

# **Studies on the cause of an encephalopathy which took place in the limited areas in Japan 2004: A possibility of poisoning by cyanide being contained in a kind of mushroom.**

**KUNIO GONMORI<sup>1,2)</sup>, KAYOKO MINAKATA<sup>1)</sup>, HIDEKI NOZAWA<sup>1)</sup>, SHIGERU SATO<sup>2)</sup>, ITARU TOYOSHIMA<sup>2)</sup>, NORIYUKI MATSUMOTO<sup>3)</sup> and OSAMU SUZUKI<sup>1)</sup>**

1) Department of Legal Medicine, Hamamatsu University School of Medicine, Japan

2) Investigative Committee of Acute Encephalopathy in Akita Prefecture, Japan

3) Niigata Prefectural Forest Research Institute, Japan

**Corresponding author:** [gonmori@hama-med.ac.jp](mailto:gonmori@hama-med.ac.jp)

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## **INTRODUCTION**

In autumn 2004, a strange encephalopathy appeared for people living in the districts along the Japan Sea (Akita, Yamagata and Niigata Prefectures); 19 died and 36 were hospitalized. Unfortunately, the cause of the encephalopathy is still not known. However, there were two clues to clarify the cause of the disorder; one is that 96% of the patients ate mushrooms of *Pleurocybella porrigens* and the other is that 85% of them were suffering from kidney insufficiency. In our previous experiments, we could identify hydrogen cyanide (HCN) in the mushrooms by gas chromatography (GC)/mass spectrometry (MS). Therefore, the aim of this study is to test if HCN in the *Pleurocybella porrigens* can be a cause of the encephalopathy by making quantitative analysis of HCN in the mushrooms harvested at various locations including the above Japan Sea coast districts.

## **METHODS**

The mushrooms of *Pleurocybella porrigens* (Figure 1) were extensively harvested at 10 locations in Akita, 8 locations in Niigata and one location in Shizuoka. The frozen mushrooms (0.5 g) were minced with scissors and put into a small glass vial, containing 1.0 ml water, 0.05 ml internal standard solution (propionitrile 0.08 mg/ml), 0.1 g ascorbic acid and 0.3 g sodium sulfate. After each vial was capped, it was heated at 55 C for 20 min. A 2.5 ml volume of the headspace vapor was injected into a GC port. The GC conditions were: instrument, HP-6890; detector, NPD; column, Supel-Q PLOT fused silica capillary (30 m x 0.32 mm i.d., Supelco); column temperatures, 50 C (1 min-hold) – 10 C/min – 120 C (6 min-hold); injection temperature, 200 C; detector temperature, 250 C; carrier gas, He (2 ml/min).

**Table 1.** Concentration of HCN in the harvested *Pleurocybella porrigens*

Prefecture	Locations	HCN (mcg/g)
AKITA	near to the patient harvested tree	14.9
		1.6
		1.9
		5.0
		0.9
		3.1
		2.1
		1.0
		12.6
		5.0
NIIGATA	patient harvested tree	66.1
	near to the patient harvested tree	14.2
	near to the patient harvested tree	41.0
	near to the patient harvested tree	4.7
		27.0
		5.9
		5.6
		3.7
SHIZUOKA		9.8

## RESULTS AND DISCUSSION

The concentration of HCN in the mushrooms ranged from 0.9 micro g/g to 66.1 micro g/g; the mushrooms harvested at the locations near to those, where the encephalopathy patients had appeared, showed higher HCN levels than those harvested at far-away locations. The mushroom harvested from the same tree as the patient's one showed even higher HCN concentration (Table 1).

In this study, we have been able to detect HCN in mushrooms *Pleurocybella porrigens*. However, their levels seem too low to ascribe the cause of the encephalopathy to poisoning by HCN only being contained in the mushrooms. Now, we are considering the influences of kidney insufficiency, because excretion disturbance may enhance the toxicity of HCN, and have just begun the experiments of such a line.



**Figure 1. *Pleurocybella porrigens***