

Original Research Article

Enzymatic Molecular Autopsy and Postmortem Thanatochemistry of Cyanide Poisoning: Multi-Organ Distribution, Cytochrome c Oxidase Inhibition and Manner of Death in Rabat, Morocco

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ABSTRACT

Background. Fatal cyanide poisoning causes histotoxic hypoxia through inhibition of cytochrome c oxidase, but postmortem interpretation is complicated by analyte volatility, redistribution and putrefactive artefact.

Objective. To describe multi-matrix cyanide distribution and cytochrome c oxidase inhibition in fatal cyanide poisoning and relate them to route and manner of death.

Methods. In this retrospective autopsy case series of 112 consecutive fatal cyanide poisonings in Rabat, Morocco, cyanide was quantified in six matrices by headspace gas chromatography–mass spectrometry, and cytochrome c oxidase activity was assayed in four tissues against matched controls. Peracute lethal outcome (death at the scene or within one hour) was analysed by logistic regression.

Results. Cyanide differed across matrices ($F(5, 666) = 4380, p < 0.001$): gastric contents 1250.0 ± 180.0 , deep brain 200.5 ± 19.5 and femoral blood 100.2 ± 9.3 mg/L. Brain cyanide was highest after oral ingestion (228.1 ± 23.6 mg/L), lowest after transcutaneous absorption (105.0 ± 13.4 mg/L) and differed by manner of death ($p < 0.001$). Enzyme inhibition was tissue-dependent (brain $88.5 \pm 5.2\%$, liver $45.6 \pm 7.8\%$; $p < 0.001$). Brain cyanide correlated with femoral blood ($r = +0.84$) and enzyme inhibition ($r = +0.79$). Brain cyanide (adjusted OR 4.82 per 25 mg/L; 95% CI 2.56–9.08) and inhibition $\geq 75\%$ (3.65; 1.88–7.10) independently predicted peracute lethal outcome; route did not.

Conclusion. Combining multi-organ thanatochemistry with enzymatic molecular autopsy may offer a more objective, corroborative basis for interpreting fatal cyanide poisoning, alongside scene and investigative evidence.

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1. Introduction

Cyanide is among the most rapidly lethal poisons encountered in forensic practice. The cyanide ion binds the ferric haem a_3 centre of cytochrome c oxidase, the terminal complex of the mitochondrial electron transport chain, arresting the reduction of molecular oxygen to water and collapsing oxidative phosphorylation within minutes. Careful enzymological work indicates that this interaction is a genuine inhibition at physiologically and toxicologically relevant concentrations rather than the biphasic stimulation once proposed, which matters directly for how postmortem enzyme measurements should be interpreted.¹ The resulting state is histotoxic hypoxia: oxygen is delivered to the tissue and remains unused, so that venous blood stays oxygenated while cells die of energy failure. Clinically the syndrome is indistinguishable at the bedside from other causes of sudden collapse, and it frequently coexists with carbon monoxide toxicity in fire victims.²

In Morocco the forensic burden of cyanide reflects a specific combination of industrial availability and domestic exposure. Cyanide salts circulate through electroplating, jewellery finishing and artisanal gold processing, while cyanogenic plant material remains an underappreciated domestic hazard; a recent Moroccan medicolegal report documents fatal poisoning in a young child after ingestion of bitter apricot kernels, illustrating how amygdalin-bearing material can deliver a lethal dose outside any occupational setting.³ Earlier work from this laboratory established the feasibility of enzymatic molecular autopsy for cyanide casework in Rabat and motivated the systematic multi-organ series reported here.⁴

Exposure routes differ in ways that shape the analytical problem. Inhalational exposure arises predominantly from structural fire effluent, where hydrogen cyanide is generated by the combustion of nitrogen-containing polymers; quantification of cyanide in fire effluent has clarified how rapidly toxic concentrations accumulate.⁵ Autopsy series of closed-space fire deaths confirm that cyanide contributes materially to mortality alongside

carbon monoxide and is frequently overlooked when only carboxyhaemoglobin is measured.⁶ Individual case documentation has further shown that a markedly elevated blood hydrogen cyanide concentration can itself function as evidence that the decedent was alive and breathing during the fire.⁷ Experimental work on the diffusion of carbon monoxide and hydrogen cyanide into muscle and blood has begun to define how these gases distribute after death, which bears directly on matrix selection.⁸

The molecular target itself is now well characterised. Cytochrome c oxidase is a multi-subunit complex whose activity is regulated allosterically, by nucleotide binding and by post-translational modification, so that baseline activity is neither fixed nor uniform across tissues.⁹ Adenosine triphosphate binding alone measurably depresses activity and alters mitochondrial reactive oxygen production, which indicates that the enzyme operates well below its maximal rate under physiological conditions.¹⁰ Structural analysis of the late maturation steps of complex IV within the human respirasome has clarified how the catalytic core is assembled,¹¹ and work on the final assembly stages under oxidative stress shows that the complex is vulnerable to perturbation during precisely the conditions that accompany dying.¹² Comparable assembly defects arise under nitrosative stress in yeast, underlining that complex IV activity is a sensitive but not wholly specific readout.¹³

Endogenous detoxification determines how long cyanide persists. The mitochondrial enzyme rhodanese, a thiosulfate–cyanide sulfurtransferase, transfers sulfur to cyanide to yield the far less toxic thiocyanate, and its distribution is weighted towards liver and kidney.¹⁴ Characterisation of purified fungal rhodanese has quantified the catalytic efficiency underlying this conversion,¹⁵ and cell-based work demonstrates that rhodanese activity is protective against the cyanide liberated from amygdalin.¹⁶ The hepatic concentration of rhodanese is the principal reason liver behaves differently from brain in the postmortem period, and it predicts that hepatic enzyme inhibition should be less severe than cerebral inhibition for the same systemic exposure.

Therapeutically, hydroxocobalamin has become the first-line antidote by binding cyanide to form cyanocobalamin, and a recent scoping review synthesises the evidence for its intravenous use in smoke-inhalation poisoning.¹⁷ Empirical administration when cyanide toxicity is suspected but unconfirmed has been evaluated in burn centres¹⁸ and is not associated with methaemoglobinaemia in inhalation injury.¹⁹ For the forensic pathologist, antidote administration before death is an important confounder, because it alters the measured analyte.

Postmortem quantification is analytically demanding. Cyanide is volatile, chemically reactive and unstable in stored specimens, so the method must capture it before loss occurs. Headspace gas chromatography–mass spectrometry has become the reference approach, and validated procedures now cover both water and postmortem biological material.²⁰ Rapid simultaneous determination of cyanide together with azide and ethanol in whole blood illustrates the throughput achievable in a routine medicolegal laboratory,²¹ while concurrent determination of cyanide and its metabolite thiocyanate in antemortem and postmortem blood provides the metabolic context required for interpretation.²² Point-of-care colorimetric platforms are emerging for rapid triage but do not yet meet forensic evidentiary requirements.²³

Kinetic approaches based on the reaction of cyanide with ninhydrin have also been applied to blood from fire victims.²⁴

Interpretation is harder than measurement. Postmortem redistribution moves many analytes down concentration gradients from solid organs into blood after death, and the magnitude of this movement varies by compound and by sampling site.²⁵ The broader difficulties of interpreting postmortem toxicology results, including site dependence and the absence of true reference ranges, are now well documented.²⁶ Cyanide adds two further complications. It can be generated *de novo* by putrefactive bacteria, so that an elevated concentration does not by itself establish antemortem exposure, and measured concentrations may also change with postmortem interval and storage conditions. Conversely, postmortem changes can generate confounding sulfur species, as shown for blood thiosulfate in the diagnosis of hydrogen sulfide poisoning, a closely analogous interpretive problem.²⁷ Toxicological investigation in bodies with extended postmortem intervals and advanced decomposition remains particularly unreliable.²⁸

These constraints have driven interest in alternative matrices. Brain-to-blood concentration ratios have been proposed as a more stable descriptor than blood concentration alone, and systematic work now supports thinking beyond blood in postmortem toxicology.²⁹ Region-specific ratios between the occipital lobe and femoral blood have been characterised for several drug classes.³⁰ Parallel work on carbon monoxide has established quantification in postmortem brain³¹ and in liver as an alternative to blood,³² demonstrating the general viability of solid-organ analysis. Determination of nitrite and nitrate in postmortem blood in sodium nitrite fatalities illustrates how matrix choice can be decisive for a specific agent.³³

The concept of molecular autopsy, in which a functional or genetic assay supplements conventional morphology, is well established in sudden cardiac death. A systematic review has summarised its diagnostic yield and current limitations,³⁴ national programmes have reported operational experience,³⁵ and next-generation sequencing has extended the approach to comprehensive gene panels.³⁶ Applying the same logic to toxicology, the enzymatic lesion produced by cyanide is a direct functional readout of the poisoning itself, and is therefore a natural candidate for an enzymatic rather than genetic molecular autopsy.

What has been missing is a systematic series that measures cyanide across several matrices and the enzymatic lesion in the same decedents, and then asks whether the combination discriminates the manner of death. Case reports and small series establish that fatal poisoning occurs across a wide range of circumstances,³⁷ quantitative autopsy analysis of other fatal poisonings demonstrates the interpretive value of pairing morphology with multi-matrix measurement,³⁸ and epidemiological work characterises the broader pattern of fatal self-poisoning,³⁹ but none supplies the quantitative interpretive framework a court requires. Accordingly, this study was designed with three objectives: to quantify cyanide across six biological matrices in a consecutive medicolegal series; to measure cytochrome c oxidase inhibition across four tissues and relate it to measured concentration; and to determine which analytical variables independently predict a peracute lethal outcome and thereby inform the interpretation of manner of death.

