CASE REPORT

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Pesticide Poisoning Initially Suspected as a Natural Death

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ABSTRACT: A pesticide poisoning victim suspected initially as having died a natural death was autopsied. The victim was a 47-year-old male. Macroscopically, signs of acute death and, in particular, general erosion in the mucosa of the airways and esophagus were observed. In the gastric contents, which had a pungent smell and a greenish-brown color, 5.00 g/L of propanil, 1.27 g/L of carbaryl, 0.38 g/L of ethylbenzene, and 0.32 g/L of xylene were detected. In the blood (serum), 21.6 mg/L of propanil, 8.1 mg/L of carbaryl, 1.7 mg/L of ethylbenzene, and 4.0 mg/L of xylene were identified. Postmortem methemoglobinemia (45%) was recognized. The cause of death was considered to have been pesticide poisoning; propanil was probably most responsible for his death. The police considered the case to be "death with illness as the suspected cause." By performing an autopsy, however, we were able to clarify that the cause of death was pesticide poisoning.

KEYWORDS: forensic science, forensic pathology, death, poisoning, propanil, carbaryl, pesticide, ethylbenzene, xylene

A pesticide poisoning case was investigated by the authors. The deceased was considered to have died from illness at first, but the cause of death was determined by autopsy to have been poisoning by pesticide [(DCPA; *N*-(3,4-dichlorophenyl) propanamide: propanil) and (NAC; 1-naphthalenol methylcarbamate: carbaryl) mixture]. Fatal propanil and carbaryl mixture poisoning cases are rare (1), and such cases are toxicologically significant. Herein, we present a fatal case of propanil and carbaryl mixture poisoning.

Case Report

The deceased was a 47-year-old male. He was found dead in his bed in the dorsal position with his eyes open, at 8 a.m., by his mother. Fine foam was seen in his nostrils and mouth. The last confirmation of signs of life was when he was heard snoring loudly at about 8 p.m. of the previous day. His medical history was not remarkable except that he had had a slight cold that had started a few days earlier. The time of his death was estimated to have been about 12 a.m. of the day he was found, on the basis of the rectal temperature and postmortem changes of the body. The autopsy was performed on the same day to investigate the cause of death.

Autopsy Findings

The deceased was 168 cm tall, 55 kg in weight, and his nutritional status was moderate. Postmortem rigidity was marked in all joints. There was moderate dark reddish-purple colored lividity on the dorsal surface of the whole body, which showed slight discoloration in response to finger pressure. The palpebral conjunctivae showed pallor on both sides, and petechiae were not observed. Both pupils were 5 mm in diameter and corneal opacity was moderate. Fine foam was abundantly seen in the nostrils and the oral cavity, and the mucosa was slightly erosional. Abnormal findings such as injury were not recognized on the body surface.

In the internal findings, the weights of the left and right lungs were 950 g and 880 g, respectively, and both lungs were congestive and edematous. A large quantity of fine foam was seen in the throat, trachea, and the esophagus, and the mucosa was erosional. Gastric contents were a greenish-brown color of approximately 350 mL in volume, and had a peculiar pungent smell, and the mucosa of the stomach was erosional. The heart weighed 330 g and contained approximately 360 mL of dark red fluid blood with no blood clotting. Slight atherosclerotic deformation in the major branch of the coronary artery was recognized. The weight of the brain was 1220 g and slight edema was recognized. No other organs showed abnormalities and were without congestion and edema. Slight atherosclerosis was recognized in the aorta and basal artery of the brain. Because clear abnormal findings by autopsy were provided in the gastric contents, we considered the possibility of some intoxication and performed various examinations.

Histological Findings

Alveolar bleeding with congestion in the lungs was recognized. Eosinophilic changes and slight fibrosis of the myocardium were also recognized. The tracheal, esophageal, and gastric mucosae were partially peeled off, and inflammatory reaction was observed in the mucosae. Remarkable changes were not observed in other organs.

Biochemical Examination

Choline esterase activity in the serum was measured at 1256 IU using a choline esterase determination kit (Wako Pure Chemical

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Industries, Ltd., Osaka, Japan). The value was within antemortem normal levels in serum (1140 to 1590 IU).

