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Distribution and Optical Purity of Methamphetamine Found in Toxic Concentration in a Civil Aviation Accident Pilot Fatality

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16. Abstract

The Federal Aviation Administration's Civil Aerospace Medical Institute conducts toxicological evaluation of postmortem biological samples collected from pilots involved in fatal civil aircraft accidents. The submitted samples are primarily analyzed for the presence of primary combustion gases, alcohol/volatiles, and drugs. Related to such an evaluation, findings of a unique aircraft accident are described in this report. Upon colliding with terrain in weather conditions of poor visibility, a 1-occupant airplane was substantially damaged, with no evidence of fire. Remains of the pilot were found outside the crashed aircraft. Pathological examination revealed multiple blunt force injuries and vascular congestion, including subdural hemorrhage of the cerebral cortex. Autopsied samples—blood, brain, gastric contents, heart, liver, muscle, spleen, urine, and vitreous fluid—were submitted for toxicological analysis. The fluorescence polarization immunoassay disclosed 8.0 µg/mL amphetamines in urine. Subsequent gas chromatographic/mass spectrometric confirmatory analysis determined the presence of methamphetamine (1.134 µg/mL in blood and 59.171 µg/mL in urine) and amphetamine (0.022 µg/mL in blood and 1.495 µg/mL in urine). Both amines were present in all the submitted sample types, except for amphetamine, which was detected neither in vitreous fluid nor in muscle. The amount of methamphetamine found in gastric contents was 575-fold higher than that of amphetamine. Stereochemical analyses of gastric contents, blood, and urine using a chiral probe, (*S*)-(–)-*N*-(trifluoroacetyl)prolyl chloride, indicated that methamphetamine detected in the sample types was not optically pure. In gastric contents and urine, this secondary amine's optical isomers were present in equal proportions. The enantiomeric excess of (+)-methamphetamine over its (–)-form was about 32% in blood. Both optical forms of amphetamine were present in the ratio of 1.2–1.5:1.0 in the 3 sample types. The blood methamphetamine concentration found was in the range sufficient to produce toxic effects. The observed variation in the ratios of amine isomer concentrations in the sample types would have been attributed to stereoselective metabolic and other pharmacokinetic processes. Findings of this study supported the conclusion of the National Transportation Safety Board that, in addition to the visibility-associated adverse meteorological conditions, the use of the controlled substance played a contributory role in the causation of the aircraft accident.

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Distribution and Optical Purity of Methamphetamine Found in Toxic Concentration in a Civil Aviation Accident Pilot Fatality

INTRODUCTION

Methamphetamine, a sympathomimetic amine (1), exists in optical isomeric forms. Its *dextrorotatory* [*dextro-;* (+)-] form has central nervous system (CNS) stimulant effects (1,2) and has been used in the treatment of obesity, while its *levorotatory* [*levo-;* (–)-] form has been used as a nasal decongestant in nonprescription inhalers (2) and as a precursor in selegiline manufacturing (3). The latter isomeric form has weaker CNS stimulant and greater peripheral sympathomimetic effects than those of the former isomeric form (1,2). Methamphetamine has been an abused drug of concern (4-6); and unless specifically excepted, its isomers are considered controlled substances under the "food and drugs" regulations of the United States (7). This secondary amine is sold in illegal drug markets in the forms of (+)-methamphetamine, (–)-methamphetamine, and (±)-methamphetamine (2, 8-10). (±)-Methamphetamine, also known as racemic methamphetamine or methamphetamine racemate, consists of *dextro-* and *levo-*forms in equal proportions. Methamphetamine mixtures of enantiomeric excess of one isomeric form over another isomeric form in variable proportions are also sold in the drug markets. These mixtures could be easily prepared by physically mixing *dextro-* and *levo-*forms together or by adding either of the isomers to methamphetamine racemate or *vice versa*.

In biological samples, a legitimate source of (–)-methamphetamine could be nasal inhalers or selegiline (11-13) and of (+)-methamphetamine could be legally prescribed Desoxyn® (14). Methamphetamine is primarily biotransformed by *N*-demethylation to the active metabolite amphetamine (2,15). Biotransformation of optical isomers of methamphetamine and amphetamine is stereospecific—*dextro*-isomers of these amines are metabolized at faster rates than their corresponding *levo*-isomers (2,15-17).

