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Application of DNA Profiling in Resolving Aviation Forensic Toxicology Issues

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16. Abstract

Biological samples from the victims of aviation accidents are submitted to the Civil Aerospace Medical Institute (CAMI) for toxicological evaluation. Body components of aviation accident fatalities are often scattered, disintegrated, commingled, contaminated, and/or putrefied at accident scenes. These situations may impose difficulties in victim identification and tissue matching, thereby in the toxicological analysis of authentic samples and the interpretation of the associated analytical results. The use of DNA typing has been exemplified in the literature to resolve the sample misidentification issue. However, the prevalence of this type of issue in relation to aviation accident forensic toxicology has not been well-established. Therefore, the CAMI toxicology database was searched for the period of 1998−2008 for those accidents/cases wherein DNA profiling was performed. During this period, samples from 3523 accidents were received by CAMI. Of these, there were 3366 aviation accidents wherein at least one fatality had occurred. Biological samples from a total of 3319 pilots were received. Of these, 3275 were fatally injured. The 3319 pilots translated into the equivalent number of aviation accidents. Of the 3319 accidents, there were only 15 (≈ 0.5%) accidents wherein DNA profiling was performed on the biological samples. Six occupants (four fatalities and two injured victims) were involved in one accident and five (two fatalities and three injured victims) in another. Three fatalities occurred in three accidents each, two fatalities in eight accidents each, and one fatality in one accident. In one accident, there were two occupants with non-fatal injuries. DNA profiling was conducted upon the requests of families in two accidents, of accident investigators in three, and of pathologists in four. In six accidents, contradictory toxicological findings—such as selective presence of analytes in samples—led the CAMI laboratory to initiate DNA profiling. The requests made by families and investigators were primarily triggered by the inconsistency between the toxicological results and the history of the use of the drugs by the victims, while by the pathologists because of the commingling of samples. In three (20%) of the 15 accidents, at least one submitted sample was misidentified or mislabeled. The low number of the accident cases requiring DNA profiling suggests that the sample-submitting agencies take extensive precautionary measures to ensure that the origin of the submitted biological samples are correctly identified. Furthermore, the present study confirms that DNA typing can be used as a tool for establishing the authenticity of the aviation biosamples, thereby their associated toxicological conclusions.

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INTRODUCTION

During aviation accident investigations, biological samples are collected from victims by local pathologists and submitted to the Federal Aviation Administration's (FAA's) Civil Aerospace Medical Institute (CAMI; Oklahoma City, OK) for toxicological evaluation.[1,2] Such shipments include samples from pilot fatalities, survived pilots, other crewmembers, and passengers. The submission of samples from passengers, however, depends upon the nature of an accident, such as an accident involving fire.

While maintaining a high degree of quality assurance and control,[3-11] acquiring accurate and authentic analytical data on the biological evidence has been the primary objective of CAMI in order to effectively assist the conclusion of aircraft accident investigations. However, the accuracy and authenticity of such collected data fundamentally depends upon the integrity of the submitted biological samples, thereby allowing the truthful interpretation of results with a high degree of confidence and judicial admissibility.

There is a potential for inherited realistic limitations in aircraft accidents, particularly in those wherein more than one fatality had occurred and, additionally, wherein multiple types of postmortem specimens were collected and submitted. In some instances, samples originating from an incorrect source would have been submitted or they were labeled with an incorrect name—that is, the sample(s) are misidentified or mislabeled, possibly due to human error. Such potential is because of the intrinsic nature of aviation accidents wherein body parts of fatally injured victims are often scattered, disintegrated, commingled, contaminated, and/or putrefied. Of course, the extent of such accident scene conditions primarily depends upon the severity and situation of a particular accident. These circumstances may impose difficulties for victim identification, tissue matching, and thereby authentic sample analysis and result interpretation. However, these issues, at least to some extent, can be resolved by DNA profiling.

The use of DNA profiling has previously been documented in resolving tissue mismatching and/or analytical result interpretation issues for two aviation accident cases.[12] One case was associated with the uncertainty of the correct identity of the tissues originated from either and/or both of the two occupants, while the other case with the selective presence of atropine. Although the application of DNA profiling has been exemplified, the prevalence of sample identity/validity and result interpretation issues in association with aviation accident forensic toxicology has not been established. Therefore, the CAMI toxicology database was searched for those aviation accident cases wherein DNA profiling was performed. The search period was from 1998 to 2008. In the present study, those DNA-related accidents/cases are described, covering the associated histories, findings, discussions, and conclusions.

