ZEQUANOX®
Invasive Mussel Control

The Zequanox Story

An in-depth review of the discovery and commercialization of a new, non-chemical alternative for invasive mussel control

by Marrone Bio Innovations

TABLE OF CONTENTS

They are tiny—the size of a person's fingernail—yet they are having a billion-dollar impact on the North American economy and causing great harm to countless freshwater ecosystems. Zebra mussels (Dreissena polymorpha) *and quagga mussels* (Dreissena rostriformis bugensis) *are highly invasive bivalves native to Eastern Europe. These tiny, freshwater species originated in the Caspian and Black Seas and were transported to North America in ballast water from a cargo ship. First discovered in the Great Lakes in the late 1980s, these mussels have now invaded waterways across the United States, causing numerous ecological impacts and creating operations and maintenance challenges for commercial facilities that draw water from infested lakes and rivers.*

By the early 1990s facility operators were employing a variety of methods to try and stave off impending damage that could be caused by the ever-expanding mussel colonies. Some methods were labor intensive, some turned out to be completely ineffective and others posed a tremendous risk to the facilities' employees and the surrounding ecosystem. Without a better alternative, operators had to tolerate these shortcomings. In 2007, however, an exciting scientific breakthrough led to the development of Zequanox®, a naturally derived molluscicide that offered a highly effective AND environmentally compatible control method for these invasive mussels. Presented herein is the story of Zequanox: how it was discovered, how the product was developed, and an overview of the science behind this innovation. This document also summarizes results that demonstrate the efficacy and safety of Zequanox and highlights the product's many advantages over traditional control options.