Several methamphetamine-abuse-associated cases of violent and irrational behaviors (blood concentration: 0.15–0.56 µg/mL), of erratic driving (blood concentration: 0.05–2.6 µg/mL), and of deaths with traumatic injuries (blood concentration: 1.4–13 µg/mL) have been reported in the literature (2,18,19). Blood concentrations of methamphetamine in the range of 0.09–18 µg/mL have been documented in fatal poisonings attributed to overdoses of this amine (2,20). Toxic and lethal levels of methamphetamine in blood have been considered to be 0.6–5.0 µg/mL and 0.23 µg/mL, respectively (21,22). Although toxic and lethal blood levels of methamphetamine have been well documented, its stereospecificity was not established in those cases—it was not known whether methamphetamine found in those cases was *dextrorotatory*, *levorotatory*, or optical isomeric mixtures of equal or non-equal proportions. Knowing the stereospecific composition of methamphetamine in biological samples could assist in concluding the source of the amine and in accurately predicting the level of the adverse effects caused by the substance.

In the present study, toxicological findings of a unique civil aviation accident pilot fatality involving methamphetamine are described. Additionally, distribution and stereospecificity of this amine and its metabolite —amphetamine—were determined in the various submitted postmortem biological samples. Also, included are pathological findings and aircraft accident case history.

Case History

A Cessna airplane was substantially damaged upon colliding with terrain at approximately 1700 hours in an isolated area. The only occupant, the pilot, was fatally injured. In the general area of the accident, heavy snow conditions were observed, and the visibility was 0.5 mile (805 m) with fog and scattered overcast. An airman's meteorological information for mountain obscuration was in effect in the area at the time of the accident. There was no sign of fire in the crash. A strong odor of gasoline was present near the wreckage. Seat belts and shoulder harnesses were not buckled. The remains of the pilot were found on the ground outside the airplane. The autopsy was conducted 69 hours after the accident.

Pathology

The pilot was a well-developed and well-nourished 44-year-old Caucasian male, weighing approximately 180 lbs (82 kg). The length of the body was about 70 in (1.8 m). The body was cold. Anatomical examination of the body revealed multiple blunt force injuries—bone fractures and internal lacerations—in the chest, abdomen, and lower back; and a small focus of subdural hemorrhage over the left parietal region of the cerebral cortex. Lacerations,

contusions, and abrasions were noted, practically all over the body. Vascular congestion was found in the kidneys, lungs, heart, spleen, and left frontal/parietal cortex. There was no evidence of medical therapy. Autopsied biological samples—blood, brain, gastric contents, heart, liver, muscle, spleen, urine, and vitreous fluid—were submitted to the Federal Aviation Administration's Civil Aerospace Medical Institute in Oklahoma City, OK, for toxicological evaluation.

MATERIALS AND METHODS

Materials

All reagents and solvents were of analytical grade and of the highest available purity. Reagents, solvents, standards, internal standards, and derivatizing agents were obtained from commercial sources. Bovine whole blood used for the preparation of calibrators and controls was obtained from a local slaughterhouse. The AxSyM® Amphetamine/Methamphetamine II assay kit and controls for the screening of amphetamine/methamphetamine in urine were purchased from Abbott Laboratories (Abbott Park, IL). This screening assay utilizes fluorescence polarization immunoassay (FPIA) technology. During the assay, 0.5, 1.5, and 4.0 µg/mL controls of (+)-amphetamine were used. Cross-reactivity of this assay with (–)-amphetamine, (+)- and (–)-methamphetamines, and other structurally related molecules has been reported by the kit manufacturer. Standards of (+)- and (–)-amphetamines and of (+)- and (–)-methamphetamines were obtained in methanolic solutions from Alltech-Applied Science Labs (State College, PA). Internal standards were supplied as racemic mixtures, (±)-amphetamine-d_8 and (±)-methamphetamine-d_8, by Cerilliant Corporation (Austin, TX). Pentafluoropropionic anhydride (PFPA) was purchased from Pierce Chemical Company (Rockford, IL). The chiral probe used in resolving enantiomers of amphetamine and methamphetamine was (*S*)-(–)-*N*-(trifluoroacetyl)prolyl chloride (TPC). Obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI), this probe was 97% enantiomerically pure and was supplied as 0.1 M solution in dichloromethane. TPC has previously been used as a probe in the stereospecific analysis of amphetamines (11-13).

