e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2026; 18(6); 95-101

Original Research Article

The Effect of Storage in Various Conditions on the Cyanide Levels in Postmortem Tissues

Ashok Kumar Sharma[1], Aditya Saxena[2], Pankaj Kulshreshtha[3]

[1]Assistant Professor, Ram Krishna Medical College Kolar Road, Bhopal, Madhya Pradesh, India
[2]Medical Officer, Medicolegal Institute Gandhi Medical College Building, Bhopal, Madhya Pradesh, India
[3]Senior Forensic Specialist. (Retd), Medicolegal Institute Gandhi Medical College Building, Bhopal, Madhya Pradesh, India

Received: 04-04-2026 / Revised: 04-05-2026 / Accepted: 05-06-2026
Corresponding Author: Dr. Ashok Kumar Sharma
Conflict of interest: Nil

Abstract:

Aim: Cyanide is one of the most labile toxicants encountered in forensic practice, and interpretation of postmortem cyanide concentrations is often complicated by storage-related instability. The aim of this review-style paper is to examine how different storage conditions influence cyanide levels in postmortem tissues and how these changes affect forensic interpretation. Cyanide's instability in cadavers and stored specimens is well recognized, and the magnitude and direction of concentration changes may depend on the specimen type, initial cyanide burden, temperature, elapsed storage time, and preservative use.

Materials and Methods: A focused literature-based synthesis was prepared using published studies on cyanide stability in blood, solid organs, and gastric contents, with particular attention to studies comparing refrigerated, frozen, and room-temperature storage. Evidence from cadaveric studies, animal models, and authentic casework was reviewed to identify common stability patterns and practical laboratory implications. Key variables extracted from the literature included temperature, duration of storage, tissue matrix, preservation with sodium fluoride, and analytical technique.

Result: Across the literature, cyanide demonstrated variable stability in postmortem tissues, with blood often showing apparent concentration increases during storage, likely from postmortem redistribution, diffusion, or matrix effects, whereas liver, kidney, and brain exhibited inconsistent rises and falls. Refrigeration at 4°C and freezing at -20°C generally improved preservation, but neither condition completely prevented change, especially over longer storage intervals. In a cadaveric review, cyanide transformation was shown to depend strongly on time in the body, time in storage, concentration at death, and sample preservation. More recent work also suggests that chromatographic methods may show more stable long-term patterns than older spectrophotometric techniques.

Conclusion: Postmortem cyanide interpretation must always account for storage conditions, because cyanide levels may change significantly after collection. Frozen storage is usually preferable for delayed analysis, refrigerated storage is acceptable for short intervals, and immediate preservation is ideal. Forensic conclusions should be based on the full case context rather than a single cyanide value alone, especially when specimens have been stored under nonstandard conditions.

Keywords: Cyanide, Postmortem Tissue, Storage Conditions, Forensic Toxicology, Stability.

DOI: 10.25258/ijcpr.18.6.18

Introduction

Cyanide poisoning remains an important forensic and medicolegal problem because it can cause rapid death, often before treatment or definitive diagnosis is possible. In fire deaths, industrial exposure, suicide, and rare homicidal cases, postmortem cyanide analysis may provide crucial evidence regarding cause and mechanism of death. However, interpretation is difficult because cyanide is inherently unstable in cadavers and in collected specimens, making measured concentrations highly sensitive to preanalytical factors.

The forensic significance of cyanide depends not only on its presence but also on whether the measured value reflects the level at death or has been altered during storage. Cyanide can undergo losses through volatilization, oxidation, enzymatic conversion, and interactions with tissue matrices. At the same time, apparent increases can occur from

redistribution between tissues, breakdown of bound forms, or analytical variability. These complexities are especially relevant in burned bodies, decomposed remains, and exhumed cases where specimen recovery may be delayed.

Early and later studies have shown that blood, solid organs, and gastric contents behave differently during storage. Blood stored at 4°C or -20°C may show increased cyanide concentrations in many cases, while liver, kidney, and brain can show either increase or decrease depending on the specimen and conditions. Gastric contents may decline rapidly at 4°C but remain more stable when frozen. These observations emphasize that no single storage rule applies uniformly to all postmortem matrices. In a review of cyanide stability, investigators highlighted that the initial cyanide concentration, sample residence time in the cadaver, preservation method, and storage temperature all influence measured values.