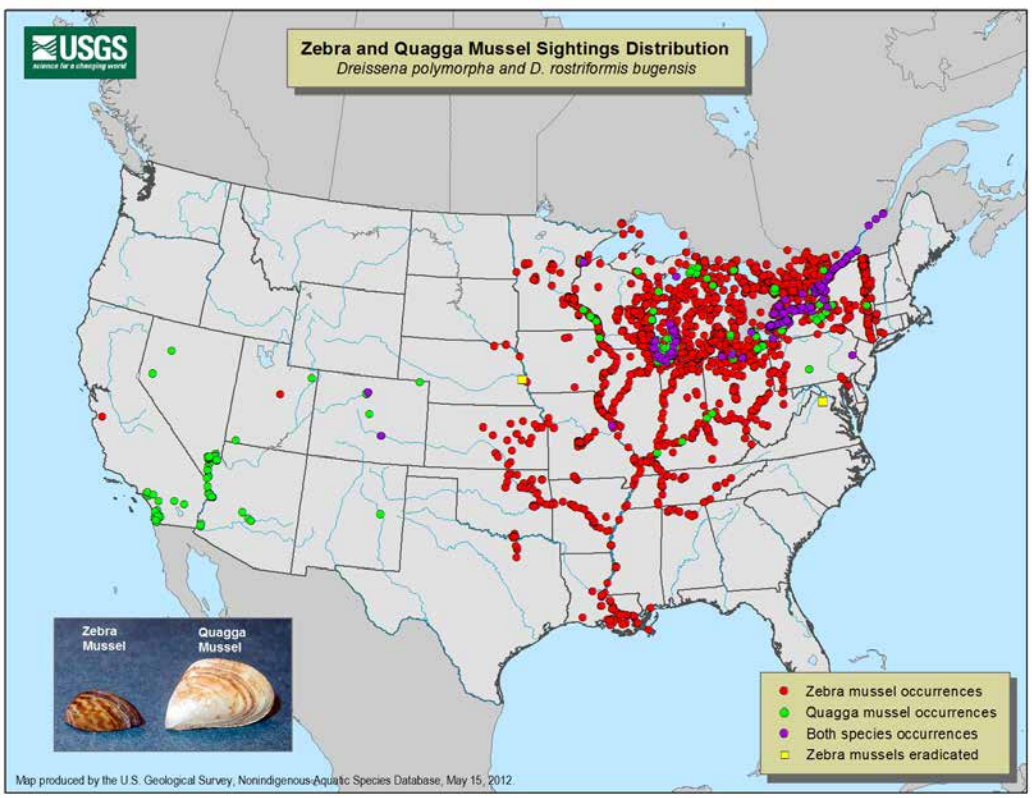
A Multibillion-Dollar Problem

Zebra and quagga mussels can attach by the millions to one another, forming dense colonies in heavy masses up to a foot thick (U.S. Department of the Interior, U.S. Geological Survey [USGS] 2008). These colonies can clog piping, filters and screens, impeding or preventing the flow of critical cooling or process water. The mussels can also cover system components and mechanical parts, causing system damage and weighing down equipment and infrastructure. These effects can hinder or in some cases shut down operations. Continual attachment of *Dreissena* can also significantly increase corrosion rates of steel and concrete (Benson and Raikow 2011), leaving equipment and infrastructure vulnerable to failure. Many of the traditional chemical control options, chlorine in particular, exacerbate the increased corrosion and pitting that invasive mussels initiate.

Six-inch process water intake pipe clogged with zebra mussels. Photo taken at an industrial facility in Michigan.

Once introduced into a new water body, the population growth of these mussels can be explosive. Very successful invaders, zebra and quagga mussels thrive in a variety of temperatures, readily find food, reproduce prolifically and rapidly, and lack natural predators. The average female zebra mussel, which is ready to reproduce in its first year of life, can release 30,000 to 40,000 eggs per year. After hatching, the planktonic larvae can not only move great distances in flowing water, but can also easily invade small places in both natural systems and industrial systems that draw from infested waters. Mussel colonies can build up very quickly. It has been reported that these mussels have been able to clog a three-foot-diameter pipe in less than three months (U.S. Department of Energy, National Energy Technology Laboratory [NETL] 2006).

An added challenge is that these mussels can rapidly disperse to other water bodies, primarily by the larval movement and their inadvertent transport by barge and boat traffic, and can survive for many days out of water, factors that have caused the zebra and quagga mussel invasion to spread to many previously uninfested waters throughout the United States. First found in North America in Lake St. Clair in 1988, zebra mussels have spread to all of the Great Lakes and into the Mississippi, Tennessee, Hudson, and Ohio River basins (USGS 2011). Quagga mussels were first sighted in the United States in Lake Erie in 1989, and have since been found in all of the Great Lakes, some inland lakes, the Mississippi River, Lake Mead, Lake Havasu, Lake Mohave and 15 Southern California reservoirs. Quagga veligers have also been reported in Colorado (USGS 2012; Benson, Richerson, Maynard, Larson and Fusaro 2012).



USGS map[1] from May 15, 2012.

1 For current sightings map, visit http://nas.er.usgs.gov/taxgroup/mollusks/zebramussel/

> *"The zebra mussel* (Dreissena polymorpha) *has become the most serious nonindigenous biofouling pest ever to be introduced into North American freshwater systems." —U.S. Army Corps of Engineers, 2002*

There is widespread agreement that zebra and quagga mussels annually cause millions of dollars in additional maintenance expenses in North America. United States Congressional researchers estimated that zebra mussels alone cost the power industry $3.1 billion during 1993–1999. The U.S. Fish and Wildlife Service estimated the economic impact during 2000–2010 at $5 billion. The mounting costs, combined with an ever-expanding geographical area of impact, have increased the need for reliable control methods that are suitable for a variety of industrial and civil applications.

The Need for a New Approach

Commercial and public entities facing zebra and quagga mussel infestations have applied a variety of methods when seeking to control mussel populations, including aqueous controls, antifouling coatings, physical removal and mechanical controls. Each of these methods has significant drawbacks.

A very common approach to mussel control is aqueous applications of chemicals such as chlorine. Chlorine-based methods using hypochlorite, chlorine gas and chlorine dioxide necessarily involve careful practices to ensure that the chemicals are safely stored, and that employees handling the chemicals are not exposed to hazards and unnecessary risk. In addition, chlorine and other oxidizing chemicals are corrosive to equipment.

Chemical treatments are toxic to other aquatic organisms and because of this non-targeted toxicity, facilities using chlorine and other chemical-based molluscicides may be required to deactivate or detoxify the treated water before discharge to meet environmental requirements (NETL 2006). Bisulfate or similar salts are used to help prevent the release of chlorine into the environment and reduce the impact on other aquatic organisms, contributing to salt loading in water bodies. Many molluscicides require the addition of clay to a treated water system to quench or deactivate the chemicals' toxicity before discharge into the environment. The ultimate fate and transport of the clay-bound molluscicides once discharged is unknown; many of these substances are nonbiodegradable and stay in the ecosystem long after discharge.