Analytical Toxicology

The submitted pilot fatality biological samples were analyzed for the presence of combustion gases (carbon monoxide and hydrogen cyanide), alcohol/volatiles, and drugs—which include a wide range of prescription, nonprescription, and abused drugs (23-27). These exogenous substances in the biological samples were analyzed according to established standard laboratory procedures, including screening and confirmatory/quantitative analyses (24). Carbon monoxide in blood was analyzed spectrophotometrically as carboxyhemoglobin (28) and hydrogen cyanide colorimetrically as cyanide (29). Alcohol/volatile analysis in vitreous fluid was performed by headspace gas chromatography. The urine sample was screened for abused drugs by FPIA and for prescription and nonprescription drugs by high-performance liquid chromatography and by gas chromatography/mass spectrometry (24).

Gas Chromatograph/Mass Spectrometer

An Agilent gas chromatograph (Model 6890)/mass spectrometer (Model 5973) system (Agilent Technologies, Wilmington, DE) was used during the confirmatory/quantitative and stereochemical analyses of methamphetamine and amphetamine. A crosslinked 100% methylsiloxane wall-coated open tubular column (12.5 m × 0.2-mm i.d.; 0.33-µm film thickness) was used in the gas chromatograph/mass spectrometer (GC/MS) system. Helium was the carrier gas with a constant flow of 1 mL/min. The injection volume was 1 µL in the splitless mode, with a splitless time of 0.5 min. The injector temperature was maintained at 250°C, and the transfer line was set at 280°C. The acquisition was in the electron impact mode of ionization using selective ion monitoring.

Confirmatory/Quantitative Analyses

Extraction

Based on the urine FPIA screening finding, each of the submitted biological sample types was analyzed for the confirmation and quantitation of amphetamines. This analysis was achieved by the solid phase extraction (SPE) technique using Bond Elut Certify™ columns (Varian Sample Preparation Products, Harbor City, CA).

Depending upon the possible anticipated amounts of the amines present in a particular sample type and its availability, different amounts of samples were used for extraction. This approach was taken to ensure that amine concentrations in the respective sample types fall within the range of the calibration curves. Amounts of samples used were 0.30, 1.0, and 3.0 mL blood; 0.030 and 3.0 mL urine; and 0.15 and 0.30 mL vitreous fluid. Homogenates of solid tissues were prepared in 1% NaF aqueous solution. Amounts of homogenates used for analysis represented 0.030, 0.30, and/or 1.0 g of a particular tissue type. Two gastric content aliquots in the amounts of 0.030 and 0.30 g were separately diluted with suitable amounts of deionized water, homogenized, and centrifuged at 770 × *g* for 5 min to obtain their respective homogeneous supernatants.

Aliquots (3.0 mL or less) of each calibrator, control, fluid specimen, diluted specimen, homogenate, and gastric content supernatant in separate 16 mm × 100 mm screw-cap culture glass tubes were separately spiked with 1.0 mL of 400 ng/mL aqueous solutions of internal standards, (±)-amphetamine-d_8 and (±)-methamphetamine-d_8. With those samples wherein less than 3.0 mL of aliquots were used, their volumes were brought to 3.0 mL by adding deionized water. To each of the tubes containing blood and homogenate, 10.0 mL of cold acetonitrile (4°C) was added to precipitate protein: Acetonitrile was not added to the tubes containing urine, vitreous fluid, and gastric content supernatant samples. All acetonitrile containing tubes were capped, shaken for 1 min, and placed on a rotary extractor until the mixture reached ambient temperature and then centrifuged at 770 × *g* for 5 min. Acetonitrile supernatants were decanted to 16 mm × 125 mm screw-cap culture glass tubes and were evaporated to less than 1 mL at 40°C, in a water bath, by using a stream of nitrogen. Subsequently, 2.0 mL of 0.10 M phosphate buffer (pH 6.00) was added to each tube, including those tubes that contained urine, vitreous fluid, and gastric content supernatant samples.