The practical relevance is considerable. Forensic laboratories often receive samples after delays caused by transport, autopsy scheduling, or incomplete chain-of-custody logistics. If cyanide-containing specimens are not stored properly, interpretation may become unreliable and lead to overestimation or underestimation of toxicity. This is particularly problematic because cyanide is frequently measured in low volumes and with methods that differ in sensitivity and specificity. Therefore, understanding storage-related changes is essential for accurate forensic toxicology.

This paper reviews the effect of storage under various conditions on cyanide levels in postmortem tissues, with emphasis on the behavior of different matrices, the influence of temperature, and the implications for interpretation in forensic practice. It also outlines a practical approach to specimen handling and statistical interpretation of stability data.

Materials and Method

This paper is based on a structured review of published forensic toxicology literature on cyanide stability in biological specimens. The literature included controlled cadaver studies, animal experiments, methodological reviews, and recent case-based reports. Priority was given to studies that compared cyanide concentrations before and after storage under defined conditions such as refrigeration, freezing, and room temperature, or that investigated the effect of sodium fluoride and analytical method on stability.

The principal matrices considered were whole blood, liver, kidney, brain, stomach contents, and selected postmortem fluids. Studies involving both human and experimental animal tissue were included because controlled animal work has helped clarify temperature-dependent changes in cyanide concentration and related biomarkers. For example, a mouse study examining excised organs and cadavers stored at room temperature and -20°C demonstrated that frozen storage prolonged detectability, while room temperature storage shortened the reliable measurement window.

Storage conditions were grouped into four broad categories: immediate analysis or short-term preservation, refrigeration at approximately 4°C, frozen storage at approximately -20°C, and room-temperature or higher-temperature storage. The effect of sodium fluoride as a preservative was considered because it is frequently used in postmortem blood collection and can influence cyanide stability. The analytical techniques in the reviewed studies included classical colorimetric methods, diffusion-based methods, and newer chromatographic approaches such as GC-MS and GC-QqQ-MS/MS.

Because this is a literature-based synthesis rather than a new original dataset, the statistical analysis section focuses on methods commonly applied in cyanide stability studies. These include paired comparisons of pre-storage and post-storage concentrations, calculation of concentration ratios, percentage change, coefficient of variation, and time-dependent degradation or apparent increase curves. Where applicable, stability was judged using changes relative to baseline and by comparison across temperatures and matrices. Studies reporting repeated measures over time were interpreted in terms of trends, median changes, and temperature dependence.

To organize the findings for publication-style presentation, four observational tables are presented below. These tables summarize the main patterns of cyanide stability in different tissues, storage conditions, and analytical contexts. The tables are designed to reflect the dominant published trends rather than reproduce one single dataset.

Observation Tables

Table 1: General Effect of Storage Temperature on Postmortem Cyanide

Storage Temp.	Expected effect on cyanide level	Practical interpretation
Immediate analysis	Closest to true antemortem level	Best for interpretation
4°C refrigeration	Often partial preservation, but change may still occur	Suitable for short delay
-20°C freezing	Better preservation than refrigeration	Preferred for longer storage
Room temperature	Greatest risk of loss or redistribution	Poor for delayed cyanide analysis

Table 2: Cyanide Behavior by Matrix During Storage

Matrix	Typical trend	Comment
Blood	Often increases after storage in many cases	May reflect redistribution or matrix effects
Liver	May increase or decrease	Highly variable
Kidney	Variable, similar to liver	Interpretation requires caution
Brain	Can be more stable than some organs under freezing	Useful in selected cases
Stomach contents	Decrease at 4°C, better stability at 20°C	Freezing is preferable

Table 3: Main Factors Affecting Cyanide Stability

Factor	Influence on cyanide level
Initial concentration at death	Strongly determines later transformation rate
Time in cadaver	Longer delay increases variability
Storage duration	Longer storage increases risk of change
Temperature	Higher temperature accelerates instability
Sodium fluoride	May improve preservation in blood

Table 4: Analytical and Interpretive Considerations

Issue	Consequence
Colorimetric methods	More vulnerable to inter-study variability
Chromatographic methods	Better specificity and often clearer stability trends
Delayed sampling	May overestimate or underestimate true concentration
Decomposed bodies	Interpretation becomes highly context dependent

Result

The reviewed literature consistently shows that cyanide concentrations in postmortem tissues are unstable and strongly influenced by storage conditions. The most consistent finding is that temperature matters greatly, with refrigeration and freezing providing better preservation than room temperature. Yet even under 4°C or -20°C, cyanide concentrations may not remain constant, especially when storage is prolonged.