MATERIALS AND METHODS

Biological Samples

Biological specimens collected from aviation accident casualties are submitted to CAMI in the FAA TOX-BOX evidence containers at the request of the National Transportation Safety Board (NTSB).[1,2] It is strongly recommended that one TOX-BOX evidence container per victim be used for shipping samples. The types of samples generally received at CAMI are blood, urine, vitreous fluid, spinal fluid, brain, lung, heart, liver, kidney, muscle, and/or other body tissues. Reference material—for example, biological samples from blood relatives and personal effects of victims—may also be received for DNA profiling.

The TOX-BOX evidence containers are received in the secured accessioning area of the CAMI's Bioaeronautical Sciences Research Laboratory. Upon receipt, the containers are opened in the presence of two (or more) authorized quality assurance/quality control staff members. Only one evidence container is processed at a time; other containers are left intact. The processing involves the opening of the container and cataloging of its contents, which entails videotaping the outside and inside of the container, including seals and labels, and documenting the details of the case, including chain-of-custody. This approach is taken according to the standard operating procedure of the laboratory. After processing a case in one TOX-BOX evidence container, the evidence container of another case is started. All videotapes are later reviewed to ensure the chain of custody of the container and its forensic contents. The policy of "one-container (case)-at-a-time" is always followed. This philosophy is also practiced during the aliquoting of samples of the cases

for the preparation of batches for analyses. Each victim of an accident from which samples are received is given a specific CAMI case number. Therefore, the "accident," "victim," and "case" words are considered related to each other and are interchangeably used in the present study, when necessary.

Analytical Toxicology

The presence of combustion gases, ethanol/volatiles, and drugs in the samples is analytically demonstrated by screening, followed by confirmation and/or quantitation.[2,13,14] All analyses are performed according to established standard operating procedures of the laboratory by using various techniques such as spectrophotometry, immunoassay, and chromatography. The combustion gases entail carbon monoxide and hydrogen cyanide; the drugs include a wide range of prescription, nonprescription, and illegal drugs. DNA profiling is performed on case samples in which there is doubt about the identity of the submitted samples. This DNA approach becomes a necessity when there are reasons to believe that the samples might have been misidentified (mislabeled), mismatched, or commingled with the samples of other victims during the collection of samples. These toxicological and DNA profiling aspects are summarized in a recent review article.[15]

Toxicology Database

Analytical toxicology results, including DNA typing findings, are electronically entered in a database at CAMI, also incorporating relevant information of the associated accidents and victims. In this CAMI toxicology database, the detailed DNA profiling findings of aviation accident cases have been stored since 1998. For the present study, the toxicology database was examined for the accidents and for the fatally/non-fatally injured pilots and other victims wherein DNA typing analyses were performed. This evaluation included the associated toxicological findings, as well. The selected period for the examination was 11 years (1998–2008). For this period, the database was also searched for the total number of all accidents, including aviation accidents and associated pilot fatalities whose postmortem biological samples were received at CAMI.

DNA Profiling

DNA profiling on the identity-related samples and/or associated reference samples (or evidence) were performed by the FAA's CAMI laboratory or by an outside laboratory. The selection of the laboratory for the profiling was governed by the accident scenario and/or by the aviation accident investigator's decision. The number and type of DNA loci evaluated were dependent upon the standard operating procedures of the laboratories from which DNA analytical services were obtained and the state-of-the-art techniques available at the time of DNA profiling. For example, DQA1 and poly-markers (PMs)—LDLR, GYPA, HBGG, D7S8, and GC—were evaluated during the 1990s.[12,16-18] However, as advancements in the technology took place during the 2000s, the number of DNA loci included in the protocols of the laboratories increased from half a dozen up to more than a dozen (D3S1358, D16S539, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, CSF1PO, TPOX, TH01, vWA, FGA, Penta E, Penta D, SRY-ZF, and amelogenin[19-24]). In some situations, an evaluation of a gender marker—for example, amelogenin[23] or SRY-ZF[19,21]—sufficed the need to deduce the origin of the tissues. This approach was particularly more realistic when there were only two occupants involved in an accident and they were of the opposite sex.

The procedure adopted in the CAMI laboratory during the 1990s for DQA1 and PMs has been reported earlier.[12] Briefly, the procedure consisted of the extraction of DNA from biological samples, followed by DNA quantitation and amplification, and polymerase chain reaction (PCR) product verification, hybridization, and detection.[16-18]

