福岡大医紀（*Med. Bull. Fukuoka Univ.*）：34(2)，49–63，2007

# A Retrospective Study of Acute Organophosphorus Poisoning Using A Rapid Blood Concentration Measurement System

Shigeki TOMOJIRI[1)], Mitsuyoshi KAGEURA[2)], Seiichi KASHIMURA[2)], Kenji HARA[2)] and Keiichi TANAKA[1)]

[1)] *Department of Emergency and Critical Care Medicine, Fukuoka University Hospital*

[2)] *Department of Forensic Medicine, School of Medicine, Fukuoka University*

**Abstract**：Objective：A retrospective evaluation of the clinical course of organophosphorus pesticide (OPP) poisoning cases was done in order to identify optimal treatment modalities by rapidly measuring the blood concentration of OPP. Design：This study was carried out at our emergency center during a 2–year and 11–month period from March 1994 to February 1997. Blood collection was obtained in 7 OPP poisoning cases. The ingested poisons in 6 cases were identified and determined to be OPP by gas chromatography with nitrogen–phosphorus detection (GC–NPD). Results：The present assay can be completed within about 1.5 hours (from solid phase extraction to GC–NPD), although the organophosphorus compound contains DDVP, GC–NPD and GC/MS failed to detect DDVP and disulfoton in our cases. In order to measure the blood concentration in cases of fenitrothion poisoning, DHP was proven to be effective. Consciousness disorders tend to appear when the blood concentration of fenitrothion reached about 0.3–0.4 μg/ml in our cases. No correlation between the plasma cholinesterase activity value and the blood concentration of fenitrothion could be identified from our findings. Conclusion：Using this measurement system the blood concentration of organophosphorus compound type agricultural chemicals in clinical elapse could be measured. This rapid blood concentration measurement system is needed to select the optimal therapy and curative effect by rapidly determining the blood concentration of OPP poisoning. Thus assay can thus be quickly applied to the diagnosis and the treatment of OPP poisoning in clinical situations.

**Key words：Organophosphorus pesticide poisoning, Rapid screening, Solid–phase extraction, GC–NPD**

## Introduction

Different types of patients in various poisoned states present at emergency centers, and the number of such deaths is not low. An accurate diagnosis of poisoning can be made relatively easily based on anamnesis in some cases. However, the diagnosis can only be deduced depending on the clinical course in others. Nonetheless, based on our experience, it is almost impossible to gather an accurate medical history from the patients themselves in most cases, and even if a medical history can be obtained, such information is often unreliable. The reason for this is that the majority of acute poisoning patients who are brought to emergency centers are the result of suicide attempts. Therefore, some cases are treated without a sufficient diagnosis. This situation is also true for organophosphorus poisoning. Acute poisoning patients who are brought to our emergency center consist of pesticide poisoning patients, and some of these patients die from such poisoning. A relatively large number of acute poisoning caused by organo-

Correspondence to：Shigeki TOMOJIRI, MD
Department of Emergency Medicine, Fukuoka Red Cross Hospital, 1–1–3 Okusu, Minami–ku, Fukuoka, 815–8555
Phone：092–521–1211 ext, 3191, FAX：092–824–4030, E–mail：tom@medical.email.ne.jp

phosphorus pesticides is common, and no rapid screening method for these pesticides has yet been established, so physicians have to treat these patients based on their symptoms and the plasma cholinesterase activity value without obtaining for the results of poison analyses. However, since the plasma cholinesterase activity value and clinical symptoms can not be used as indicators in some patients, physicians often have a difficult time in determining the optimal treatment plans for such patients. Whether or not the type of organophosphorus pesticides can be quickly identified can thus greatly influence the clinical course of some patients. In the last decade, many reports on analytical methods for organophosphorus pesticides have been published in the fields of environmental chemistry[1)-11)] and forensic toxicology[12)-14)], however, few studies have been developed for human body materials. We therefore attempted to modify the clean-up method described in a Bond Elut Certify Instruction Manual (Varian Sample Preparation Products, 1991) regarding its application to organophosphorus pesticides in the whole blood, before an analysis by gas chromatography with nitrogen-phosphorus detection[15)] (GC-NPD) in order to chronologically measure the blood concentrations of pesticides in acute organophosphorus poisoning patients.

## Materials and Methods

Seven patients with acute organophosphorus poisoning who were brought to our emergency center over a two year and eleven month period between March 1994 and February 1997 served as the subjects (Table 1). Blood samples that were collected

**Table 1.** Human Cases of Acute Poisoning with Several Organophosphates

| Case No. | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | No. 6 | No. 7 |
|---|---|---|---|---|---|---|---|
| Age (Sex) | 41 (M) | 58 (M) | 49 (M) | 55 (F) | 71 (M) | 26 (M) | 64 (M) |
| Trade name & Contents | fenitrothion 10% (emulsion) | fenitrothion 10% (emulsion) | fenitrothion 10% (dusting powder) | fenitrothion 10%, DDVP (unknown) | malathion 50% (emulsion) | diazinon 3%, DDVP 2% (emulsion) | disulfoton 3% (granules) |
| Quantity of intake | about 30 ml | about 1,000 ml | about 70 g | unknown | a small quantity | about 180 ml | about 300 mg |
| Intake form | ingestion (suicide attempt) | ingestion (suicide attempt) | ingestion (suicide attempt) | ingestion (suicide attempt) | ingestion (suicide attempt) | ingestion (suicide attempt) | ingestion (suicide attempt) |
| Starting time* | unknown | unknown | about 3hr | about 5hr | about 6hr | about 3.5hr | about 72hr |
| Consciousness level on admission | semicoma | coma | clear | semicoma | clear | clear | clear |
| Miosis | (+) 2.5 mm | (+) 1.5 mm | (−) 4 mm | (−) 3 mm | (−) 3 mm | (−) 4 mm | (+) 1.5 mm |
| Other objective sympton | vomiting | perspiration | vomiting | chemical burn | non | non | non |
| OPP† | fenitrothion | fenitrothion | fenitrothion | fenitrothion | marathion | diazinon | nd‡ |
| The lowest value of chE (IU/L) | 29 | 0 | 10 | 18 | 156 | 1 | 1 |
| The highest value of chE (IU/L) | 72 | 14 | 159 | 75 | 163 | 80 | 71 |
| Psychosis | non | non | Depression | Depression | non | Schizophrenia | non |
| Other anamnesis | Acute appendicitis | Liver Cirrhosis, HCC | Myasthenia gravis | Hypertension | Diabetes mellitus | non | Hypertension, Stenocardia |
| Therapy to admission | non | volume infusion<br>intubation | volume infusion, diuresis | volume infusion, diuresis<br>lavage§, charcoal‖, catharics<br>atropin¶ 0.5 mg, PAM 500 mg | volume infusion | non | volume infusion, diuresis<br>lavage§, atropin¶ 0.5 mg |
| Therapy after admission | volume infusion, diuresis<br>atropin¶ 2.5 mg, PAM 500 mg<br>lavage§, charcoal‖, catharics<br>DHP** | volume infusion, diuresis<br>atropin¶ 166 mg, PAM 500 mg<br>lavage§, charcoal‖, catharics<br>DHP**, CHDF††<br>S-G catheter, respirator | volume infusion, diuresis<br>atropin¶ 2.5 mg, PAM 500 mg<br>lavage§, charcoal‖, catharics | volume infusion, diuresis<br>respirator | volume infusion, diuresis<br>charcoal‖, catharics | volume infusion, diuresis<br>atropin¶ 1.5 mg<br>lavage§, charcoal‖ catharics | volume infusion, diuresis<br>atropin¶ 17.5 mg, PAM 1,000 mg<br>charcoal‖, |
| The admission days | 5 | 17 | 7 | 12 | 3 | 3 | 4 |
| Clinical course | discharge | death | remission | remission | discharge | remission | remission |

