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Case Report

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An Unusual Autopsy Case in Which the Consumption of Organophosphate Insecticide Was Not the Direct Cause of Death

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Abstract

In acute poisoning cases involving the ingestion of organophosphate insecticides such as fenitrothion and malathion, serum cholinesterase (ChE) activity is remarkably decreased, thus representing a useful indicator of the direct cause of death. In the present case, a man in his early 70s tried to committed suicide via the oral ingestion of both fenitrothion and malathion. Fenitrothion and malathion concentrations in cardiac blood were 2.63–2.98 and 0.31–0.58 μg/mL, respectively. However, the serum ChE level was 200 IU/L, which was not considerably lower than the normal range in males (242–495 IU/L). Conversely, we confirmed a positive reaction for *Streptococcus pneumoniae* using a urinary antigen detection kit. Moreover, histopathological analysis of both the left and right lungs revealed extensive inflammatory cell infiltration into the alveolar space. The autopsy and histopathological findings indicated that the direct cause of death was severe bacterial pneumonia caused by the infection of *S. pneumoniae*. This is an unusual autopsy case in which the oral ingestion of both fenitrothion and malathion was not the direct cause of the death, and might have rapidly exacerbated respiratory decline.

Keywords: Bacterial pneumonia, Organophosphate insecticide, Fenitrothion, Malathion, Cholinesterase activity

Introduction

Organophosphates (OPs) have been used as agricultural insecticides globally [1–3]. In humans, the inhibition of acetylcholinesterase (AChE) by OP insecticides, such as fenitrothion and malathion, causes the accumulation of acetylcholine (ACh) at the cholinergic synapses, and leads to the overstimulation of the muscarinic receptors that induces miosis and emesis [2, 4]. Moreover, the inhibition of AChE causes respiratory distress via the depression of respiratory centers in the brainstem [2, 4]. Although patients' pupils were miotic (1–2 mm) in clinical emergency cases of OP poisonings [5, 6], in forensic autopsy cases, miosis was not always been observed owing to the elapsed time after death [7–9]. Conversely, the serum cholinesterase (ChE) activity markedly decreased in cases of fatal OP poisonings [7–9]. Given that the serum ChE can be measured faster and easier than AChE, the measurement of serum ChE has been established as a screening test for the exposure of OP insecticides in the clinical field [10]. Therefore, the measurement of the deceased's serum butyrylcholinesterase (BuChE) levels is used to determine the direct cause of death in acute poisoning cases caused by the ingestion of OPs [10]. In Japan, pneumonia is the fifth leading direct cause of death, and death attributable to pneumonia most commonly occurs in the elderly [11. 12]. In elderly Japanese patients, *Streptococcus pneumoniae*

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(*S. pneumoniae*) is the most frequently detected pathogenic species [13]. Extensive inflammatory cell infiltration into the alveolar space is a typical histopathological finding in the lungs of patients with bacterial pneumonia caused by *S. pneumoniae* [14, 15]. In this study, we report an unusual autopsy case of a man in his early 70s who died of bacterial pneumonia caused by *S. pneumoniae*, and the oral ingestion of both fenitrothion and malathion was believed to have hastened his death.

Case History

One day, a man in his early 70s was diagnosed with dementia in a hospital. Following the diagnosis, he called his wife and expressed suicidal ideation, and went missing on the same day. Five days after his disappearance, he was found dead on a forest road approximately 17 km away from his house. The OP insecticide, which consists of both fenitrothion and malathion, was not found both at the forest road and at his house.