During the 2000s, several new genetic markers were incorporated in the protocol of the CAMI laboratory.[25] In general, DNA profiling on samples was performed by comparing PCR amplicon lengths generated from nine autosomal loci and one or two gender determination loci by electrophoretic mobility.[25] The short tandem repeat (STR) loci are part of the Federal Bureau of Investigation's Combined DNA Index System—that is, CODIS.[26] The commonly used loci in CAMI's laboratory were D3S1358, D5S818, D7S820, D13S317, D16S539, FGA, THO1, TPOX, and vWA. These STR loci are variable four-nucleotide tandem repeats. Reactions for each autosomal locus were individually performed. There was no multiplexing. For gender determination, amelogenin[23] was initially used. Subsequently, this marker was replaced with a duplex reaction for establishing the presence of the Y-chromosome-specific SRY locus and the homologous zinc finger protein genes, ZFX/ZFY, of the X and Y chromosomes.[27] PCR amplicon lengths were compared by micro-fluidic electrophoresis on an Agilent BioAnalyzer 2100 (Santa Clara, CA) by using DNA1000 series-2 chips that were processed following the Agilent's DNA1000 series-2 protocol for this instrument. At the expected fragment lengths for these loci, the instrument has four-nucleotide resolution.

CASE HISTORIES

Case 1

Scenario

An experimental aircraft crashed during a personal pleasure flight, killing both occupants—the pilot and the passenger. This flight was being conducted to demonstrate the performance capabilities of the aircraft to the passenger. The aircraft was destroyed. This accident occurred in 1998. Autopsies on both victims were performed, and postmortem samples from the victims were submitted to CAMI.

Submitted Samples

Pilot: Bile, brain, gastric, heart, kidney, liver, lung, muscle, spleen, and urine

Passenger: Bile, brain, gastric, heart, kidney, liver, lung, muscle, and spleen

Toxicological Findings

Pilot: Negative

Passenger: Diphenhydramine 0.075 µg/mL in liver fluid and 0.08 µg/mL in kidney fluid

Reason for DNA Profiling

Upon the request of the passenger's family, DNA typing of the passenger's "so called" brain sample was performed by an external laboratory in relation to the reference samples—hair from the passenger and blood from the passenger's daughter. The DNA typing of the hair and blood samples did not exclude the passenger as the biological father of the daughter, but the DNA profile of the brain suggested that it did not originate from the passenger.

The above sample ambiguity led the CAMI laboratory to perform additional DNA analysis on all submitted solid tissue samples from the pilot, as well as from the passenger. Except for brain samples, all other sample types originated from two different sources. The brain samples labeled as "pilot" and as "passenger" matched with each other. These findings, in conjunction with the earlier reference sample analysis, concluded that both submitted brain samples originated from the same individual—that is, the pilot, not the passenger. Therefore, it was concluded that all samples, except brain samples, were correctly labeled at the time of autopsy as "pilot" and as "passenger," and the toxicological findings of the presence of diphenhydramine in liver and kidney were correctly associated with the passenger, not with the pilot. No toxicological analysis was performed on the brain samples.

DNA Loci Evaluated

The passenger's hair, daughter's blood, and brain samples were evaluated for D2S44, D18S27, D4S163, D7S21, DQA1, LDLR, GYPA, HBGG, D7S8, and GC by an external laboratory; and all solid samples from the pilot and the passenger for DQA1, LDLR, GYPA, HBGG, D7S8, and GC by the CAMI laboratory.

Case 2

Scenario

In 1998, a two-occupant aircraft on a maintenance test flight crashed, killing the pilot and the copilot. The aircraft was destroyed. At the time of accident, visual meteorological conditions prevailed. A muscle sample collected from the pilot was submitted to CAMI.

Submitted Samples

Pilot: Muscle

Copilot: No sample

Toxicological Findings

Pilot: Negative

Copilot: Not applicable

Reason for DNA Profiling

CAMI was requested to conduct DNA analysis on the muscle sample, since the pathologist was unsure whether the muscle remains belonged to the pilot or to the copilot. Reference items (hair dryer, hair brush, toothbrush, and two dental picks from the pilot; electric razor and toothbrush from the copilot) were later submitted to CAMI for the DNA examination. The DNA profiling of the muscle sample and of the hair sample taken from the hair dryer labeled as "pilot" suggested the genotype of these samples were the same. DNA found on the toothbrush labeled as "copilot" did not match with that of the muscle. Based upon the CAMI laboratory analysis, it was concluded that the source of the muscle was indeed the pilot.

DNA Loci Evaluated

The DNA loci examined were DQA1, LDLR, GYPA, HBGG, D7S8, and GC.

Case 3

Scenario

A float-equipped airplane sustained substantial damage when it collided with the ground. There were two occupants in the plane, the first pilot/flight instructor and the student pilot. Both occupants received serious injuries in this 2000 non-fatal accident. The first pilot/flight instructor was a male, and the student pilot was his daughter. The accident-investigator-in-charge coordinated with the local authorities the shipment of the biological samples

collected from the male pilot to CAMI. Samples from the female pilot were not submitted, as it was determined that the aircraft was flown by the male pilot.