*Starting time of treatment at our hospital after ingetion; †Organophosphorus pesticides which was detected by GC-NPD; ‡Not detected; §Gastric lavage; ‖Activated charcoal; ¶Atropin sulfate
**Direct hemoperfusion (plasma adsorption with activating charcoal column); ††Continous hemodiafiltration

from these patients at various times were retrospectively analyzed. All seven patients had attempted suicide by orally ingesting organophosphorus pesticides: three patients took fenitrothion, one took fenitrothion and Dichlorovos(DDVP), one took daiazinon and DDVP, one took malathion, and one took disulfoton (emulsion, powder and granules). The age of the subjects ranged from 26 to 71 years with an average of 46.1 years. They included six men and one woman. At the time of admission, the following conditions were observed: coma (1 case), semicoma (2 cases), asymptomatic (3 cases) and miosis (3 cases). Their underlying diseases were:hypertension(2 patients), stenocardia (1 patient), diabetes (1 patient), myasthenia gravis (1 patient), liver cirrhosis(1 patient), hepatocellular carcinoma (1 patient), schizophrenia (1 patient), and depression (2 patients) (multiple answers). The minimum cholinesterase activity value after hospitalization ranged from 0 to 163 IU/L (normal range: 220–470 IU/L) with an average of 60.6 IU/L. Five patients had been treated by a physician before being brought to the emergency center, while the other two patients were directly brought by an ambulance. At our emergency center, atropine sulfate was administered to five patients (maximum dose: 166 mg), pyridine-2-aldoxime methiodide (PAM) was administered to three patients, and acute blood purification was performed on two patients. The average length of hospitalization was 7.3 days. Two patients were discharged, four patients were transferred to another hospital after their conditions improved, and one patient died.

DDVP, salithion (2-methoxy-5,6-benzo-4*H*-1,3,2-dioxaphosphorin-2-sulfide), dimethoate, diazinon, disulfoton, IBP (*S*-benzyl-*O*,*O*-diisopropyl thiophosphate), malathion, phenthoate (PAP), methidathion (DMTP), ethion, edifenphos (EDDP), EPN, phosalone and parathion (IS) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Bond Elut Certify® (cartridges contain mixed-mode bonded silica gel, which consisting of hydrophobic and cation exchange functional groups) cartridges (3 ml/300 mg), were obtained from Varian Sample Preparation Products (Harbor City, CA, USA). All other chemicals used were of analytical grade. The blood used was supplied by volunteers who had not taken any medication, for at least one week. The extraction method has been described in the literature.[15]

The blood samples of the seven acute organophosphorus poisoning patients who were brought to our emergency center were collected at various times. Furthermore, in cases 1 and 2, blood samples were collected before and after plasma adsorption with the coated activated charcoal procedure (DHP) and continuous hemodiafiltration (CHDF), and effluent was also collected.

To eliminate any drugs that might induce consciousness disorders and poisons that might cause other symptoms, samples were screened by automatic high performance liquid chromatography (REMEDi-HS: BIO-RAD Ltd.) first and then subjected to GC/MS.

## Results

After solid phase extraction, the following organophosphorus compound were identified and quantified by GC-NPD: fenitrothion in four patients, malathion in one patient, and daiazinon in one patient (Table 2).

**Table 2.** Clinical data of 7 cases with organophsphorus pesticide poisoning

| Case | Contents of OPP[a] | Detection of OPP[a] by GC-NPD | Concentration of OPP[a] on admission (μg/ml) |
|---|---|---|---|
| 1 | fenitrothion 10% | fenitrothion | 0.6877 |
| 2 | fenitrothion 10% | fenitrothion | 0.7523 |
| 3 | fenitrothion 10% | fenitrothion | 0.6599 |
| 4 | fenitrothion, DDVP | fenitrothion (DDVP: ND[b]) | 0.3361 |
| 5 | malathion 50% | malathion | 0.7763 |
| 6 | diazinon 3%, DDVP 2% | diazinon (DDVP: ND[b]) | 0.8219 |
| 7 | disulfoton 5% | ND[b] | — |

a; organophosphorus pesticides b; not determined

**1. The clinical course and the results of a quantitative analysis of the four fenitrothion patients**

〈Case 1〉

Figures 1 and 2 show the changes in the activity of the plasma cholinesterase activity value and the time profile for fenitrothion concentration in the blood. Blood specimens were collected at 0, 2, 4, 6, 8, 10, 12 hours after his admission. The dotted lines indicate the time when DHP was performed. The fenitrothion concentration was 0.608 μg/ml in the blood collected on admission and 0.443 μg/ml and 0.011 μg/ml immediately before and after DHP, respectively. However, it rose to 0.186 μg/ml after six hours, and fell to 0.022 μg/ml after twelve hours. No other drugs were detected in the blood samples that were collected from this patient during hospitalization in addition to the above findings. Figure 3 shows a chromatogram of the patient's blood before and after DHP respectively.

