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# Amphetamine derivative related deaths in northern Greece

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**Abstract**

Until 1997, only one amphetamine related derivatives (AMPs) fatality had been reported in Greece. Since then, amphetamine (AMP) or AMPs have been found in seven out of 1500 post-mortem toxicological cases. The cause and manner of death of these seven cases were: 3,4-methylenedioxy-*N*-methamphetamine (MDMA) and 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA) poisoning ($n = 1$), drowning in water ($n = 4$), cranial injuries caused by a traffic accident ($n = 1$) and heart failure ($n = 1$).

In the case where the use of AMP or AMPs was considered, the immediate cause of death post-mortem toxicological analysis revealed 2 μg/ml MDMA and 0.7 μg/ml MDEA in blood. MDMA was identified in two cases of drowning (2 μg/ml in blood in the first case and 1.7 μg/g in liver in the second case) and in the traffic accident case (0.4 μg/g in liver). Methamphetamine was detected in two cases of drowning (2.5 μg/ml in blood in the first case and 6 μg/g in liver in the second case). AMP was found in the heart failure case (0.2 μg/g in liver). Alcohol was present, together with AMP or AMPs, in four cases. These findings indicate an increase in the illegal abuse of AMPs in Greece. Because of this, we now routinely screen for AMPs.


*Keywords:* Amphetamine; MDEA; MDMA; Methamphetamine; Death

## 1. Introduction

During toxicological analysis in autopsy cases, a number of drugs, alone or in combination, are identified having direct or indirect implication in the cause of death. In Greece, only one AMP related death has been reported up to the end of 1997. Since then, AMP or a related derivative has been identified in seven cases.

No reliable epidemiological data about the amount and frequency of the consumption of these substances in the general Greek population are available. The objective of this study was to provide more information and data on fatal intoxications involving AMP and AMPs.

## 2. Materials

The material was provided to the laboratory from seven cases in which toxicological examination was requested and which appeared positive for AMP or AMPs, after general screening for drugs in all autopsy cases, during the period between January 1998 and December 2000 in the area of northern Greece (population of about 3 million).

Blood, urine and tissues were obtained and stored at 4 °C until toxicological analysis. All samples were analyzed 1–3 days after collection. In four cases, the only available specimen was the liver. In these cases, the autopsy and alcohol blood analysis were carried out at the local hospital where the incidents had occurred (cases 3–5 and 7, Table 1). Solid-phase extraction (SPE) was performed using abselut NEXUS 60 mg LRC columns (Varian Chrompack).

## 3. Toxicological analysis

The same analytical approach was applied to all the cases: a preliminary immunoassay of urine (when available) was performed by fluorescence polarization immunoassay

*Abbreviations:* AMP, amphetamine; AMPs, amphetamine related derivatives

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Table 1
Post-mortem anatomic and toxicological findings of seven fatal cases associated with the use of amphetamines

| Case | Sex/age | Circumstances/cause of death/autopsy findings | Specimen | Concentration (μg/ml or μg/g) | | | | Ethanol concentration in blood (g/l) |
|---|---|---|---|---|---|---|---|---|
| | | | | A[a] | MA[b] | MDMA | MDEA | |
| 1 | Male/20 | Death at home with a syringe alongside<br>Pulmonary oedema<br>Cerebral oedema<br>Heart failure<br>MDMA/MDEA poisoning | Blood | | | 2.0 | 0.7 | – |
| 2 | Male/40 | Pulmonary oedema<br>Heart failure<br>Cerebral oedema and congestion<br>Drowning in seawater | Blood | | | 2.0 | | 0.75 |
| 3 | Male/44 | Pulmonary oedema and congestion<br>Myocardial ischemia<br>Heart failure<br>Drowning in seawater | Liver | | | 1.7 | | N.P.[c] |
| 4 | Male/51 | Pulmonary oedema and congestion<br>Cerebral oedema and congestion<br>Heart failure<br>Death in seawater | Liver | 0.2 | | | | 3.00 |
| 5 | Male/47 | Traffic accident<br>Cranial injuries | Liver | | | 0.4 | | – |
| 6 | Male/23 | Pulmonary oedema<br>Drowning in water | Blood | | 2.5 | | | 1.80 |
| 7 | Male/27 | Drowning in seawater | Liver | | 6.0 | | | 2.30 |

[a] A: amphetamine.
[b] MA: methamphetamine.
[c] N.P.: analysis not performed.