Submitted Samples

Pilot: Blood, serum, and urine
Student Pilot: None

Toxicological Findings

Diphenhydramine 0.018 µg/mL in blood
Bupropion metabolite present in blood
Acetaminophen 14.3 µg/mL in urine
Diphenhydramine, bupropion, and lidocaine present in urine

Reason for DNA Profiling

Based upon the toxicological findings, it was viewed that the pilot was flying while using unapproved medications. The male pilot claimed that the analyzed samples belonged to his daughter and not to him; accordingly, he declared that it was his daughter who was on the medications and not him. Thus, the wrong specimens were analyzed. The NTSB accident investigator requested DNA analysis to determine the gender and origin of the source. The blood sample was sent to an outside laboratory for the DNA analysis. The amelogenin locus was used for the gender identification of the origin of the blood sample and found that the sample was from a single male human source. Therefore, it was concluded that the toxicologically tested samples originated from a male (the pilot), not from a female (the student pilot—daughter).

DNA Locus Evaluated

The locus was amelogenin.

Case 4

Scenario

In 2000, an aircraft collided with the terrain, fatally injuring the pilot and two passengers. The airplane was destroyed. Bodies of all the three victims were severely fragmented and commingled. Several pieces of tissue samples were shipped to CAMI in one TOX-BOX evidence container.

Submitted Samples

Pilot/Passengers: Body tissue, kidney, lung, and muscle

Toxicological Findings

Pilot: Negative
Passengers: No analysis

Reason for DNA Profiling

The NTSB accident investigator requested DNA profiling because samples from the three fatalities were severely fragmented. The DNA profiling was conducted by an external laboratory. Based upon the DNA profiling findings and the accident scene sector numbers, the samples were labeled with the respective sector numbers and were accordingly identified as to be from pilot (body tissue, kidney, lung, and muscle), passenger I (body tissue), and passenger II (body tissue and muscle). All samples were submitted to CAMI in one TOX-BOX evidence container. Knowing the source of the samples, the toxicological analysis was performed on the samples originated from the pilot. Samples from passengers were not toxicologically evaluated.

DNA Loci Evaluated

The loci were D3S1358, VWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, CSF1PO, TPOX, TH01, D16S539, and amelogenin.

Case 5

Scenario

In 2001, a cargo plane impacted the terrain following an uncontrolled descent and was totally destroyed. One of the two occupants of the plane died at the scene of the accident. The second occupant was seriously injured but died later. Postmortem samples were submitted to CAMI.

Submitted Samples

Pilot/Copilot: Three pieces of muscle

Toxicological Findings

Pilot: Negative
Copilot: Negative

Reason for DNA Profiling

The three pieces of muscle were submitted in one TOX-BOX evidence container. The samples were commingled, and the origin of the muscles could not be positively established by the pathologist. Because of the source uncertainty, samples from the muscles were subjected to DNA profiling. The profiling was performed at an external laboratory. Based upon the DNA results, the muscles were separated in two groups—one muscle piece belonged to one person and the remaining two pieces to the second person. However, in the absence of any reference sample, it was not possible to link the origin of those groups specifically to the pilot or copilot. Since toxicological findings were negative in both cases, the origin association was not relevant.

DNA Loci Evaluated

The loci were D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, amelogenin, vWA, D8S1179, TPOX, and FGA.

Case 6

Scenario

An aircraft piloted by a private pilot was destroyed on impact in a pasture. This flight was associated with a pheasant hunting expedition. In this 2004 accident, there were three occupants (the pilot and two passengers), all of whom were fatally injured. Body parts of victims were badly fragmented and were also commingled among approximately 27 kg of pheasant. Although there were three fatalities, biological samples, presumably from the pilot, were submitted to CAMI.

Submitted Samples

Pilot: Bone, muscle, skin, and lung

Toxicological Findings

Pilot: Propoxyphene 0.308 µg/g in muscle and 0.341 µg/g in skin
Norpropoxyphene 0.671 µg/g in muscle and 0.123 µg/g in skin
Bupropion present in muscle
Dextromethorphan and tramadol present in muscle and skin
Passenger (Post-DNA Analysis): Pseudoephedrine present in lung

Reason for DNA Profiling

The pathologist requested that CAMI perform DNA profiling on the submitted samples to ensure that they truly originated from one human being—that is, the pilot. Muscle, skin, and lung specimens were subjected to the DNA analysis by the CAMI laboratory. The analysis revealed that the muscle and the skin came from one victim, while the lung from a second victim. These DNA findings warranted creating a new case for the second victim—that is, one of the two passengers—and the lung sample was transferred to that case. These two groupings were further supported by the toxicological findings. The drugs found in the muscle and skin specimens were consistent with each other, whereas the drug in the lung was not, supporting the DNA results that the muscle and skin came from one individual, while the lung from a different individual.