He was semicomatose and his consciousness level was determined to be III–200, by based on the Japan Coma Scale (JCS) criteria and his pupils were contracted to 2.5 mm in diameter. In spite of conventional treatment, as time passed his consciousness deteriorated and the miosis did not improve. We therefore had to perform DHP. The blood concentration remarkably decreased after DHP, and an amelioration in his consciousness was also seen since the blood concentration dropped by about 0.3 μg/ml (about 20 minutes after DHP). Thanks to

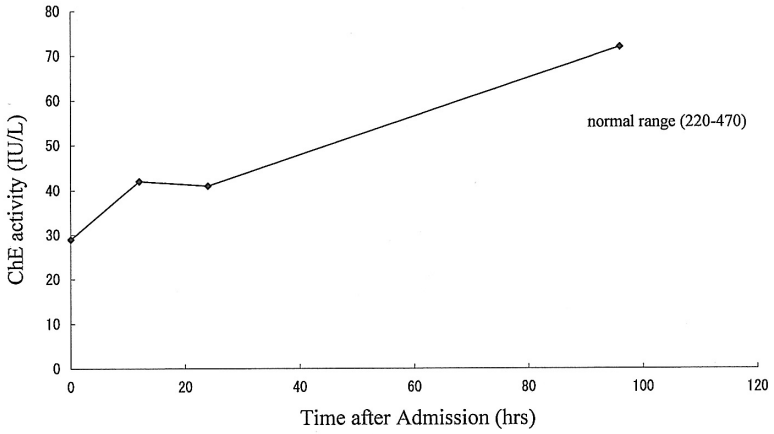


**Figure. 1.** Changes in the activity of plasma ChE in a patient poisoned by fenitrothion (Case 1).

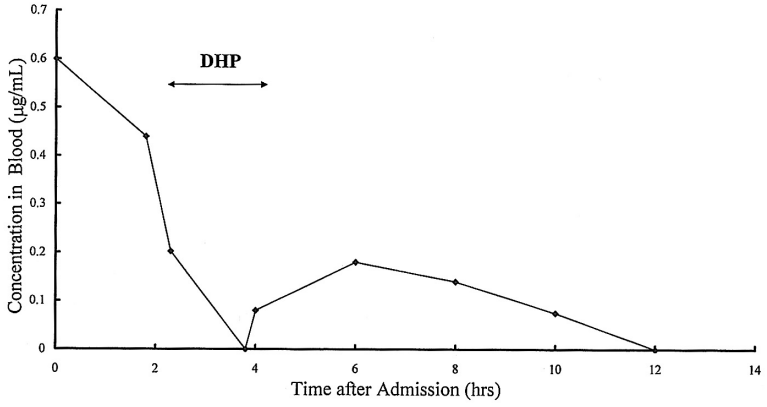


**Figure. 2.** The time profile for fenitrothion concentration in the blood of the patient (Case 1).

the DHP, the length of hospitalization, the consciousness level and the patient's general condition did not become exacerbated.

Since the consciousness condition improved in line with the decrease in the fenitrothion concentration in the blood by DHP was admitted, some correlation is thus suggested to exist between the blood concentration and the clinical sign. There have been several studies on hemoperfusion to organophosphorus poisoning.[16)-19)] Among the various organophosphorus pesticides, fenitrothion in particular penetrates heavily into adipose tissue, so there are reports that DHP has little effect in poisoned patients once a long period has elapsed since the pesticides was ingested. In the current case, one reason why prompt DHP was found to be an effective treatment is that penetration into adipose tissue was minimal since only a small amount of the pesticide was ingested and a short time had elapsed since its ingestion.

〈Case 2〉

Figures 4 and 5 show the changes in the activity of the plasma cholinesterase activity value and the time profile for the fenitrothion concentration in the whole blood. Blood specimens were collected a

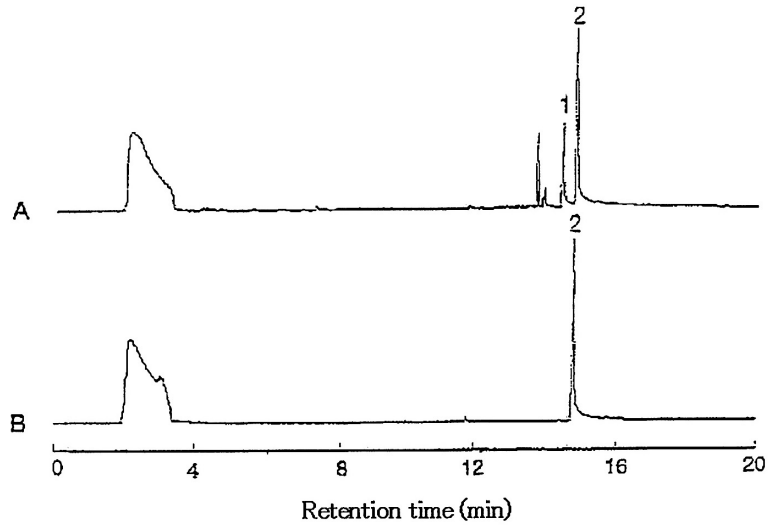


**Figure. 3.** Gas chromatograms of the extract from the patient's blood (Case 1). A : before plasma adsorption (1.8 hrs after admission) B : after plasma adsorption (3.8 hrs after admission) 1 : fenitrothion ; 2 : parathion (IS)

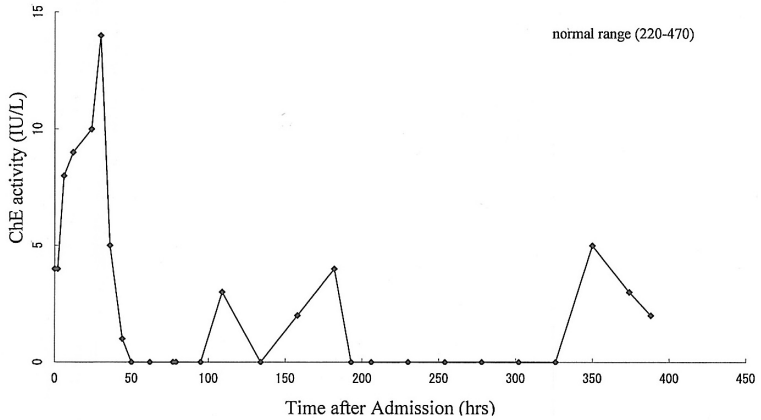


**Figure. 4.** Changes in the activity of plasma ChE in patient poisoned by fenitrothion (Case 2).

total of fourteen times : on admission, and at 2, 4, 5, 6, 12, 24, 36, 48, 72, 96, 240, 312, and 360 hours after admission. In addition, the concentration of fenitrothion was measured in the blood samples both before and after DHP and CHDF and in the effluent (Table 3). Even though the blood concentration of fenitrothion on admission was 0.752 μg/ml, it increased to 0.811 μg/ml two hours after admission, and later reached 1.049 and 1.375 μg/ml four (soon after the completion of DHP) and six hours, respectively, after admission. DHP was performed a total of seven times and the blood concentration of fenitrothion markedly decreased over time : it decreased to 0.302 μg/ml (lowest after admission) just before the patient died.

