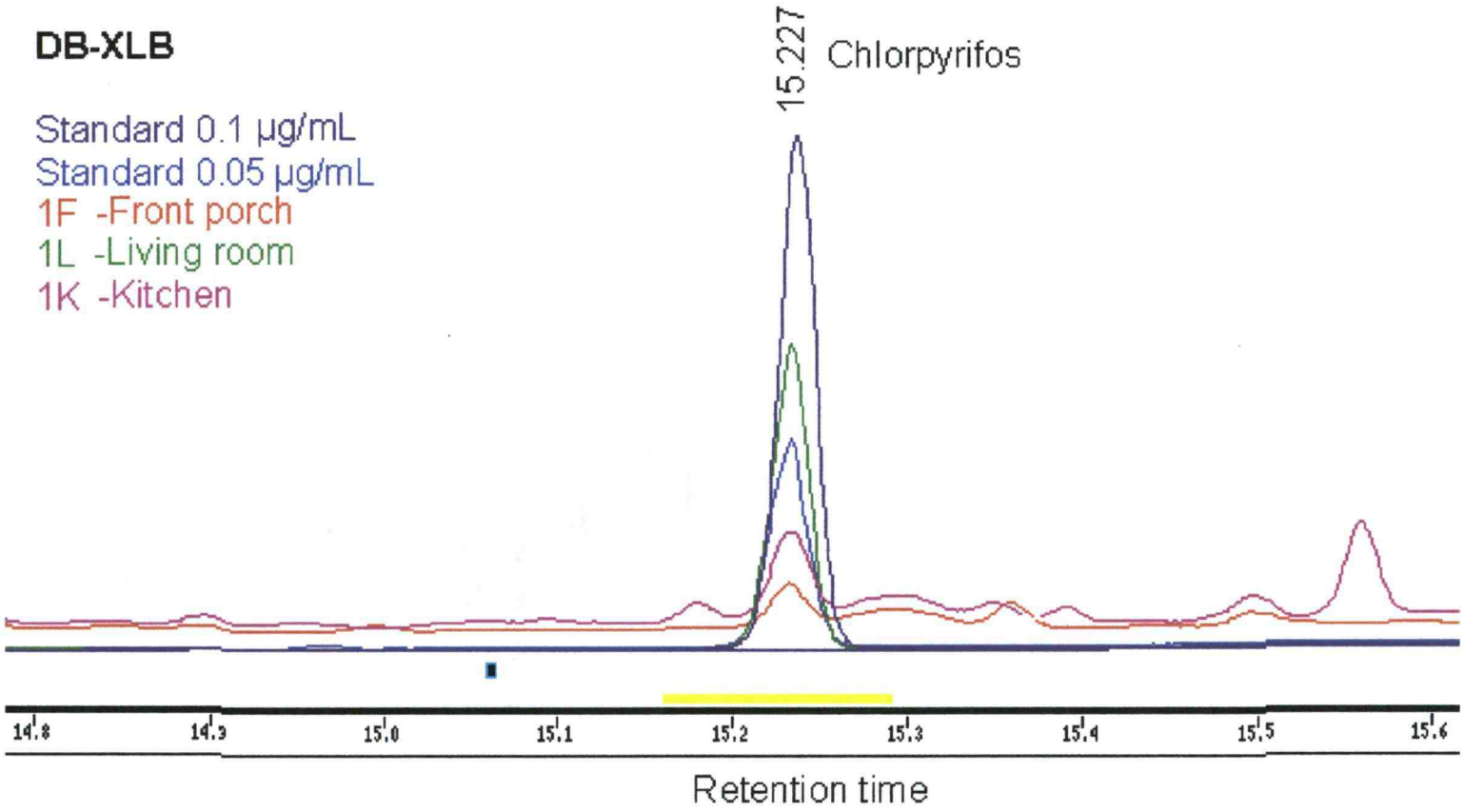

Figure 3.13b: Comparison of dust analysis results from house 1 on DB-XLB column

Figure 3.14: Comparison of dust analysis results from house 2

2F - Dust from front porch
2L - Dust from living room
2K - Dust from kitchen

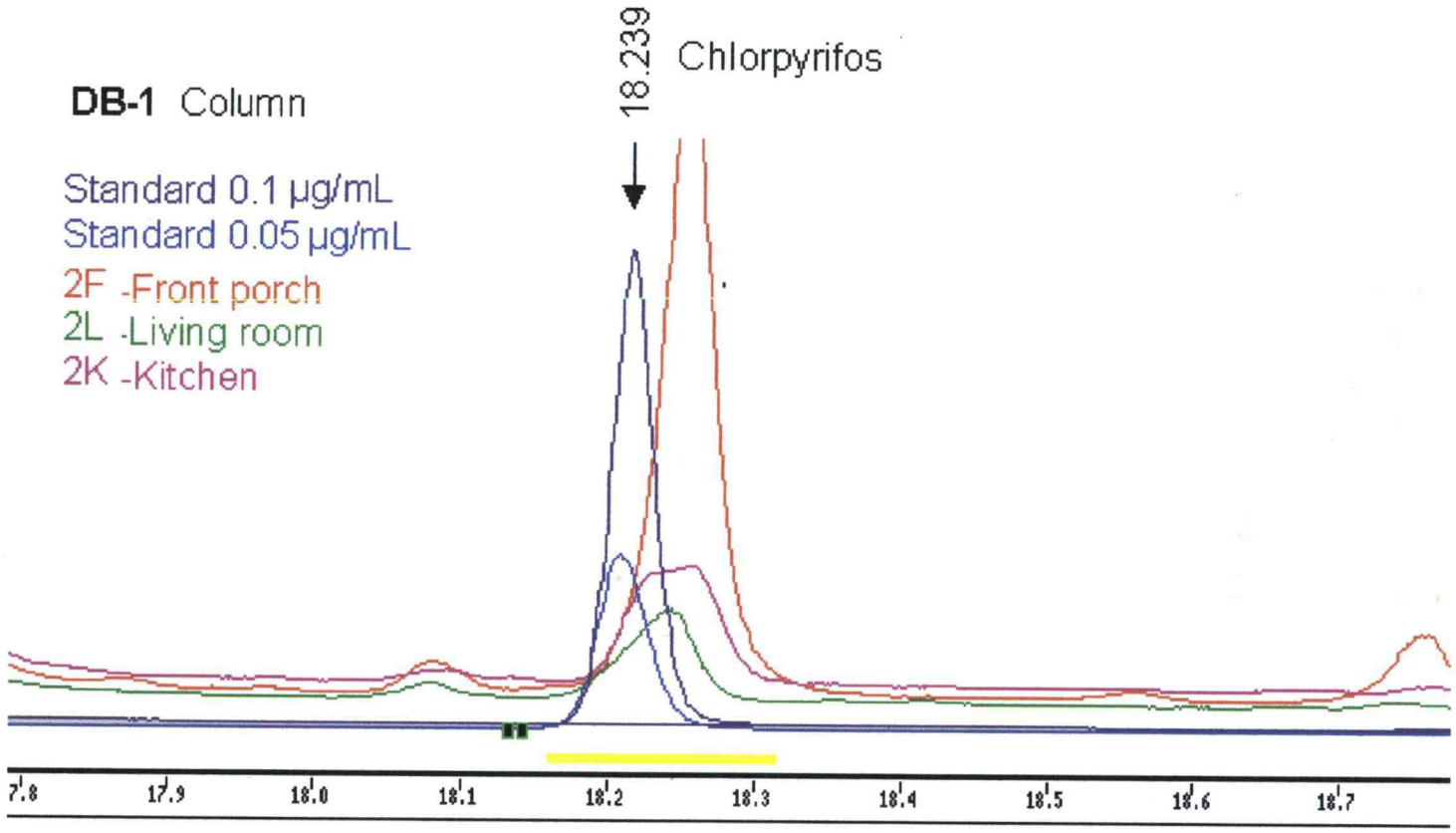

Figure 3.14a: Comparison of dust analysis results from house 2 on DB-1 column

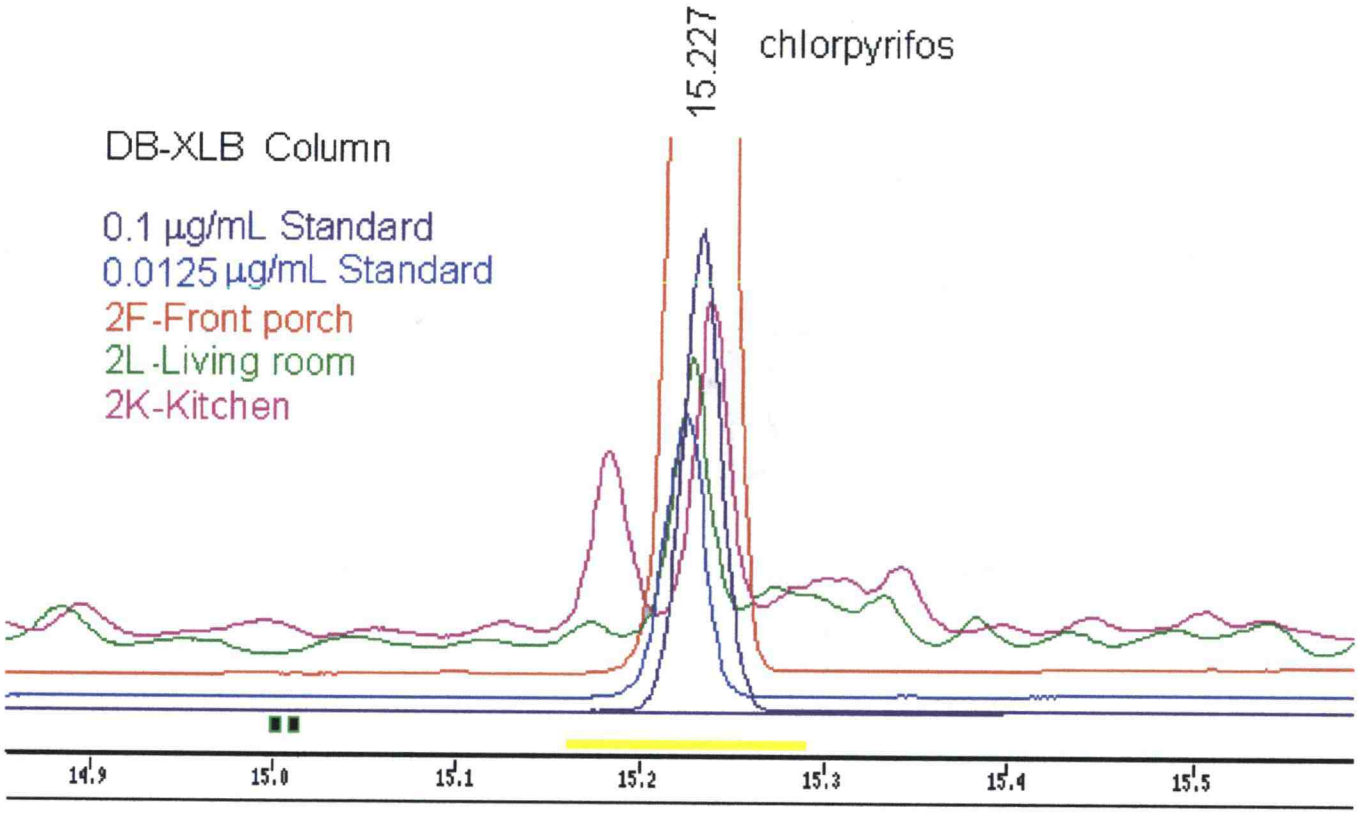