An additional disadvantage of using chlorine is that the mussels perceive the chlorinated water as a threat, causing them to shut their valves for so long that very long application times are necessary to achieve results. The formation of harmful by-products is yet another area of concern; when chlorine combines with organic compounds in water, potentially carcinogenic substances such as trihalomethanes, haloacetic acids and dioxins are formed (U.S. Environmental Protection Agency [EPA] 1999; Thornton 2000).

Traditional methods also include antifouling coatings, which are effective only where they can be applied, and physical removal, which is effective only for places reachable by pressure washers, specialized scraping machines or divers. Some facilities also use heated water to control invasive mussel populations. These methods have limited applicability, and are also limited by their labor intensiveness and the risk of equipment damage. Further, these methods typically require a system shutdown or bypass for application.

Pressure washing (aka "pigging") is one method of removal of mussel colonies. Photo credit Zebra Mussel Information System, 2005.

Microfiltration and UV light treatments have also been applied, but are in limited use. These methods are not hazardous to employees or the environment, but they typically involve hefty capital investments as well as installation, operation and continual maintenance of specialized equipment. In addition, microfiltration and UV light address only veliger control and they provide control only at the equipment location. Any temporary failure or shutdown of this equipment—even a nonfunctioning UV lamp or a micro tear in a filter—could allow veligers to move downstream in the system and settle. Further, these methods can be compromised by seasonal cloudy, murky water or high solids from algae blooms.

A Biological Breakthrough

The need for a new control method drove extensive research that led to an industry-changing discovery. Faced with the threat of zebra mussels fouling electric power facilities within New York State, a research consortium of New York State's electric power generation companies contracted with New York State Museum Field Research Laboratory in 1991 for the screening of bacteria as potential biological control agents. The use of microbial, natural product compounds already had a clear record of commercial success and environmental safety in the control of invertebrate pests in North America, as well as globally (Rodgers 1993).

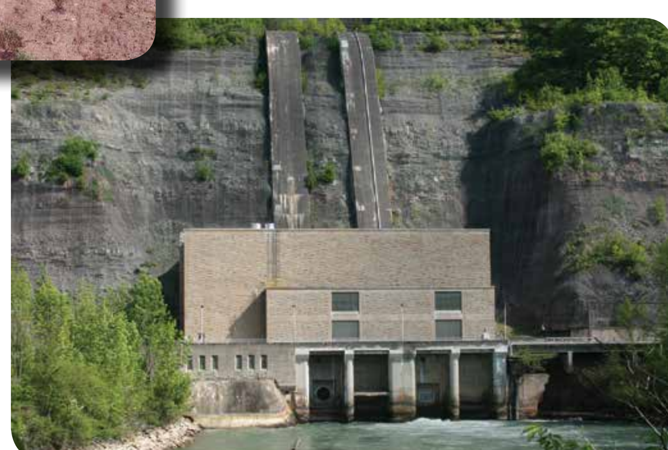
Extensive laboratory screening trials of more than 700 bacterial strains identified a North American isolate, strain CL145A of *Pseudomonas fluorescens*, to be lethal to zebra and quagga mussels (Molloy 2002). A patent for this purpose was issued in both the United States (Molloy 2001, patent number 6,194,194) and Canada (Molloy 2004, patent number 2,225,436). *Pseudomonas fluorescens* is worldwide in distribution and is present in all North American water bodies. In nature, it is a harmless bacterial species that is found protecting the roots of plants from diseases.

