

PAPER

CRIMINALISTICS

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Stable Carbon and Nitrogen Isotope Ratios of Sodium and Potassium Cyanide as a Forensic Signature

ABSTRACT: Sodium and potassium cyanide are highly toxic, produced in large amounts by the chemical industry, and linked to numerous high-profile crimes. The U.S. Centers for Disease Control and Prevention has identified cyanide as one of the most probable agents to be used in a chemical terrorism event. We investigated whether stable C and N isotopic content of sodium and potassium cyanide could serve as a forensic signature for sample matching, using a collection of 65 cyanide samples. Upon analysis, a few of the cyanide samples displayed nonhomogeneous isotopic content associated with degradation to a carbonate salt and loss of hydrogen cyanide. Most samples had highly reproducible isotope content. Of the 65 cyanide samples, >95% could be properly matched based on C and N isotope ratios, with a false match rate <3%. These results suggest that stable C and N isotope ratios are a useful forensic signature for matching cyanide samples.

KEYWORDS: forensic science, potassium cyanide, sodium cyanide, isotope ratio mass spectrometry, sample matching

Cyanide salts have properties that make them attractive to criminals or potential terrorists. They are highly toxic and release toxic hydrogen cyanide gas when mixed with dilute acid. They are relatively stable solids and therefore easily stored and transported. Cyanide salts have been used in a number of high-profile product-tampering crimes such as the Tylenol[®] poisonings that killed seven people in Chicago in 1982 and the Excedrin[®] poisonings that killed two in the state of Washington in 1986, as well as in individual poisoning cases (1). Cyanide has been used as an instrument of mass murder, most infamously as hydrogen cyanide (in the form of Zyklon B pellets) in the gas chambers of the Nazi regime during World War II, and more recently in the more than 900 deaths among the followers of Reverend Jim Jones caused by drinking cyanide-laced Kool-Aid[®] in 1978 (1). Cyanide is therefore a well-known and highly publicized poison.

The toxicity of cyanide is a consequence of its high affinity for many transition metals (2). It exerts its lethal effect by binding to iron within the enzyme cytochrome *c* oxidase, a component of the electron transport chain required for aerobic respiration in mammalian cells. In so doing, the cyanide anion inhibits the enzyme, preventing transport of electrons to oxygen and the generation of adenosine triphosphate for energy, blocking aerobic respiration (3). The potency of the cyanide ion as a metal ligand makes it a key

tool in gold and silver mining, its primary industrial application. In the so-called cyanide process, ores are exposed to a solution of sodium cyanide, and the cyanide ion complexes the precious metals, forming soluble derivatives and enabling extraction of the metals (4). Cyanide salts are also used in electroplating and other various industries and in scientific laboratories.

Cyanide is produced in large amounts by the chemical industry (4) and is therefore potentially available to criminals or terrorists. Cases have been reported involving the theft of significant amounts of cyanide salts or the discovery of caches of such material (1). The Centers for Disease Control and Prevention includes cyanide as one of the most probable agents of chemical terrorism (5). Forensic methods are therefore needed for sample matching and tracing of these toxic industrial chemicals.

Stable isotopic content is of proven utility as a signature for sample matching and has been applied to various sample types for this and other forensic applications (6–8). Substances synthesized from isotopically distinct starting materials (such as natural gas or petroleum from different reservoirs) or by different processes acquire different stable isotopic content despite chemical identity. Sodium cyanide and potassium cyanide are synthesized by neutralization of hydrogen cyanide with either sodium or potassium hydroxide. Six processes for synthesizing hydrogen cyanide are presently of economic significance. Each of these uses ammonia as the source of nitrogen, but the carbon source varies (9). The most widely used manufacturing method is the Andrussov process, which uses methane as the source of carbon. The BMA (DeGussa) process also uses methane, while the Fluohmic process typically uses propane or butane. Hydrogen cyanide can also be produced by the less common formamide, acrylonitrile, or methanol processes. In these cases, the sources of carbon are, respectively,

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carbon monoxide and methanol, propylene, and methanol (4,9). The carbon stable isotope ratios of methane are known to vary (see for example [10]), and given the other potential sources of carbon atoms in cyanide salts, significant variation in carbon stable isotope ratios of cyanide is expected, with less variation in stable nitrogen isotope ratios.