Autopsy Findings

On external examination, the man was 167 cm tall, and he weighed 57.2 kg. The diameters of his left and right pupils could not be measured because of severe corneal opacity. On internal examination, a volatile odor typical of organic solvents was detected from the tongue and bronchi. On the trachea and both the left and right bronchial mucosa, a milky white liquid was attached. A 275-mL of coagulated cardiac blood was noted to be dusky red in color. In the stomach, 250 mL of a greenish white muddy fluid with a volatile odor were found. The left and right lungs weighed 730 and 390 g, respectively, and the inferior lobes of both lungs were muddy white in color. The brain weight was 1477 g, and convolutional atrophy was observed. On the cut surface of the brain, both the left and right lateral ventricles were enlarged. The remaining organs exhibited no pathological findings highlighting the direct cause of death. Hematoxylin–eosin (HE) staining of both the left and right lungs revealed the extensive infiltration of inflammatory cells into the alveolar space of the inferior lobes (Fig. 1A). In the superior lobes of both the left and right lungs, and in the intermediate lobe of the right lung, the infiltration of inflammatory cells into the alveolar spaces of these lobes was also observed. Additionally, detachment of the bronchiolar mucosal epithelial cells was observed in the lungs (Fig. 1B). In the remaining organs, there were no histopathological findings associated with the cause of death.

Serum sample from the autopsy was sent to the SRL laboratory (Tokyo, Japan) and the serum ChE level was 200 IU/L (normal range in males: 242–495 IU/L). For the detection of *S. pneumoniae* antigens in urine to diagnose *S. pneumoniae* infection, an IMMUNOCATCH™ *S. pneumoniae* rapid diagnostic test kit (Eiken Chemical Co., Ltd, Tokyo, Japan) was used, and a positive result was obtained. The alcohol concentration in the cardiac blood and urine was <0.1 mg/mL, as determined by gas chromatography. Toxicological evaluation of the urine was performed with the immunochemical drug screening kit Triage® DOA (Alere, Waltham, MA, USA), which produced negative results for phencyclidine, benzodiazepines, cocaine metabolites, stimulants, cannabis, morphine-based drugs, barbiturates, and tricyclic antidepressants. Furthermore, drug screening using cardiac blood was performed with the LC/MS/MS Rapid Toxicology Screening System Ver. 3 (Shimadzu, Kyoto, Japan). The results revealed the presence of caffeine, fenitrothion, and malathion.

Toxicological Analyses

Chemicals and Reagents

Fenitrothion, fenthion, and malathion were obtained from Sigma–Aldrich (Tokyo, Japan). Acetonitrile, ammonium formate, formic acid (abt. 99%), methanol, and ultrapure water were all analytical grade, and they were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). A methanolic solution of fenthion was used as the internal standard (IS) for both fenitrothion and malathion. Stock solutions of fenitrothion, fenthion, and malathion (mg/mL) were prepared in methanol. All solutions were stored at –20 °C in the dark when not in use. Working standard solutions of the compounds were prepared by combining the aliquots of each primary solution and diluting them with methanol.

Sample Preparation

For drug analyses at the time of autopsy, whole blood was obtained from both the left and the right cardiac chambers. Blank whole human blood, which was purchased from KAC Co., Ltd. (Kyoto, Japan), was screened for fenitrothion, fenthion, and malathion, none of which was detected in the samples. Calibration curves were prepared by spiking whole blood (100 µL) with appropriate volumes of the previously mentioned working standard solutions to produce calibration curve points equivalent to 0.2, 0.5, 1, 5, 10, and 100 µg/mL for fenitrothion and to 0.0005, 0.005, 0.01, 0.05, 0.1, and 0.5 µg/mL for malathion. Quality control (QC) samples at 0.2 (lower limit of quantification; LLOQ), 0.4 (low), 2 (medium), and 50 (high) µg/mL for fenitrothion were prepared in bulk by spiking the appropriate working standard solutions into whole blood (100 µL). Malathion QC samples were also prepared at 0.0005 (LLOQ), 0.001 (low), 0.02 (medium), and 0.2 (high) µg/mL. All samples were extracted using a Micro Volume QuEChERS Kit for LC/MS (Forensic) (Shimadzu). The extraction procedure for QC, calibration curve, and autopsy samples were prepared as described previously [16]. The extracted methanolic solution (1 µL) was injected into the ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) system.