2. Methods

2.1. Study design, setting and ethical framework

This retrospective autopsy case series examined 112 consecutive fatal cyanide poisonings investigated at the Forensic Toxicology Laboratory of the National School of Public Health, Rabat, Morocco. All cases underwent complete medicolegal autopsy under judicial authority. The protocol conformed to the ethical principles of the Declaration of Helsinki; ethical approval, consent and data handling are described in the Declarations.

2.2. Eligibility criteria

Cases were eligible when cyanide poisoning was established as the cause of death by the combination of scene investigation, autopsy findings and confirmatory toxicological analysis. Cases were excluded when the discovery-to-autopsy interval exceeded 72 hours, when advanced putrefaction was present, when the body had been embalmed, or when insufficient material remained for multi-matrix sampling. These criteria were applied because putrefactive cyanogenesis and progressive analyte loss make interpretation unreliable beyond this window. Autopsy series of fire-related deaths informed the approach to cases with combined carbon monoxide and cyanide exposure.⁶

2.3. Sampling and specimen handling

Sampling followed a fixed sequence to minimise site-dependent variation. Femoral venous blood was aspirated from a clamped vessel before evisceration, in accordance with the principle that peripheral blood is less affected by redistribution than cardiac blood.²⁶ Deep brain parenchyma was taken from the basal ganglia and adjacent white matter, liver from the central right lobe, renal cortex from the mid-pole, urine from the bladder by direct puncture, and gastric contents after measurement of total volume. Solid-organ sampling was designed to permit brain-to-blood comparison, an approach validated for other analytes in postmortem casework.²⁹

All specimens were placed immediately into gas-tight borosilicate vials with minimal headspace, sealed with polytetrafluoroethylene-lined caps, and held at 4 °C with analysis completed within 48 hours of sampling. This cold chain was adopted because cyanide concentrations fall measurably during storage and because postmortem processes can generate confounding sulfur species that complicate interpretation.²⁷

2.4. Cyanide quantification

Cyanide was quantified by headspace gas chromatography–mass spectrometry following a validated procedure for postmortem material.²⁰ Tissue was homogenised in phosphate buffer, acidified within the sealed vial to liberate hydrogen cyanide into the headspace, and equilibrated at 60 °C for 20 minutes before injection. Quantification used deuterated internal standardisation with calibration across the working range and duplicate analysis of every specimen. The approach parallels validated methods for the simultaneous determination of cyanide with other volatile toxicants in whole blood.²¹ Thiocyanate was determined in parallel to document the extent of metabolic conversion, using an approach validated for concurrent cyanide and thiocyanate measurement.²² Kinetic colorimetric confirmation was applied to a random subset as an orthogonal check.²⁴

2.5. Cytochrome c oxidase assay

Cytochrome c oxidase activity was measured spectrophotometrically in mitochondrial fractions prepared from brain stem and cerebral cortex, left ventricular myocardium, femoral skeletal muscle and hepatic parenchyma. Activity was determined from the first-order rate of oxidation of reduced cytochrome c at 550 nm and normalised to mitochondrial protein, with results expressed as units per milligram. Because baseline activity differs between tissues and is subject to allosteric regulation,⁹ each tissue was referenced to its own matched control obtained from non-poisoned medicolegal autopsies of comparable postmortem interval, and inhibition was expressed as the proportional reduction against that control. Assay conditions followed established practice for measuring nucleotide-sensitive complex IV activity.¹⁰

2.6. Additional toxicological analyses

Carboxyhaemoglobin saturation was determined in every case to identify combined exposure, and quantification in solid organs was available as a supplementary measure where blood was compromised.^{31,32} Nitrite and nitrate were screened to exclude alternative asphyxiant poisoning, following methodology developed for sodium nitrite fatalities.³³

2.7. Adjudication of manner of death

The manner of death was adjudicated independently by two forensic pathologists blinded to the enzymatic results, using scene findings, police investigation, psychiatric and occupational history, autopsy morphology and toxicological results. Disagreements were resolved by consensus with a third assessor. This structured approach mirrors the retrospective methodology applied to distinguishing homicide from suicide in other modalities of violent death.⁴⁰ Cases in which exposure was documented but the circumstances remained unresolved after this process were classified as undetermined or natural.

2.8. Statistical analysis

Analyses were performed in SPSS version 28.0 and R version 4.3.1. Continuous variables were assessed for normality by the Shapiro–Wilk test and are reported as mean ± standard deviation with median and interquartile range where distributional shape warranted. Differences across biological matrices, manner-of-death strata and tissues were tested by one-way analysis of variance with Tukey honestly significant difference contrasts. Associations between continuous variables were quantified by Pearson product-moment correlation with two-tailed significance testing. Multivariable binary logistic regression identified independent predictors of a peracute lethal outcome, defined as death at the scene or within one hour of exposure without an effective therapeutic interval; model calibration was assessed by the Hosmer–Lemeshow test. Adjusted odds ratios are reported with 95% confidence intervals, and two-tailed *p* values below 0.05 were treated as significant.

3. Results

3.1. Demographic and forensic profile

The series comprised 112 consecutive fatal cyanide poisonings whose demographic and forensic characteristics are set out in Table 1 and displayed in Figure 1. Males predominated, accounting for 67 decedents (59.8%) against 45 females (40.2%), a ratio of 1.49:1. The mean age was 34.2 ± 12.8 years with a range of 8 to 74

years. Young adults aged 18 to 39 years constituted by far the largest stratum at 58 cases (51.8%), followed by middle-aged adults at 32 (28.6%), individuals aged 60 years or above at 14 (12.5%) and paediatric decedents at 8 (7.1%). The concentration of fatalities in adults of working age may reflect occupational and self-harm exposure,

although occupation is not reported here. The mean discovery-to-autopsy interval was 21.4 ± 11.2 hours, with a maximum of 72 hours, placing the entire series within the window in which putrefactive cyanogenesis remains limited.

Table 1. Demographic and forensic profile of the fatal cyanide poisoning series.

Demographic or forensic parameter	Decedents, n	Proportion or summary statistic
Biological sex: male	67	59.8%
Biological sex: female	45	40.2%
Age stratum: Paediatric (0–17 years)	8	7.1%
Age stratum: Young adult (18–39 years)	58	51.8%
Age stratum: Middle-aged (40–59 years)	32	28.6%
Age stratum: Elderly (≥ 60 years)	14	12.5%
Age in years, mean $\pm$ SD (range)	112	34.2 ± 12.8 (8–74)
Discovery-to-autopsy interval in hours, mean $\pm$ SD (range)	112	21.4 ± 11.2 (6–72)

Notes: All proportions are calculated against the full series of $n = 112$ medicolegal autopsies. The postmortem interval is the documented interval from discovery of the body to commencement of autopsy.

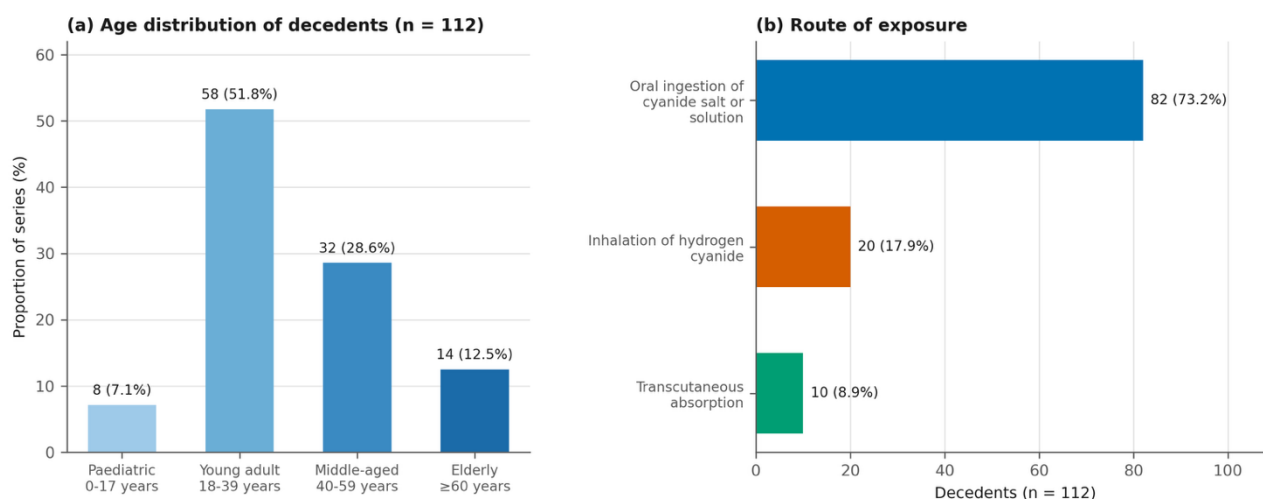


Figure 1. Demographic composition and route of exposure in the Rabat fatal cyanide series ($n = 112$). (a) Age distribution, with counts and proportions above each bar. (b) Route of exposure assigned from scene investigation, witness account and matrix distribution. Values correspond to Tables 1 and 2.

