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World Class Science for the Marine and Freshwater Environment

Cyanide in the aquatic environment and its metabolism by fish

A desk-based review

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Table of contents

Figures

Tables

Executive Summary

Cyanide (CN) is a rapidly acting chemical which occurs in many forms, the most toxic and common of which are free cyanide (CN^-) and hydrogen cyanide or hydrocyanic acid (HCN). These are toxic to aerobic organisms through the disruption of oxygen transfer in cells and can be found in the aquatic environment as a result of a number of natural and anthropogenic pathways. Cyanide is produced naturally by bacteria, fungi, algae, plants and some invertebrates, although the greatest natural input of cyanide is through the burning of biomass such as in forest fires, although these natural inputs are unlikely to occur in concentrations considered toxic to the aquatic environment.

Cyanide is also used in a wide range of industrial processes globally including: iron and steel production, metal electroplating, the extraction of gold and silver from ores, petroleum refineries, the manufacture of synthetic fibres, plastics, fertilisers and pesticides, along with its use for eradicating pest species from fish farm ponds. Cassava (yuca) production and metal processing and mining activities are considered to be the greatest anthropogenic sources of cyanide entering the natural environment. During cassava production, hydrogen cyanide is released in waste waters at concentrations of up to 200000 micrograms per liter (µg l^{-1}), thereby demonstrating its potential as an important but localised source of cyanide entering the aquatic environment. Furthermore, over 90 percent of all gold excavated globally is extracted using cyanide with concentrations of cyanide up to 280 µg l^{-1} recorded several kilometres downstream of a metal processing plant.

Anthropogenic cyanide can also enter the aquatic environment as a result of cyanide fishing. This illegal fishing technique is an efficient and profitable method used by some fishers to harvest live reef fish for the aquarium trade and live fish for food trade (LRFFT). During this process, fish are exposed to a cyanide and seawater solution ranging in concentration between 2 g l^{-1} and 120 g l^{-1} which is absorbed via the gills and intestine before being distributed around the body via the blood stream, concentrating in tissues and organs with the highest blood flow, such as the liver and spleen. Cyanide is then metabolised within cells where it is transformed into other metabolites which are more easily excreted. Thiocyanate is the major metabolite produced accounting for up to 80 percent of the cyanide excreted from the organism.

There is limited information on the other products of cyanide metabolism in fish. However, known metabolites in mammals include: thiocyanate, ATCA (2-amino-2-thiazoline-4-carboxylic acid) and ITCA (2-Iminothiazolidine-4-carboxylic acid), cyanocobalamin (vitamin B12), α-ketoglutarate cyanohydrin (α-KgCN) and protein adducts. As major metabolic

pathways are highly complex between different taxonomic groups, further research on these products (specificially in fish and particularly marine fish) may help us better understand biological pathways. As such, it is recognised that an experimental approach is required to provide the necessary understanding of the mechanisms for cyanide metabolite production and retention in fish, thereby allowing an approach for the detection of cyanide to be implemented which is reliable for evaluating whether the levels detected are within or beyond those expected to be present in accordance with natural background exposure levels.

There remain a number of evidence challenges which need to be addressed before a robust and practical method for detecting cyanide fishing can be implemented. Background levels of cyanide and its compounds in un-exposed fish samples must be determined at different life stages, species, sexes and sizes of target fish species, as well as establishing the background concentrations in the aquatic environment in key collection regions. Ultimately any method developed should have the ability to discriminate between natural and anthropogenic sources of exposure in marine fish in order for it to be used as a reliable and enforceable management tool in those areas where cyanide fishing is believed to be most prevalent.

1. Overview

Live fish for the aquarium trade and live fish for the food industry have been harvested since the 1930s using small nets, hooks and lines and traps on tropical coral reefs (McAllister et al., 1999; Halim, 2002). It was not until the early 1960s that the incidence of cyanide fishing was first documented when a local fish collector in the Philippines began to spray sodium cyanide on reefs to stun fish, facilitating their capture (Rubec, 2001). In the 1990s, efforts to mitigate the use of cyanide began when the Philippines government, in collaboration with the International Marine Life Alliance, implemented a permitting system and cyanide testing initiative establishing a number of Cyanide Detection Laboratories (CDL) to test reef fish at the point of export (Barber and Pratt, 1997; Barber, 1999; Rubec et al., 2001). However, as the reliable detection of cyanide is particularly difficult and the method employed at the testing units required fish samples to be sacrificed, the accuracy and robustness of the testing units was challenged and as a result only four test laboratories are currently functional in the Philippines (Mak et al., 2005; Bruckner and Roberts, 2008; *pers.comm* Rose-Liza Eisma-Osorio, Professor of Environmental Law, University of Cebu, Philippines July 2017). Complex supply chains, rapid metabolism of cyanide following exposure and other inputs of anthropogenic cyanide into the aquatic environment, present challenges for law enforcement when gathering robust evidence to regulate illegal cyanide fishing activity (Dee et al., 2014).

Nevertheless, the live fish industry remain committed to ensuring that their suppliers are providing sustainable and legally collected fish which are harvested in line with currently accepted best practice. It is therefore essential that any test developed for detecting the use of cyanide fishing is sensitive and robust as the detection of positive results has implications for livelihoods of fish collectors in some of the world's poorest countries where the largest exporters of live fish are located.

The aim of this study is to review current understanding of background levels of cyanide in the aquatic environment and pathways of uptake and metabolism by key fish species which are traditionally harvested live for the aquarium trade and also as live fish for food. Specifically this report will:

1. Identify and review the potential natural and anthropogenic pathways for cyanide entering aquatic systems.
2. Review current understanding of the key metabolic pathways involved in metabolism of cyanide by fish and the key metabolites and transformation products that could be used as indicators for exposure to cyanide.

3. Identify remaining gaps in our understanding and provide recommendations on how these could be addressed in the development of robust methods for cyanide detection specifically for identifying the occurrence of cyanide fishing.

2. Cyanide: the target compound

2.1. Chemical properties of free cyanide

Cyanide (CN) is a rapidly acting chemical consisting of one carbon atom joined to one nitrogen atom via a triple bond. It is highly reactive and occurs in many different forms, the most toxic and common of which is free cyanide, comprising of the cyanide ion (CN^-) and hydrogen cyanide, also known as hydrocyanic acid (HCN). Free cyanide can readily react with many different elements to produce a wide range of different compounds as illustrated in Figure 1. It will form simple water-soluble compounds when in contact with elements such as sodium or potassium (NaCN and KCN respectively) and will easily dissolve to release free cyanide (CN^- and HCN).

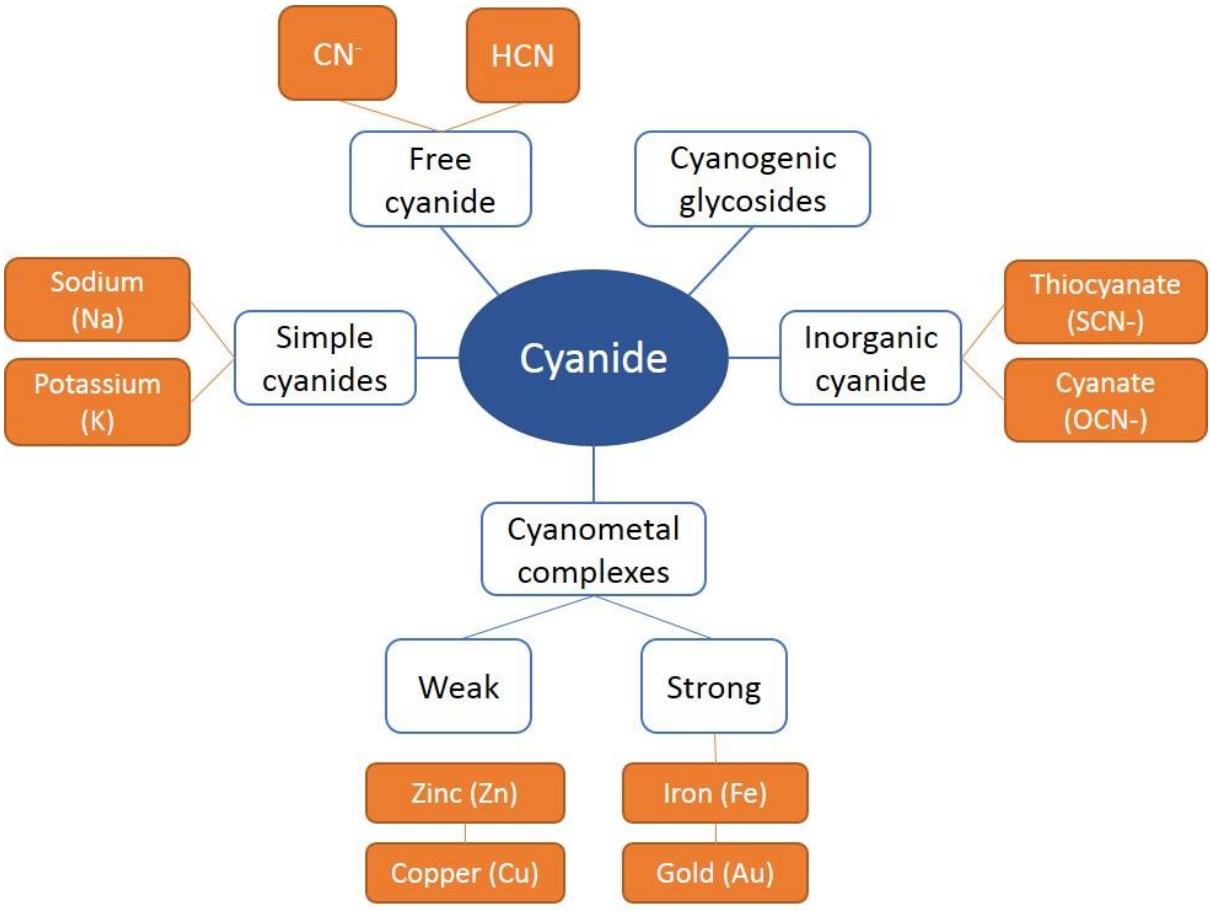

Figure 1. The range of cyanide compounds.

In the presence of metals, cyanide will form a variety of metal complexes of varying strengths. Under certain conditions these metal complexes will also dissociate to release free cyanide (Sorokin et al., 2008). The strong affinity of cyanide for metals is one of the

properties which makes it highly versatile in many industrial processes such as a lixiviant in metal extraction (Young and Jordan, 1995). pH is the primary factor influencing the stability of cyanide metal complexes although some will also degrade under certain wavelengths of light. Metals such as zinc and copper form weak complexes (weak acid dissociable) and can dissociate and release free cyanide under weakly acidic conditions (Dash et al., 2009a; Dash et al., 2009b). Stronger cyanide metal complexes, such as those formed with iron and gold, are much more stable and are degraded by stronger acids or exposure to light (Moran, 1999; Moran, 2001).

Cyanide will also form inorganic compounds such as cyanate and thiocyanate. Oxidation of cyanide produces cyanate (OCN-) which is less toxic than hydrogen cyanide and readily hydrolyses to ammonia and carbon dioxide (Dash et al., 2009a). Thiocyanate is the major product of cyanide metabolism within aerobic organisms although it can also be produced externally when cyanide combines with sulphur, such as in the tailings ponds associated with mining activities.

2.2. Toxicity to aerobic organisms

Cyanide is toxic to aerobic organisms as it disrupts oxygen transfer in mitochondria, essentially suffocating the organism despite the availability of oxygen in external medium and maintenance of a 'functioning' respiratory system. This is known as histotoxic hypoxia. Cyanide causes histotoxic hypoxia due to its affinity with binding to the cytochrome c oxidase enzyme within the mitochondria. Cytochrome c oxidase is the terminal electron acceptor in the respiratory electron transport chain. It converts oxygen molecules into water creating an electrochemical gradient which facilitates the production of ATP (Adenosine triphosphate). Cyanide bound to cytochrome c oxidase disrupts electron transport between the enzyme and molecular oxygen (Egekeze and Oehme, 1980; Ramzy, 2014). Tissues become starved of oxygen, ATP production decreases and cellular respiration ceases (Jackson and Logue, 2017).

Cyanometal complexes are much less toxic than free cyanide, and the level of toxicity of these complexes is determined by how readily they release free cyanide; CN- and HCN (Dzombak et al., 2006). Pablo et al., (1997b) exposed two species of marine fish, Australian bass (*Macquaria novemaculeata*) and black bream (*Acanthopagrus butcheri*), to sodium cyanide and two iron cyanide complexes; ferricyanide and ferrocyanide. They found that the 96-hour LC_{50} (Lethal Concentration resulting in 50% mortality) of each cyanide derivative was correlated with free cyanide and not total cyanide. The observed differences in toxicity

of ferricyanide and ferrocyanide are likely due to their differing rates of dissociation to free cyanide, which accounts for the majority of the toxicity (Pablo et al., 1997b).

The Lethal Concentration resulting in 50% mortality (LC_{50}) of different cyanide derivatives in a variety of aquatic organisms as reported in the peer-reviewed literature is shown in Table 1 and Table 2. Free cyanide is acutely toxic to aquatic organisms with median lethal dose concentrations generally described in micrograms per litre (µg l^{-1}). Exposure to cyanometal complexes such as iron cyanide is considerably less toxic with median lethal dose concentrations described in milligrams per litre (mg l^{-1}). There is variability between different taxonomic groups in their tolerance to cyanide although there are no clear patterns to suggest which marine or freshwater species are more vulnerable or tolerant to exposure. Toxicity to cyanide has also been found to be correlated with body size in a study on clownfish with smaller fish more susceptible to sodium cyanide than larger fish (Da Silva., 2015). As a result, both intraspecies and interspecies variation can be expected in relation to cyanide tolerances.

2.3. Analytical techniques to determine cyanide exposure

Due to the toxicity of cyanide to aerobic organisms, many analytical techniques have been developed to determine exposure to the compound but most of these have focused on the analysis of blood, urine, saliva, expired air and tissue samples in humans. During the past 20 years there have been a number of peer-reviewed review articles on these methods including: the analysis of cyanide in blood (Lindsay et al., 2004); biosensors for cyanide analysis (Mak et al., 2005); the analysis of cyanide metabolites (thiocyanate, ATCA and cyanide adducts) (Logue et al. 2010); developments in cyanide detection (Ma and Dasgupta, 2010); developments in quantifying cyanide and hydrogen cyanide (Randviir and Banks, 2015); and the latest rapid and portable techniques for the diagnosis of cyanide exposure (Jackson and Logue, 2017). Most recently, a study on methods of detecting cyanide, thiocyanate and other by-products in seawater provided a systematic approach for evaluating the quality of analytical tests for cyanide detection (Losada & Bersuder, 2017 unpublished report).

The majority of sample types used for the development of tests in humans can be difficult to obtain in aquatic organisms such as fish. For example, the analysis of blood in fish is possible but the method would need to be very sensitive due to the low volumes that could be safely extracted. There are only a small number of studies which have focused on the development of methodologies for the detection of thiocyanate or other cyanide metabolites in water. A summary of these studies including the target compound, the matrix tested, recoveries and accuracy is presented in Table 3.

Table 1. Summary of median lethal dose (LC_{50}) data of different cyanide derivatives to a variety of aquatic organisms (1 of 2).