The patient was comatose and his consciousness level was determined to be III-300, by based on the JCS criteria and his pupils were contracted to 1.5 mm in diameter. After hospitalization, endotracheal intubation was performed because of poor respiration, and catecholamine was administered to treat unstable hemodynamics. Next, through the use of a Swan-Ganz catheter, atropine sulfate and PAM were administered. Gastric lavage was performed using 5,000 ml of saline, and activated charcoal and a laxative were administered through a nasal tube. Nonetheless, since the miosis and the consciousness level of the patient did not improve, DHP was performed using an activated charcoal column. After DHP, even though the patient responded to name calling by opening his eyes, the consciousness level thereafter again deteriorated. To stabilize the hemodynamics and to improve metabolic acidosis, CHDF was used and meiron-sam was continuously administered. During seventeen days of hospitalization, a total of seven DHP were performed, and a total of 166 mg of atropine sulfate was administered. However, the consciousness level of the patient improved and worsened repeatedly, and the plasma cholinesterase activity value only increased to 14 IU/L (normal range : 220-470 IU/L). We recognized an amelioration of his consciousness condition when the blood concentration dropped to about 0.38 μg/ml degree (about 340 hours after admission). At the time of admission, DIC (disseminated intravascular coagulation syndrome) and aspiration pneumonitis were confirmed, and the patient eventually developed multiple organ failure (MOF) and died seventeen days after hospitalization.

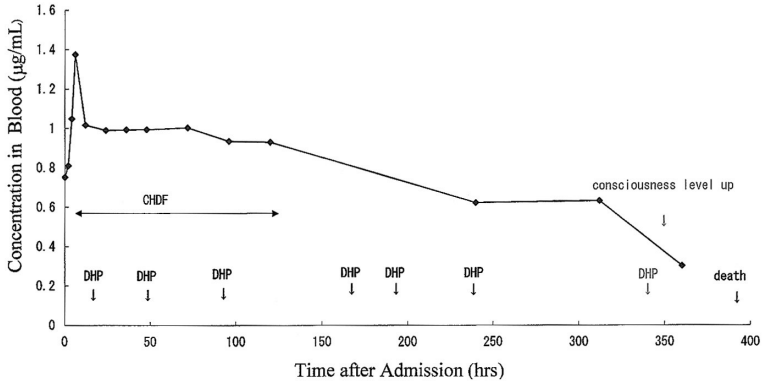


**Figure. 5.** The time profile for fenitrothion concentration in the whole blood of the patient (Case 2).

**Table 3.** Concentrations of Fenitrothion in blood pre- and post columns

| | | | Concentration of fenitrothion (μg/ml) |
|---|---|---|---|
| PRE | DHP* | column | 0.905 |
| POST | DHP* | column | 0.030 |
| PRE | CHDF† | column | 0.866 |
| POST | CHDF† | column | 0.878 |
| Effluent | | | 0.184 |

*Direct hemoperfusion (plasma adsorption with an activating charcoal column)
†Continous hemodiafiltration

This patient also suffered from cirrhosis and hepatocellular carcinoma.

It is increasing with 0.811 μg/ml and 1.049 μg/ml when comparing the blood concentration of fenitrothion at 2 hour and 4 hour after hospitalization. It seems that DHP has no therapeutic effect, however, it actually does have a therapeutic effect. The concentrations of fenitrothion before and after the DHP column were compared. The fenitrothion concentration was 0.905 μg/ml before the column, 0.030 μg/ml after the column as shown in Table 3. In addition, the concentrations of fenitrothion before and after the CHDF column and in waste fluid were compared. The fenitrothion concentration was 0.866 μg/ml before the column, 0.878 μg/ml after the column, and 0.186 μg/ml in waste fluid as shown in Table 3.

As is apparent, CHDF is thus expected to have almost no therapeutic effect.

The case was serious in that patient had ingested about 1,000 ml of fenitrothion, and precisely when the patient had ingested the pesticide was unclear. In addition, the patient had a past history of cirrhosis, so plasma cholinesterase activity value was unable to serve as a therapeutic guide. The results here suggested that, in cases with a short time since the fenitrothion was ingested and with a high its concentration in blood, the preferable approach is perform plasma exchange before fenitrothion penetrates into adipose tissue from the blood and then continuous DHP in order to quickly remove fenitrothion from the blood.

〈Case 3〉

Figures 6 and 7 show the changes in the activity of the plasma cholinesterase activity value and the

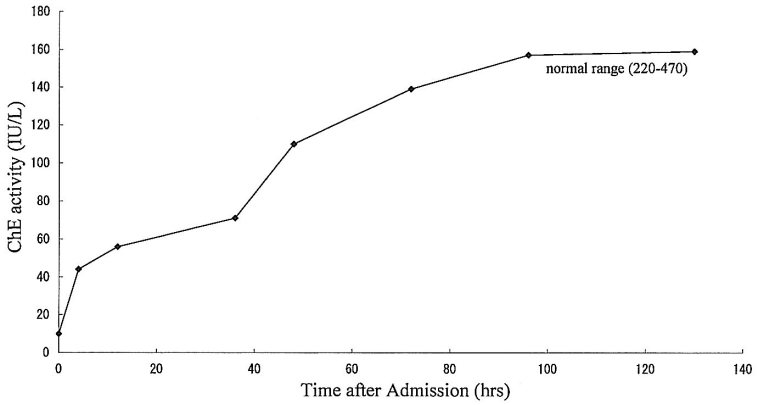


**Figure. 6.** Changes in the activity of plasma ChE in patient poisoned by fenitrothion (Case 3).

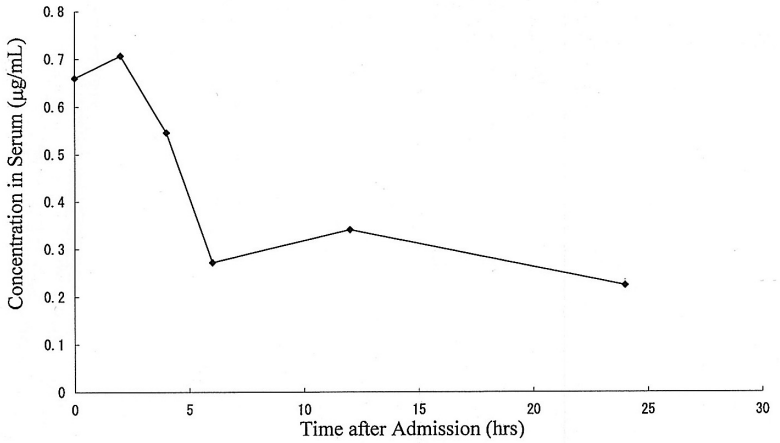


**Figure. 7.** The time profile for fenitrothion concentration in the whole blood of the patient (Case 3).

time profile for the fenitrothion concentration in whole blood. Blood specimens were collected for a total of seven times : at admission, and at 2, 4, 6, 12 and 24 hours after admission. The blood concentration of fenitrothion was 0.660 μg/ml at the time of admission, but it later increased to 0.707 μg/ml two hours after admission, and thereafter it markedly decreased. The blood concentration of fenitrothion was 0.272 μg/ml six hours after admission, and even though it increased to 0.340 μg/ml twelve hours after admission, it decreased to 0.224 μg/ml twenty four hours after admission.

