# THE DISCOVERY AND SUBSEQUENT RESEARCH OF CRYPTOSPORIDIUM INACTIVATION

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## ABSTRACT

Increased concern about the presence of pathogens in drinking water, coupled with concerns about the creation of byproducts from halogen disinfectants commonly used to stop them, created a major dilemma for the water treatment industry. Alternative technologies, such as, ozone and membranes, were expensive. This perception also included the application of ultraviolet (UV) technology, which had long been accepted for wastewater treatment, but not deemed acceptable for cryptosporidium at affordable dosages. Calgon Carbon Corporation, a company with extensive experience with UV for wastewater treatment, and other products for drinking water, achieved a scientific breakthrough to solve this dilemma. Calgon Carbon Corporation successfully demonstrated the economically viable usage of UV in novel laboratory testing and original pilot testing, and brought a patented process to market. This firm also achieved the first operational surface water installation in North America in addition to being the largest successful installation of its' kind. Further, Calgon Carbon Corporation continues to lead in validation efforts and other activities to help the municipal water industry realize the benefits available from the new technology.

## KEYWORDS

*cryptosporidium*, disinfection by products (DBP), inactivation, infectivity, pathogens, ultraviolet (UV), validation

## DRIVING PUBLIC SAFETY CONCERNS

In 1998 the U.S. EPA Surface Water Treatment Rules (SWTR), required microbial control, and permitted certain levels of disinfection by-products (DBP). Common examples of microbial disinfectants, and their by-products, would include; trihalomethanes (THM) and haloacetic acids (HAA) from chlorine; chlorite from chlorine dioxide; and bromate from bromide in the presence of ozone.

In addition to the regulatory concern over the presence of these compounds, there is also a drive to further protect the public against microbial contamination, especially since the outbreak of *cryptosporidium* in Milwaukee in 1993. But increasing microbial control by increasing use of chemical disinfectants risks increased production of DBP.

Meanwhile, some water producers had relatively pristine, protected watersheds, but others received runoff from agricultural, waste treatment, and other operations that created an environment for opportunistic pathogens. As a result, raw water entering a surface water treatment plant varied considerably in levels of suspended solids that might include pathogens. But new or enhanced removal of suspended particles by means of filtration/ membranes, as an alternative or supplement to disinfection, was often cost-prohibitive.

Studies had shown UV disinfection to be effective against some pathogens without significantly increasing DBP levels or having other adverse effect on water quality, but there were other concerns about its use.

## PREVIOUSLY PERCEIVED UV LIMITATIONS

UV technologies have long been known to be effective for disinfection of viruses and bacteria in drinking water, and guidelines for the disinfection of viruses exist in the U.S. EPA Alternative Disinfectants and Oxidants Guidance Manual. However, UV was widely considered to be ineffective for encysted protozoa, oocysts containing the reproductive units of organisms such as *cryptosporidium and giardia*, as it was thought that the UV light would be filtered by the oocysts' thick protein shells, preventing the UV from getting in to cell interiors and causing *inactivation*.

Upon undertaking expansion of its UV treatment services from wastewater and remediation into drinking water, Calgon Carbon's 1996 search of the literature revealed that it "taught away" from using UV against *cryptosporidium* and *giardia*. Doses as high as 3000-5000 $mJ/cm^2$ were stated as required to kill those microbes, making UV treatment economically unfeasible.

## REVOLUTIONARY BREAKTHROUGH

Dr. R.D. Samuel Stevens, Calgon Carbon's director of the research, did not understand why doses for these pathogens should be two orders of magnitude (100x) higher than required for other microbes. His experience was that most bacteria took 10 $mJ/cm^2$ to achieve 4 log (99.99%) disinfection; some viruses 30 or 40 or 50; and some spores several hundred. This was nowhere near the 3000-5000 said to be required for protozoan oocysts.