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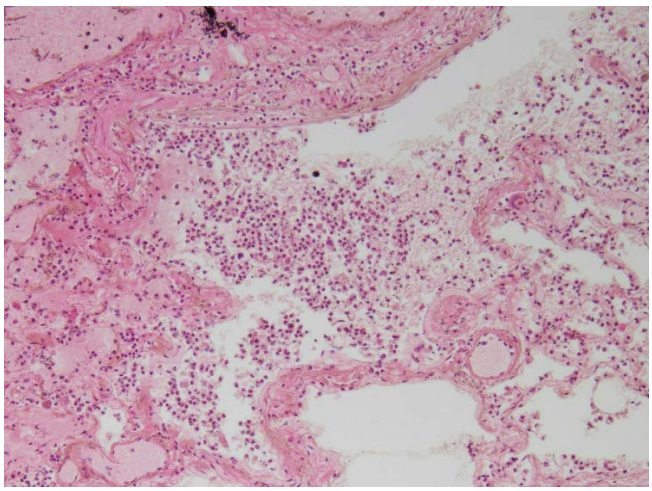
a



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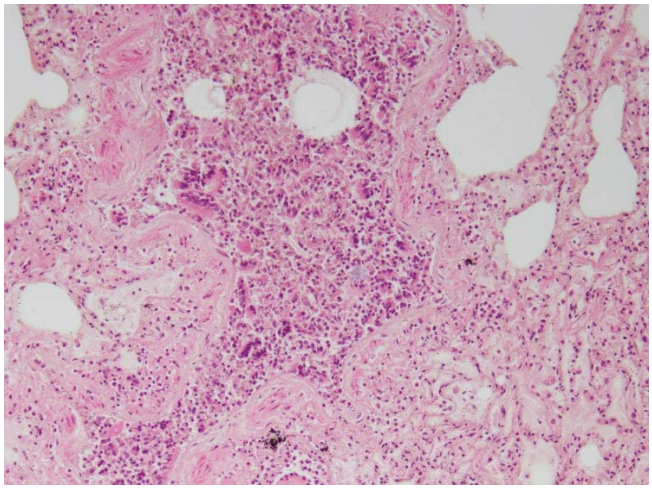

Fig: 1

a. Hematoxylin–eosin (HE) staining shows the inflammatory cell infiltration, distended capillaries and congestion with prominent neutrophilic infiltration, and erythrocytes into the alveolar space of the left lung inferior lobes (×100).

b. HE staining shows the detachment of the bronchiolar mucosal epithelial cells in the left lung (×100).

UHPLC–MS/MS conditions

Qualitative and quantitative analyses were performed using a Nexera X2 HPLC system coupled with an LCMS-8045 triple quadrupole mass spectrometer (Shimadzu). Chromatographic separation was achieved using a Kinetex® XB-C18 column (100 × 2.1 mm i.d.; particle size, 2.6 µm; Phenomenex, Torrance, CA, USA) with a Security Guard ULTRA cartridge system (UHPLC C18 for 2.1 mm ID column; Phenomenex) maintained at 40°C. The mobile phases consisted of 10 mM ammonium formate with 0.1% formic acid in water (A) and in methanol (B). The flow rate was 0.4 mL/min. The elution gradient was 5%–95% B (0–6.0 min), 95% B (6.0–7.5 min), 95% –5% B (7.5–7.6 min), and 5% B (7.6–10 min). The mass spectrometer was operated on the positive mode with an electrospray ionization interface. The ionization source conditions were conducted as described previously [16]. The multiple reaction monitoring (MRM) mode was used to detect analytes. Two product ions (*m/z*), namely a quantifier and a qualifier, were monitored for each compound in the MRM transitions (Table S1). Both the product ions and collision energy were optimized via the post-column infusion of each compound's methanolic solution (Table S1). Labsolutions Insight Ver. 3.10 SP1 software (Shimadzu) was used for the quantitative analysis of all data.

