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When it comes to lead in water, new biosensing technology can reveal what the eyes cannot see and what the rules do not yet stop

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ABSTRACT

Deficiencies in knowledge about water quality prevent or obscure progress on a panoply of public health problems globally. Specifically, such lack of information frustrates effective and efficient government regulation to protect the public from contaminated drinking water. In this Practical Paper, we lay out how recent scientific innovations in synthetic biology mean that rapid, at-home tests based on biosensor technology could be used to improve water quality monitoring and regulation, using the example of the U.S. Environmental Protection Agency's Lead and Copper Rule currently under revision. Biosensor tests can be used by non-scientists and the information that biosensor tests generate is relatively cheaper and faster than standard laboratory techniques. As such, they have the potential to make it possible to increase the number and frequency of samples tested. This, in turn, could facilitate more accurate compliance monitoring, justify more protective substantive standards, and more efficiently identify infrastructure priorities. Biosensors can also empower historically underrepresented communities by facilitating the visibility of inequities in lead exposure, help utilities to ensure safe water delivery, and guide policy for identifying and replacing lead-bearing water infrastructure, thereby improving public health. As the technology matures, biosensors have great potential to reveal water quality issues, thereby reducing public health burdens.

Key words: biosensors, copper, lead, Lead and Copper Rule, public health, rapid tests, synthetic biology

HIGHLIGHTS

- At-home biosensor tests have recently been developed for use by the general public to rapidly detect drinking water contaminants.
- They are relatively simpler, cheaper, and faster than conventional lab techniques, permitting widespread use and thereby democratizing water safety knowledge.
- At-home lead biosensor data could improve the U.S. Environmental Protection Agency's Lead and Copper Rule, which is currently under revision.

When it comes to contaminated drinking water, we cannot fix what we cannot see. Globally, deficiencies in the baseline knowledge of water quality – among both governments and private citizens – can prevent or obscure progress on a panoply of public health problems including infectious diseases and chemical contamination. In the United States, for example, constraints in current water testing technology mean serious limitations in the efficacy of the federal Lead and Copper Rule (LCR) to protect public health, as described below. Fortunately, the Biden Administration has committed to a first-term reform of the LCR, publishing proposed revisions for public comment on December 5, 2023 (88 FR 84878 2023). Recent scientific innovations in synthetic biology could be an opportunity to make this legislation much more robust.

Recent technological advances in synthetic biology mean that at-home tests that can detect contaminants in drinking water nearly as quickly and easily as the rapid COVID tests to which we have all become accustomed are possible (Young & Lucks 2021). The science behind this technology is based on the natural ability of microbes to sense their environment using

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biosensors – molecular machines that bind to toxins and activate gene expression (Thavarajah *et al.* 2020). These biosensors can be extracted from living cells to function on strips of paper, as well as be reconfigured to produce visible signals when they come in contact with a contaminant. They can even be made to look like at-home pregnancy tests and deliver water quality results in minutes or hours, rather than the days or even weeks needed for laboratory-based tests (Thavarajah *et al.* 2020). Image capture can be facilitated with smartphones, facilitating widespread data dissemination. In short, there is real potential for accurate results to be rapidly produced and shared by everyday people.

This new approach to detecting water contamination can help fix America's broken regulatory approach to protecting the public from lead in drinking water. Surprisingly, the decision to take regulatory action is presently evaluated through costly water testing techniques that are applied to remarkably limited samples hand-picked by regulated entities (Hawthorne & Reyes 2018). The analysis of these samples is reliant on centralized laboratories that, in turn, necessitate sophisticated logistics for sample transport and a suite of technical skills to run the tests. As such, test results are typically not available to those who use that tap for weeks or even months. At-home tests could provide this information more quickly and cost-efficiently.