DNA Loci Evaluated

The samples were examined for the following loci: amelogenin, CSF1PO, D5S818, D7S820, D13S317, D16S539, TH01, TPOX, and vWA.

Case 7

Scenario

A single-engine aircraft was destroyed during impact with terrain following a loss of control during an approach. The two occupants—the flight instructor and the student pilot—did not survive the crash. There was no fire. Postmortem samples from both victims were submitted to CAMI.

Submitted Samples

Flight Instructor: Bile, blood, brain, gastric, heart, kidney, liver, lung, muscle, spleen, and urine

Student Pilot: Bile, blood, brain, gastric, heart, kidney, liver, lung, muscle, spleen, urine, and vitreous fluid

Toxicological Findings

Flight Instructor: Atenolol detected in blood and urine

Student Pilot: Cyanide 3.7 µg/mL in blood

Reason for DNA Profiling

There was no fire in this accident. The blood from the instructor was negative for cyanide, but the blood from the student was found to be positive for cyanide. The blood cyanide concentration was in the lethal range. Such selective presence of cyanide led the CAMI laboratory to perform DNA profiling on the cyanide-positive blood sample, along with the associated sample types used for other toxicology tests. These other sample types were liver and kidney. The DNA analysis disclosed that the blood, liver, and kidney were from the same individual. Although the DNA analysis was able to ensure that these three samples were of one origin, the reason for the selective presence of cyanide in the high concentration could not be toxicologically deduced, particularly when there was no fire and the carboxyhemoglobin level was negative.

DNA Loci Evaluated

The loci used for the DNA typing were amelogenin, D3S1358, D5S818, D7S820, D13S317, D16S539, TH01, TPOX, and vWA.

Case 8

Scenario

In 2006, an aircraft was destroyed when it impacted water and light stanchions while approaching the airport. There were two pilots and three passengers. The pilots

were fatally injured, but the passengers received only minor injuries. Postmortem samples from the pilots were submitted to CAMI.

Submitted Samples

Pilot: Bile, blood, brain, gastric, heart, kidney, liver, lung, muscle, spleen, urine, and vitreous fluid
Copilot: Bile, blood, brain, gastric, heart, kidney, liver, lung, muscle, spleen, urine, and vitreous fluid

Toxicological Findings

Pilot: Cocaine 0.202 µg/mL in urine
Benzoylecgonine 0.445 µg/mL in urine
Ecgonine methyl ester and quinine present in urine
Copilot: Ephedrine, pseudoephedrine, and phenylpropanolamine present in urine

Reason for DNA Profiling

DNA analysis was performed because cocaine was found in urine but not in the pilot's blood. It was suspected that the blood might have originated from the copilot. To confirm the suspicion, the DNA typing was performed by the CAMI laboratory on the blood samples from both victims. The DNA profiling results concluded that the submitted blood samples originated from two different sources. Thus, the blood that was analyzed for cocaine was truly negative for cocaine.

DNA Loci Evaluated

The DNA loci entailed SRY-ZF, D3S1358, D5S818, D7S820, D13S317, D16S539, TH01, TPOX, and vWA.

Case 9

Scenario

An aircraft collided with terrain during a circling instrument approach and was destroyed. This accident took place in 2007. Both the pilot and a passenger were killed and their bodies were extensively fragmented. Postmortem samples from both victims were submitted to CAMI.

Submitted Samples

Pilot: Blood, lung, muscle, and spleen
Passenger: Blood, gastric, kidney, liver, lung, muscle, and spleen

Toxicological Findings

Pilot: Negative
Passenger: Negative

Reason for DNA Profiling

The pathologist informed CAMI that organs of the aviation accident victims were commingled. A doubt was raised whether the submitted blood samples collected from a disaster pouch originated from a single individual or if they were mixtures of blood originating from both individuals. CAMI performed DNA profiling on Blood 1, Blood 2, and muscle samples from the pilot (Group 1) and on Blood 1, muscle, and liver samples from the passenger (Group 2). The DNA analysis of the three sample types from the pilot (Group 1) confirmed that they originated from one individual. The muscle and liver of Group 2 belonged to a different individual, which was deduced to be of the passenger. The "so called" Blood 1 sample from the passenger was determined to be a mixture of two different genetic origins—that is, from the pilot and the passenger. Therefore, Blood 1 was not used for toxicological evaluation.

DNA Loci Evaluated

The DNA profiling was established by using the following markers: amelogenin, D3S1358, D5S818, D7S820, D13S317, D16S539, TH01, TPOX, and vWA.

