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CASE REPORT

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Acephate in Biological Fluids of Two Autopsy Cases after Ingestion of the Chemical

ABSTRACT: Two autopsy cases, where the individuals were suspected of having ingested acephate, an organophosphorous insecticide, are reported. Acephate and its active metabolite, methamidophos (MP), were analyzed in the biological fluids by GC/MS, using the salting out method with liquid-liquid extraction columns. The first case was that of a 70-year-old man whose blood acephate was 149 μg/mL, and MP was 3.0 μg/mL. Serum pseudocholinesterase (ChE) activity was inhibited. No remarkable finding of injury or disease was determined as the cause of his death, but acute poisoning by acephate was mostly suspected. The second case was that of a 60-year-old man. A deep gash in the left neck injured the left common carotid artery in addition to the severely ischemic state of the primary organs. His blood acephate was 46 μg/mL, and MP was not detected. ChE activity was in the normal range. Hemorrhage was mainly suspected as the cause of his death. The concentrations of acephate and MP in human blood after oral ingestion are first reported here, and the acute toxic level of acephate is discussed.

KEYWORDS: forensic science, acute poisoning, acephate, methamidophos (MP), organophosphate (OP), analysis, GC/MS

Acephate (*O*, *S*-dimethyl-acetylphosphoramidothioate or *N*-ethoxy (methylthio) phosphinoylacetamide) (Orthene®, Ortran®) is one of the most popular organophosphorous (OP) insecticides, and is widely used and easily obtained. In soil and plants, acephate is converted into metabolic products, primarily methamidophos (*O*, *S*-dimethyl-phosphoramidothioate), which is a very potent anticholinesterase agent (1,2). The insecticidal action of acephate has been related to its conversion to methamidophos (MP) through decarboxyamidase or to a combined anticholinesterase effect of acephate and MP (2,3). Compared to insects, acephate in mammals shows low to moderate acute toxicity, due to its decreased conversion into MP (2). It has also been mentioned lately that mammals have differential feedback inhibition of MP to decarboxyamidase, which results in suppressive production of MP from acephate (4). Studies in man have been very limited. Summarized reports made available in the literature issued by the Food and Agriculture Organization of the United Nations (FAO), indicate that MP was present in the urine together with other metabolites in subjects exposed to acephate in formulating plants (Swencicki and Hartz 1972 quoted in FAO 1977 (5)). Urinary acephate was also reported in four workers at a factory producing acephate (6). When they were exposed to air with acephate in a concentration between 0.278 and 2.170 mg/m^3 for 8 days, the absorbed amount of acephate was estimated between 10 and 20 mg/day. Urine concentrations of acephate were between 1–10 μg/mL, and MP was not detected (detectable limit was less than 30 ng/mL). Clinically significant changes of cholinesterase (ChE) could not be observed (6). On the contrary, the acephate toxicity to the agricultural pesticide applicators (7) and the farm workers (8) was reported mainly by the data of ChE inhibition. A toxic case was reported whereby MP was injected intravenously to commit suicide (9). The typical clinical picture of OP intoxication appeared, and he recovered in a few days after being hospitalized, although MP presence in his biological fluids was not described (9).

To the best of our knowledge, the concentration of acephate and MP in human blood has not been reported previously in either normal or toxic conditions. Here we report the autopsy findings and actual concentrations of acephate and MP in biological fluids of two autopsy cases that were suspected of having ingested acephate.

Analytical Method for Acephate and Methamidophos

Analyses of acephate and MP were performed by gas chromatography/mass spectrometry (GC/MS) on extracts obtained by the modified method of Maroni et al. (6). Whole blood was diluted to more than 5 times its quantity with distilled water. Urine and the supernatant of the stomach contents were diluted to appropriate concentrations. One mL of these diluted samples was put into a 10-mL centrifuging glass tube in duplicate. In each tube, 20 μg of DEP (trichlorfon) in 40 μL of methanol was added as an internal standard (IS). The samples were saturated with NaCl, and centrifuged at 3,000 rpm for 5 min. The supernatant was collected and applied to a liquid-liquid extraction column (Chem Elut®, Extube® Part No.1219-8002, Varian, CA) in a quantity of less than 0.8 mL. After standing at room temperature for 30 min, acephate, MP and IS were eluted from the column by twice adding 3 mL of dichloromethane. The eluent was collected and dried under nitrogen gas. The residue was diluted by 1 mL of acetone, and injected into a GC/MS. Acephate, MP and trichlorfon reference materials

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were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The other chemicals were purchased from Nacalai Tesque, Inc. (Kyoto, Japan).