Figure 3.14b: Comparison of dust analysis results from house 2 on DB-XLB column

Figure 3.15: Comparison of dust analysis results from house 3

3F - Dust from front porch
3L - Dust from living room
3K - Dust from kitchen

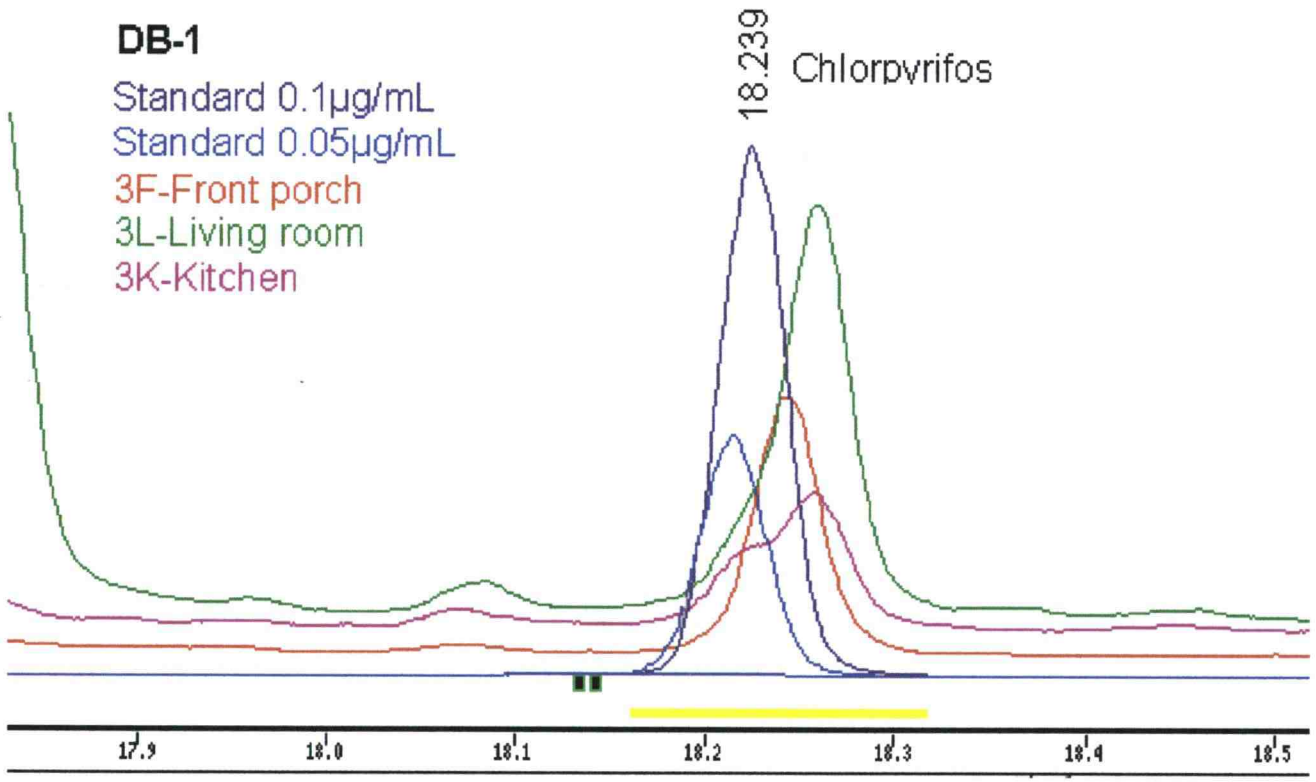

Figure 3.15a: Comparison of dust analysis results from house 3 on DB-1 column

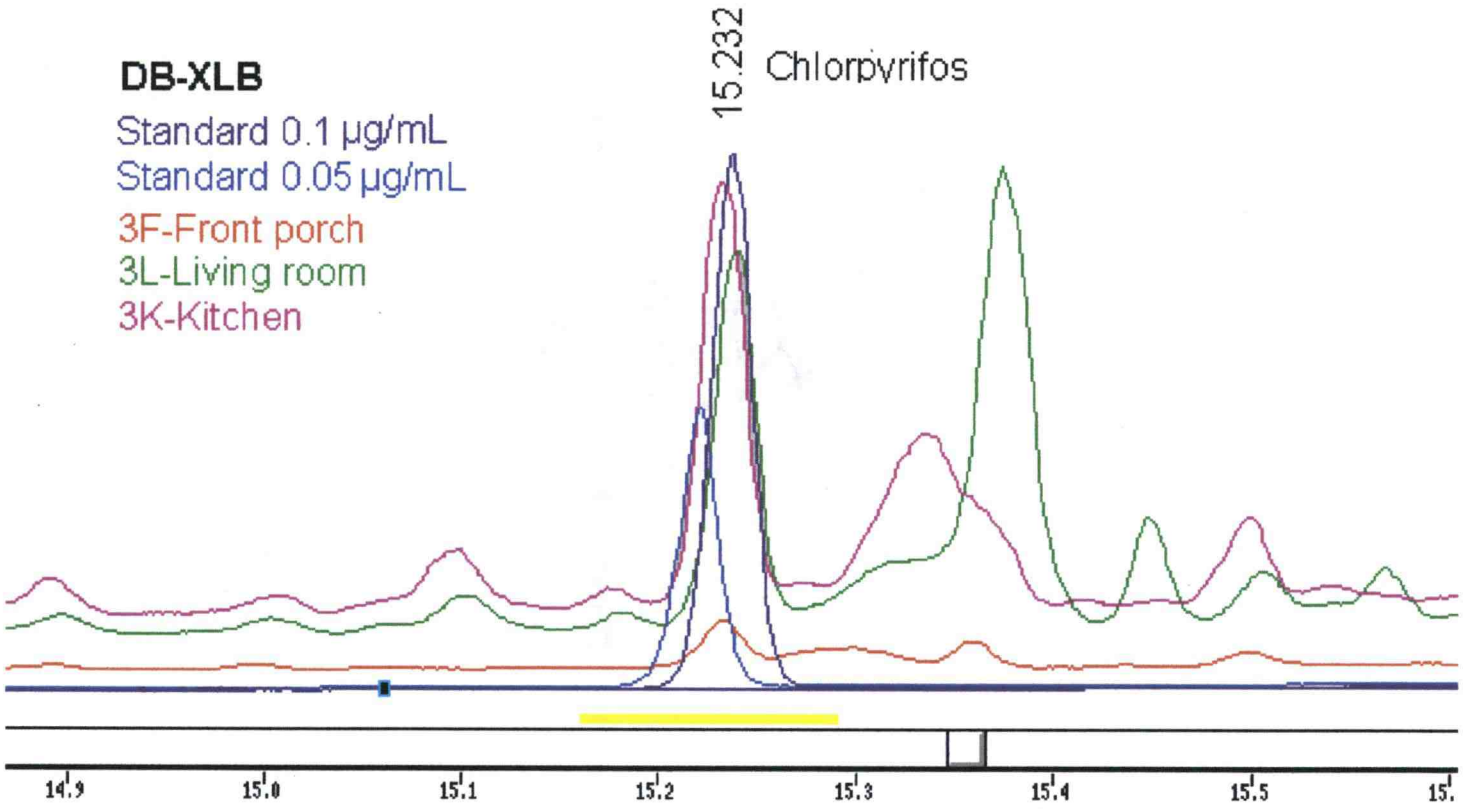

Figure 3.15b: Comparison of dust analysis results from house 3 on DB-XLB column

