

## SHORT COMMUNICATION

# Potentiometry for Cyanide Detection Applied to Fisheries Regulation

Paul Bryan Karlo R. Cudia\* , Marc Lawrence J. Romero

National Fisheries Laboratory Division, Bureau of Fisheries and Aquatic Resource, Quezon City, Philippines

### ABSTRACT

Illegal cyanide fishing is practiced by some fishers to capture agile and otherwise inaccessible reef fishes easily. Economic incentives had prevented discontinuation of the practice despite regulations present. Aside from the well-known toxicity of cyanide, it is a concern whether the fish is for ornamental purposes or human consumption that cyanide is being used because of the environmental damage it can cause by killing off non-target species. Currently, the cyanide content of fish is determined using an ion-selective electrode (ISE), with distillation as the mode of extraction of cyanide from tissues. This paper reports a modification of ASTM Method 500-CN-E, a method originally used to test for cyanide content in the wastewater. This paper outlines the process of determining the applicability of the method modification for analysis of fish tissue samples, in which no standard method was designed specifically for the matrix mentioned above. Although percent recoveries for cyanide at 0.05-10 mg/L range in spiked distilled water matrices are in line with the American Society of Testing and Materials (ASTM) results (90-105%), cyanide recoveries in spiked fish tissue matrices at the same concentration range are appreciably lower (~60-80%). For regulatory purposes, it serves as a temporarily acceptable method to detect cyanide-laden fish until a suitable method can be validated on international standards. However, to be accepted as a standard method, additional modifications may be needed or proven in inter-laboratory tests that the recovery of cyanide in fish is consistently low.

\*Corresponding Author: [paulbryan.cudia@gmail.com](mailto:paulbryan.cudia@gmail.com)  
Received: April 12, 2021  
Accepted: March 24, 2022

**Keywords:** *potentiometry with ion-selective electrodes, cyanide, live fish trade, distillation*

Cyanide (CN<sup>-</sup>) is a toxin that targets key electron transport chain enzymes crucial for adenosine triphosphate (ATP) production by cells in their mitochondria (Beasley and Glass 1998). Manifestations of cyanide in fish, as observed by David et al. (2015), indicate fish exposed to lethal concentrations of sodium cyanide (NaCN) has produced the loss of coordination, causing irregular and jerky movements and tendency to sink towards the bottom, and the disruption of their schooling behavior.

Calado et al. (2014) summarized the observations of Rubec et al. (2001) with regards to the common illicit practices of Indo-Pacific fishers as follows: Either potassium cyanide (KCN) or NaCN solids are dissolved in seawater and stored in squirt bottles for easy dispensing of cyanide to target fish. Then, reef fishes are targeted by squirting the bottle at short pulses within reef crevices, and after the fish is

stunned, it is immediately captured and placed in CN<sup>-</sup> free seawater. Ecological concerns resulting from such practices include effects on untargeted species, such as losing the ability to avoid predation or death by poisoning. Coral death, especially, is a major concern due to its importance in maintaining a healthy marine ecosystem of that locality.

Despite regulations in effect, cyanide use is still rampant due to its effectiveness, and surveys conducted by Vaz et al. (2017) of live reef fish exported to the European Union (EU) had observed that some of it was poisoned, evidenced by their secretions of thiocyanate (SCN<sup>-</sup>) ions. Indirect determination of cyanide poisoning through thiocyanate detection is based on the activity of an enzyme called rhodanese that converts CN<sup>-</sup> ions to SCN<sup>-</sup> ions through a transfer reaction between cyanide and thiosulfate (or any other suitable sulfur donor) (Baghshani and Aminlari 2012).

The method currently used by the Bureau of Fisheries and Aquatic Resources' (BFAR) numerous laboratory arms is an adaptation by the International Marinelife Alliance (IMA) and BFAR of ASTM Method 500-CN-E, developed by the American Society of Testing and Materials (APHA-AWWA-WPCF 1992) originally for analysis of wastewater samples. The main focus of this study is to preliminarily demonstrate the analytical fitness of the modified ASTM Method 500-CN-E to analyze fish samples for cyanide fishing monitoring in support of the implementation of Section 92 of RA 10654, or the amended Philippine Fisheries Code of 1998, which sanctions the use of noxious substances (including cyanide) to catch any fishery species in Philippine waters. In this adaptation, cyanide content in fish is determined through the acid distillation of specific internal organs, the liver, and gills. These organs were chosen because a study determined that cyanide accumulates in those organs due to high blood flow through them (Bellwood 1981).