Because his consciousness level was determined to be I−1, by based on the JCS criteria, no miosis was recognized and since the time after the ingestion of fenitrothion was long, we did not perform DHP. After hospitalization, central venous line insertion and gastric lavage using 2,500 ml of saline were performed, and after intravenously administering atropine sulfate and PAM, activated charcoal and laxative were administered. From the time when the patient was admitted, the patient was conscious, and no symptoms indicative of organophosphorus poisoning were seen, and it was thus determined that drip infusion and diuresis would be used and that the patient would be merely monitored. The general condition of the patient did not worsen and the patient did not develop any complications. In addition, since the plasma cholinesterase activity value markedly increased to 159 IU/L seven days after hospitalization, the patient was thereafter transferred to another hospital.

During the admission period, it became clear that his anamnesis included myasthenia gravis and he took anticholinergic agents more than 3 years ago.

In this case, after admission, as for the concentration of fenitrothion in the 2 hour blood, 0.707 μg/ml was high concentration.

Though the plasma cholinesterase activity value showed a low, it was scarce about the subjective symptoms. Because, as for this patient, the to that depending influence was sufficiently thought of with the medical history having myasthenia gravis and an anticholinesterase contained in the common use medicine. In the recovery of the plasma cholinesterase activity value, as for being rapid, it thought that it is cause compared with the other fenitrothion patient. The plasma cholinesterase activity value did not become the index of the treatment in case of this case, too.

〈Case 4〉

Figures 8 and 9 show the changes in the activity of the plasma cholinesterase activity value and the time profile for the fenitrothion concentration in the whole blood. Blood specimens were collected a total of seven times : at admission, and at 2, 4, 6, 12, 24, and 36 hours after admission. Even though the blood concentration of fenitrothion was 0.336 μg/ml at the time of admission, it decreased to 0.305 μg/ml two hours after admission and then continued to markedly decrease after that. The blood concentration of fenitrothion decreased to

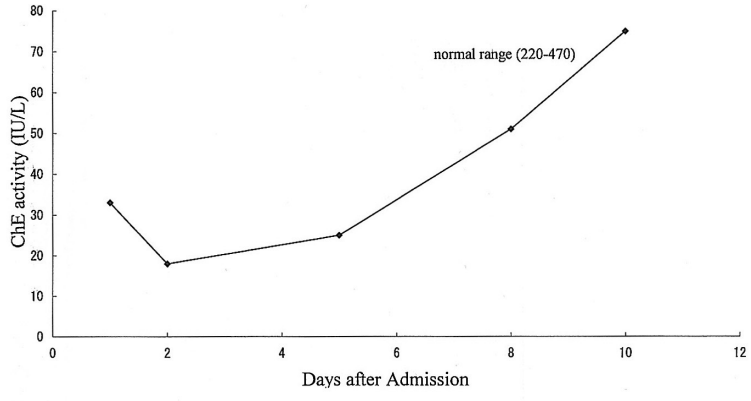


**Figure. 8.** Changes in the activity of plasma ChE in patient poisoned by fenitrothion (Case 4).

0.024 μg/ml thirty six hours after admission.

She was semicomatose and her consciousness level was determined to be III-200, by based on the JCS criteria, and no miosis was recognized. After hospitalization, artificial respiration and PAM administration were performed. Thereafter the patient responded when called by name and opened his eyes about two hours after admission, it was thus determined that drip infusion and diuresis would be used, and that the patient would be monitored. The consciousness level of the patient thereafter gradually improved, and the tube was removed three days after hospitalization. The general condition of the patient did not worsen and the patient did not experience any complications. The plasma cholinesterase activity value markedly increased, reaching 75 IU/L nine days after hospitalization. The patient's condition improved, and the patient was transferred to another hospital twelve days after hospitalization.

In this case, it was unclear in the quantity and the taking it time which took fenitrothion. It thinks that the early treatment in the hospital before hospitalized to this hospital hindered absorption to the fenitrothion blood inside about increasing the concentration of fenitrothion in the blood. In addition, the organophosphorus pesticide ingested by the patient also contained DDVP, but it was not detected by either GC-NPD or GC/MS. The reason for this is because the absorbed DDVP is quickly broken down in the body. Therefore, among the 13 types of organophosphorus pesticides, DDVP in particular is difficult to quantify.

**2. The clinical course and the results of a quantitative analysis of the one malathion patient.**

〈Case 5〉

Figures 10 and 11 show the changes in the activity of the plasma cholinesterase activity value and the time profile for malathion concentration in the whole blood. Blood specimens were collected for a total of seven times : at admission, and at 2, 4, 6, 12, 24, and 36 hours after admission. Even though the blood concentration of malathion was 0.776 μg/ml at the time of admission, it increased to 0.845 μg/ml two hours after admission. It thereafter decreased to 0.723 μg/ml four hours after admission, and continued to markedly decrease after that. The blood concentration of malathion decreased to 0.111 μg/ml twenty four hours after admission. Nonetheless, it increased again to 0.169 μg/ml thirty six hours after admission. Figure 12 shows a chromatogram of blood two hours after admission and standard sample.

Because his consciousness level was determined to be I-1, by based on the JCS criteria, no miosis was recognized and since the time after the ingestion of malathion was long, gastric lavage, and activated charcoal and laxative administrations were performed. Nevertheless, no atropine sulfate or PAM were administered intravenously to this patient. The patient was conscious on admission,