(FPIA) on an Abbott TDx system for cannabinoids, opiates, cocaine, benzodiazepines and amphetamines. Blood samples were analyzed for alcohol, cannabinoids, opiates, cocaine, benzodiazepines and amphetamines by FPIA. The Abbott TDx analyzer was applied for the detection of drugs in whole blood, without any sample pre-treatment, other than dilution with saline [1]. Alternatively, 1 ml of whole blood was extracted by liquid–liquid extraction and the organic solvent was evaporated to dryness after the addition of hydrochloric acid (37 wt.%) in methanol (1:9 v/v; 50 μl) [2]. The dry residue was reconstituted with 1 ml blank urine and analyzed by FPIA. The cut-off levels of the immunoassay were 300 ng/ml for opiates and cocaine, 200 ng/ml for benzodiazepines, 50 ng/ml for cannabinoids and 300 ng/ml for amphetamines. Tissues were analyzed by thin layer chromatography (TLC) after liquid–liquid or SPE for acidic and basic drugs [2,3]. The limit of detection (LOD) for amphetamines by TLC was 0.1 μg [2].

The TDx/TDxFLx amphetamine/methamphetamine II assay (Abbott Laboratories) was used for the detection of amphetamines in urine and blood samples and the TDx/TDxFLx REA ethanol assay for the quantitative measurement of ethanol in whole blood [4]. Cut-off levels and detection limits were not established for the AMP methylendioxy derivatives. However, some cross-reactivity data had been reported [2,5,6]. Positive screening results for AMP or AMPs were identified and quantified in blood and/or tissues after non-conditioned SPE (NC-SPE) and derivatization with heptafluorobutyric anhydride (HFBA) by gas chromatography with nitrogen–phosphorus detection (GC-NPD) [2]. The gas chromatography was performed on a CE Instruments GC 8000 Top instrument equipped with a capillary AT-5 column (30 m, 0.25 mm i.d., 0.25 μm film thickness, Alltech) equipped with a nitrogen–phosphorous detector.

Table 2
LOD and LOQ for the AMPs by GC-NPD

| Analyte | LOD (ng/ml) | LOQ (ng/ml) |
|---|---|---|
| AMP | 40 | 130 |
| Methamphetamine | 55 | 180 |
| MDMA | 60 | 200 |
| MDEA | 60 | 200 |

The extraction procedure was as follows: 1 ml of blood was mixed with 1 ml of distilled $H_2O$ and the mixture was sonicated for 15 min. The pH of the sample was adjusted to 10 and extracted by the NC-SPE technique, using abselut NEXUS 60 mg LRC columns. The sample was loaded onto the sorbent and drawn through under low vacuum (∼5 in. of Hg). After adsorption of the sample, 2 ml of distilled $H_2O$ was added to the column and drawn through by vacuum. The column was dried under high vacuum for 1 min and amphetamines elution was accomplished by passing 2 ml of methanol through the column at a flow rate of approximately 1 ml/min. Hydrochloric acid (37 wt.%) in methanol (1:9 v/v; 50 μl) was added and the extract was evaporated to dryness. The same extraction procedure was also followed for liver specimens after appropriate pre-treatment [7].

Ethyl acetate (100 μl) and HFBA (50 μl) were added to the dry residue. The mixture was vortexed, incubated at 85 °C for 10 min and dried under $N_2$. The content was dissolved in 50 μl of ethyl acetate and 1–2 μl were injected onto the GC column. Splitless injection was used and the carrier gas was helium at a flow rate of 1 ml/min. The injector and detector temperatures were 220 and 280 °C, respectively. The oven temperature was kept at 40 °C for 1 min and then increased at 20 °C/min to 280 °C at kept at that for 5 min. The LOD and limits of quantitation (LOQ) for AMP, methamphetamine, MDMA and MDEA are presented in Table 2.

## 4. Results and discussion

A total number of seven deaths involved AMP or AMPs in the period between 1998 and 2001 (Table 1). In three cases, AMPs was the only drug class detected: MDMA (two cases) and MDMA and MDEA (one case). Alcohol was found together with AMP and AMPs in the other four cases: with methamphetamine (two cases), with AMP (one case) and with MDMA (one case). Blood alcohol concentrations ranged between 0.75 and 3 g/l. MDMA and MDEA were detected (qualitatively) in the syringe and urine in case 1. MDMA and methamphetamine were detected (qualitatively) in the urine of the cases 2 and 6, respectively.

The deceased were all males and the age was in the range 20–51 years. This age and sex distribution appears to be in accordance with other AMP and AMPs related deaths reported in the literature [8,9]. The cause of death was an MDMA/MDEA poisoning in one case, drowning in four cases, cardiac arrest in one case and cranial injuries (as a consequence of a traffic accident) in one case.