Form of cyanide	Marine or freshwater	Taxonomy	Species	Life stage	LC_{50} ($\mu g\ l^{-1}$)	Duration	Reference
Free cyanide (CN^-, HCN)	Freshwater	Fish	Rainbow Trout *(Onchrynchus mykiss)*	Adult	45	96-h	Barber et al., 2003
Potassium cyanide (KCN)	Freshwater	Fish	Rainbow Trout *(Onchrynchus mykiss)*	Adult	43	96-h	McGeachy and Leduc, 1988
UV light & Cobalt cyanide complex, $K_3Co(CN)_6$	Freshwater	Fish	Rainbow Trout *(Onchrynchus mykiss)*	Fingerling	383.2	96-h	Little et al., 2007
No UV light & Cobalt cyanide complex, $K_3Co(CN)_6$	Freshwater	Fish	Rainbow Trout *(Onchrynchus mykiss)*	Fingerling	112900	96-h	Little et al., 2007
Sodium cyanide (NaCN)	Marine	Fish	Australian Bass *(Macquaria novemaculeata)*	Adult	109	96-h	Pablo et al., 1997b
Iron-cyanide complexes, $k_3Fe(CN)_6$, $k_4Fe(CN)_6$	Marine	Fish	Australian Bass *(Macquaria novemaculeata)*	Adult	2830 - 285000	96-h	Pablo et al., 1997b
Sodium cyanide (NaCN)	Marine	Fish	Black Bream *(Acanthopagrus butcheri)*	Adult	70	96-h	Pablo et al., 1997b
Iron-cyanide complexes, $k_3Fe(CN)_6$, $k_4Fe(CN)_6$	Marine	Fish	Black Bream *(Acanthopagrus butcheri)*	Adult	1730 - 20500	96-h	Pablo et al., 1997b
Sodium cyanide (NaCN)	Freshwater	Fish	Nile Tilapia *(Oreochromis niloticus)*	Adult	387	96-h	Ramzy, 2014
Sodium cyanide (NaCN)	Marine	Fish	Clownfish *(Amphiprion ocellaris)*	Adult	28450 – 50000	96-h	Da Silva, 2015
Sodium cyanide (NaCN)	Freshwater	Fish	Rohu *(Labeo rohita)*	Fingerling	33	96-h	Prashanth et al., 2011a
Sodium cyanide (NaCN)	Freshwater	Fish	Rohu *(Labeo rohita)*	Fingerling	320	96-h	Dube and Hosetti, 2010
Sodium cyanide (NaCN)	Freshwater	Fish	Rohu *(Labeo rohita)*	Fingerling	190	96-h	David et al., 2010
Sodium cyanide (NaCN)	Freshwater	Fish	North African Catfish *(Clarias gariepinus)*	Fingerling	1600	96-h	Al-Ghanim and Mahboob, 2012
Potassium cyanide (KCN)	Freshwater	Fish	North African Catfish *(Clarias gariepinus)*	Fingerling	361000	96-h	Oseni, 2016
Sodium cyanide (NaCN)	Freshwater	Fish	Guppy *(Poecilia reticulata)*	Adult	9.13	96-h	Prashanth et al., 2011b
Sodium cyanide (NaCN)	Freshwater	Fish	Catla *(Catla catla)*	Fingerling	110	96-h	David et al., 2010
Copper cyanide (CuCN)	Freshwater	Fish	Catla *(Catla catla)*	Fingerling	760	96-h	Basaling and Praveen, 2011
Sodium cyanide (NaCN)	Freshwater	Fish	Mrigal Carp *(Cirrhinus mrigala)*	Fingerling	330	96-h	David et al., 2010
Sodium cyanide (NaCN)	Freshwater	Fish	Common Carp *(Cyprinus carpio)*	Fingerling	1000	96-h	David et al., 2010
Sodium cyanide (NaCN)	Freshwater	Fish	Mozambique Tilapia *(Oreochromis mossambicus)*	Fingerling	420	96-h	Basaling and Praveen, 2011
Sodium cyanide (NaCN)	Freshwater	Fish	Mozambique Tilapia *(Oreochromis mossambicus)*	NA	44.33	96-h	Prashanth, 2012
Free cyanide (CN^-, HCN)	Marine	Fish	Cobia *(Rachycentron canadum)*	Larvae (1 day)	35	96-h	Dung et al., 2005
Free cyanide (CN^-, HCN)	Marine	Fish	Cobia *(Rachycentron canadum)*	Juvenile (20 days)	57	96-h	Dung et al., 2005
Free cyanide (CN^-, HCN)	Marine	Fish	Cobia *(Rachycentron canadum)*	Juvenile (40 days)	30	96-h	Dung et al., 2005

Table 2. Summary of median lethal dose (LC50) data of different cyanide derivatives to a variety of aquatic organisms (2 of 2).

Form of cyanide	Marine or freshwater	Taxonomy	Species	Life stage	LC_{50} ($\mu g\ l^{-1}$)	Duration	Reference
Sodium cyanide (NaCN)	Marine	Invertebrate	Graceful Rock Crab *(Cancer gracilis)*	First stage zoea	135 – 153	96-h	Brix et al., 2000
Sodium cyanide (NaCN)	Marine	Invertebrate	Dungeness Crab *(Cancer magister)*	First stage zoea	51 - 92	96-h	Brix et al., 2000
Sodium cyanide (NaCN)	Marine	Invertebrate	Pygmy Rock Crab *(Cancer oregonensis)*	First stage zoea	111 – 154	96-h	Brix et al., 2000
Sodium cyanide (NaCN)	Marine	Invertebrate	Red Rock Crab *(Cancer productus)*	First stage zoea	107 - 219	96-h	Brix et al., 2000
Cyanide ion (CN^-)	Marine	Invertebrate	Mysid Shrimp *(Mysidopsis bahia)*	24-h old	113	96-h	Lussier et al., 1985
Sodium cyanide (NaCN)	Freshwater	Invertebrate	Water Flea *(Daphnia magna)*	NA	19	96-h	Jaafarzadeh et al., 2013
UV light & Cobalt cyanide complex, $K_3Co(CN)_6$	Freshwater	Invertebrate	Water Flea *(Daphnia magna)*	Adult	<250	96-h	Little et al., 2007
No UV light & Cobalt cyanide complex, $K_3Co(CN)_6$	Freshwater	Invertebrate	Water Flea *(Daphnia magna)*	Adult	502	96-h	Little et al., 2007
UV light & Cobalt cyanide complex, $K_3Co(CN)_6$	Freshwater	Invertebrate	Water Flea *(Ceriodaphnia dubia)*	Adult	125	96-h	Little et al., 2007
No UV light & Cobalt cyanide complex, $K_3Co(CN)_6$	Freshwater	Invertebrate	Water Flea *(Ceriodaphnia dubia)*	Adult	2289	96-h	Little et al., 2007
Sodium cyanide (NaCN)	Marine	Invertebrate	Giant Tiger Prawn *(Penaeus monodon)*	Post-larvae	110	96-h	Pablo et al., 1997a
Potassium cyanide (KCN)	Marine	Invertebrate (coral)	Cauliflower Coral *(Pocillopora damicornis)*	Coral cells	5100	3-h	Downs et al., 2010

Table 3. Summary of methods for the analysis of cyanide and thiocyanate. Abbreviations: SCN: Thiocyanate; CN: Cyanide; HS: Head space; SPME: Solid phase microextraction; N/M: Not mentioned; HPLC: High performance liquid chromatography; UV: Ultraviolet detector; SERS: Surface enhanced Raman scattering; LC: Liquid chromatography.

Target compound	Matrix	Sample prep	Sample size	Recoveries (in %)	Internal Standard	Analysis	Linearity	Interferences	LODs	Validated	RSD (in %)	Accuracy	Time	Portability	References
SCN-	Water	No	N/M	N/M	N/M	Electrochemical (metalloporphyrins-porphyrins)	N/M	N/M	1-2 ppb (1-2 µg l^{-1})	N/M	N/M	N/M	~10 min		Murphy and Rhyne 2017
SCN-	Seawater	Filtration	~ 20 µL	102 - 106	No	HPLC—UV	6.0 µg l^{-1} – 2.32 µg l^{-1}	N/M	2 µg l^{-1}	N/M	3	N/M		Potential	Rong 2005
SCN-	Seawater	Pre-concentration	50 mL	N/M	N/M	Spectroscopy (SERS)	N/M	N/M	< 5ppb (<5 µg l^{-1})	N/M	N/M	N/M		Potential	Dashti 2016
SCN-	Seawater	Filtration, 4°C	20 µL	N/M	No	LC-Optic fibre	4-400 µg l^{-1}	None for iodide and iodate	3 µg l^{-1}	HPLC-UV	N/M	0.4%	<6 min	Potential	Silva, 2011
SCN-	Artificial seawater	No	20 µL	N/M	No	LC-Optic fibre	4-400 µg l^{-1}	N/M	3.16 µg l^{-1}	N/M	N/M	0.4%	<6 min	Potential	Vaz, et al., 2012
CN-, SCN- (as CN-)	Waste water, saliva	HS-SPME with Hg	N/M	N/M	N/M	UV-Spectrophotometer	1-50 µmol l^{-1}	N/M	0.34 µmol l^{-1} CN- (9 µg l^{-1} CN-)	Colorimetric method	N/M	N/M	N/M		Al-Saidi 2016

Not only are there fewer studies on the detection of cyanide or cyanide metabolites in seawater, there are also some conflicting results, particularly when different research groups have attempted to replicate the work of others or when methods have been applied to artificial and natural seawater samples. Vaz et al., (2012) applied a fiber optic detector to artificial seawater holding live fish following the methods developed for natural seawater by Silva et al. (2011), reporting a limit of detection for thiocyanate of 3.16 µg/L. However, Herz et al. (2016) then tried to replicate the methods described by Vaz et al., using natural seawater samples but reported the tests were unsuccessful suggesting that natural seawater is too complex to be analyzed using the method developed by Vaz et al., (2012). Importantly Herz et al., (2016) did not specify the detection technique used which was bespoke in the approach developed by Vaz et al. (2012). Recent studies by Dashti (2016) and Murphy and Rhyne (2017) show promise, reporting low limits of detection for thiocyanate in seawater at 5 ng l-1 and 2 µg l^{-1} respectively. However, data from both studies are yet to be published in the peer-reviewed literature so it is not possible to fully evaluate the suitability and robustness of these methods for operational use.

KEY POINTS:

- Cyanide (CN) is a rapidly acting chemical, the most toxic and common of which is free cyanide also known as hydrocyanic acid (HCN).
- Cyanide is toxic to aerobic organisms by disrupting oxygen transfer in cells, essentially suffocating the organism.
- Many analytical techniques have been developed to detect and measure exposure to cyanide but most have focused on tissue samples in humans.
- Only a small number of studies have focused on the the detection of thiocyanate or other cyanide metabolites in water and to date, a number of these have shown conflicting results.

3. Cyanide in the aquatic environment

In aqueous solution at a pH of 7, more than 99 percent of cyanide exists as hydrogen cyanide. If the solution becomes more alkaline (increasing pH), hydrogen cyanide will dissociate to the free cyanide ion. At a pH of 9.3, hydrogen cyanide and the free cyanide ion exist in equilibrium (50:50 ratio) and under still more alkaline conditions at a pH of 11, all cyanide will exist as the free cyanide ion (Sorokin et al., 2008; Ramzy 2014). In seawater (pH 7.5 – 8.4) this ratio will be weighted in the favour of hydrogen cyanide, although both forms of free cyanide are likely to be present (Figure 2). If it does not interact with metal ions already present in the water, hydrogen cyanide will readily volatise from solution at temperatures greater than 25°C due to its low boiling point (Dzombak et al., 2006). The rate of volatisation increases with greater mixing of surface waters, increasing temperature and decreasing pH (Johnson, 2015). Therefore, it is to be expected that the rate of hydrogen cyanide volatisation will be greater in more energetic aquatic environments such as rapidly flowing rivers and larger water bodies during stormy weather. The free cyanide ion persists longer in water with a half-life of 15 days compared to hydrogen cyanide which, depending on temperature, has a half-life of a matter of hours to a few days (Sorokin et al., 2008).

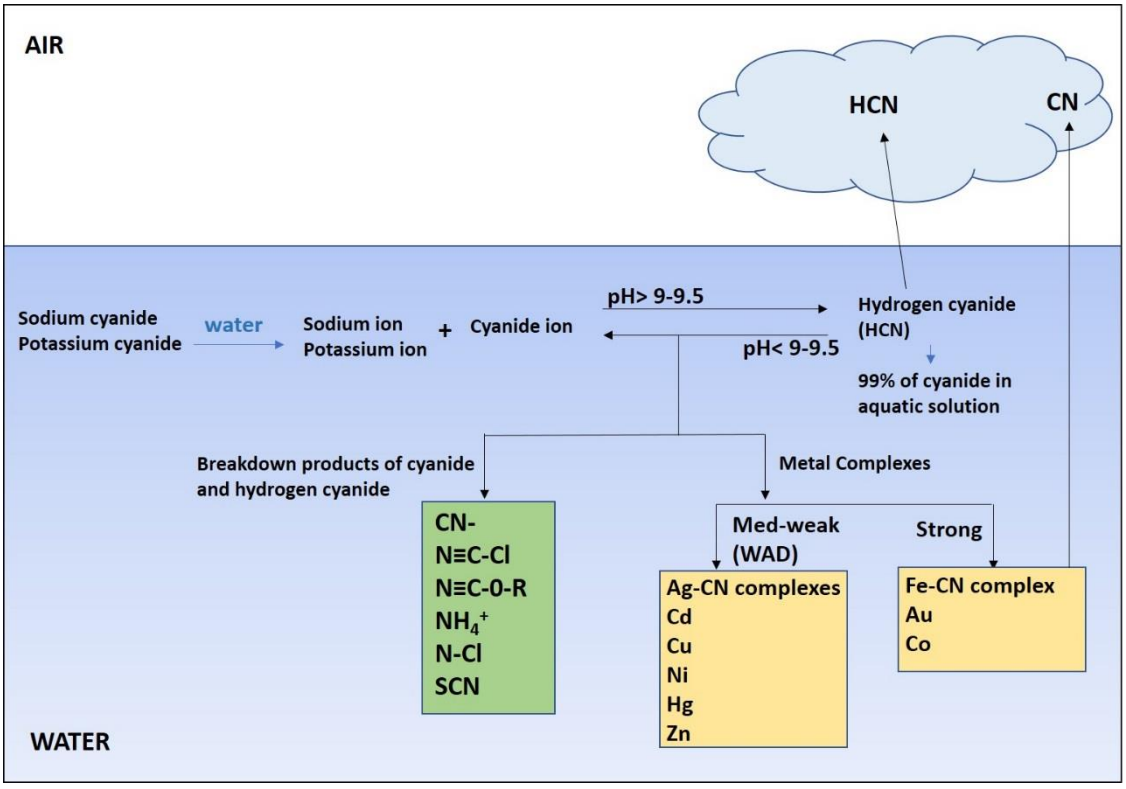

Figure 2. Cyanide pathways and reactions in the aquatic environment. NaCN (Sodium cyanide); KCN (Potassium cyanide); Na^+ (Sodium ion); K^+ (Potassium ion) CN^- (Cyanide ion); HCN (Hydrogen cyanide); N≡C-Cl or N≡C-O-R (Nitriles); NH_4^+ (ammonia); N-Cl (chloramines); SCN^- (Thiocyanate); Ag (Silver); Cd (Cadmuim); Ni (Nickel); Hg (Mercury); Zn (Zinc); Fe (Iron); Au (Gold); Co (Cobalt).

Photolysis (the separation of molecules by the action of light) may also be an important factor acting on the fate of free cyanide (CN−) and ferrocyanide (K4Fe(CN)6) in the aquatic environment. Dzombak et al., (2006) reported that while free cyanide in the presence of potassium cyanide decreased rapidly from 20 µg l-1 to below the detection limit (0.5 µg l-1) in three days while it took six days for free cyanide to drop below the detection in the presence of ferrocyanide from a starting concentration of 21.4 µg l-1.

Whilst oceanic concentrations of atmospherically derived cyanide are largely unknown, they are predicted to be less than 1 µg l^{-1} (Li et al., 2000; Dzombak et al., 2006). Table 4 summarises concentrations of cyanide recorded in the aquatic environments and reported within the peer-reviewed literature. For context, it should be noted that the Association of Southeast Asian Nations (ASEAN) proposed a marine water quality criteria for cyanide of 7 µg l^{-1} to protect aquatic life (ATSDR, 2006).

Table 4. Measured concentrations of cyanide in the aquatic environment reported in peer-reviewed literature.