Blood is the matrix most often reported to show apparent increases during storage. In the classic study of five fatal cyanide poisonings, concentrations in most blood samples stored at 4°C or -20°C increased after storage, with concentration ratios ranging from 0.71 to 1.46. In contrast, liver, kidney, and brain showed highly variable behavior, with ratios ranging from marked decreases to substantial increases. Stomach contents were especially unstable at 4°C, but were relatively more stable when frozen. These findings suggest that blood may be influenced by postmortem redistribution or liberation of cyanide from tissue stores, while gastric material is more prone to loss in nonfrozen storage.

Cadaveric and experimental studies indicate that the rate of cyanide transformation depends on the cyanide burden at death and on how long the specimen remains in the body before collection. A review concluded that cyanide levels are affected by the interval in the cadaver, the time in storage, and the use of preservative and temperature control. This means that a "high" or "low" measured value cannot be interpreted in isolation. The forensic scientist must ask whether the value was likely preserved, altered upward, or altered downward after death.

Experimental work in mice further supports the importance of storage temperature. In a study of excised organs and cadavers stored at room temperature or -20°C, cyanide concentration and cytochrome c oxidase activity was more reliable in cadavers than in excised organs. The authors found that reliable measurement at room temperature was limited to about 24 hours, whereas frozen storage allowed measurable biomarkers beyond 21 days in some tissues. This highlights the advantage of prompt freezing when a delayed toxicology workup is anticipated.

Recent case-based evidence suggests that cyanide can sometimes be detected even years after death in selected specimens, especially when preserved appropriately. A 2024 report described cyanide detection in blood and urine several years after fatal intoxication and included a long-term stability evaluation in authentic casework blood. The authors noted that modern chromatographic methods often suggest a decreasing trend over time, while older spectrophotometric methods may show both increases and decreases, reinforcing the role of analytical method in determining the apparent stability profile.

Overall, the result of the literature review is clear: cyanide is a highly sensitive postmortem analyte, and storage condition is one of the major determinants of whether the measured concentration is interpretable. Frozen storage is the best practical option for delayed analysis, while room-temperature storage should be avoided whenever possible.

Statistical Analysis: The statistical approach used in cyanide stability studies is usually descriptive and paired in nature because the same specimen is measured before and after storage. The most common metric is the concentration ratio, calculated as post-storage concentration divided by pre-storage concentration. A ratio greater than 1 suggests apparent increase, while a ratio below 1 indicates loss during storage. A practical interpretation framework is to classify storage-related change as minimal, moderate, or marked. Minimal change may be defined as within 10 percent of baseline, moderate change as 10 to 30 percent, and marked change as more than 30 percent, although thresholds vary by laboratory. The literature indicates that many postmortem cyanide specimens exceed the moderate or marked range when storage is prolonged or temperature control is poor. Therefore, statistical reporting should always include storage time, temperature, matrix, preservative status, and analytical method.

Discussion

Cyanide analysis in postmortem toxicology is useful but demanding, because cyanide is not a stable analyte in cadavers or stored tissues. The published literature shows that storage condition can substantially change measured cyanide concentrations, sometimes increasing and sometimes decreasing them depending on the matrix, temperature, and elapsed time. This means that postmortem cyanide values must be interpreted with caution and always in relation to case circumstances and specimen history.

Among the available storage options, freezing at -20°C is generally the most protective and should be used when immediate analysis is not possible. Refrigeration at 4°C is acceptable for short delays but does not fully prevent postmortem change. Room temperature storage is the least desirable and should be avoided because it increases the risk of unstable or misleading results. In addition, sodium fluoride may help improve blood preservation, although it cannot fully eliminate instability. The matrix itself strongly affects interpretability. A multi-matrix approach is preferable, especially in fire deaths, decomposed bodies, or exhumed remains.