Methemoglobin concentration in the blood was approximately 45%, detected by the method of Matsubara (2) (antemortem normal level: less 1%).

Toxicological Examination

Chemicals

Propanil and carbaryl were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and *o*-, *m*- and *p*-xylene were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The other reagents were of analytical grade and were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Conditions of GC/MS and GC

Gas Chromatography/Mass Spectrometry (GC/MS) Ionization—Shimadzu QP1000EX type gas chromatograph with electron impact mass spectrometer. Column: a slightly polar fused silica capillary column (15 m × 0.25 mm inside diameter, film thickness 0.25 μm, DB-5). Data processing was performed with a NIST/EPA/NIH mass spectral database (NIST) in the scan mode monitoring m/z ions from 30 to 500. The GC/MS was operated in the splitless mode for 1.0 min with a helium carrier gas linear velocity of 1 mL/min. Propanil and carbaryl analysis: initial oven temperature was 70°C for 3 min with an injection port temperature of 220°C. The temperature was ramped at 20°C/min to a final temperature of 250°C, which was held for 20 min. Ethylbenzene and xylene analysis: the oven temperature was constant at 40°C with an injection port temperature of 150°C.

GC Instrument—Shimadzu gas chromatograph (GC) 14 A (detector: hydrogen flame ionization). Column: an intermediately fused silica capillary column (15 m × 0.53 mm inside diameter, film thickness 1.5 μm, DB-1701). Propanil and carbaryl analysis: initial oven temperature was 100°C for 3 min with an injection port temperature of 220°C. The temperature was ramped at 8°C/min to a final temperature of 200°C, which was held for 5 min. Ethylbenzene and xylene analysis: the oven temperature was constant at 40°C with an injection port temperature of 150°C.

Propanil and Carbaryl

Qualitative Analysis—The preparation used 1 mL for gastric contents and blood (serum). As preliminary treatment, a Sep-Pak C_{18} cartridge was activated with methanol and added to each preparation (3). After washing with pure water, the samples were eluted with a solvent of dichlormethane-methanol (9:1). After evaporation, the residue was dissolved in 1 mL of methanol. An aliquot (1 μL) was injected into the GC/MS.

Quantification—After each sample of gastric contents and serum was taken, 200 μL (diluted with 800 μL of water), 10 μg of malathion (internal standard: I.S.), and 10 mL of dichloromethane were further added, and were stirred using a blender and shaken for 15 min at room temperature. The sample mixtures were filtered with a glass filter, and the dichloromethane layer was extracted after centrifugation at 10 000 g for 10 min. The sample mixtures were concentrated and exsiccated under decompression. The residues were added to 5 mL of hexane and 5 mL of acetonitrile, and the acetonitrile layer was collected with a separating funnel, and extracted with acetonitrile two more times. After concentration and exsiccation were done under the decompression of acetonitrile extract, the residue was dissolved in 1 mL of acetone, and 1 μL of the sample was injected into the GC (4). Ten μg of malathion (I.S.) and 10 mL of dichloromethane were added to separate 1.0 g samples of the brain, lungs, liver, kidneys, spleen, heart, pancreas, and fatty tissue (venter). The samples were then homogenized in a mini-adapted Waring blender. The sample mixtures were obtained with the same method as mentioned above, and 1 μL was injected into GC.

Xylene and Ethylbenzene

Qualitative Analysis—Gastric contents (0.1 mL) were added to a 10 mL vial, and 0.5 mL of 20% olive oil and 2.0 mL of 1.0 N NaCl were added. The vial was sealed and shaken at 35°C for 2 h, and underwent injection of 0.1 mL of the gas phase in GC. Each gas phase of standards (*o*-, *m*-, *p*-xylene and ethylbenzene) was injected in GC (5).

Quantification—One mL of whole blood and gastric contents, added to separate 1.0 g samples of the brain, lungs, liver, kidneys, spleen, heart, pancreas, and fatty tissue (venter), was collected and measured as in the qualitative analysis.