Water analysis for TCP: The Method detection limits for TCP in water was 167 ng/L and the mean recovery from spiked water samples was 86.5%. Retention time for TCP was 12.499 min on the DB-1 column. Two out of three wells were located at the center of the farm field, and the levels of TCP in those two were 9 and 10 ng/mL, respectively. The third well was located about 300m away from the cultivated area, and the level of TCP was 0.6 ng/mL. TCP in water was either from TCP leaching from soil into the well or hydrolysis of chlorpyrifos in the drinking water wells. Chromatographs for TCP analysis in drinking water samples, TCP spiked blank water (49 ng TCP in 15 mL water), and TCP standard are given in Figure 3.16 (a-e).

Figure 3.16: Chromatograms for water analysis for TCP in drinking water

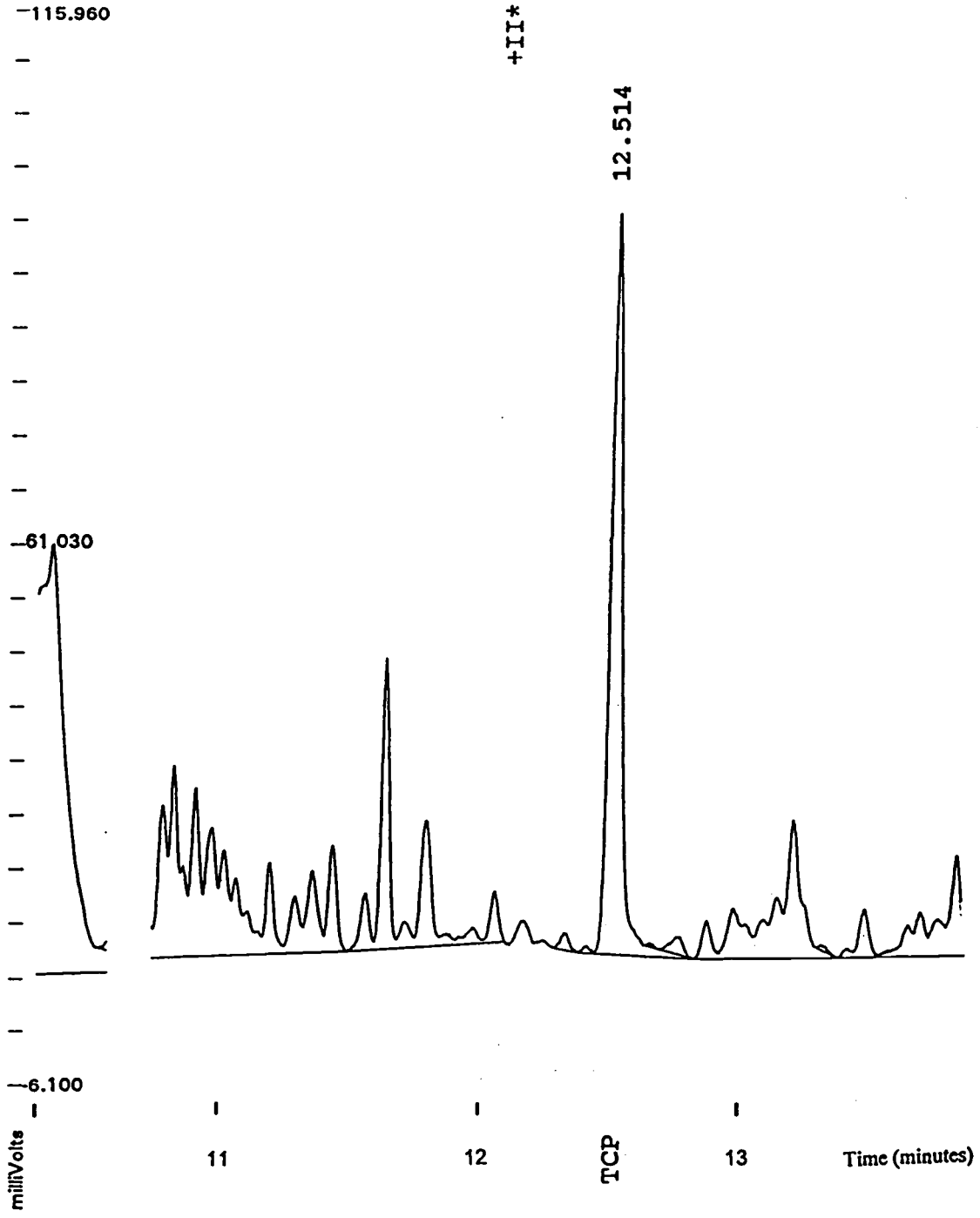

Figure 3.16a: Chromatogram of 10ng/mL TCP standard (attenuation 50)