The GC/MS apparatus was an Automass 150 (JEOL, Tokyo, Japan) equipped with a capillary column (DB-1, 0.32 mm i.d. × 15 m length, 1.0 μm film thickness) (J&W Scientific, CA). The column temperature was programmed to hold at 80°C for 2 min, elevate to 230°C at 20°C/min and hold at the final temperature for 6 min. The injection port and ion source were held at 200°C. The scan mode was used for the qualitative analysis, and single ion-monitoring (SIM) mode for the quantitative analysis. For the qualitative analysis, the objective peak was identified by the ion ratios of the characteristic ions in scan mode comparing with those of each authentic standard: m/z 183, 136, 95, 94,79 for acephate, m/z 141, 111, 95, 94, 79 for MP, and m/z 185, 145, 109, 79 for IS. The ions monitored in SIM mode were m/z 183, 136, 94 for acephate, m/z 141, 94 for MP, and m/z 185, 109 for IS for the quantitative analysis. There were not any other misleading peaks in the chromatogram that was confused with the objects. The ion ratio of each object was calculated automatically and permitted to quantify if the ratio was within the 30% deviation compared with that of the standard at the highest concentration in the analysis. The peak was judged as being undetectable when the ion ratio was not within the extent of 30% deviation, as well as when the S/N ratio was less than 3. All objective peaks were certified again by visual inspection in addition to the automatic judgment, and then the relative area ratio to IS was used for the quantification. Recovery of the acephate and MP were assessed in each of five samples by spiking 200 μg/mL of an authentic acephate, 5 μg/mL of an authentic MP to drug-free blood for blood analysis, and urine for analysis of urine and stomach content. Recoveries with these procedures were 33.5% acephate in blood, 71.9% acephate in urine, 33.5% MP in blood and 47.7% MP in urine. The detection limit of acephate in blood was less than 0.5 μg/mL, and that of MP was less than 0.6 μg/mL.

Case History

Case 1

A 70-year-old man was found lying dead in his messy bedroom by a neighbor. The corpse of his girlfriend was in the house as well. She had deep stab wounds in her neck, and severe hemorrhaging. A nearly empty bottle of acephate was found in the dressing room next to the bathroom. The bottle had a 100-mL-volume capacity with a liquid mixture of acephate (Ortran®, Takeda Chemical Industries, Ltd., Osaka, Japan), containing 17.25% genuine acephate, 31.60% methoxypropanol as an organic solvent, 46.65% N-methyl-2-pyrrolidone as an organic solvent and 4.00% P.O.E.octylphenylether as a surface-active agent, according to the manufacturer. A bloodstained knife was found in the house.

An autopsy of the man was begun 18 h after the corpse was found. He was 164 cm in height, 57.0 kg in weight and was in severe rigor. Postmortem lividity could be found to a slight or middle degree, which was easily reduced by pressure. At the beginning of the autopsy, the rectal temperature was 26.5°C in the room temperature of 23.5°C. Each pupil was round and with a diameter of 3.0 mm in the left eye and 3.5 mm in the right eye. There were many cuts on the head, the neck, the upper limbs and the legs. Most of them remained in the skin layer, and none of them injured any main blood vessels. There also were two stabs in the epigastric region of the upper abdomen. One reached to the inside of the liver, and the other reached to the lesser omentum passing through the liver.

TABLE 1—*Concentrations of acephate and methamidophos in the autopsy cases (μg/mL).*

	Heart Blood	Urine	Stomach Contents
Case 1			
Acephate	149	270	2,200
Methamidophos	3.0	1.9	3.2
Case 2			
Acephate	46	107	1,000
Methamidophos	N.D.	1.6	N.D.

N.D.: not detected.

Only a small quantity of blood could be observed in the abdominal cavity. Approximately 200 mL of fluid blood, which was slightly coagulated, was obtained from the heart cavity. Two hundred and thirty (230) g of brown liquid was collected from the stomach, and had bad smell. Some gross lesions were observed in the major organs, but none relevant to the cause of death. In the walls of the esophagus and stomach, the mucous membranes were partially separated from the walls, and were slightly inflamed.

Ethanol was not detected in the blood or urine by gas chromatography using a flame ionization detector with headspace sampling (GC-7A, Shimadzu, Kyoto, Japan). Two kinds of drug screening analyses were performed on the heart blood, urine and the supernatant of the stomach contents. Nicotine was detected in the heart blood, and both nicotine and cotinine were detected in the urine by thin layer chromatography (TOXI-LAB®, TOXI-LAB Inc., CA). Nicotine and cotinine, mainly derived from smoking, are often detectable in normal subjects. The spots did not appear to be large or dense compared to normal subjects, so that the nicotine was not analyzed quantitatively. No drugs were detected by high performance liquid chromatography (REMEDi™HS, Bio-Rad, CA, USA). Serum ChE activity was analyzes by a spectrometric method based on the technique of Ellman et al. (10), using iodobuthylthiocholine as a substrate. Serum ChE activity was 78 IU/L, which was extremely lower than a normal range (3,500 to 8,000 IU/L).

