



**There's Something  
in the Water:  
*N*-nitrosodimethylamine**

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Professor Bevin Page Engelward

# THERE'S SOMETHING IN THE WATER: N-NITROSODIMETHYLAMINE

*N-nitrosodimethylamine* (NDMA for short) is a worryingly prevalent, potentially potent carcinogen found in food, water, cigarettes and medical drugs. Researchers at the Massachusetts Institute of Technology, USA, are working to better understand what NDMA does and how cells can defend themselves from its effects, with important implications for public health.

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## A Once Little-known Carcinogen

Millions of people worldwide have been exposed to what was once a little-known carcinogen. *N-nitrosodimethylamine* (or NDMA) is becoming a household name as a result of nearly a dozen recalls on critical medications that were contaminated with NDMA and taken by people on a daily basis. Valsartan (for blood pressure), Metformin (for diabetes), and Zantac (for gastric reflux) have all been recalled due to the presence of NDMA, leaving people both concerned and without critical medicines, and costing billions of dollars to the pharmaceutical industry.

NDMA has also been found in drinking water consumed by millions of people as a result of chemical reactions that occur when water containing organic materials is treated via chloramination. In fact, a survey by the United States Environmental Protection Agency revealed that as many as 10% of municipal water sources contain NDMA.

Currently, the impact of NDMA exposure is uncertain and depends on many factors, including how long people were exposed, at what age, at what level, and whether or not they are genetically predisposed to be susceptible to NDMA's carcinogenic potential.

Professor Bevin Engelward is the Director of the MIT Superfund Research Program (SRP) and she is joined by Professors Desiree Plata and John Essigmann, who serve as Deputy-Directors. The MIT SRP team is currently studying NDMA. While the team cannot yet answer the question of 'how much NDMA is too much?', they are making important advances toward understanding genes that likely impact susceptibility to cancer. They also provide convincing reasons to believe that exposure during development is more problematic than exposure as an adult.

## What Does *N-nitrosodimethylamine* Do?

A key issue for the MIT SRP team is to determine what exactly NDMA does, and to identify how cells can defend themselves from its effects.



For a small stretch of DNA – let's say 10 base pairs reading ATCGTATATG – there are 97 carbon atoms, 73 nitrogen atoms and 20 oxygen atoms. Professor Engelward explains that adding just one additional carbon to a place it does not belong can create a 'DNA lesion' with dire biological consequences. The problem is that an extra carbon (a 'methyl' group) at certain sites on DNA bases like adenine can 'jam up' DNA polymerases, which replicate DNA, causing them to get stuck.

If there is one critical thing that a cell has to do every time it divides, it is to accurately replicate the entire genome. If there are 3-methyladenines in the way, polymerases can bumble over the damage, creating a risk of putting in the wrong nucleotide, and thus causing a mutation. While one mutation in the context of the 6 billion base



pairs may seem trivial, it all depends on where it happens. A mutation in a gene called p53, for example, puts cells one step closer to cancer. Given that NDMA is very good at creating 3-methyladenine lesions, it is no surprise that it can cause cancer.

Work led by Dr. Jennifer Kay with support from Mr. Joshua Corrigan, Dr. Amanda Armijo, and other members of the MIT SRP team, has demonstrated for the first time that 3-methyladenine lesions caused by NDMA are indeed carcinogenic in animal models. By genetically engineering mice that are not able to remove 3-methyladenine, the team has demonstrated that these mice are highly prone to NDMA-induced mutations and cancer.

The enzyme responsible for repairing 3-methyladenine is called AAG. This enzyme scans and hops along the DNA searching for 3-methyladenine lesions (and other kinds of damage). When it finds a lesion, it swivels the damaged base into its active site and chops it off. While this is a good thing, since it gets rid of the 3-methyladenine problem, it also creates a new problem: a missing base.

As you can imagine, not having a base is a problem, since the cell won't know how to read that piece of DNA. But there's an even more serious problem. To resolve the empty site where the damaged base was removed, the cell needs to cut the DNA backbone so that replication machinery can get in and fill in the missing information. While this is generally a good thing, it can be a problem if there are too many repair patches. Professor

Engelward provides the analogy of having a bump in the road. The bump itself is a problem, but digging out the bump and repaving the road is also a problem until everything is fixed.

This is where the story gets even more interesting. Dr. Kay and the MIT SRP team used mice that have very high levels of AAG to see what would happen when they were exposed to NDMA. Not surprisingly, the mice had fewer instances of cancer, since there were lower levels of 3-methyladenine and therefore lower levels of mutations that could promote cancer. However, the mice had a new problem: cells were dying. A lot of cell death means that the damaged tissues cannot function properly and in some cases, the mice could not survive. But how does this all relate to the problem of people being exposed to NDMA?

