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Cross-border disaster victim identification after the Struma Motorway bus fire: 45 victims identified within 10 days

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Abstract

Background

Mass-fatality road incidents involving severely burned foreign nationals require rapid integration of scene recovery, post-mortem examination, ante-mortem information and reconciliation. This report describes the locally assembled response to the Struma Motorway bus crash and fire in Bulgaria on 23 November 2021 and compares it element by element with the INTERPOL Disaster Victim Identification (DVI) Guide.

Results

Authorities organized three parallel workstreams for scene recovery, autopsy and laboratory investigation. Scene examination began at 06:05 in darkness and rain. Twenty decedents were autopsied by 21:00 on the first day and the remaining 24 the next day. When reconciliation showed that one passenger remained unaccounted for, a repeat scene examination on 26 November recovered the 45th decedent from the front-left luggage compartment. DNA profiles were obtained from all 45 sets of remains and compared with profiles from 34 relatives sampled in North Macedonia. DNA, odontology and corroborating individual features resolved a dizygotic twin pair and several sibling groups. All 45 victims were identified within 10 days. Fatal thermal injury was found in all cases; methemoglobin ranged from 3% to 38%. Carboxyhemoglobin, reconstructed for all 45 decedents together with the recovery position of each, was not detected in seven, reached 49%, and increased from the front of the saloon towards the rear: median 9%, 16% and 23% across three longitudinal zones (Spearman rho = 0.47, p = 0.001). Of 32 Guide elements assessed against the case record, 18 were fulfilled by a documented local equivalent, 8 were fulfilled with partial documentation, 3 were not applicable to an event of this type and scale, and 3 are capabilities or formats identified for pre-adoption.

Conclusions

The principal innovation was organizational rather than technological: early cross-border collection of comparative material, parallel post-mortem work and reconciliation that actively redirected scene examination. Because the INTERPOL Guide is written as a flexible baseline rather than a fixed checklist, the case demonstrates that its core functions can be delivered by existing local institutions, and it offers a transferable model for jurisdictions that must mobilize such capacity before a fully standardized DVI structure is available.

Background

Disaster victim identification (DVI) is an interdisciplinary process that links scene examination, post-mortem (PM) data, ante-mortem (AM) data and reconciliation. INTERPOL describes friction-ridge analysis, comparative dental analysis and DNA as primary identifiers, with unique serial numbers of

medical implants as a further reliable means; personal description, medical findings, clothing and other evidence found on the body are secondary means that normally support, rather than independently establish, identity, and identification from photographs should be avoided as a sole means (INTERPOL 2023). The process is particularly demanding when heat has damaged external features, when close relatives are travelling together, and when victims and their families are located in different countries (Lessig and Rothschild 2012; Schuliar and Knudsen 2012; Anderson et al. 2017; Montelius and Lindblom 2012; Prinz et al. 2007; Alonso et al. 2005; Weedn and Baum 2011).

The 23 November 2021 Struma Motorway incident combined all of these challenges. A post-collision fire rapidly involved a bus carrying travellers from North Macedonia. Of the 52 occupants – 50 passengers and two drivers – 45 died. The seven survivors, two women and five men, were in the rear of the saloon; they broke a side window, escaped through it and were taken to hospital. Many bodies were severely burned, the rear door was mechanically obstructed by a road barrier, and the identification operation required immediate coordination between Bulgarian investigative and forensic services and North Macedonian prosecutors, forensic specialists and families.

Although bus and coach occupants represent a small proportion of all European road deaths, severe coach crashes can produce a disproportionate forensic and rescue burden. The ECBOS in-depth database described 31 crashes involving M3 buses and 1,341 occupants; 1,015 were casualties, including 264 severely injured persons and 119 fatalities (UNECE 2004). A later literature review estimated that bus and coach occupants accounted for only 0.3–0.5% of European traffic fatalities overall, but identified intrusion, ejection and smoke inhalation as important mechanisms in severe events (Albertsson and Falkmer 2005). Fire adds a short evacuation window, destroys visual identifiers and personal effects, and may convert a road crash into a DVI operation.

Selected mass-fatality bus and coach fires from 1982 to 2023 are summarized in Table 1. The comparison is intentionally descriptive rather than systematic, and it follows the source hierarchy set out in the Methods: official accident and investigation reports were used where available, and authoritative contemporaneous reports were used only where no public technical report or forensic publication could be located. The pattern across events is recurrent - rapid fire development, compromised exits, severely burned victims and, in cross-border or family-group travel, the need for formal reconciliation rather than visual recognition alone (Swedish National Board of Health and Welfare 2011; Anadolu Agency 2014; NBC News 2015; BEA-TT 2020; ANSA 2020; Ministry of Foreign Affairs and Trade of Hungary 2017; Bavarian State Government 2017; Anadolu Agency 2018; Associated Press 2023; Dawn 2023; Corpo Nazionale dei Vigili del Fuoco 2023).

Table 1

Table 1
Selected mass-fatality bus and coach fires, 1982–2023

Date and location	Reported deaths	Fire and evacuation features	Identification or DVI relevance
31 Jul 1982, Beaune, France	53	Multi-vehicle motorway collision followed by a coach fire; a published reconstruction reported complete black-smoke filling within approximately 90 seconds.	Historic European benchmark for rapid tenability loss; the cited safety report does not detail the identification method (Swedish National Board of Health and Welfare 2011).
22 Jul 2014, Lice, Turkey	26	An overturned LPG tanker exploded; two passing passenger buses and other vehicles were engulfed.	Illustrates a fuel-fed, multi-vehicle closed-disaster scene and the need to reconcile victims from more than one vehicle (Anadolu Agency 2014).
10 Apr 2015, Tan-Tan, Morocco	>=33	Coach-truck collision followed by fire; many bodies were completely carbonized and many victims were children.	Contemporaneous reports explicitly described difficulty with visual identification and contact with the bus operator (NBC News 2015).
23 Oct 2015, Puisseguin, France	43	Coach and articulated lorry collision; immediate fire, toxic black smoke, difficult access and emergency-exit operation.	The official investigation linked the fatal outcome to fuel, interior materials, smoke extraction, exits and emergency lighting (BEA-TT 2020).
20 Jan 2017, Verona, Italy	16 initially; 18 final	Coach struck a motorway bridge support and burned; most passengers were Hungarian students and accompanying adults.	Sixteen burned victims were identified by DNA comparison with relatives; the final toll later rose because of delayed deaths (ANSA 2020; Ministry of Foreign Affairs and Trade of Hungary 2017).
3 Jul 2017, A9, Bavaria, Germany	18	Coach carrying 48 people collided with a heavy goods vehicle and caught fire.	The official response specifically identified rapid recovery and identification as operational priorities (Bavarian State Government 2017).
29–30 Mar 2018, Igdir-Kars road, Turkey	17	Overcrowded minibus carrying migrants struck a lighting pole and burned; occupants included Afghan, Pakistani and Iranian nationals.	A cross-national, vulnerable-population event in which passenger documentation and AM data may be limited (Anadolu Agency 2018).
19 Jul 2023, Tamanrasset, Algeria	34	Bus-car collision followed by fire; the fire service reported that the victims burned to death.	Shows the continuing occurrence of high-fatality post-collision bus fires; public reporting contains little identification-method detail (Associated Press 2023).
20 Aug 2023, Pindi Bhattian,	18	Passenger bus collided with a pickup carrying diesel	Burned bodies were reported as unrecognizable and samples were

Date and location	Reported deaths	Fire and evacuation features	Identification or DVI relevance
Pakistan		drums and was rapidly engulfed.	referred for DNA identification (Dawn 2023).
3 Oct 2023, Mestre, Italy	21	Electric shuttle bus fell from a viaduct and caught fire on impact; victims represented several nationalities.	Multinational victim group requiring coordinated AM information and repatriation; the fire service confirmed 21 deaths (Corpo Nazionale dei Vigili del Fuoco 2023).

Counts reflect the cited sources and may differ from early media totals. The table compares operational context; it does not imply that all events used the same DVI protocol or published equivalent forensic detail.

This retrospective operational case study has three aims: to report the verified recovery, pathology, toxicology and identification findings from all 45 decedents; to describe the case-specific local workflow that delivered them; and to compare that workflow element by element with the INTERPOL DVI Guide in order to identify which adaptations are transferable and which capabilities should be adopted in advance of the next event. The focus is not a novel laboratory assay, but a practical model for converting existing scene, mortuary and laboratory capacity into a coordinated cross-border identification operation.

Methods

Study design and source material

We conducted a retrospective descriptive review of the judicially mandated medico-legal investigation. Source material comprised scene-examination records, recovery numbering, autopsy reports, toxicology and DNA reports, information supplied by the North Macedonian partner laboratory, AM dental and family information, and the final identification outcomes. Only facts documented in the primary case record were retained. No inferential analysis was performed because a complete validated case-level dataset was not available in the source material.

External sources were used in a fixed hierarchy. Normative and official documents were consulted first, followed by peer-reviewed literature. Contemporaneous non-scientific reports were used only for the descriptive incident comparison in Table 1, only where no official technical report or forensic publication could be located, and they are identified as such in that table and in the reference list. No conclusion in this study rests on a non-peer-reviewed source. Official and normative documents are institutional publications and, like the news reports, are not peer-reviewed; the three classes are distinguishable in the reference list. Where a wire-service item cited in an earlier draft could not be retrieved, the retrievable report actually consulted is cited instead, and any discrepancy between contemporaneously reported casualty figures is stated in the reference entry.

Incident setting and local operational model

The bus left the roadway, damaged the outer barrier, crossed toward the median and became wedged between two central barriers. Fire began in the front of the vehicle and spread throughout the passenger compartment within minutes. The front door was no longer available and the rear door could not be opened because it was compressed externally by a barrier. Bodies were concentrated in the middle of the bus and around the rear exit. The resting position and the obstructed exit are documented in the scene record.

A case-specific command decision created three concurrent workstreams. Team 1, composed of scene investigators, forensic physicians and recovery staff, documented and recovered the remains. Team 2 remained at the central forensic medicine facility in the capital and began autopsies as soon as the first transports arrived. Team 3, composed of biologists and chemists working with the forensic physicians, selected and processed samples for DNA and toxicology. Ten specialized mortuary transport vehicles were mobilized, carrying five body bags each. In parallel, Bulgarian prosecutors requested urgent comparative samples, dental information and other AM data from North Macedonia through mutual legal assistance.