The samples used in this study are *Plectropomus leopardus* submitted through the National Fisheries Laboratory Division of BFAR that were kept in retention (i.e., stored in a freezer) after official analysis. After a prescribed 2-month retention period, the homogenized retained internal organs were used as the source of the fish tissue for the experiment. The body cavity is opened up by cutting either through the dorsal side or ventrally through the cloaca. The liver and gills were harvested and then homogenized collectively into a single bulk sample. This homogenized bulk sample was analyzed before any spiking to set a "blank" value that will be subtracted to the value obtained from the analysis of the spiked sample. From the single homogenized bulk, around 15 g portions were spiked with different amounts of  $\text{CN}^-$  (from KCN stock solution), specifically to make 0.05, 0.1, 1, and 10 mg/kg  $\text{CN}^-$  samples, to evaluate recovery at different concentrations. The spiked sample was then analyzed for cyanide using the method described below. The percent recovery is then assessed by comparing the amount of cyanide distilled out and cyanide spiked. Recovery tests were also carried out on a distilled-water-only matrix spiked with the same quantity of  $\text{CN}^-$  as above to see if there was a matrix effect owing to biochemical molecules and to compare and verify the standard procedure, which used water as its original matrix.

The spiked portions were weighed and homogenized with 100 mL water with a 2 mL ionic strength adjustor (10 M sodium hydroxide (NaOH)). The resulting homogenized material was

then subjected to the distillation process and diluted with additional water until around ~500 mL total volume. The distillation process involves heating the homogenized sample under acidic conditions through a distillation setup. Figure 1 shows how the distillation apparatus is set up for cyanide acid distillation, consisting of customized Pyrex glassware and Azzota SHM-1000 heating mantle. The receiving flask was prepared by mixing 10 mL 1.0 M NaOH with 40 mL distilled water and adding a pinch of lead (II) carbonate ( $\text{PbCO}_3$ ). The following reagents were added sequentially with time (in parenthesis) between adding the next reagent to facilitate mixing: 20 mL 510 g/L magnesium chloride hexahydrate (1 min wait), 15 mL 130 g/L sulfamic acid (3 min wait), 50 mL 1:1 concentrated sulfuric acid/water solution (start heating). The flask was heated for 60 minutes to ensure all bound  $\text{CN}^-$  were converted to free  $\text{CN}^-$  ions. The heat was then turned off, and the apparatus was set aside for 15 minutes. After that, the contents of the receiving flask were filtered with Whatman No. 1 filter paper into a 250 mL amber volumetric flask. The flask was then diluted to mark.

Calibration standard solutions were prepared with 0.05, 0.1, 1, and 10 mg/L  $\text{CN}^-$  concentrations by serial dilution from a 1000 mg/L stock solution to create a calibration curve. Each calibration standard's potential (in mV) was then measured using a LAQUA F-73 pH/ion meter, and a plot of mV vs  $\log[\text{CN}^-]$  was then constructed based on measured data. The slope of the plot is then evaluated; an acceptable slope, as specified in the manual of the Orion™ cyanide ISE used, is between -54 and -60. The fitness of the curve is evaluated through the coefficient of correlation ( $r^2$ ). Seven calibration trials were performed to assess the linearity of the method. The limit of quantification was also determined through 7 blank reagent measurements and was calculated according to Eurachem (2014).