BRINGING THE SOLUTION TO MARKET

NYS M NEW YORK State Museum

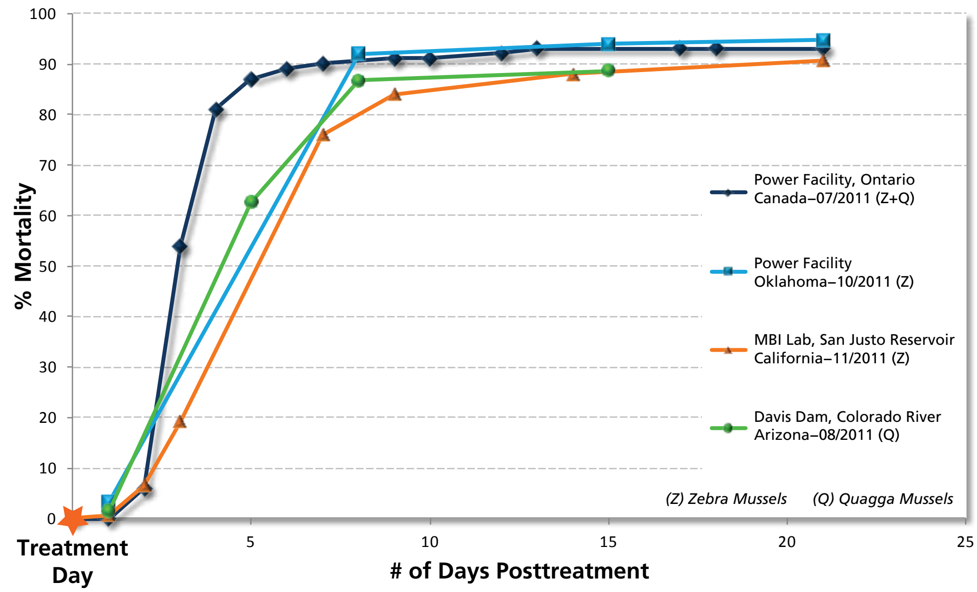
In 2007 Marrone Bio Innovations (MBI) entered into a commercial partnership with the New York State Museum to bring this naturally occurring soil microorganism to market for the control of zebra and quagga mussels. The result was Zequanox—the industry's first aqueous, environmentally compatible molluscicide. The EPA registered Zequanox on July 29, 2011, and while the research through 2011 focused on controlling invasive mussels within cooling water systems in industrial and power facilities, other future applications include, but are not limited to, aquaculture, fish hatcheries, agricultural facilities, and ultimately reservoirs and recreational waterways.

Beginning in 2009, MBI in cooperation with the U.S. Bureau of Reclamation (Reclamation) conducted field trials of Zequanox under a Cooperative Research and Development Agreement. The product was tested at Reclamation's Davis Dam on the lower Colorado River, where supply lines were heavily infested. MBI also teamed with Ontario Power Generation of Ontario, Canada, to perform testing at the DeCew II Generating Station Facility. Ontario Power Generation, which had a 20-year history of chlorine control that had reached its maximum optimization potential and wanted to help bring a more sustainable mussel control solution to the market, assisted MBI in its commercial development of Zequanox (Van Oostrom, Peterson-Murray and Dow 2010).



Bureau of Reclamation's Davis Dam (left above) and the DeCew II Generating Station operated by Ontario Power Generation (right).

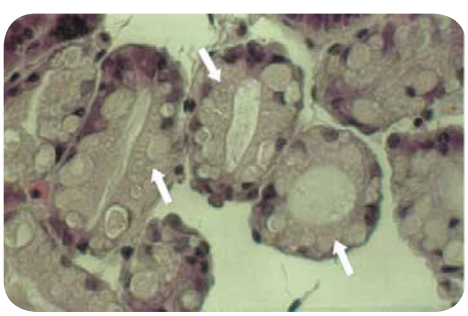
In 2011 MBI conducted a number of demonstration and full-scale Zequanox treatments throughout North America. The chart below summarizes test results of adult mussel treatments—conducted in different locations with varying water qualities—on both zebra and quagga mussels. Typical treatments ranged from six to eight hours and mortality was scored from two weeks up to one month posttreatment.



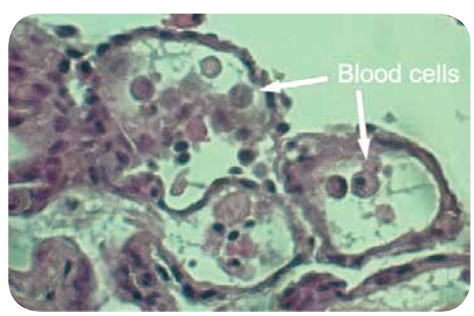
Summary of test results for Zequanox

How Does Zequanox Work?