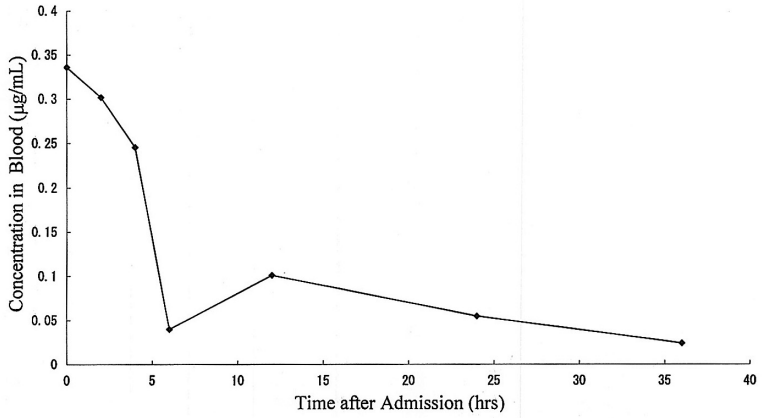


**Figure. 9.** The time profile for fenitrothion concentration in the blood of the patient (Case 4).

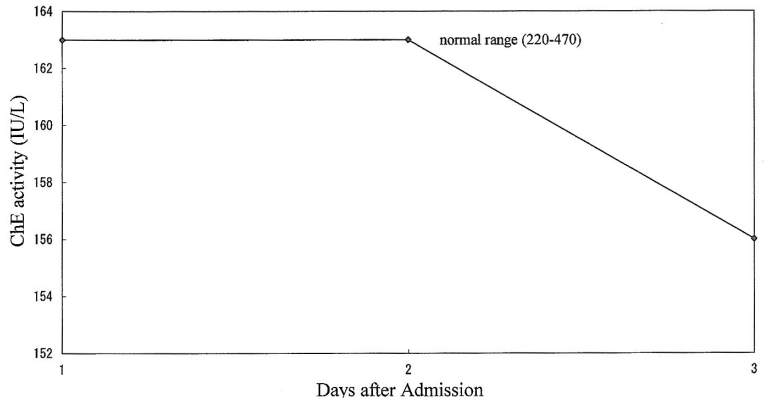


**Figure.10.** Changes in the activity of plasma ChE in humans poisoned by malathion (Case 5).

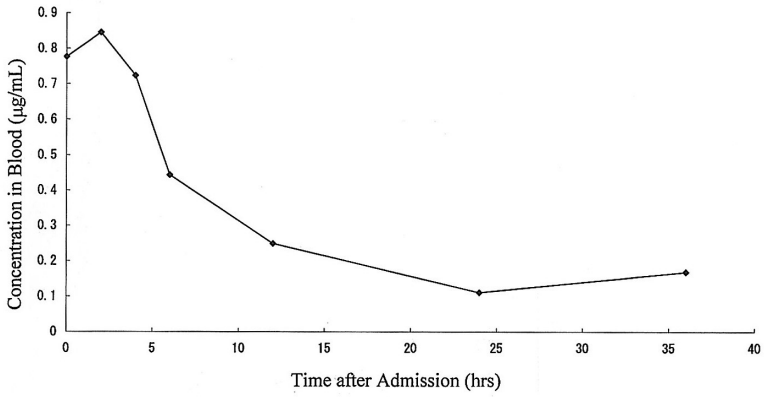


**Figure.11.** The time profile for malathion concentration in the blood of the patient (Case 5).

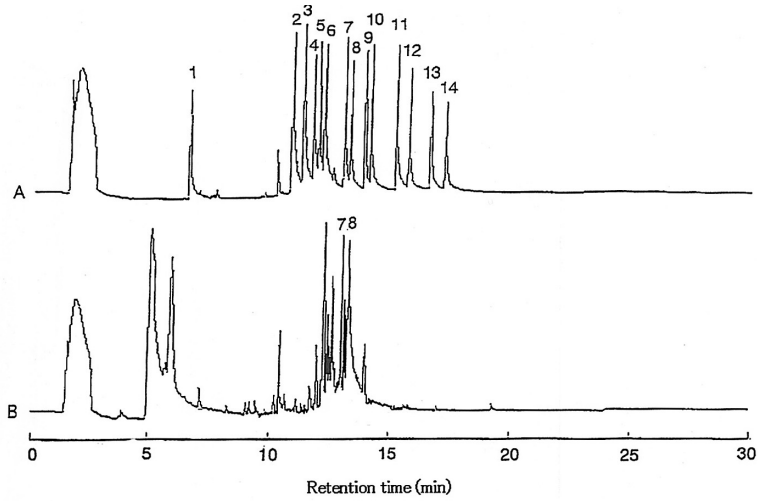


**Figure.12.** Gas chromatograms of the extract from the patient's blood and standard sample (Case 5). A : standard sample ; B : blood sample (2 hrs after admission) ; 1=dichlorovos (DDVP) ; 2=salithion ; 3=dimethoate ; 4=diazinon ; 5=disulfoton ; 6=IBP ;7=malathion ; 8=parathion (IS) ; 9=phenthoate ; 10=methidathion; 11=ethion ; 12=edifenphos ; 13=EPN ; 14=phosalone.

and because no symptoms indicative of organophosphorus poisoning were detected, it was determined that drip infusion and diuresis would merely be used and the patient would be monitored. The general condition of the patient did not exacerbate and the patient did not worsen complications. Furthermore, the plasma cholinesterase activity value did not markedly fluctuate, and the patient was discharged three days after hospitalization.

In this case, after admission, the concentration of malathion in the 2 hour blood was high concentration in 0.845 μg/ml. It was high concentration when comparing with the concentration level of fenitrothion in the blood but the plasma cholinesterase activity was 163 IU/L. It is necessary to choose a treatment after based on type of toxicity and the organophosphorus compound in addition to the blood concentration. It is thus important to quickly identify the organophosphorus compound quickly with this measuring method and to measure a blood concentration.

### 3. The clinical course and the results of a quantitative analysis of the one daiazinon patient.

〈Case 6〉

Figures 13 and 14 show the changes in the activity of plasma cholinesterase activity value and the time profile for daiazinon concentration in the whole blood. Blood specimens were collected a total of seven times : on admission, and at 2, 4, 6, 12, 24, and 36 hours after admission. Even though the blood concentration of daiazinon was 0.822 μg/ml at the time of admission, it significantly decreased to 0.222 μg/ml two hours after admission, but then further increased to 0.299 μg/ml four hours after admission. However, it continued to markedly decrease thereafter, and the blood concentra-

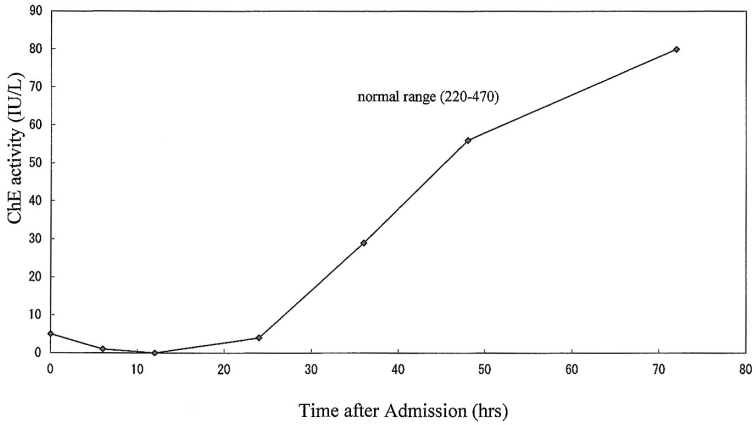


**Figure.13.** Changes in the activity of plasma ChE of patient poisoned by diazinon (Case 6).

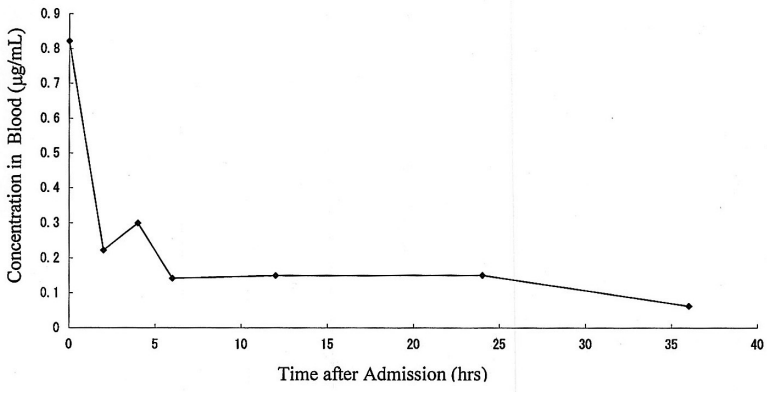


**Figure.14.** Time profile for diazinon concentration in the blood of the patient (Case 6).

tion of daiazinon decreased to 0.063 μg/ml thirty six hours after admission. Although the organophosphorus compound contains DDVP, GC－NPD and GC/MS failed to detect DDVP in this case. Figure 15 shows a chromatogram of the blood 2 hours after admission and standard sample.