To assess the method's reproducibility in fish samples, the same procedure as the recovery test was also applied to two additional groups of three samples spiked with 0.05 mg/L  $\text{CN}^-$  were analyzed. Each group was analyzed on different days from other groups, but each sample group was analyzed within the same day. Days were chosen so that the analyses occur at least a day apart to maximize variance in environmental conditions. The results were then evaluated using a one-way analysis of variance (ANOVA) to determine the method's reproducibility with varying laboratory conditions. The percent recoveries obtained in the collaborative studies published by APHA-AWWA-

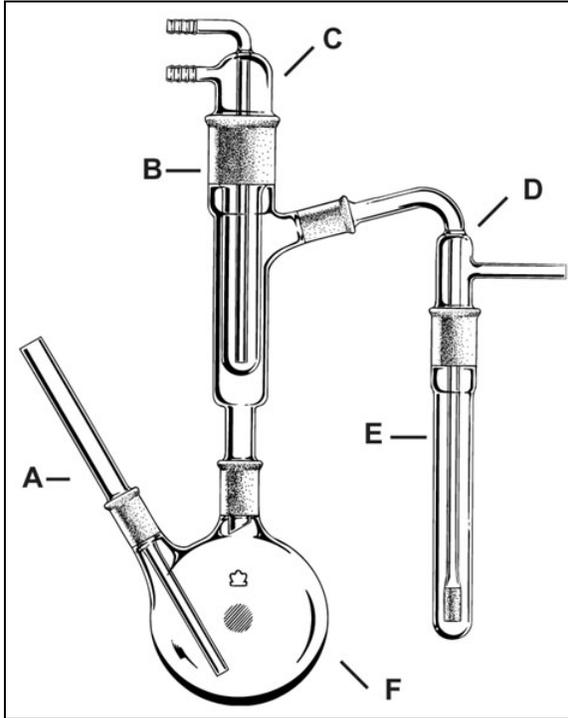


Figure 1. Cyanide distillation apparatus. Image lifted from Laboratory Sales & Service LLC's (2009) site. (A) Inlet tube, (B) condenser jacket, (C) Cold finger condenser, (D) Dispersion tube, (E) Absorber tube, (F) 1,000 mL double-headed round bottom flask (D) connects to a vacuum pump through rubber tubing and the other two tubes in (C) are connected to a water reservoir to feed water into the condenser jacket. Not shown in the diagram is a heating mantle beneath (F).

WPCF (1992) indicate that the method is appropriate for analysis of cyanide in water as the recoveries obtained in this study for water-only matrix (Figure 2) are in line with those of the collaborative study and within the acceptable range (90-110%) for the concentration (0.05 mg/L to 10 mg/L). This verifies the standard method for this study and preliminarily demonstrates that the method can recover cyanide in the study conditions. Since the modified method only replaced the sample matrix used, it would mean that deviations in recovery would be primarily attributed to the change in the matrix rather than systematic errors.

The reliability of the potentiometric ISE detection method set by the ASTM had been demonstrated by Ghosh et al. (2006) for aqueous solutions. The problem is then more rooted in cyanide recovery in fish tissue through distillation. Cytokinetics also play a significant role in the determination of cyanide due to various factors playing on the

conversion of  $CN^-$  to  $SCN^-$  by the action of rhodanese. Leduc's (1984) study on the conversion of hydrogen cyanide to  $SCN^-$  shows an apparent dependency on the sulfur availability for an individual fish. Brown et. al (1995) had obtained in their study the pharmacokinetics of the elimination of plasma  $SCN^-$ , the first-rate constant of 0.29 to 0.34  $day^{-1}$ , upon calculation would yield a half-life of around four days. There is also toxicokinetic evidence that the action of rhodanese is animal dependent, with human rhodanese being similar to rabbit rhodanese (Bhandari et al. 2014). While there was a study conducted by Baghshani and Aminlari (2012) regarding the interspecies dependency of rhodanese levels that demonstrated no apparent difference between cyprinid fishes, there were no further studies conducted that compared the differences between fishes of different families.

Rubec et al. (2008) has mentioned other possible methods for detecting cyanide in fish applicable to monitoring purposes, such as GC-MS (Murphy et al. 2006; Eaton 2009). Thiocyanate had been sought as the target marker for cyanide poisoning by some studies (Vinnakota et al. 2012; Vaz et al. 2017) due to being a by-product of cyanide detoxification. Plenty of methods exist for the detection of  $SCN^-$  (Leduc 1984; Saussereau et al. 2007) that can be adapted to fish monitoring, but there is still a need to establish a definite relationship between  $CN^-$  and  $SCN^-$  concentration properly for quantitative measurement of  $CN^-$  through interspecies comparison across different fish families and investigation of possible varying biochemical mechanisms in cyanide detoxification.

