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Accumulation of EPN in Adipose Tissue Following Oral Ingestion

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Abstract

Introduction: We present here an autopsy case involving ingestion of ethyl *p*-nitrophenyl phenylphosphonothionate (EPN), an organophosphate pesticide.

Objectives: To examine whether adipose tissue is useful for identification of lipophilic chemicals.

Results: Toxicological analysis using liquid chromatography tandem mass spectrometry quantified EPN at concentrations of 75 ng/ml in femoral venous blood and an extremely high 51,155 ng/g in adipose tissue.

Conclusion: We concluded that the cause of death was respiratory insufficiency due to EPN poisoning. As EPN accumulates in adipose tissue, this tissue may be useful for identification of EPN at long intervals after ingestion.

Keywords: adipose tissue EPN; insecticide; poisoning;

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INTRODUCTION

Ethyl p-nitrophenyl phenylphosphonothionate (EPN) is an organophosphate cholinesterase inhibitor that has been used as an insecticide since 1950 (1). As a highly toxic organophosphate compound, a case of fatality involving edifenphos and EPN has previously been reported (2). In cases of poisoning, toxicity can be difficult to evaluate from blood concentrations after more than 10 days has elapsed, and the distribution of EPN has not been well described. Here we report an autopsy case following EPN ingestion, and discuss the accumulation of this chemical in tissues.

CASE REPORT

A male in his eighties (height, 159 cm; weight, 55.5 kg) was found unconsciousness in an irrigation canal and was transported to hospital for treatment. Since serum cholinesterase activity was below the lower limit of measurement, acute organophosphate poisoning was suspected. He died approximately 10 days after admission, despite intensive therapy. Medico-legal autopsy revealed no evidence of external injury. The heart weighed 379 g, and the left and right lungs weighed 449 g and 590 g, respectively, with severe congestion and atelectasis of the lower lobes. The brain weighed 1398 g, and was slightly edematous. Histological examination revealed congestion of each organ. Pleural effusion was collected from the thoracic cavity (left, 60 ml; right, 250 ml). Stomach contents comprised approximately 80 ml of greenish liquid, without food residue and solvent odor. No residual urine was present in the bladder. Samples of blood (femoral vein, right heart chamber and aorta), pleural effusion (left and right thoracic cavities), tissue specimens (brain, lung, liver and adipose tissue), cerebrospinal fluid, bile and stomach contents were collected for toxicological examinations.

A headspace gas chromatography mass spectrometry (HS-GC/MS) system (QP-2010 Plus; Shimadzu, Kyoto, Japan) was used to screen for volatile compounds (3-5). Quantitation of ethanol was performed using headspace gas chromatography. Subsequent toxicological analysis using liquid chromatography tandem mass spectrometry (LC-MS/MS) was performed as described previously (6). Briefly, liquid chromatography separations were carried out using ekspert^{TM} ultraLC 100-XL (Eksigent part of Sciex, Framingham, MA). An L-column2 ODS (1.5 mm × 150 mm, 5.0 μm particle size; Chemicals Evaluation and Research Institutes, Tokyo, Japan) was used with a mobile phase of solvent A (5% methanol containing 10 mM ammonium formate) and solvent B (95% methanol containing 10 mM ammonium formate) with a flow rate of 0.1 mL/min. A QTrap$^{®}$ 4500 tandem mass spectrometer (Sciex) was used to obtain mass spectra.

RESULTS AND DISCUSSION

In the present case, isomers of xylene were identified by HS-GC/MS from adipose tissue and blood, suggesting ingestion of insecticides or

pesticides (3-5). Toxicological analysis using LC-MS/MS identified EPN in blood and other postmortem specimens. Table 1 shows the quantitation of EPN in each sample.

Table 1. Concentrations of EPN in each sample (ng/ml or ng/g)

	EPN
Femoral venous blood	75
Blood in aorta	85
Heart blood (right)	38
Pleural effusion (left)	31
Pleural effusion (right)	26
Cerebrospinal fluid	BDL
Bile	BDL
Stomach contents	BDL
Brain	247
Lung	95
Liver	BDL
Adipose tissue	51155

BDL: below the detection limit

The femoral blood concentration of EPN in the present case was very low, possibly due to metabolism. However, the EPN concentration in adipose tissue was extremely high compared to other specimens. As EPN is highly lipophilic (7), accumulation will occur in adipose tissue.

Adipose tissue acts as a reservoir, and redistribution of EPN from adipose tissue to blood would occur when blood levels decrease (8,9). These physicochemical properties may result in protracted intoxication by highly lipophilic chemicals. Since lipophilic chemicals such as butane or methidathion accumulate in adipose tissue (10,11), and are thus somewhat protected from metabolism (9), samples of adipose tissue are useful for identification of chemicals (10) and fat biopsy can be performed for toxicological diagnosis (11).

EPN concentration was lower in pleural effusion than in blood. As concentrations of chemical in pleural effusion are unrelated to the blood concentration (12), pleural effusion may be not worth sampling for quantitative analyses. The distribution of EPN in each tissue may depend on the fat content of that tissue. EPN would thus accumulate in brain.

Based on the autopsy findings and the results of subsequent examinations, including toxicological investigations, we concluded that the cause of death was respiratory insufficiency due to EPN ingestion. Accumulation of EPN in adipose tissue was quantified approximately 10 days following ingestion. Since chemicals in adipose tissue are relatively protected from metabolism and thus remain stable (9), this tissue may represent a valuable sample for confirming EPN ingestion.

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Conflict of Interest Statement: The authors declare that they have no conflict of interest.

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