Form of cyanide	Aquatic environment	Location	Concentration (µg l^{-1})	Reference
Free cyanide	Seawater	Jiaozhou Bay, China	0.02 - 0.46	Yang et al., 2015; Yang et al., 2016
Free cyanide	Cassava processing wastewater	Nigeria	7890	Okafor et al., 2001
Free cyanide	Freshwater downstream of mining activities	Ecuador	280	Guimaraes et al., 2011
Thiocyanate	Seawater, offshore (>6km)	Aveiro, Portugal	<3.3	Silva et al., 2011
Thiocyanate	Seawater, inshore (<6km)	Aveiro, Portugal	5.1 - 22.7	Silva et al., 2011
Thiocyanate	Seawater	Japan	8.1 - 15.0	Rong et al., 2005; cited in Silva et al., 2011
Potassium cyanide	Seawater	Black Sea	0.13 – 3.01	Güven et al., 2001
Potassium cyanide	Seawater	Igneada, Turkey	0.11 – 0.34	Güven et al., 2001
Potassium cyanide	Seawater	Istanbul Straight	0.25 – 1.56	Güven et al., 2001
Potassium cyanide	Seawater	Kilyos and Rumeli Feneri, Turkey	0.09 – 1.74	Güven et al., 2001
Cyanide	Seawater	Port Kembla Harbour, Australia	1200	Moran and Grant, 1993
Sodium cyanide or Potassium cyanide	Cyanide fishing seized squirt bottles	NA	2×10^6 – 1.2×10^8	Jones et al., 1999
Free cyanide	Saltmarsh pore-waters	Delaware Great Marsh, USA	82.6	Kamyshny et al., 2013

3.1. Natural pathways

Cyanide can enter the aquatic environment via both natural and anthropogenic pathways. Under natural conditions, cyanogenic compounds are produced by bacteria, fungi, algae, vascular plants and some invertebrates (Kamyshny et al., 2013). Cyanide produced via these sources has the potential to enter both marine and freshwater habitats as described in the following section.

Only three species of cyanogenic algae have been identified to date, these are *Chorella vulgaris*, *Anacystis nidulans* and *Nostroc muscorum* (Dzombak et al., 2006). Known examples of cyanogenic bacteria are equally uncommon. Hydrogen cyanide is produced by bacteria but only aerobically during transition from the log phase of growth to the stationary phase as its production is limited by the presence of glycine (Knowles 1976; Dzombak et al., 2006). Hydrogen cyanide may also be produced by certain species of algae and bacteria as a by-product of metabolic processes as opposed to intentional synthesis, for example as a deterrent from predation. A greater number of microorganisms are actually able to biodegrade cyanide as it provides a source of carbon and nitrogen (Ebbs, 2004). Therefore, the small quantities of cyanide produced by bacteria and algae are unlikely to reach toxic concentrations in the aquatic environment.

Many fungal species and life stages are able to directly produce hydrogen cyanide, cyanogenic compounds, or produce enzymes capable of liberating cyanide from cyanogenic glycosides within vascular plants (Knowles 1976; Dzombak et al., 2006). Knowles (1976) hypothesised that cyanide is produced by fungi to (1) reduce competition from adjacent organisms through damaging or limiting their growth, (2) reduce the likelihood of predation or (3) aid the parasitism of adjacent cells or organisms. Some terrestrial invertebrates such as species of centipede and millipedes can also produce hydrogen cyanide as a form of chemical defence to deter predators (Dash et al., 2009a; Vujisić et al., 2013).

Cyanogenic organisms such as bacteria and fungi are capable of biodegrading free cyanide but are often unable to break apart strongly bonded cyanometal complexes (Oudjehani et al., 2002). Cyanide is assimilated in the organism or biodegraded via several key pathways. Oxidation produces ammonia and carbon dioxide, reduction produces ammonia and methane and hydrolysis produces formate and ammonia (Johnson, 2015). *Pseudomonas putida*, a soil bacterium, is one such organism which uses cyanide as a source of carbon and nitrogen. It biodegrades cyanide by oxidation producing ammonia (NH_3) and carbon dioxide (CO_2) as products of this pathway (Chapatwala et al., 1998). However, high concentrations of free cyanide can cause toxicity to these organisms resulting in reductions in growth rates and mortality (Dash et al., 2009a).

Cyanogenic glycoside compounds have been documented in over 2,500 species of plant across many different taxonomic groups including: cassava; apricots; cherries; apples and sweet potatoes (Vetter, 2000). Cyanide concentrations of 2,360,000 and 3,000,000 µg kg^{-1} have been recorded in cassava and lima beans respectively (WHO, 2004). Catabolic degradation of cyanogenic glycosides results in the formation and potential release of hydrogen cyanide into the wider environment (Poulton, 1990). These resultant products of natural degradation can enter the atmosphere, soil, water and pass directly into organisms by ingestion. The natural breakdown of cyanogenic glycosides within plants forming hydrogen cyanide is generally assumed to not occur in quantities sufficient to cause high levels of cyanide in the surrounding marine environment (Dzombak et al., 2006). However, a study by Kamyshny et al., (2013) recorded concentrations of both free and complexed cyanide up to 6.94 micromole per litre (µmol l^{-1}) (1.92 µmol l^{-1} or 82.6 µg l^{-1} free cyanide) in saltmarsh sediments and pore-waters, which were previously perceived to be non-polluted. The observed cyanide levels were hypothesised to be a result of cord grass (*Spartina alterniflora)* decomposition. The roots were found to contain 50 µmol kg^{-1} cyanide at the time of sampling. The availability of elements able to react and form complexes with cyanide may have reduced the atmospheric release of hydrogen cyanide from the saltmarsh sediments and pore-waters. This input of cyanide to the saltmarsh sediments probably persisted, primarily through two pathways; the formation of iron cyanide complexes; and the formation of thiocyanate via the oxidative reaction with polysulphides. Of these two pathways, the formation of thiocyanate was identified as the most important. It is possible that other aquatic habitats such as mangroves forests are also capable of accumulating cyanide compounds in sediments. However, no literature confirming the presence of cyanide in these other habitats was identified.

The burning of biomass for example during forest fires is perhaps the greatest natural input of hydrogen cyanide into the atmosphere. High temperatures attained during burning events causes pyrolysis, which liberates hydrogen cyanide from amino acids, nitrogen heterocycles, dicarboxylic acids and cyanogenic glycosides (Johnson and Kang, 1971; Barber et al., 2003). Biomass burning releases an estimated 1.4 – 2.9 teragrams of nitrogen per year (Tg N yr^{-1}), and after an atmospheric lifetime of around five months, the ocean is reported to be the primary sink, capable of absorbing 1.1 – 2.6 Tg N yr^{-1} (Li et al., 2000; Li et al., 2003). Whilst much of the cyanide from biomass burning is released into the atmosphere, both gaseous and particulate cyanide contained within ash deposits may also diffuse or be washed into aquatic environments through wet and dry deposition and precipitation run off (Barber et al., 2003).

KEY POINTS:

- Cyanide compounds are produced naturally by bacteria, fungi, algae, plants and some invertebrates but the greatest natural input of cyanide into the atmosphere is through the burning of biomass, for example in forest fires.
- Cyanide produced by bacteria, algae and plants is unlikely to occur in concentrations considered toxic to the aquatic environment.
- Cyanide released during biomass burning has an atmospheric lifetime of around five months with the ocean being reported to be the primary sink.

3.2. Anthropogenic pathways

Due to its high degree of reactivity and affinity for precious metals, such as gold and silver, cyanide is used in a wide range of different industrial processes around the world (Boening & Chew, 1999; Mak, 2003). It is used in iron and steel processing plants, metal electroplating, the extraction of gold and silver from ores, petroleum refineries, the manufacture of synthetic fibres (such as nylon), plastics, fertilisers and pesticides, and also for the eradication of pests species from fish farm ponds (Eisler, 1991; Halim, 2002). As a result, cyanide enters the aquatic environment via waste water effluent associated with many chemical and industrial processes, and densely urbanised areas. Whilst cyanide naturally occurs in the environment as described in section 3.1, concentrations are often elevated in the vicinity of anthropogenic activites and if left untreated, cyanide containing wastewaters are a major anthropogenic source of cyanide entering the natural environment (McPherson et al., 1999). Cyanide, as a by-product, can also be produced in chlorine treated wastewaters (Redman and Santore, 2012). Cyanide pollution is most commonly monitored through the analysis of water samples. In 1983, a survey was conducted in Jiaozhou Bay, China to determine cyanide concentrations within the bay. The two main inputs of cyanide were reported to be riverine and from industrial activities within the bay itself. These activities caused low, but detectable concentrations of cyanide within the bay of 0.02 - 0.46 µg l^{-1}. Cyanide was less concentrated in the mouth of the bay as distance from the assumed cyanide sources increased (Yang et al., 2015; Yang et al., 2016).

For this review we examined the three key anthropogenic sources of cyanide input to the aquatic environment: 1) cassava processing; 2) mining; and 3) cyanide fishing.

3.2.1. Cassava processing

Cassava (yuca) is a staple annual crop able to grow in poor soil conditions which is cultivated throughout much of the tropics and subtropics as a key source of carbohydrate. During cassava production, cyanogenic glycosides are released and degrade into hydrogen cyanide resulting in waste water concentrations of cyanide up to 200,000 µg l^{-1} (Siller and Winter, 1998; Simeonova and Fishbein, 2004). Cyanide in concentrations of up to 7,890 µg l^{-1} have been detected in waters adjacent to cassava processing plants in Nigeria (Okafor et al., 2001). Therefore, waste water from cassava processing plants has the potential to be an important but localised source of hydrogen cyanide entering the aquatic environment.

3.2.2. Metal processing and mining

Metal processing and mining activities are considered to be the greatest anthropogenic sources of cyanide entering the natural environment (Dash et al., 2009). In the mining industry cyanide has been used to extract precious metals from their ores for over a hundred years. It is particularly prevalent in gold and silver mining, with over 90 percent of all gold excavated globally being extracted using cyanide (Mudder and Botz, 2004). Gold is extracted in a hydrometallurgical process whereby a cyanide solution is used to dissolve the gold and isolate it from the ore.

When cyanide is used to extract gold from ores it will typically bind with other common metals also present to produce a huge range of different cyanometal complexes. These complexes vary in strength depending upon the metal in question. Weak complexes (weak acid dissociable) typically breakdown in acidic conditions, while stronger complexes, such as those formed with iron and gold are much more stable and are degraded by exposure to light at particular wavelengths (Moran, 1999; Moran, 2001). Cyanometal complexes are much less toxic than either the free cyanide ion or hydrogen cyanide, however as they break down they release free cyanide. The breakdown products of cyanide whilst less toxic than free cyanide may be more persistant in the natural environment. Some of these require specific analytical methods to accurately assess their concentration and as a result are often underestimated during routine analysis of environmental samples (Moran, 1999).

Thiocyanate, the major product of cyanide metabolism in aerobic organisms, can also be produced as a result of mining activities, although the formation of cyanometal complexes interferes with this pathway and may also inhibit the formation of thiocyanate (Johnson, 2015). Cyanide reacts with free sulphur and sulphide minerals (e.g. chalcopyrite, chalcocite and pyrrhotite), which are sometimes present in the natural environment and mining

materials to form thiocyanate (Kuyucak and Akcil, 2013). While less toxic than free cyanide, thiocyanate has been shown to be toxic to various aquatic organisms including fish (Rubec, 1986; Lanno and Dixon, 1996). It is also more stable than free cyanide and so persists in the environment for longer with the potential to disperse over a wider area (Gould et al., 2012).

Mining activities have been associated with large scale pollution events which can introduce huge quantities of cyanide compounds into the environment over a short time period. In 1995, almost three million cubic meters of cyanide contaminated wastewaters were released into the Omai river when a dam breached in Guyana, and another dam breach in Romania in 2000 resulted in 100 tonnes of cyanide being released into the Danube (Hilson and Monhemius, 2006). Such pollution events are associated with large scale fish kills in rivers all over the world, however, they are infrequent, with generally no more than two spills per year (Ketcheson and Fingas, 2000).

Rivers are the major pathway for the transfer of cyanide from inland mining activities to the marine environment. In Ecuador, water samples taken from the Puyango river showed undetectable levels of free cyanide upstream of gold and silver mining activities, whilst a cyanide concentration of 280 µg l^{-1} was detected several kilometres downstream and remained at detectable levels for up to 100 km downstream from the source (Guimaraes et al., 2011). Of the cyanide detected, half of it was dissolved and half was associated with suspended particles. The distance down river that cyanide can still be detected is likely to depend on factors such as the volume and concentration of cyanide introduced, the species of cyanide present, the volume and flow rate of the river and the pH of the water.

Some of the most abundant cyanometal complexes associated with gold mining effluent are those formed with iron, such as ferricyanide and ferrocyanide (Moran, 1999). This reflects the abundance of iron in the natural environment. This reaction is reversible and under acidic conditions these complexes will dissociate to release free cyanide. Whilst iron cyanide complexes are relatively stable at an environmentally relevant pH, they will undergo photo dissociation in response to light exposure. Measurements taken by Johnson et al., (2002) showed a decrease in iron cyanide concentration downstream of mining activities during daylight hours accompanied by an increase in Weak Acid Dissociable (WAD) cyanide in the order of 0.5 mg l^{-1} (500 µg l^{-1}). The greatest measured iron cyanide concentration was recorded during the hours of darkness and WADs during daylight, suggesting a diurnal pattern in the presence of different forms. As a result, diurnal peaks in free cyanide and WADs may be expected to occur in watercourses downstream of mining activities. Strongly bonded cyanometal complexes such as the iron cyanides existing in non-acidic conditions, in the absence of light, may be present in mining effluent for many years. Both cyanide and

cyanometal complexes have some affinity for organic carbon and inorganic constituents in soils and sediments, such as aluminium, iron and manganese oxides. However, it has not been determined how much cyanide is deposited in this manner.

Due to the dissociative effect of light exposure on iron cyanide complexes it is expected that the rate of free cyanide release will be greatest in shallow, sunlit waters with low turbidity. In these environments, light can penetrate the water column to a greater depth and iron cyanide complexes would be expected to more rapidly dissociate during daylight hours. In deep, dark, turbid waters, where light is only able to penetrate the surface layers, it would be expected that the rate of free cyanide release will be reduced. In these environments iron cyanide complexes would be expected to remain more stable, potentially persisting until either the turbidity or pH decreases sufficiently to release free cyanide.

Some cyanometal complexes are insoluble and form solid compounds which precipitate in aqueous solutions. One of the most common of which is another iron cyanide complex, $Fe_4[Fe(CN)_6]_3$ also called Prussian blue (Dzombak et al., 2006). It has been recorded as present in sediments associated with mining activities. Under increasingly alkaline conditions it becomes more water soluble and will dissociate to produce ferricyanide and ferrocyanide ions (Johnson, 2015). In summary, mining activities utilising cyanide have the potential to introduce a wide range of different cyanide compounds into the surrounding water and sediments.

3.2.3. Cyanide fishing

Cyanide, in the form of either sodium cyanide (NaCN) or potassium cyanide (KCN), is one of the methods used to collect live fish from coral reefs for both the aquarium trade and the live reef fish food trade (LRFFT) (Bruckner & Roberts, 2008). The technique is perceived to facilitate the capture of live fish when compared to use of traditional methods such as nets and traps, making it more efficient and financially rewarding (Halim, 2002). Its use has been reported in at least 15 countries including the Philippines, Indonesia, Malaysia, Maldive Islands, Papua New Guinea, Sri Lanka, Thailand and Vietnam (Jones et al., 1999) and is now spreading to other parts of the world (Giuliani et al., 2004). However, it is possible that the technique occurs wherever fish are collected for the live fish industry given that it is considered to be a more efficient and financially rewarding method of collection.