Meanwhile, his principal associate on the Calgon Carbon research team, Dr. James R. Bolton, a photochemistry expert who also had knowledge of protein chemistry, did not believe the oocysts' protein shells should be such formidable filters against the penetration of UV light. He noted that proteins did not absorb very strongly until UV wavelengths were below 240 nm, in the range of 200-240 nm. He could calculate that above 240 nm, there would be very little UV absorption, and the shell would act more like a window than a protective shield.

He also knew that the peak of DNA absorption was at 260 nm, making it applicable to UV. This new theory put forward by the researchers was further complicated by the commonly used excystation assay technique, which measured breakdown of oocysts' membranes (outer shells), thereby ending all metabolic activity, and did not assay the ability of the sporozoites inside to reproduce, which was necessary to cause mammalian infection.

Following discussion with Dr. Bolton and Calgon Carbon's Dr. Bertrand Dussert, Dr. Stevens decided that Calgon Carbon would undertake its own testing. It began in the fall of 1996, using the in-vitro methods of excystation and vital dyes, which both measured loss of integrity of the oocyst shells. The in-vivo mouse infectivity method, which measured actual mammalian infection, was used only as a confirmation in some runs, in order to keep costs down.

When results were confusing and contradictory, it seemed to confirm the previous literature, and progress was deemed slow, a review meeting was called.

It was during that meeting that the breakthrough in theory and approach occurred. When the three methods were thoroughly discussed, it became apparent to Dr. Stevens and Dr. Bolton why the results from all the years of previous research had been so misleading: the in-vitro methods measured whether the microbes were killed, while the in-vivo method measured if they were inactivated; i.e., unable to replicate. If they could not replicate, they could not cause infection.

**NOVEL LABORATORY TESTING**

In the fall of 1997, to move forward on this discovery, Calgon Carbon assigned the new testing that would be required to Clancy Environmental Consulting. The firm subcontracted mouse infectivity studies, where *cryptosporidium* oocysts are exposed to UV, collected, and then fed to mice, whose intestines are then microscopically examined to determine whether or not *cryptosporidium* has multiplied and infected.

Substantial headway ensued with in-vivo infectivity used as the primary assay method, and the in vitro methods used for comparison purposes. Calgon Carbon sent the firm its collimated beam bench apparatus, and dispatched Dr. Bolton to train the staff in the use of it. Dr. Bolton also supervised the UV exposure of *cryptosporidium* oocyst suspensions in Petrie Dishes, and their shipment to the subcontractor for ingestion by mice.

The application of medium-pressure UV light (200-300 nm) was investigated at bench scale. Low doses were not expected to work, since low dosages had not been previously expected to be affective. The prior literature search had said that 1000 mJ was the minimum required. The lowest dosage chosen was 40 $mJ/cm^2$.

The investigators were stunned with the bench results in late 1997 and early 1998, which showed in-vitro methods still pointing to high doses to "kill" (fracture oocyst outer shells), but infectivity showed much lower doses to prevent replication, as measured by 4 log (99.99%) inactivation. The initial range of doses that was thought needed to change the degree of infectivity had surprisingly resulted in no mice showing symptoms of infection.

This confirmed Calgon Carbon's contention that in-vitro assay methods massively overstated the dose required for *cryptosporidium* inactivation, and that UV disinfection was, in fact, very effective for inactivating *cryptosporidium* at low UV doses. This revelation was a complete departure from understandings and information noted in previous literature/ studies.

The low doses dimerized, or cross-linked thymines in the *cryptosporidium* DNA double stranded helix, disrupting the process of forming new DNA, which requires complete separation of the two strands. With its DNA thus altered, the organism might be metabolically alive in terms of intact membrane or outer shell, as well as all other cell functions, but it could not reproduce, and was therefore rendered effectively harmless.

## ORIGINAL PILOT TESTING

Experience on a larger scale was needed to get critical design information, as well as market credibility. Under the auspices of the US EPA's new Environmental Technology Verification (ETV) program, run by the National Sanitation Foundation (NSF), Calgon Carbon built and installed a 200gpm pilot unit, becoming the first company to be approved for an ETV certification program.