3.2. Route of exposure and route-stratified burden

Routes of exposure, summarised in Table 2 and shown in panel (b) of Figure 1, were dominated by oral ingestion, which accounted for 82 cases (73.2%). Inhalation of hydrogen cyanide accounted for 20 cases (17.9%), arising from structural fire effluent or from release in electroplating and jewellery workshops, and transcutaneous absorption during plating work accounted for the remaining 10 cases (8.9%). Route assignment drew on scene investigation and witness account together with the observed distribution across matrices, in which a markedly elevated gastric concentration was taken as evidence of ingestion; the comparison of concentrations by route is therefore partly circular (see Limitations).

Cyanide burden differed sharply by route, as the concentration columns of Table 2 record. Oral ingestion produced by far the highest values, with a mean brain

concentration of 228.1 ± 23.6 mg/L and femoral blood of 114.0 ± 11.8 mg/L. Inhalational exposure produced 135.0 ± 17.2 mg/L in brain and 67.5 ± 8.6 mg/L in blood, and transcutaneous absorption the lowest values at 105.0 ± 13.4 mg/L and 52.5 ± 6.7 mg/L. One-way analysis of variance across the three routes gave $F(2, 109) = 249.3$, $p < 0.001$ for brain concentration, with oral ingestion differing from both inhalation and transcutaneous absorption on Tukey contrasts ($p < 0.001$ for each) and the two non-oral routes differing from one another ($p = 0.002$). The gradient is toxicokinetically coherent: a swallowed bolus delivers the entire dose to the portal circulation over minutes, whereas inhalational uptake is limited by ambient concentration and respiratory minute volume, and transcutaneous absorption is slower still and constrained by the area of skin in contact. The case-weighted means of the three routes reproduce the whole-series means, and the ratio of the group means for brain to blood is approximately 2.00 within every route stratum.

Table 2. Route of exposure, route-stratified cyanide burden and circumstantial profile.

Route of exposure	Decedents, n (%)	Brain cyanide, mean ± SD (mg/L)	Femoral blood cyanide, mean ± SD (mg/L)	Circumstantial profile
Oral ingestion of cyanide salt or solution	82 (73.2%)	228.1 ± 23.6	114.0 ± 11.8	Domestic or occupational salt, dissolved in a beverage or swallowed directly
Inhalation of hydrogen cyanide	20 (17.9%)	135.0 ± 17.2	67.5 ± 8.6	Structural fire effluent or electroplating and jewellery workshop release
Transcutaneous absorption	10 (8.9%)	105.0 ± 13.4	52.5 ± 6.7	Prolonged skin contact with alkaline cyanide solution during plating work
Whole series	112 (100.0%)	200.5 ± 19.5	100.2 ± 9.3	ANOVA $F(2, 109) = 249.3, p < 0.001$ for brain

Notes: Route was assigned from scene investigation, witness account and the distribution of cyanide across the sampled matrices, with a markedly elevated gastric concentration taken as evidence of ingestion.

3.3. Multi-organ cyanide distribution

Quantification across the six sampled matrices showed a markedly non-uniform distribution, detailed in Table 3 and displayed on a logarithmic scale in Figure 2. One-way analysis of variance across matrices gave $F(5, 666) = 4380, p < 0.001$. Gastric contents carried by far the highest burden at 1250.0 ± 180.0 mg/L, 12.48 times the femoral blood concentration, reflecting the unabsorbed residue of an ingested bolus. Among the parenchymal matrices, deep brain parenchyma was highest at 200.5 ± 19.5 mg/L, approximately twice the femoral blood mean, followed by liver at 145.8 ± 16.2 mg/L (1.46 times blood), femoral blood itself at 100.2 ± 9.3 mg/L, renal cortex at 88.6 ± 8.7 mg/L (0.88 times blood) and urine at 50.4 ± 4.8 mg/L (0.50 times blood).

The ordering of these matrices is mechanistically coherent. The elevated cerebral concentration reflects the high metabolic demand and lipid content of brain tissue together with its status as the principal target organ, while the hepatic value is consistent with first-pass exposure modulated by rhodanese-mediated conversion to thiocyanate. The low urinary concentration is characteristic of peracute death, in which circulatory arrest precedes meaningful renal elimination; in the small number of cases with a documented survival interval, urinary values were correspondingly higher. The brain-to-blood ratio of approximately 2.00 (a ratio of group means) may offer an internal plausibility check for an individual blood result, although individual-level ratios are not reported.

Table 3. Multi-organ postmortem cyanide distribution across sampled biological matrices.

Biological matrix	Mean ± SD (mg/L)	Median (IQR) (mg/L)	Ratio to femoral blood	Toxicological significance
Gastric contents	1250.0 ± 180.0	1240.0 (1120.0–1380.0)	12.48	Unabsorbed bolus; indicates the ingestion route
Deep brain parenchyma	200.5 ± 19.5	201.2 (188.4–213.6)	2.00	Primary target organ; least affected by postmortem redistribution
Liver parenchyma	145.8 ± 16.2	146.1 (135.2–156.4)	1.46	First-pass metabolism and rhodanese-mediated detoxification
Femoral whole blood	100.2 ± 9.3	100.8 (93.5–106.4)	1.00	Peripheral reference matrix; international reporting standard
Renal cortex	88.6 ± 8.7	88.9 (82.4–94.6)	0.88	Reflects systemic perfusion up to circulatory arrest
Urine	50.4 ± 4.8	50.2 (47.1–53.8)	0.50	Renal elimination; low in peracute death, higher with survival interval
One-way ANOVA across the six matrices	$F(5, 666) = 4380$	$p < 0.001$	—	Distribution across matrices is markedly non-uniform

Notes: Gastric values are expressed per litre of total gastric content. The ratio column is referenced to the femoral blood mean of 100.2 mg/L, the international reporting standard for postmortem toxicology.

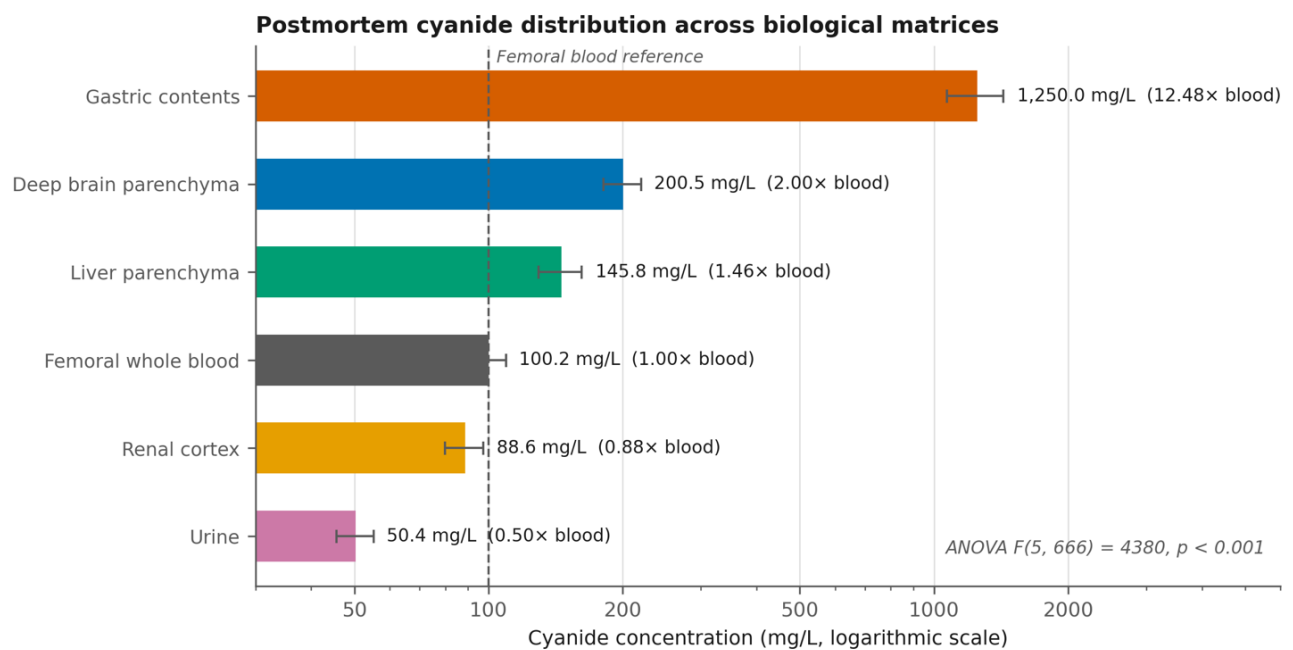


Figure 2. Postmortem cyanide concentration across the six sampled biological matrices, plotted on a logarithmic scale because gastric contents exceed the parenchymal matrices by more than an order of magnitude. Bars show the mean, error bars one standard deviation, and each annotation the ratio to the femoral blood mean.

3.4. Cyanide concentration by manner of death

Stratification by adjudicated manner of death, presented in Table 4 and compared graphically in panel (a) of Figure 3, revealed a clear concentration gradient. Suicide was the commonest manner at 42 cases (37.5%), with a mean brain concentration of 251.2 ± 24.8 mg/L and femoral blood of 125.5 ± 12.4 mg/L. Homicide accounted for 30 cases (26.8%) at 220.8 ± 22.1 mg/L in brain and 110.3 ± 11.0 mg/L in blood. Accidental exposure, whether occupational

or fire-related, accounted for 25 cases (22.3%) at 150.7 ± 18.6 mg/L and 75.3 ± 9.3 mg/L respectively, and undetermined or natural deaths with documented exposure accounted for 15 cases (13.4%) at 100.8 ± 14.2 mg/L and 50.4 ± 7.1 mg/L. One-way analysis of variance across the four strata gave $F(3, 108) = 236.2, p < 0.001$ for brain concentration, with suicide differing significantly from accidental and from undetermined deaths on Tukey contrasts ($p < 0.001$ for each).