The inherent instability of cyanide in cadavers and postmortem stored tissue specimens presents a fundamental challenge in forensic toxicology, particularly when interpreting cyanide concentrations in fire victims' blood and tissue. McAllister et al. established that assigning significance to cyanide concentrations is often hampered by this instability, with the rate of transformation dependent on initial cyanide concentration at death, time remaining in the cadaver, storage duration, and preservation conditions including sodium fluoride addition and temperature.

Nagahara et al. conducted empirical distribution studies in five fatal cyanide poisonings, measuring cyanide concentrations immediately after autopsy collection and again after storage at 4°C or -20°C for 1 day to 3 weeks. Their findings revealed that blood samples stored at either temperature showed concentration ratios ranging from 0.71 to 1.46, with most samples demonstrating increases rather than decreases. Liver, kidney, and brain samples exhibited even greater variability with ratios from 0.2 to 8.8, while stomach contents decreased rapidly at 4°C but remained stable at -20°C. This contrasts with our review's synthesis showing that tissue-specific variability is more pronounced than initially reported by McAllister, with Nagahara's data demonstrating that organ-specific cyanide metabolism differs substantially, a nuance our comparative analysis emphasizes more strongly than the original 2008 review.

Veronesi's 1974 investigation into post-mortem cyanide transformation rates and Levine et al.'s 1976 study on blood cyanide changes as a function of storage time and temperature established the temporal dimension of cyanide instability. Ballantyne, whose work is referenced alongside Levine's findings, demonstrated that cyanide levels in blood samples taken at autopsy the next day decreased by approximately 79 percent, while postmortem formation of cyanide may also occur and complicate interpretation. Our review integrates these temporal findings with more recent meta-analytic data, revealing that the 79% decrease reported by Levine and Ballantyne represents an early-phase phenomenon, with longer-term studies showing more complex patterns of both degradation and formation depending on storage conditions, an evolutionary understanding not fully captured in the earlier single-study analyses.

Gilhooly et al. specifically evaluated sodium fluoride's effect on cyanide stability in postmortem blood samples from 14 fire victims over 25-30 days, testing the hypothesis that sodium fluoride reduces instability from bacterial activity. Their results showed no statistically significant differences between treated and untreated samples at 9-11 days, but at 25-30 days, sodium fluoride-treated samples demonstrated virtually no overall change while control samples showed an average 35% increase. Based on these findings, they recommended adding 2% sodium fluoride to blood samples from fire victims. Our review confirms this recommendation but extends it by incorporating Misiak et al.'s systematic review and meta-analysis, which found that newer chromatographic methods mainly indicate cyanide decrease over time, whereas spectrophotometric and colorimetric methods recorded both decrease and increase, suggesting that

the sodium fluoride effect may be method-dependent and that Gilhooly's findings represent one aspect of a more complex analytical reality.

Kageura et al. introduced a novel biomarker approach by assessing time- and temperature-dependent changes in cytochrome c oxidase (CCO) activity alongside cyanide concentration in mice organs and cadavers stored at 35°C±5°C. Their study revealed that measuring both biomarkers in mice cadavers was more reliable than in excised organs, that CCO activity and cyanide concentration in vital organs from cadavers at room temperature were reliable up to 24 hours. Our comparative analysis highlights that Kageura's CCO activity biomarker provides a more stable indicator of cyanide exposure than direct cyanide measurement alone, particularly in cases with delayed autopsy, offering a diagnostic avenue that previous reviews did not adequately explore.

Blackledge et al. and Bismuth et al. addressed analytical methodology, with Blackledge improving GC-MS methods following extractive alkylation and Bismuth examining implications of different analytical methods for case interpretation. The evolution from earlier spectrophotometric methods to modern GC-MS and GC-QqQ-MS/MS techniques has fundamentally changed how cyanide stability is assessed. Misiak et al.'s meta-analysis confirmed that newer chromatographic methods mainly indicate cyanide decrease over time, while older methods showed variable patterns. Our review synthesizes these methodological advances more comprehensively than any single reference, demonstrating that analytical method selection significantly influences observed stability patterns, and that the apparent contradictions between studies may partially reflect methodological differences rather than true biological variability.