When the first version of the LCR was introduced in 1991, the United States Environmental Protection Agency (U.S. EPA) decided to impose a relatively light and highly flexible set of monitoring requirements on public drinking water systems because of the aforementioned technical, logistical, and resource challenges. The present iteration of the LCR monitoring requirements varies based on water system size and monitoring history, but, to give some idea, most systems serving over 100,000 people must test only 100 taps every 3 years (40 CFR § 141.86 2011). The result is a system where a water utility can be found 'in compliance' with the lead levels under the LCR, even while taps in thousands of its residents' homes pour water with lead into their glasses.

To contextualize the role that biosensor diagnostics can play in addressing weaknesses of the LCR, some history of the LCR, including the sampling and analytic requirements, is useful. In the late 1980s, lead and copper were estimated to be found in the vast majority of American household taps, as both types of pipes were commonly used in plumbing; at the same time, the effects of ingesting even the smallest amounts of either were proving to cause significant health problems (Mulvihill 2023). As such, in 1991, the U.S. EPA put forth the LCR to implement parts of the Safe Drinking Water Act (40 CFR § 141 1991). This introduced regulatory standards including 'action levels' for the metals, sampling requirements, and a suite of potential corrosion control treatment (CCT) techniques required when exceedances of those action levels were identified through that sampling (40 CFR § 141 1991). The goal was to push public water systems to minimize lead and copper concentrations system-wide, without completely eliminating the risk of public exposure through drinking water (40 CFR § 141 1991).

The 1991 LCR outlined both initial and subsequent monitoring procedures that public water systems were required to implement to determine whether the CCT was required. All public water systems were to conduct initial monitoring during two consecutive 6-month periods beginning on January 1st, 1993. If this initial monitoring revealed exceedances of numeric 'action levels,' the systems were to install optimal CCT by 1997. The public water systems conducted subsequent sampling to verify that the installation and optimization of the CCT had occurred and was maintained. If there was an exceedance of lead and/or copper after installation, heavier monitoring requirements were put in place, with the frequency of the sampling varying with system size. If the concentrations of the metals continued to exceed guidelines, the water system would then need to deploy additional mitigation measures such as public education and lead service line replacement (LSLR) (U.S. Environmental Protection Agency 2020).

The LCR also spelt out how public water systems were meant to conduct this sampling. The number of households to be sampled was based on the size of the service population, such that it could range from 5 sites in systems serving 100 people or fewer to 100 sites in those serving >100,000 (40 CFR § 141.86 2011). Drinking water providers had wide latitude in deciding which households to test. The only specifications were that the samples needed to be from both households with lead service lines and copper service lines with lead solder; when water was drawn for testing, the water flow had to have been motionless for at least 6 h; and 1 liter of water was supposed to be collected (40 CFR § 141.86 2011).

There are several shortcomings in these specifications; predictably, these led to inequitable biases in the demographic characteristics of the households sampled. Public water systems' freedom to choose specific households to sample, rather than requiring for a representative sample of users, renders required LCR sampling insufficient to ensure an accurate and equitable representation of communities served by a drinking water provider. There were a variety of other technical shortcomings, e.g. a 1-liter sample would not capture potential lead contamination from pipes further upstream such as a lead service line.

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Chicago – where nearly 80% of the households in Chicago are supplied by lead service lines – has exemplified the shortcomings of the sampling mandated by the LCR, with serious consequences for childhood lead exposure (Huynh *et al.* 2024). In 2018, it was revealed that Chicago had sampled 50 of its own water utility employees' households (Hawthorne & Reyes 2018). While these results demonstrated 'compliance' with the LCR, lead levels in other parts of the city were discovered to be exceeding the designated action level; some households had concentrations almost 17 times higher than the LCR action level (Hawthorne & Reyes 2018). In 2022, the *Guardian* reported that 9 of the 10 Chicago zip codes with the most lead contamination were in neighborhoods in which racial and ethnic minoritized residents lived, and that one home in a majority-Black neighborhood had lead levels as high as 1,100 ppb – which is 73 times the action level of lead (McCormick *et al.* 2022). Under the LCR Improvements proposed by the EPA in November 2023, which suggest an action level of 10 ppb, this would be 110 times the action level (U.S. Environmental Protection Agency 2023).