Table 4. Brain and femoral blood cyanide concentration stratified by forensic manner of death.

Manner of death	Cases, n (%)	Brain cyanide, mean $\pm$ SD (mg/L)	Femoral blood cyanide, mean $\pm$ SD (mg/L)	Toxicological and judicial profile
Suicide (intentional self-poisoning)	42 (37.5%)	251.2 $\pm$ 24.8	125.5 $\pm$ 12.4	Oral ingestion in all 42 cases (Table 5)
Homicide (criminal administration)	30 (26.8%)	220.8 $\pm$ 22.1	110.3 $\pm$ 11.0	Oral ingestion in all 30 cases (Table 5)
Accidental (occupational or fire)	25 (22.3%)	150.7 $\pm$ 18.6	75.3 $\pm$ 9.3	Electroplating release or synthetic polymer combustion effluent
Undetermined or natural, with exposure	15 (13.4%)	100.8 $\pm$ 14.2	50.4 $\pm$ 7.1	Cardiovascular collapse with secondary environmental exposure
Whole series	112 (100.0%)	200.5 $\pm$ 19.5	100.2 $\pm$ 9.3	ANOVA $F(3, 108) = 236.2, p < 0.001$ for brain

Notes: Case-weighted means of the four strata reproduce the whole-series means of 200.5 mg/L for brain and 100.2 mg/L for femoral blood. Between-stratum differences were tested by one-way analysis of variance with Tukey honestly significant difference contrasts.

Table 5 cross-tabulates the two partitions and Figure 3 sets them side by side on a shared axis. The manner-of-death gradient in panel (a) largely tracks the route gradient in panel (b), because suicide and homicide are both overwhelmingly oral whereas accidental deaths are predominantly inhalational or transcutaneous, so the concentration difference between manners is in part a

difference in how the dose was delivered. Route cannot, however, explain differences between suicide and homicide, which were both entirely oral (Table 5). The overlap between these two strata is too great for concentration alone to classify individual cases, and concentration must be combined with circumstantial evidence.

Table 5. Cross-tabulation of adjudicated manner of death against route of exposure.

Manner of death	Oral ingestion, n	Inhalation, n	Transcutaneous, n	Stratum total, n (%)
Suicide	42	0	0	42 (37.5%)
Homicide	30	0	0	30 (26.8%)
Accidental	2	16	7	25 (22.3%)
Undetermined or natural	8	4	3	15 (13.4%)
Route total, n (%)	82 (73.2%)	20 (17.9%)	10 (8.9%)	112 (100.0%)

Notes: Cross-tabulation of the two partitions of the same series. Every deliberate administration, whether self-inflicted or criminal (72 of 72 cases), was enteral, whereas all 30 inhalational and transcutaneous exposures arose from accident or unresolved circumstance.

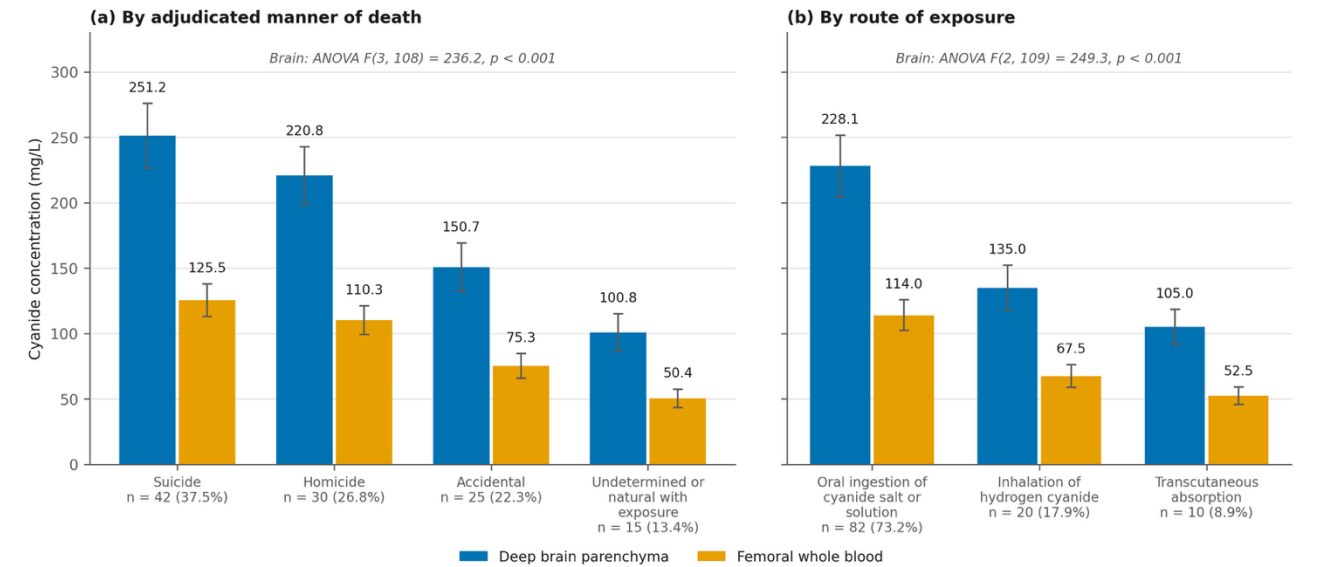


Figure 3. Brain and femoral blood cyanide concentration under two partitions of the same cohort, drawn on a shared value axis. (a) By adjudicated manner of death. (b) By route of exposure. Bars show the mean and error bars one standard deviation; stratum size is given beneath each label. Values correspond to Tables 2 and 4.

3.5. Cytochrome c oxidase inhibition

Enzymatic analysis, reported in Table 6 and displayed in Figure 4, showed marked and strongly tissue-dependent inhibition, with $F(3, 444) = 965, p < 0.001$ across the four sampled tissues. Cerebral tissue showed the most severe lesion, with activity reduced to 0.14 ± 0.04 U/mg against a matched control of 1.22 U/mg, corresponding to $88.5 \pm 5.2\%$ inhibition. Left ventricular myocardium followed at 0.26 ± 0.06 U/mg against a control of 1.19 U/mg, or $78.2 \pm 6.4\%$ inhibition. Femoral skeletal muscle was inhibited by $52.4 \pm 8.1\%$ (0.58 ± 0.11 U/mg against 1.22 U/mg) and hepatic parenchyma least, by $45.6 \pm 7.8\%$ (0.66 ± 0.12 U/mg against 1.21 U/mg). Cerebral inhibition exceeded

both hepatic and myocardial inhibition on Tukey contrasts ($p < 0.001$ for each).

The relative sparing of liver is the most mechanistically informative feature of this gradient. Hepatic tissue carries the highest endogenous concentration of rhodanese, and the observation that the organ with the greatest sulfurtransferase capacity sustains the least enzymatic injury despite the second-highest parenchymal cyanide concentration is compatible with a protective role of hepatic rhodanese, although rhodanese activity was not measured. Skeletal muscle, with intermediate perfusion and low metabolic rate at the time of death, occupied an intermediate position.

Table 6. Cytochrome c oxidase activity and inhibition across anatomical tissues.

Anatomical tissue	Measured activity (U/mg)	Matched control activity (U/mg)	Inhibition (%)	Mechanistic interpretation
Brain stem and cerebral cortex	0.14 ± 0.04	1.22	88.5 ± 5.2	Marked inhibition of oxidative phosphorylation
Left ventricular myocardium	0.26 ± 0.06	1.19	78.2 ± 6.4	Marked myocardial inhibition
Skeletal muscle (femoral)	0.58 ± 0.11	1.22	52.4 ± 8.1	Moderate peripheral inhibition; intermediate tissue
Hepatic parenchyma	0.66 ± 0.12	1.21	45.6 ± 7.8	Partial protection by endogenous mitochondrial rhodanese activity
One-way ANOVA across the four tissues	—	—	$F(3, 444) = 965, p < 0.001$	Inhibition is strongly tissue-dependent

Notes: Inhibition is calculated as the proportional reduction in cytochrome c oxidase activity relative to the matched non-poisoned control for the same tissue; control activities averaged 1.21 U/mg across the four sites. U denotes enzyme units.