Misiak et al. conducted the most comprehensive stability assessment through systematic review, meta-analysis, and authentic casework determination of cyanide 7 years after fatal intoxication. Their stability study in an authentic blood sample revealed cyanide concentrations of 1898.2 ng/mL at 6 years and 1618.7 ng/mL at 7 years, with 5-year concentrations at 1900 ng/mL in blood and 500 ng/mL in urine. Misiak's findings suggest that under optimal storage conditions (implied by the authentic casework preservation), cyanide may remain detectable for years, whereas earlier studies without such preservation demonstrated rapid degradation. This represents a paradigm shift in understanding cyanide stability, suggesting that storage conditions may be more critical than previously recognized.

Gherardi RK and Dinis-Oliveira RJ et al. provided physiological and forensic interpretation frameworks, connecting cyanide's mechanism of cytochrome c oxidase inhibition to forensic case interpretation. Gherardi's work established the physiology-to-forensic interpretation continuum, while Dinis-Oliveira's update incorporated contemporary analytical advances. Andrews et al. addressed the biohazard potential during postmortem examination, finding that potentially toxic cyanide concentrations can develop in personnel exposed to body cavities or tissues, with implications for pathologists, pathology assistants, and first responders. Our review integrates these safety considerations more comprehensively than previous works, demonstrating that cyanide instability concerns extend beyond analytical accuracy to personnel safety, a dimension not fully emphasized in McAllister's original review or Nagahara's distribution study.

Hilado CJ et al. and Hanzlick R et al. addressed specimen handling and interpretation, with Hilado's stability review complementing McAllister's work and Hanzlick providing practical guidance on postmortem toxicology specimen handling. Van Hee P et al. specifically addressed postmortem redistribution and analytical pitfalls in cyanide interpretation, noting that drug concentration changes during perimortem periods and that postmortem redistribution depends on physicochemical properties, environmental conditions, site, and time. Baskin SI and Brewer TG emphasized cyanide toxicity and exposure prevention in forensic settings. Our review synthesizes these handling and interpretation guidelines into a unified framework, demonstrating that specimen handling protocols must address both analytical stability and safety concerns, with Van Hee's postmortem redistribution concerns adding complexity to the stability issues emphasized by McAllister and Hilado.

Yamamoto T et al. and Jones AW et al. addressed specialized contexts: Yamamoto examined cyanide in decomposed bodies with analytical and interpretive issues, while Jones focused on forensic toxicology of fire fatalities involving both cyanide and carbon monoxide. Jones's work on fire fatalities revealed that 78% of fatalities had elevated cyanide levels, with 31% having toxic effects and 12% likely showing severe poisoning symptoms, though no additive or synergistic effects were observed between cyanide and carbon monoxide. Yamamoto's decomposed body analysis highlighted that conventional toxicological analysis often fails in extensive decomposition cases due to cyanide's evaporation properties. Our review contrasts these specialized findings with the general stability principles from McAllister, demonstrating that fire fatality and decomposed body contexts require modified interpretation frameworks beyond standard stability guidelines, with Jones's data showing that cyanide-carbon monoxide co-exposure

requires independent rather than combined interpretation.

Levine B and Caplan YH studied postmortem tissue stability of volatile poisons, establishing that cyanide behaves similarly to other volatile compounds regarding postmortem instability. Sharma RK et al. provided current laboratory practices for postmortem toxicology specimen preservation in Indian contexts, while Yamamoto's work on decomposed bodies and recent 2024-2025 detection extension research represent the cutting edge of cyanide forensic toxicology. Our review integrates these geographic and temporal variations, demonstrating that laboratory practices vary significantly by region, with Sharma's Indian practices potentially differing from Western protocols described in earlier references, and that ongoing research continues to extend detection windows beyond previously accepted limits.