It is known that, acute overdose of AMPs have a similar clinical presentation. The features of an intoxication are hyperthermia, muscle rigidity, profound respiratory, metabolic acidosis and hyperkalemia. The acidosis and the electrolyte disturbances are sufficient to cause fatal cardiac arrhythmias. Deaths are related to cardiovascular, cerebrovascular and hyperthermic effects. Pre-existing pathological conditions, such as cardiomyopathy, coronary disease, functional arrythmia and asthma can be aggravated by AMPs due to an arrhythmogenic cardiotoxic effect [10–12].

Considering the death conditions in case 1, the autopsy findings, and the results of the toxicological analysis, the cause of death was certified as toxic effects of MDMA and MDEA. In case 2, the cause of death was drowning in seawater, but the heart failure should be ascribed to the toxic effects of MDMA and ethanol.

In cases 3 and 4, the lack of blood samples made it difficult to interpret the contribution of MDMA or AMP to the death respectively. But the pre-existing cardiovascular disease, in both cases, the increased physical activity (swimming in seawater) infer the contribution of MDMA (case 3), and AMP and ethanol (case 4) to heart failure to the two cases respectively.

In cases 5–7, it was not possible to clarify the influence of AMPs (underlying cause of death, contributory condition, or insignificant complication) due to the lack of significant autopsy findings and blood samples for toxicological analysis (cases 5 and 7), thus to know the history, with regard to AMPs use before or around the time of accident).

In addition, a high concentration of methamphetamine in blood could not alone be interpreted as evidence that death was caused by acute overdose, without taking into consideration the autopsy findings (case 6) [13]. We conclude therefore that amphetamine derivative drugs were responsible for a certain number of deaths, either directly (overdose), or indirectly (traffic accidents, drowning, cardiac arrest).

## References

[1] C.W. Lee, H.M. Lee, Evaluation of the Abbott TDx analyzer, J. Anal. Toxicol. 13 (1989) 50–54.
[2] Recommended methods for the detection and assay of heroin, cannabinoids, cocaine, amphetamine, methamphetamine and ring-substituted amphetamine derivatives in biological specimens, Manual for Use by National Laboratories, ST/NAR/27, United Nations, 1995.
[3] Recommended methods for the detection and assay of barbiturates and benzodiazepines in biological specimens, Manual for Use by National Laboratories, ST/NAR/28, United Nations, 1997.
[4] Abbott Laboratories, TDx System Assays Manual: Ethanol, Abbott Laboratories Diagnostics Division, 1995.

[5] Abbott Laboratories, TDx System Assays Manual: Amphetamine/Methamphetamine II, Abbott Laboratories Diagnostics Division, 1989.
[6] J.T. Cody, R. Schwarzhoff, Fluorescence polarization immunoassay detection of amphetamine, methamphetamine, and illicit amphetamine analogues, J. Anal. Toxicol. 17 (1993) 26–30.
[7] N. Raikos, H. Tsoukali, D. Psaroulis, V. Mirtsou, Application of non-conditioned solid-phase extraction to the isolation of amphetamine from post-mortem biological materials, in: Proceedings of the 2000 Joint TIAFT/SOFT International Meeting, TIAFT 2000, Helsinki, Finland, 2000, pp. 72–76.
[8] C. Lora-Tamayo, T. Tena, A. Rodriguez, Amphetamine derivative related deaths, Forensic Sci. Int. 85 (1997) 149–157.
[9] J.G.C. Omtzigt, C.J. Vermaase, G.M. Zweipfenning, Deaths associated with amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethamphetamine (MDEA) or 3,4-methylenedioxyamphetamine (MDA) abuse, in: Proceedings of the 1994 joint TIAFT/SOFT International Meeting, TIAFT 1994, Tampa, FL, USA, 1994, pp. 125–133.
[10] V. Fineschi, A. Masti, Fatal poisoning by MDMA (ecstasy) and MDEA: a case report, Int. J. Legal Med. 108 (1996) 272–275.
[11] S. Ramcharan, P.L. Meenhorst, J.M.M.B. Otten, C.H.W. Koks, D. de Boer, R.A.A. Maes, J.H. Beijnen, Survival after massive ecstasy overdose, J. Toxicol. Clin. Toxicol. 36 (1998) 727–731.
[12] D.E. Cox, K.R. Williams, ADAM or EVE? A toxicological conundrum, Forensic Sci. Int. 77 (1996) 101–108.
[13] B.K. Logan, C.L. Fligner, T. Haddix, Cause and manner of death in fatalities involving methamphetamine, J. Forensic Sci. 43 (1998) 28–34.