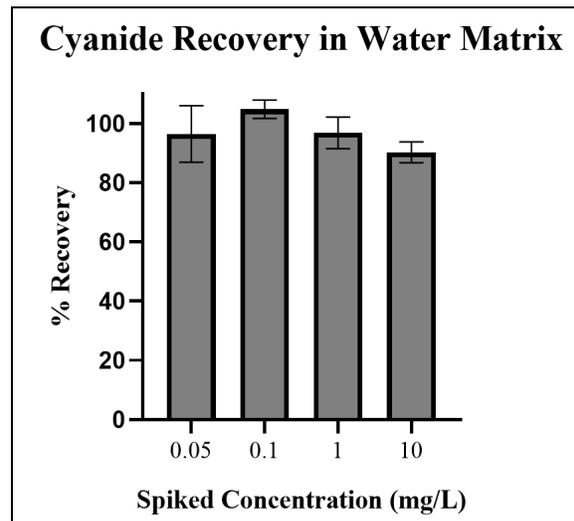


Figure 2. Cyanide recovery in distilled water only matrix. Error bars represent the standard deviation of the three trials.

The recovery test for each corresponding amount of spiked cyanide was done in three trials on different days. Figure 3 shows the percent recovery of cyanide from spiked fish tissue using the distillation method. When dealing with minute analyte concentrations, especially at the mg/L concentration level, it is usually the case that recoveries are relatively low. AOAC International's (2002) guidelines recognize this fact and the range of analytically-acceptable recovery limits increases as analyte concentration decreases. It is to note that at 0.1 mg/kg of analyte in a fish matrix, the recovery is below 50%, even though at 0.05 mg/kg analyte, the recovery is significantly higher than it. Matrix effects are evident due to overall lower recovery amounts in fish matrix compared to water-only matrix, which is given by the results of the verification analysis (i.e., the recovery analysis of water matrices) in Figure 3.

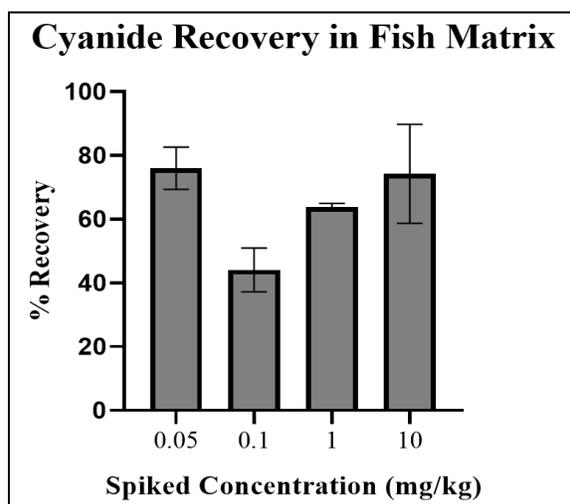


Figure 3. Cyanide recovery in the fish tissue matrix. Error bars represent the standard deviation of the three trials.

Regarding the linearity and range of the calibration curves, an average  $r^2$  value of  $0.9990 \pm 0.0006$  was obtained from seven calibrations. The limit of quantification, determined through seven blank sample measurements, was at 0.039 mg/kg.

Evaluation of the reproducibility data, shown in Figure 4, using ANOVA revealed a significant ( $p = 0.0484$ , confidence interval set at 95%) difference between the groups. Upon performing Tukey's multiple comparisons test, comparing the Day 1 group and Day 8 group show no significant difference (adjusted  $p = 0.1840$ ) and between Day 8 and Day 12 group (adjusted  $p = 0.5183$ ). However, a comparison between the Day 1 and Day 12 groups revealed a significant difference (adjusted  $p = 0.0429$ ).

The recoveries obtained from fish matrices were lower than those of water matrices. This may be due to numerous interferants present in the fish tissue matrix. From the presentation of O.I. Analytical (2009) at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, numerous chemical species have been identified as interferants in cyanide detection, with relevant interferants shown in Table 1.

