Abstract: Sunlight has been known to inactivate bacteria for more than a century, and has also been found to reduce the infectivity of viruses and parasites. These photoinactivation processes are important in both natural and engineered systems. Photoinactivation is an important factor in the fate and transport of microorganisms in sunlit natural environments, and has applications to drinking water and wastewater treatment in developing country settings. One such application, solar disinfection of drinking water (SODIS), has been widely promoted as a technology for Household Water Treatment and Storage (HWTS) in the last decade. However, the mechanisms by which photoinactivation occurs are not yet fully understood. We explored the photoinactivation of bacteria and viruses in a series of laboratory and field studies. *E. coli* inactivation rates in SODIS field trials were found to depend on a number of factors, including container material and the addition of low-cost, nontoxic additives. In addition, bacteria derived from wastewater were found to be much more resistant to sunlight than bacteria of the same species grown under laboratory conditions. Laboratory studies further elucidated the wavelength dependence of the photoinactivation of bacteria and viruses by simulated sunlight. In addition, these studies illustrated the importance of iron, hydrogen peroxide, and superoxide in the photoinactivation of *E. coli*. These findings have important implications for the effectiveness of SODIS as an HWTS technology, and may have implications for pathogen inactivation in other natural and engineered systems as well.