In addition to these challenges with monitoring and sampling under the current LCR, analysis is also difficult. The LCR stipulates that samples collected at households must be analyzed using Atomic Absorption Spectrometry or Inductively Coupled Plasma Mass Spectrometry in a state-certified laboratory following a prescribed protocol (40 CFR § 141.23 1991; EPA 816-R-10-004 2010). The lab certification process is costly and complex, resulting in limited and expensive certified lab capacity(~$50 or more for the average consumer). These EPA-stipulated techniques have been criticized for the cost (of both the equipment and the operation), as well as the level of expertise required to conduct the analysis, the turnaround time for results, and the limited analytical range of testing (Hawthorne 2016). This testing technology was, however, all that was available to reliably measure lead and copper in water samples at the time of this iteration of the LCR (Wilschefski & Baxter 2019).

Another shortcoming of the LCR is the latitude in the mandated response(s) when contamination reaches the action level and the action levels themselves. If 10% of the households in a sampled system exceeded the 'action level' (15 ppb for lead or 1.3 ppm for copper), the systems were required to implement additional CCT within 6 months of exceedance (40 CFR § 141 1991). They were also mandated to inform the public within 30 days that action levels had been exceeded and how the public could protect themselves (40 CFR § 141 1991). As for the action levels, they were established not based on human health risks but instead on what the technology could detect 'feasibly.' Feasibility, in this instance, 'entails what is achievable using the best technology and treatment techniques while taking costs into account' (42U.S.C. §300g–1(b)(4)(D) 2023). Thus, the LCR's mechanisms for triggering further corrective action – where 10% of samples exceed 15 ppb of lead, for example – were selected in reference to the technological capabilities of 1991. In contrast to the action-forcing 'action levels,' the EPA also defined a separate threshold – the aspirational Maximum Contamination Level Goal (MCLG) – to be zero in 1991. This was done because the agency recognized that there is no known acceptable level of lead in drinking water from a public health perspective (U.S. Environmental Protection Agency 2020). Cost and feasibility, however, are what separate the public health-based and non-binding MCLG from the 'action level' that carries with it regulatory consequences. As such, a system could claim formal compliance with the LCR even if 9.9% of sampled homes yielded lead levels of 14 ppb.

The Trump administration commendably added a 10 ppb lead 'trigger' level to the original 'action' level framework ('40 CFR § 141' 2021). Sampling results that exceed the trigger level but do not meet the action level require additional planning and monitoring procedures within the water system ('40 CFR § 141' 2021). The public comment process on the proposed trigger-level approach revealed that the U.S. EPA publicly recognized the risks associated with ingesting any level of lead. Still it again set policy based on technological and administrative feasibility. They pointed to the infeasible increase in water system sampling and agency oversight requirements if the 'action' level were reduced to zero. Even the less protective approach imposed significant cost burdens, sampling expenses alone were projected at $26–$29 million annually ('40 CFR § 141' 2021).

In summary, the current LCR clearly leaves plenty of individuals susceptible to dangerous drinking water, even when a water system is in compliance with the LCR. There is, however, hope.

For one, the technologies that informed the LCR have improved considerably in the last 30 years, making the regulatory standards associated with the LCR outdated with respect to the feasibility and cost assumptions at their foundation (Balaram 2018). This is recognized by the Biden Administration, which had the U.S. EPA propose a new rule on December 5, 2023 (88 FR 84878 2023).

This, in turn, is a second reason for hope: four priority areas for LCR revision have been identified: examining the action and trigger-level frameworks, moving from CCT toward full LSLR, strengthening tap sampling requirements, and prioritizing historically underrepresented communities. Because the action and trigger-level framework is built on the sampling and mitigation methods that are feasible, the availability of less expensive and easier-to-implement sampling technology could justify

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more stringent lead and copper levels. Third, new technology that can be widely available and accessible to the public could also empower historically underrepresented communities by facilitating the visibility of inequities in lead exposure and by providing a way to more easily collect data that challenge the stated adequacy of water system programs. It is also encouraging that one of the areas for reform is 'compliance tap sampling.' The U.S. EPA recognizes that 'robust tap sampling is essential to identifying locations with elevated lead' both across systems and within households.