Scene examination and recovery

The first examination began at 06:05 on 23 November 2021 in darkness, light rain and limited visibility, using generator-powered floodlights and vehicle lights. Recovery proceeded from front to rear. Investigators documented the original position of each body or fragment, its relation to other remains and associated personal effects. Because the floor was extensively burned and bodies were trapped among seat frames, firefighters cut seats and side panels with angle grinders and pneumatic shears. Each set of remains was placed in a numbered bag and then in a second bag bearing the same standardized label before transport.

Two detached lower legs with feet were recovered near the rear of the aisle and were packaged separately for later attribution. After 44 decedents from the first scene examination had been autopsied, reconciliation of the deceased, survivors and available passenger information showed that one person remained missing. A second scene team - prosecutor, forensic photographer, chemist and forensic physician - returned at 11:45 on 26 November. Static examination of the soot-covered front-left luggage compartment identified rib-like bone. After careful brush removal of soot and debris, the team recovered a charred male child in prone position. No further human remains were found after a complete reinspection.

Post-mortem examination and sampling

External examination recorded sex, stature, preserved tattoos, jewellery, clothing, footwear and other distinctive features. Deep incisions of the trunk and limbs were used to detect occult haemorrhage,

fracture or soft-tissue injury. Internal examination used the Virchow method in most cases, the Letulle-Shore en bloc method where appropriate, and in-situ examination in the most extensively charred bodies. Brain sectioning and cardiac examination were adapted to tissue preservation. Dental status and female pelvic or other individual anatomical findings were recorded when recoverable.

The forensic physicians assessed the preservation of blood and tissue separately in every body and body part. Because flame exposure was intense but short, blood protected in deep vessels or body cavities was selected for DNA profiling and toxicology whenever its condition was suitable, and preserved tissue was used when blood was unavailable or questionable (Watherston et al. 2018). From the two detached lower legs and feet, the team collected one blood sample on a sterile swab and one fragment of ankle-joint cartilage so that the parts could later be attributed.

Toxicological analysis

The toxicological programme examined blood for carboxyhemoglobin (COHb), methemoglobin (MetHb), ethanol and other volatile substances, common drug classes and combustion-related organic compounds. For COHb, 0.2 mL of blood was diluted to 25 mL with 0.1% ammonium hydroxide and sodium dithionite was added to the test and blank preparations. Samples were scanned from 450 to 650 nm in quartz cuvettes on a Pharmacia Biotech Ultrospec 3000 UV/visible spectrophotometer, and COHb was calculated from the absorbance ratio at the 540- and 579-nm maxima.

For MetHb, 200 microlitres of blood were mixed with 6 mL distilled water, left for 5 minutes, combined with 5 mL phosphate buffer and centrifuged at 3,000 rpm for 10 minutes. Two 3-mL supernatant aliquots were used in four parallel preparations containing distilled water or 4% potassium ferricyanide with phosphate buffer or supernatant. Absorbance at 630 nm was read before and after adding 30 microlitres of 5% potassium cyanide and allowing 2 minutes for reaction, and the four readings were entered into the laboratory calculation procedure for MetHb percentage.

Ethanol and volatile substances were analysed by headspace GC-FID on a Thermo Scientific Trace 1310 system fitted with a TRACE GOLD TG ALC column (30 m x 0.32 mm x 1.8 micrometres) and a TriPlus 300 autosampler. Each 20-mL headspace vial contained 100 microlitres of sample and 500 microlitres of internal standard, with calibration against a previously established internal-standard curve. Headspace conditions were 85 degrees C for the oven, 100 degrees C for the transfer line and 85 degrees C for the manifold; GC conditions were an isothermal 40 degrees C oven, 220 degrees C injector, 240 degrees C detector and nitrogen carrier gas at 2 mL/min.

Broad screening covered volatile reducing substances, alkaloids and opioids, benzodiazepines, barbiturates, tricyclic antidepressants, phenothiazines and plasticizers. Blood was extracted with ethyl acetate under the laboratory screening procedure; the extract was evaporated, reconstituted in ethyl acetate and injected at 1 microlitre in splitless mode. GC-MS used a Thermo Scientific Focus GC with an ISQ single-quadrupole detector in electron-impact mode at 70 eV. The oven programme was 55 degrees

C for 2 minutes, 10 degrees C/min to 170 degrees C with a 1-minute hold, then 8 degrees C/min to 290 degrees C with a 20-minute hold. Mass spectra were searched against the NIST library.

Reconstruction of the scene scheme and spatial analysis

The case record contains a hand-annotated schematic of the passenger saloon on which each set of remains is marked with its scene body/group number and, next to it, the corresponding blood carboxyhemoglobin value. The schematic exists only as a formatted table in the original document, and no underlying data file accompanies it. It was therefore transcribed rather than redrawn from memory: the body numbers are encoded as circled-digit symbol glyphs and the toxicology values as red numerals, and each cell was read as an ordered sequence of these runs, so that every number is recovered together with the value written beside it. Two adjacent circled digits denote one two-digit identifier (for example 4 followed by 5 is group 45) and not two separate sets of remains. All 45 pairs were recovered; no value was interpolated, averaged or otherwise reconstructed.

Positions were recovered from the geometry of the same table. The longitudinal (front-to-rear) coordinate is the row position and the transverse coordinate is the horizontal offset of the marker within its cell, obtained from the column widths, merged-cell spans, paragraph alignment and indents of the source table. The interior of the vehicle is delimited in the source by cell borders rather than by text: the front and rear walls and both side walls are drawn as double borders, the driver station is labelled in the front left corner, the front door appears as a dashed border segment in the right wall at the front row and the rear door as a heavy composite border segment in the right wall, with its label placed in a narrow strip outside that wall. Transverse positions were therefore normalized to the true interior width between the two side walls, so that 0 denotes the left wall and 1 the right wall, on which both doors are located; the longitudinal coordinate was normalized between the front and rear walls. The reconstruction preserves relative order, left-right relationships and clustering; it is ordinal and proportional, not metric, and marker positions denote recovery positions rather than assigned seats.

Three longitudinal zones were defined from the structure of the schematic itself and not from the toxicology results: the front and middle section, the row at which the rear door is drawn, and the rear section behind it. Transverse position was summarized in thirds of the interior width and, separately, as distance to each of the two doors in units of vehicle width, computed on the normalized coordinates. Distributions are reported as median and interquartile range. The association between carboxyhemoglobin and longitudinal position was tested with the Spearman rank correlation, differences between the three zones with the Kruskal-Wallis test and the comparison between the left and right halves of the saloon with the Mann-Whitney U test, all two-sided. Values reported as not detected were treated as 0% and no imputation was used; carboxyhemoglobin is available for all 45 decedents, so all analyses are complete case. The analysis is exploratory and descriptive. Five comparisons were computed on a single set of 45 observations, p values are reported unadjusted for multiplicity, and neighbouring recovery positions are not independent observations; the p values therefore quantify how strong the pattern is within this data set and are not tests of a hypothesis in a sample drawn from a

wider population. Analyses were performed in Python 3 with the SciPy library. The reconstructed data set is provided as Additional file 1 so that Fig. 1 and every statistic reported here can be recomputed from it.

One discrepancy was identified during transcription and is reported rather than reconciled. The schematic places the marker for group 45 in the front left area of the saloon, which corresponds to the set of remains recovered from the front left luggage hold during the second search of the vehicle on 26 November, whereas one passage of the case narrative assigns that bag number to a different set of remains. The figures follow the schematic, since it is the primary contemporaneous scene record, and the point is flagged here because it illustrates precisely the kind of numbering ambiguity that a standardized DVI numbering and reconciliation format is designed to prevent.

DNA profiling and cross-border comparison

Extraction, quantification and amplification

DNA was extracted on an AutoMate Express Forensic DNA Extraction System with the PrepFiler Express Forensic DNA Extraction Kit (Thermo Fisher Scientific) (Watherston et al. 2018). Extract quantity and quality were assessed on a 7500 Real-Time PCR System (Life Technologies) with the Quantifiler Trio DNA Quantification Kit and HID Real-Time PCR Analysis Software v1.2. Amplification was performed in 25-microlitre reactions on a SimpliAmp Thermal Cycler. NGM Detect was used for the following autosomal STR and sex-defining markers: D2S1338, SE33, D16S539, D18S51, TH01, D12S391, D3S1358, FGA, Y-indel, Amelogenin, vWA, D21S11, D1S1656, D2S441, D8S1179, D19S433, D22S1045 and D10S1248.

Male-lineage profiling used the Yfiler Plus PCR Amplification Kit in 25-microlitre reactions on the same platform, covering DYS576, DYS389I, DYS635, DYS389II, DYS627, DYS460, DYS458, DYS19, Y-GATA-H4, DYS448, DYS391, DYS456, DYS390, DYS438, DYS392, DYS518, DYS570, DYS437, DYS385a/b, DYS449, DYS393, DYS439, DYS481, DYS387S1 and DYS533. Y-STR results were used where paternal-line information could support or resolve the proposed kinship structure; they were not treated as individualizing evidence by themselves.

Fragment analysis and quality assurance

Amplified fragments were separated by eight-capillary electrophoresis on a 3500 Series Genetic Analyzer for Human Identification using 3500 POP-4 polymer and laser detection. Alleles were analysed in GeneMapper v1.2 and reported using the kit nomenclature. Each analytical series included DNA Control 007 as the positive control and a negative control. Instrument and sizing controls comprised Matrix Standard DS-37 (FAM, VIC, TED, TAZ, SID and LIZ dyes), Matrix Standard DS-36 (FAM, VIC, NED, TAZ, SID and LIZ dyes), GeneScan 600 LIZ Size Standard v2.0, the IQCS and IQCL internal quality-control markers, the NGM Detect allelic ladder and the Yfiler Plus allelic ladder. Results were accepted only after the relevant controls and internal markers met the laboratory criteria.

Ante-mortem reference collection and comparison

Buccal samples from hospitalized survivors were collected on sterile foam-tipped applicators and separately sealed and documented so that their profiles could be excluded from the pool of unidentified remains. Seven occupants survived the fire and were hospitalized. The number of survivor samples entered into the comparison is not stated here because the source narrative and the specimen-number range are not concordant on that figure; the discrepancy does not affect the 45 PM profiles or the 34 family-reference profiles.