A method for C and N isotope ratio analysis of cyanide was published by C. Johnson in 1996 (10). In a subsequent publication, he and co-workers analyzed the fate of process solution cyanide in three mining operations using C and N isotope ratios (11). These two papers reported variations in cyanide carbon isotope ratios and detectable variations in nitrogen isotope ratios in a small number of samples.

The development of isotopic analysis of NaCN and KCN as a forensic tool requires a survey of isotope ratios of a large number of commercially available samples to determine whether sufficient variation exists to permit use of isotope ratios as an effective sample matching tool. If so, it would also be desirable to develop working cyanide reference standards. Here, we present a survey of the C and N stable isotope content of 65 samples of commercially purchased sodium or potassium cyanide and the calibration of three potassium cyanide working standards. Analyses were carried out at Oak Ridge National Laboratory (ORNL) and Pacific Northwest National Laboratory (PNNL).

Materials and Methods

Safety Considerations

Both sodium and potassium cyanide are highly toxic. The purchased samples were all granular in nature and were safely handled with standard laboratory precautions. The stable isotope analyses combusted samples to CO₂ and N₂ and therefore left no toxic residue.

Isotope Ratio Analysis

At PNNL, C and N isotope ratios of survey samples were analyzed using a Costech Analytical Technologies (Valencia, California) model 4010 Elemental Analyzer (EA) equipped with a zero-blank autosampler coupled to a Thermo-Finnigan (Bremen, Germany) Delta V Plus isotope ratio mass spectrometer. For C analysis 1300–1400 µg and for N analysis 300–400 µg NaCN or KCN were weighed into a tin capsule (Costech Analytical Technologies) and introduced into the instrument via the autosampler. At ORNL, survey samples were analyzed using a Costech model 4010 Elemental Analyzer (EA) coupled to a Thermo-Finnigan MAT252 isotope ratio mass spectrometer. At both laboratories, analytical sample sets included United States Geological Survey (USGS) 40 and USGS 41 glutamic acid isotopic standards, and data were corrected to these standards using the point-slope method (12).

Stable isotope content is measured as a ratio, R (¹³C/¹²C or ¹⁵N/¹⁴N), and reported as a delta (δ) value where $\delta = (R_A/R_{Std}-1) * 1000\text{‰}$, and R_A and R_{Std} are the isotope ratios of the sample and an internationally recognized standard, respectively. The standard for C isotope ratio analysis is Vienna PeeDee Belemnite and for N is air. Each sample was analyzed a minimum of three times. If the standard deviation of the measurements was <0.2 ‰, the value was accepted. We found that our samples could be grouped into one large set and one small one. The overwhelming majority of the samples yielded highly reproducible measurements with standard deviations meeting the acceptance criteria. A few of the samples, however, yielded measurements of poor reproducibility, and

application of Dixon's Q test (13) to determine whether any values could be rejected showed that the lack of reproducibility was not because of a single outlier value. These samples also showed a lower-than-stoichiometric yield of nitrogen for a cyanide salt, and analysis of one of them showed it had undergone partial degradation to sodium carbonate. We concluded that these samples were inherently heterogeneous.

Results

Samples

We acquired samples of KCN and NaCN either directly from vendors or from existing supplies at PNNL, ORNL, or Lawrence Livermore National Laboratory. In all, we collected 40 samples of KCN and 25 of NaCN. The samples originated from 14 different chemical suppliers. The purity of each of the samples obtained varied from 96.0% to 99.9%.