Under a positive pressure manifold, the SPE columns for each of the tubes were first conditioned with 2.0 mL of methanol, followed by 2.0 mL of the phosphate buffer. The previously buffered sample solutions were transferred onto the conditioned columns and allowed to pass through the columns. Then, the columns were rinsed with 1.0 mL of 1.0 M acetic acid and dried with 25 lb of pressure for 5 min. Subsequently, 6.0 mL of methanol was passed through the columns, and they were then allowed to dry for 2 min with 25 lb pressure. Analytes were eluted from the columns into 10-mL screw-cap conical glass tubes by using 4.0 mL of 2% NH_4OH in ethyl acetate. Hydrogen chloride was bubbled through the collected eluates to minimize the loss of amphetamines.

PFPA Derivatization

The obtained eluates were evaporated to dryness at 40°C, in a water bath, by using a stream of nitrogen. To each tube was added 50.0 µL of ethyl acetate, and each tube was vortexed for 15 sec. Subsequently, 50.0 µL of PFPA was added to the tubes. These reaction mixtures were incubated for 20 min at 70°C in a heating block, allowed to cool to ambient temperature, and evaporated to dryness in a 40°C water bath by purging with nitrogen, followed by the addition of 50.0 µL of ethyl acetate for reconstitution. The reconstituted mixture from each tube was then transferred into 200-µL inserts of autosampler vials, and 1.0 µL of the mixture from the inserts was injected onto the GC/MS system for characterization, confirmation, and quantitation of the derivatized products of amphetamines.

GC/MS Conditions for PFPA Derivatives

The initial GC oven temperature of 70°C was increased to 150°C at 15°C/min and then to 290°C at 40°C/min with no hold times. The retention times (± 0.01 min) for PFPA derivatives of (±)-amphetamine-d_8, (±)-amphetamine, (±)-methamphetamine-d_8, and (±)-methamphetamine were 3.72, 3.75, 4.54, and 4.57 min, respectively. The ions (m/z) monitored were 193, 126, and 96 for PFPA-(±)-amphetamine-d_8; 190, 118, and 91 for (±)-amphetamine; 211, 163, and 123 for PFPA-(±)-methamphetamine-d_8; and 204, 160, and 118 for PFPA-(±)-methamphetamine. The first of the 3 ions of each of these analytes was used as their quantifying ions, while the remaining 2 ions of each of the analytes were used as their qualifying ions. Calibration curves for amphetamine and methamphetamine in bovine blood were constructed. Calibrators of both amines for obtaining the curves were 0.025, 0.050, 0.100, 0.200, and 0.400 µg/mL. The regression analysis disclosed that both curves were linear ($r \geq 0.9990$) in the stated concentration range. Since PFPA is a non-chiral compound, the stereospecificity of amphetamines in the submitted samples could not be established by this analysis.

Stereochemical Analyses

Extraction

To establish enantiomeric purity, amphetamines were extracted from re-accessioned gastric content, blood, and urine samples by the liquid-liquid extraction method and were then derivatized by the TPC chiral probe. Because amphetamines were present in varying amounts in these sample types, different amounts of samples were used for the extraction so that amine concentrations fall close to a 0.200-µg/mL control, the mid-point concentration on the quantitative analysis calibration curve for each amine. A mixture control, in bovine blood, consisting of 0.200 µg (200.0 µL of 1.0 µg/mL) of (+)-amphetamine, (−)-amphetamine, (+)-methamphetamine, and (−)-methamphetamine each was analyzed. Individual controls of each of these analytes at 0.200 µg (200.0 µL of 1.0 µg/mL) in bovine blood were also separately analyzed. Bovine blood was used as a negative control. As was done during the quantitative analyses, 2 supernatants of the gastric content sample in deionized water were prepared; 200.0 µL of the supernatants represented 0.010 and 0.820 g of gastric contents. The 0.010-g gastric content supernatant was used for methamphetamine analysis, while the 0.820-g gastric content supernatant for amphetamine analysis. Amounts of blood and urine used were also 200.0 µL.