Sulfides, in particular, are heavily present in biological systems due to their role in defining key protein structures. They can be released in proteins as hydrogen sulfide ( $H_2S$ ) under strongly acidic conditions present in the distillation process. The issue of sulfide interferants was dealt with in the ASTM method using  $PbCO_3$ . However, since the original method deals with water samples with significantly lower protein masses compared to the dissolved mass of animal tissue, it may be possible that the prescribed amount of  $PbCO_3$  is insufficient to sequester all of the produced  $H_2S$  gas in the reaction flask. There is also a problem with the amount of  $PbCO_3$  being subjective because the required amount is a "pinch" of solid material (APHA-AWWA-WPCF 1992). Theoretically, the accuracy of the amount of  $PbCO_3$  dispensed into the absorber tube is not that crucial as it only needs to be in excess to precipitate all of the sulfides out of the absorber tube. Practically, however, too much carbonate can produce foams that may pose risks to the distillation setup integrity.

Thiocyanates are also a significant interferant present in fish tissue samples, especially those exposed to cyanide and naturally-occurring cyanogenic compounds. This was an issue when fish samples submitted for monitoring processes were not killed immediately after assumed exposure to cyanide, as rhodanese can convert cyanides to thiocyanates while the fish is still alive. In the context of this experiment, though, it is improbable that rhodanese would still be functional after being subjected to relatively harsh conditions (i.e., below-freezing storage temperatures), and the thiocyanate already present in the stored fish tissue were already accounted for upon subtracting the initial cyanide content from the calculated spiked content.

Although nitrates and nitrites can form oximes with some organic compounds, which decompose to cyanide under distillation conditions (EPA 2014), the addition of sulfamic acid neutralizes the nitrates and nitrites. Tissue specimens are also not expected to have significant concentrations of nitrates as high concentrations are toxic to most animals.

Table 1. Pertinent interferants for the analysis of cyanide content (lifted from O.I. Analytical (2009))

Interferant	Description of Interference/Action
Oxidizers	Oxidizes cyanide into cyanogen
Sulfide	Reacts with cyanide to form thiosulfate, detected as CN <sup>-</sup> in ISE
Sulfite/sulfur dioxide	Reacts with cyanide
Thiosulfate	Decomposes to sulfur dioxide
Thiocyanate	Decomposes to sulfur dioxide
Carbonate	Excessive foaming and possible violent release of carbon dioxide
Misc. Organics + Nitrate or Nitrile	Decompose to form cyanide
Thiocyanate + Nitrate or nitrile	Decompose to form cyanide

EPA (2014) noted that, possibly, free cyanide could combine with iron to form precipitable complexes under the distillation conditions or adhere to suspended solids. It may be a concern for this experiment as it is expected that there will be high amounts of suspended solid and iron, from fish hemoglobin, in the sample upon acid reflux. It is not possible to perform the alkaline extraction method suggested by EPA (2014), as most of the cyanide is not superficial and is most likely be within the tissue matrix, along with blood. The best possible way to

minimize this interference is to exclude blood from the sample as much as possible.

In comparison with other methods used for determining cyanide poisoning in fish (Table 2), the method offers a direct determination of cyanide, in which biomarkers such as thiocyanate may not be able to consistently determine if a fish was exposed to cyanide due to lack of understanding of detoxification mechanisms between fish species. Also, it may be valuable for developing countries that need to monitor the use of cyanide in ornamental fishes

Table 2. Comparison of methods used for cyanide detection in fish

Method	Advantages	Disadvantages
Modified ASTM Method 500-CN-E (method used in this study)	Direct determination of cyanide	Sample is destroyed
	Relatively inexpensive to perform	Low recovery
Thiocyanate determination (various detection methods)	High half-life in some species (Vaz et al. 2012)	Low sample throughput
	Validated in similar matrices, such as plasma (Murphy et al. 2006)	Needs understanding of biochemistry of various fish species as consistency cannot be established to be used as a standard method to determine cyanide poisoning (Breen et al. 2018)
	Non-destructive, can analyze excretions and/or plasma	Requires relatively advanced and expensive instrumentation (e.g., chromatography with mass spectrometry detection)
Soundararajan method* (Rubec and Soundararajan 1991)	Easy and inexpensive to perform	Highly inaccurate due to matrix effects (Rubec et al. 2008)

as it is inexpensive to perform compared to other methods, but provides more accuracy than previous methods such as the Soundararajan method (Rubec and Soundararajan 1991) due to the addition of the distillation step. While there is no regulatory limit set by Fisheries Administrative Orders from the BFAR for cyanide that is specific for fish samples, a fish sample was likely poisoned with cyanide if the concentration in the tissues exceeds the limit set by the Department of Environment and Natural Resources (DENR) for effluent water (0.20 mg/L) (DENR 2016). This limit is within this method's identified useful range (0.039 to 10 mg/kg).