His consciousness level was determined to be I－1, by based on the JCS criteria, no miosis was recognized and the time after the ingestion of daiazinon was long. After hospitalization, gastric lavage using 2,500 ml of saline was performed. After administering 1.5 mg (3A) of atropine sulfate intravenously, activated charcoal was administered. The patient was conscious on admission, and because no symptoms indicative of organophosphorus poisoning were detected, it was thus determined that drip infusion and diuresis would be used and the patient would merely be monitored. The general condition of the patient did not worsen and the patient did not experience any complications. Furthermore, the plasma cholinesterase activity value increased to 80 IU/L seventy two hours after hospitalization, and the patient was transferred to another hospital on the same day.

In this case, the blood concentration of daiazinon was 0.845 μg/ml at the time of admission. It was high concentration when comparing with the concentration level of fenitrothion in the blood. The plasma cholinesterase activity value was 1 IU/L.

The subjective symptoms of this patient disappeared after admission in this hospital. The treatment was only the prescribing of drip infusion and diuresis. At 36 hours after admission, the concentration of daiazinon in the blood decreased with 0.063 μg/ml. The patient was transferred to another hospital on three days after hospitalization.

When considering these things, it is dangerous to choose a treatment only in the fact that the plasma cholinesterase activity value merely shows decrease. It is important to choose in the treatment after identifying the type of the organophosphorus compound. It is critical to identify the type of the organophosphorus compound quickly with this measuring method and to measure the blood concentration.

**4. The clinical course and the results of a quantitative analysis on in disulfoton patient.**

〈Case 7〉

Since disulfoton could not be detected by GC－NPD following solid phase extraction, it could not

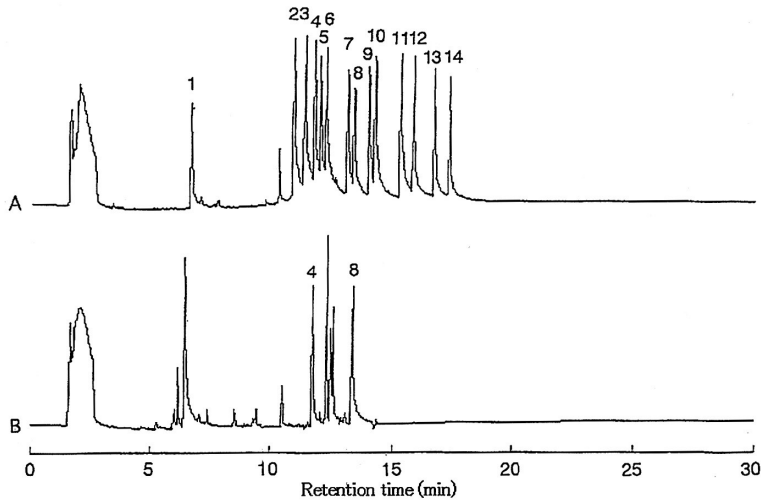


**Figure.15.** Gas chromatograms of the extract from the patient's blood and standard sample (Case 6). A：satdard sample；B：blood sample (2 hrs after admission)；1=dichlorovos (DDVP)；2=salithion；3=dimethoate；4=diazinon；5=disulfoton；6=IBP；7=malathion；8=parathion (IS)；9=phenthoate；10=methidathion；11=ethion；12=edifenphos；13=EPN；14=phosalone.

be quantified.

The patient consciousness level was determined to be I-1, based on the JCS criteria, no miosis was recognized. After hospitalization, gastric lavage was performed. After administering atropine sulfate 2.5 mg (5A) and PAM (2A) intravenously, activated charcoal was administered. The patient was conscious on admission, and because only contracted pupils were detected as a symptom indicative of organophosphorus poisoning, it was determined that a total of 17.5 mg (25A) of atropine sulfate would be administered, drip infusion and diuresis would be used, and the patient would be merely monitored. The general condition of the patient did not worsen and the patient did not experience any complications. The plasma cholinesterase activity value 1 IU/L on admission markedly increased to 71 IU/L four days after hospitalization, and thus the patient was transferred to another hospital on the same day.

Since disulfoton could not be detected by GC-NPD following solid phase extraction, it could not be quantified. As for the reason why disulfoton could not be measured, that it is easy for disulfoton to resolve at short time is cause and it thinks that resolve is the thing that the elapse to the hospitalization of this patient was long, too. As for the measurement of disulfoton with this method, it was found to be difficult, similar to DDVP.

## Discussion

In the field of emergency medicine, it is not possible to estimate the type, time and dosage of poisons based only on the symptoms, questions and other evidence, so the swift identification and determination of poisons are crucial in order to make a quick and accurate diagnosis. Of the various poisons, acute poisoning caused by organophosphorus compound occurs frequently in Japan. Depending on the dosage and type of pesticide ingested, some people die of acute poisoning, and then early treatment is an important key in saving the lives of such patients.

Commercially available organophosphorus pesticides often contain more than one compound, so when treating acute organophosphorus poisoning, it is necessary to select a therapy based not only on the blood concentration, but also on the toxicity of organophosphorus compound.

In general, when patients suspected of suffering from acute organophosphorus poisoning are brought into an emergency center, conventional therapies are usually selected consisting of : checking the vital signs, securing a drip infusion line, collecting blood samples, performing X-ray and gastric lavage, inserting a stomach tube, administrating emetics, antagonists, activated charcoal and laxatives. Depending on the severity, it takes about one to two hours to complete these therapies. Nonetheless, as long as columns and standard chemicals are ready, the present assay can be completed within about 1.5 hours (from solid phase extraction to GC–NPD), and thus the present assay is considered to be useful for identifying organophosphorus compound and measuring their blood concentrations.

There have been several studies on the adsorption rate of activated charcoal in adsorption columns.[16)-19)] As far as fenitrothion is concerned, the results of fenitrothion measurements before and after DHP using an activated charcoal column showed this to be a useful therapy. It is thus suggested that to swiftly eliminate organophosphorus compound from the body, continuous DHP should be performed on patients who ingested the pesticides not long before coming to the hospital and who have high blood concentrations. Furthermore, according to an analysis on consciousness disorders in acute organophosphorus poisoning,[20)] consciousness disorders tend to appear when the blood concentration of fenitrothion reached about 0.3-0.4 μg/ml in our cases.