Under the mutual-legal-assistance request, the partner laboratory in Skopje collected blood and buccal samples from 34 relatives and recorded the stated relationships. DNA isolation used the AutoMate Express/PrepFiler Express workflow; fragments were separated on a 3500 Genetic Analyzer and analysed in GeneMapper ID-X. Bulgarian investigators received 34 certified profile tables, each linked to the documented relationship. After the two laboratories confirmed marker, allele-nomenclature and platform compatibility, PM profiles were jointly compared with the nominated relatives. Complete interpretable profiles were reported from all 45 sets of human remains. The case record did not report case-level likelihood ratios or combined paternity indices; accordingly, none are introduced here.

Identification decisions

Identity decisions integrated DNA kinship findings with dental comparison and non-genetic features that did not conflict with the proposed identity. Consistent with the INTERPOL identifier hierarchy, DNA and comparative dental analysis carried the identification, while personal description, stature, jewellery, clothing and family photographs were used only to discriminate between candidates already constrained by primary evidence. No identification rested on visual recognition or on photographs alone (INTERPOL 2023).

Framework for comparison with the INTERPOL DVI standard

The comparison uses the INTERPOL DVI Guide (version 2023) as the primary normative reference, including Part A and the Part B annexures on emergency responders, scene, PM, AM, reconciliation and numbering (INTERPOL 2023). Two features of that document determine how a single-event case can legitimately be assessed against it. First, the Guide presents itself as a source of broad-based baseline standards and states that it provides sufficiently flexible guidelines and structures to accommodate differences and variations in jurisdictional legal systems, policies and practices. Second, it states that the descriptors and designations relevant to each nation or jurisdiction may vary and that, regardless of the terminology used, what matters is that the functions and roles are adequately catered for. The Guide is therefore a functional framework rather than a conformity checklist, and it does not require that a jurisdiction reproduce INTERPOL nomenclature, team titles or forms in order to conduct a sound DVI operation.

Accordingly, each element was assessed for whether the underlying function was performed, by whatever local instrument, and each was assigned one of four descriptors: fulfilled through a documented local equivalent; fulfilled with partial documentation, where the function is evidenced by outcome but the corresponding record was not available to us; not applicable, where the element addresses a scale, terrain or condition that this event did not present; and pre-adoption priority, where a capability or standardized format would strengthen a future response. No element is scored as non-conformity, because a flexible baseline framework does not generate that category for a jurisdiction that achieved the intended outcome by other documented means. The assessment is based solely on actions documented in the case record; where a record was unavailable, the element is reported as partially documented rather than as not performed. Elements were extracted before the case material was scored, and scoring was restricted to the documented record.

For forward-looking recommendations only, the pre-adoption items were also considered against the ISO 21043 series on forensic sciences, whose Part 1 vocabulary was reissued and whose interpretation and reporting parts were published in 2025, after this operation (ISO 2025; Berger 2025; Morrison et al. 2025). The series is used here as a prospective quality reference, not as a benchmark against which a 2021 response could have been measured.

Results

Operational timeline and recovery

The parallel model shortened the interval between recovery and examination. Twenty of the initially recovered decedents had been autopsied by approximately 21:00 on 23 November; the other 24 were autopsied on 24 November. Reconciliation then revealed the missing passenger, leading to the targeted second examination and recovery of the 45th decedent on 26 November. This feedback from reconciliation to scene work was the key quality-control event in the operation (Table 2).

Table 2

Table 2
Operational timeline of the response

Date/time	Documented action	DVI relevance
23 Nov, 06:05	First scene examination began in darkness and light rain; recovery proceeded front to rear.	Scene phase and controlled recovery numbering.
23 Nov, by ~ 21:00	First 20 decedents autopsied while scene recovery continued.	Parallel PM processing reduced delay.
24 Nov	Remaining 24 decedents from the initial recovery autopsied.	PM data and samples available for comparison.
25–26 Nov	Comparison of deceased, survivor and passenger information showed one person unaccounted for.	Reconciliation identified a recovery gap.
26 Nov, 11:45	Repeat inspection recovered the 45th decedent from the front-left luggage compartment.	Reconciliation redirected the scene phase.
Days 7–10	All 45 identities confirmed and remains released for return and burial.	Completed reconciliation and humanitarian outcome.

The 7-10-day interval is reported in the primary case record.

Post-mortem and toxicological findings

All 45 decedents had fatal thermal injury from intense dry-flame exposure. Common findings included burns of variable extent, charring, pugilistic posture, destruction of bone and internal organs, bright-red blood or musculature, soot in the trachea and bronchi, petechial haemorrhages beneath the pleura and epicardium, and occasional heat-related post-mortem epidural haematomas. One male decedent had an additional vital traumatic injury not attributable to heat: rupture of the T3-T4 intervertebral disc with haemorrhage, consistent with forceful hyperextension.

COHb was not detected in seven decedents; the 38 detected values recorded in the analytical chart ranged from 1% to 49%, while the prose summary of the case record gives the detected range as 2–49%. MetHb ranged from 3% to 38% and was not detected in ten victims. Di-octyl terephthalate and di-iso-octyl phthalate were detected by GC-MS in blood samples. These common plasticizers were considered ancillary evidence of exposure to products released from burning interior materials; they were not used for identification and should not be interpreted without considering possible environmental or analytical contamination. The aggregate findings are summarized in Table 3.

Table 3

Table 3
Aggregate forensic and identification findings

Measure	Documented result
Fatalities	45
PM DNA profiles	45 interpretable profiles from human remains
Reference relatives	34 relatives sampled in North Macedonia
COHb	Not detected in 7; 38 detected values, 1–49% as charted (2–49% in the source prose)
MetHb	3–38%; not detected in 10 victims
Primary fatal injury	Fatal thermal injury in all 45 decedents
Additional vital trauma	One T3-T4 intervertebral disc rupture with haemorrhage
Plasticizers detected	Di-octyl terephthalate and di-iso-octyl phthalate
Identification outcome	All 45 victims identified within 10 days

COHb, carboxyhemoglobin; MetHb, methemoglobin; PM, post-mortem. The two reported COHb lower bounds are given as they appear in the analytical chart and in the narrative summary of the case record.

Spatial distribution of carboxyhemoglobin and recovery positions

Figure 1 reconstructs the annotated scene schematic and preserves the one-to-one link between each scene body number and the carboxyhemoglobin value measured in that decedent. Across the 45 decedents the median saturation was 14% (interquartile range 5–22%, range 0–49%), and the result was reported as not detected in seven decedents (groups 1, 2, 3, 4, 24, 26 and 27), four of them recovered in the front and middle section and three at the rear-door level.

Values increased from the front of the saloon towards the rear. The median was 9% in the front and middle section (interquartile range 2–14%, n = 19), 16% at the level of the rear door (10–21%, n = 15) and 23% in the rear section behind the rear door (14.5–29.5%, n = 11). The association with longitudinal position was moderate and statistically significant (Spearman rho = 0.47, p = 0.001), and the three zones differed significantly (Kruskal-Wallis H = 8.20, p = 0.017). The five highest values, 49%, 44%, 42%, 34% and 32%, belonged to groups 29, 37, 35, 36 and 12; the first four were recovered at or behind the rear door.

The transverse distribution shows where in the vehicle the remains were found relative to the walls and the two doors. Most sets of remains were not against the side walls: 28 of 45 lay in the central third of the interior width, which contains the aisle, 11 in the left third and 6 in the right third, on which both doors are located. Only 4 of 45 lay within 15% of the interior width of a side wall (three at the left wall, one at

the right). Carboxyhemoglobin did not differ between the left and right halves of the saloon (median 13.5%, n = 28 versus 16%, n = 17; Mann-Whitney p = 0.673), and it was not associated with the distance to the nearer of the two doors (Spearman rho = -0.10, p = 0.522). Grouping decedents by which door was nearer gives a marked difference in median saturation, 16% for the 37 nearer the rear door against 2.5% for the 8 nearer the front door, but this contrast is collinear with the front-to-rear gradient described above and cannot be interpreted as an independent effect of proximity to an exit.

The single most crowded part of the vehicle was the row at which the rear door is drawn in the source schematic, where 15 sets of remains were recorded. Within that row the remains spanned almost the whole interior width (transverse position 0.01 to 0.92, median 0.51), 8 of the 15 lay in the door half of the saloon, and two sets, groups 32 and 33, lay immediately against the wall in which the rear door is placed. This is the door that the external guardrail held shut, and the remains at that level were photographed in situ after firefighters had forced it open; those photographs belong to the case file and are not reproduced here.

Figure 2 sets these values against the fire and egress conditions documented at the scene. The fire originated in the front section and involved the whole vehicle within minutes; the driver station, the dashboard and the tachograph were destroyed, the insulation of the entire electrical installation was burnt away, seats, wall linings and upholstery were consumed, floor panels were burnt through in places, all glazing was lost and roof hatches and roof sections were missing. The front right door was absent at examination and the rear door structure was displaced outwards onto the guardrail. The only egress route that was actually used was the rear windows, broken by the few passengers who were awake. The direction of flame spread is an inference from the documented area of origin and the damage pattern, whereas the carboxyhemoglobin gradient is an independent laboratory measurement; their agreement therefore corroborates the reconstruction of the event in a vehicle that was otherwise almost completely destroyed, rather than one finding being derived from the other.

Series that report carboxyhemoglobin for every decedent together with the position at which each was recovered appear to be scarce. A search of Europe PMC on 30 August 2026 returned six records for bus, coach or vehicle fire combined with carboxyhemoglobin, six for disaster victim identification combined with bus and fire, 34 for carboxyhemoglobin combined with body or seat position and fire, and three for mass fatality combined with fire and carboxyhemoglobin; on title and abstract screening none reported a complete victim group of a single bus fire mapped in this way. The four search strings are given in Additional file 2. The reconstruction reported here therefore adds a data set of a type that is rarely available, which is also why it is reported in full as a supplementary data file.

Two features of the toxicology results limit how far they can be taken. The highest measured saturation was 49%, below the range conventionally regarded as lethal in isolation, and 17 of 45 values were below 10% (Ernst and Zibrak 1998; Anderson et al. 1981). In closed-vehicle fires with rapid full involvement, death results from the combined effect of heat, oxygen depletion and a mixture of toxic gases, and low or non-detectable carboxyhemoglobin is compatible with a very short survival interval in the smoke

(Anderson et al. 1981); absent carboxyhemoglobin has been reported in victims of flash fires, in whom death followed exposure too rapidly for measurable uptake (Hirsch 1969). Hydrogen cyanide, which is generated by burning nitrogen-containing polymers and is found at elevated concentrations in smoke-inhalation victims (Baud et al. 1991), and which current practice recommends be assessed together with carboxyhemoglobin and methemoglobin in fire deaths (Ferrari and Giannuzzi 2015; Chaturvedi et al. 2001), was not requested or analysed in this case; its contribution here is therefore unknown and is not estimated. The gradient is therefore reported here as a measure of relative smoke exposure by position and as a corroboration of the fire reconstruction, not as a determination of the cause of death in individual decedents, which rested on the autopsy findings.