Method Validation

The method was validated according to the guidelines of the US Food and Drug Administration (FDA) [17]. The method's linearity was expressed as the standard curve's correlation coefficient (*r*). The limit of detection (LOD) and LLOQ, which corresponded to signal/noise ratios of ≥3 and ≥10, respectively, were calculated as the concentrations of analytes in QC samples. The matrix effect and recovery were expressed as the mean ± standard deviation. The analytes' matrix effect was determined by comparing the peak area of each analyte extracted from whole-blood samples spiked with working solutions after extraction with that of a neat solution without this extraction. Analyte recovery was determined by comparing the peak area of each analyte extracted from whole-blood samples spiked with working solutions before extraction with that of the compound spiked after extraction. Accuracy and precision were determined by analyzing the QC samples at each analyte concentration over 3 days (six QC sample replicates per day). The inter-day accuracy and precision of the QC samples were obtained from three replications of the intra-day assay. The accuracy of QC sample quantification at each concentration was determined as the percentage of the calculated concentrations obtained from the standard curve. Precision was calculated as the percentage of replicates' coefficient of variation at each concentration used in the inter- and intra-day analyses.

Results

Method Validation

The calibration curve, correlation coefficient, concentration range, LOD, and LLOQ of each analyte are presented in Table S2. Overall, the results obtained with this method using whole blood were linear and sensitive for each analyte. Using this method, the means of the matrix effects and recovery rate ranged 80%–120% for all QC analyte concentrations, and the accuracy and precision validation data (intra- and inter-day combined) were within ±15%,

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excluding LLOQ, which had accuracy and precision values within ±20% (Tables S3 and S4). These validated data met the criteria indicated in the FDA guidelines [17].

Quantification of Fenitrothion and Malathion

Table 1 summarizes the quantitative results for fenitrothion, and malathion, which were detected in left and right cardiac blood from the autopsy. The total amounts of fenitrothion and malathion detected in the stomach were 7.58 and 4.47 g, respectively.

Table 1

		Concentration (μg/mL)	
	Site of the collected blood or serum	Fenitrothion	Malathion
Our case	Right heart blood	2.63	0.31
	Left heart blood	2.98	0.58
Inoue et al. [21]	Serum (Not specified)	2.4 ± 1.2	—
Groszek et al. [22]	Serum (Not specified)	0.47-8.35	—
Yonemitsu et al. [23]	Blood (Not specified)	7.22	—
Yonemitsu et al. [23]	Heart blood	—	2.14
Thompson et al. [24]	Blood (Not specified)	—	1.8
Suzuki et al. [25]	Blood (Not specified)	—	1.89

Discussion

Generally, both fenitrothion and malathion are rapidly metabolized, and their elimination half-lives in blood are 0.8–4.5 h and 3–6 h, respectively [18, 19]. In addition, 10 μg/mL concentration of fenitrothion or malathion decreased by >25% or 100%, respectively, after 24 h at room temperature [20]. Therefore, in forensic autopsy cases of fenitrothion or malathion poisoning, the detected blood concentrations of these compounds may be lower than the ingested antemortem blood concentrations because a certain period has passed since death. Several previously reported fenitrothion or malathion fatal poisoning cases based on cadaveric blood or serum collected from the femoral vein, or the left and right cardiac chambers [21–25] were not clearly determined (Table 1). Therefore, it was difficult to determine the fatal cardiac blood concentrations of fenitrothion or malathion. However, the cardiac blood concentrations of fenitrothion in our case was ranged from 2.63 to 2.98 μg/mL. In view of the time elapsed since his death, we thought that these values were within the range of fenitrothion intoxication concentrations. Conversely, the cardiac blood concentrations of malathion in this case were relatively low compared with the findings in other cases of fatal malathion poisoning (Table 1). In our case, the outside temperature of the area in which he was found was 24.5 °C, and the minimum and maximum temperatures of the area for 5 days until he was found were 18 °C and 23 °C, respectively. Therefore, malathion might have decomposed after his death. However, malathion was detected in his stomach content, and the oral ingestion of malathion was thought to have a modest contribution to his death. Although the intake route for the compounds before his death was unknown, it was speculated that he may have ingested the emulsion given that a mixture of fenitrothion and malathion (named as Sumison emulsion) is been sold as an insecticide in Japan. In clinical emergency OPs poisoning cases, hemorrhagic ulcers of the upper gastrointestinal tract or small intestine perforations have been observed [5, 26]. In fatal OPs poisoning cases, the mucosa of both esophagus and stomach turn brown due to denaturation, and small bleeding spots are observed on the gastric mucosa [27]; however, in our case, we did not observe hemorrhagic ulcer in the stomach mucosa or small intestine perforations.