Inter-laboratory Consistency

To test whether the isotope ratio measurements made at PNNL and ORNL were directly comparable, the laboratories exchanged aliquots of their internal working glutamic acid standards (three from PNNL and one from ORNL). The C isotope ratios of these working glutamic acid standards spanned a range of approximately 50 ‰, and the N isotope ratios, approximately 150 ‰. The laboratories measured the C and N isotope ratios of these glutamic acids using USGS40 and USGS 41 as measurement standards. For every working glutamic acid standard, values from the two laboratories for both C and N matched within one standard deviation.

Survey of Cyanide Stable Isotope Ratios

The C and N isotope ratios of 25 samples of NaCN and 40 samples of KCN are presented in Fig. 1. The measured C isotope ratios spanned a range of approximately 20 ‰, while the measured N isotope ratios displayed a range of approximately 7.5 ‰. Most samples yielded highly reproducible isotope ratios, but a few did not, suggesting heterogeneity in those particular samples. These samples also showed a lower-than-expected N content. Sodium cyanide degrades to sodium carbonate in the presence of CO₂ and moisture via the following displacement reaction (14):



One of the low-N, isotopically heterogeneous samples was characterized by X-ray diffraction and found to be predominantly NaCO₃, explaining both its reduced N content and variable isotope measurements.

Calibration of Working Reference Materials

International isotope reference standards are in limited supply. Consequently, laboratories develop working reference materials, calibrated to international standards, for everyday use. Best practice is for working standards to have the same chemical composition as the samples to be analyzed. Therefore, following an initial survey of the C and N isotope ratios of purchased cyanide samples (which were thus available in large quantities), we selected three KCN samples as potential working standards, designated CN1, CN5, and CN6. CN1 and 6 represented the widest range of N isotope ratios among the purchased samples, while CN5 and 6 spanned most of the C isotope ratio range.

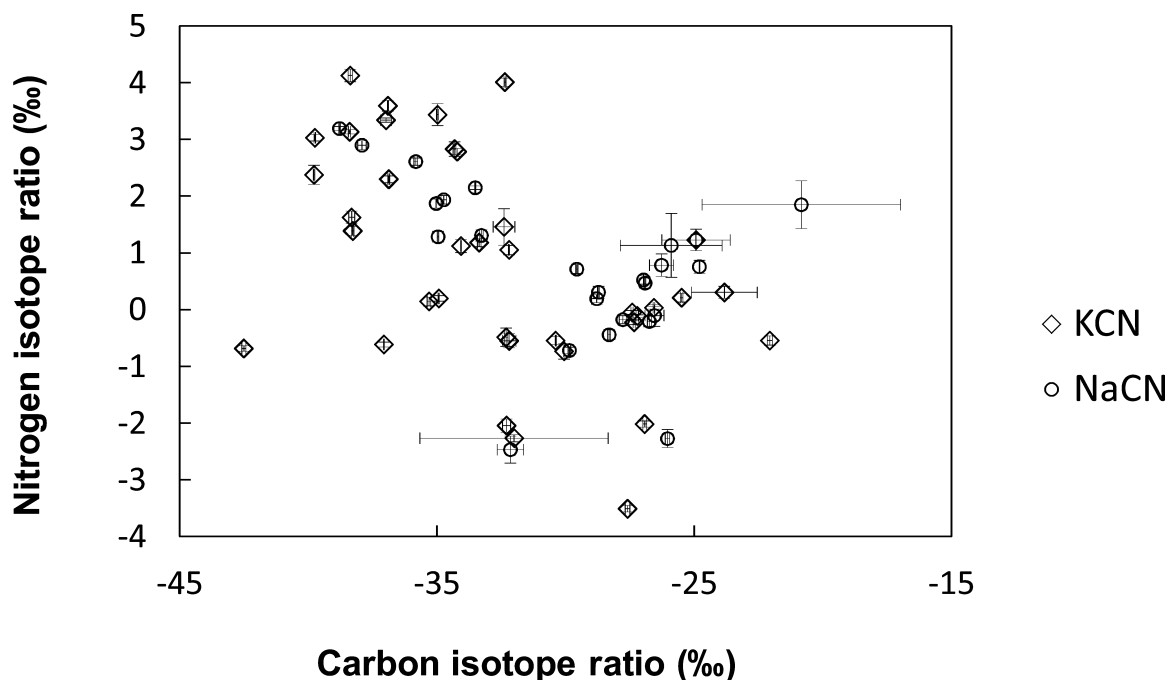


FIG. 1—C and N isotope ratios of sodium and potassium cyanide samples. Error bars represent one standard deviation of repeated measurements.