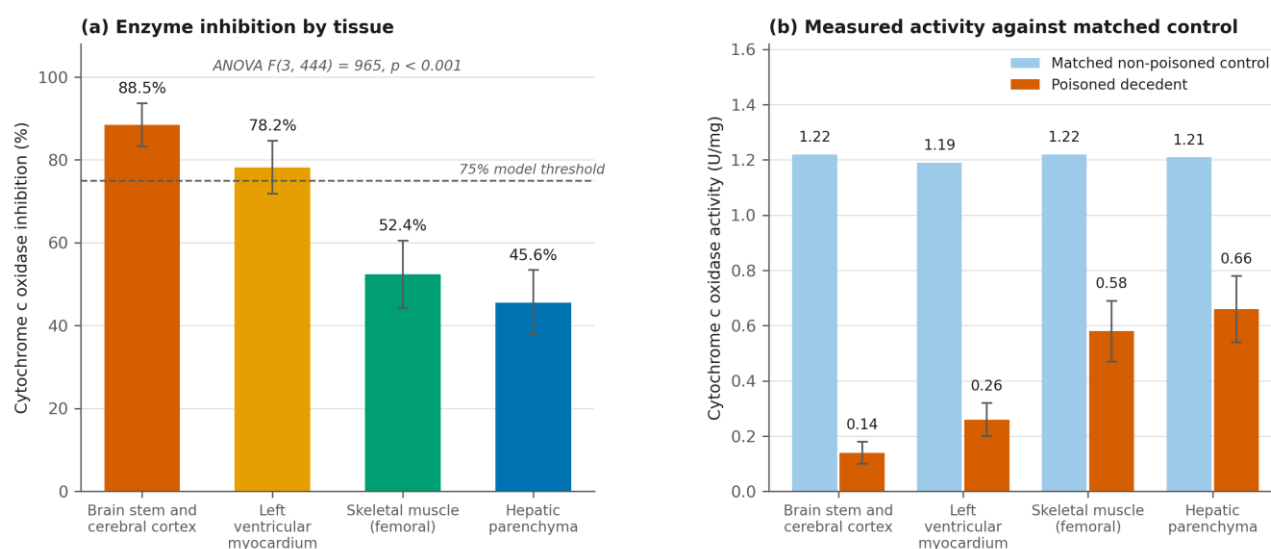


Figure 4. Cytochrome c oxidase across four anatomical tissues. (a) Percentage inhibition, with the 75% threshold used in the regression model marked. (b) Measured activity in poisoned decedents against the matched non-poisoned control for the same tissue, from which the inhibition percentages are derived.

3.6. Correlation structure

The correlation structure among the principal analytical variables is set out in Table 7 and displayed as a matrix in Figure 5. Brain cyanide correlated strongly with femoral blood cyanide ($r = +0.84$, $p < 0.001$), suggesting that peripheral blood is a useful proxy for central target-organ burden in specimens collected under these conditions. Brain concentration also correlated closely with cerebral cytochrome c oxidase inhibition ($r = +0.79$, $p < 0.001$), and blood concentration somewhat less closely ($r = +0.71$, $p < 0.001$), which is the ordering that would be expected if the enzymatic lesion is driven by local tissue concentration rather than by circulating concentration.

Urine correlated moderately with blood ($r = +0.58$, $p < 0.001$), consistent with variable survival intervals across the series, and gastric contents correlated least ($r = +0.46$, $p < 0.001$), reflecting heterogeneous absorption from the ingested bolus. The discovery-to-autopsy interval correlated inversely with femoral blood concentration ($r = -0.32$, $p < 0.001$), consistent with progressive analyte loss during the interval before autopsy and suggesting that the discovery-to-autopsy interval should be treated as a covariate rather than ignored when a blood concentration is interpreted.

Table 7. Correlation structure between cyanide concentrations, enzyme inhibition and postmortem interval.

Variable pair	Pearson r	p value	Interpretation
Brain cyanide vs Femoral blood cyanide	+0.84	$p < 0.001$	Peripheral blood is a useful proxy for central target-organ burden
Brain cyanide vs Cytochrome c oxidase inhibition	+0.79	$p < 0.001$	Target-organ concentration tracks the enzymatic lesion directly
Femoral blood cyanide vs Cytochrome c oxidase inhibition	+0.71	$p < 0.001$	Blood concentration predicts enzyme inhibition, though less tightly than brain
Urine cyanide vs Femoral blood cyanide	+0.58	$p < 0.001$	Moderate association; depends on the survival interval before death
Gastric cyanide vs Femoral blood cyanide	+0.46	$p < 0.001$	Weakest association; reflects variable absorption from the bolus
Discovery-to-autopsy interval vs Femoral blood cyanide	-0.32	$p < 0.001$	Inverse association consistent with progressive postmortem loss

Notes: All coefficients are Pearson product-moment correlations over $n = 112$ cases with two-tailed significance testing. Cytochrome c oxidase inhibition refers to the cerebral measurement.

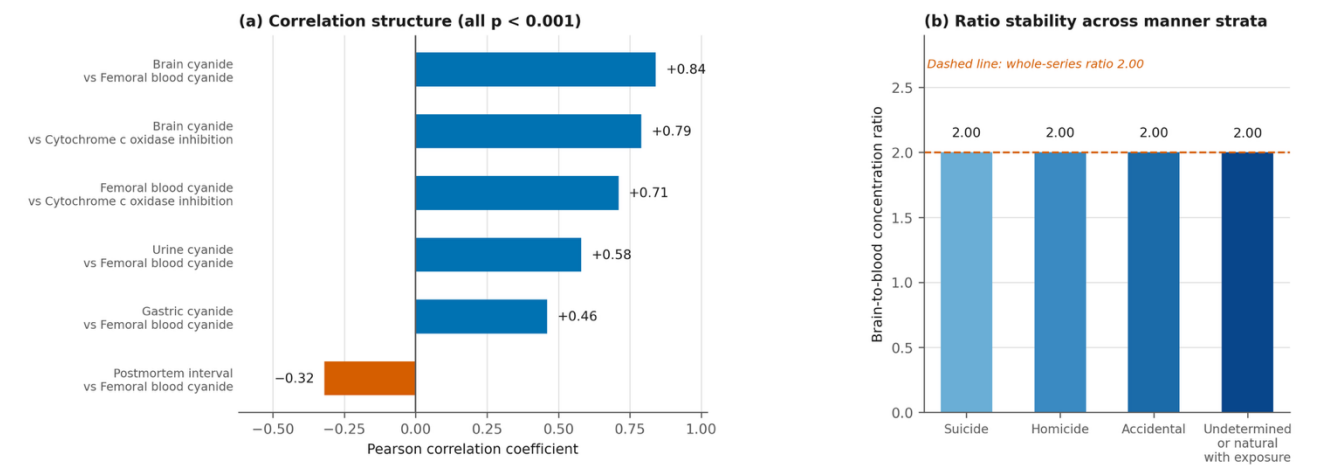


Figure 5. Correlation structure and internal consistency. (a) Pearson coefficients for the six assessed variable pairs, ordered by magnitude; all were significant at $p < 0.001$. (b) The brain-to-blood concentration ratio computed within each manner-of-death stratum, showing ratios of group means close to the whole-series value of approximately 2.00.

3.7. Independent predictors of peracute lethal outcome

Univariable and multivariable logistic regression is presented in Table 8, with adjusted effect sizes displayed as a forest plot in Figure 6. Two variables remained strongly independent after mutual adjustment. Each 25 mg/L increment in brain cyanide concentration carried an adjusted odds ratio of 4.82 (95% CI 2.56–9.08, $p < 0.001$), and cerebral cytochrome c oxidase inhibition of 75% or more carried an adjusted odds ratio of 3.65 (95% CI 1.88–

7.10, $p < 0.001$). Each 25 mg/L increment in femoral blood cyanide remained independently predictive at 2.45 (95% CI 1.48–4.05, $p < 0.001$). Neither a discovery-to-autopsy interval within 24 hours (adjusted 1.72, 95% CI 0.92–3.22, $p = 0.088$) nor the oral ingestion route (adjusted 1.52, 95% CI 0.84–2.76, $p = 0.165$) retained significance after adjustment. Model calibration was acceptable (Hosmer–Lemeshow $p = 0.612$). The pattern suggests that target-organ concentration and the enzymatic lesion it produces, rather than exposure route or autopsy timing, are the main determinants of a peracute lethal course.

Table 8. Univariable and multivariable logistic regression for peracute lethal outcome

Predictor in the lethal-outcome model	Crude OR (95% CI)	Univariable p value	Adjusted OR (95% CI)	Multivariable p value
Brain cyanide, per 25 mg/L increment	4.15 (2.42–7.12)	$p < 0.001$	4.82 (2.56–9.08)	$p < 0.001$
Cerebral cytochrome c oxidase inhibition $\geq 75\%$	3.24 (1.82–5.78)	$p < 0.001$	3.65 (1.88–7.10)	$p < 0.001$
Femoral blood cyanide, per 25 mg/L increment	2.28 (1.45–3.58)	$p < 0.001$	2.45 (1.48–4.05)	$p < 0.001$
Discovery-to-autopsy interval ≤ 24 hours	1.85 (1.04–3.28)	$p = 0.035$	1.72 (0.92–3.22)	$p = 0.088$
Oral ingestion route (vs inhalation or transcutaneous)	1.64 (0.95–2.84)	$p = 0.076$	1.52 (0.84–2.76)	$p = 0.165$

Notes: OR denotes odds ratio and CI denotes confidence interval. The model estimates the odds of a peracute lethal toxicological outcome. Model calibration was acceptable (Hosmer–Lemeshow $p = 0.612$). Peracute lethal outcome was defined as death at the scene or within one hour of exposure.