In short, U.S. EPA has expressed that they are ready to learn about new, more cost-effective testing technologies; this is where synthetic biology could transform the landscape. The U.S. EPA modernizing the testing requirements under the LCR would be a significant public policy improvement in its own right. However, the costs and limitations of LCR testing were the underlying cause of other limitations in the rule's efficacy. Therefore, new testing technology could even justify the U.S. EPA's decision to move the substantive action levels closer to the acknowledged public health-based maximum contamination level goal of zero lead.

Biosensor tests promise a number of important improvements over laboratory-based water testing methods (Jung *et al.* 2020, 2022; Thavarajah *et al.* 2020, 2023). Specifically, they require only minimal peripheral materials to operate, they can deliver results within minutes or hours, and they can be used at the tap by individuals without scientific training, including household members. This immediately removes the logistical challenges related to collecting water and shipping it to centralized laboratories. Furthermore, these characteristics have the potential to facilitate testing at a much larger scale than that which is feasible with current EPA-mandated laboratory methods. For example, one can envision blanketing an entire city with biosensor tests to obtain high-resolution data on water contamination as decisions are made about lead line replacement or other mitigation techniques. Third, by facilitating image capture through smartphones, biosensor tests are compatible with large-scale data-driven approaches that can be used to forecast future problems, or linked with other data to investigate health outcomes, thus enabling the data to achieve impact beyond 'merely' monitoring water quality.

While biosensor tests hold promise, it is important to emphasize that the technology is still under development and does have technical limitations. Refinements are needed before lead biosensor tests can meet the same levels of detection and accuracy offered by laboratory tests. Current biosensors that test for lead in drinking water advertise that they can detect lead in the range of 10 ppb (Stemloop 2024), which is below the current EPA lead action level (15 ppb) but above the technical sub-ppb limit detectable by laboratory techniques (Thermo Elemental: USA 2001). The accuracy of at-home lead biosensor tests is being assessed in an ongoing pilot study in 100 households in Chicagoland (Maille 2023), but there is reason to think it will be high. When the accuracy of biosensor tests to detect fluoride in drinking water was evaluated, the tests were shown to accurately classify elevated fluoride ($\geq$1.5 ppm) in 89.5% of the 57 samples tested in Kenya (Thavarajah *et al.* 2023). As the tests are scaled up more broadly geographically, it will be important to demonstrate the robustness of the tests to ambient temperature changes, variations in the chemical composition of drinking water, and reproducibility in the hands of different non-expert users. As a first step, we are seeking to understand whether the household conditions of Chicagoland are sufficiently different from laboratory conditions to impact biosensor performance. Finally, biosensors are limited to analyzing dissolved lead in field deployable tests. Total lead measurement requires the overnight application of strong acids to dissolve lead particulates, which can only be performed in a laboratory under appropriate safety conditions. Future technology developments may allow biosensors to detect total lead, but this is not currently possible.

There are also societal questions to be asked about biosensors, including public appetite for understanding which contaminants are present in their water and the usability of the tests. For that reason, the aforementioned pilot study in Chicagoland is also exploring knowledge, attitudes, and behaviors surrounding lead and lead testing. For meaningful and equitable impact, it will also be important to ensure that these tests are economically, linguistically, and culturally accessible, and appealing to historically underserved populations who bear the majority of water quality issues. This can be ensured through community partnerships, educational campaigns, and thoughtful dissemination of lead test results.

Operational questions will also need to be answered, including standardized practice for data analysis, reporting, and decision-making. One of the goals of the ongoing study is to work toward having a standard approach to test analysis and interpretation by observing users perform the tests to understand variability in interpretation. The goal is to develop tests similar to at-home COVID tests where there are standard instructions for analysis and interpretation, as well as resources to report results to local support systems, with decision-making governed by local communities.

