**Abstract**

In this proposal, we propose to develop novel applications of the Ames Test as a possible diagnostic tool for detecting medical waste in the environment. Specifically, we are looking to pilot our method using formaldehyde first, but perhaps expanding to more targets depending on the efficacy of our test. Formaldehyde is used prevalently as a disinfectant, but its status as a carcinogen has curtailed its usage. [2] However, many rural hospitals have struggled to switch to newer or more expensive methods, and instead use whatever chemicals they can access. This can lead to improper use or disposal of dangerous chemicals, potentially causing problems in the surrounding ecosystems of areas in question.[8] To remedy this, our team seeks to exploit the mutagenic properties of formaldehyde using a biological assay called the Ames test to assess environmental concentrations of medical waste. While the Ames test is traditionally used to assess genotoxicity [6] we propose to use it as a tool to determine net concentration rather than toxicity. Based on previous observations, we predict that the genotoxicity of a substance scales linearly with its concentration.[6] By performing many versions of the Ames tests with different known concentrations of formaldehyde, we can create a reference curve that can be used to back-calculate the concentration of an unknown sample via how genotoxic it is. A novel method like this provides a low cost alternative to traditional analysis techniques such as NMR, as well as not requiring such extensive technical training.[5] If this method was demonstrated to be effective for formaldehyde, we could generalize the process, and develop similar curves for other potentially problematic genotoxic agents as well. Once the initial curve was created, the cost of performing additional tests and comparing to the reference would be trivial, providing cheap diagnostics to areas that are the most in need.

**Background**

Chemicals like formaldehyde have seen wide use, both as a synthetic precursor molecule and a hospital grade disinfectant.[1] In recent years, it has been steadily phased out in favor of other chemicals due to increased evidence of its negative effect on health. Formaldehyde is a known carcinogen, and improper exposure to it has been linked to nasal and other cancers. [2] Many rural or developing communities do not have the resources to switch away from formaldehyde-based materials. As such, continued use of formaldehyde runs the risk of both exposing those who handle it, as well as leaking out and contaminating the environment of surrounding communities. Further, formaldehyde has several well-studied genotoxic properties that can damage the DNA of microorganisms to varying degrees. Formaldehyde can induce tandem base substitutions, particularly at the 5’-GG and 5’-GA sequences.[3] [4] While these point mutations are detrimental, they are the type of damage most easily repaired by the nucleotide excision repair pathway. Formaldehyde can also induce intra-strand crosslinks between purine residues of DNA. Crosslinks are a much more serious type of mutation, which is often fatal to cells if not properly excised. These mutagenic capabilities cause formaldehyde to pose a serious risk to those who work with it, and damage within the environment in the case of leaks.

Even if every hospital was able to switch to less dangerous chemicals, accidents will always happen. We propose developing cheap biological assays that can be used to gauge levels of mutagenic chemicals. While traditional chemical analysis methods like NMR have existed for a long time, they are often costly to implement and require lots of additional training to use effectively.[5] In contrast, many biological assays can be run quickly and easily in the field and are simple to interpret. Economically speaking, they don’t require a large initial investment, as bulky NMR machines do.

**Approach**

After surveying methods of measuring chemical concentrations, we intend to apply the Ames Salmonella/microsome mutagenicity assay (Ames test) to detect the levels of formaldehyde in medical waste. This bacterial reverse mutation test uses the amino acid-dependent TA98 strain of *Salmonella typhimurium*, which has a pre-existing frameshift mutation on HisD3052 which eliminates the accurate DNA excision repair mechanism, leaving a defective lipopolysaccharide(LPS) layer coating the bacterial surface.[6] This mutation thus causes the bacteria to be both more permeable to bulky chemicals and unable to synthesize the required histidine operon, and therefore unable to grow and form colonies in its absence. [7] Mutagens like formaldehyde, when introduced to the bacteria, can cause new mutations near the site of preexisting TA98 mutations and potentially restore the gene’s function, allowing the cells to synthesize histidine normally. The Ames test functions by culturing the TA98 colonies on surfaces without histidine. If colonies can grow, additional mutations must have occurred. The size and numbers of colonies observed is correlated with mutagenic capabilities of a compound it has been exposed to.