Results

Propanil and Carbaryl

Qualitative Analysis—The EI mass spectra of the authentic standard of propanil and carbaryl gave molecular ion peaks of m/z 217 and 201, and fragmentations of m/z 161, 57, and 32, and m/z 144, 115, 57, and 32, respectively (Fig. 1*b*,*c*). The EI mass spectrum of each peak of the gastric contents provided on the total ion chromatogram (TIC) was identified with propanil and carbaryl by NIST/EPA/NIH mass spectral database (Fig. 1*d*,*e*). The EI mass spectra of the gastric contents and the serum showed the same EI mass spectra of propanil and carbaryl authentic standard, respectively (Fig. 1*d*–*g*), and thus propanil and carbaryl were detected from the serum and the gastric contents, respectively.

Quantification—Propanil, carbaryl, and malathion (I.S.) were well separated at the retention times of 12.813 min for propanil, 13.083 min for carbaryl, and 14.163 min for malathion (Fig. 2). Each propanil, carbaryl, or malathion standard was detected as a single peak at the assay condition, and the peaks of degradations were not recognized at all. The calibration curve was made using propanil or carbaryl-spiked blood and tissues obtained from a drug-free cadaver, which produced fairly straight lines against known quantities of propanil in the 2 to 30 mg/L range ($r = 0.998$) and carbaryl in the 2 to 30 mg/L range ($r = 0.998$). Homologous peaks of propanil and carbaryl standards were detected from the gastric contents and the serum. Similarly, homologous peaks were detected from each organ and fatty tissue. The quantitative results of propanil and carbaryl are summarized in Table 1. It was revealed that propanil and carbaryl existed in concentrations of approximately 2.5 to 4.4:1 in all samples. The brain had the highest levels of propanil and carbaryl of all collected organs.

Xylene and Ethylbenzene

As a result of qualitative analysis, peaks I-VI were detected on GC chromatograms from the gastric contents and the blood, as