In humans, there are two types of ChE, AChE and BuChE [10, 28]. AChE eliminates ACh, and is primarily found in red blood cells and in the central and peripheral nervous systems. BuChE, also known as serum ChE, is produced in the liver and is secreted in to serum [10, 28]. The measurement of serum ChE has been used as a screening test for the exposure of OP insecticides in the clinical field [10]. Although the toxicities of both fenitrothion and malathion (following oral administration) are known to be relatively low in mammals, acute poisoning of these compounds in humans is often fatal and life-threatening, and the serum ChE activities were remarkably reduced to a few % of the normal values [18, 21]. Cadaveric ChE levels based on blood samples collected within 24 h after death were slightly decreased [29]. However, in fatal OP poisoning cases, serum ChE levels were decreased considerably at levels below the normal range [7–9]. In our case, the serum ChE level was 200 IU/L, which was not markedly reduced compared with that in previous reported cases of OPs acute poisoning. Generally, serum ChE levels in males gradually decrease with age, ranging 200–450 IU/L in 70-year-olds [30]. Considering the age of the studied subject, this finding appeared to reflect age-related declines in serum ChE activity rather than OP-related decreases.

In Japan, the causative microorganisms of pneumonia in the elderly are more diverse than those in the young [14, 31]. As a parameter indicating the severity of pneumonia, lung weight on autopsy has been considered useful [32]. Patient cases in which pneumonia was the direct cause of death exhibited significantly higher lung weights (520 ± 240 g) than those cases in which non-pneumonia condition was the cause of death (377 ± 144 g) [32]. In the present case, the left lung weight on autopsy was 730 g, which was more obviously weighted than that of non-pneumonia.

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To identify the causative pathogens in fatal autopsy cases of pneumonia, histopathological examination using lung tissue can identify specific pathogens and corroborate the microbiological diagnosis [33]. In autopsy cases of patients who died of pneumonia, the inferior lobes of the left and right lungs were the most common sites of pneumonia [34]. Additionally, as microscopic findings in the patients with pneumococcal pneumonia, intra-alveolar fibrinous exudates with neutrophils and mononuclear cells and marked capillary congestion have been observed [15]. In this case, we observed white turbid inferior lobes in both lungs during the autopsy. Additionally, the remarkable inflammatory cell infiltration, distended capillaries, and congestion with prominent neutrophilic infiltration and erythrocytes were found within the alveolar space of both lung lobes. Because the presence of desquamated bronchiole mucosal epithelial cells in the lungs on histopathology was observed (Fig. 1B), it was believed to reflect the oral ingestion of foreign substances, which were fenitrothion and malathion, and these compounds were aspirated intratracheally. In OP poisoning, the impairment of the diaphragm and thoracic skeletal muscles cause respiratory paralysis, and high ACh concentrations in the central nervous system cause respiratory depression [2]. Therefore, in our case, it may be rapidly exacerbated respiratory decline following the oral ingestion of fenitrothion and malathion.