Two-hundred-µL aliquots of each control and sample were transferred into separate 16 mm × 150 mm screw-cap glass test tubes. To each tube were added 1.0 mL of 400 ng/mL aqueous solution of the internal standards

(±)-amphetamine-d_8 and (±)-methamphetamine-d_8, 4 drops of concentrated NH_4OH, and 10.0 mL of *n*-butyl chloride. Each tube was vigorously shaken for 2 min and then centrifuged at 770 × *g* for 5 min. The organic phase from the tubes was transferred into 16 mm × 150 mm screw-cap glass test tubes. After adding 4.0 mL of 1.0 N HCl to each of the tubes, they were vigorously shaken for 2 min and centrifuged at 770 × *g* for 5 min. The separated organic phase was discarded, and 1.0 mL of concentrated NH_4OH was slowly added to each of the tubes, kept in ice, while swirling. After 15 min, tubes were vortexed for 15 sec, and 4.0 mL of chloroform was added to each tube. Subsequently, tubes were shaken and centrifuged, and the upper aqueous phase from each tube was removed by aspiration. The chloroform phase from each tube was decanted into 10-mL screw-cap conical glass tubes, and hydrogen chloride was bubbled through each chloroform solution.

TPC Derivatization

The hydrogen chloride-bubbled chloroform solutions were evaporated to dryness at 40°C, in a water bath, by using a stream of nitrogen. Tubes were allowed to come to room temperature, and then 50.0 µL of the TPC solution was added to each tube. After a brief mixing, the obtained reaction mixtures were incubated for 30 min at 70°C in a heating block, allowed to cool to ambient temperature, and evaporated to dryness in a 40°C water bath by purging with nitrogen. To each tube was added 50.0 µL of ethyl acetate to reconstitute the residue. All tubes were vortexed for 15 sec, and then the ethyl acetate solutions were transferred into 200-µL inserts of autosampler vials and 1.0 µL of each solution was injected onto the GC/MS system.

GC/MS Conditions for TPC Derivatives

The GC oven temperature was set at 70°C; it was then increased to 290°C at a rate of 30°C/min with a final hold time of 2.67 min. The retention times (± 0.01 min) of the TPC-derivatized diastereomers, (–)-amphetamine-d_8, (–)-amphetamine, (+)-amphetamine-d_8, (+)-amphetamine, (–)-methamphetamine-d_8, (–)-methamphetamine, (+)-methamphetamine-d_8, and (+)-methamphetamine, were 4.94, 4.95, 5.01, 5.02, 5.40, 5.42, 5.45, and 5.47 min, respectively. The ions (m/z) monitored were 240, 241, and 242 for TCP-(–)-amphetamine-d_8 or TCP-(+)-amphetamine-d_8; 237, 238, and 239 for TCP-(–)-amphetamine or TCP-(+)-amphetamine; 258, 259, and 260 for TCP-(–)-methamphetamine-d_8 or TCP-(+)-methamphetamine-d_8; and 251, 252, and 253 for TCP-(–)-methamphetamine or TCP-(+)-methamphetamine. The first of the 3 ions of each of these analytes was used as a quantifying ion for the determination of enantiomeric percentages, while the remaining 2 ions of each of the analytes were used as qualifying ions.

The enantiomeric percentages were determined by separately calculating ratios of TCP-(–)- and TCP-(+)-amine peak areas to the peak areas of respective isomeric-d_8 internal standards and then dividing each of the obtained ratios with the sum of both ratios as expressed in the following formula.

$$[\{PA_{\text{TCP-(– or +)-amine}} \div PA_{\text{TCP-(– or +)-amine-}d8}\} \times 100] \div [\{PA_{\text{TCP-(–)-amine}} \div PA_{\text{TCP-(–)-amine-}d8}\} + \{PA_{\text{TCP-(+)-amine}} \div PA_{\text{TCP-(+)-amine-}d8}\}]$$

Where, PA = peak area of amphetamine or methamphetamine TCP derivatives.

Table I. Distribution of Methamphetamine and Amphetamine and Their Concentration Ratios in Various Biological Samples From the Pilot Fatality.

Sample Type	Methamphetamine (µg/mL or µg/g)	Amphetamine (µg/mL or µg/g)	[Methamphetamine] / [Amphetamine]
Blood	1.134	0.022	51.5
Urine	59.171	1.495	39.6
Brain	1.527	0.038	40.2
Vitreous fluid	0.229	Not detected	–
Heart	1.060	0.017	62.4
Liver	5.534	0.133	41.6
Spleen	3.349	0.084	39.9
Muscle	0.327	Not detected	–
Gastric contents	5.753 mg*	0.010 mg*	575.3

*Total present in 78.0 g of the submitted gastric content sample.