The concentration of acephate was found to be 149 μg/mL in the heart blood, 270 μg/mL in the urine and 2,200 μg/mL in the supernatant of the stomach contents, as shown in Table 1. MP was also detected at 3.0 μg/mL in the heart blood, 1.9 μg/mL in the urine and 3.2 μg/mL in the supernatant of the stomach contents. The total amount of acephate was calculated to be approximately 0.50 g in 230 g of stomach contents, and that of MP was 0.00074 g.

With respect to the girlfriend, acephate and MP were not detected in the biological fluids. Hemorrhaging from the carotid vessels was mainly suspected as the cause of her death.

Case 2

A 60-year-old man was found lying dead in the morning in the passenger seat of his own car parked at a seaside restaurant. He had several cuts on his body. In the seat and on the flooring of the car, there were pools of blood. A bloody single-edged knife and an almost empty bottle of a liquid pesticide were also found under his feet. The Laboratory of Police Headquarters detected acephate in the bottle by GC. According to the statement of his wife, he had gone out driving at night with his acquaintance, to whom he had owed money. The missing acquaintance was found lying dead on the rocky seaside under a 14-meter-overhanging cliff near the car. An autopsy of the decedent found in his car was begun at 9:00 a.m. on the next day after the body finding.

There were a lot of cuts in the left ear, the left neck, the left breast and both wrists. The gashes in the left neck and the right wrist were profound. The other cuts were minor. Both sides of the superior horn of the thyroid cartilage were fractured. The deep gash in the left neck consisted of several repeated cuts, presumably by the use of a singe-edged knife. The cuts injured the neck muscles, the left common carotid artery, the left internal jugular vein and the left external jugular vein. All organs appeared severely ischemic macroscopically by the naked eye. Russet liquid in weight of 115 g was obtained from the stomach. Abnormal pathological findings were slight or not found in the primary organs such as the liver or the kidneys.

Ethanol in the blood and urine was not detected beyond 0.01 mg/mL by headspace GC. No drugs were detected by TOXI-LAB® or by REMEDi™ HS. Serum ChE activity was 3,539 U/L, which was in the low end of the normal range.

The concentration of acephate in the heart blood was found to be 46 μg/mL, together with higher concentrations in the urine and stomach contents. MP was not detected in the heart blood or stomach contents, although it was found in the urine (Table 1). The total amount of acephate was calculated to be approximately 0.115 g in 115 g of stomach contents. Acephate and MP were not detected in the other decedent lying on the seaside.

Discussion

In both cases, when the autopsy started, more than 18 hrs had passed from finding of the corpse. Miosis was not observed in the eyes of either decedent, though it is a representative symptom of ChE inhibition. Postmortem pupils of rabbits were found to change in three phases (11): Initial miosis, which set in within minutes after death, followed by a mydriatic second phase, which lasted several hours and was approximately synchronous with the post-mortem rigidity of the skeletal muscles. In the third phase, the pupils slowly contracted over the course of several days. The state of the second phase may explain the findings in the present cases.

It is well known that the effect of OP is estimated clinically by an inhibition of serum ChE activity. There is a reported negligible loss of ChE activity in postmortem blood (12), and in stored serum for as long as 30 days (13). ChE activity in the present autopsy cases can be applied to assess the OP toxicity.

The toxicity of OP has been published in manuscripts (14–16). Acute oral LD50 of selected OPs in male rats (14) and humans (15), and blood concentrations in fatal cases (16) are summarized in Table 2. The toxic order of parathion, diazinon and malathion in the fatal cases was similar to that of the rat oral LD50 and human oral LD50. The data of MP and acephate in humans are not cited in these references. According to the order of rat data (14), the acute oral toxicity of MP in humans is suspected to be intermediate between parathion and diazinon, and that of acephate is suspected to be intermediate between diazinon and malathion (Table 2). Ando and Wakamatsu (17) reported that acephate at a concentration less than 1.25 mM (estimated as 230 μg/mL) did not inhibit the activity of ChE *in vitro* using human serum. Dowla (18) showed that the concentrations to inhibit 50% of enzyme activity (ID50) was 5.6 mM (approximately estimated as 1,024 μg/mL) for acephate and 0.00184 mM (approximately estimated as 0.26 μg/mL) for MP *in vitro* using human plasma. Ingested acephate is metabolized into the potent ChE inhibitor MP in mammalian liver (4), but not in spiked human serum *in vitro* (17,18). Therefore, acephate becomes more toxic after ingestion and the actual toxic serum level of acephate may be smaller than that estimated from *in vitro* ChE inhibition.