The isotopic content of the working standards was measured in multiple sample runs spanning several weeks at PNNL. The mean values of these analyses had a precision of ≤ 0.2 ‰, the nominal instrument precision. A statistical prediction interval was then calculated for the C and N isotope ratios of each working standard (15). A prediction interval is an estimate of an interval in which future observations will fall, with a certain probability, given what has already been observed. Portions of the three working standards were sent to ORNL, where each standard was analyzed in quadruplicate. The ORNL measurements were compared to the prediction intervals for the samples. Every C and N measurement for each of the three candidate standards fell into the prediction intervals generated from the PNNL data. The ORNL and PNNL measurements for each sample were then pooled, any values greater than 2σ from the mean were discarded from each data set (16), and an average and standard deviation were calculated for each candidate standard. No values measured at either PNNL or ORNL were rejected for either CN1 or CN5 (all measurements fell within 2σ of the mean), and one measurement of the C isotope ratio and one of the N isotope ratio were rejected from the data used to calculate the average values for CN6. The average values and standard deviations of the working standards are presented in Table 1.

Sample Matching Using Stable Isotope Ratios

The data were used to explore the potential of isotope ratios as a signature for sample matching. For this exercise, samples determined to be heterogeneous as a result of inconsistent isotope ratios and low nitrogen yield were discarded from the database. To evaluate the performance of the sample matching algorithm, all C and N values from a given sample were paired with each other to represent possible sets of C and N values from that sample. A pair of C, N values was selected, and an interval about those C and N values was created by adding and subtracting multiples of the root mean squared error (RMSE, the pooled standard deviation) derived from fitting the mixed effects model

TABLE 1—Carbon and nitrogen stable isotope ratios of potassium cyanide working standards.

	$\delta^{15}\text{N}$, ‰			$\delta^{13}\text{C}$, ‰		
	Average	SD	<i>n</i>	Average	SD	<i>n</i>
KCN1	3.00	0.09	17	-33.95	0.10	9
KCN5	0.06	0.16	13	-42.68	0.19	17
KCN6	-0.93	0.11	18	-29.07	0.15	19

$Y_{ijkl} = \beta_0 + \beta_1 * \text{lab}_i + \beta_2 * \text{cation}_j + \beta_3 * \text{id}_{k(i,j)} + \epsilon_{ijkl}$, where $i = 1, 2$ (PNNL, ORNL); $j = 1, 2$ (Na, K); $k = 1:49$, the number of CN samples; and $l = 1:n_k$, the number of IR values for each sample. The RMSE was estimated using the C and N data from which all values from the sample had been excluded, so as not to bias the estimate. Lab and cation were modeled as fixed effects, and id as a random effect. If another set of values from the sample was simultaneously within the C and N interval, then a match was declared, otherwise a nonmatch. In a like manner, each set of C and N values was tested against all other sets from all of the other samples. Sensitivity (true positive rate) is defined as $\# \text{matches} / (\# \text{matches} + \# \text{nonmatches})$ for the situation where samples should match (sets from the same source), and specificity (true negative rate) is defined as $\# \text{nonmatches} / (\# \text{matches} + \# \text{nonmatches})$ for the situation where samples should not match (sets from a different source). Table 2 shows the sensitivity and specificity for each multiple of the RMSE. Figure 2 is a plot of these data, where the y-axis is sensitivity and the x-axis is 1-specificity. On these scales, a value of (0,1) represents perfection in identifying matches and nonmatches. For multiples of 4, 5, and 6 * RMSE, both sensitivity and specificity were better than 0.95. Two of the samples in our collection were from the same manufacturer and lot number (although we obtained them from separate donors), and all of the pairs of C, N values from both of these samples matched one another within the four RMSE interval.