RESULTS AND DISCUSSION

Analysis of the submitted blood sample failed to disclose the presence of carbon monoxide, cyanide, or ethanol in a detectable amount. However, the FPIA technology-based assay revealed the presence of 8.0 µg/mL amphetamines in urine. Because of cross-reactivity, this screening assay does not correctly distinguish optical isomeric forms of amphetamine or methamphetamine and also does not distinguish amphetamine from methamphetamine. Since the former is a metabolite of the latter and the cross-reactivity with other abused drugs structurally similar to amphetamine has been reported with the FPIA assay, a more specific alternate GC/MS-based analytical method was used for confirming the FPIA analytical result. The GC/MS analysis revealed the presence of methamphetamine in all the submitted sample types and of amphetamine in all the sample types but in vitreous fluid and muscle (Table I). This information suggested that methamphetamine and its metabolite—amphetamine—were distributed throughout the body, including the brain, of the pilot. As expected, the higher amounts of amines were present in urine, liver, or spleen than the amounts in vitreous fluid or in muscle—in fact, amphetamine was not detected in these 2 sample types. The levels of amphetamine were significantly lower in all the sample types than the levels of methamphetamine in the corresponding sample types. The blood methamphetamine level of 1.134 µg/mL was in the range sufficient to produce toxic effects, including erratic behavior (2,18,19). This level overlapped the lethal concentration range of methamphetamine (0.09–18 µg/mL) reported in the literature (2,20). Although 0.022 µg/mL of amphetamine in blood is a subtherapeutic concentration (15,21), its contributory role cannot be ruled out in enhancing the overall effects of methamphetamine in the deceased. The 575-fold higher amount of methamphetamine found in the gastric contents than that of amphetamine suggested that methamphetamine might have been taken orally. This conclusion is further supported by the fact that the methamphetamine to amphetamine concentration ratio (575) in the gastric contents is drastically higher than the ratios (39.6–62.4) in the 6 different body compartments wherein amphetamine was metabolically generated (Table I). Basic drugs have a tendency to accumulate in the stomach because of the pH gradient between plasma and gastric juice (30). Therefore, the relatively small amount of amphetamine found in the gastric contents was potentially the result of the low-pH associated ion trapping of the methamphetamine metabolite in the stomach. Since only a portion of the gastric contents was submitted for the analysis, the total amounts of both amines present in the stomach could not be calculated.

Unless specifically excepted, optical isomers of amphetamine and of methamphetamine are considered controlled substances under the U.S. regulations of food and drugs (7). However, (–)-methamphetamine and its metabolite could legitimately be found in biological samples of individuals who use nasal inhalers or the anti-Parkinson's medication selegiline (11-13). Therefore, there is a genuine need for analytically determining stereospecificity—*levorotatory, dextrorotatory,* or optical isomeric mixture—of methamphetamine and its metabolite (amphetamine) for establishing the degree and type of biological effects and the source of the amines. Such determination is of significance because a particular optical isomer may produce a selective biological effect, may be more potent than the other isomer (1,2,15), or could be considered a controlled substance (7).

Blood methamphetamine concentrations reported in the literature do not specifically mention stereospecificity of the amine (2,18-20). The amine could be in the (+)- or (–)-optical form or could be a mixture consisting of both optical forms in equal or non-equal proportions.

Table II. Optical Purity of Methamphetamine and of Amphetamine in Biological Samples From the Pilot Fatality

Sample Type	Methamphetamine		Amphetamine	
	(+)-Form %	(–)-Form %	(+)-Form %	(–)-Form %
Gastric contents	50	50	60	40
Blood	66	34	54	46
Urine	48	52	58	42

Since pharmacological effects of methamphetamine are stereospecific [(+)-form has stronger central stimulant effects than the (–)-form], a racemic methamphetamine [(±)-methamphetamine]—that is, (+)-methamphetamine and (–)-methamphetamine in equal proportions—would have pharmacological effects falling somewhere between the degree of effects caused by each of the optical isomers alone. Therefore, the concentration of the amine found in the biological samples could not necessarily be accurately correlated with the degree of toxicity, provided the stereospecificity of methamphetamine has been established.