Case 10

Scenario

Following a descent from cruising altitude, an aircraft with two occupants was destroyed on impact with terrain. Both occupants—the pilot and the pilot-rated passenger—sustained fatal injuries in this 2007 crash. Postmortem samples were submitted to CAMI.

Submitted Samples

Pilot: Lung and muscle
Passenger: Kidney and liver

Toxicological Findings

Pilot: Ethanol 43 mg/hg in muscle and 59 mg/hg in lung
Atenolol present in lung and muscle
Passenger: Ethanol 26 mg/hg in liver and 25 mg/hg in kidney
Atenolol present in liver and kidney

Reason for DNA Profiling

Lung and muscle samples were submitted together in one single plastic bag. Upon receiving the bag, these two tissue types were separated and placed individually in two different bags. Similarly, liver and kidney samples from the second victim were submitted in one single plastic bag; these sample types were separated and placed in two different bags. Because of the sample submissions of two sample types in one bag and the presence of ethanol and atenolol in samples of both victims, there was a suspicion whether the submitted samples originated from one individual. The four samples—lung and muscle from the pilot and liver and kidney from the second victim—were

subjected to DNA profiling at the CAMI laboratory. The DNA results suggested that lung and muscle originated from one male subject, while liver and kidney from a female subject. The presence of ethanol and atenolol in the sample types from both subjects was determined to be coincidental.

DNA Loci Evaluated

The DNA loci were SRY-ZF, D3S1358, D5S818, D7S820, D13S317, D16S539, TH01, TPOX, and vWA.

Case 11

Scenario

This 2007 accident involved an aircraft that was destroyed upon colliding with terrain after takeoff. The sole occupant of the aircraft did not survive the crash. Postmortem samples from the victim were submitted to CAMI.

Submitted Samples

Pilot: Blood, brain, gastric, heart, kidney, liver, lung, muscle, spleen, and vitreous fluid

Toxicological Findings

Pilot: Negative

Reason for DNA Profiling

An external laboratory detected hydrocodone in the victim's blood, but the drug was absent in urine. CAMI's toxicological evaluation failed to disclose the presence of any drugs/alcohol and, therefore, the case was considered as negative. The discrepancy in the toxicological findings—positive *versus* negative—from the two laboratories, led the CAMI laboratory to subject the blood, kidney, and muscle samples for DNA profiling. These samples were found to be from the same male. This conclusion was consistent with the fact that the pilot was the sole male occupant of the crashed aircraft. It could thus be deduced that the blood sample analyzed by the outside laboratory might have originated from a person other than the pilot. The analyzed blood was possibly misidentified as from "pilot," leading to the reporting of a "false" positive for hydrocodone in the pilot's blood by the external laboratory.

DNA Loci Evaluated

The loci evaluated were SRY-ZF, D3S1358, D5S818, D7S820, D13S317, D16S539, TH01, TPOX, and vWA.

Case 12

Scenario

An aircraft was substantially damaged when it impacted the ground while on approach to the airport. Both occupants—the pilot and the passenger—sustained fatal injuries. Postmortem biological samples from the victims were submitted to CAMI.

Submitted Samples

Pilot: Blood, brain, gastric, heart, liver, lung, muscle, and spleen

Passenger: Blood, brain, heart, kidney, liver, lung, muscle, spleen, and urine

Toxicological Findings

Pilot: Atenolol present in liver and lung

Passenger: Atenolol present in liver and urine

Reason for DNA Profiling

Both victims' toxicology suggested the presence of atenolol in their systems and their medical records indicated that they were not on any medication, so it was decided to perform DNA typing to ensure that the samples did not originate from the same individual. The DNA profiling was performed at the CAMI laboratory. The samples were liver and lung from the pilot (Group 1), and also liver and lung from the passenger (Group 2). The DNA profiling findings concluded that the Group 1 samples originated from one individual, while the Group 2 samples from another individual. Therefore, the presence of atenolol in the systems of both victims was coincidental.

DNA Loci Evaluated

The DNA loci used for the typing were SRY-ZF, D3S1358, D5S818, D7S820, D13S317, D16S539, TH01, TPOX, and vWA.

Case 13

Scenario

An amateur-built aircraft was destroyed when it impacted trees and terrain after missing an approach to landing. Both the pilot and the copilot were fatally injured. Postmortem samples from both victims were collected by the pathologist and submitted to the CAMI laboratory. This aviation accident occurred in 2008.