The within laboratory precision seems to be poor, with the recovery rate progressively decreasing with repeated measures. Although Day 8 recovery rates have no significant difference with the Day 1 group, the higher standard deviation and lower mean value suggest a worsening trend. By Day 12, there is a significant reduction of the recovery rate compared to the Day 1 group. It was not intended to study any time factor in this study, but it is suspected that the sample is possibly degraded. Although this has yet to be confirmed by us through more rigorous testing, residual cyanide in the sample prior to spiking (from background sources) might have been degraded over time, reflecting upon measuring the sample's cyanide content. In the context of analytical measurements, this would mean that analyzing the sample within 1-2 days of sampling would be recommended, based on the recovery data to minimize degradation of the cyanide that is possibly in the sample, as there is an apparent decrease of 3-4% in recovery per day based on these results.

The method, as is, was demonstrated to be effective in the laboratory setting based on the results of the verification experiment with the water matrix (Figure 2). However, the method shows some problems when dealing with fish tissue matrices. Due to the complexity of biological samples, there are numerous possibilities of interferences in the analysis. Further modifications to the method may be necessary to achieve better recovery rates. However, further study on the nature of the unexpected interferences and interactions resulting from the distillation conditions is needed to refine the method. Comparison with other laboratories is recommended to determine if the low recoveries in the obtained fish matrix are reproducible.

In terms of detecting cyanide-laden fish, it is an acceptable method as it can recover a very significant amount (more than 50%) of cyanide from the tissue

matrix, albeit not in amounts that make it acceptable by AOAC standards. For regulatory purposes, this modified method would suffice as there is currently no method standardized by any scientific organization concerning analytical chemistry to determine cyanide in fish tissue, as also noted by Breen et al. (2018), even considering the methods for indirect determination through thiocyanate analysis. However, the method's limitations should be noted when used in fish samples, particularly its time-dependency as cyanide in fish may decay rapidly due to biological actions. Despite the results not fitting the criteria established by major analytical bodies such as the ASTM or AOAC, this is a step for developing such a method. We hope that inter-laboratory studies can be arranged to validate further the method through collaboration.

## ACKNOWLEDGMENTS

We want to acknowledge the Bureau of Fisheries and Aquatic Resources' National Fisheries Laboratory Division for providing the necessary resources for conducting this experiment.

## AUTHOR CONTRIBUTIONS

**Cudia PBKR:** Methodology, Validation, Formal Analysis, Investigation, Writing—Original Draft, Visualization. **Romero MLJ:** Conceptualization, Resources, Writing—Review and Editing, Supervision.

## CONFLICTS OF INTEREST

To the best of our knowledge, no conflict of interest exists in writing this paper.

## ETHICS STATEMENT

No animal or human studies were carried out by the authors.

## REFERENCES

- APHA-AWWA-WPCF. 1992. Method 4500-CN-E. In: Greenberg A, Clesceri L, Eaton A, Franson M. Standard Methods for the Examination of Water and Wastewater, 18th ed. Washington, DC: American Public Health Association.
- AOAC International. 2002. AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and