S. pneumoniae is the most common causative microorganism of community-acquired pneumonia (CAP) in the elderly [13, 15, 31, 32]. The diagnosis of pneumonia caused by *S. pneumoniae* is traditionally obtained through culture-based investigations; however, *S. pneumoniae* is difficult to isolate, and bacteremic pneumococcal pneumonia comprises only one-fourth of all cases of CAP [34]. For these reasons, an *S. pneumoniae* urinary antigen detection kit is recommended for identifying the causative agent in patients with CAP [34, 35]. The IMMUNOCATCH™ *S. pneumoniae* pneumococcal urinary antigen test is a useful tool for the qualitative detection of *S. pneumoniae* capsular antigen [34]. We confirmed a positive reaction for *S. pneumoniae* with this kit using urine collected at autopsy and identified *S. pneumoniae* as the causative agent of bacterial pneumonia.

In conclusion, we determined that the direct cause of death in the present case was severe bacterial pneumonia caused by *S. pneumoniae* infection, and the oral ingestion of both fenitrothion and malathion was further and rapidly exacerbated respiratory function, hastening death. This unusual case provides that organophosphorus pesticide intoxication was not necessarily the direct cause of death.

Ethics Declarations

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with animals performed by any of the authors.

Informed consent

The article does not include participants from whom informed consent was required.

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Supplementary Tables

S1

Retention time (RT), precursor ion, product ion, and collision energy (CE) of fenitrothion, malathion, and internal standard (IS).

* Quantifier ion is described as boldface and the qualifier ion is described with plain fonts.

Compound	RT (min)	Precursor ion (*m/z*)	Product ion * (*m/z*)	CE (eV)
Fenitrothion	5.44	278.05	**136.10**	8.6
			65.10	20.4
Malathion	5.57	331.00	**127.10**	12.6
			285.00	7.8
Fenthion (IS)	6.00	279.05	**169.05**	16.4
			105.15	24.2

S2

Calibration curve, correlation coefficient (r^2), concentration range, limit of detection (LOD), and lower limit of quantification (LLOQ) of the fenitrothion, and malathion.

Compound	Calibration curve	Correlation coefficient (r^2)	Concentration range (μg/mL)	LOD (μg/mL)	LLOQ (μg/mL)
Fenitrothion	y = 0.0578 x - 0.00114	0.999	0.2 - 100	0.1	0.2
Malathion	y = 52.9 x + 0.00547	0.999	0.0005 - 0.5	0.0002	0.0005

S3

Matrix effect and recovery of fenitrothion, and malathion in the whole blood at four quality control concentrations.

LLOQ: lower limit of quantification

Compound	LLOQ		Low		Medium		High	
	Matrix effect (%)	Recovery (%)	Matrix effect (%)	Recovery (%)	Matrix effect (%)	Recovery (%)	Matrix effect (%)	Recovery (%)
Fenitrothion	96.1 ± 2.1	97.5 ± 7.7	95.7 ± 0.8	94.0 ± 2.5	88.1 ± 3.4	100.5 ± 2.1	92.5 ± 1.8	88.5 ± 1.0
Malathion	91.8 ± 4.3	105.6 ± 1.3	91.1 ± 1.2	107.0 ± 2.4	107.6 ± 0.6	99.9 ± 0.7	90.4 ± 1.2	99.3 ± 0.6

S4

Intra- and inter-day accuracy and precision of fenitrothion, and malathion in the whole blood at four quality control concentrations.

LLOQ: lower limit of quantification; CV: coefficient of variation

Compound	LLOQ				Low				Medium				High			
	Intra-day		Inter-day		Intra-day		Inter-day		Intra-day		Inter-day		Intra-day		Inter-day	
	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)
Fenitrothion	110.6	1.6	108.5	4.9	104.7	1.8	104.6	9.2	103.8	1.6	107.0	3.8	107.0	2.2	109.0	4.3
Malathion	94.0	1.1	97.0	1.7	101.5	1.6	95.7	1.7	100.8	3.3	104.3	3.2	104.6	2.5	106.5	4.8