Identification outcomes and complex family groups

All 45 victims were identified. DNA established the relevant family relationships, while odontology, anthropometry, jewellery, clothing and photographs resolved cases in which several close relatives were plausible candidates. These secondary findings were used in combination with, and never in contradiction to, the primary identification evidence.

Two male children were reported as twins. DNA showed that they were dizygotic. Both were approximately 98–101 cm tall. One child had no recoverable dentition because of charring; the other retained deciduous teeth 15, 25, 35 and 45, diastemata between 22–23, 32–33 and 11–21, and a characteristic incisal curve across teeth 11–13. Comparison with family photographs allowed the twins to be differentiated.

For a pair of sisters, DNA established sibling relationships. A thermally altered ring with a heart-shaped ornament, reported by relatives as worn by only one sister, provided the discriminating feature. A second family group contained three sisters. One decedent measured 122–124 cm and had deep caries on tooth 25 and an anterior open bite; a second measured 153–155 cm, had approximately 50 cm of chestnut hair, upper and lower orthodontic appliances with pink elastics and a preserved white Nike shoe; the third measured at least 157 cm and had photopolymer restorations on teeth 16, 36 and 46. The combined AM and PM characteristics allowed individual attribution within the sibling group.

Correspondence with the INTERPOL DVI standard

Table 4 summarizes how the local workflow delivered each phase of the INTERPOL DVI process, including Phase 5, Review of Actions and Preparation, which this study itself addresses. Table 5 gives the element-by-element assessment.

Table 4

Table 4
Local implementation across the five phases of the INTERPOL DVI process

DVI phase	Local implementation	Transferable lesson
1. Scene	Sequential front-to-rear documentation and recovery; cutting of seats and panels; double-bagging under one label; scene numbering carried through the whole process; repeat search after reconciliation.	Recovery records must remain traceable and responsive to later discrepancies.
2. PM	Immediate parallel autopsies at a permanent central facility; examination adapted to charred remains; dental recording; deep-tissue DNA and toxicology sampling.	Mortuary work can begin before scene recovery ends if numbering and transport are controlled.
3. AM	Urgent mutual-legal-assistance request to North Macedonia; sampling of 34 relatives in their own country; collection of dental records, photographs and descriptions.	Cross-border AM collection should start before PM profiling is complete.
4. Reconciliation	Joint comparison by two laboratories using compatible markers, nomenclature and platforms; DNA-led resolution supported by dental and personal features; release under prosecutorial authority.	Reconciliation is an active quality-control process, not merely the final administrative step.
5. Review of actions and preparation	Retrospective reconstruction of the operation, identification of the functions delivered by local equivalents, and a defined list of capabilities and formats to adopt in advance.	A documented post-event review converts a single response into institutional readiness.

The mapping is retrospective. It does not imply that INTERPOL nomenclature, governance titles or forms were used during the 2021 operation.

Table 5

Table 5
Element-by-element assessment of the response against the INTERPOL DVI Guide (version 2023)

INTERPOL DVI Guide element	Locator	Documented implementation in this case	Assessment
Identification is owned by the coroner or equivalent legal authority, which authorizes formal identification and release of the deceased	Part A 5.1, 5.4	Judicially mandated medico-legal investigation under prosecutorial authority; identities confirmed and remains released for return and burial	Fulfilled - documented local equivalent
Distinct operational units are formed, clearly identifiable and assigned to specific duties, with a flexible mind-set when integrating DVI into a multi-agency response	Part A 3.1	Command decision created three concurrent, separately staffed workstreams for scene, mortuary and laboratory work	Fulfilled - documented local equivalent
Designations may vary between jurisdictions provided the functions and roles are adequately catered for	Part A 1	No INTERPOL DVI titles were used; the corresponding commander, phase-coordination and reconciliation functions were performed by existing officeholders	Fulfilled - documented local equivalent
Disaster classification determines how quickly comparative AM data can be obtained	Part A 2.4	Event had closed-disaster characteristics: single vehicle, available passenger information and identified survivors, giving a bounded missing-person list within days	Fulfilled - documented local equivalent
International cooperation where victims and families are in different countries	Part A 3	Mutual-legal-assistance channel between Bulgarian and North Macedonian prosecutors; partner forensic laboratory engaged directly	Fulfilled - documented local equivalent
A search plan adapted to the area; grid or sector subdivision of the site	Annexure 3	Confined vehicle interior recovered by a sequential front-to-rear, compartment-based plan; grid and sector subdivision address open terrain and were not applicable	Not applicable to this event
The exact location of remains within the scene and in relation to other remains and evidence must be recorded	Annexure 3	Original position of every body and fragment, its relation to other remains and associated personal effects recorded; the resulting annotated scheme underlies Fig. 1	Fulfilled - documented local equivalent
Human remains and body bags labelled with the same number and sealed	Annexure 3	Each set of remains placed in a numbered bag inside a second bag bearing the same standardized label before transport	Fulfilled - documented local equivalent

INTERPOL DVI Guide element	Locator	Documented implementation in this case	Assessment
Body parts may be recovered separately with the possible association recorded for mortuary follow-up	Annexure 3	Two detached lower legs packaged separately; swab blood and ankle-joint cartilage taken specifically to permit later attribution	Fulfilled - documented local equivalent
Medical and dental experts attend the scene; separation and handling of remains only by authorized forensic medical experts	Annexure 3	Forensic physicians embedded in both the first and the second scene teams, the latter with prosecutor, photographer and chemist	Fulfilled - documented local equivalent
Photographic and written documentation of remains and property on INTERPOL DVI victim-recovery forms	Annexure 3	Photographic and written documentation performed on national scene-examination records; use of the INTERPOL recovery form is not documented	Fulfilled - documentation partial
A unique number allocated at the scene stays with the remains and is used on all documentation and samples	Annexure 9	Scene recovery number retained through autopsy, toxicology and DNA records and used to link every COHb result to its body group	Fulfilled - documented local equivalent
Recommended PM number format combining a PM prefix, country code, sequential number and scene reference	Annexure 9	Locally sequential numbering was used; the INTERPOL composite format, which becomes important once foreign teams or multiple scenes are involved, is a format to adopt in advance	Pre-adoption priority
Mortuary provides reception, autopsy, dental examination, fingerprinting, DNA collection, evidence and property processing, and quality control	Annexure 4	Permanent central forensic medicine facility used; reception, autopsy, dental recording, DNA and toxicology sampling, property recording and documented laboratory controls all present	Fulfilled - documented local equivalent
Medical imaging, including whole-body CT where possible, which may also obviate separate anthropological procedures	Annexure 4	Post-mortem imaging is not documented in the case record; complete DNA coverage carried the identifications. Imaging is the single highest-value capability to add for future responses	Pre-adoption priority
Friction-ridge examination as one of the three primary identifiers	Annexure 4, Part A 6	Not reported; identification rested on the two remaining primary identifiers, which the Guide accepts as a function of the condition of the remains	Not applicable to this event
Dental status recorded by an odontology team producing a radiographic and photographic record	Annexure 4	Dental status recorded and individual dental features were decisive within sibling groups; the multi-person odontology team	Fulfilled - documentation partial

INTERPOL DVI Guide element	Locator	Documented implementation in this case	Assessment
		structure and PM dental radiography are not documented	
DNA samples collected by the PM team from the best-preserved available material	Annexure 4	Preservation assessed body by body; deep-vessel or cavity blood preferred, preserved tissue and cartilage used where indicated, with the selection rationale documented	Fulfilled - documented local equivalent
Reconstruction of the body to a condition acceptable for presentation to the family	Annexure 4	Not applicable given the documented degree of thermal destruction; remains were released for return and burial	Not applicable to this event
Quality-assurance check of identified remains before release	Annexure 4	Release followed confirmed identification and analytical control criteria; a separate documented pre-release sign-off step is not described	Fulfilled - documentation partial
AM data collected as quickly as possible, with a personal file and checklist per missing person	Annexure 5	AM request issued through prosecutors within days and 34 relatives sampled abroad; the per-person AM file and checklist structure is not documented	Fulfilled - documentation partial
AM DNA references limited to first-degree relatives, more than one where possible, with the status of biological relative established	Annexure 5	Relationships stated by families and then verified genetically; 34 references collected, several per family group. The degree distribution of relatives is not reported in the case record	Fulfilled - documentation partial
The set of loci is agreed with the scientific community of the countries mainly involved	Annexure 5	Autosomal NGM Detect and Yfiler Plus sets; the two laboratories confirmed marker compatibility before joint comparison	Fulfilled - documented local equivalent
All laboratories observe ISFG nomenclature and a standard data-exchange format such as the INTERPOL XML format	Annexure 5	Allele nomenclature and platform compatibility confirmed bilaterally and 34 certified profile tables exchanged; a standardized machine-readable exchange format is a format to adopt in advance	Fulfilled - documentation partial
AM data collected on the INTERPOL yellow AM form by briefed personnel, preferably in face-to-face interviews	Annexure 5	Equivalent content collected in person by the partner laboratory and investigators; use of the yellow form is not documented	Fulfilled - documentation partial
Language and cultural barriers may reduce willingness of relatives to give samples	Annexure 5	Sampling performed in the victims' home country, in the families' own language and environment, by their national laboratory	Fulfilled - documented local equivalent

INTERPOL DVI Guide element	Locator	Documented implementation in this case	Assessment
The reconciliation team compares AM and PM findings, with a section to receive, log, record and file AM and PM information	Annexure 6	Joint comparison of 45 PM profiles against nominated relatives, integrated with dental and personal data; certified profile tables logged against documented relationships	Fulfilled - documented local equivalent
Primary identifiers carry identification; secondary means normally support it; photographic and visual identification are unreliable in disfigured victims and should not stand alone	Part A 6	DNA and comparative dental analysis carried every identification; stature, jewellery, clothing, orthodontic appliances and family photographs were used only to discriminate between candidates already constrained by primary evidence, and never contradicted it. No identification rested on visual recognition	Fulfilled - documented local equivalent
A comparison report and certificate of identification are provided for each set of remains, followed by release authority	Annexure 6	Identification confirmed and release authorized within the judicial process; the per-case report and certificate formats are not described in the material available	Fulfilled - documentation partial
The DVI Identification Board is convened to approve identifications	Annexure 6	Decisions were taken within the judicially led process; no standing multidisciplinary board is documented. A formally constituted identification commission, as used after the 1996 Spanish bus fire, is a governance element to adopt in advance	Pre-adoption priority
Reconciliation is a continuous quality-control function throughout the operation	Annexure 6	Reconciliation detected that one passenger was unaccounted for after 44 autopsies, redirected the scene phase, and led to recovery of the 45th decedent on 26 November followed by complete reinspection	Fulfilled - documented local equivalent
Phase 5 review of actions and preparation for future responses	Part A 5.5	Delivered by this retrospective study: reconstruction of the operation, identification of the functions carried by local equivalents, and a defined pre-adoption list	Fulfilled - documented local equivalent

Locators refer to Part A sections and Part B annexures of the INTERPOL DVI Guide, version 2023 (INTERPOL 2023). The Guide provides broad-based baseline standards and explicitly flexible structures that accommodate differences in jurisdictional legal systems, policies and practices, and it requires that functions and roles be catered for regardless of local terminology; assessment therefore addresses fulfilment of function and includes no non-conformity category. "Documentation partial" means that the function is evidenced by the operational outcome while the corresponding record was not available for

this review, and it is not a statement that the action was omitted. AM, ante-mortem; ISFG, International Society for Forensic Genetics; PM, post-mortem.