- Botanicals [Internet]. [updated n.d.; cited 2020 October 12]. [https://s27415.pcdn.co/wp-content/uploads/2020/01/64ER20-7/Validation\\_Methods/d-AOAC\\_Guidelines\\_For\\_Single\\_Laboratory\\_Validation\\_Dietary\\_Supplements\\_and\\_Botanicals.pdf](https://s27415.pcdn.co/wp-content/uploads/2020/01/64ER20-7/Validation_Methods/d-AOAC_Guidelines_For_Single_Laboratory_Validation_Dietary_Supplements_and_Botanicals.pdf)
- Baghshani H, Aminlari M. 2012. Tissue distribution of the enzyme rhodanese in four cyprinid fish species. *Comp Clin Pathol.* 21(5):719-723. <https://doi.org/10.1007/s00580-010-1164-5>
- Bhandari R, Oda R, Petrikovics I, Thompson D, Brenner M, Mahon S, Berbarta V, Rockwood G, Logue B. 2014. Cyanide Toxicokinetics: The Behavior of Cyanide, Thiocyanate and 2-Amino-2-Thiazoline-4-Carboxylic Acid in Multiple Animal Models. *Journal of Analytical Toxicology.* 38(4):218-225. <https://doi.org/10.1093/jat/bku020>
- Beasley D, Glass W. 1998. Cyanide poisoning: pathophysiology and treatment recommendations. *Occupational Medicine.* 48(7):427-431.
- Bellwood DR. 1981. Cyanide...An investigation into the long term histological effects of sodium cyanide doses upon the gastro-intestinal tract of *Dascyllus trimaculatus*, Part Two. *Freshwat Mar Aquar Mag.* 4(12):7-9.
- Breen NE, Lowenstein J, Metivier R, Andrade L, Rhyne AL. 2018. Can excreted thiocyanate be used to detect cyanide exposure in live reef fish?. *PLOS ONE.* 13(10):e0205552. <https://doi.org/10.1371/journal.pone.0196841>
- Brown DG, Lanno RP, van den Heuvel MR, Dixon DG. 1995. HPLC Determination of Plasma Thiocyanate Concentrations in Fish Blood: Application to Laboratory Pharmacokinetic and Field-Monitoring studies. *Ecotoxicol Environ Saf.* 30(3):302-308. <https://doi.org/10.1006/eesa.1995.1034>
- Calado R, Leal MC, Vaz MCM, Brown C, Rosa R, Stevenson TC, Cooper CH, Tissot BN, Li Y, Thornhill DJ. 2014. Caught in the Act: How the U.S. Lacey Act Can Hamper the Fight Against Cyanide Fishing in Tropical Coral Reefs. *Conservation Letters.* 7(6):561-564. <https://doi.org/10.1111/conl.12088>
- David M, Jeyabalan S, Harish E. 2015. Sodium cyanide induced alteration in the whole animal oxygen consumption and behavioural pattern of freshwater fish *Labeo rohita*. *J Environ Biol.* 36:405-408. PMID: 25895263.
- [DENR] Department of Environment and Natural Resources. 2016. DENR Administrative Order No. 2016-08. Water Quality Guidelines and General Effluent Standards of 2016. Available from: [https://emb.gov.ph/wp-content/uploads/2019/04/DAO-2016-08\\_WATER-QUALITY-GUIDELINES-AND-GENERAL-EFFLUENT-STANDARDS.pdf](https://emb.gov.ph/wp-content/uploads/2019/04/DAO-2016-08_WATER-QUALITY-GUIDELINES-AND-GENERAL-EFFLUENT-STANDARDS.pdf)
- Eaton J. 2009. Method ME355.01: Determination of Cyanide in Drinking Water by GC/MS Headspace Analysis. Revision 1.0 [Internet]. [updated n.d.; cited 2018 Apr 30]. [https://www.nemi.gov/methods/method\\_pdf/10443/](https://www.nemi.gov/methods/method_pdf/10443/)
- [EPA] Environmental Protection Agency. 2014. Method 9013A: Cyanide Extraction Procedure for Solids and Oils [Internet]. [updated n.d.; cited 2020 May 18]. <https://www.epa.gov/sites/production/files/2015-12/documents/9013a.pdf>
- Eurachem. 2014. Method Performance Characteristics. In: Magnusson B, Örnemark U, editors. *Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics.* 2nd ed. Available from [https://www.eurachem.org/images/stories/Guides/pdf/MV\\_guide\\_2nd\\_ed\\_EN.pdf](https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf)
- Ghosh RS, Dzombak DA, Drop SM, Zheng A. 2006. Analysis of cyanide in water. In: Dzombak DA, Ghosh RS, Wong-Chong GM, editors. *Cyanide In Water And Soil: Chemistry, Risk, and Management.* Florida: Taylor & Francis Group. p. 123-153
- Laboratory Sales & Service LLC. 2009. Cyanide Distillation Glassware (Kontes Style). [accessed 2021 Oct 23]. <https://laboratorysales.com/images/products/detail/1490.gif>
- Leduc G. 1984. Cyanides in water: toxicological significance. In: Weber LJ, editor. *Aquatic Toxicology.* 2:153-244. New York: Raven Press.