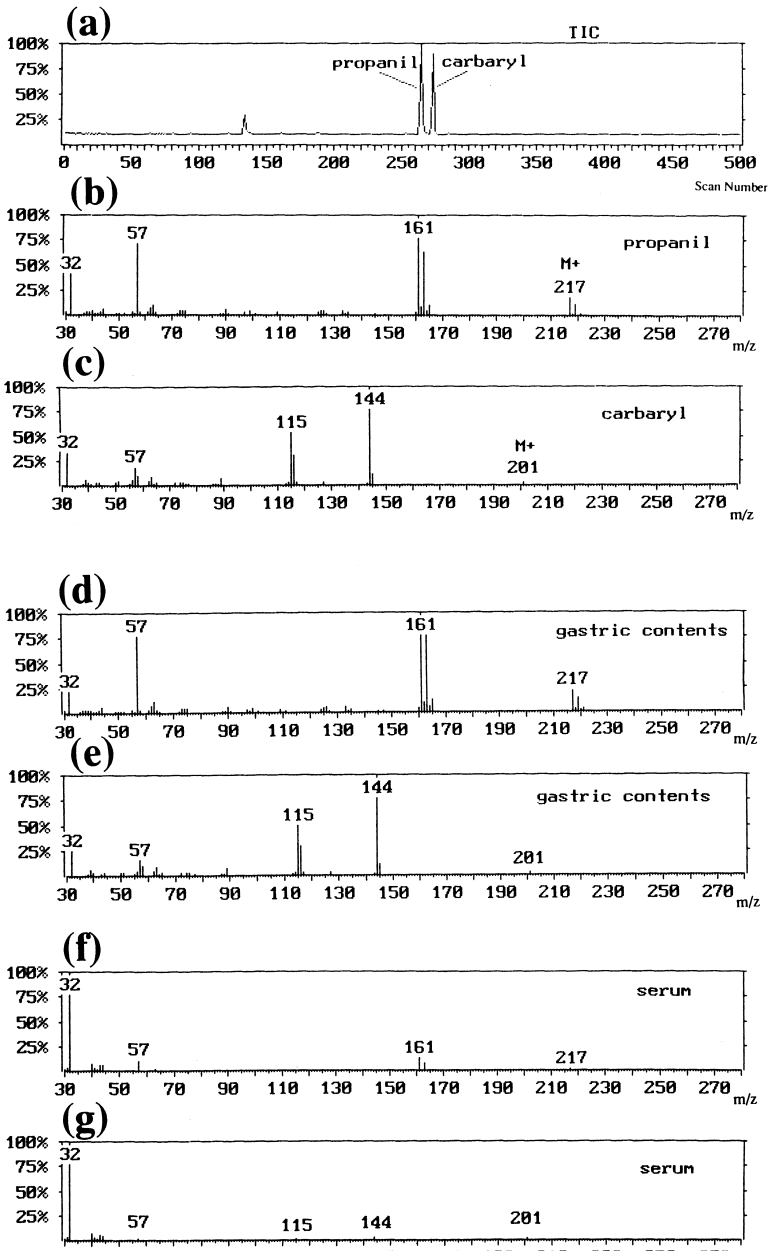

FIG. 1—*Total ion chromatogram obtained from propanil and carbaryl authentic standards* (a) *EI mass spectra of extract from propanil and carbaryl authentic standards* (b, c); *gastric contents* (d, e); *and serum* (f, g).

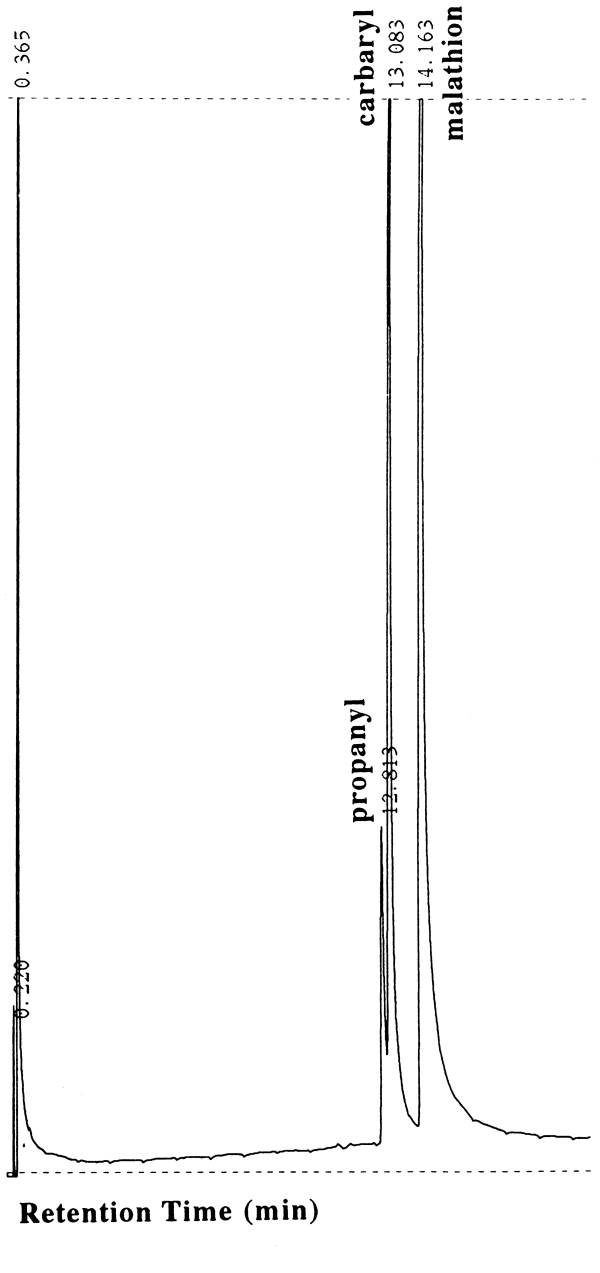

FIG. 2—*Gas chromatograms of authentic standards of propanil (10 ng), carbaryl (10 ng), and malathion (I.S.; 10 ng).*

shown in Fig. 3*b* and *c*. These peaks were assigned to be ethylbenzene (I, VI), *m*- or *p*-xylene (II, V), and *o*-xylene (III, VI) of the relative retention times (Fig. 3*a*). The existence of ethylbenzene, *m*-, *p*-xylene, and *o*-xylene was confirmed from the gastric contents.

The results of quantification are shown in Table 1. Both xylene and ethylbenzene were most abundantly detected in the liver.