However, to investigate whether or not the clinical symptoms correlate to shifts in blood concentrations, many studies have been conducted to analyze the relationships between the blood concentration of toxins and plasma/serum cholinesterase activities,[21)-23)] in some cases, subjective and objective symptoms and the plasma cholinesterase activity value can not be used as indicators of the severity of acute poisoning, such as in patients with an illness that lowers plasma cholinesterase activity value or those on anticholinergic agents. Therefore, it is important to measure the blood concentrations of toxins.

In any type of acute organophosphorus poisoning, the character of each organophosphorus compound should be known, and it is thus important to do a qualitative analysis, to determine the organophosphorus compound quickly as possible as described above. In the future, by studying more such cases, it will be necessary to determine what type of therapy is most appropriate for treating organophosphorus poisoning patients who have high blood concentrations and those patients who ingested pesticides a long time prior to admission.

## Acknowledgments

This work was supported in part by a fund from the Central Research Institute of Fukuoka University.

## References

1) Tsiropoulos NG, Likas DT, Karpouzas DG. : Liquid chromatographic determination of fosthiazate residues in environmental samples and application of the method to a fosthiazate field dissipation study. *J AOAC Int* 2005 ; 88 : 1827–33.
2) Olsson AO, Nguyen JV, Sadowski MA, *et al*. : A liquid chromatography/electrospray ionization–tandem mass spectrometry method for quantification of specific organophosphorus pesticide biomarkers in human urine. *Anal Bioanal Chem* 2003 ; 376 : 808–15.
3) Pico Y, Louter A. J., Vreuls J. J, Brinkman UA. : On－line trace－level enrichment gas chromatography of triazine herbicides, organophosphorus pesticides, and organosulfur compounds from drinking and surface waters. *Analyst* 1994 ; 119 : 2025–2031.
4) Psathaki M, Manoussaridou E, Stephanou E. G. : Determination of organophosphorus and triazine pesticides in ground and drinking water by solid–phase extraction and gas chromatography with nitrogen－phosphorus or mass spectrometric detection. *J Chromatogr A* 1994 ; 667 : 241–248.
5) Driss M. R, Hennion M. C, Bouguerra M. L. : Determination of carbaryl and some organophosphorus pesticides in drinking water using on–line liquid chromatographic preconcentration techniques. *J Chromatogr* 1993 ; 639 : 352–358.
6) Carabias M. R, Rodriguez G. E, Amigo M.M.J, Hernandez M. J. : Sensitive method for the determination of organophosphorus pesticides in fruits and surface waters by high－performance liquid chromatography with ultraviolet detection. *J Chromatogr* 1992 ; 607 : 37 –45.
7) Molto J. C, Pico Y, Font G, Manes J. : Determination of triazines and organophosphorus pesticides in water samples using solid–phase extraction. *J Chromatogr* 1991 ; 555 : 137–145.
8) Ting, K.－C, Kho P. : GC/MIP/AED method for pesticide residue determination in fruits and vegetables. *J Assoc Off Anal Chem*, 1991 ; 74 : 991–993.
9) Mallet C, Mallet V. N. : Conversion of conventional packed－column gas chromatograph to accommodate megabore columns. *J Chromatog* 1989 ; 481 : 37–44.
10) Neicheva A, Kovacheva E, Marudov G. : Determination of organophosphorus pesticides in apples and water by gas－liquid chromatography with electron－capture detection. *J Chromatogr* 1988 ; 437 : 249–253.
11) Sasaki, K., Suzuki, T. and Saito, Y. : Simplified cleanup and gas chromatographic determination of organophosphorus pesticides in crops. *J Assoc Off Anal Chem* 1987 ; 70 : 460–464.
12) Teixeira H, Proenca P, Alvarenga M, *et al*. : Pesticide intoxications in the Centre of Portugal : three years analysis. *Forensic Sci Int* 2004 ; 143 : 199－204.
13) Kojima T, Yashiki M, Ohtani M, Chikasue F, Miyazaki T. : Determination of dimethoate in blood and hemoperfusion cartridge following ingestion of formothion : a case study. *Forensic Sci Int* 1990 ; 41 : 79–88.
14) Liu, J., Suzuki, O., Kumazawa, T. and Seno, H. : Rapid isolation with Sep–Pak $C_{18}$ cartridges and wide－bore capillary gas chromatography of organophosphorus pesticides. *Forensic Sci Int* 1989 ; 41 : 67–72.
15) Tomojiri S, Kageura M, Kashimura S, *et al*. Analysis of organophosphorus pesticides in whole blood by Bond Elut Certify extraction and gas chromatography with nitrogen–phosphorus detection. *Jpn. J. Forencic Toxicol*. 1997 ; 15 : 194–202
16) Monhart V, Fusek J, Brndiar M, *et al*. Use of hemoperfusion in experimental intoxication with nerve agents. *Artificial Organs* 1994 ; 10 : 770－2.
17) Martinez Chuecos J, Jurado MC, Gimenez MP, Martinez D, Menendez M. Experience with hemoperfusion for organophosphate poisoning. *Crit Care Med* 1992 ; 20 : 1538–1543.
18) Okonek S, Tonnis HJ, Baldamus CA, Hofmann A. Hemoperfusion versus hemodialysis in the management of patients severely poisoned by organophosphorus insecticides and bipyridyl herbicides. *Artificial Organs* 1979 ; 3 : 341–345.
19) Okonek S. Probable progress in the therapy of organophosphate poisoning. *Arch Toxicol* 1976 ; 35 : 221 –227.
20) Fukuzako T, Yamamoto I, Nakano K, *et al*. EEG monitoring in acute organophosphorus intoxication. *Kyukyuigaku* 1989 ; 13 : 389–390.
21) Thiermann H, Szinicz L, Eyer P, Zilker T, *et al*. Correlation between red blood cell acetylcholinesterase

activity and neuromuscular transmission in organophosphate poisoning. *Chem Biol Interact* 2005 ; 157–158 : 345–7.

22) Yamanaka S, Yoshida M, Yamamura Y, *et al*. A study on acute organophosphorus poisoning — Changes in the activity and isozyme patterns of serum cholinesterase in human poisoning—. *Jpn J Hyg* 1993 ; 48 : 955–965.

23) Shimada E, Yoshida M, Yamanaka S, *et al*. Erythrocyte and plasma cholinesterase activities on poisoning of disyston, fenitrothion and malathion. *Jpn j Ind Health* 1986 ; 28 : 368–369.

(Received on November, 2, 2006,
Accepted on March, 30, 2007)