Cyanide fishing used for aquarium trade collection is achieved by mixing one or two crushed cyanide pellets that can be easily obtained from chemical stores, with seawater in “squirt” bottles to produce a hydrogen cyanide (HCN) solution ranging in concentration between 2 g

l^{-1} and 120 g l^{-1} (2 x 10^6 - 1.2 x 10^8 µg l^{-1}) according to bottles seized by authorities during enforcement activities (Jones et al., 1999; Rubec et al., 2001). The solution acts by interfering with oxygen metabolism in fish, blocking the key enzyme system cytochrome oxidase (Mak et al., 2005) thereby allowing divers to target and stun desirable fish which would otherwise be difficult to collect. Once at the surface on board the fishing vessel, the fish are transferred to clean, cyanide-free seawater for recovery (Wabnitz et al., 2003). The technique used for the LRFFT differs and is described in Calado et al., (2014) as being on a larger-scale with a greater impact to the surrounding non-target environment. Fishermen fill 100–200 L plastic/metal drums with a cyanide solution and load them onto small fishing boats. Once at the fishing site, divers set up gill or barrier nets around the coral reef before the boat circles the reef releasing the cyanide solution, stunning fish in the area and allowing them to be easily collected by hand-netting from boats or by divers. Due to the illegal, unreported and unregulated (IUU) nature of cyanide fishing, it is impossible to quantify the number of fish captured for the ornamental fish industry using this method. However, an estimate of approximately 70 percent by Mak (2003), though not necessarily applicable today, does show how prevalent it has been in the recent past.

Cyanide is toxic to fish and although they are able to detoxify sub-lethal doses (Eisler, 1991), exposure can irreversibly effect their condition potentially resulting in death (Way et al., 1988). Mortality rates of fish collected using cyanide for the aquarium trade are reported to range between 5 to 75 percent within hours post-collection (Wabnitz et al., 2003) to an estimate exceeding 90 percent mortality from reef to the retailer (Rubec, 2001). Acquiring recent, robust data on cyanide-related mortality of traded live reef fish is however difficult given the illegal, unreported and unregulated nature of the method. Not only have the population status of moderately abundant target fish species, such as *Cheilinus undulates* (humphead wrasse), been reduced with increasing prevalence of cyanide fishing in Southeast Asia (Hodgson, 1999), cyanide has also been reported to impact non-target organisms including reef invertebrates and corals. Corals exposed to cyanide have been found to have a reduction or cessation of respiration, with the most obvious response being the dissociation of the coral-algal symbiosis which results in discolouration or bleaching (Jones et al., 1999). The ecological consequences of this includes a reduction in phototrophic potential, a decrease in growth rates and a decrease in fecundity with recovery of symbiosis taking between six months to one year or more (Jones, 2006). Cyanide fishing also poses a risk to human health as fishermen deploying the solution are regularly diving in close proximity to the poison, and the technique is often associated with “hookah compressor” or compression diving (oxygen pumped from the surface through a hosepipe to the diver below) (Halim, 2002; *pers.comm* Rose-Liza Eisma-Osorio, Professor of

Environmental Law, Philippines, July 2017). Given what is known of the impacts of cyanide fishing on target species, associated reef habitats and species and human health, use of the technique is banned in most exporting countries with 77 percent of 18 key exporters having anti-cyanide laws (Dee et al., 2014).

Both sodium and potassium forms of cyanide which are used for fishing would be expected to rapidly dissociate to release free cyanide, which at the typical pH of seawater (between pH 7.5 and 8.4), would predominantly be in the form of hydrogen cyanide. The cyanide ion is less abundant than hydrogen cyanide at this pH and is less volatile and more persistent as a limited amount is lost into the atmosphere. In the presence of cyanide metabolising organisms, some of the free cyanide will be transformed into cyanide metabolic products such as ammonia, carbon dioxide and thiocyanate and if seawater temperatures are above 25°C, hydrogen cyanide will immediately start evaporating into the atmosphere. The rate of this volatisation is dependent on temperature and the degree of mixing. Some hydrogen cyanide may also react with metal ions already present in the seawater to form various cyanometal complexes which would dissociate at a rate according to the species of the bonded metal. Combined with rapid dilution, it is expected that these processes would rapidly transform the original input of cyanide from the cyanide fishing event to the point where it is no longer detectable as free cyanide.

KEY POINTS:

- Cyanide is used in a wide range of industrial processes around the world including among others, the extraction of gold and silver from ores, petroleum refineries, the manufacture of synthetic fibres such as fertilisers and pesticides, and poisoning pests in fish farm ponds.
- Metal processing and mining activities are considered to be the greatest anthropogenic source of cyanide entering the natural environment. Cyanide concentrations of 280 µg l^{-1} have been recorded several kilometres downstream of a metal processing plant in Ecuador.
- Cyanide fishing is used for collecting live fish for the aquarium trade and live fish for food trade. Fish are exposed to a cyanide and seawater solution ranging in concentration between 2 g l^{-1} and 120 g l^{-1}.
- The rate of cyanide breakdown in the aquatic environment is dependent on water temperature and the degree of mixing. Cyanide may also react with metal ions already present in the water to form compounds which would breakdown at a rate depending on which metal was present.
- In dynamic aquatic environments, rapid dilution and reaction with metals present in the water is expected to rapidly transform the original input of cyanide to the point where it is no longer detectable as free cyanide.

3.3. Anthropogenic cyanide in the coral triangle: a high priority regional case study

Many of the anthropogenic pathways for cyanide entering the aquatic environment, which are described above, are of particular relevance in the coral triangle region. The coral triangle situated in the western Pacific Ocean is a biodiversity hot spot exhibiting some of the richest marine flora and fauna in the world. The area encompasses Indonesia, Malaysia, the Philippines, Brunei, Timor L'Este, Papua New Guinea and the Solomon Islands (Figure 3) and contains the highest diversity of reef building corals and coral reef fishes found anywhere in the world with 76 percent and 37 percent of species known to science respectively (Figure 4) (Green et al., 2008). Three hundred and seventy million people live within this region with the majority of the population directly reliant on the marine environment for the provision of food and employment (Foale et al., 2013).

Production of cassava in Asia is increasing year on year, from around 20 million tons in 1965 to over 80 million tons in 2010 (Cock, 2011) and in 2008/2009, Asia accounted for 35 percent of global production (81,620,000 tons) with several counties in the coral triangle amongst the highest Asian producers. Indonesia is the largest producer, however Malaysia, Philippines and Timor L'Este all produce cassava on a large industrial scale, with coral triangle countries contributing 24,560,000 tons of the Asian total in 2008/09 (Cock, 2011).

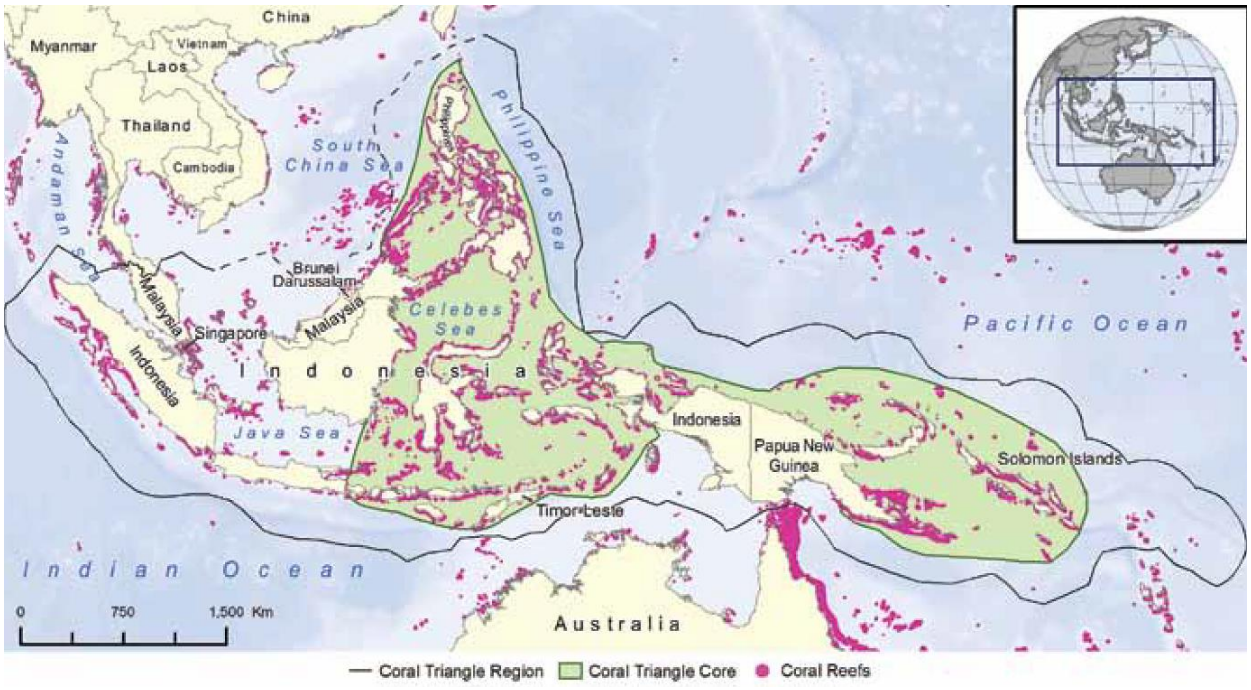

Figure 3. A map of the coral triangle region and locations of coral reef habitat locations (Burke *et al.,* 2011).

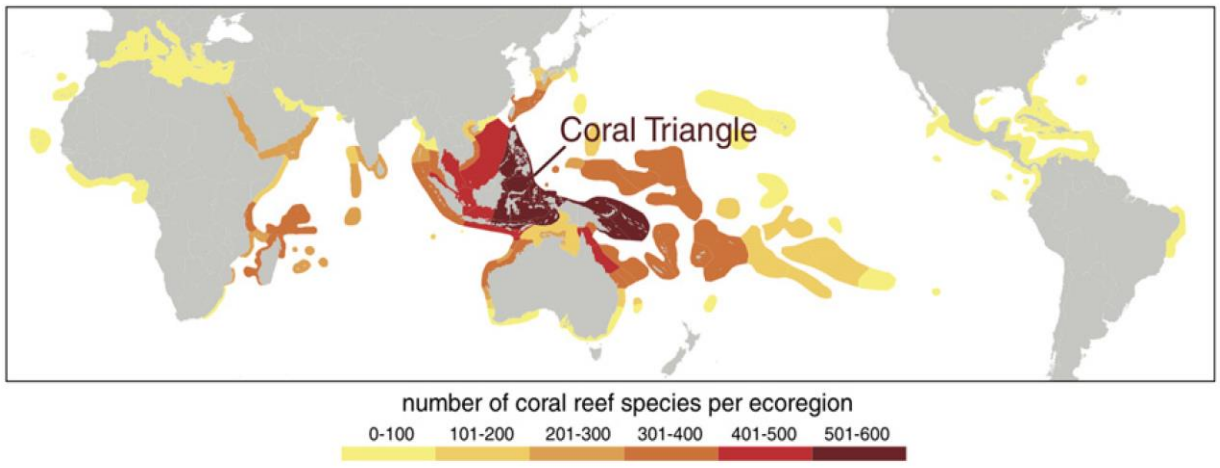

Figure 4. Biodiversity of reef building corals, showing the location of the Coral Triangle. Colours indicate total species richness per eco-region (Foale et al., 2013, adapted from Veron et al., 2009).

Gold mining is also a major industry in the coral triangle with Indonesia and Papua New Guinea accounting for 6 percent and 3 percent of global gold production respectively in 2004

(Mudder and Botz, 2004). In poorly regulated mines, and typically in developing countries, mercury is often used as an amalgam to extract the precious metals from ores in place of or in addition to cyanide. Like cyanide, mercury is toxic to humans and wildlife and as such it has its own environmental implications when introduced into the wider environment. Cyanide and mercury are frequently used in combination to increase the gold recovery rate which is more efficient when compared with using either chemical on its own (Castilhos et al., 2006; Guimaraes et al., 2011).

In the past two decades, the region of Diwalwal on the island of Mindanao in the Philippines has experienced a gold rush with many small-scale mines appearing. This led to high instances of human mercury intoxication in the miners themselves, as well as intoxication of people living in the local area downstream from the mines (Drasch et al., 2001), providing evidence that mining waste waters are not adequately contained and are reaching the wider environment most likely through river systems. As mercury and cyanide are used in combination for mining gold, it is reasonable to suggest that cyanide contaminated waste waters are also being released into the wider environment in effluent. The Buyat-Ratatotok mining district in North Sulawesi, Indonesia is another example of an area of intense gold mining activity which has been linked to heavy metal contamination of surrounding coral reefs and marine sediments (Edinger et al., 2008). A study by Lu in 2014 specifically tested for the presence of cyanide in the Benguet mining district of the Philippines and found that 92 percent of water samples collected from different freshwater sources in the area contained detectable concentrations of cyanide (mean 5.4 µg l^{-1}) (N = 98) . However, none of these samples exceeded the maximum allowable limit for free cyanide (200 µg l^{-1}) according to the US Environmental Protection Agency (USEPA) standards.

In the small island states of the coral triangle, mining activities have to be situated close to the ocean. The intensively mined Buyat-Ratatotok district in North Sulawesi, Indonesia, is situated no more than 15 km from the ocean and the mining district Diwalwal in the Philippines is located only 30 km from the coast. When riverine inputs of cyanide reach the ocean, rapid dilution will reduce its concentration considerably and it is likely that this cyanide input will largely be contained within a plume, on entry, the direction of which will be determined by physical parameters such as wind strength and direction, tidal activity, and local currents. With this in mind, coastal reefs, particularly those close to estuaries with rivers in the catchment area of mining activities, are at increased risk of cyanide exposure through metal and mining industry.

As the epicentre of marine biodiversity, the coral triangle region includes the two largest players in the export of live reef fish destined for the marine aquarium trade, Indonesia and

the Philippines. Marine ornamental fish were first exported from the Philippines in 1957 when fishes were caught using small hand nets and traps on coral reefs (McAllister et al., 1999). It was not until the early 1960s that the incidence of cyanide fishing was first documented when a local fish collector began to spray sodium cyanide on reefs to stun fish making them easy to capture (Rubec, 1988). It has been hypothesised that the Filipino fisher may have learned about the action of cyanide on fish from the 1958 US Fish and Wildlife Service scientific report by Bridges or through the use of cyanide as a method of milkfish pond decontamination in the Philippines, and adapted its use as a method of fishing (McAllister et al., 1999). By the 1970s, the practice had spread throughout Southeast Asia and into the Pacific Islands, not only to collect live fish for the aquarium trade but also for the live reef fish food trade (LRFFT) by the 1980s (Halim, 2002; Bruckner & Roberts, 2008).

Estimates suggest that by the mid-1980s, more than 80 percent of all fish harvested in the Philippines to supply the aquarium trade were collected using cyanide and by the mid 1990s, about 90 percent of vessels transporting live fish in the eastern islands of Indonesia had cyanide on board (Rubec, 1986; Rubec, 2001; Wabnitz et al., 2003). Efforts to mitigate against the use of cyanide in the Philippines began in the 1990s, when the government, in collaboration with the International Marine life Alliance, implemented a permitting system and cyanide testing initiative. They established a number of Cyanide Detection Laboratories (CDL) to test reef fish at export (Barber and Pratt, 1997; Barber, 1999; Rubec et al., 2001). The method employed by the testing labs was time-consuming and required the fish to be sacrificed, and therefore the accuracy and robustness of the testing units was challenged (Mak et al., 2005; Bruckner and Roberts, 2008). Currently, only four test laboratories are operational in the Philippines and these are not purely used for cyanide detection but also for other testing purposes (*pers.comm* Rose-Liza Eisma-Osorio, Professor of Environmental Law, University of Cebu, Philippines July 2017). Complex supply chains, the problematic nature of detecting cyanide with increasing time post collection, and the input of cyanide in the marine environment via other anthropogenic pathways such as mining, present priority challenges for law enforcement when gathering robust evidence of cyanide fishing activity (Dee et al., 2014).