The quantitative relationship between numbers of revertant colonies and formaldehyde included in a given sample of medical waste would still be unknown. Thus, before applying this method to test a sample waste concentration, repeated experiments are required to build a reference curve. By introducing varying known concentrations of formaldehyde, numbers of induced revertant colonies could be recorded, and used to construct an appropriate relationship for reference. With this reference curve, the concentration of formaldehyde in medical waste can now be measured by using the Ames test and resulting colonies grown, compared against the created reference to obtain the result concentration.

**Risks/Justification**

Previous techniques and methods of assessing concentrations of chemicals are already in existence, such as NMR. Additionally, using analytical chemistry as the centerpoint of analysis provides some information that our test cannot, such as the specific identity of the molecules in question. These previous techniques don’t require investigation to prove their efficacy, and are known to be reliable. In spite of this, we believe our method would still be beneficial to the STEM community at large. Compared to purchasing an NMR machine, once our reference curve was established, our method would be incredibly cheap. [5] The only costs would be collecting samples from the environment, purchasing the strains of TA98, and the nutrient broth needed to culture them. In contrast, an NMR machine is very expensive to install, and requires advanced training to use and interpret properly. Besides, the strain of TA98 is a safe experimental material which is classified under the lowest level of risk group 1. [7]

The created reference curve would provide a method of quantifying both concentration of waste present as well as potential mutagenic risk. As it is based on the disinfectant formaldehyde, this curve would be most applicable in testing areas surrounding under-funded hospitals or medical facilities, but could also be useful in synthetic plants where formaldehyde is commonly used as a precursor molecule. As previously discussed, formaldehyde is still commonly used in developing countries due to its abundance and low cost, despite its carcinogenic effects. The Dominican Republic in particular has been shown to experience a significant level of overexposure to formaldehyde [8]. For example, improper storage, handling and disposal of formaldehyde contribute to this issue for the Hospital General de la Plaza de la Salud, however unplanned leaks from sterilizing equipment is a main cause. The reference curve could be used to assess potentially hazardous levels of formaldehyde waste in the community surrounding this particular medical facility.

One of the challenges we may encounter in doing this project would be to finely control the samples collected when applying this test to medical waste samples gathered in the field. This could prove difficult due to the variety of objects formaldehyde may be preserved on, as well as the varying environmental conditions it could be exposed to. Several previous formaldehyde emission test methods revealed that conditions, such as test temperature and sample handling, can greatly impact results. [9] Further, in our project, material included in or impacted by the medical waste may vary from plastics to different fabrics. Since previous research suggests that formaldehyde emission can be reduced by fibrous products, it is reasonable to conclude that the stability of formaldehyde may vary on different materials.[10] Overall, it is important to consider the handling of samples when calculating the reference curve, and to handle and tests samples in the field following the same protocol. The materials included in the medical waste is a variable that could be difficult to control. It is unlikely, however, that this would have a significant enough effect on the results of the Ames test results to cause a discrepancy between the actual concentration of environmental formaldehyde, and that suggested by the more controlled reference curve.

Once demonstrated to be successful, our method of developing reference curves based on the Ames test could have applications beyond just formaldehyde. Once funding is secured, many different curves for different genotoxic agents only need to be produced a single time; once the curve is established, any laboratory can compare their Ames test results to the reference for quick feedback on a variety of chemicals. This could be particularly useful for hospitals or facilities with known waste management issues. A tailored set of reference curves and Ames test strains could be used based on the specific needs of the facility to quickly and effectively check the environmental concentrations of chemicals. Thus, our proposed method would be particularly useful when paired in tandem with internal waste management investigations at different facilities, suggesting that our method works best when the identity of the contaminant is already known. However, even if a user was unsure of the identity of the contaminant, our Ames test could still indicate high levels of genotoxicity This would thus provide valuable information, still confirming that an environmental leak had, in fact, occurred.

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**Individual Contributions**

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