Discussion

Propanil is a typical aniline pesticide. When propanil is hydrolyzed by acylamidase in the liver, it produces 3′,4′-dichloroaniline (one of the aniline derivatives), and the product produces methemoglobinemia (6). It is suggested that 3′,4′-dichloroaniline metabolizes to *N*-hydroxy-3′,4′-dichloroaniline, which is a highly reactive product by microsomal *N*-hydrolysis. Therefore, methemoglobinemia by propanil intoxication suggests that 3′,4′-dichloroaniline is formed by hydrolysis of propanil by acylamidase and follows metabolism by microsomal oxidase (7). The LD_{50} value of propanil in *i.o.* in rats is 1384 mg/kg (8). Propanil has mucosal irritant action, and it is assumed that it also has central nerve inhibitory action (1).

On the other hand, carbaryl is a carbamate ester, and its toxicity depends on acetylcholine accumulation by inhibition of choline esterase activity for the carbamyl group of the carbaryl noncovalent bond in choline esterase (9). However, the time at which the carbamyl group of carbaryl is removed from choline esterase and the esterase activity is recovered is relatively short compared to that of organophosphorus esters, and the half-life of recovery is 30 to 60 min (9). The LD_{50} value of carbaryl by *i.o.* of rats is 233 to 850 mg/kg (9). Regarding clinical symptoms of carbaryl intoxication, miosis, bronchoconstriction, and lung edema occur as muscarine action. Subsequently, contraction, tachycardia, and elevation of blood pressure appear as nicotine-like actions. Headache, convulsion, coma, and respiratory depression appear as central nervous system depressive symptoms (9). Carbaryl is known to suppress not only choline esterase activity but also acylamidase activity (9). When propanil and carbaryl are mixed, degradation of propanil would be delayed as a result of suppression of acylamidase activity by carbaryl (1,9). Therefore, the pesticide's toxic effect is increased.

After the existence of propanil and carbaryl in the gastric contents by toxicological examination was confirmed, the police investigated the deceased's home again. A 100 mL bottle of a propanil and carbaryl mixture was found on a shoe cabinet in the deceased's home, and the residual quantity was about 50%. The bottle of the pesticide contained 25% (v/v) propanil, 5% (v/v) carbaryl, and 70% (v/v) organic solvent and/or emulsifier, according

TABLE 1—*Distribution of the pesticides and organic catalyzers in body fluids and organs of the victim.*

Organ (mg/kg or L)	Propanil	Carbaryl	Ethylbenzene	*m, p*-Xylene	*o*-Xylene	Xylene (Total)
Lung	38.4	12.4	1.5	2.3	1.3	3.6
Liver	93.3	21.0	22.5	20.4	8.3	28.7
Kidney	77.8	23.3	10.2	8.8	3.5	12.3
Brain	129.7	34.8	4.9	6.0	2.6	8.6
Spleen	69.8	20.7	1.5	2.5	1.3	3.8
Pancreas	81.5	19.7	16.2	12.5	4.9	17.4
Heart	107.4	34.6	6.8	6.1	2.5	8.6
Fatty tissue	51.4	20.2	14.2	10.0	3.6	13.6
Gastric contents	5003.0	1271.0	381.3	245.3	76.0	321.3
Blood (serum)	21.6	8.1	1.7	2.7	1.3	4.0