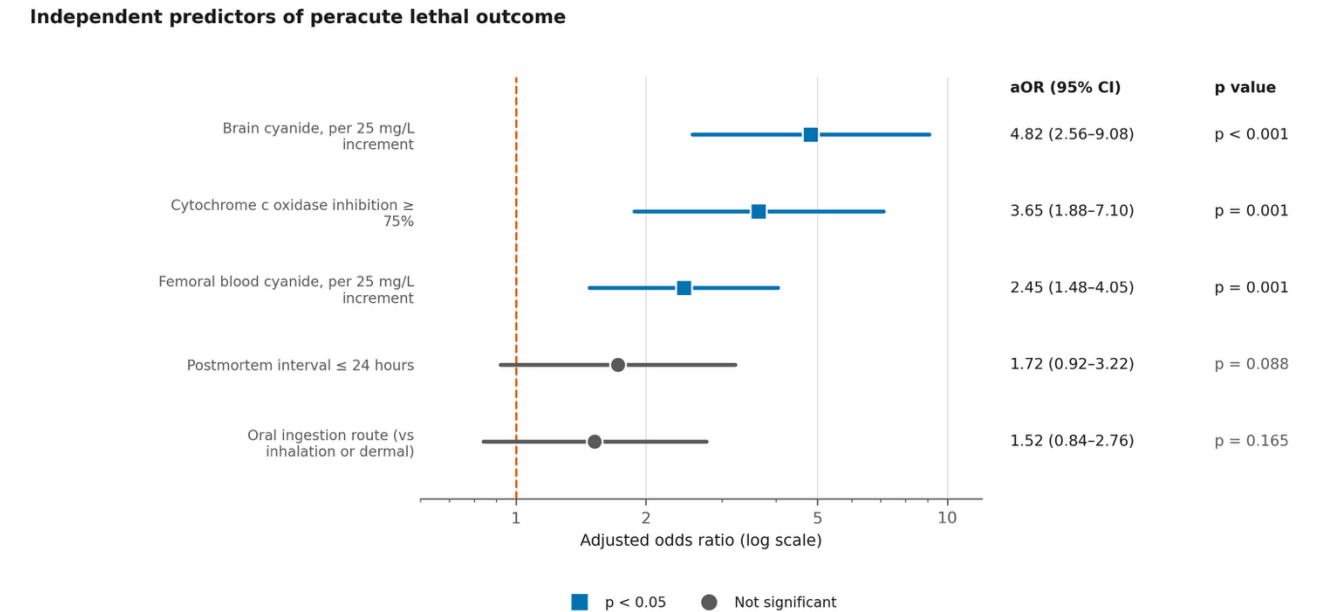


Figure 6. Adjusted odds ratios with 95% confidence intervals for peracute lethal outcome. Square markers denote predictors significant at $p < 0.05$ after mutual adjustment; the dashed line marks the null.

4. Discussion

4.1. Principal findings

This consecutive medicolegal series suggests that pairing multi-organ cyanide quantification with direct measurement of the enzymatic lesion may provide an interpretive framework more robust than blood concentration alone, although no comparison of blood-only and combined models was made. Three findings carry the argument: distribution across matrices is highly ordered and reproducible, with a brain-to-blood ratio of approximately 2.00; cytochrome c oxidase inhibition is severe, tissue-dependent and consistent with an inverse relationship to endogenous detoxification capacity; and target-organ concentration together with enzyme inhibition independently predict a peracute lethal course after adjustment for discovery-to-autopsy interval and exposure route.

4.2. Brain concentration as a target-organ measure

The primacy of brain tissue in this series merits emphasis. Deep brain parenchyma carried twice the femoral blood concentration, and its correlation with the enzymatic lesion was closer than that of blood. This is consistent with the growing recognition that postmortem toxicology should look beyond blood, and that brain-to-blood ratios offer a more stable descriptor than blood alone in the presence of redistribution.²⁹ Region-specific ratios have been characterised for several drug classes,³⁰ and parallel work on carbon monoxide has established that solid organs are analytically tractable alternatives to blood.^{31,32} The practical implication for cyanide casework is that a brain specimen should be collected routinely, not reserved for cases in which blood proves inadequate.

4.3. Distribution across matrices

The ordering of concentrations across matrices is mechanistically interpretable rather than merely descriptive. The gastric burden of 1250.0 mg/L, 12.48 times blood, marks the unabsorbed residue of an ingested bolus and is the single most useful discriminator of route; in the inhalational and transcutaneous subgroups this relationship was absent. The hepatic value of 145.8 mg/L, intermediate between brain and blood, reflects first-pass exposure modulated by conversion to thiocyanate through mitochondrial rhodanese.¹⁴ The low urinary concentration of 50.4 mg/L is the expected signature of peracute death in which circulatory arrest precedes renal elimination, and its moderate correlation with blood ($r = +0.58$) is explained by the small number of cases with a survival interval.

4.4. Enzymatic findings

The enzymatic findings provide supportive evidence that the measured concentrations are toxicologically meaningful rather than artefactual. Cerebral inhibition of 88.5% is consistent with binding of cyanide at the ferric haem a_3 centre of complex IV, and the demonstration that physiological cyanide concentrations genuinely inhibit rather than stimulate the enzyme supports reading such a measurement as evidence of poisoning.¹ The tissue gradient is equally informative. Liver sustained the least inhibition at 45.6% despite carrying the second-highest parenchymal concentration, which is the dissociation expected where sulfurtransferase capacity is greatest; characterisation of purified fungal rhodanese kinetics supports this reading,¹⁵ as does cell-based evidence that rhodanese protects against cyanide liberated from amygdalin.¹⁶ The series is therefore compatible with a

protective role of hepatic rhodanese in humans, a mechanism previously characterised largely *in vitro*; rhodanese activity was not measured, and this interpretation remains inferential.

Interpretation of the enzyme measurement nonetheless requires caution, because complex IV activity is not a wholly specific marker. Baseline activity is allosterically regulated and varies between tissues,⁹ nucleotide binding alone depresses it measurably,¹⁰ assembly of the catalytic core is a staged process,¹¹ and both oxidative and nitrosative stress perturb the final assembly steps.^{12,13} These considerations are why the present protocol referenced every tissue to its own matched control from non-poisoned autopsies of comparable postmortem interval, rather than to a published reference value. Without that internal control, a depressed activity could as easily reflect agonal hypoxia or postmortem change as cyanide binding.

4.5. Manner of death

The manner-of-death gradient is the finding of greatest immediate forensic utility, but it must be stated carefully. Brain concentration separated the four strata clearly in aggregate ($F(3, 108) = 236.2, p < 0.001$), ranging from 251.2 mg/L in suicide to 100.8 mg/L in undetermined deaths. Yet the suicide and homicide distributions overlap substantially, and the present data do not support using concentration alone to distinguish them. What the analysis does support is a narrower and more defensible claim: a concentration in the upper range, combined with severe enzymatic inhibition, is consistent with deliberate administration of a concentrated dose, leaving the question of who administered it to be settled by investigation. This is the same structure of reasoning applied to distinguishing homicide from suicide in other modalities of violent death,⁴⁰ where the physical findings constrain rather than determine the manner.

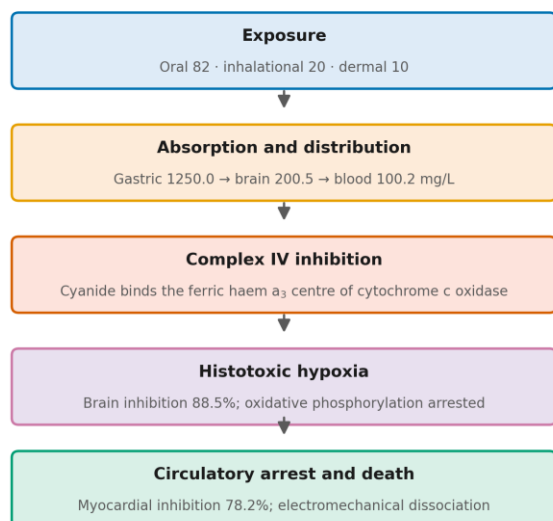
4.6. Route of exposure

Route of exposure emerged as the dominant determinant of absorbed burden, and the way it behaves in the regression is instructive. Oral ingestion produced a mean brain concentration of 228.1 mg/L against 135.0 mg/L for inhalation and 105.0 mg/L for transcutaneous absorption, a gradient that reflects delivery kinetics rather than intrinsic toxicity: a swallowed bolus presents the whole dose to the portal circulation within minutes, whereas inhalational uptake is bounded by ambient concentration and minute volume, and transcutaneous absorption is bounded further by contact area and stratum corneum permeability. Yet once brain concentration entered the multivariable model, the oral route lost independent predictive weight (adjusted odds ratio 1.52, 95% CI 0.84–2.76, $p = 0.165$). This pattern is compatible with mediation rather than absence of effect: route may influence outcome because it determines concentration, so conditioning on concentration would be expected to remove the route effect, although no formal mediation analysis was performed. The practical corollary for casework is that route should be used to assess whether a measured concentration is plausible for the circumstances alleged, not as an independent indicator of severity. A femoral blood concentration near 114.0 mg/L is consistent with ingestion; the same value in a decedent recovered from a structural fire, where the inhalational group mean in this series was 67.5 mg/L, would warrant scrutiny of whether ingestion also occurred; group means are not reference ranges, and the inhalation group combined fire and workshop exposures.

