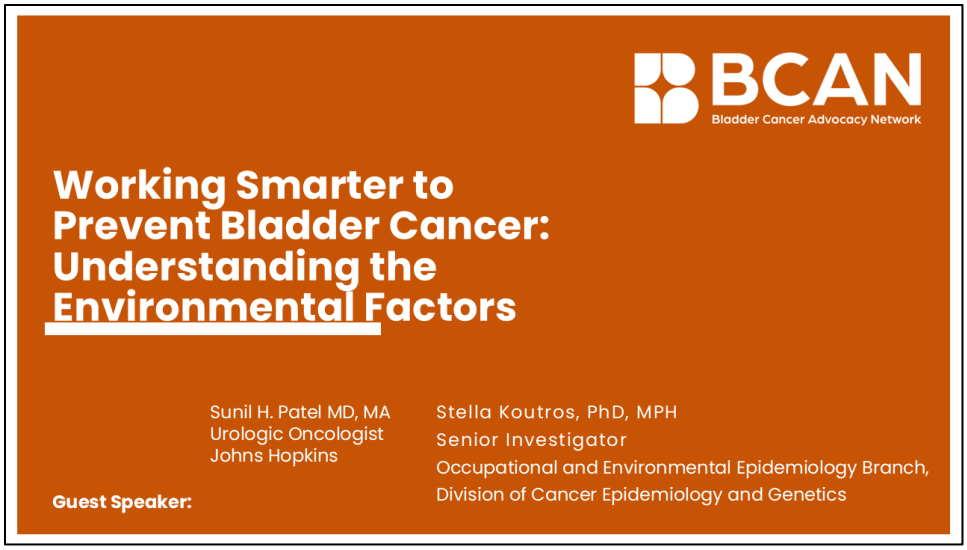
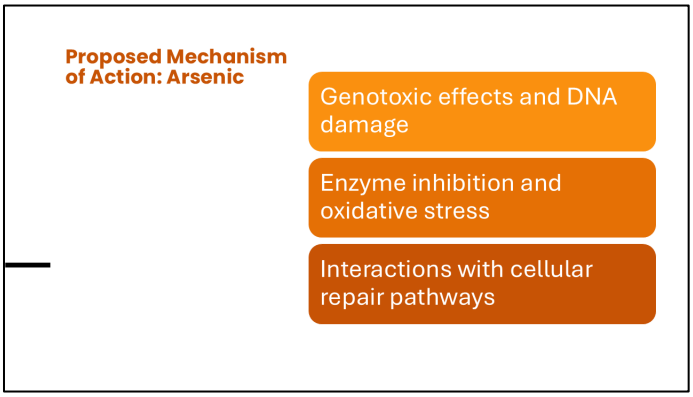


Dr. Sunil Patel:



And so when we talk about arsenic and we talk about the strong association, we still do not have a really good example or good finalized mechanism of how this is carcinogenic. It's been hypothesized that there are genotoxic effects and DNA damage, it can alter enzymes and create oxidative stress, which would promote carcinogenesis or interact with certain or repair pathways. I was talking to one of my colleagues recently and we're in developing another study in environmental carcinogenesis, and we talk about almost the thought of evolution and how we've became from single cell organisms to what we are right now and how we've developed and all living things have developed mechanisms to protect themselves from the environment or the exposures they are exposed to.

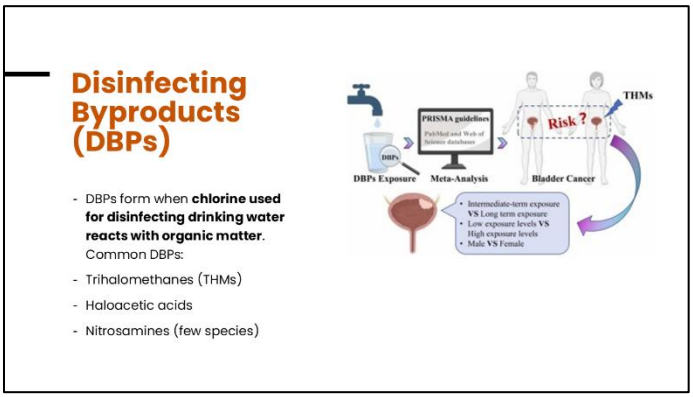
And the human body has a lot of pathways and mechanisms to correct them, namely, a lot of our liver enzymes are actually meant to help combat a lot of these toxins.