Zequanox is composed of dead cells of the *Pseudomonas fluorescens* microorganism. The cells contain natural compounds that, when ingested, are lethal to zebra and quagga mussels during all life stages (veliger to adult). The mussels perceive Zequanox as a nonthreatening food source and readily consume the product along with their normal phytoplankton diet. As mentioned previously, this feeding mechanism contrasts with biocides such as chlorine, which mussels sense as threatening, causing them to quickly shut their valves to guard themselves against the chemical. While susceptibility increases with water temperature, with more than 90% mussel mortality when Zequanox is used in water temperatures greater than 14°C, high mortality is achievable even in very cold waters.



In healthy mussels, epithelial cells (arrows) appear as a thick layer lining the tubules of the digestive gland.

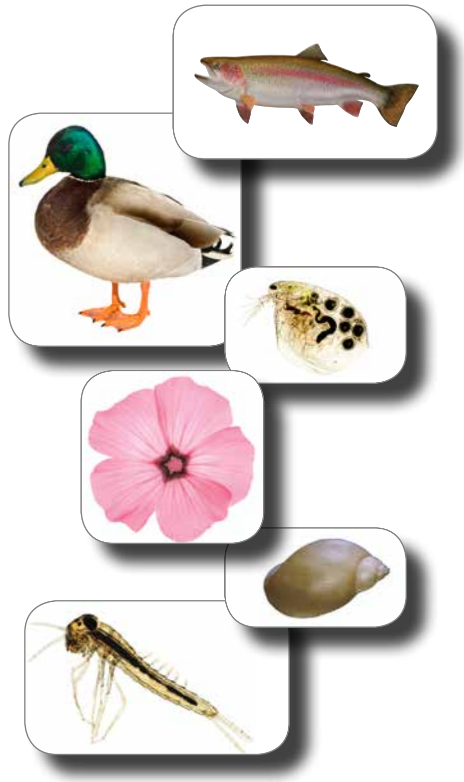


Following bacterial treatment, epithelial cells are destroyed. Blood cells are abundant as the digestive gland hemorrhages.

EXTENSIVE TOXICOLOGY STUDIES DEMONSTRATE SELECTIVITY

As previously noted, *P. fluorescens* is worldwide in distribution and is present in all North American water bodies. In nature it is a harmless bacterial species. Extensive toxicology studies have been conducted with Zequanox and the findings demonstrate that, unlike chemical molluscicides, Zequanox is highly selective toward zebra and quagga mussels and is of inherently low toxicity to non-target organisms (including other mussel species).

A number of fish species from representative taxonomic groups have been tested. Several high-dose, multi-day toxicity studies on either Brown or Rainbow trout, which are often noted to be the most sensitive species in ecotoxicological studies, indicate minimal toxicity to trout. The results of studies on more hardy species—the fathead minnow of the cyprinid family, striped bass, and suckers—also show that the fish would be safe at the concentrations and exposure durations that they would experience in water bodies near facilities undergoing Zequanox treatments. Considering the relatively short treatment times using Zequanox, the immediate dilution in receiving waters and the rapid environmental breakdown of the product, no toxic effects to non-target fish species are expected or likely.



In addition to fish species, a broad range of invertebrate taxa has also been tested. No effects were noted on daphnia (a common, small, free-swimming crustacean) or the sediment-dwelling amphipod crustacean *Hyalella azteca*. Based on the results of studies conducted on two benthic insects—mayfly nymphs and chironomids—no effects would be expected on free-swimming and benthic invertebrates.

A 14-day study conducted with the mallard duck (a common, representative aquatic avian species) showed no mortality, no clinical signs of toxicity, no effect on body weight or feed consumption, and no pathological findings in all cases and at all concentrations tested. It is expected that exposure to water containing maximum treatment concentrations would not pose a threat to aquatic birds.

Six common species of aquatic plants (common water plantain, small-flower umbrella sedge, nightshade, bindweed, mallow and curly dock) were immersed in water containing Zequanox at a concentration higher than that typically used for treatments. After six days of immersion, no signs of phytotoxicity were observed in any of the plants.

A range of freshwater mussel species (in the unionid family) was exposed to the maximum Zequanox concentrations. There was a complete absence of mortality in all cases, while in the same studies mortality in the zebra and quagga mussels consistently approached 100%. In addition, no mortality was observed in the native freshwater anadonta mussel. Based on these studies, no risks to native mussels are expected.

How Does Zequanox Compare with Alternative Solutions?