Submitted Samples

Pilot: Kidney, liver, and muscle

Passenger: Kidney, liver, and muscle

Toxicological Findings (Pre-DNA Analysis)

Pilot: Ethanol 15 mg/hg in liver
Ethanol not present in muscle
Amlodipine present in liver and kidney
Losartan and diphenhydramine present in liver
Copilot: Ethanol 24 mg/hg in muscle

Toxicological Findings (Post-DNA Analysis)

Pilot: Negative
Copilot: Ethanol 15 mg/hg in liver and 24 mg/hg in muscle
Amlodipine present in liver and kidney
Losartan and diphenhydramine present in liver

Reason for DNA Profiling

Upon receiving the toxicological report, an uncertainty was raised by the family members of the pilot that there was something wrong in the sample collection and/or toxicological evaluation processes. To the best of the family members' knowledge, the pilot was not on the medications mentioned in the toxicology report. The NTSB suspected that there was a sample mix-up at the time of autopsy and asked for DNA analysis to be performed by the CAMI laboratory. It appeared that the samples were mislabeled. A reference DNA sample from the pilot's son was collected as buccal swabs and submitted to CAMI. With reference to the son's DNA typing, the submitted liver, kidney, and muscle samples labeled as "pilot" and the muscle sample labeled as "copilot" were subjected to DNA typing. The DNA findings concluded that the liver and kidney samples originally labeled as "pilot" originated from the copilot, instead of the pilot, but the submitted muscles were correctly labeled as to their respective origins. Thus, both toxicological reports were accordingly corrected (see the post-DNA analysis toxicological findings mentioned earlier). The supplemental report findings were consistent with the medical histories of the pilot and of the copilot. In the copilot's medical certification records, the use of amlodipine and losartan was documented. In addition to the modifications in the reports, the originally submitted samples were correspondingly labeled correctly and transferred to the respective case storage bins in the CAMI laboratory. Necessary changes in the folders of both cases were also made and documented to rectify the mislabeling of specimens during autopsy.

DNA Loci Evaluated

The DNA loci examined were SRY-ZF, D3S1358, D5S818, D7S820, D13S317, D16S539, FGA, TH01, TPOX, and vWA.

Case 14

Scenario

In an aviation accident that occurred in 2008, three occupants—the pilot and two passengers—were fatally injured. One of the occupants was female. This accident happened when the aircraft impacted water following a loss of control. The wreckage came to rest approximately 12 m below the surface of the water. Under the water, bodies of the victims were completely fragmented, and their parts were scattered and commingled. Because of the water depth, it was difficult for the investigators to properly retrieve the remains of the victims and to correctly establish the identity of the recovered body parts. Body parts did not have anatomically identifying features. Considering the complexity of the accident, the recovered samples were packed in three TOX-BOX evidence containers and shipped to the CAMI laboratory. Each of the three containers was intended to contain samples originating from one of the three victims, but the potential for the presence of the body parts from the other victims in any given evidence container was very strong. Therefore, the misidentification of the submitted body tissues and their commingling could not be ruled out.

Submitted Samples

Pilot/Passengers: Large pieces of body parts

Toxicological Findings

Pilot: Negative
Female Passenger: Negative
Male Passenger: Diazepam, nordiazepam, and zolpidem present in body tissue

Reason for DNA Profiling

Because of the complexity of the case and the potential of the misidentification of the body parts, the accident investigator requested that DNA profiling be performed by the CAMI laboratory. Reference samples—hairbrush, toothbrush, razor, and comb belonging to each victim—were provided for the DNA analysis. Buccal swabs from the female victim's son were also submitted.

To ensure sufficient amounts of samples available for the postmortem toxicology, three large pieces of the body parts were taken out from each evidence container and were properly marked with unique identification numbers. Small portions of these nine body parts were subjected to the DNA analysis, along with the respective reference samples and the buccal swab. Based upon the DNA typing findings, the nine body parts were separated and grouped into three. Such separation and grouping was based upon the DNA profile matching of the nine tissue samples with that of the reference samples and buccal swab, including

the DNA-based gender identification. These three groups were identified as the pilot, the female passenger, and the male passenger. After this DNA-based identification and grouping, the samples from the three victims—the three groups of body tissues—were toxicologically evaluated. The toxicological findings are summarized in the previous subsection of this case.

DNA Loci Evaluated

The loci were SRY-ZF, D3S1358, D5S818, D7S820, D13S317, D16S539, FGA, TH01, TPOX, and vWA.

Case 15

Scenario

In a 2008 accident, an aircraft overran the runway while taking off and crashed. Tire debris and portions of airplane components were found along the runway. The beginning of the takeoff roll appeared normal and then sparks were observed. The crew attempted to reject the takeoff, but was unable to stop the airplane. The plane continued beyond the runway and crashed through airport lighting, navigation facilities, fence, and a roadway, and came to stop. There was a postcrash fire. The two crewmembers and two of the four passengers were fatally injured. The remaining passengers suffered serious injuries. Postmortem samples from the crewmembers—the pilot and the copilot—were submitted to CAMI.