And so we hypothesize that they're potentially not just arsenic, but other exposures and other environmental toxicants could just have... Our bodies have not developed the protected mechanism. So we don't really know for sure which way arsenic can, and it's been hypothesized it can interact with a Sonic Hedgehog gene and create mutations there at the basal level, and that's what creates carcinogenesis and bladder cancer. There was a study

done in China about three years ago that showed it with a small study or a small sample size, but that was done all ex vivo.

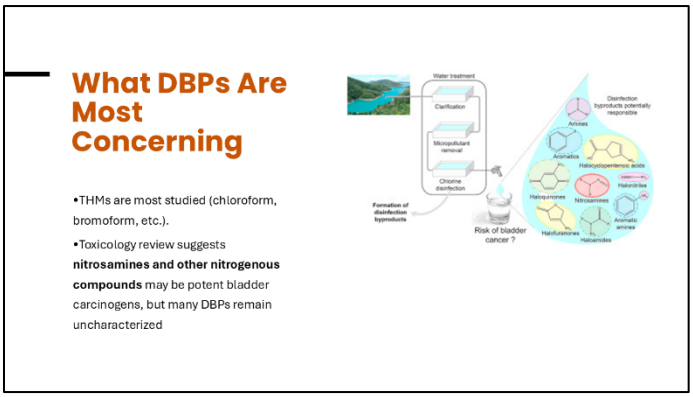
But again, there's multiple pathways and there's a lot of hypothesis, and I think that's going to be the next step of how, if we can figure out a mechanism of this carcinogenesis or potentially this co-mutagen, then we can figure out a way to target it or combat it. Next slide.

Dr. Sunil Patel:



And now shifting over to disinfecting byproducts, again, another environmental risk factor that's becoming more and more evident and/or being studied more. And so DBPs, it's a little tongue twister, 401 chlorine is used for disinfecting drinking water and when they react with organic matter. The most common ones are trihalomethanes, so THMs, haloacetic acids, and nitrosamines. And so those are the three most common ones. We're going to focus a little bit on the trihalomethanes because those have been studied the most. Next slide.

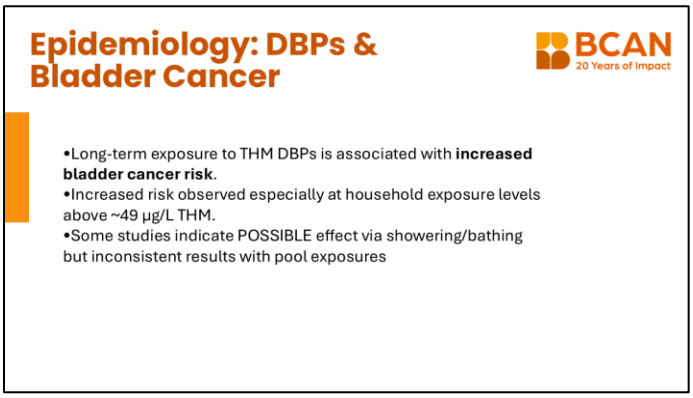
Dr. Sunil Patel:



So again, what DBPs are the most concerning? Trihalomethanes are the most studied, so chloroform, bromaform, et cetera. Again, a lot of key component of disinfecting byproducts. There are also some suggestions that nitrosamines and other nitrogenous compounds may be known bladder cancer carcinogens, but we still don't have a good grasp of all of the DBPs out there. Next slide.