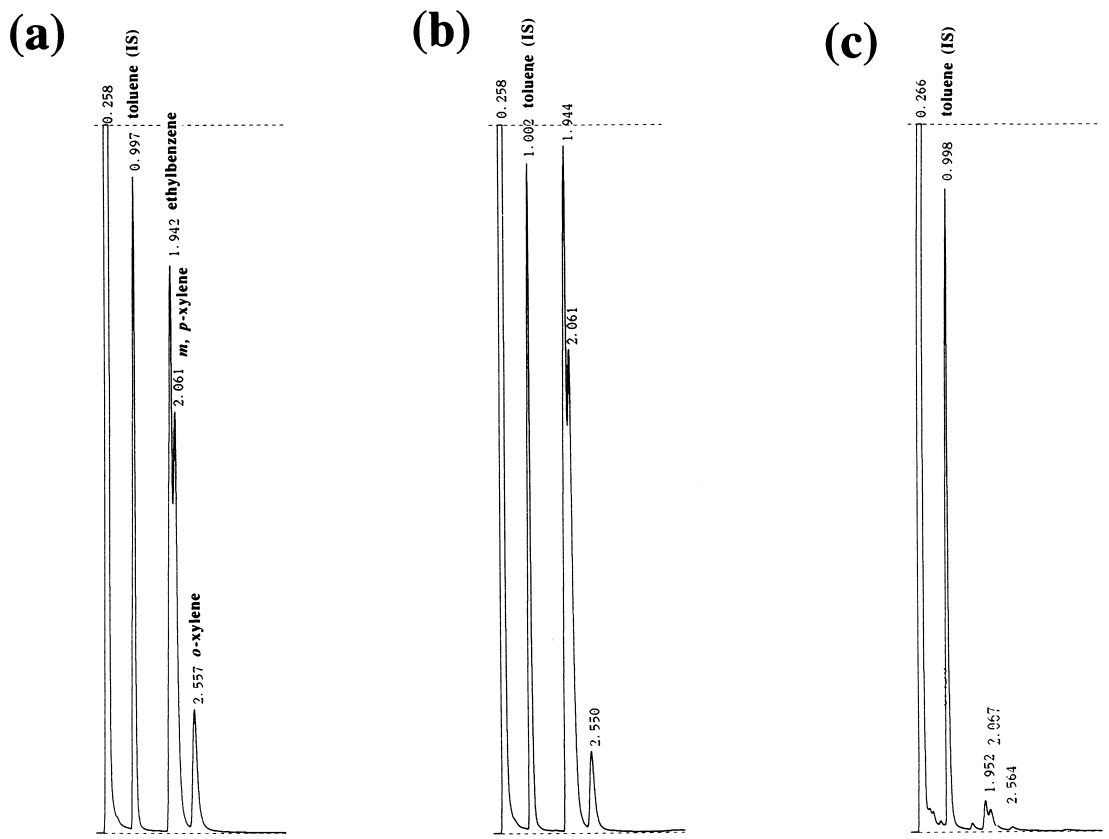

FIG. 3—(a) *Gas chromatograms of authentic standards of ethylbenzene (10 ng),* m- *and* p-*xylene (10 ng),* o-*xylene and toluene (I.S.; 10 ng).* (b) *Gas chromatograms of the gastric contents. Peaks: (I) ethylbenzene, (II)* m-, p-*xylene and (III)* o-*xylene.* (c) *Gas chromatograms of the serum. Peaks: (IV) ethylbenzene, (V)* m-, p-*xylene and (VI)* o-*xylene. Concentrations are given in Table 1.*

to the list of ingredients. Therefore, it seemed that propanil, carbaryl, xylene, and ethylbenzene, which were detected in the gastric contents, were not contradicted as the cause of death, for each was in this bottle of pesticide.

Unfortunately, a suicide note the deceased left was not found by the police scene investigations. The deceased did not show signs of emotional change in the several weeks before his death. A possible motivation for ingesting the pesticides was still unclear after a close police investigation.

As for the causes of depression of the central nervous system in this case, (1) the hypoxial state of the organs with high methemoglobinemia, (2) the toxicity of propanil itself, (3) neurological disorder with carbaryl intoxication, and (4) the influence of an organic solvent are considered.

Conclusions

(1) Generally speaking, clinical symptoms of methemoglobinemia are shown when methemoglobin concentration in the blood is 15 to 20%, cyanosis becomes clear, and at 20 to 45%, headache, drowsiness, dizziness, and dyspnea are produced, and central nervous system depression is worsened at 45 to 50%, and arrhythmia, shock, and coma appear at 55 to 70% (1,10). In our case, 45% methemoglobin was detected in the blood, and this level is definitely capable of producing methemoglobinemia. However, the lack of stability of methemoglobin (11) is an important factor in evaluating postmortem methemoglobinemia. Both formation and reduction of methemoglobin can occur with postmortem time and after sampling of cadaverous blood (12). The methemoglobin formed is rapidly reduced to hemoglobin by intraerythrocytic methemoglobin reductase when blood samples are stored as whole blood without freezing (12). Methemoglobin formation can also occur by autoxidation or by putrefaction after sampling (12). Therefore, to prevent both formation and reduction of methemoglobin, the concentrations of it are determined as soon as possible (12). In our case, the samples of the whole blood were stored at −80°C. Sato et al. (13) showed that methemoglobin formation by autoxidation was inevitably observed in whole blood samples stored at −30°C, whereas methemoglobin concentrations were stable stored at −80°C or −196°C. They stressed that the storage at −80°C or −196°C was suitable for long-term storage of blood samples from autopsy cadavers for methemoglobin determination. Therefore, it seems that the detected value is a considerably reliable one. Indeed, the value itself doesn't indicate the direct certification of advanced methemoglobinemia at the time of death, but the possibility of advanced methemoglobinemia at the time of death cannot be denied.