KEY POINTS:

- The coral triangle region hosts some of the richest marine flora and fauna in the world but it is also the home for many industries which use cyanide in their production, making it a perceived high risk area for cyanide entering the aquatic environment.
    - Cassava production and metal mining both utilise cyanide. Asia accounts for around 35 percent of global cavassa production (81,620,000 tons) while Indonesia and Papua New Guinea account for 6 percent and 3 percent of global gold mining production.
    - Release of cyanide waste water from industry is therefore of high risk in the region.
    - Industrial cyanide is also readily available to supply these industries in the region.
    - Capture of live reef fish destined for the marine aquarium trade and live fish for food industry is prevelant in the region given its diversity of reef fish. Use of cyanide as an illegal method of harvesting live fish began in the region and its use continues today.
- Complex supply chains, the problematic nature of detecting cyanide with increasing time post collection, and the input of cyanide in the marine environment via other anthropogenic pathways such as mining, present priority challenges for law

3.4. Detecting cyanide fishing

In light of the challenges in gathering robust evidence to demonstrate the use or absence of cyanide fishing, the USA as the world's largest importer of marine aquarium fish (Rhyne et al., 2012) hosted the International Cyanide Detection Testing Workshop, funded by the NOAA Coral Reef Conservation Program and Kingfisher, in Florida in 2008. The workshop brought together a range of cyanide technical specialists including forensic chemists, government and non-government representatives from the USA and three of the biggest marine fish exporting countries: the Philippines, Indonesia and Vietnam. The aim was to encourage best practise in live fish harvesting and to mitigate the use of cyanide to capture reef fish for both the marine aquarium trade and the live reef food fish trade. The outcome of the workshop was the development of nine priority recommendations (Table 5) for further research as required to implement a robust method for detecting cyanide fishing activities (Bruckner & Roberts, 2008). The following sections of this report will focus specifically on Recommendation One and our current scientific understanding of cyanide metabolism in

marine fish and the determination of environmental background levels. This report will not, however, address the effect of cyanide detection following the freezing of samples as it is deemed beyond the remit of this report and covered in the recent Cefas report by Losada & Bersuder (2017 unpublished report).

Table 5. Summary recommendations from the Proceedings of the International Cyanide Detection Testing Workshop in the USA in 2008 (Bruckner & Roberts, 2008).

	Recommendation	Key challenges
1	Determine the pharmaco-kinetics of cyanide	Determine detectable level of cyanide and metabolites in coral reef fish and how levels change over time following exposure. Determine detectable environmental background levels. Establish the effect of cyanide detection following freezing of samples
2	Validation of the Ion Selective Electrode (ISE) cyanide detection test	Establish the sensitivity, specificity, accuracy and precision of the ISE method which was commonly used in the Philippines between 1993 to 2001.
3	Establish networks of Cyanide Detection Testing (CDT) laboratories at points of export	Develop standardized testing at export points (airports). Include poison-free capture testing in export licenses to ensure compliance and self finance the cost of testing.
4	Obtain funding for establishment and maintenance of CDT networks in exporting countries	Obtain funding from, for example, World Bank, Asian Development Bank or international NGOs.
5	Establish training, quality assurance and proficiency programs for CDT labs and chemists	Development of a training program. Development of an ISO accredited quality assurance program.
6	Explore options for a simple field test at points of collection	Develop a field test to detect presence of cyanide (not concentration) at collection points and holding facilities.
7	Testing and accreditation plan for cyanide free fish in exporting countries	Establish a certification scheme funded by suitable export license fees. Develop a cyanide testing database and link license renewal to the testing program.
8	Implement complementary legislation against cyanide fishing in importing countries	
9	Develop a cyanide testing program in the United States	

4. Metabolism of cyanide in fish

Recommendation One from the Cyanide Detection Testing Workshop 2008 highlighted the requirement for further research to determine the pharmaco-kinetics of cyanide. Specifically, the need for increased understanding of how fish metabolise cyanide, as well as determining detectable background levels of cyanide in the environment in a bid to differentiate background environmental cyanide signals from those resulting from targeted cyanide fishing. Ultimately, filling in knowledge gaps in these areas could lead to the identification of a biomarker capable of determining if a fish has been caught using cyanide fishing techniques. Results of literature mining (including the key search terms used) on the topic of cyanide metabolism by fish since the 2008 workshop are shown in Table 6. Literature from both before and after the workshop are considered in the following section.

Table 6. Literature available since the 2008 cyanide detection workshop addressing the recommendations for further research to determine the pharmaco-kinetics of cyanide.

Search term	Search engine	Literature cited
Cyanide metabolism fish	Google Scholar / Science direct	Logue et al., 2010; Dube and Hosetti, 2011; Shwetha et al., 2012; Dube and Hosetti, 2010; Dube et al., 2013; Vaz et al., 2012; Emuebie et al., 2010; David et al., 2010; Al-Ghanim and Mahboob, 2012; Sadati et al., 2013; Prashanth et al., 2011a/b; Ramzy, 2014; Nath et al., 2013; David and Kartheek, 2016; Dube and Hosetti, 2012; Baghshani and Aminlari, 2012; Authman et al., 2013; Dube et al., 2014; Manjunatha et al., 2015; Prashanth et al., 2012; David et al., 2008; Shwetha et al., 2012;
Cyanide toxicokinetics	Google Scholar / Science direct	Jackson and Logue, 2017; Mitchell et al., 2013a; Bhandari et al., 2014;
Cyanide toxicity fish	Google Scholar / Science direct	Guimaraes et al., 2011
Thiocyanate fish	Google Scholar / Science direct	Gould et al., 2012; Herz et al., 2016; Jack et al., 2015
Rhodanese fish cyanide	Google Scholar / Science direct	Calado et al., 2014;
Cyanide fish ATCA	Google Scholar / Science direct	Bruckner & Roberts, 2008;

Cyanide which enters the aquatic environment may exist in the water or sediment, forming various compounds until it eventually degrades or is taken up by organisms within the ecosystem. Fish, both marine and freshwater, are particularly sensitive to cyanide exposure (Table 1 and Table 2). If taken in by an organism, cyanide can be biologically transformed

and metabolised. Metabolism describes a two-phased process in which chemical transformations within cells of living organisms occur to change compounds into products called metabolites which are easier to excrete (Figure 5). The key purposes of metabolism are the conversion of food/fuel into energy or building blocks such as for proteins and to eliminate waste. Basic metabolic pathways are strikingly conserved even between distinctly different taxonomic groups.

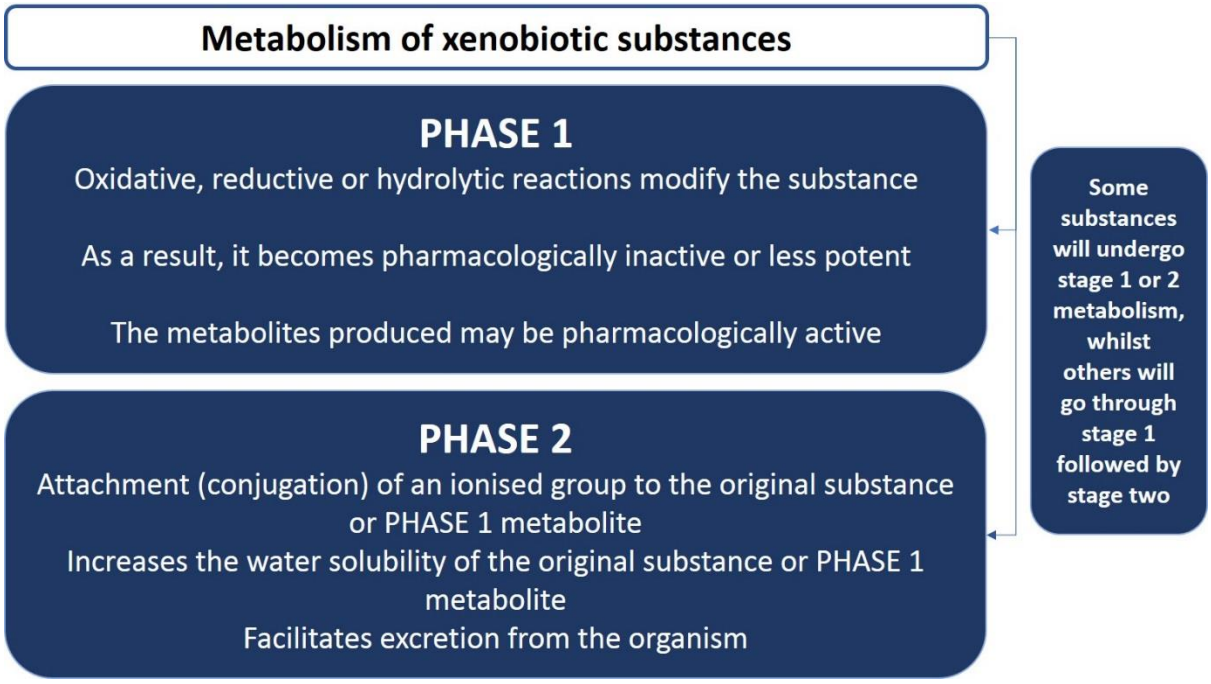

Figure 5. Phase one and two of the process of metabolism.

At low doses, cyanide temporarily stuns the fish making them sluggish or immobile and therefore easier to capture whilst at higher doses it can rapidly cause mortality. If the concentration of cyanide is insufficient to cause immediate death, a range of sub-lethal effects are associated with cyanide exposure. Cyanide can be absorbed via the gills and the intestine. Hydrogen cyanide in seawater is readily absorbed through the epithelial tissues present in abundance in the gill structures of marine organisms and are therefore thought to be the primary absorption site (Bellwood 1981b; cited in Rubec, 1986; Mak et al., 2005). Cyanide which enters the fish is then distributed around the body via the blood stream. It can easily cross cell membranes where at the physiological pH inside the cell it is converted to free cyanide, before concentrating in tissues and organs with the highest blood flow such as the liver and spleen (Bellwood 1981b; cited in Rubec, 1986; Pablo et al., 1997b; Logue et al., 2010). At low exposure concentrations cyanide is quickly detoxified by specific metabolic pathways (Ramzy, 2014). It does not bioaccumulate in an organisms fatty tissues due to its solubility in water and low log kow value (ratio of solute concentration between water and

octanol which is used as a measure of hydrophobicity), therefore it can be readily metabolised and excreted. The persistence of cyanide in living organisms is determined by the exposure concentration and duration and the rate of detoxification (Ramzy, 2014).

4.1. Thiocyanate

The major metabolic pathway for cyanide detoxification is its conversion to the metabolite thiocyanate (SCN) and subsequent excretion in urine. This accounts for up to 80 percent of cyanide removal (Logue et al., 2010; Ramzy, 2014). The rapidity at which cyanide is converted to thiocyanate means that cyanide has a short half-life once absorbed (Logue et al., 2010). Ramzy (2014) reported a half-life of cyanide in Nile Tilapia (*Oreochromis niloticus*) of approximately one hour. In contrast, the half-life of thiocyanate is considerably longer and is to some extent dependent on the excretion rate of the organism in question. The enzyme rhodanese, also called thiosulfate sulfurtransferase which is found in mitochondria facilitates the production of thiocyanate via the conjugation of cyanide and sulphur from a sulphur donor such as thiosulfate (Figure 5) (Rubec, 1986). The limiting factors in this metabolic pathway are therefore the abundance of rhodanese enzymes and the availability of sulphur donors. The formation of thiocyanate is a reversible reaction which is facilitated by the enzyme thiocyanate oxidase, however the pathway is strongly weighted in the direction of forming thiocyanate (Egekeze and Oehme, 1980; Logue et al., 2010). Thiocyanate is less toxic than free cyanide although it does have a small amount of accompanying toxicity itself. It is an example of a phase two metabolic process whereby the conjugation of cyanide with ionised sulphur increases the water solubility of the resulting metabolite enabling its excretion via the urinary system.

A number of studies carried out over three decades ago documented increasing levels of thiocyanate in fish tissues following exposure to cyanide, such as those by Raymond (1984) and Bois (1988). Although thiocyanate is rapidly formed following exposure to cyanide, the rate of excretion is considerably slower and it therefore has the potential to bioaccumulate in living organisms (Raymond, 1984; Bois, 1988; Brown et al., 1995; Lanno and Dixon, 1996) supporting observations in those early studies. Brown et al., (1995) exposed rainbow trout (*Oncorhynchus mykiss*) to a waterborne thiocyanate concentration of 39800 µg l^{-1} and observed a bio concentration factor of 1.6 in a 20-day exposure. The authors showed that rainbow trout with a blood plasma thiocyanate concentration of 64000 µg l^{-1} could return to pre-exposure concentrations after 8-16 days of depuration once exposure to the waterborne thiocyanate ceased. These rates of thiocyanate depuration corrobarated with what Bois (1988) had reported in rainbow trout (7-15 days after a 30-day exposure to 10 and 20 µg l^{-1} hydrogen cyanide). Lanno and Dixon (1996), exposed juvenile rainbow trout (*Oncorhynchus*

mykiss) to 69,957 µg l^{-1} (720 µmol) potassium thiocyanate (KSCN) for 16 weeks and observed mild toxicity characterised by diffuse colloid goitre, slightly reduced growth, decreased hepatosomatic and splenosomatic indices and haematocrit. Exposure to 5,169 µg l^{-1} (89 µmol l^{-1}) thiocyanate, 20.8 and 63.8 µg l^{-1} (0.32 and 0.98 µmol l^{-1}) potassium cyanide elevated plasma thiocyanate levels above control values but did not affect any of the other parameters measured. Thiocyanate bioaccumulation has been linked to the occurrence of sudden death syndrome (SDS) in fish which appears to be stimulated by stress or high energy movements like those potentially experienced during shipment (Rubec, 1986).

Bois (1988), Brown et al., (1995) and Lanno and Dixon (1996) examined the effects of long-term exposure of freshwater fish to low concentrations of thiocyanate. Such levels of exposure are more likely to occur if a fish inhabits waters polluted by cyanide or thiocyanate, as opposed to the direct, high concentration, short-term exposure that would be expected as a result of cyanide fishing. Thiocyanate is present in seawater, particularly in inshore waters, and fish living in these environments may have long term exposure to low concentrations of thiocyanate in the range of 5 – 22 µg l^{-1} (Rong et al., 2005; Silva et al., 2011). The source of this thiocyanate input in seawater is not known and may be as a result of natural and/or anthropogenic inputs. Prolonged exposure to thiocyanate as a result of capture using cyanide fishing techniques may also occur if fish are kept in relatively small volumes of seawater post capture. If water is changed infrequently, thiocyanate concentrations will be elevated due to excretion.

Thiocyanate bioaccumulation in marine fish has not been described in the same detail as the studies described above on rainbow trout (*Oncorhynchus mykiss*). There are considerable differences in the osmoregulatory physiological demands between fish living in marine and freshwater environments, therefore, urinary excretion rates of thiocyanate are likely to be higher in freshwater fish resulting in a reduction in the associating half-life when compared to marine fish (Ramzy, 2014). As a result there is potential for marine fish to bioaccumulate thiocyanate to a greater extent with increased depuration times compared to freshwater fish.

In 2012, Vaz et al. exposed clownfish (*Amphiprion clarkii*) to concentrations of 12.5 and 25 mg l^{-1} (12500 and 25000 µg l^{-1}) of cyanide for 60 seconds and measured thiocyanate concentrations of up to 6960 and 9840 µg l^{-1} respectively in the artificial tank water after 28 days following exposure. These data support Rubec et al.'s (2003) observation that clown fish do not rapidly metabolize cyanide into thiocyanate, and that thiocyanate is excreted in the days and weeks following exposure rather than the first few hours. Importantly, Vaz et al., (2012) provide an indication that thiocyanate concentrations in fish holding seawater can be expected to be in the lower µg l^{-1} range. It should be noted that studies by Vaz et al.,

(2012) used artificial seawater without renewal during the test period which may have enhanced the persistence of thiocyanate and hence increased its exposure. Herz et al., (2016) attempted to repeat these studies but using daily renewal of test solutions and could not detect thiocyanate.