Of the 32 Guide elements assessed, 18 were fulfilled through a documented local equivalent, 8 were fulfilled with partial documentation, 3 concerned conditions this event did not present, and 3 are capabilities or standardized formats identified for pre-adoption. All elements bearing directly on evidential integrity - unique numbering carried from scene to laboratory, recorded position and association of remains, controlled sample selection, analytical quality control, the primary-identifier hierarchy and reconciliation as continuous quality control - were fulfilled. The elements not fulfilled in INTERPOL form were matters of documentation format and governance nomenclature, and the elements assessed as not applicable concerned open-terrain search methodology, friction-ridge comparison in carbonized remains and cosmetic reconstruction of the body.

Discussion

The practical contribution: organization before technology

The distinctive contribution of this case is the rapid conversion of existing local services into a functional DVI pathway. The three parallel workstreams did not reproduce the INTERPOL phases as separate organizational units. Instead, they created the capacity to perform scene, PM and laboratory work concurrently, while prosecutors initiated AM collection abroad. Reconciliation linked these streams and, critically, sent the team back to the scene when the numbers did not close. The recovery of the 45th decedent demonstrates why reconciliation should begin early and continue throughout the operation (INTERPOL 2023; Lessig and Rothschild 2012; Schuliar and Knudsen 2012; Anderson et al. 2017).

This model is relevant to jurisdictions where formal DVI teams, dedicated mortuary surge facilities or integrated data systems are still developing. Its core requirements are achievable with existing resources: a single numbering system, predefined handoffs, protected parallel laboratory capacity, rapid legal channels for AM data, and a reconciliation function empowered to challenge apparent completeness. The case therefore supports capacity building through interoperable procedures as much as through acquisition of new technology.

What the case demonstrates about the DVI standard

Read against the Guide as its authors framed it, this operation is not an approximation of a standard that was unavailable, but an instance of the standard working as intended. The Guide asks that functions and roles be catered for and expressly accommodates jurisdictional variation; a response built in hours from a national forensic medicine institute, a prosecution service, a police scene capability and a foreign partner laboratory did cater for them. Three findings follow.

First, the functions that protect evidential integrity transferred completely. A single number allocated at the scene and carried through autopsy, toxicology and DNA reporting, recorded position and association

of every body and fragment, same-label double bagging, separate recovery of detached limbs with dedicated attribution samples, and documented analytical controls together delivered the chain of traceability that Annexures 3, 4 and 9 exist to secure. These are procedural rather than capital-intensive requirements, which is why they were achievable immediately.

Second, the identifier hierarchy was applied strictly, and this is where the case has the clearest didactic value. In a victim group containing a twin pair and two sibling groups, kinship DNA can establish that remains belong to a family cluster without assigning each individual. The operation resolved these clusters with comparative dental analysis and with secondary features - stature, orthodontic appliances, a heart-shaped ring, preserved footwear, restorations, family photographs - used only within the constraint already imposed by primary evidence. The Guide warns that identification from photographs is notoriously unreliable as a sole means and that visual identification is unreliable in disfigured victims; in a fire with this degree of charring, the discipline of never allowing a secondary feature to originate an identification is the difference between a defensible and an unsafe result.

Third, reconciliation performed the function the Guide assigns it, and did so beyond the minimum. Reconciliation is often described as the terminal administrative step. Here it operated as a control loop: after 44 autopsies, comparison of the deceased against survivors and passenger information showed a deficit of one, the scene phase was reopened, and a charred child was recovered from a luggage compartment on 26 November, followed by complete reinspection. An operation that had closed at 44 would have produced 44 correct identifications and one undiscovered victim. This is the most transferable single lesson of the case, and it cost nothing but the authority to reopen a closed phase.

The cross-border component shows the same pattern. The Guide requires common nomenclature and a standard exchange format precisely so that profiles can move between jurisdictions; the two laboratories achieved the substance of that requirement bilaterally by verifying marker sets, allele nomenclature and platform compatibility before comparison, and by exchanging certified profile tables linked to documented relationships. Sampling relatives in their own country, in their own language, also addressed the Guide's observation that language and cultural barriers influence willingness to provide reference material, and it prevented AM collection from becoming the rate-limiting step.

Priorities for pre-adoption before the next event

The elements that were fulfilled only in substance, or that this event did not require, define a short and inexpensive readiness agenda. Post-mortem imaging is the first priority: Annexure 4 lists medical imaging, including whole-body computed tomography where possible, among the mortuary functions and notes that it may obviate separate anthropological procedures. In carbonized remains it would document skeletal trauma, dental status and radiopaque foreign material non-destructively before dissection, and would provide a durable archive for later review - an advantage in exactly the situation this case presented, where one vital T3-T4 injury had to be found within extensive thermal destruction. The remaining items are formats and governance rather than capability: the composite PM numbering format of Annexure 9, which becomes necessary as soon as foreign teams or multiple scenes are

involved; the INTERPOL PM and AM form sets and a machine-readable profile-exchange format; a per-person AM file with the checklist structure of Annexure 5; a documented pre-release quality-assurance step; and a formally constituted multidisciplinary identification board, of the kind used after the 1996 Spanish bus fire (Martin-de las Heras et al. 1999), to approve identifications and issue comparison reports and certificates.

Adopting these items in advance would also align a future response with the ISO 21043 series on forensic sciences, whose vocabulary part was reissued in 2025 and whose interpretation and reporting parts were published in the same year, after this operation (ISO 2025; Berger 2025; Morrison et al. 2025). Because that series addresses the collection, analysis, interpretation and reporting chain rather than disaster response specifically, it complements the INTERPOL Guide: the Guide defines the operational functions, while ISO 21043 shapes how the resulting examinations, interpretations and reports should be documented. A jurisdiction that pre-adopts the INTERPOL forms and numbering and the ISO reporting requirements would enter its next mass-fatality event with the documentation layer already in place - which, on the evidence of this case, is the only layer that was not improvised successfully.

Comparison with published bus-fire identification cases

Detailed publications on the identification component of fatal bus fires are uncommon; most public reports concentrate on crash causation, fire behaviour or rescue. The cases in Table 6 were therefore selected because they provide enough PM, AM or reconciliation detail for a methodological comparison. They are not a systematic sample, and differences in case size, preservation, available records and national law prevent ranking of performance.

Table 6

Table 6
Methodological comparison with published identification operations after bus fires

Case	PM and AM material	Principal identification approach	Operational outcome and transferable lesson
Spain, 1996; 28 fatalities (Martin-de las Heras et al. 1999)	External examination, photographs, dental examination, intraoral/extraoral and general radiographs, biological methods including DNA; AM dental and medical records on INTERPOL forms.	Manual AM-PM comparison by a multidisciplinary Identification Commission; dental evidence established identity in 57%, with complementary methods for the remainder.	Demonstrated the value of standardized forms, a designated multidisciplinary commission and dental/radiographic preservation in almost completely incinerated victims.
Malaysia, 1996; 13 fatalities (Lim et al. 2000)	Twenty-one PM blood, bone-marrow and tissue samples; 24 reference blood samples from six families.	Four-locus HaeIII RFLP kinship analysis in a closed system, supported by PM sex and family/passenger information.	All 13 identified within nine days of the first samples; highlighted coordinated sampling and intergovernmental collection of references.
Induruwa, Sri Lanka, 2007; 19 fatalities (Ruwanpura et al. 2012)	Autopsy, whole-body radiography, anthropology, dental recording, personal effects and retained tissue for DNA; police AM databank using INTERPOL protocol.	Three-stage comparison combining provisional AM information, sex/age assessment and odontological, personal-effect and circumstantial evidence.	All victims identified despite extensive burning and obstruction of both exits; shows the usefulness and limitations of non-DNA modalities when AM records vary.
Verona, Italy, 2017; 16 burned victims requiring identification (ANSA 2020; Ministry of Foreign Affairs and Trade of Hungary 2017)	PM DNA profiles and reference samples collected from relatives in Hungary; cross-border governmental and forensic coordination.	DNA comparison with family references for the burned victims.	All 16 burned victims identified; direct comparator for urgent cross-border AM sampling and repatriation.
Struma, Bulgaria, 2021; 45 fatalities	Forty-five interpretable PM DNA profiles; 34 relatives sampled in North Macedonia; dental, anthropometric and personal data; autopsy and toxicology.	Autosomal STR and Y-STR kinship analysis with multimodal confirmation; parallel scene, PM and laboratory work; reconciliation-triggered repeat search.	All 45 identified within 10 days; demonstrates a locally assembled cross-border workflow at larger scale and active reconciliation as operational quality control.

AM, ante-mortem; DNA, deoxyribonucleic acid; PM, post-mortem; RFLP, restriction fragment length polymorphism; STR, short tandem repeat. Outcomes are reported as stated by the cited sources; different evidentiary standards and case conditions preclude direct performance ranking.

Relative to these published comparators, the Struma operation combined a larger number of fatalities, complete PM profiling, a substantial AM family-reference programme abroad, contemporary autosomal and Y-chromosomal STR systems, and simultaneous recovery, autopsy and laboratory processing. Its distinctive contribution is not analytical novelty: it is the organizational integration of validated methods, cross-border data compatibility and a reconciliation process capable of reopening the scene phase when one passenger remained unaccounted for.