Dr. Sunil Patel:

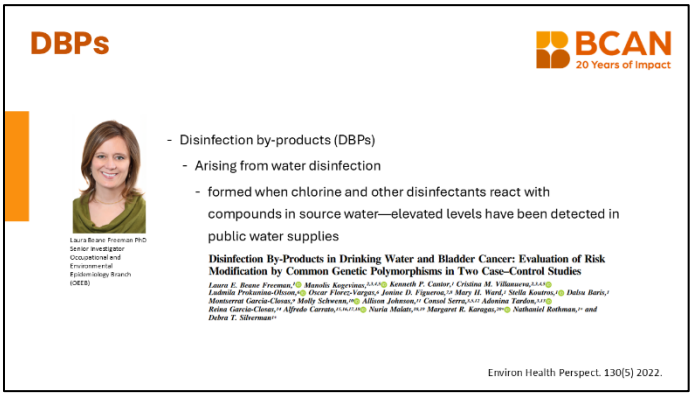
So from the epidemiologic literature, we do know that long-term exposure to these trihalomethanes is associated with increased bladder risk. Again, the risk is obviously, again, time-dependent, dose-dependent variables. There is an increased risk observed, especially household exposure levels, which is above 49 micrograms per liter.



There's been some conflicting data, but there has been some good data showing that some studies do indicate that there's a possible effect with showering and bathing, but I think the studies have been really conclusive in modern era and I think recently done at the NCI, showing that pool exposures really do not increase the risk. So it's thought to be due to the ingestion, but they're all potentially mechanisms of how we can be exposed to these. Next slide.

Dr. Sunil Patel:

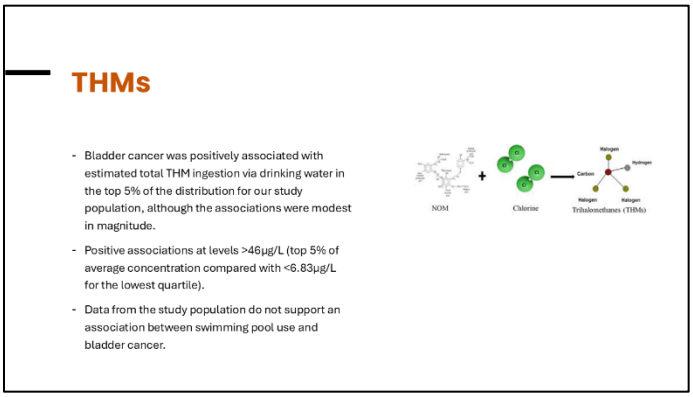
And again, Dr. Freeman, another excellent researcher over at the NIH, NCI, really focuses on this and really found that it's, again, what's formed from chlorine and other disinfectants that clearly showed that there's been elevated levels in these. And again, this was another two case control study. So it's a very large study done by big case controls with thousands of patients.



And again, this study really highlights, and a lot of the other ones we just talked about really highlights how from the epidemiologic literature we find these associations and potential causal links to cancer development. And so Dr. Freeman did a beautiful job of this study, and I urge all of you guys to take a look at this study because it really highlights a good way to do these big large population studies. Next step or next slide, excuse me.

Dr. Sunil Patel:

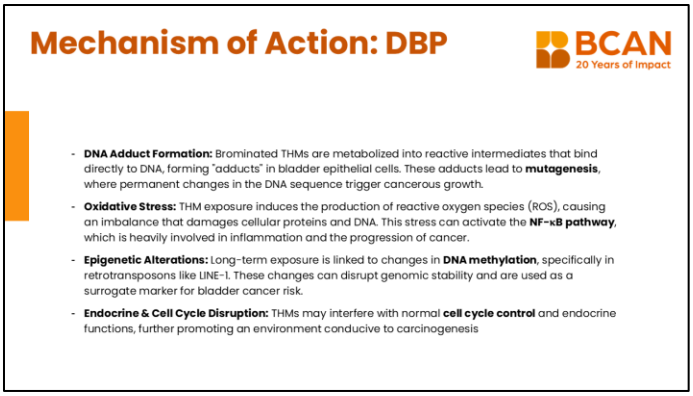
And so in that study, Dr. Freeman found that bladder cancer was positively associated with total THM or trihalomethane ingestion via drinking water in the top 5% of the distribution study. There were positive associations with higher than 46 micrograms, so the top 5% compared to the lowest quartile, which is less than six.