It is important to note that in fish, thiocyanate may not be exclusively produced following exposure to cyanide. Brown et al., (1995) documented rainbow trout blood plasma thiocyanate concentrations of 2000 - 3300 $\mu g\ l^{-1}$, in both control fish and individuals not yet exposed to either cyanide or thiocyanate. Several possibilities exist to explain this observation; either the fish were unintentionally exposed to cyanide, sample cross contamination occurred, or thiocyanate is also produced *in vivo* via metabolic pathways in addition to cyanide. Brown et al., (1995) reported that the observed thiocyanate concentrations were similar to those reported in control rainbow trout by Heming and Blumhagen (1989), so it is unlikely that this observation is a result of sample cross contamination. The presence of thiocyanate in control fish raises an important issue, namely the potential for false positives, detectable by analytical tests designed to determine cyanide exposure. However, exposure to both cyanide and thiocyanate has been shown to drastically elevate thiocyanate concentrations above this baseline value (Raymond, 1984; Bois, 1988; Brown et al., 1995; Lanno and Dixon, 1996). Therefore, presence of thiocyanate in fish is not necessarily indicative of cyanide exposure and the observed concentration must be significantly raised above baseline levels to signify recent cyanide exposure. Baseline thiocyanate concentration setting in different geographical areas and in different species of fish is necessary to determine if the level of thiocyanate is above background levels.

The analysis of thiocyanate appears to be a suitable indicator of cyanide exposure in marine and freshwater fish but it is not without its flaws. Specifically, limited information on the retention times of thiocyanate following an exposure akin to cyanide fishing practices, the variability of background concentrations of thiocyanate in fish and in the environment, and whether inputs of thiocyanate from other anthropogenic activities induce a similar response to targeted cyanide fishing remain gaps in our knowledge. As a consequence of *in vivo* production of thiocyanate not associated with direct cyanide exposure (Heming and Blumhagen, 1989; Brown et al., 1995), and the background levels of thiocyanate observed in inshore seawater habitats (Rong et al., 2005; Silva et al., 2011), it is critical that we understand the baseline concentrations of thiocyanate in different (1) species of fish, both marine and freshwater (2) sizes and life stages of fish (3) sexes of fish (4) aquatic environments (5) matrices taking into account seasonal variations.

In contrast to the formation of thiocyanate very little is known about the alternative pathways of cyanide metabolism in fish. Key aspects of these pathways such as; their timeframes, persistence and behaviour following cyanide exposure are lacking. Further work is required to comprehensively evaluate the gap in additional idiosyncratic metabolic pathways in fish. Fish species are both numerous and very diverse and there may be considerable variation between different taxonomic groups inhabiting the same habitats.

4.2. Other biological indicators of cyanide exposure

As a result of its mode of toxicity, and providing the exposure is not lethal, cyanide elicits a range of sub-lethal impacts on fish such as alterations in enzyme activity, behavioural changes and tissue damage (Bellwood, 1981a; Rubec, 1986; Dube and Hosetti, 2012; Shwetha et al., 2012). These could provide another avenue for the identification of a biomarker which is capable of determining whether a fish has been caught using cyanide fishing techniques. Known products and activities which are altered in marine and freshwater fish by exposure to cyanide are illustrated in Figure 6 and described in the following section.

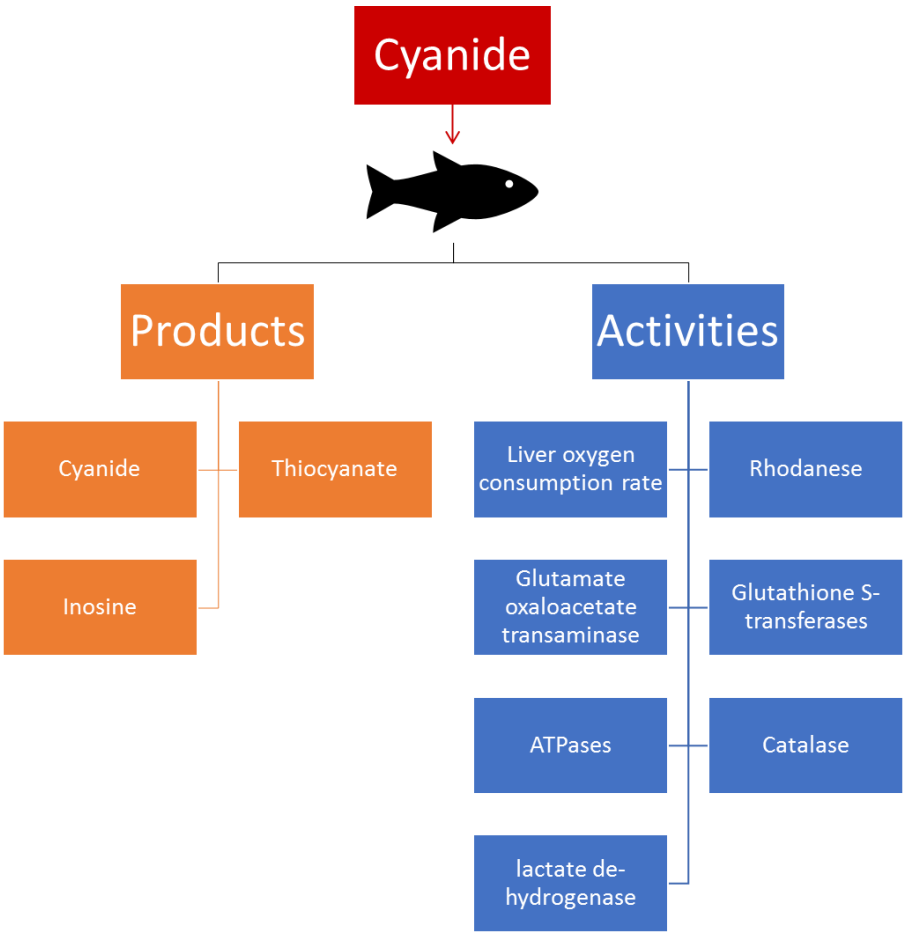

Figure 6. Known products and activities in marine and freshwater fish significantly altered by exposure to cyanide.

An early study by Bellwood (1981a), cited in Rubec (1986) on the chronic effects of short term cyanide exposure to Domino Damsels (*Dascyllus trimaculatus*), was designed to mimic cyanide fishing. The authors observed a loss of inner stomach lining; gastric mucosa followed by cell damage to the stomach and intestines of exposed fish, and it was hypothesised that cyanide caught fish may be incapable of digesting their food which may explain observed occurences of starvation following cyanide exposure (Bellwood, 1981a; cited in Rubec, 1986). However, another study mimicking cyanide fishing, this time exposing Neon Damselfish (*Pomacentrus coelestis*) to cyanide, found that exposure had no detectable effects on the intestinal mucosa (Hall and Bellwood, 1995). This study also examined the effect of stress in combination with cyanide exposure, however, up to 25 percent mortality was observed in the control fish suggesting caution is required when interpreting their results. Raymond (1984) examined the chronic effects of a 20-day cyanide exposure at three concentrations (10, 20 and 30 µg l^{-1} hydrogen cyanide) to Rainbow Trout (*Oncorhynchus mykiss*). They found that thiocyanate levels in blood plasma rapidly increased within 24 hours of exposure to cyanide and bioaccumulated for the duration of the study. The Hepatosomatic Index (HSI) which is the ratio of liver weight to body weight and provides an indication of energy reserves in an animal was reduced by cyanide exposure, and a 60-80 percent reduction in liver cytochrome oxidase activity was observed and attributed to cyanide exposure.

More recent studies investigating the effect of sub-lethal cyanide exposure have provided additional detail. Inosine, for example has been identified as a possible biomarker of cyanide exposure in zebrafish (*Danio rerio*). It has been documented to briefly increase following exposure to cyanide, before decreasing after 60 minutes of recovery (Nath et al., 2013). Exposure to cyanide can have sex-specific impacts. Male and female African catfish (*Clarias gariepinus*) both showed increased levels of glutamate oxaloacetate transaminase (Authman et al., 2013) following cyanide exposure, however, female fish exhibited reduced serum levels of glutamate pyruvate transaminase, total plasma protein, phosphoprotein phosphorus, vitellogenin gene expression and estrogen receptors, whereas male fish showed a significant increase in phosphoprotein phosphorus and vitellogenin gene expression (Authman et al., 2013). In addition, the activities of both ATPase and catalase have been shown to be depressed by cyanide exposure (David et al., 2008; Dube and Hosetti, 2012; Shwetha et al., 2012; David and Kartheek, 2016). Sadati et al., (2013) examined sub-lethal cyanide exposure to common carp (*Cyprinus carpio*) in relation to biochemical blood parameters that are indicative of cell damage. Exposure to 100 and 200 µg l^{-1} potassium cyanide for a two-week period caused significant increases of aspartate aminotransferase and lactate dehydrogenase activities and creatinine concentration when

compared to a control group. The observed increase in serum and tissue levels of lactate dehydrogenase (LDH) activities are typical of lactic acidosis which is caused by the cyanide induced inhibition of aerobic metabolism (Sadati et al., 2013). An increase in LDH activity has also been documented in other species of carp (*Labeo rohita, Catla catla* and *Cyprinus carpio*), catfish (*Clarias gariepinus*) and tilapia (*Oreochromis niloticus*) (David et al., 2010; Al-Ghanim and Mahboob, 2012; Dube et al., 2013; Dube et al., 2014; Manjunatha et al., 2015). Such alterations in the biochemical blood parameters examined are indicative of damage to the liver and kidney.

Rhodanese, also called thiosulfate sulfurtransferase, is a mitrochondrial enzyme which plays a central role in cyanide detoxification facilitating the conversion of cyanide to thiocyanate (Saidu, 2004; Emuebie et al., 2010). Baghshani and Aminlari, (2012) investigated the distribution of rhodanese in liver, kidney, brain, gill and intestine tissues of four carp species; the common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), and bighead carp (*Aristichthys nobilis*). The liver and kidneys of all four species were associated with the highest rhodanese activity across all tissues tested although all tissues showed some degree of rhodanese activity. Hanawa et al., (1998) examined both the acute and long-term effects of cyanide exposure on Whitetail *dascyllus* (*Dascyllus aruanus*). Fish were exposed to 25000 and 50000 µg l^{-1} cyanide for 10, 60 and 120 seconds followed by a two and a half week recovery period. They measured blood haemoglobin content, the percent blood oxygen content, liver rhodanese activity and the liver oxygen consumption rate. Sixty and 100 percent (96h) mortality was observed after a 120 second exposure to 25000 and 50000 µg l^{-1} respectively. Of the physiological parameters tested, only liver oxygen consumption rate was altered by cyanide exposure. Whilst no significant impact on liver rhodanese activity was detected in this study, significant differences in liver rhodanese activity was observed between treatments but were not related to cyanide exposure (Hanawa et al., 1998; Baghshani and Aminlari, 2012).

Da Silva (2015) exposed 60 small and 60 medium sized clown fish (*Amphiprion ocellaris*) to different concentrations of cyanide 6.25, 12.5, 25, 50 and 100 mg l^{-1} (6250, 12500, 25000, 50000 and 100000 µg l^{-1}) for 60 seconds in artificial seawater. Ninety six hours after exposure to cyanide, fish were studied for biomarkers. They found that the induction levels of catalase activity in the liver and glutathione S-transferases (GST) activity in muscle had a relationship with the exposure concentrations to cyanide. Other biomarkers like lipid peroxidation (LPO), glutathione peroxidase (GPx) or acetylcholinesterase (AChE) activities did not show a clear relationship with exposure nor did they show different activity between the different fish sizes. All small fish showed loss of respiratory activity and sank to the

bottom of the tank after exposure to all concentrations (6250, 12500, 25000, 50000 and 100000 µg l^{-1}) and the time of recovery increased with increasing concentrations with 100 percent survival at the lowest concentration, decreasing to 70, 40, 20 and 0 percent with the increasing concentration. Medium sized fish were more tolerant. This study confirmed that vulnerability to cyanide poisoning may vary intraspecifically (e.g., specimen size) and interspecifically (even in species which are closely related phylogenetically). The clownfish (*A. ocellaris*) displayed the highest tolerance to cyanide poisoning compared to other studies with pomacentrids (Da Silva, 2015). The levels of cyanide the fish are exposed to during collection is reported to be as low as 6250 µg l^{-1} which is enough to affect behaviour and allow their easy capture.

Use of biosensors which are based on cyanide degrading enzymes such as rhodanese, represents a promising method of determining cyanide exposure in fish, (Martínková et al., 2015). However, the ubiquitous nature of rhodanese suggests that its physiological role is not exclusively confined to the detoxification of cyanide (Baghshani and Aminlari, 2012). Rhodanese located in the colon is an important enzyme in the detoxification of hydrogen sulphide, the synthesis of sulfur containing macromolecules and in the prevention of inorganic sulphide formation (Picton et al., 2002; Jack et al., 2015). For a biosensor to be used as a robust method for assessing cyanide exposure in fish, the enzyme must be exclusively activated by cyanide. Activation by other commonly occurring substances will undermine its use in this capacity.

In summary, the studies discussed above have increased our understanding to some extent regarding the 2008 cyanide detection workshop recommendation to determine the detectable levels of cyanide and cyanide metabolites in coral reef fishes and to understand how these levels change over time following exposure (Bruckner and Roberts, 2008). However, none are able to describe the metabolic pathways of cyanide in marine fish conclusively. More targeted studies are needed to understand the behaviour of different cyanide derived compounds in different fish species, and to discriminate between background levels (biological production or environmental exposure) and levels present following direct exposure during cyanide fishing.

KEY POINTS:

- Organisms can transform and metabolise cyanide into other products called metabolites which they can more easily excrete.
- In fish, cyanide is absorbed via the gills and intestine and is then distributed around the body via the blood stream, concentrating in tissues and organs with the highest blood flow such as the liver and spleen.
- Thiocyanate (SCN) is the major metabolite produced and is excreted in urine accounting for up to 80 percent of cyanide removal. It is rapidly produced following exposure to cyanide but the rate of excretion is much slower, therefore it can become concentrated in living organisms.
- Thiocyanate can also be produced within the fish, therefore, the presence of thiocyanate alone is not necessarily indicative of cyanide exposure. Concentrations of thiocyanate are however much higher following direct exposure to cyanide.
- Sub-lethal impacts on fish such as changes in enzyme activity, behaviour and tissue damage could provide a biomarker to determine if a fish has been collected using cyanide fishing. However, studies so far have demonstrated that these impacts tend to vary between different species, sexes and sizes of fish.
- More targeted studies are needed to understand the behaviour of different cyanide derived compounds in different fish species, and to discriminate between background levels (biological production or environmental exposure) and levels present following direct exposure during cyanide fishing.

5. Metabolism of cyanide in other organisms and their potential application to fish

Where mechanisms of cyanide metabolism in fish remain poorly understood, a number of studies have focused on the physiology of other organisms which may bear significant insight to biological pathways in fish. For example, major metabolic pathways are highly conserved between fish and mammals, so it is reasonable to assume that the process of cyanide metabolism is similar between the two taxonomic groups. The following section provides a summary of cyanide metabolism in such other organisms for wider consideration.

Known products of cyanide metabolism which are reported in organisms other than fish, include thiocyanate, the tautomer's ATCA (2-amino-2-thiazoline-4-carboxylic acid) and ITCA (2-Iminothiazolidine-4-carboxylic acid), cyanocobalamin (vitamin B12), α-ketoglutarate cyanohydrin (α-KgCN) and protein adducts (Figure 7) (Logue et al., 2010; Mitchell et al., 2013a; Jackson and Logue, 2017).