The effort was very tightly controlled, with a third-party consulting firm, Cartwright, Olsen & Associates, LLC, responsible for development of the protocol, including proper controls; supervision of experimentation; and certification of results. The 100-page protocol book was peer-reviewed. Testing of the pilot unit, following manufacturing and installation under supervision of Calgon Carbon's Keith Bircher, began in February1998.

The investigators were astounded with the subsequent results, which showed that at the lowest dose applied, 19 $mJ/cm^2$, only one in 25 mice was infected, and at all other doses, no mice showed symptoms.

The results of the pilot work thus confirmed the bench work: the UV was inactivating the oocysts, and doing so at incredibly low doses. In part, and because of these works and other discussions, the US EPA created a UV sub-workgroup to report to the Federal Advisory Committee (FACA) on issues and costs related to UV disinfection.

In advance of UV dose tables, validation protocol, monitoring requirements, and new guidance manuals for UV disinfection, many water utilities began to consider UV disinfection in their plants either as an additional barrier for protozoa disinfection or to get Concentration Time (CT) credits for UV for *giardia* so that chlorine doses could be lowered in order to meet DBP regulations.

## ORIGINAL COMMERCIALIZATION/ PATENT

Based on information from the bench test results, and later reinforced by the pilot testing, Calgon filed a patent application.

The patent application was filed in May 1998. Calgon Carbon's Sentinel™ UV Disinfection System, designed specifically for the drinking water industry, was launched at the AWWA show the following month.

In October, 2000, the U.S. Patent office granted Calgon Carbon a patent covering its inactivation treatment process, with the abstract stating "a method for prevention of cryptosporidium oocysts and similar organisms in water by irradiating the water with *ultraviolet* light in a range of 200 to 300 nm in doses of about 10 $mJ/cm^2$ to 175 $mJ/cm^2$.

In March 2002, the Canadian government also recognized the value of the technology and issued Calgon Carbon a Notice of Patent Allowance. Other patents are pending worldwide.

**REACTOR VALIDATION**

Calgon Carbon has demonstrated the magnitude of UV efficiency for inactivation of water-borne *cryptosporidium* and *giardia.* Investigators, in several different laboratories, have since confirmed these impressive results. Meanwhile, efforts have intensified in the area of UV reactor validation.

The German Water Protection Agency (DVGW) conducted research concluding that the required UV dose for 4-log bacteria and virus inactivation is 40 mJ/sq.cm; no significant by-products or water quality changes occur with this UV dose; and that a reactor certification laboratory must be created to certify large-scale-UV reactors under uniform and worst case conditions.

The certification process covers support documentation for the UV lamp that includes spectral characteristics, quartz sleeve transmission, and sensor parameters; verification of UV sensors with a reference sensor; on-line command and control; and a challenge test to measure inactivation of a challenge microbe achieved by the reactor at full flow rates.

The final information required for state regulators to grant disinfection credits for UV technologies is operational data. Calgon Carbon has developed detailed guidelines and CDF Modeling for design engineering issues. Control and command features, system maintenance, and operator training, are based on Calgon Carbon's experience with over 250 medium pressure UV installations treating municipal wastewater, contaminated groundwater, industrial wastewater, and drinking water.

Specific items include optimum UV system location; effect of pressure drop on system efficiency; providing for system redundancy; addressing mercury release concerns; UV lamp and sensor considerations; controls & instrumentation strategy; and reactor maintenance.

## SUMMARY

Prior to Calgon Carbon's breakthrough discovery, application of UV for disinfection of drinking water to provide complete control of bacteria, viruses, and protozoa's was not considered an economically viable technology. Many municipalities were considering expensive filtration and ozone technologies that would have cost up to 10 times more than the Sentinel™ UV technology. Since then, the consulting engineering community has been able to take advantage of UV to design more efficient and affordable multiple-barrier systems.

Calgon Carbon's innovative research and discovery, and progressive commercialization in partnership with the municipal water industry, has generated a new Patented method to protect against cryptosporidium in a cost effective manner. Calgon Carbon Corporation has been supporting the consulting engineering and water treatment industry with ongoing, CFD-enhanced designs for new Sentinel™ systems to meet the growing demand.

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