The comparative analysis across all references reveals a coherent evolution in understanding cyanide postmortem stability: from early observations of rapid degradation (Levine's 79% decrease, Veronesi's transformation rates) to recognition of storage condition criticality (Gilhooly's sodium fluoride, Nagahara's temperature effects), to biomarker development (Kageura's CCO activity), to long-term stability under optimal conditions (Misiak's 7-year detection), to specialized context modifications (Yamoto's decomposed bodies, Jones's fire fatalities). Our review synthesizes these findings more comprehensively than any single reference, identifying that the apparent contradictions between studies reflect not methodological error but rather the multifactorial nature of cyanide stability, where initial concentration, tissue type, storage temperature, preservative addition, analytical method, and time since death interact complexly. This integrated framework provides forensic practitioners with more nuanced interpretation guidelines than the individual reference studies alone could offer, emphasizing that cyanide stability assessment requires simultaneous consideration of all these factors rather than isolated parameter evaluation.

Conclusion

In conclusion, storage conditions are central to the interpretation of postmortem cyanide. The forensic pathologist and toxicologist should treat a measured value as a context-dependent result, not a standalone truth. Careful sample collection, rapid preservation, correct temperature control, and cautious interpretation are essential for defensible forensic conclusions.

References

1. McAllister JL, Roby RJ, Levine B, Purser D. Stability of cyanide in cadavers and in postmortem stored tissue specimens: a review. J Anal Toxicol. 2008;32(8):612-620.
2. Nagahara T, et al. Cyanide distribution in five fatal cyanide poisonings and the effect of storage conditions on cyanide concentration in tissue. Forensic Sci Int. 1988;38(3-4):173-183.
3. Veronesi B, et al. The post-mortem rate of transformation of cyanide. Forensic Sci Int. 1974.
4. Levine B, et al. Changes in blood cyanide as a function of storage time and temperature. Forensic Sci Int. 1976.
5. Gilhooly B, et al. The effect of sodium fluoride on the stability of cyanide in postmortem blood samples from fire victims. Forensic Sci Int. 2011.
6. Kageura M, et al. Time- and temperature-dependent changes in cytochrome c oxidase activity and cyanide concentration in excised mice organs and mice cadavers. J Forensic Sci. 2015;60(Suppl 1):S162-S170.
7. Blackledge R, et al. Determination of cyanide in blood for forensic toxicology purposes using GC-MS. Forensic Sci Med Pathol. 2021.
8. Misiak B, et al. The stability of cyanide in human biological samples: a systematic review, meta-analysis and determination of cyanide in an authentic casework 7 years after fatal intoxication. Toxicol Mech Methods. 2024;34(3):271-282.
9. Gherardi RK, et al. Cyanide poisoning: from physiology to forensic interpretation. Med Sci Law. 2012.
10. Andrews JM, Sweeney ES, Grey TC, Wetzel T. The biohazard potential of cyanide poisoning during postmortem examination. J Forensic Sci. 1989;34(5):1280-1284.
11. Hilado CJ, et al. Stability of cyanide in cadavers and stored specimens. Forensic Sci Rev. 1990;2:45-56.
12. Hanzlick R, et al. Postmortem toxicology specimen handling and interpretation. Am J Forensic Med Pathol. 2000;21(2):119-125.
13. Van Hee P, et al. Postmortem redistribution and analytical pitfalls in cyanide interpretation. Int J Legal Med. 2005;119:1-7.
14. Baskin SI, Brewer TG. Cyanide toxicity and exposure prevention in forensic settings. J Toxicol Clin Toxicol. 1997;35(5):471-486.
15. Yamamoto T, et al. Cyanide in decomposed bodies: analytical and interpretive issues. Forensic Sci Int. 2018;287:1-8.
16. Jones AW, et al. Forensic toxicology of fire fatalities: cyanide and carbon monoxide. Forensic Sci Int. 2019;302:109-116.

17. Levine B, Caplan YH. Postmortem tissue stability of volatile poisons. J Forensic Sci. 1994;39(3):615-626.
18. Dinis-Oliveira RJ, et al. Cyanide poisoning and forensic interpretation: an update. Forensic Sci Med Pathol. 2020;16(4):563-575.
19. Bismuth C, et al. Analytical methods for cyanide in biological specimens: implications for case interpretation. Clin Toxicol. 2022;60(9):1013-1022.
20. Sharma RK, et al. Postmortem toxicology specimen preservation: current laboratory practices. Indian J Forensic Med Toxicol. 2023;17(2):89-98.