And again, that study that she did did not support the association between swimming pool use and bladder cancer development. Next slide.

Dr. Sunil Patel:

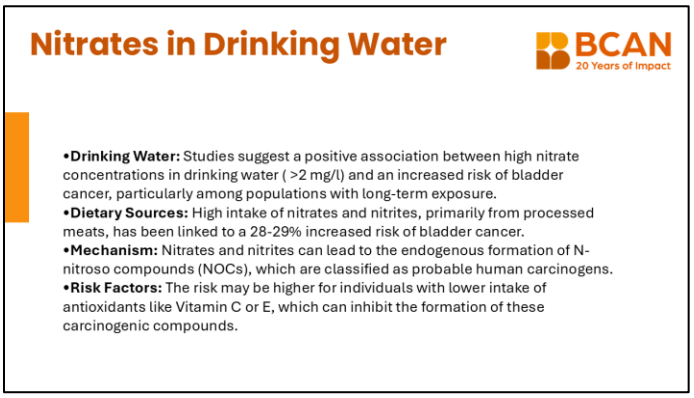
And again, the mechanism of action, again, is all hypothetical at this point, but brominated THMs are metabolized and the reactive intermediates bind to DNA and they favor bladder epithelial cells, and they can lead to mutagenesis, again, causing DNA damage and then triggering a sequence and how a lot of cancers develop this repetitive sequence without being checked on and causing cancer's growth. Oxidative stress, again, potentially... TSMs can create reactive oxygen species.



Again, another way that we think of sometimes even patients in obesity and cancer development, especially in kidney cancer, causing an imbalance of cellular proteins. And it's hypothesized it can affect the NFKB pathway, again, which is heavily involved in regulation of cancer development. And then we look at the epigenetic alterations as well, causing DNA methylation. And then another aspect of cancer development, which I think we're learning more and more about is endocrine disruption.

And as we know, endocrine and hormonal functions is very important and potentially can be protective as well. And THCMs may alter normal cell cycle and endocrine function, which can potentially create bladder cancer as well. Next slide.

Dr. Sunil Patel:

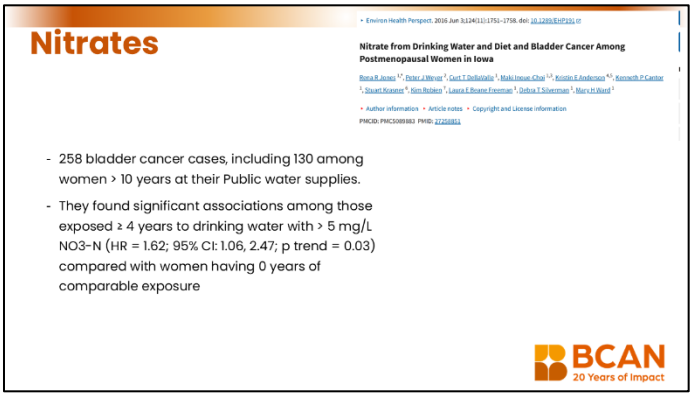


And then shifting into nitrates. So nitrates is another environmental risk factor that we've been looking into as well, we, meaning the whole bladder cancer community. And studies show that there is a positive association between higher nitrate concentrations in drinking water and an increased bladder risk, again, potentially amongst patients and people exposed to long-term. Again, so I think the key hallmark, there is some sort of longevity, with this like Dr. Silverman's really show that there's around a latency about 40 years.