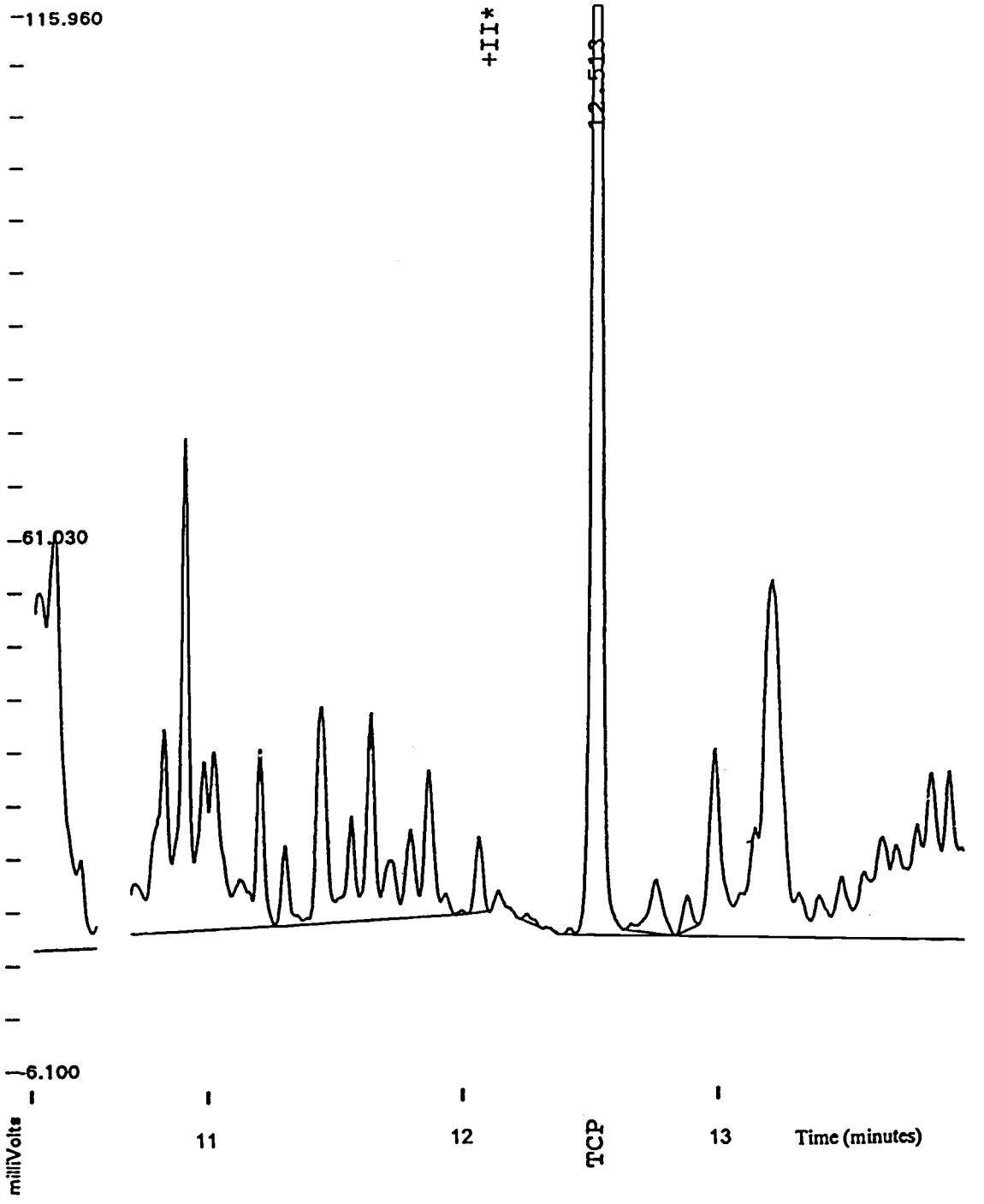

Figure 3.16b: Chromatogram of blank water spiked with 49 ng of TCP (attenuation 50)