4.7. Integrated interpretive framework

Figure 7 consolidates these findings into an integrated interpretive framework. The schematic traces the mechanistic pathway from exposure through absorption, distribution and complex IV inhibition to histotoxic hypoxia and death, and sets alongside it the analytical sequence by which each step is evidenced: gastric

(a) Mechanistic cascade from exposure to death



(b) Corresponding evidentiary chain

No single measurement carries the whole evidentiary weight

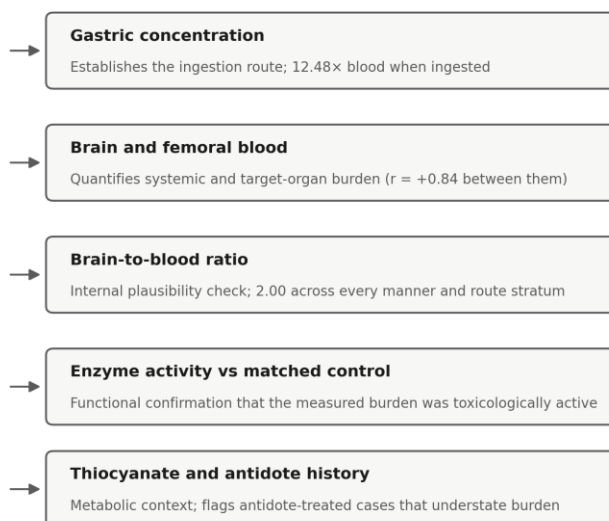


Figure 7. Integrated interpretive framework for fatal cyanide poisoning. (a) The mechanistic cascade from exposure through distribution and complex IV inhibition to death, annotated with the values observed in this series. (b) The corresponding evidentiary chain, in which each analytical measurement addresses one step of the cascade so that no single result bears the whole evidentiary weight.

4.8. Postmortem interval and analytical considerations

The inverse correlation between discovery-to-autopsy interval and blood concentration ($r = -0.32$, $p < 0.001$) is consistent with a problem that has long been recognised qualitatively. Postmortem redistribution moves analytes along concentration gradients after death in a compound-specific and site-specific manner,²⁵ and the general difficulty of interpreting postmortem toxicology without true reference ranges is well documented.²⁶ For cyanide specifically the problem is bidirectional: concentrations fall through volatilisation and chemical reaction, while putrefactive bacteria can generate cyanide de novo, so that a given value may be either an underestimate or an artefact depending on the interval. The analogous interpretive difficulty created by postmortem thiosulfate in hydrogen sulfide casework illustrates how readily a postmortem artefact can be mistaken for evidence of exposure.²⁷ Restricting the present series to intervals within 72 hours, with a mean of 21.4 hours, was a deliberate design choice that limits but does not abolish this effect; series with extended intervals and advanced decomposition remain substantially less interpretable.²⁸

Analytical choices were consequential. Headspace gas chromatography–mass spectrometry was selected because it captures the volatile analyte within a sealed system before loss can occur, and validated procedures exist for postmortem material.²⁰ Simultaneous determination alongside other volatile toxicants is achievable at routine throughput,²¹ and parallel thiocyanate measurement may supply metabolic context.²² Point-of-care colorimetric platforms are advancing rapidly and may eventually support scene triage, but do not presently meet the evidentiary standard required for court testimony.²³

4.9. Fire-related deaths and antidote exposure

The fire-related subgroup warrants separate comment. Cyanide is generated by combustion of nitrogen-containing polymers and contributes to mortality in closed-space fires, yet is frequently unmeasured when only carboxyhaemoglobin is assessed.⁶ Routine dual measurement of carboxyhaemoglobin and cyanide in every fire death is therefore recommended, and screening for nitrite and nitrate similarly guards against misattribution to an alternative asphyxiant.³³

Where a therapeutic interval existed, antidote administration may have complicated interpretation, because hydroxocobalamin binds cyanide to form cyanocobalamin and therefore alters the measured analyte.¹⁷ Forensic laboratories should therefore record antidote exposure explicitly, since a treated decedent may show a concentration that understates the original burden while retaining the enzymatic signature of severe poisoning.

4.10. Enzymatic and genetic molecular autopsy

Positioned within the broader molecular autopsy literature, the present approach differs instructively from its cardiac counterpart. In sudden cardiac death the molecular autopsy is genetic, identifying a predisposing variant whose relationship to the fatal event must then be inferred; systematic review has documented both its yield and its interpretive limitations,³⁴ national programmes have reported operational experience,³⁵ sequencing panels have extended its reach,³⁶ and reviews in the young have set out how a molecular finding must be reconciled with morphology. The enzymatic molecular autopsy described here measures the lesion itself rather than a predisposition, and is therefore closer to a direct functional

demonstration of mechanism. That distinction may strengthen its evidentiary value in court.

4.11. Limitations and future directions

Several limitations bound these conclusions. The study is retrospective and single-centre, so the manner-of-death distribution reflects the referral pattern of one Moroccan medicolegal institute and should not be read as a population incidence; epidemiological characterisation of fatal self-poisoning requires a different design.³⁹ Matched enzyme controls were drawn from non-poisoned autopsies of comparable interval but could not be matched on every relevant covariate, and agonal hypoxia of other causes may depress complex IV activity to a degree not fully excluded here. Exclusion of cases beyond 72 hours and of putrefied bodies improves internal validity at the cost of generalisability to the decomposed cases that forensic practice frequently encounters.²⁸ The peracute outcome definition relies partly on witness accounts of collapse timing, which are imprecise. Individual-level covariates such as chronic hepatic disease, which would be expected to reduce rhodanese capacity, were not available in sufficient detail to model. The comparisons across matrices and tissues used independent-groups analyses although the same decedents supplied every measurement, and manner of death and route were assigned partly from information that included toxicology, so the gradients across these strata are partly circular. The number of cases screened and excluded is not reported, there was no cyanide-negative comparison group for tissue values, the interval was measured from discovery of the body rather than from death, and thiocyanate and carboxyhaemoglobin results are not reported. Use of group means as reference values in casework requires external validation.

Future work should proceed along three lines. A prospective multi-centre series would establish whether the brain-to-blood ratio of approximately 2.00 observed here is stable across populations and sampling protocols, since a reproducible ratio would function as a quantitative plausibility check in routine casework. Systematic measurement of hepatic rhodanese activity alongside enzyme inhibition would test the detoxification hypothesis directly rather than inferentially. And controlled characterisation of enzyme inhibition across a range of postmortem intervals and storage conditions would define how long the enzymatic signature remains interpretable, which is the question that most constrains the method's application to the decomposed cases where conventional toxicology is least reliable. Fatal case documentation from unusual sources, such as potassium gold cyanide, continues to extend the range of circumstances against which any such framework must be tested.³⁷

5. Conclusion

This consecutive forensic series suggests that multi-organ thanatochemistry combined with enzymatic molecular autopsy may provide a more objective basis for interpreting fatal cyanide poisoning. Cyanide distributed in a highly ordered fashion across the six sampled matrices, with gastric contents marking the ingestion route and deep brain parenchyma carrying approximately twice the femoral blood concentration. Cytochrome c oxidase inhibition was severe and strongly tissue-dependent, reaching 88.5% in brain while sparing the liver at 45.6%, a gradient that is compatible with a protective role of endogenous sulfurtransferase capacity.

Brain cyanide concentration and cerebral enzyme inhibition were the strongest independent predictors of a

peracute lethal outcome after adjustment (femoral blood cyanide was also independently associated), while exposure route and discovery-to-autopsy interval were not. Concentration separated the manner-of-death strata clearly in aggregate but overlapped between suicide and homicide, which indicates that thanatochemistry constrains rather than determines the manner and must be integrated with scene and investigative evidence. Routine collection of a brain specimen alongside femoral blood, measurement of enzyme activity against matched controls, and explicit recording of antidote exposure and discovery-to-autopsy interval together constitute a practical protocol that may strengthen the evidentiary standing of cyanide casework.

Declarations

Use of generative artificial intelligence. None. The authors declare that no generative artificial intelligence tool was used in the conception, research, analysis, drafting, revision or preparation of this manuscript.

Author contributions. KM: Conceptualization, Methodology, Investigation, Formal analysis, Supervision, Writing – original draft. MY: Investigation, Data curation, Validation, Writing – review and editing. Both authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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Conflict of interest. None. The authors declare no competing financial, personal or professional interests.

Ethical approval. The study was approved by the institutional forensic review committee of the National School of Public Health, Rabat, in accordance with the Declaration of Helsinki (approval no. 2023-EC-205) All material derived from judicially mandated medicolegal autopsies, for which individual consent is not applicable; all data were de-identified before analysis.

Consent for publication. Not applicable. No individually identifiable material is reported.

Data availability. The de-identified analytical dataset is available from the corresponding author on reasonable academic request, subject to the authorisation of the relevant judicial authority.