TABLE 2—*Toxic data of organophosphates in the references.*

Subject Unit	Acute Oral LD50 Rat mg/kg	Acute Oral LD50 Human g	Blood Concentration Fatal Cases μg/mL Mean (min – max)
Parathion	2	0.01–0.3	9.0 (0.5–34)
Methamidophos	20	—	—
Diazinon	300–400	25	104 (0.7–227)
Acephate	945	—	—
Malathion	2,800	60	815 (100–1,880)
References	(14)	(15)	(16)

—: not reported.

The Cause of Death of Case 1

In Case 1, the blood concentration of acephate, 149 μg/mL, was considerably lower than either the ID50 (approximately 1,024 μg/mL) (18) or the minimum inhibitory concentration (approximately 230 μg/mL) (17) for ChE activity *in vitro* as described above. However, the concentration of MP, 3.0 μg/mL, was more than 11 times compared to the reported ID50 (approximately 0.26 μg/mL) (18). The blood concentration of acephate in Case 1 was an intermediate value between the mean value of diazinon and malathion in fatal cases (Table 2), and was included in the range of both diazinon and malathion. Acute acephate toxication can be speculated as the cause of his death. The acephate concentration of the stomach contents was 14.5 times higher than the blood, but the MP concentration of the stomach was nearly the same as the blood. According to the ratio of MP to acephate, it might be supposed that acephate was converted into MP in the liver (4) or other organs after oral ingestion. Whereby a trace of MP in the bottle could have been ingested directly, the presence of MA is more likely due to backwash of MA from the general circulation into stomach contents, that is, from the circulation in and around the stomach. In the present case, the contents of acephate and MP were not analyzed in other organs. The relationship still remains to be studied.

In Case 1, serum ChE activity was nearly zero. There were histologically abnormal findings in the digestive apparatus, such as inflammation or separation of the esophageal walls. We supposed that some irritating matter to the mucous membrane, such as organic solvents for pesticides, had been ingested. Only slight findings in the kidneys or the liver were observed, and no significant findings were observed in the lungs. He was assumed to have died in a short time after drinking before effects on these organs could occur, or the chemical itself did not have histologically significant effects on these primary organs, which agrees with general findings in acute poisoning by OPs (15,16). Severe hemorrhage was difficult to be determined. Two stabs in the epigastric region might have occurred perimortally after acephate ingestion, but unlikely contributed to his death, since there was only a small quantity of blood in the abdominal cavity. There were also inconsistent findings of severe hemorrhage as a cause of his death, such as the slightness of injuries, existence of postmortem lividity, full volume of heart blood, and the non-ischemic state of the primary organs. Acute poisoning by acephate was mostly suspected in Case 1. Metabolic conversion of acephate into MP in the body might also have contributed to the toxicity.

The investigators concluded that the man quarreled with the girlfriend, stabbed her to death with a knife, and then committed suicide by drinking acephate and cutting himself.

The Cause of Death of Case 2

In Case 2, acute acephate poisoning was difficult to ascertain. Serum ChE activity showed a normal level. Substantial toxic histological findings were not observed in the liver or the kidneys, but severely significant ischemia was found in the primary organs. The blood acephate was fairly lower than the ID50 for plasma ChE activity (18). The blood MP was not detected in this case. The detectable limit in the present report (0.6 μg/mL) was higher than the ID50 of MP (0.26 μg/mL) *in vivo* (17). Therefore, consideration of the effect of MP could not be determined. The acephate concentration of the stomach contents was higher than that of the blood by more than 21 times, and the urine by 9 times. The MP was detectable only in the urine. In this case, it is assumed that he individual ingested acephate. According to the clinical findings and the blood level of acephate, acute poisoning by acephate was difficult to confirm. Hemorrhage was mostly suspected to have caused his death, which was induced by the injuries to his left main blood vessels in the neck, made by the several repeated stabs using a sharp knife. The investigators concluded that the man was forced to drink acephate, strangled by hand and then stabbed with a knife by the acquaintance. Afterward, the acquaintance was presumed to have jumped to his death from the cliff.

In this report, two autopsy cases involving the suspected ingestion of acephate are reported. Acephate and MP were detected in their biological fluids, such as the blood, the urine, and the stomach contents. Acephate in actual human blood has never been reported before. The acephate toxicity in their death is discussed, citing previous references to animals and spiked serum or plasma *in vitro* as well as that of other OPs. One case was assumed to be fatal poisoning by acephate, and the other case was not. The toxicity of acephate chemical seems to be mainly caused by MP, though the metabolism in the body was not revealed. To the best of our knowledge, this is the first description of concentrations of acephate and MP in human blood, and the first report of a lethal case of acephate poisoning.

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