Submitted Samples

Pilot: Bile, blood, brain, gastric, heart, kidney, liver, lung, muscle, spleen, and urine
Copilot: Bile, blood, brain, gastric, heart, kidney, liver, lung, muscle, spleen, and vitreous fluid

Toxicological Findings

Pilot: Carboxyhemoglobin 20%
Cyanide 1.80 µg/mL in blood
Diphenhydramine 0.030 µg/mL in blood
Diphenhydramine present in liver
Copilot: Carboxyhemoglobin 25%
Cyanide 2.07 µg/mL in blood
Diphenhydramine 0.036 µg/mL in blood
Diphenhydramine present in liver and urine
Ibuprofen present in urine

Reason for DNA Profiling

Because the toxicological results were similar for both victims, DNA profiling was performed by the CAMI laboratory. The samples tested for the profiling were blood and liver from the pilot, as well as from the copilot. The results revealed that all specimens tested were indeed from the respective female (pilot) and male (copilot) victims, as indicated by the specimen labels provided by the pathologist.

DNA Loci Evaluated

The DNA loci were SRY-ZF, D3S1358, D5S818, D7S820, D13S317, D16S539, FGA, TH01, TPOX, and vWA.

RESULTS AND DISCUSSION

During the 11-year period (1998–2008), biological samples from the casualties of a total of 3523 accidents were submitted to CAMI for toxicological evaluation. Of these, 3366 were fatal aviation accidents—that is, at least one fatality had occurred in each of these accidents. Biological samples from a total of 3319 pilots were submitted; of these pilots, 3275 were fatally injured. The 3319 pilots translated into the same number of aviation accidents. Out of the 3319 accidents, there were only15 (≈ 0.5%) accidents—one non-fatal and 14 fatal—wherein DNA profiling was performed to resolve the issue of the identity of the submitted samples. In some instances, additional evidence—such as biological samples from blood relatives and tooth brush and hairs of the victims—was included in the DNA typing to resolve the identity issue.

In these 15 accidents, the number of DNA loci evaluated ranged from one to 16. Depending upon the nature of an aviation accident and the number of occupants, the DNA loci examined had sufficient power of discrimination to resolve the origin issue of the accident case samples. For example, if there were two occupants of opposite sex in an accident, then one DNA locus of a genetic marker for the determination of gender sufficed the need for establishing the origin of the samples. In other situations wherein occupants were of the same sex, the higher number (six to 16) of DNA loci was desirable for establishing the source of the samples with an acceptable, high degree of certainty. This acceptability was decisive because the number of occupants in any given accident was small (one to six) and well-defined by DNA analysis. This approach was within the realm of the application of DNA typing for a small number of individuals in a population.

Of the 15 accidents, six occupants (four fatalities and two injured victims) were involved in one accident and five (two fatalities and three injured victims) in another. Three fatalities occurred in three accidents each, two fatalities in eight accidents each, and one fatality in one accident. In one accident, there were two occupants with non-fatal injuries. The DNA profiling of the collected biological specimens from these 15 accidents was conducted upon the requests of families in two accidents, of accident

investigators in three, and of pathologists in four. In six accidents, contradictory toxicological findings—such as selective presence of analytes in samples or presence of same analytes in the samples from each occupant—led the CAMI laboratory to initiate the DNA profiling to ensure the identity of the submitted samples of the victims. The requests made by families and investigators were also primarily triggered by the inconsistency between the toxicological results and the aeromedical history of the use of the drugs by the victims, while the pathologists were concerned because of the commingling of samples. In three (20%) of the 15 accidents, at least, one submitted sample was determined to be misidentified or wrongly labeled, presumably due to human error during autopsy.

Because of the fundamental nature of the seriousness of aviation accidents consisting of commingling, scattering, disintegration, and putrefaction of bodies and their parts at the accident scenes, the potential for the samples to be misidentified with respect to their origin is a real challenge for the accident investigators and pathologists. In spite of these limitations, the findings of this study suggests that the sample submitting agencies take extensive precautionary measures to make certain that the origin of the submitted biological samples are correctly identified, though the potential for misidentification/mislabeling of samples still exits. This conclusion is supported by the fact that there were only ≈ 0.5% of the accidents wherein the DNA profiling was requested and the mislabeling of samples was found in only two (13%) of the 15 accidents. The present study confirms that the DNA typing could be used[12] as a tool for establishing the authenticity of the submitted samples, and thereby their associated toxicological conclusions. Based on the findings of the present study, it is concluded that mislabeling of specimens appears to occur rarely.

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