Stereochemical analyses of the gastric contents, blood, and urine disclosed that methamphetamine and amphetamine were present in the samples in an optical mixture form (Table II). As is given in the table, (+)- and (–)-forms of methamphetamine were present in almost equal proportions in gastric contents and in urine, but the percent concentration ratio of (+)-methamphetamine to its (–)-form was 2:1 in blood. On the other hand, (+)- and (–)-amphetamines were present in the gastric contents, blood, and urine in the ratios of 1.5:1, 1.2:1, and 1.4:1, respectively. The biotransformation of methamphetamine and amphetamine is stereoselective; their (+)-form is metabolized faster than their (–)-form (2,15-17). Therefore, the (+)-form should be present in the lesser amounts than the (–)-form in various body compartments. However, *in vivo* stereoselective pharmacokinetic processes other than metabolism may prevent these isomers from reaching such an anticipated concentration pattern in a particular body compartment. Since biotransformation is essentially negligible in the stomach, the stereoselective effect of the process will also be minimal on the concentrations of optical isomeric forms of methamphetamine. Thus, the concentration ratio found in the gastric contents more likely reflect the optical purity of methamphetamine ingested by the deceased. However, the isomeric concentration ratios of both amines in other sample types could also be the resultant of the effectiveness of other stereoselective pharmacokinetic processes, leading to the different isomeric concentration ratios in different sample types.

Considering the isomeric ratio of methamphetamine in blood, concentrations of its (+)- and (–)-forms could be calculated as to be 0.748 and 0.386 µg/mL, respectively. Therefore, the net biological effect on the deceased was caused by both isomers, and the intensity of the effect would have been less than that would have been caused by only *dextro*-methamphetamine at 1.134 µg/mL. The possibility for reaching the *levo*-isomer's concentration at the calculated value from a legitimate use of a non-prescription inhaler or from selegiline is remote, particularly in the presence of the *dextro*-isomer and in the absence of selegiline itself.

Toxicological findings suggested that the deceased took the racemic methamphetamine orally. This suggestion was supported by the presence of a relatively large amount of (±)-methamphetamine in the gastric contents in relation to amphetamine. The origin of (±)-methamphetamine could have been a chemically synthesized product from phenylacetone and *N*-methylformamide (2, 8-10) or a physically prepared product from mixing (+)- and (–)-forms of methamphetamine in equal amounts, as these chemical and physical routes would produce methamphetamine racemate. The oral ingestion allowed the blood methamphetamine concentration to reach a level sufficient to produce some degree of central stimulant effects, causing performance impairment. Findings of this study supported the National Transportation Safety Board's determination of factors of the accident as "meteorological conditions obscuring the pilot's visibility and incapacitation due to illegal substances."

REFERENCES

1. Hoffman BB, Lefkowitz RJ. Catecholamines, sympathomimetic drugs, and adrenergic receptor antagonists. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman, AG, Eds. Goodman & Gilman's *The pharmacological basis of therapeutics*, 9th ed. New York, NY: McGraw-Hill; 1996: 199–248.
2. Baselt RC. *Disposition of toxic drugs and chemicals in man*, 6th ed. Foster City, CA: Biomedical Publications; 2002:646–50.
3. Baselt RC. *Disposition of toxic drugs and chemicals in man*, 6th ed. Foster City, CA: Biomedical Publications; 2002:953–5.
4. Foltz RL, Fentiman, AF, Jr, Foltz RB. GC/MS assays for abused drugs in body fluids. Rockville, MD: U.S. Department of Health and Human Services, Alcohol, Drug Abuse, and Mental Health Administration; 1980 Aug. National Institute on Drug Abuse Research Monograph 32.
5. DEA briefing book. Arlington, VA: U.S. Department of Justice, Drug Enforcement Administration; 1999 Oct.
6. DEA briefing book. Arlington, VA: U.S. Department of Justice, Drug Enforcement Administration; 2001 Nov.
7. Code of Federal Regulations (CFR). Title 21—Food and drugs, Chapter II, Part 1308—Schedules of controlled substances. Washington, DC: U.S. Government Printing Office, 2002.

8. Allen A, Cantrell TS. Synthetic reductions in clandestine amphetamine and methamphetamine laboratories: A review. *Forensic Sci Int* 1989; 42: 183–99.

9. Jirovský D, Lemr K, Ševčík J, Smysl B, Stránský Z. Methamphetamine—properties and analytical methods of enantiomer determination. *Forensic Sci Int* 1998; 96:61–70.