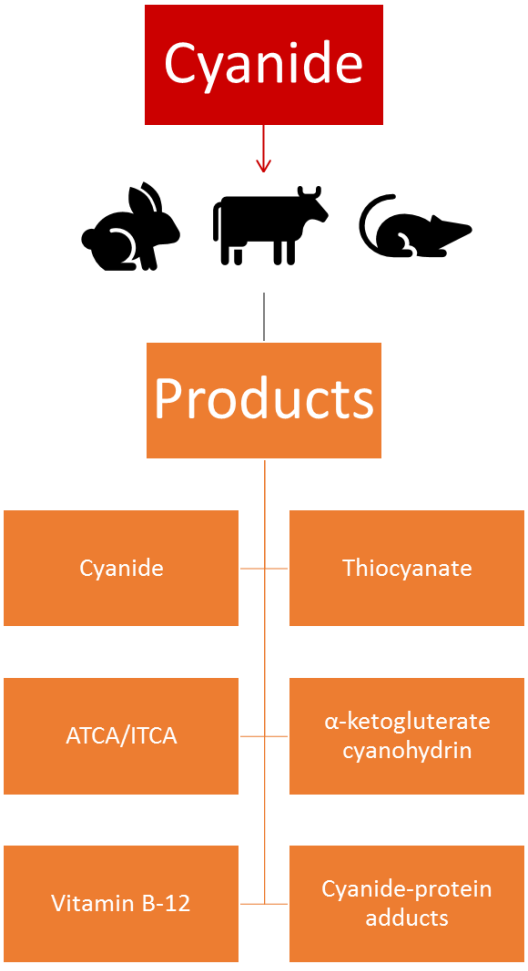

Figure 7. Metabolic products of cyanide in other organisms.

After the formation of thiocyanate, the production of 2-amino-2-thiazoline-4-carboxylic acid (ATCA) and its tautomer 2-iminothiazolidine-4-carboxylic acid (ITCA) are the second most important metabolic pathways, accounting for 15 percent of cyanide detoxification in rats (Egekeze and Oehme, 1980). The conversion of the cyanide ion into ATCA is thought to be particularly important in tissues deficient in rhodanese or when endogenous supplies of sulphur donors are exhausted, essentially the two limiting factors for the production of thiocyanate, as discussed previously (see Rubec et al., 2008). Once absorbed, cyanide reacts with L-cystine via ß- thiocyanoalanine, which has been hypothesised as an intermediary, to form ATCA (Egekeze and Oehme, 1980; Logue et al., 2010; Bhandari et al., 2014).

The reaction of cyanide with hydroxocobalamin to form cyanocobalamin (vitamin B_{12}), which is then excreted in urine, is widely reported to be one of the minor metabolic pathways of cyanide (Logue et al., 2010) although no further information on this pathway could be found during this literature review. It has also been reported that cyanide can react with endogenous a-ketoglutarate (a-Kg) to form α-ketoglutarate cyanohydrin (α-KgCN). This pathway may be used when the more established pathways are inaccessible (Mitchell et al., 2013a; Jackson and Logue, 2017).

The creation of cyanide protein adducts is a relatively recent discovery (Logue et al., 2010). In living organisms, cyanide can react with disulphide bonds in proteins to form protein-bound thiocyanate (Youso et al., 2010). In humans, cyanide has been shown to form covalent adducts with two commonly occurring blood plasma proteins, immunoglobulin G and serum albumin (Fasco et al., 2007). The half-lives of these adducts is expected to be similar to that of the parent protein if they are stable (Logue et al., 2010). The half-lives of both immunoglobulin G and serum albumin are reported to be in the region of 20 days.

Bhandari et al., (2014) examined the behaviour of cyanide and its two major metabolites, thiocyanate and ATCA in blood in three different mammals; rats (250–300 g), rabbits (3.5–4.2 kg) and pigs (47–54 kg) following cyanide exposure. All animals were intravenously administered cyanide in the form of sodium or potassium cyanide. Rats received 2 - 6 mg kg^{-1} potassium cyanide, rabbits received 10 mg sodium cyanide dissolved in 60 ml of 0.9 percent sodium chloride, swine received an average of 1.7 mg kg^{-1} potassium cyanide, or until the cessation of breathing occurred. The observed concentrations were consistent with the rapid absorption of cyanide and its subsequent conversion to thiocyanate and ATCA. The mean elimination half-life of cyanide injected subcutaneously in rats was 1.04 days, whereas cyanide intravenously injected into rabbits and swine was 177 and 26 minutes respectively. The mean elimination half-life of thiocyanate in rats ranged from 1.75 to 2.09

days, longer than the 5.8 hours previously reported by Sousa et al., (2003). The mean elimination half-life of thiocyanate in rabbits and swine could not be calculated because the thiocyanate concentrations were continuing to rise at the end of the study. The longer elimination times of cyanide and thiocyanate observed in rats is possibly a reflection of the differing study lengths and modes of cyanide delivery. Ingested cyanide, absorbed through the stomach and intestines is expected to have a slower rate of release than intravenously injected cyanide. The concentrations of ATCA following cyanide exposure were found to be highly variable, with up to a 40-fold increase observed in rabbits and a 3-fold increase observed in swine while ATCA in rats did not significantly rise above the baseline (Figure 8) (Bhandari et al., 2014). This may be as a result of physiological differences between the organisms or conceivably fewer available sulphur donors in rabbits to facilitate the formation of thiocyanate, necessitating ATCA to play a more important role in cyanide metabolism. The variability between mammalian species presented in this study highlights a substantial problem when trying to extrapolate these results to fish.

Mitchell et al., (2013a) compared the post exposure behaviour of cyanide, its two major metabolites (thiocyanate and ATCA) and a minor metabolite (α-ketoglutarate cyanohydrin) in blood plasma over the course of 60 minutes by intravenously injecting swine with 4 mg/mL of potassium cyanide. The study found that compared to thiocyanate and ATCA, only a small amount of α-ketoglutarate cyanohydrin was produced post exposure but suggested this pathway may play a more signifant role when the production of thiocyanate and ATCA are impaired. Whilst the production of α-ketoglutarate cyanohydrin was small, this represented a >100-fold increase over baseline concentrations before rapidly decreasing post exposure.

Literature on the metabolism of cyanide in other organisms has identified new products which may have potential as possible biomarkers of cyanide exposure. However, the variability of the pathways observed in mammalian studies makes the behaviour and retention of cyanide and all of its transformation products difficult to predict for fish. One method which could potentially be used to further our understanding of this process is isotopic or radio-labelling. The labelled cyanide ion could be administered to a test organism, enabling the identification of its metabolic pathways and biological retention times of different transformation products. This is perhaps the most viable avenue of research that could increase our understanding of this process.

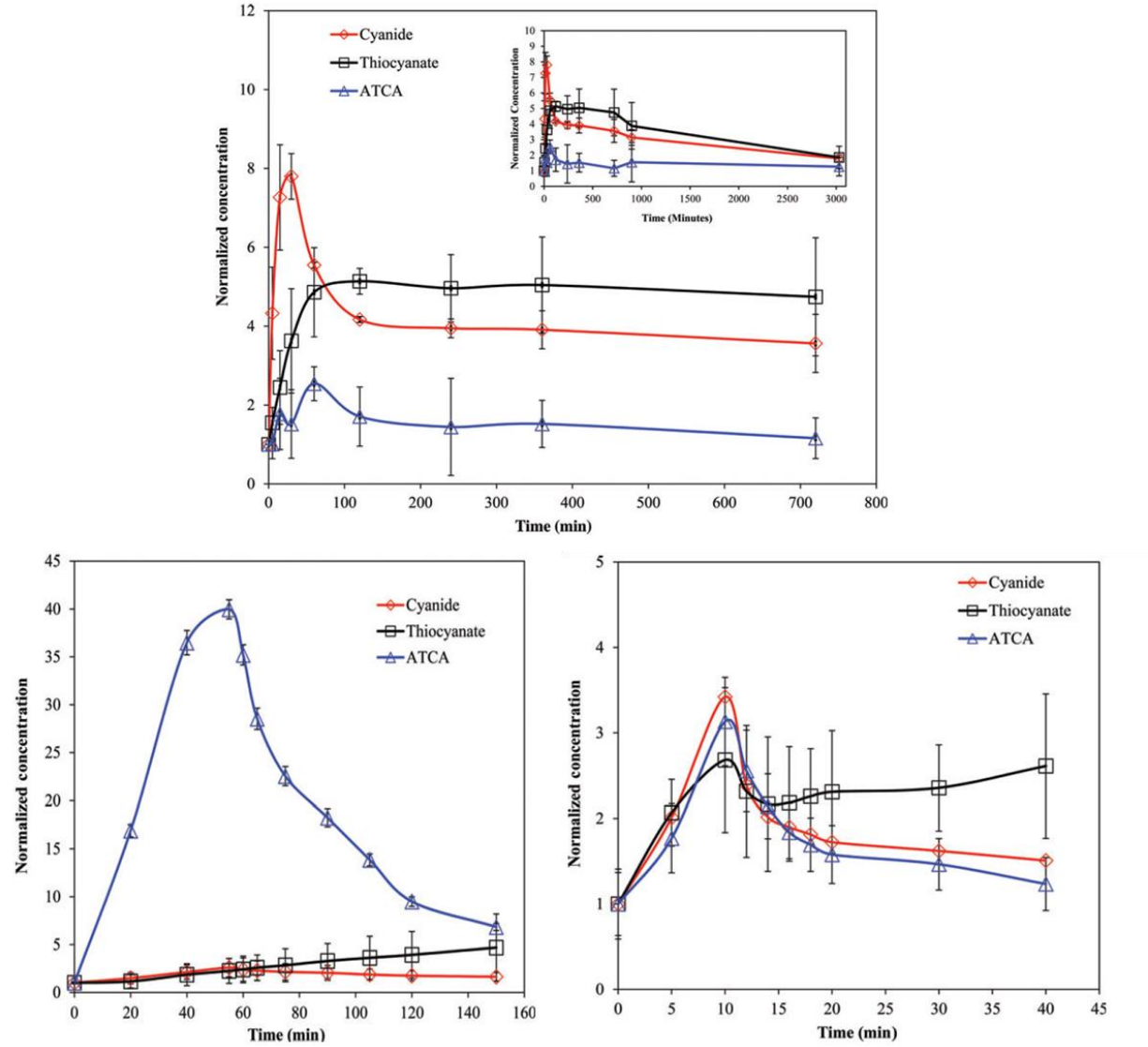

Figure 8. (Top) Rat whole blood cyanide, thiocyanate and plasma ATCA normalized concentrations after cyanide exposure (6 mg/kg body weight KCN injection subcutaneously to rats). Error bars are plotted as standard error of mean (SEM) (N = 3). Inset: Full time course up to 50.5 h post-injection of KCN. (Left). Rabbit plasma cyanide, thiocyanate and ATCA normalized concentrations after 10 mg NaCN infusion. (Right). Swine plasma cyanide, thiocyanate and ATCA normalized concentrations during and after intravenous dose (0.17 mg/kg/min until apnea; ~10 min) (Bhandari et al., 2014).

KEY POINTS:

- Major metabolic pathways are highly conserved between fish and mammals so research in mammals may help us understand biological pathways in fish where information is still lacking.
- Known metabolites of cyanide metabolism in mammals include:
    - Thiocyanate
    - ATCA (2-amino-2-thiazoline-4-carboxylic acid) and ITCA (2-Iminothiazolidine-4-carboxylic acid)
    - cyanocobalamin (vitamin B12)
    - α-ketoglutarate cyanohydrin (α-KgCN)
    - protein adducts
- After thiocyanate, ATCA and ITCA are the second most important pathways accounting for 15 percent of cyanide detoxification in rats. These pathways are particularly important in tissues deficient in rhodanese or where supplies of sulphur are limited.
- Concentrations of ATCA following cyanide exposure in rats, rabbits and swine were found to be highly variable. This highlights a substantial problem when trying to extrapolate these results to fish. This variability observed in mammalian studies makes the behaviour and retention of cyanide and all of its transformation products difficult to predict for fish.
- Experimental isotopic or radio-labelling in fish exposed to cyanide could be used as a method to track the production of metabolites in fish and how long the different products remain in fish.

6. Summary

Since the late 1980s there have been efforts to develop a method to reliably detect the use of cyanide fishing for the collection of live marine fish as a tool for enforcing and regulating anti-cyanide legislation, and to reassure the live fish industry that they are sourcing sustainable fish that are harvested in line with currently accepted best practice. While potentially promising methods for the detection of cyanide and its associated metabolites have been developed (as reviewed by Losada and Bersuder, 2017, unpublished report), there remain a number of critical knowledge gaps in our understanding on the background levels of cyanide in the environment at live fish collection sites around the world; and in the way different species, life stages and sexes of fish metabolize cyanide in response to the varying concentrations they may be exposed in the marine environment.

Cyanide compounds are generally found naturally in low concentrations in the marine environment with inshore waters more likely to contain higher concentrations than offshore waters due to coastal input of natural and anthropogenic sources. Fish inhabiting some inshore waters may be regularly, if not constantly, exposed to low concentrations of thiocyanate in the range of 5 – 22 µg l^{-1} (Rong et al., 2005; Silva et al., 2011). Regardless of the source of this input, studies by Bois, (1988); Brown et al., (1995) and Lanno and Dixon, (1996) have shown that fish are able to bioaccumulate thiocyanate during long-term exposure to low concentrations of both cyanide and thiocyanate and as a result, fish inhabiting waters that have elevated concentrations of both compounds may register detectable concentrations during testing despite not being directly exposed to cyanide fishing practices.

The cyanide compound does not bioaccumulate in living organisms and is rapidly metabolised at sub-lethal concentrations (Ramzy, 2014). Its persistence in marine fish may be influenced by the organism's physiological characteristics (i.e., life stage), the exposure concentration and mode of exposure i.e. absorbed through gills or taken up in the gut after ingestion. These factors affect the rate of cyanide absorption into the blood which can impact the toxicity, and potentially the persistence of free cyanide in the organism. Considering any variation in the half-life of free cyanide due to these factors, its use as a biomarker of cyanide exposure may be limited to a short period following exposure and is therefore, of limited practicality.

Thiocyanate is the primary detoxification metabolite accounting for up to 80 percent of cyanide removal. It is a phase-two metabolic process, whereby the conjugation with ionised sulphur increases the water solubility of the resulting metabolite and enables its excretion via

the urinary system. In contrast to free cyanide, the half-life of thiocyanate is considerably longer but is to some extent dependent of the excretion rate of the given organism. Marine fish are adapted to the considerable osmoregulatory and physiological demands of living in saline waters including a reduced urinary excretion rate when compared to freshwater fish. Persistence of thiocyanate in marine fish may therefore be greater than that reported by freshwater fish. Its status as the primary metabolite means that it is consistently produced following exposure to cyanide, and is less variable than ATCA production. It is also likely to be present in sufficient concentration to not require acutely sensitive analytical techniques for detection which is an important practical consideration for sample collection and analysis. Thiocyanate has been shown to bioaccumulate in rainbow trout (*Oncorhynchus mykiss*) following long term exposure to waterborne cyanide and thiocyanate (Raymond, 1984; Bois, 1988; Brown et al., 1995; Lanno and Dixon, 1996) and marine fish may be able to bioaccumulate thiocyanate to a greater magnitude with longer depuration times due to a reduced urinary excretion rate when compared to freshwater fish. Based on currently available evidence, it is reported that cyanide can stay in fish tissues for up to three weeks while there is a progressive increase in thiocyanate in plasma over multiple days, followed by a decline until a period where it was no longer detectable (from 16 days to 16 weeks or more) (Bruckner and Roberts, 2008; Vaz et al., 2012).

Despite this, thiocyanate as a biomarker has its limitations. Its mean elimination time is still relatively short for practical field sampling and would rely upon fish samples which have potentially been exposed to cyanide being rapidly examined after capture. Thiocyanate is also formed naturally when free cyanide combines with sulphur, often as a by-product of the mining industry, and is likely to be present in natural water samples and mining effluent, and thiocyanate is also naturally found in the biological fluids of unexposed fish making it difficult to determine low-level cyanide exposure (Logue et al., 2010). It is not possible to differentiate between different commercial sources of cyanide (Mansfeldt and Hohener., 2016) to allow the presence of cyanide or its metabolites to be attributed to specific activities such as mining or fishing. Therefore, the presence of elevated thiocyanate concentrations in fish cannot be assumed to be exclusively the result of cyanide fishing activities representing a potential problem concerning its use as a biomarker.