Cross-border DNA interoperability and multimodal identification

Early sampling of 34 relatives in North Macedonia prevented AM DNA collection from becoming a late bottleneck. Because the two laboratories used compatible markers, extraction platforms and capillary-electrophoresis systems, certified profiles could be compared without transporting all relatives to Bulgaria. Comparable validation, allele nomenclature, quality assurance and documented kinship remain essential whenever profile data cross borders (Montelius and Lindblom 2012; Prinz et al. 2007; Alonso et al. 2005; Weedn and Baum 2011; Lin et al. 2006).

The twin and sibling groups illustrate a recurrent DVI problem. Family DNA may establish that remains belong to a close family cluster without, by itself, assigning each individual. Dental features, photographs, jewellery, stature, orthodontic appliances and clothing then become decisive corroborative evidence (INTERPOL 2023; Brannon and Kessler 1999; Prajapati et al. 2018). The locally developed approach respected this hierarchy by using DNA and odontology as primary evidence and personal features to distinguish candidates within genetically related groups.

Pathology and toxicology in the DVI operation

The pathology and toxicology results established cause and circumstances of death rather than identity. Soot in the trachea and bronchi, a qualitative confirmatory sign that respiration continued during the fire, together with COHb and MetHb, indicated breathing during the fire in many cases, while the absence of measurable COHb in seven victims demonstrates that no single marker should be applied as a universal vitality threshold. COHb interpretation is influenced by exposure duration, combined toxicants, sampling and individual factors (Eckert and Perper 1981; Michiue et al. 2015; Wirthwein and Pless 1996). The detected plasticizers were consistent with the burned vehicle environment but should remain ancillary observations. The isolated vital T3-T4 injury also shows that mechanical trauma must be sought even when thermal destruction dominates the scene.

Humanitarian and ethical relevance

Complete identification within 10 days allowed the remains to be released and returned for burial. Speed is not a substitute for certainty, but reducing avoidable delay has humanitarian value for families and supports legal processes. Respectful handling, privacy, transparent communication and control of graphic images are integral to DVI practice (INTERPOL 2023; Caenazzo et al. 2013; Leider et al. 2017).

Images of identifiable human remains are published here only within the legal and ethical authority recorded in the Declarations.

Limitations

This is a retrospective report of one incident. The source material did not provide a complete digitized case-level dataset, exact identification timestamps for each victim, or case-by-case DNA statistical values, so no time-to-event analysis of the identification process was possible and the statistical analysis is confined to the reconstructed carboxyhemoglobin data set. The reconstructed COHb map preserves the group identifiers and their relative vertical, left-right and rear-door relationships from the annotated scheme in the case record, but the locations are recovery positions rather than verified seats or metric coordinates; labels in the dense cluster were minimally displaced for legibility without changing group order or local spatial relationships. The three longitudinal zones follow the geometry of the schematic - the row at which the rear door is drawn - and were fixed from that geometry rather than from the values; no alternative boundaries were tested. The tests are exploratory and unadjusted, and neighbouring positions are not independent. Movement before death, fire suppression and recovery could alter spatial relationships, so the gradient is a description of the positions in which the decedents were recovered and not a measurement of where each of them was during the fire. Hydrogen cyanide was not requested in this case, which limits toxicological interpretation of the lower values.

The comparison with the INTERPOL Guide has three specific limits. It is retrospective, and the contemporaneous use of particular INTERPOL forms is not documented. It is based on the written case record, so an element recorded as partially documented may have been performed more fully than the available material shows; absence of a record is not evidence of absence of the action. And it was performed by authors who took part in the operation, which is a source of favourable bias that we have limited by extracting the Guide elements before scoring, by scoring only documented actions, and by reporting the pre-adoption items explicitly. Some AM dental records were incomplete, and several individualizing details rely on family-supplied photographs or descriptions. These limitations do not affect the documented outcome of 45 identifications, but they restrict causal claims about which component shortened the total duration.

Conclusions

A locally assembled, cross-border response identified all 45 victims of the Struma Motorway bus fire within 10 days, with complete PM DNA profiling, no reliance on visual identification, and resolution of a twin pair and two sibling groups by combined genetic, odontological and secondary evidence.

Assessed element by element against the INTERPOL DVI Guide, the response met the Guide on the Guide’s own terms. Of 32 elements, 18 were fulfilled through documented local equivalents and 8 in substance with partial documentation; every element bearing on evidential integrity was fulfilled. The gaps were not operational failures but a documentation and governance layer - INTERPOL numbering

and forms, a machine-readable exchange format, a formal identification board - together with post-mortem imaging as the one substantive capability to add.

Three findings are transferable to any jurisdiction that must build a DVI response from existing institutions. Traceability functions are procedural and can be delivered immediately at no capital cost. The primary-identifier hierarchy must be enforced most strictly precisely where families travelled together and secondary features are tempting. And reconciliation must be empowered to reopen a closed phase: in this case that authority was the difference between 44 and 45 victims identified. The scene document, at no cost at the time, preserved an analysis that could still be performed years later and that becomes impossible once the vehicle is released: because the schematic recorded both the recovery position and the group number under which each sample was taken, the toxicology results could be mapped onto the interior of the bus. Recording position and sample identifier together is therefore recommended as routine practice in vehicle-fire mass-fatality scenes. The INTERPOL framework, applied as the flexible baseline it declares itself to be, was sufficient to guide an effective response assembled in hours - and the same framework defines precisely what should be prepared before the next event.

Abbreviations

AM: ante-mortem; COHb: carboxyhemoglobin; CT: computed tomography; DVI: disaster victim identification; GC-FID: gas chromatography with flame-ionization detection; GC-MS: gas chromatography-mass spectrometry; IQR: interquartile range; ISFG: International Society for Forensic Genetics; ISO: International Organization for Standardization; MetHb: methemoglobin; PM: post-mortem; STR: short tandem repeat; Y-STR: Y-chromosomal short tandem repeat.

Declarations

This manuscript is submitted for double-anonymised peer review. In accordance with the journal instructions and the technical check, the ethics statement, the funding statement, the data availability statement, competing interests, the authors' contributions and the acknowledgements are provided in the corresponding sections of the submission system and are therefore not reproduced here. The supplementary files are listed above.

Clinical trial number: not applicable.

Consent for publication

No individually identifiable person is described or shown. Decedents are referred to only by the scene body or group numbers used in the case record, no names, dates of birth or other identifying particulars are reported, and no relative is identifiable from the ante-mortem information described. Publication of the anonymized case material is covered by the authorization of the competent prosecutor referred to

above; consent of relatives is not the applicable legal basis for the medico-legal material in this jurisdiction. No photographs of the scene or of human remains are reproduced in this article; the two figures are a data reconstruction and a schematic drawing.

Use of generative artificial intelligence

A generative artificial-intelligence assistant was used for English-language editing of the authors' text, for formatting of the references, and for the code that produced Additional file 1, and Figures 1 and 2. No content originated with the assistant and it generated no case finding: all case material and interpretations are those of the authors, who verified every value, figure, citation and statement and take full responsibility. It is not credited with authorship..

Competing Interests

Yanko Kolev is a Guest Editor of the special issue to which this manuscript is submitted. He has taken and will take no part in the peer review of, or the editorial decision on, this manuscript, which is handled independently by another Editor in accordance with the journal’s procedure for editor-authored submissions. The remaining authors declare that they have no competing interests.

Author Contribution

AA conceived the report, conceptualized and drafted the manuscript; EA performed the genetic analyses; BM and AC performed the post-mortem examinations and prepared the draft; HM supervised the laboratory workflow; YK conceptualized the report, developed its structure and prepared the manuscript for publication, including the comparison with the INTERPOL DVI Guide and the submission materials

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Data Availability

The reconstructed, de-identified data set underlying Figure 1 and the spatial analyses is provided as Additional file 1 (CSV); the per-decedent listing is Supplementary Table S1 in Additional file 2. All other de-identified aggregate data supporting the findings are included in the article and its supplementary information files. The case-level medico-legal, genetic and family data are not publicly available because they form part of a judicial case file and contain sensitive personal and kinship information; access is

subject to authorisation by the competent judicial authority and to applicable data-protection requirements.