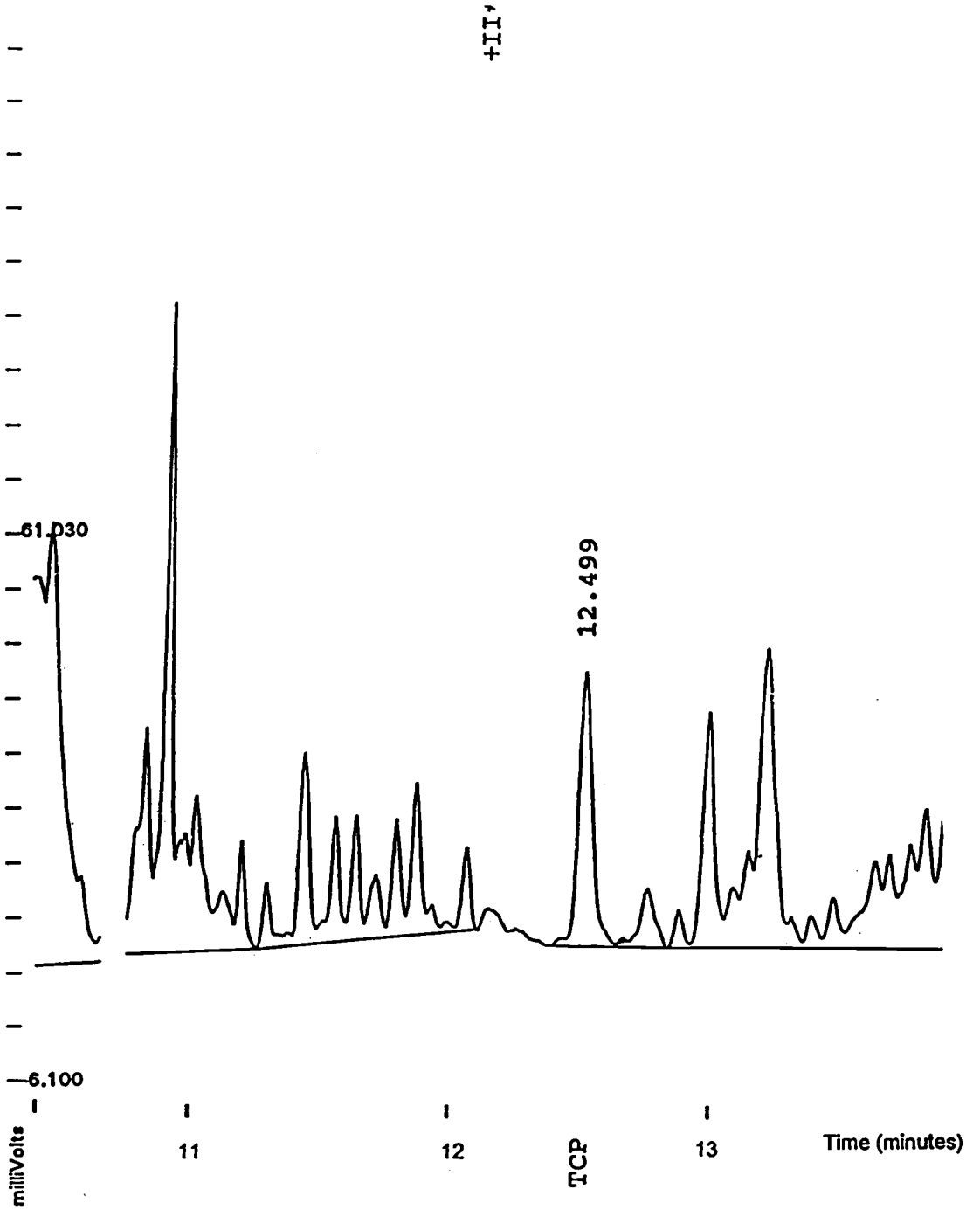

Figure 3.16c: Chromatogram of drinking water (from well 1) analysis for TCP (attenuation 50)