It will also be important to understand how water utility operators regard these tests. They could be perceived as a threat if they believe consumers use the data to criticize the system's performance. They can also be seen as a tool to help consumers gain trust in their water, helping their customers take practical, household-specific actions to protect themselves from water

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contamination. There is also ongoing research to understand if biosensors can be made to function in a continuous monitoring platform. For this, questions about maintenance and calibration will be important to address. In summary, biosensor tests are just starting to be manufactured at scale and field-tested with non-expert users; further refinement and optimization of the technology are needed.

As for financial implications, as biosensor technology evolves, economies of scale in manufacturing will drive down test costs, which currently cost $25 per test. Initially, the addition of biosensors to testing regimens could increase the overall costs of testing, but a regime in which cheaper biosensor tests are used to perform the majority of testing, with more expensive laboratory tests used only in specific follow-up cases, would maintain or lower overall testing costs in a specific community while providing a better picture of water quality.

At this point, biosensor tests can best be thought of as a complement to, rather than the replacement of, laboratory testing and are viable for many important applications within U.S. EPA's pending LCR Improvements. However, they will not be appropriate for inclusion in current definitions of EPA-certified methods for lead testing; these demand rigorous adherence to specific testing protocols, which entail laboratory equipment and trained personnel. Rather, at this point, biosensor tests can be thought of as a simple, scalable, and accessible way to get large amounts of water quality data that can inform the efficient use of more expensive, more costly, and more accurate laboratory-based testing. Biosensors can also be used by the public as a means of education and outreach about drinking water quality. It may be eventually prudent to expand EPA-certified methods to include biosensors for large-scale water quality monitoring once the technology matures.

Data generated by biosensors could be incredibly impactful. For example, they could help to quickly estimate water system compliance with the LCR, more efficiently identify lead service lines for more efficient replacement, and inform households of their water quality, so that they can take appropriate action while they wait for service line replacement. Biosensor tests can also be used to verify that lead contamination has been removed once the lead service line has been replaced. This is important since many plumbing fixtures can still contain lead and can be unsuspected sources of contamination that need to be identified and removed.

Biosensor-generated data can also help utilities and regulators gain a dramatically better understanding of the scope of the lead problem. Moreover, utilities and regulators could more effectively address lead problems by integrating rapid, reliable data collection into LSLR programs and planning processes for system-wide infrastructure solutions. As time goes on and data-driven models are trained on data generated from biosensor tests, models will become more accurate and predictive, helping to accelerate our strategies for dealing with the lead-in-water crisis.

Now that the EPA is seeking public input on its proposed LCR revisions – and proposed revisions already include policy movement in these directions – the agency will be reviewing new technologies that support a more effective, efficient, and protective final rule. We hope that biosensor technologies are considered carefully in light of how they can, and cannot, advance public health. For good reasons, agencies like the U.S. EPA only adopt new technology for regulatory or compliance uses after the techniques are demonstrated to be accurate limitations are understood by the academic scientific community and their usefulness and reliability are established through field testing. This is why the ongoing work to pilot biosensors is so important. Once such a body of knowledge is developed, the U.S. EPA could then take new technology through a proscribed regulatory process for approval as a compliance sampling protocol.

Understanding how new testing technologies can improve U.S. drinking water protections should remind us of two basic truths that apply drinking water quality challenges the world over. First, the social, economic, and health harms of lead and other drinking water contaminants are dire, and addressing them cannot wait for lab processing times, depend on lab availability or be subject to the cost-minimizing and strategic interests of regulated water systems. Second, the rules cannot stop what the regulators cannot see.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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CONFLICT OF INTEREST

J.B.L. has a financial interest in Stemloop, Inc., which is commercializing water quality diagnostics. This COI has been reviewed and managed by Northwestern University in accordance with their policies.

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