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References

1. Giamogante F, Cali T, Malatesta F. Physiological cyanide concentrations do not stimulate mitochondrial cytochrome c oxidase activity. *Proc Natl Acad Sci U S A*. 2021;118(39):e2112373118. doi:10.1073/pnas.2112373118
2. Jaiswal A. Effects of Carbon Monoxide and Cyanide Poisoning On Human Health. *Public Health Open Access*. 2021;5(1). doi:10.23880/phoa-16000182
3. Bouchalta M, Belkaid B, Abouzaher H, et al. Fatal Cyanide Poisoning in A Toddler After Ingestion of Bitter Apricot Kernels: A Medico-Legal Case Report. *Moroccan Forensic Law J*. 2026;4(1):1–5. doi:10.63126/okzh9524
4. Hansen-Bruhn I, McKenna ST, Hull TR. Quantification of hydrogen cyanide in fire effluent. *J Fire Sci*. 2023;41(5):224–237. doi:10.1177/07349041231193756
5. Okudan M, Tufekci D, Ofluoglu F, et al. Examining the role of cyanide poisoning in the autopsies of closed-space fire deaths in Istanbul (Türkiye). *J Forensic Leg Med*. 2026;118:103071. doi:10.1016/j.jflm.2026.103071
6. Tabian D, Drochioiu G, Damian SI, et al. Toxic Blood Hydrogen Cyanide Concentration as a Vital Sign of a Deceased Room Fire Victim—Case Report. *Toxics*. 2021;9(2):36. doi:10.3390/toxics9020036
7. Baj J, Buszewicz G, Przygodzka D, et al. Diffusion of Carbon Monoxide and Hydrogen Cyanide to Muscles and Blood—An

- Experimental Study. *Toxics*. 2022;10(11):707. doi:10.3390/toxics10110707
8. Ramzan R, Kadenbach B, Vogt S. Multiple Mechanisms Regulate Eukaryotic Cytochrome C Oxidase. *Cells*. 2021;10(3):514. doi:10.3390/cells10030514
 9. Ramzan R, Dolga AM, Michels S, et al. Cytochrome c Oxidase Inhibition by ATP Decreases Mitochondrial ROS Production. *Cells*. 2022;11(6):992. doi:10.3390/cells11060992
 10. Nguyen MD, Sierra-Magro A, Singh V, et al. Structural basis for late maturation steps of mitochondrial respiratory chain complex IV within the human respirasome. *Nat Commun*. 2026;17(1):1550. doi:10.1038/s41467-025-68274-3
 11. Sierra-Magro A, Ortega R, Barrientos A. Final stages of mitochondrial respiratory chain complex IV assembly in physiological and oxidative stress conditions. *Free Radic Biol Med*. 2025;233:S52. doi:10.1016/j.freeradbiomed.2025.05.183
 12. Biswas S, Sengupta A, Das S, et al. Nitrosative stress affects mitochondrial respiratory chain complex II and complex IV assemblies in *Saccharomyces cerevisiae*: S-nitrosylation of complex II. *Biochim Biophys Acta Gen Subj*. 2025;1869(10):130845. doi:10.1016/j.bbagen.2025.130845
 13. Buonvino S, Arciero I, Melino S. Thiosulfate-Cyanide Sulfurtransferase a Mitochondrial Essential Enzyme: From Cell Metabolism to the Biotechnological Applications. *Int J Mol Sci*. 2022;23(15):8452. doi:10.3390/ijms23158452
 14. Lawal OT, Sanni DM. Physicochemical and thermodynamic properties of purified rhodanese from *A. welwitschiae* LOT1 and the cyanide detoxification potential of the enzyme. *World J Microbiol Biotechnol*. 2024;40(11):355. doi:10.1007/s11274-024-04164-y
 15. Alwan AM, Afshari JT. Investigating the Protective Role of Rhodanese Enzyme against Cyanide, the Cytotoxic by-product of Amygdalin, in HDF and L929 Cell Lines. *Lett Drug Des Discov*. 2023;20(9):1295–1307. doi:10.2174/1570180819666220610101055
 16. Dunne R, Goodloe JM, Augustine JJ, et al. Intravenous Hydroxocobalamin for Cyanide Poisoning From Smoke Inhalation: A Comprehensive Scoping Review. *J Am Coll Emerg Physicians Open*. 2026;7(2):100340. doi:10.1016/j.acepo.2026.100340
 17. Kamta J, Maynard K, Schult RF, et al. Evaluation of hydroxocobalamin use for the treatment of suspected cyanide toxicity secondary to smoke inhalation. *Burns*. 2024;50(1):157–166. doi:10.1016/j.burns.2023.08.020
 18. Stanton EW, Wang S, Han K, et al. Hydroxocobalamin is not associated with methemoglobinemia in patients with inhalation injury and suspected cyanide toxicity and a proposed algorithm for hydroxocobalamin administration. *Burns*. 2024;50(7):1746–1751. doi:10.1016/j.burns.2024.04.007
 19. Destanoğlu O, Ateş İ. Headspace gas chromatography-mass spectrometry method for the determination of total cyanide concentration in water and postmortem blood samples. *J Serb Chem Soc*. 2021;86(1):77–90. doi:10.2298/jsc200422063d
 20. Murakami T, Iwamuro Y, Sakamoto Y, et al. Rapid Simultaneous Determination of Cyanide, Azide, and Ethanol in Whole Blood Using Headspace Gas Chromatography-Mass Spectrometry. *Chromatographia*. 2023;86(11–12):701–706. doi:10.1007/s10337-023-04289-6
 21. Alluhayb AH, Severance C, Hendry-Hofer T, et al. Concurrent determination of cyanide and thiocyanate in human and swine antemortem and postmortem blood by high-performance liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem*. 2023;415(26):6595–6609. doi:10.1007/s00216-023-04939-6
 22. Sheini A, Aseman MD, Bordbar MM. Origami paper analytical assay based on metal complex sensor for rapid determination of blood cyanide concentration in fire survivors. *Sci Rep*. 2021;11(1):3521. doi:10.1038/s41598-021-83186-0
 23. Tabian D, Bulgaru Iliescu D, Diac MM, et al. Evaluation of Hydrogen Cyanide in the Blood of Fire Victims Based on the Kinetics of the Reaction with Ninhydrin. *Appl Sci*. 2022;12(5):2329. doi:10.3390/app12052329
 24. Abdelaal GMM, Hegazy NI, Eteawa RL, et al. Postmortem redistribution of drugs: a literature review. *Forensic Sci Med Pathol*. 2023;20(4):1483–1490. doi:10.1007/s12024-023-00709-z
 25. Stephenson L, Van Den Heuvel C, Scott T, et al. Difficulties associated with the interpretation of postmortem toxicology. *J Anal Toxicol*. 2024;48(6):405–412. doi:10.1093/jat/bkae052
 26. Suzuka M, Jin S, Takeuchi A, et al. Influence of blood thiosulfate produced by postmortem changes for the diagnosis of hydrogen sulfide poisoning in forensic autopsy. *Asian Biomed*. 2024;18(6):281–286. doi:10.2478/abm-2024-0035
 27. van der Hulst R, Borra LCP, Oostra RJ, et al. Postmortem toxicological investigations in cases with an extended postmortem interval and advanced decomposition. *Int J Legal Med*. 2026. doi:10.1007/s00414-026-03948-1
 28. Skov KØ, van der Meer M, Nedahl M, et al. Thinking beyond blood: Brain concentrations and brain-blood ratios in postmortem toxicology. *J Anal Toxicol*. 2026;bkag083. doi:10.1093/jat/bkag083
 29. Behnke G, Gray TR. Occipital lobe to femoral blood ratios of amphetamine, methamphetamine, cocaine, benzoylecgonine, cocaethylene, and fentanyl in postmortem cases. *J Anal Toxicol*. 2026;50(7):bkag068. doi:10.1093/jat/bkag068
 30. Mori K, Yoshida J, Morioka F, et al. Quantification of carbon monoxide (CO) in postmortem human brain tissues after CO poisoning. *Sci Rep*. 2025;15(1):32072. doi:10.1038/s41598-025-15661-x
 31. Velez AA, Phipps RA. Detection and quantification of carbon monoxide in postmortem liver tissues via headspace GC-MS as an alternative to blood. *J Anal Toxicol*. 2026;50(9):bkag076. doi:10.1093/jat/bkag076
 32. Kim M, Kim S, Yang W, et al. Determination of nitrite and nitrate in postmortem whole blood samples of 10 sodium nitrite poisoning cases: The importance of nitrate in determining nitrite poisoning. *Forensic Sci Int*. 2022;335:111279. doi:10.1016/j.forsciint.2022.111279
 33. Tomassini L, Ricchezza G, Fedeli P, et al. New Insights on Molecular Autopsy in Sudden Death: A Systematic Review. *Diagnostics*. 2024;14(11):1151. doi:10.3390/diagnostics14111151
 34. Yamamoto T, Emoto Y, Murase T, et al. Molecular autopsy for sudden death in Japan. *J Toxicol Pathol*. 2024;37(1):1–10. doi:10.1293/tox.2023-0080
 35. Fadoni J, Santos A, Amorim A, et al. Sudden Cardiac Death: The Role of Molecular Autopsy with Next-Generation Sequencing. *Diagnostics*. 2025;15(4):460. doi:10.3390/diagnostics15040460
 36. Seol I, Lee S, Kim H, et al. A fatal case of potassium gold cyanide poisoning. *J Forensic Sci*. 2024;70(1):387–391. doi:10.1111/1556-4029.15654
 37. Weisheng H, Shuquan Z, Weiwei Z, et al. Fatal poisoning due to aconite: Autopsy findings and postmortem quantitative analysis. *Forensic Sci Med Pathol*. 2023;20(3):999–1004. doi:10.1007/s12024-023-00728-w
 38. Ahmad F, Ali K. Para-suicide by Self-Poisoning: Profile of Toxic agents used in Aligarh District of India. *Indian J Forensic Med Toxicol*. 2024;18(3):66–72. doi:10.37506/xkg8c383
 39. Lupi Manso N, Ribeiro IP, Inácio AR. Sharp force fatalities: Differentiating homicide from suicide through a retrospective review (2012–2019) of autopsy findings in Lisbon (Portugal). *Forensic Sci Int*. 2021;327:110959. doi:10.1016/j.forsciint.2021.110959
 40. Salzillo C, Sansone V, Napolitano F. Sudden Cardiac Death in the Young: State-of-the-Art Review in Molecular Autopsy. *Curr Issues Mol Biol*. 2024;46(4):3313–3327. doi:10.3390/cimb46040207