### **The Consequences of Human Exposure**

The biggest challenge in discerning the risks of NDMA exposure is that we really don't know what happens to people who are exposed to relatively low levels of NDMA for a long period of time. Professor Engelward notes that, if we were to take an educated guess, we would hypothesize that exposure *in utero* would be more problematic than exposure as an adult, because there needs to be a lot of cell division to go from one fertilized egg to the trillion cells that are necessary to create a baby. Every time a cell divides, there is a risk of a mutation. If you now layer onto that risk the additional DNA damage caused by exposure to NDMA, there is certainly a reason to be concerned that NDMA could promote mutations that eventually give rise to childhood cancer.



Remarkably, an epidemiological study (the study of the distribution and determinants of disease) showed a connection between NDMA exposure *in utero* and an increased risk of cancer in children. This came about because of a Superfund site located in Wilmington, Massachusetts, where waste products reacted with one another to create extremely high levels of NDMA that eventually made their way into the town's drinking water wells. The tragedy that ensued was intolerable. Nearly two dozen children in the town with a population of under 20,000 got cancer, and some of them did not survive.

The people of Wilmington have been struggling for years to find out what was causing cancer in their children. A study by the Massachusetts Department of Public Health that pointed to an association between NDMA exposure *in utero* and childhood cancer provided some relief since it validated the townspeople's beliefs, but at the same time, it caused a resurfacing of extreme grief. Professor Engelward explains that while there is nothing that can be done about the past, the people of Wilmington want to make sure that nobody else has to suffer the way that they did.

It is important to recognise that this is just one study. In most cases, researchers compile data from multiple epidemiological studies in order to gain confidence in the results. Clearly, much more work needs to be done to piece together the missing information on the extent to which NDMA in drinking water is a risk for cancer.

In pursuit of that goal, Dr. Robert Croy and Professor John Essigmann, who are members of the MIT SRP team, have developed a novel way to determine the biological effects of NDMA in drinking water. They developed a sensitive method to measure the products of DNA damage by NDMA (e.g., O<sup>6</sup>-methylguanine, 3-methyladenine and 7-methylguanine) in the genomes of animals that have been given small amounts of NDMA in drinking water over several weeks. At least one of these DNA-derived products, O<sup>6</sup>-methylguanine, is a widely known biomarker of genetic damage by environmental chemicals. Their work meshes seamlessly with the additional genetic studies, and the development of sensors to detect NDMA, going on as other parts of the MIT SRP.



### Critical Next Steps

When asked about her next steps, Professor Engelward explains, 'We see a path forward with two elements. The first thing is that we want to understand what happens when animals are exposed to NDMA in drinking water, the way that people are exposed'. She further elaborates, 'In our earlier studies, mice received a high dose of NDMA, much higher than what people experience. We are now setting off to do long-term drinking water studies with much more realistic levels.'

Professor Engelward and the team's ongoing work including DNA repair-deficient mice in their studies is important because AAG levels vary from person to person, and people with low AAG may be at increased risk. When thinking about the risk of exposure in the population, it is critical to consider the risk of those who are most vulnerable. As her earlier research pointed to AAG as a susceptibility factor, Professor Engelward now aims to find out if it in fact defends against cancer caused by contaminated drinking water.

There is much work to be done by researchers, and a lot of work to be done by people in the pharmaceutical industry to make sure that people's drugs are free from NDMA. Professor Engelward reminds us that 'The good news is that we have a lot of knowledge about NDMA and we have ideas about its potential biological effects; now it is time to do the hard work and get the research done.'

For people who already have been exposed, there are steps that can be taken. On this, Professor Engelward concludes, 'All of the good advice that you get from your nurse or doctor about exercise, eating right, and avoiding exposure to chemicals, is good advice, and can go a long way toward offsetting any possible increased risk of cancer caused by NDMA.'

With a team of highly talented students and more senior researchers, as well as support from the National Institute of Environmental Health's Superfund Research Program, we can be confident that the MIT SRP will soon present a much better understanding of the risks that NDMA poses and the implications for public health.

# Meet the researchers



**Professor Bevin Page Engelward**  
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Professor Bevin Page Engelward graduated from Yale University and then received her doctoral degree from the Harvard School of Public Health. In 1997, she became an Assistant Professor at the Massachusetts Institute of Technology (MIT). She is now a Professor in the Department of Biological Engineering and the Director of the MIT Superfund Research Program. With an overarching commitment to improving public health, Professor Engelward's research focuses on gene-environment interactions that modulate disease susceptibility through the development of novel tools for studying exogenously induced genetic changes in animals and human cells.

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## FURTHER READING

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**Dr. Jennifer Kay**  
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Dr. Jennifer Kay received her bachelor's degree from the University of Pittsburgh and went on to complete her PhD and postdoctoral training in Professor Bevin Engelward's laboratory in the Biological Engineering Department at MIT. As part of the SRP, Dr. Kay helped lead research projects using mice genetically engineered to have different DNA repair capacities and reporter genes for measuring mutations. She also served as the MIT SRP Research Translation Core director, bringing

MIT SRP research advances to community groups, government agencies, other academics, and the general public. Dr. Kay is now a Research Scientist at Silent Spring Institute, studying environmental exposures that can lead to breast cancer. Dr. Kay's goals are to understand what chemicals do to cause cancer so that people can be better protected from potentially dangerous exposure.

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