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Figures

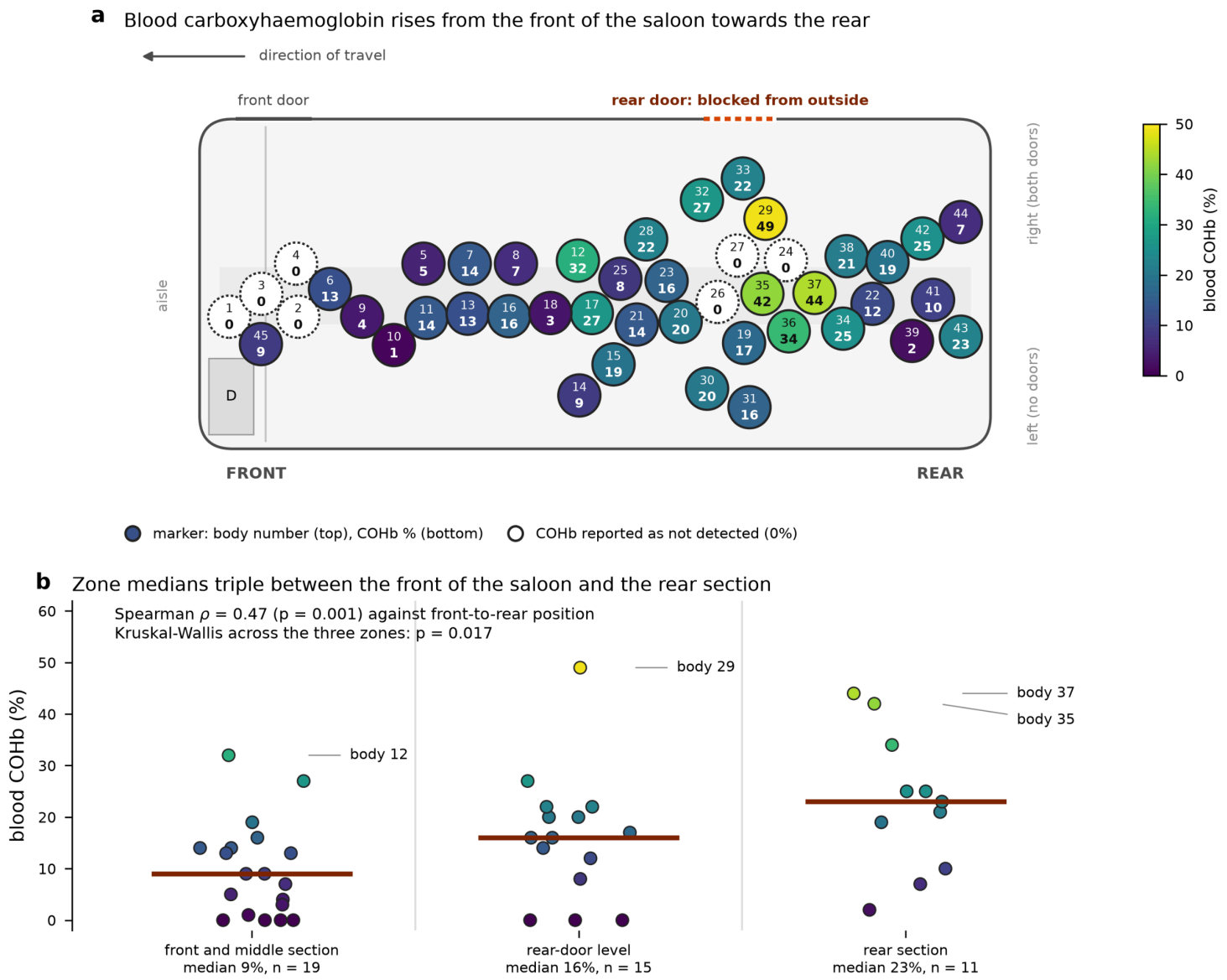


Figure 1

Reconstructed recovery map of the passenger saloon with post-mortem blood carboxyhemoglobin values. (a) Plan of the saloon, front to the left; the vehicle travelled in the direction of the arrow. Each marker is one set of remains: the upper number is the scene body/group number and the lower number the blood carboxyhemoglobin percentage in that decedent, with marker colour encoding the same value. Dotted markers are the seven results reported as not detected. All 45 number/value pairs are transcribed from the annotated schematic in the case record. The walls, the driver station, the front door and the blocked rear door are taken from the border formatting of the source schematic; the aisle band is shown in the position in which it is labelled there. Transverse positions are normalized between the two side walls and are reproduced as decoded; to keep every label legible, markers were displaced only along the vehicle axis, by at most 2.2 of the 12 length units of the plan. Positions denote recovery positions, not assigned seats, and are ordinal and proportional rather than metric. (b) Distribution of carboxyhemoglobin by longitudinal zone, one point per decedent, with the zone median as a horizontal

line; the zones are defined from the schematic, the rear-door zone being the row at which the rear door is drawn. Test statistics are given in the panel.

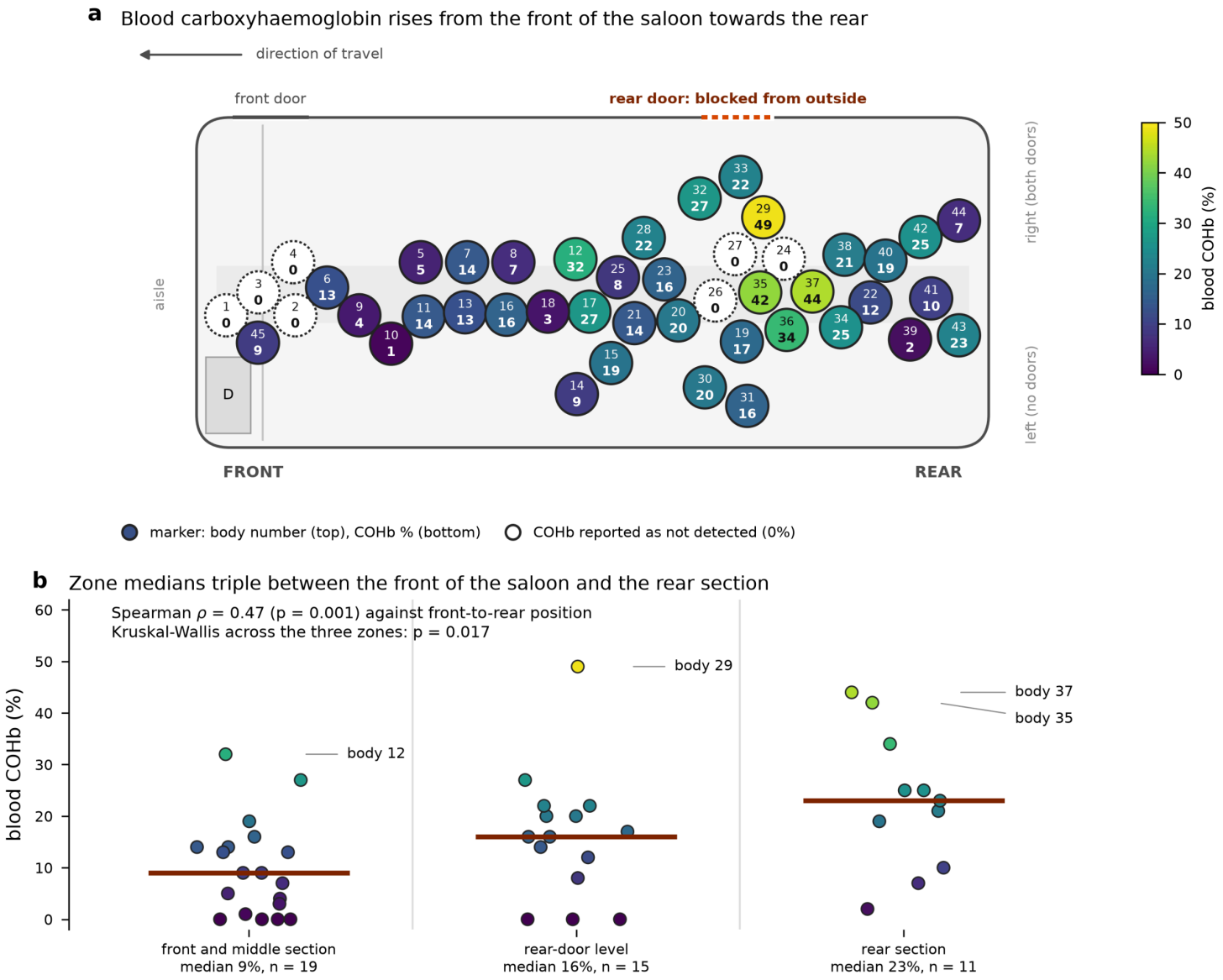


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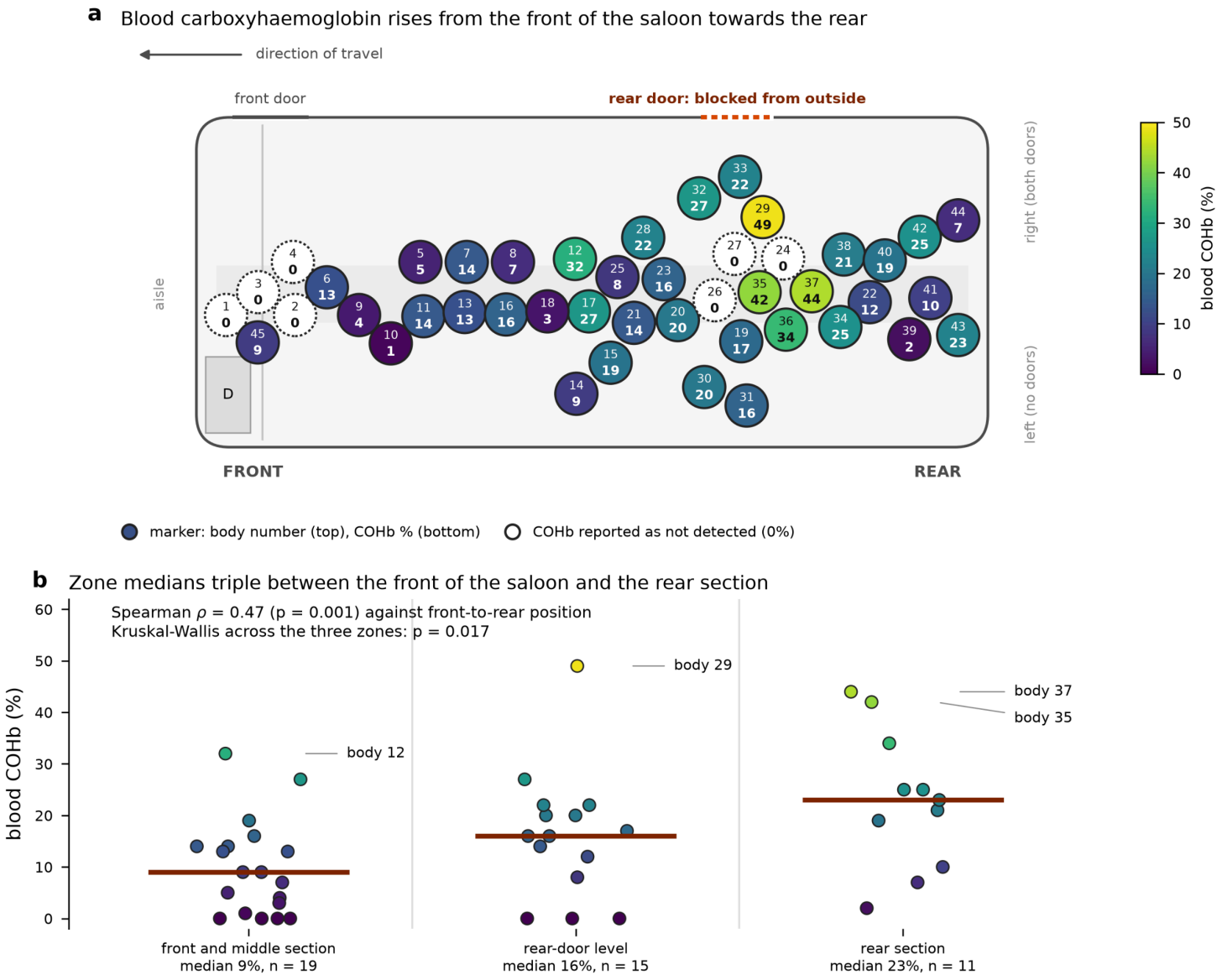