10. Cheng W, Lee W, Chan M, Tsui P, Dao K. Enantiomeric separation of methamphetamine and related analogs by capillary zone electrophoresis: Intelligence study in routine methamphetamine seizures. *J Forensic Sci* 2002; 47:1248–52.

11. Fitzgerald RL, Ramos JM, Jr, Bogema SC, Poklis A. Resolution of methamphetamine stereoisomers in urine drug testing: Urinary excretion of *R*(–)-methamphetamine following use of nasal inhalers. *J Anal Toxicol* 1988; 12:255–9.

12. Romberg RW, Needleman SB, Snyder JJ, Greedan A. Methamphetamine and amphetamine derived from the metabolism of selegiline. *J Forensic Sci* 1995; 40:1100–2.

13. Kupiec TC, Chaturvedi AK. Stereochemical determination of selegiline metabolites in postmortem biological specimens. *J Forensic Sci* 1999; 44: 222–6.

14. *Physicians' desk reference*, 57th ed. Montvale, NJ: Thomas PDR; 2003:441-2.

15. Baselt RC. *Disposition of toxic drugs and chemicals in man*, 6th ed. Foster City, CA: Biomedical Publications; 2002:64–6.

16. Wan SH, Matin SB, Azarnoff DL. Kinetics, salivary excretion of amphetamine isomers, and effect of urinary pH. *Clin Pharmacol Ther* 1978; 23:585–90.

17. Beckett AH, Rowland M. Urinary excretion kinetics of methylamphetamine in man. *J Pharm Pharmacol* 1965; 17(Suppl):109S–14S.

18. Lebish P, Finkle BS, Brackett JW, Jr. Determination of amphetamine, methamphetamine, and related amines in blood and urine by gas chromatography with hydrogen-flame ionization detector. *Clin Chem* 1970; 16:195–200.

19. Logan BK. Methamphetamine and driving impairment. *J Forensic Sci* 1996; 41:547–64.

20 Logan BK, Flinger CL, Haddix T. Cause and manner of death in fatalities involving methamphetamine. *J Forensic Sci* 1998; 43:28–34.

21. Winek CL. Drug & chemical blood-level data 1985. Fisher Scientific, Pittsburgh, PA, 1985.

22. Repetto MR, Repetto M. Habitual, toxic, and lethal concentrations of 103 drugs of abuse in humans. *Clin Toxicol* 1997; 35:1–9.

23. Chaturvedi AK, Smith DR, Canfield DV. Blood carbon monoxide and hydrogen cyanide concentrations in the fatalities of fire and non-fire associated civil aviation accidents, 1991–1998. *Forensic Sci Int* 2001; 121:183–8.

24. Chaturvedi AK, Smith DR, Soper JW, Canfield, DV, Whinnery JE. Characteristics and toxicological processing of postmortem pilot specimens from fatal civil aviation accidents. *Aviat Space Environ Med* 2003; 74:252–9.

25. Soper JW, Chaturvedi AK, Canfield DV. Prevalence of chlorpheniramine in aviation accident pilot fatalities, 1991–1996. *Aviat Space Environ Med* 2000; 71:1206–9.

26. Canfield D, Flemig J, Hordinsky J, Birky M. Drugs and alcohol found in fatal civil aviation accidents between 1989 and 1993. Washington, DC: U.S. Department of Transportation, Federal Aviation Administration; 1995 Nov. Report No: DOT/FAA/AM-95/28.[1]

27. Canfield DV, Hordinsky J, Millett DP, Endecott B, Smith D. Prevalence of drugs and alcohol in fatal civil aviation accidents between 1994 and 1998. *Aviat Space Environ Med* 2001; 72:120–4.

28. Canfield DV, Smith M, Ritter RM, Chaturvedi AK. Preparation of carboxyhemoglobin standards and calculation of spectrophotometric quantitation constants. *J Forensic Sci* 1999; 44:409–12.

29. Chaturvedi AK, Sanders DC, Endecott BR, Ritter RM. Exposures to carbon monoxide, hydrogen cyanide and their mixtures: Interrelationship between gas exposure concentration, time to incapacitation, carboxyhemoglobin and blood cyanide in rats. *J Appl Toxicol* 1995; 15:357–63.

30. Evans MA, Baselt RC. Principles of toxicant disposition. In: Cravey RH, Baselt RC, Eds. *Introduction to forensic toxicology*. Davis, CA: Biomedical Publications; 1981:41–68.

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