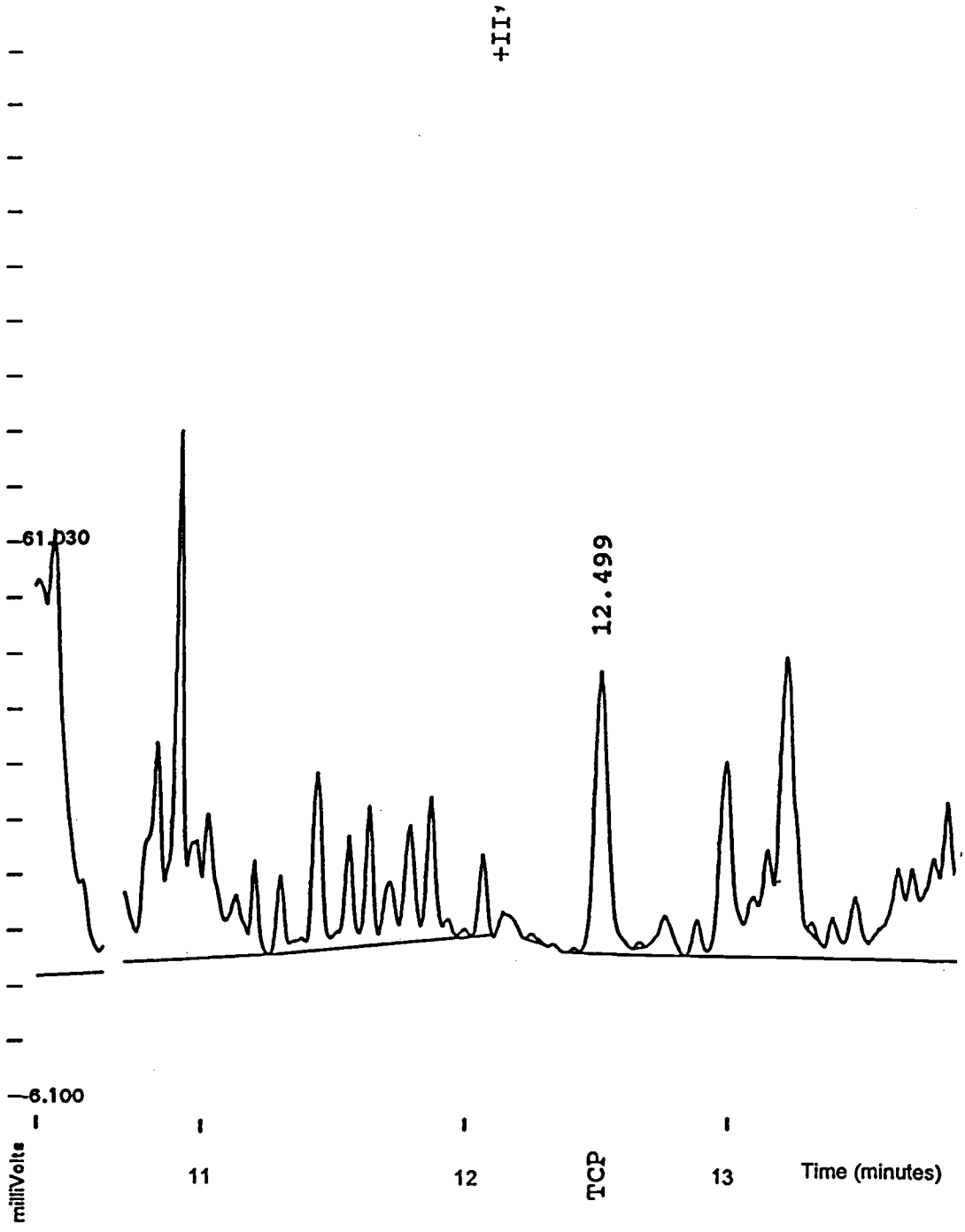

Figure 3.16d: Chromatogram of drinking water (from well 2) analysis for TCP (attenuation 50)

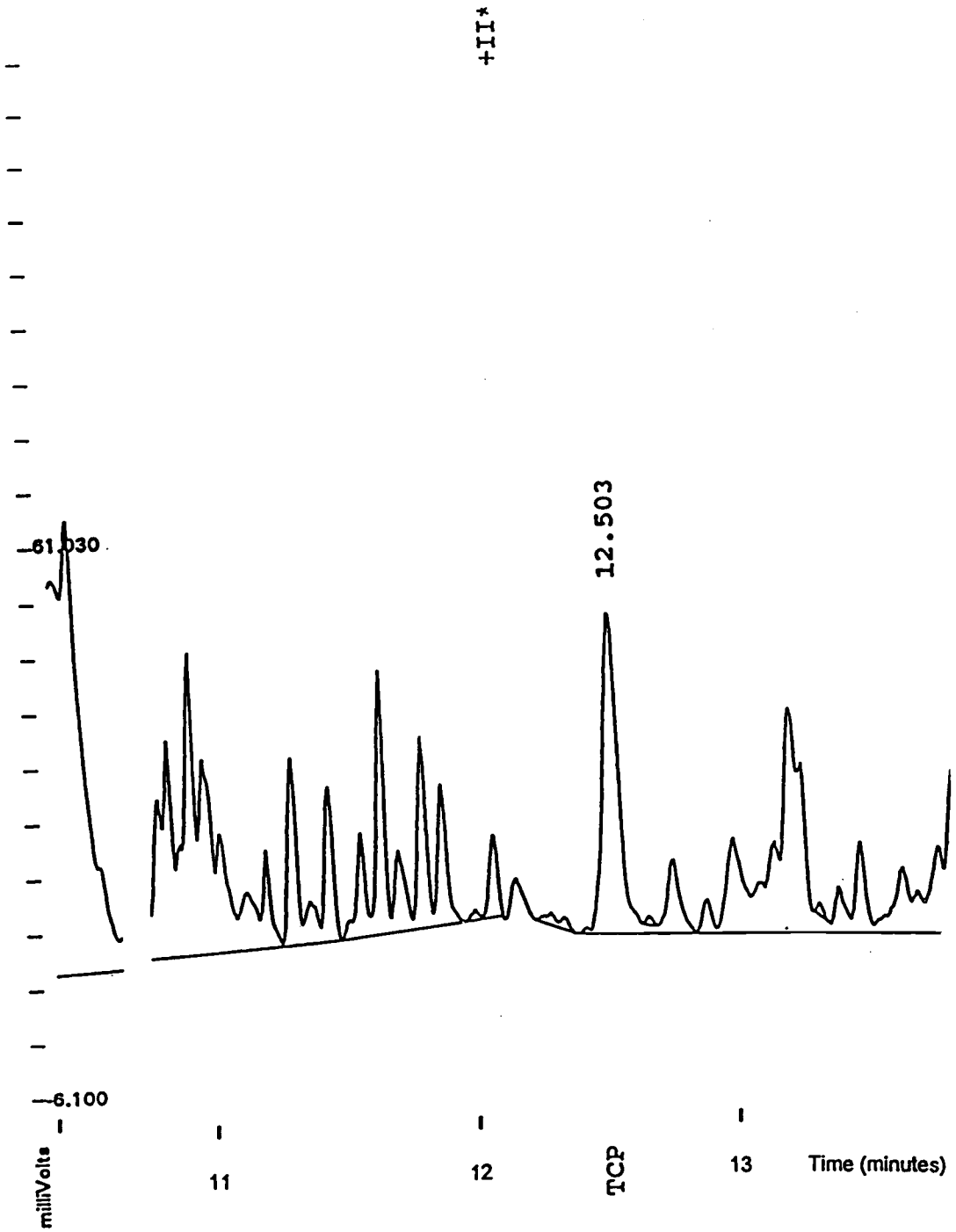

Figure 3.16e: Chromatogram of drinking water (from well 3) analysis for TCP (attenuation 50)

DISCUSSION

Assuming the source of TCP in well water is contamination from spray operation, and one TCP mole was generated from one mole of chlorpyrifos, the average TCP level is equivalent to 12.69 ng/mL of chlorpyrifos in water. Assuming the parent was present and assuming a 70 kg adult drink 2L of water a day, the internal dose by drinking water would be 0.36 ng/kg/day. RfD for chlorpyrifos is 0.003 mg/kg/day, and, hence, hazard quotient and margin of safety of drinking water is 0.121 and 83.3, respectively. One would conclude that any contribution from water or house dust to the farmer's overall exposure would be minimal. This exposure would not represent a significant increment for the farmer over and above the exposure (0.0021-0.0084 mg/kg) he received during application of the chlorpyrifos.

One might have expected to detect more chlorpyrifos in wells located in the middle of the treated fields. However, the areas treated were relatively small, and the back-pack sprayers would not produce aerosols susceptible to drift. In addition, high soil moisture and pH (~7) would enhance degradation. It is most likely that the TCP leached into the well after hydrolysis of the chlorpyrifos in the soil.