- Murphy K, Schantz M, Butler T, Benner B, Jr., Wood L, Turk G. 2006. Determination of cyanide in blood by isotope-dilution gas chromatography-mass spectrometry. *Clin Chem.* 52(3):458-467. <https://doi.org/10.1373/clinchem.2005.061002>
- O.I. Analytical. 2009. An Overview and Comparison of Methods for Cyanide Analysis [Internet]. College Station, Texas: O.I Analytical. [updated n.d.; cited 2020 May 18]. [https://www.waterboards.ca.gov/coloradoriver/water\\_issues/programs/npdes/docs/oi\\_analysis.pdf](https://www.waterboards.ca.gov/coloradoriver/water_issues/programs/npdes/docs/oi_analysis.pdf)
- Rubec PJ, Soundararajan R. 1991. Chronic toxic effects of cyanide on tropical marine fish. In Chapman P, et al., editors. *Proceedings of the Seventeenth Annual Toxicity Workshop: November 5-7, 1990, Vancouver, B.C.* Canadian Technical Report of Fisheries and Aquatic Sciences. 1774(1):243-251
- Rubec P, Cruz F, Pratt V, Oellers R, McCullough B, Lallo F. 2001. Cyanide-free Net-caught Fish for the Marine Aquarium Trade. *Aquarium Sciences and Conservation.* 3:37-51. <https://doi.org/10.1023/A:1011370106291>
- Rubec P, Frant M, Manipula B. 2008. Methods for Detection of Cyanide and Its Metabolites In Marine Fish [Internet]. [updated n.d.; cited 2018 Apr 30]. Available from: [https://www.researchgate.net/publication/303543261\\_Methods\\_for\\_Detection\\_of\\_Cyanide\\_and\\_its\\_Metabolites\\_in\\_Marine\\_Fish?enrichId=r-greq716491b988946893d9ac7b415bc68683XXX-&enrichSource=Y292ZXJQYWdlOzMwMzU0MzI2MTtBUzozNjYwODUyNzE1MDY5NDZAMTQ2NDI5MjkzODQxOA%3D%3D&el=1\\_x\\_2&\\_esc=publicationCoverPdf](https://www.researchgate.net/publication/303543261_Methods_for_Detection_of_Cyanide_and_its_Metabolites_in_Marine_Fish?enrichId=r-greq716491b988946893d9ac7b415bc68683XXX-&enrichSource=Y292ZXJQYWdlOzMwMzU0MzI2MTtBUzozNjYwODUyNzE1MDY5NDZAMTQ2NDI5MjkzODQxOA%3D%3D&el=1_x_2&_esc=publicationCoverPdf)
- Saussereau E, Goullé J, Lacroix C. 2007. Determination of thiocyanate in plasma by ion chromatography and ultraviolet detection. *Journal of Analytical Toxicology.* 31(7): 383-387. <https://doi.org/10.1093/jat/31.7.383>
- Vaz MCM, Rocha-Santos TAP, Rocha RJM, Lopes I, Pereira R, Duarte AC, Rubec PJ, Calado R. 2012. Excreted Thiocyanate Detects Live Reef Fishes Illegally Collected Using Cyanide—A Non-Invasive and Non-Destructive Testing Approach. *PLOS ONE.* 7(4):e35355. <https://doi.org/10.1371/journal.pone.0035355>
- Vaz MCM, Esteves VI, Calado R. 2017. Live reef fish displaying physiological evidence of cyanide poisoning are still traded in the EU marine aquarium industry. *Sci Rep.* 7:6566. <https://doi.org/10.1038/s41598-017-04940-x>
- Vinnakota C, Peetha N, Perrizo M, Ferris D, Oda R, Rockwood G, Logue B. 2012. Comparison of cyanide exposure markers in the biofluids of smokers and non-smokers. *Biomarkers* 17(7): 625-633. <https://doi.org/10.3109/1354750X.2012.709880>



© 2022 The authors. Published by the National Fisheries Research and Development Institute. This is an open access article distributed under the [CC BY-NC 4.0](https://creativecommons.org/licenses/by-nc/4.0/) license.