Zequanox offers several advantages over chlorine and other chemical pesticides, including safety, flexibility and ease of use. First and foremost, Zequanox poses very limited to no risk to workers, non-target species and the environment. As a reduced-risk pesticide, Zequanox is safe to store, handle and apply; only minimal personal protective equipment is needed. In contrast, chlorine and other chemical pesticides are toxic to aquatic life and the environment (i.e., they often fall into the level 1 pesticide, or other high-risk category). These products require special handling, safety warning placards, sophisticated permitting, tracking and monitoring. If not properly managed, chlorine and other hazardous chemicals can cause serious (even fatal) harm to humans, and can cause irreparable harm to the environment. And as mentioned previously, the corrosive nature of oxidizing chemicals can limit the life span of valuable equipment or create the need for additional maintenance.

Zequanox at a Glance

- Minimal risk to humans and non-target species
- Requires only minimal personal protective equipment
- Noncorrosive and nonvolatile
- Minimizes permitting and monitoring hassles
- Detoxification not needed before discharge
- Short treatment times
- Applies with standard equipment
- Control not vulnerable to equipment failure
- Effective in a broad range of water conditions and temperatures
- Customizable treatment programs

To comply with the National Pollutant Discharge Elimination System, chlorine and harmful chemicals require special permitting, tracking, monitoring and detoxifying before discharge. The use of Zequanox carries none of these requirements, and detoxification is not required before discharge of the treated water.

Applications of Zequanox are less labor intensive and less operationally disruptive than chemical methods. Zequanox treatments can be done during normal facility operations and typically occur within a six- to eight-hour period. This timeframe is in contrast to chlorine treatments, which can require several weeks of around-the-clock treatment, and often require special procedures to ensure worker safety during the treatments. Zequanox offers additional flexibility in that it is proven effective in a broader range of water conditions and temperatures than chlorine, thus expanding the "treatment season" during which Zequanox treatments can be effective.

Zequanox also offers a number of advantages when compared with UV and microfiltration solutions. First, Zequanox can be applied using standard injection equipment, so facility operators can implement a Zequanox control program quickly and easily. Zequanox can be employed without having to undergo an arduous capital budgeting process and equipment installation, and without incurring the additional overhead of ongoing equipment maintenance. The aqueous formulation of Zequanox provides the added benefit of being able to reach and treat even the smallest of crevices in the water system, whereas mechanical solutions offer control only at a fixed location.

Zequanox also offers the unmatched ability to tailor the treatment regimen to achieve the desired balance of mussel control, application frequency and shell debris management.

A Comparison of Mussel Control Methods

	Chlorine and Other Chemical Pesticides	Microfiltration/UV	Zequanox
Application Time	Days to weeks	Continuous	6–8 Hours
Start-up Investment	Limited	High	Limited
Worker Safety Requirements	High—special precautions, storage and handling required	Minimal	Minimal
PPE Requirements	Maximum protection needed	Minimal	Minimal
Discharge Requirements	Detoxification may be required	NA	Detoxification not required
Risk to Environment and Non-target Species	High risk	No risk	No risk when used as directed
Equipment Corrosion Risk	High with oxidizing chemicals	None	None
Equipment Maintenance	NA	High	NA
Water Temperature/ Quality Impacts on Control	Efficacy of oxidative chemicals might be compromised when excessive organic matter and algae are present in the water. Limited efficacy when water temp is below 8°C.	Efficacy might be compromised in cloudy, murky water, or when excessive organic matter and algae are present.	Limited
Regulatory Restrictions	High	NA	Very low

Conclusion

Throughout North America and Europe, zebra and quagga mussels are crippling industrial and commercial operations by restricting water flow in heat exchangers, condensers, fire suppression systems, and service and cooling water systems, as well as by damaging other infrastructure and equipment. Unfortunately the battle against these invasive, destructive mussels rages on, intensified by their unrelenting spread and complicated by increasing regulatory pressures, such as stricter discharge permits. Today facility operators are faced with what appear to be conflicting goals—controlling mussels while managing shell debris, and achieving a high level of efficacy without harming the environment or putting the facility or employees at risk.

The first biological mussel control solution, Zequanox offers what no other mussel control solution does—a highly effective, flexible method that requires little or no capital investment and that can be used without putting employees or the environment at risk from harsh chemicals. Using Zequanox for invasive mussel control allows facility owners to support environmental stewardship while protecting their operations and assets.

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