So we potentially are thinking about the same thing for these other risk factors. Dietary sources, I think that that is one that, it's another way that we can ingest and get nitrates. However, that's not been truly a hundred percent proven, but high intake of nitrates and nitrates, essentially processed meats have been linked to increased risk, but we don't really know the mechanism.

Again, and then the mechanism that we think can lead to it is just the endogenous formation of NOCs, which are probable carcinogens. And then another risk factor for nitrate in development is lower intake of antioxidants, vitamin C, E, which can inhibit the formation of some of these. Next slide.

Dr. Sunil Patel:



So this was a study done looking at the nitrate from drinking water in diets and bladder cancer amongst post-menopausal women, specifically in the state of Iowa. And they looked at around 250 bladder cancer cases, including 130 among women, and they looked at their public water supplies for greater than 10 years. And they found that there was a significant association with the exposure, so greater than four years of that drinking water with greater than five milligrams per liter compared to women who had zero years of that comparable exposure.

Again, another really good way to study this is the long-term or longitudinal studies of looking at patients and following them for several years and understanding their water supplies. And I think that's a brilliant way that our epidemiologists really learned about these, is that understanding, getting these really good data from public water municipalities and getting good data for that.

So the study really did show that there is an association with dose, but also time. Next slide.

Dr. Sunil Patel:

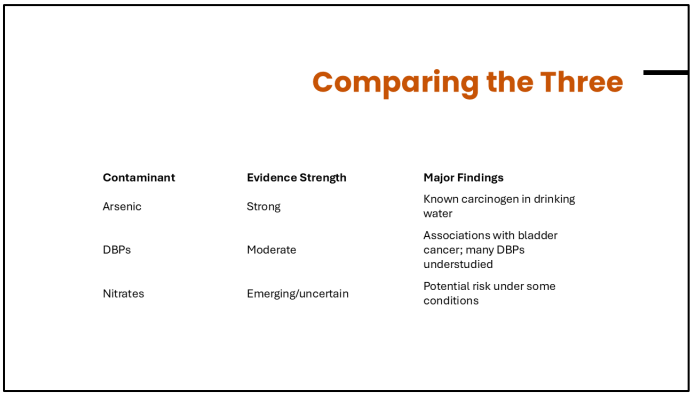
And this, again, is another key study, again, done by Dr. Ward. And again, looking at ingested nitrates and nitrides in bladder cancer, again, and this is mainly through water supplies. And we believe that this is primarily from agricultural sources. So nitrogen and nitrates can be used and are commonly found in fertilizers and manure and human waste.

So we do know that they're often used in our agricultural communities. So in Dr. Ward's study, they found that the average drinking water nitrate concentration was above the 95th percentile compared to the lower ones in patients who developed bladder cancer.

So again, another kind of concentration variable seen and seen that there is an increased association with that as well. Next slide.

Dr. Sunil Patel:

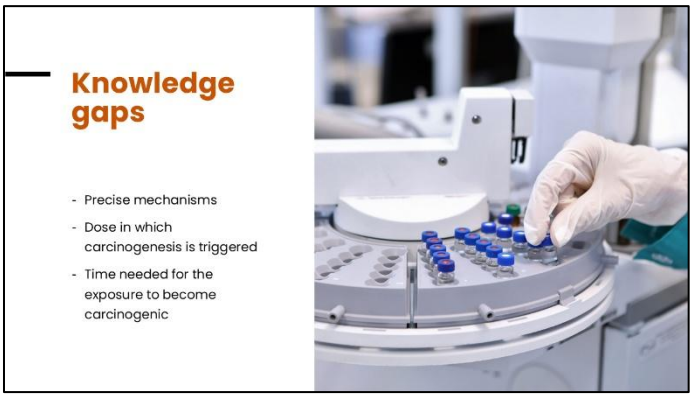
Again, so if we really look at comparing the three that we highlighted today, we have arsenic, DBPs and nitrates. So arsenic, I would say we probably have the strongest evidence in terms of it being a carcinogen. Again, I think the mechanism is yet to be fully described. That's something that we're actually actively working on, Dr. Koutros and I. And really this was actually, commend BCAN for allowing this in patients from BCAN as well as all the researchers to create this almost a task force out of the think tank to really study this. And that's one of our research goals is to figure out a mechanism for this.