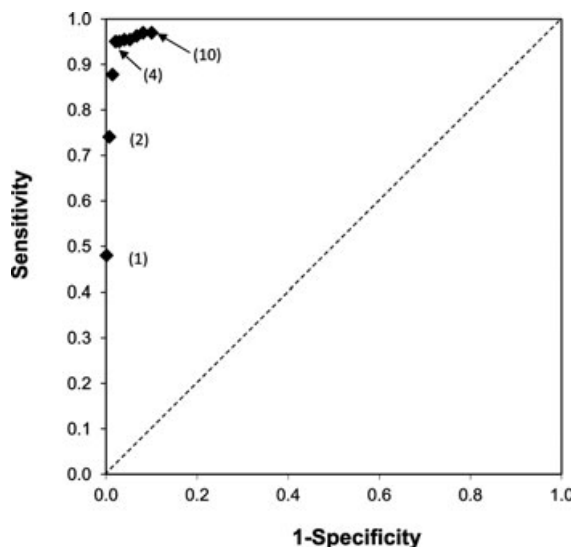


FIG. 2—Sensitivity versus specificity of CN sample matching using the indicated multiples of root mean squared errors (number in parentheses) around both C and N isotope ratios as the match interval. The dotted diagonal line denotes an equal proportion of true and false matches.

TABLE 2—Proportion of true pairwise matches (sensitivity) and nonmatches (specificity) obtained using increasing multiples of the root mean square error (RMSE) for $\delta^{13}\text{C}$ measurements and $\delta^{15}\text{N}$ measurements to define matching intervals.

RMSE Multiple	Sensitivity	Specificity
1	0.480	0.999
2	0.741	0.993
3	0.878	0.986
4	0.951	0.979
5	0.951	0.971
6	0.955	0.960
7	0.955	0.948
8	0.963	0.933
9	0.970	0.919
10	0.970	0.900

Discussion

The data presented here establish that significant variation in both the C and N stable isotope ratios among samples of commercially available cyanides exists, while the average within-sample variability is small ($\text{RMSE}_\text{C} = 0.198$; $\text{RMSE}_\text{N} = 0.095$). We obtained a true match rate of >95% with a simultaneous false match rate of <3% in a matching exercise using the measured C and N stable isotope ratios of our collection of NaCN and KCN samples. These results were obtained with samples that varied in initial purity from 96.0% to 99.9%. In fact, the initial purity of the cyanide should not be a factor in a sample matching exercise, as any C or N impurities in a given batch of cyanide would contribute to its overall isotope ratio. In addition, the contribution of C or N to the small percentage of impurities in these samples is likely small; therefore, the influence of C or N impurities on the overall isotopic content of the sample would likely be negligible. Stable carbon and nitrogen isotope ratios can therefore be used as a forensic signature of high sensitivity and specificity for cyanide sample matching.

Our data underscore the need for proper handling and storage of samples, both those recovered for analysis and samples stored for routine laboratory usage. We assume that any samples recovered

during the course of an investigation would be characterized chemically, so that chemical composition and degree of purity at the time of recovery would be known. Even in the absence of such characterization, our analyses showed that conversion of cyanide to carbonate can result in isotopic heterogeneity within a sample. Further, degraded samples could easily be distinguished from non-degraded samples via their isotopic heterogeneity and N content that was lower than stoichiometric for cyanides (the elemental analyzers typically used in conjunction with isotope ratio mass spectrometers for C and N isotope ratio analysis also provide C and N content data). As the degradation reaction requires exposure to both moisture and CO_2 (14), it is imperative that samples be stored in dry conditions prior to analysis. Studies of isotopic fractionation during degradation of cyanide to carbonate might enable the association of even degraded samples with their nondegraded counterparts.

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