It should be noted that there have also been some conflicting results in the studies available on the detection of thiocyanate in seawater, especially when methods have been applied to both artificial and natural seawater samples. Herz et al. (2016) was unable to detect thiocyanate in natural seawater samples when following the methods described by Vaz et al., (2012). Two points to note are that Herz et al., (2016) did not specify the detection

technique used which was bespoke in the Vaz et al. method and the authors used daily renewal of test solutions whereas Vaz et al., recirculated the test solution over 28 days. Still, an experimental comparison between the detection of thiocyanate in natural and artificial seawater would be needed to identify the source of these inconsistent results, and prove or disprove Herz et al., conclusion that natural seawater is too complex for the detection of thiocyanate. One factor to consider is the process of photolysis and the work by Dzombak et al., (2006) which reported that while free cyanide in the presence of potassium cyanide decreased rapidly below the detection limit in three days, it took six days for free cyanide to drop below the detection in the presence of ferrocyanide. Ferrocyanide (found in industrial anti-caking agents) can find their way into the aquatic environment through run-off (Kelting and Laxon, 2010). In aqueous solution, they can dissociate under exposure to UV light to release free cyanide (Kuhn and Young, 2005). There is anecdotal evidence to suggest that similar anti-caking compounds based upon ferrocyanides are present in some marine salts used to create artificial seawater. If this is the case there may be potential for the release of free cyanide in aquaria under exposure to UV light. An experimental approach to test this hypothesis is required as if free cyanide is released from artificial marine salts under UV, this may lead to false positive readings in any analytical tests on fish held in quarantine facilities.

Major metabolic pathways governing the detoxification of cyanide are highly conserved between fish and mammals, so while there is limited information on other biomarkers in fish specifically, some potential biomarkers have been identified in mammals. In rats, ATCA production is the second most important metabolic pathway accounting for up to 15 percent of cyanide detoxification although this can be very variable with more recent studies suggesting it is less important than previously thought and may account for only 0.10 - 9.19 percent (Egekeze and Oehme, 1980; Mitchell et al., 2013a; Bhandari et al., 2014; Jackson and Logue, 2017). The concentration of ATCA post exposure is expected to be more variable than thiocyanate and this inconsistency potentially limits its usefulness as a biomarker. However, ATCA has not yet been documented in fish specifically and would need targeted experiments in target fish species to fully evaluate its potential use.

Inosine has more recently been identified as a possible biomarker of cyanide exposure in zebrafish (*Danio rerio*). It is only present in elevated concentrations for a small time window following cyanide exposure (Nath et al., 2013) which may limit its practical use as a biomarker as it would require immediate testing post capture. Elevated inosine in response to cyanide exposure is a relatively recent discovery, and further research on this product may bring to light information which promotes its usefulness as a biomarker.

Epigenetics is a relatively new area of science but one which may enable the development of new antidotes to cyanide or identify *in-vivo* genetic biomarkers of cyanide exposure (ATSDR, 2004). Kiang et al., (2003) documented alterations of both stress-related gene expression and caspase-3 cellular activity (human intestinal epithelial T84 cells and Jurkat T cells) in humans as a result of sodium cyanide exposure. However, this avenue of research is very much in the research and development stage and much more work is needed to investigate if cyanide has a specific pattern of epigenetic markers which are distinguishable from other chemicals.

One of the critical factors involved in accurately and consistently detecting cyanide exposure is the length of time taken to transport the fish from the site of exposure to a testing facility. The rapid metabolism of cyanide may result in fish caught using cyanide fishing techniques to return to pre-exposure levels before reaching a testing facility. Determination of cyanide exposure in fish should therefore take place as soon as possible, in the country of origin and prior to export. To provide evidence of fish caught using cyanide which will stand up beyond all reasonable doubt in a court of law, the test must be independently verifiable.

KEY POINTS:

- A number of promising methods for the detection of cyanide and its associated metabolites have been developed over the last 10 years to address the mitigation of cyanide fishing, however, there remains a number of critical knowledge gaps:
    - Understanding the background levels of cyanide in the environment at live fish collection sites around the world;
    - how different species, life stages and sexes of fish metabolize cyanide in response to the varying concentrations they may be exposed in the marine environment.
- Studies have shown that fish are able to bioaccumulate thiocyanate during long-term exposure to low concentrations of cyanide and thiocyanate which fish in inshore water nears industry outflow may be exposed and at detectable levels.
- Cyanide can stay in fish tissues for up to three weeks but thiocyanate can increase over multiple days, followed by a decline until a period where it was no longer detectable (from 16 days to 16 weeks or more).
- The half-life of thiocyanate is dependent of the excretion rate of the organism, and as marine fish have a reduced urinary excretion rate for living in saline waters, its persistence in marine fish may be greater than is reported in freshwater fish.
- Thiocyanate is also likely to be present in sufficient concentrations to not require acutely sensitive analytical techniques for detection - an important practical consideration for sample collection and analysis.
- A critical factor in accurately detecting cyanide exposure is the length of time taken to transport the fish from the site of exposure to a testing facility. Testing in fish should therefore take place as soon as possible, in the country of origin and prior to export.

7. Recommendations

There remain a number of evidence challenges which need to be addressed before a robust and practical method for detecting cyanide can be implemented. Any method developed should have the ability to discriminate between natural and anthropogenic sources of exposure in marine fish, and be used as a management tool in areas of high-risk cyanide locations. Specific recommendations on how to address these evidence challenges are provided below for both determining the sources of cyanide in the aquatic environment and for furthering our understanding of how fish metabolise cyanide.

7.1. Environmental pathways of cyanide in the aquatic environment

1. A risk-based approach should be taken to identify locations globally where cyanide is at a high-risk of being introduced into the aquatic environment both through natural and anthropogenic pathways and which overlap with collection of marine aquarium fish. In partnership with the ornamental industry, aquarium fish collection sites would be identified before a detailed desk study on the sites local industry and ecology would be undertaken to flag areas of high risk.

2. In the high-risk areas identified, an assessment should be made on the existence of a water quality monitoring programme, undertaken by industry (mining, cassava industry etc) or by government, to determine if aquatic cyanide concentrations are currently monitored and if not, if there is an exisiting programme measuring other parameters which could include cyanide in the future.

3. In the absence of current cyanide monitoring, background levels of free cyanide and cyanide derived compounds should be determined at fish collection sites. Both water samples and fish tissue samples would be required to produce a robust baseline. Samples for this baseline should be collected throughout the year, during different tidal states and include areas which cover a range of reef habitats (e.g. reefs fringing densely populated areas; reefs in close proximity to estuaries with known mining or other anthropogenic activities upstream; reefs situated further offshore). Control sites determined by a low-risk rating to environmental cyanide should be included in the programme.

4. Baseline results should be used to set regional action levels for cyanide and its metabolites against which, the results of live fish testing can be compared.

7.2. Key metabolic pathways in fish

1. Background levels of cyanide and its compounds in un-exposed fish samples must be determined at different life stages, species, sexes and sizes of target fish species. These background levels should also include different biological matrices (e.g. tissues, blood, urine).

2. Experiments focused on increasing our understanding of the metabolic pathways of cyanide in fish are required to provide key information on the appropriate timeframe by which a test should be conducted following exposure. A suggested approach is to perform isotopic tracer experiments using both natural and artificial seawater.

   A labelled form of cyanide would be administered to both marine and freshwater experimental fish and then its pathway tracked through cells and tissues to provide information on the full range of cyanide pathways and products in fish and their retention times. It is possible that this technique could identify a more persistent metabolite which may be stored in tissues for extended periods post exposure (for example cyanide protein adducts are expected to have a half-life similar to that of the parent protein. In humans it is documented that they form covalent adducts with two commonly occurring blood plasma proteins).

   Such experiments should be performed on a range of target species which are collected for the live fish for food industry and the aquarium trade and by using both marine and freshwater species, the pathways of cyanide metabolism between fish living in different aquatic environments can be compared. Knowledge of the background levels of cyanide and its compounds in different collection areas will enable the creation of specific action levels for each region. Cyanide concentrations detected above these threshold values will enable cyanide fishing activities to be distinguished from the normal background levels in that region thus reducing the potential for false positive results attributable to background exposure to cyanide. Ultimately, the results of these experiments would improve our understanding of the wider environmental context surrounding cyanide in the aquatic environment and support the development of a verifiable analytical test which can be used in the field by agencies to mitigate the use of cyanide fishing.

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Glossary

Acetylcholinesterase (AChE): an enzyme present in nervous tissue, muscle and red blood cells that hydrolyzes the neurotransmitter acetylcholine, its action is blocked by certain drugs.

Adduct: the product of combining two or more distinct molecules which results in a single product which contains all atoms of the original molecules.

Aerobic: relating to, involving, or requiring free oxygen.

Anthropogenic: (chiefly of environmental pollution and pollutants) originating in human activity.

Aspartate aminotransferase: an enzyme that is normally present in liver and heart cells. AST is released into blood when the liver or heart is damaged.

ATPase: An enzyme that causes the breakdown of ATP into ADP and a free phosphate ion, thereby releasing energy for use in another biochemical reaction.

Bioaccumulation: accumulation of substances which occurs when an organism absorbs a substance faster than is lost by metabolism and excretion.

Biodegradation: is the process by which organic substances are decomposed by micro-organisms (mainly aerobic bacteria) into simpler substances such as carbon dioxide, water and ammonia.

Biodiversity: the variety of plant and animal life in a particular habitat, a high level of which is usually considered to be important and desirable.

Biomarker or biological marker: generally refers to a measurable indicator of some biological state, condition or process (such as metabolic process).

Biosensor: a device which uses biological materials (living organism or biological molecules, especially enzymes or antibodies) to detect the presence of chemicals. It converts a biological response into an electrical signal.

Catabolic: destructive metabolism; the breaking down in living organisms of more complex substances into simpler ones, with the release of energy (opposed to anabolism).

Catalase: an enzyme that catalyses the reduction of hydrogen peroxide.

Chemosensor or molecular sensor: a molecule that interacts with an analyte to produce a detectable change.

Chromatography: analytical technique used to separate proteins, nucleic acids, or small molecules in complex mixtures.

Colloid goiter (nodules): benign, noncancerous enlargement of thyroid tissue.

Conjugation: a compound formed by the joining of two or more chemical compounds.

Creatinine: a compound which is produced by metabolism of creatine and excreted in the urine.

Cyanocobalamin (vitamin B12): a complex red crystalline compound, containing cyanide and cobalt and occurring in the liver: lack of it in the tissues leads to pernicious anaemia.

Cyanogenic: (of a plant) capable of cyanogenesis, or containing a cyanide group in the molecule.

Cyclic voltammetry: an electrochemical detection technique (electrochemical) that measures the current in a cell.

Degradation: a change of a chemical compound into a less complex one, often breaking it down and deteriorating it.

Depuration: the action or process of freeing something of impurities.

Detoxification: the proccess of removing toxic substances.

Dissociation: (with reference to a molecule) split into separate smaller atoms, ions, or molecules, especially reversibly.

Electrochemical gradient: the electrical potential that acts on an ion to drive the movement of the ion in one or another direction.

Fecundity: producing or capable of producing an abundance of offspring or new growth; highly fertile.

Gastric mucosa: the mucous membrane lining the interior of stomach.

Glutamate-Oxaloacetate Transaminase (GOT): an enzyme which catalyzes amino group of an amino acid with keto group of keto acid.

Glutathione peroxidase (GPx): a selenium-containing enzyme that protects tissues from oxidative damage by removing peroxides resulting from free radical action, linked to oxidation of glutathione; part of the body's antioxidant protection.

Glutathione S-transferases (GST): a family of Phase II detoxification enzymes that catalyse the conjugation of glutathione (GSH) to a wide variety of endogenous and exogenous electrophilic compounds.

Haematocrit: the ratio of the volume of red blood cells to the total volume of blood.

Half-life: time required for any specified property (e.g., the concentration of a substance in the body) to decrease by half.

Head space: a technique based on the collection (with a syringe or fibre) of the volatile compounds released from a liquid or a solid, usually on an enclosed space, like a sealed vial.

Hepatosomatic index (HSI): the ratio of liver weight to body weight. It provides an indication on status of energy reserve in an animal. In a poor environment, fish usually have a smaller liver (with less energy reserved in the liver).

Histotoxic hypoxia: the inability of cells to take up or utilize oxygen from the bloodstream, despite its availability in cells and tissues.

Hydrolysis: the break down of a compound by chemical reaction with water.

Hydroxocobalamin: an analog of cyanocobalamin (vitamin B12) having exceptionally long-acting hematopoietic activity. Called also vitamin B12b.

Hypoxia: diminished availability of oxygen to the body tissues.

Immunoglobulin G: a class of immunoglobulins including the most common antibodies circulating in the blood that facilitate the destruction of microorganisms foreign to the body.

Inorganic compounds: A compound that does not contain hydrocarbon groups.

Interspecific: characteristic occurring or existing between different species.

Intraspecific: characteristic occurring or existing within a species or individuals from a single species.

Intravenous: into or connected to a vein.

Lactate dehydrogenase (LDH): an enzyme that catalyzes the conversion of lactate to pyruvate. This is an important step in energy production in cells.

Lethal Concentration 50% (LC_{50}): the concentration of a toxin that will kill 50% of organisms.

Lipid peroxidation (LPO): the oxidative degradation of lipids, polyunsaturated fatty acids are highly susceptible to lipid peroxidation, which compromises the integrity and function of the cell membrane in which they reside.

Liquid chromatography (LC): separates molecules in a liquid mobile phase using a solid stationary phase.

Lixiviant: liquid medium used in hydrometallurgy to selectively extract the desired metal from the ore or mineral. It assists in rapid and complete leaching. The metal can be recovered from it in a concentrated form after leaching.

Macromolecule: a molecule containing a very large number of atoms, such as a protein, nucleic acid, or synthetic polymer.

Metabolisation: chemical processes occurring within a living cell or organism that are necessary for the maintenance of life. During metabolism, some substances are broken down to yield energy, some are synthesized and some are transformed into less toxic substances.

Microgram: a unit of mass equal to one millionth of a gram.

Mitochondria: an organelle found in large numbers in most cells, in which the biochemical processes of respiration and energy production occur.

Nanogram: a unit of mass equal to one billionth of a gram

Ore: a naturally occurring solid material from which a metal or valuable mineral can be extracted profitably.

Osmoregulation: the maintenance of constant osmotic pressure in the fluids of an organism by the control of water and salt concentrations.

Oxidation: the loss of electrons or an increase in oxidation state by a molecule, atom, or ion.

Pharmacokinetics: a branch of pharmacology concerned with the movement of drugs within the body.

Phosphoprotein: A protein that contains phosphorus (other than in a nucleic acid or a phospholipid).

Photolysis: decomposition or separation of molecules by the action of light.

Polysulphides: a compound containing two or more sulphur atoms bonded together as a group.

Porphyrin: a group of heterocyclic pigments containing four pyrrole rings, sometimes with a central metal atom. E.g.: heamoglobin and chlorophyll.

Preconcentration: to increase the concentration of a sample prior to analysis or detection.

Pyrolysis: decomposition brought about by high temperatures.

Serum albumin: the most abundant protein of blood serum: it is synthesized by the liver and serves to regulate osmotic pressure and to carry certain metabolic products.

Spectroscopy: use of the absorption, emission, or scattering of electromagnetic radiation by matter to qualitatively or quantitatively study the matter or to study physical processes. The matter can be atoms, molecules, atomic or molecular ions, or solids.

Splenosomatic index: spleen size as a percentage of body weight.

Teragram: a unit of mass equal to one trillion of a gram.

UV-Vis spectroscopy: ultraviolet–visible spectroscopy refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent ranges.

Vitellogenin: a protein present in the blood from which the substance of egg yolk is derived.

Volatile: a substance that is readily vaporizable at relatively low temperature.

Xenobiotic: a chemical compound that would not otherwise be found in animals (e.g. drugs) or in the environment (e.g. industrial pollutants).

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