DBPs, again, I think there's moderate strength. I think there are good associations with bladder cancer, but we don't have a true understanding of a lot of them. Again, I think the trihalomethanes have been studied the best.

And then nitrates as well, it's still emerging. I guess there's some uncertainty, I think there is clear an association. We're trying to help understand that a little bit more as well. Next slide.

Dr. Sunil Patel:



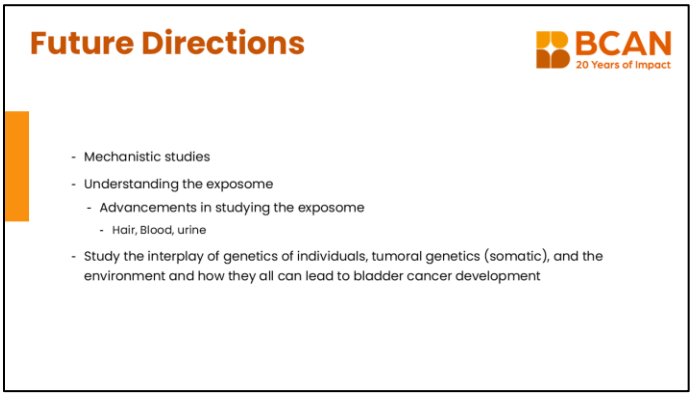
And so the knowledge gaps in this are, I think I alluded to this several times, is precise mechanisms. I think finding the mechanism of how these are either mutagens or co-mutagens or how they disrupt our normal body's checkpoints is going to be key in order to understanding bladder cancer development.

I think we made a lot of progress, and again, namely due to our brilliant epidemiologists at the NCI who really figured out and found dose associations, but I do believe that we can do better in this. And when is that ultimate trigger? We've hypothesized creating almost this calculation of a dose plus the time, and maybe that's the number that we need to figure out, and that's going to trigger someone to be an increased risk in maybe potential introduce screening.

And again, going to that time, the time needed. I think we're learning that more.

And I think, again, Dr. Soleman's study with the New England water study was really pivotal in that, but I think for a lot of these other environmental risk factors, even a lot that we did not cover today, that's something that's absolutely key. These three things, the mechanism, the dose and the time. Next slide.

Dr. Sunil Patel:



So future directions, like I said, mechanistic studies I think are going to be key. And another thing is really understanding the exposome. So the exposome is, again, everything that we are exposed to, and we're having more developments in actually studying the exposome. So it's really hard to study exposures.

And a lot of this are retrospective studies where we're going back or we're doing longitudinal studies and we're following patients for X amount of years, but we're maybe getting epidemiologic studies or information from that saying their water quality, their duration of exposure, potentially the dose of what they're exposed to. But sometimes that's a very generalizable thing. Sometimes we don't know the individual.

Also, we have not been able to really correlate that to in terms of that individual's cancer. So what we've been trying to do here at Hopkins, and actually Dr. Koutros and I are doing a study together, which is currently in progress, is identifying a small cohort of patients in studying their hair, blood, and urine, which can potentially hold a little bit of more of what they've been exposed to, comparing that to what their environmental exposures that were known through public data and epidemiologies and looking directly at their tumor.

And that kind of gets to the last bullet point for this is really study the interplay of the genetics of the individual, whether that's mainly their germline. And there's been a lot of germline hole-wide association studies that have been coming out and tumoral genetics, so the somatic aspects of these tumors, and again, their environment and their exposome, and really creating a way to look at this from three different ways. And I think that would be the ultimate goal in environmental carcinogenesis. If we can really study this wholly, that would lead us to more advancements in bladder cancer.

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