Failure to detect chlorpyrifos in house dust is not consistent with observations in the USA (Davis and Ahmed, 1998; Gurunathan et al., 1998). This distinction may also reflect differences in areas treated and persistence. The farmers in Sri Lanka treat only small area in contrast to the many acres that may be involved in a larger operation. With higher soil temperatures in tropical areas and a soil pH of about 7, the chlorpyrifos would be less persistent than in temperate zones.

CONCLUSION

Chlorpyrifos was not present with detection limits of 7 ng/L in drinking water wells located near fields treated with this organophosphate. Small quantities of trichloropyridinol metabolite (9, 10, and 0.6 ng/mL) were detected in well water. House dust collected in houses close to treated fields did not contain chlorpyrifos. Neither well water nor house dust contributed to the farmers' exposure to chlorpyrifos.

CHAPTER 4

CONCLUSION

Farmers applying chlorpyrifos showed a consistent excretion pattern of the metabolite, TCP, characteristic for this organophosphate. The excretion half-life ranged from 24.8 to 37.6hr with an average value 31.3hr. The cumulative TCP excreted over 120hr was used to calculate the internal dose of chlorpyrifos, which ranged from 0.0021 to 0.0084 mg/kg. It was assumed that major exposure route was skin, and a dose of 0.4 to 1.2 mg/kg was estimated, based on 1% dermal uptake. This dose was considered to give a marginal risk with hazard quotients range from 0.7 to 2.7 and margin of safety from 4 to 14. Statistical analysis established that the internal dose was determined, in large part, by the amount of chemical applied. In addition, it was demonstrated that faulty spray equipment and the amount of skin exposed also was associated with an increase in the internal dose. Analysis also indicated that wearing long pants could increase the internal dose, although the reason for this unexpected response is not clear. This study provides quantitative information for that program, which can be used to train farmers in the use of safer application practices.

Chlorpyrifos was not present with detection limits of 7 ng/L in drinking water wells located near fields treated with this organophosphate. Small quantities of trichloropyridinol metabolite (9, 10, and 0.6 ng/mL) were detected in well water. House dust collected in houses close to treated fields did not contain chlorpyrifos. Neither well water nor house dust contributed to the farmers' exposure to chlorpyrifos.

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APPENDIX

APPENDIX

QUESTIONNAIRE FOR FARMERS

Appendix

Serial No:

Weight:

Height:

Division of Health Service:

1) Name:
2) Address:
3) Educational Qualifications:
    a) Up to 5 years
    b) Up to year 6- 8
    c) Year 8 or above
4) Occupation:
    a) Fulltime spray operator
    b) Fulltime farmer
        i) Self application of pesticides
        ii) Applicator is not the farmer
    c) Part time farmer:
        i) Self application of pesticides
        ii) Applicator is not the farmer

5) Using integrated pest management systems: Y/N

6) Number of family members:

a) Less than 1 year
b) Between 1-5 years
c) Between 5-12 years
d) Between 12-18 years
e) Between 18-40 years
f) Over 40 years

7) Pregnant woman:

8) Pesticides are used on :
a) paddy
b) vegetables
c) Other

9) Cultivation:
a) Seasonal
b) Throughout the year

10) Days between last application and harvest:

11) Frequency of pesticide application:
a) Hours per week
b) Tanks per week
c) Land area

12) Distance to the field from the house

13) Distance to the closest agricultural land from your house

14) Time of pesticide application: start and end

15) After applying pesticides:

a) Bathing — After how long

b) Washing hands, legs, face — After how long

16) Amount of concentrated pesticides per tank:

17) Pesticides used during the past week:

Pesticide	Method of application	Concentration

18) Does anyone help you apply pesticides:

If yes, who

19) When applying pesticides:

a) Do you use alcohol

b) Chewing beetles

c) Other food

20) Safety measures used when applying pesticides

a) Face cover

i) Face mask

ii) Face cover

iii) Handkerchief

iv) Other face cover device

b) Banian

c) Shirt — Long-sleeved — Short-sleeved

d) Pants — Long — Short

e) Saron — Up to the knee — Full

f) Gloves

g) Sleepers/ Shoes

21) Weaknesses in application of pesticides:

a) Tank is leaking

b) Damaged gloves

c) Damaged shoes

d) Damaged clothing

22) Spray tank:

Good condition

Leaking

Blocked nozzles

Cleaning the tank

What time

Who

Where

23) Pesticide storage at home:

a) Kitchen

b) Roof

c) Field

d) Garden

e) Other

24) Source of drinking water

a) Tap

b) Tube well

c) Well

d) Stream

25) Distance between source of drinking water and closest field:

Less than 10m

Between 10-20m

More than 20m

26) Alcohol consumption

Daily/ occasional

27 Smoking: how many

26) Health related problems in the family:

a) Children: yes no

b) If no children:

i) Married for how long:

ii) Ages of male and female:

iii) Number of years pesticides applied:

c) Are there any other married family members without children:

If yes who:

27) Are you or any your family members suffering from following diseases:

a) Cough

b) Short of breath

c) Asthma

d) Angina pectoris

e) Palpitation

f) Faintness

g) Swelling of ankle

h) Nausea

i) Vomiting

j) Loss of weight

k) Constipation

l) Diarrhea

m) Abdominal pain

n) Dysuria

o) Polyuria

p) Urinary incontinence

q) Urgency

r) Muscle ache/mayalgia

s) Arthalgia

t) Arthritis

u) Headache

v) Visual defects

w) Hearing defects

x) Adomnia

y) Giddiness

z) Stammering

aa) Dysphagia

ab) Ataxia

ac) Loss of consciousness

ad) Numbness

af) Shivering

ag) Itching

ah) Burning sensation of the eye