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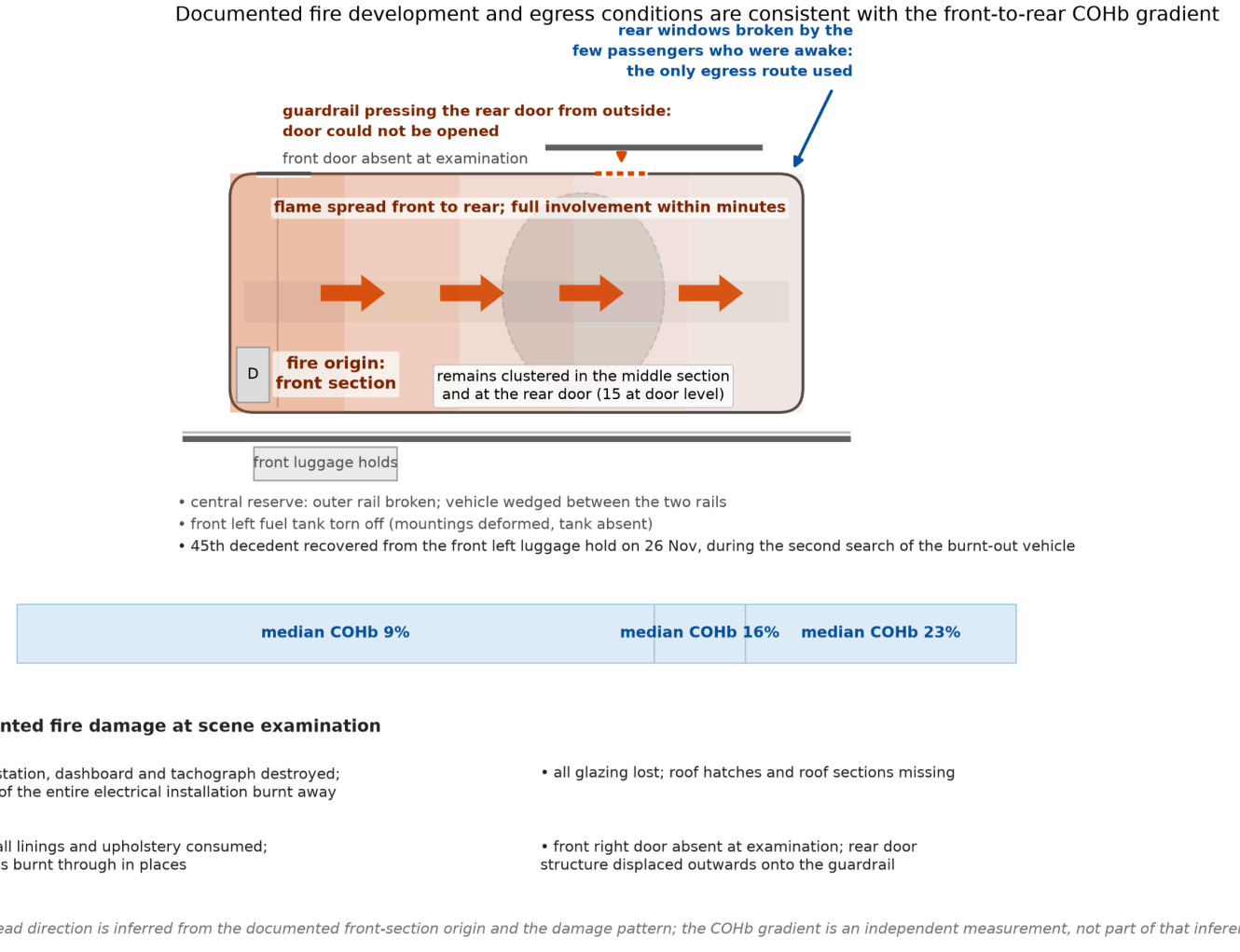


Figure 2

Documented fire development, egress conditions and the measured carboxyhemoglobin gradient. The plan of the saloon carries the area of origin in the front section, the inferred front-to-rear direction of flame spread, the zone of greatest clustering of remains, the rear door held shut by the external guardrail, the rear windows that were the only egress route used, the front luggage holds and the position of the vehicle against the central-reserve rails. The strip below the plan gives the median carboxyhemoglobin value for each of the three longitudinal zones on the same horizontal scale, so that the biochemical gradient can be read against the fire and egress geometry. The inventory below lists the fire damage

recorded at scene examination. The direction of flame spread is inferred from the documented area of origin and the damage pattern; the carboxyhemoglobin values are independent measurements and are not part of that inference.

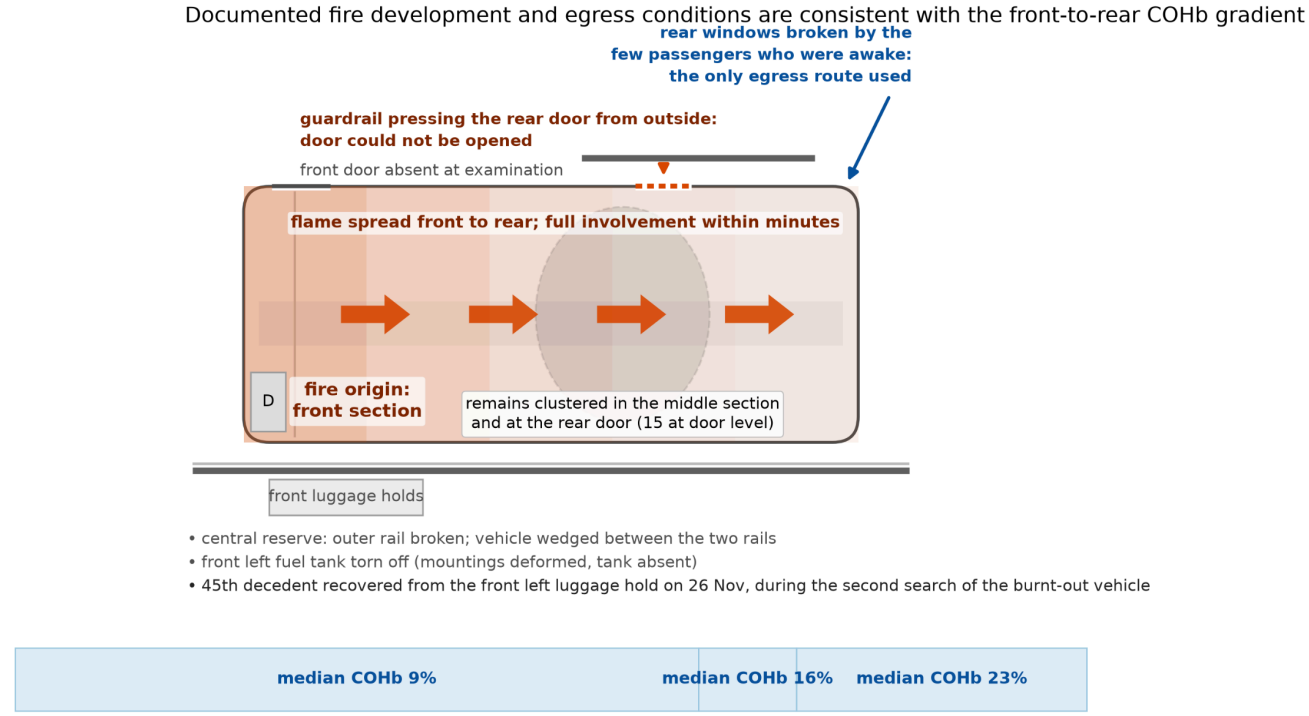


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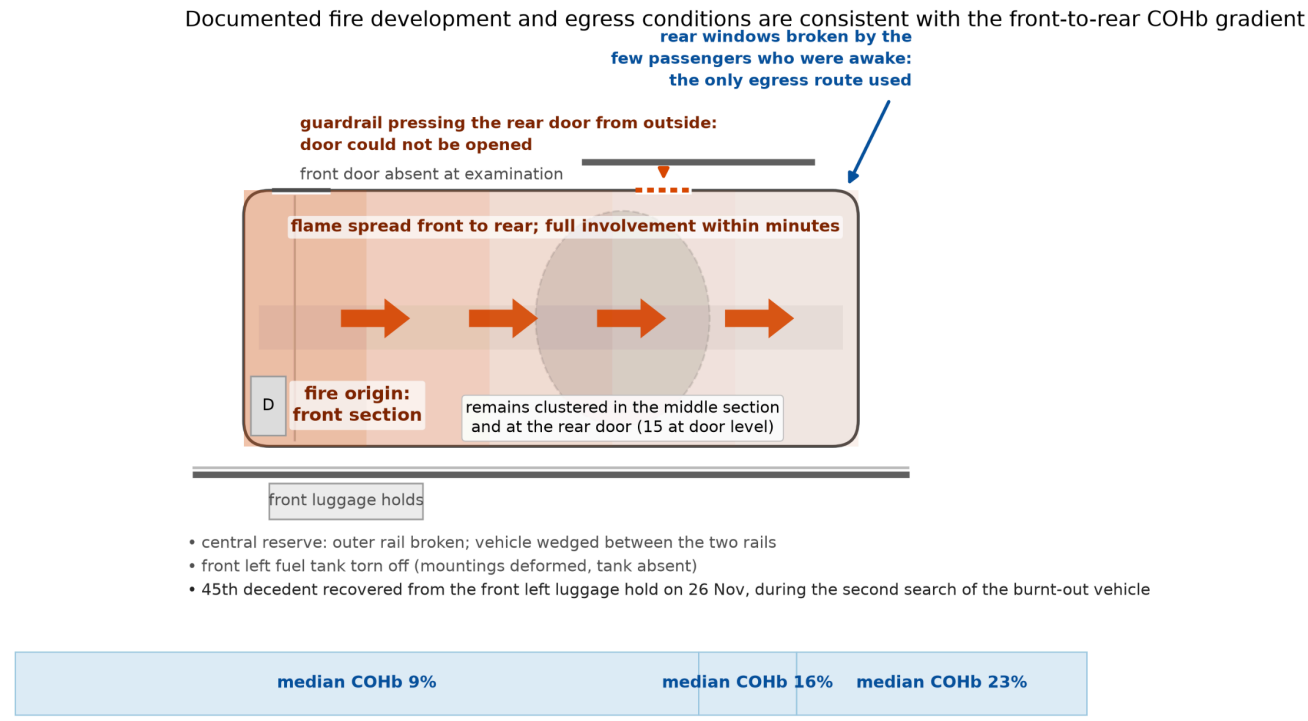


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Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- EJFSbusCOHbdataset.csv

- EJFSbusCOHbdataset.csv
- EJFSbusCOHbdataset.csv
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