(2) There is some suppression of inhibitory effects on the central nervous system by propanil itself. There are some cases in which loss of consciousness is seen in spite of absence of methemoglobinemia (1). Also, there have been a few fatal propanil poisoning cases even at relatively low concentrations of methemoglobinemia (14). Furthermore, when propanil is administered after having suppressed acylamidase in animal experiments,

methemoglobinemia can be prevented, but the death rate cannot be decreased (7).

(3) Serum choline esterase activity in our case did not show any differences compared to the activities of antemortem normal levels. It is known that postmortem hematocyte and serum choline esterase activities are extremely stable (15). Therefore, this value seems to reflect the level at the time of death. It is supposed that there is little influence of choline esterase inhibition by carbaryl when it is considered that the amount of carbaryl in the pesticide is relatively low (5% of contents in the bottle).

(4) As other possible substances which lead to poisoning, xylene and ethylbenzene were detected from gastric contents, blood, and various tissues. Both xylene and ethylbenzene have anesthetic effects and depress the central nervous system. The LD_{50} value of xylene (three kinds of isomer mixtures) of rats by *i.o.* administration is 4.3 g/kg (16), and it is considered that the lethal concentration of xylene in human blood is 3 to 40 mg/L (17). Because 4.0 mg/L of xylene was detected in the blood, it seems that the possibility of xylene poisoning cannot be completely denied. Because the LD_{50} value of ethylbenzene of rats by *i.o.* administration is 3.5 g/kg (18), it is assumed that the toxicity of ethylbenzene is comparatively low. There have not been any reports of fatal ethylbenzene poisonings, and the lethal level of ethylbenzene in blood is unclear. Therefore, it could be thought that there was little possibility of ethylbenzene poisoning, in consideration of the LD_{50} value of rats.

By the above-mentioned considerations, regarding central nervous system depression by the pesticides, it is suspected that the most influential cause was the effect of propanil (methemoglobinemia and the toxicity of propanil itself).

With anatomical and histological findings, congestion was recognized in each organ, and advanced congestion and edema were especially recognized in the lungs. The anatomical findings of typical sudden death were observed: congestion of various organs, petechiae under the caput subcutis, the pelvis renalis tunica mucosa, and serosa of various organs, heart blood of dark reddish-color (chocolate-like color) and flowability. These anatomical findings are thought to have been caused by anoxemia by central nervous system depression and methemoglobinemia by these pesticides. The patient finally would have died from respiratory and circulatory failure. Therefore, the cause of death was determined to have been poisoning by having ingested a pesticide, and in particular, propanil was most probably responsible for the death.

Fatal cases of propanil intoxication are relatively rare, and there are few reports of such cases (14). Serious illness levels by propanil poisoning do not always run parallel with the dose of the pesticide (14). In other words, species specificity and individual difference of metabolic rates of propanil in people are regarded as major factors, in addition to the quantity of emesis and the presence of care (14).

Approximately 1.75 g of propanil existed in the gastric contents of the deceased. Because propanil was detected in all organs inspected, as well as fatty tissue and serum, the sum of propanil in the deceased was estimated to have been about 2.42 g. The dose of the pesticide was estimated to be at least more than approximately 9.7 mL, on the basis of the concentration ratio. In consideration of postmortem or antemortem degradation of propanil in the body, or both, the estimated dose is not contradicted by the condition of the bottle found, that contained about 50% of the original quantity.

The family did not notice that the patient had drunk the pesticide, and the police treated the death as a "death suspected to have been caused by an illness" at first. By performing an autopsy, we were able to make clear that the cause of death was pesticide poisoning. Our case indicates the importance of autopsy to determine the cause of death (19).

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