

The One and Only MS2 UV Dose Response Curve

Thomas Hargy, Senior Scientist
Tetra Tech – Clancy Environmental Consultants, St Albans, VT 05478
802 527-2460 ext. 20

Keywords: MS2 ATCC 15597 B1, Bioassay, UV dose Response, UV Guidelines

The UV inactivation kinetics of microbial species are predictable, within a range of variability, in waters free of interfering particles, and when the UV absorbance is accounted for in the measured irradiance through the UV light path. While some organisms, particularly bacterial spores, show a lag period of relatively low sensitivity at low UV doses followed by steeper kinetics at higher UV doses, others, such as vegetative bacteria and viruses exhibit first order kinetics from low to higher levels of inactivation. At levels above 4- to 5-log₁₀, most microorganisms exhibit a tailing of the UV dose response. Given the growing body of data generated by UV reactor bioassays, the UV dose response of many studied microorganisms is quite predictable within species or strains. However, measured UV dose responses will vary arising from experimental variability, water quality influences, and microbiological variability. For applications to UV reactor bioassays, acceptable UV dose response bounds for coliphage MS2 have been prescribed by several sets of guidelines, including the USEPA's UV Disinfection Guidance Manual (UVDGM, 2006), and the NWRI UV Guidelines (2000, 2003 and 2012). This paper reviews the history of the NWRI MS2 UV dose response bounds through the three editions from 2000 to 2012, and assesses the newest edition's standardized UV dose response relationship as well as the data with which it was developed.

Editions 1, 2 and 3 of the NWRI Ultraviolet Disinfection Guidelines for Drinking Water and Water Reuse (National Water Research Institute, 2000, 2003, 2012) provide guidance for the design, installation, operation and maintenance of UV systems, and include a protocol for performance validation by bioassay. This protocol specifies the bioassay surrogate microorganism as coliphage MS2 (ATCC 15597 B1), and that the UV dose response of the stock microbial suspension must fall within defined upper and lower bounds. A significant change made in the third edition is the assignment of a fixed, or standardized, UV dose response of the MS2 used in all bioassays,

as opposed to the conventional use of the specific UV dose response, developed by collimated beam (CB), of MS2 in feed water samples collected each day of bioassay testing. The purpose for this change was to “minimize the impact of experimental variability” (NWRI, 2012), and the drive for this was to avoid the possibility that reactors that had been validated prior to installation might be de-rated in a post-installation spot check if the variability in the measurement of the MS2 UV dose response returned a lower reduction equivalent dose (RED) than was predicted by pre-installation test results.

In all three editions, the NWRI protocol requires that the UV dose response of stock MS2 suspensions used in bioassays be determined by the CB method, using exposures to produce UV doses of 20 to 150 mJ/cm². The linear regression of the resulting data points is determined and compared to upper and lower bound equations (Eq 1 and 2). To be acceptable, 80% of all data points from 20 to 150 mJ/cm² must fall within these bounds.

$$[1] \quad -\log_{10} (N/N_0) = (0.040) (\text{UV dose, mJ/cm}^2) + 0.64$$

$$[2] \quad -\log_{10} (N/N_0) = (0.033) (\text{UV dose, mJ/cm}^2) + 0.20$$

These NWRI UV dose response bounds were derived from an undefined body of MS2 UV dose response data that had been generated prior to the first (2000) edition, prior to the publication of standardized CB methodology (Bolton and Linden, 2003). Neither this body of data nor the methodology(ies) used to generate it has been available for review. The linear equations were developed with data from 20 to 150 mJ/cm², and do not incorporate any data for 0 log inactivation at 0 mJ/cm² data point (zero UV dose).

In editions 1 and 2, the bounds were applied in two ways. First, stock MS2 UV dose response was measured after being produced in a laboratory, and if it was within these

bounds, it could be used in a bioassay. Second, the UV dose response of the MS2 in the seeded reactor feed water in a bioassay was also measured by CB and used as the basis for determining the RED. This UV dose response must also lie within the NWRI bounds, or the bioassay must be repeated. Edition 3 retains the same bounds, and stock MS2 UV dose response must again fall within them to qualify for use in a bioassay. Likewise, the UV dose response of MS2 seeded to the reactor feed must meet the bounds, or the bioassay is repeated. The third edition differs, however, from its predecessors and from conventional bioassay protocol, in that the UV dose response curve of the seeded MS2 is not used in the determination of RED. Rather, a UV dose response relationship calculated from a large body of more recent collimated beam test results, generated by three independent laboratories involved in NWRI and UVDGM validations, is used as the default curve to which all reactor inactivations are compared to determine the RED achieved during any bioassay. The data used to produce this relationship, given in Equation 3, are shown in Figure 1, along with the upper and lower NWRI UV dose response bounds.

$$[3] \quad -\log_{10}(N/N_0) = (0.0368)(\text{UV dose, mJ/cm}^2) + 0.5464$$

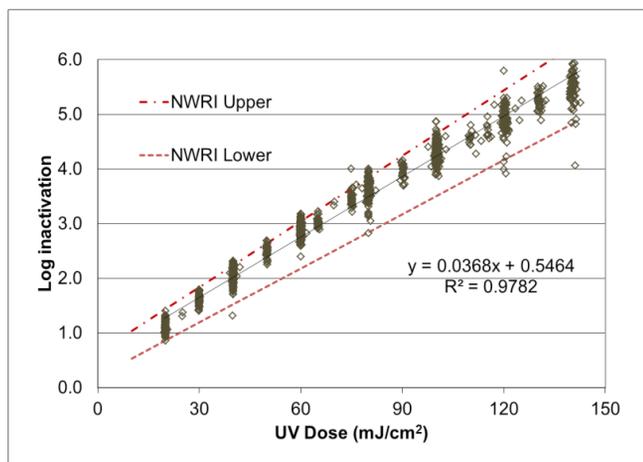


Figure 1. MS2 data used in NWRI 3rd Edition linear standardized UV dose response (dataset courtesy A. Salvesson, K. Bourgeois, Carollo Engineers)

As in the manner in which the upper and lower bounds were described, this standardized UV dose response relationship is a linear expression, and does not include zero-UV dose data points, or any data points below 20 mJ/cm², even though data at 10 mJ/cm² and 15 mJ/cm² were present in the full dataset available. The 3rd edition (p 46) explicitly states that “the ‘zero UV dose’ point is not to be included in the regression analysis because MS-2 UV dose-response is not linear all the way to a UV dose of zero. The linear regression must only be applied to the

linear portion of the UV dose-response curve.” This requirement affects how well the standardized curve represents the MS2 UV dose response, and influences whether actual CB data can be used in a bioassay. The difficulty of describing a straight line through these data from the zero UV dose point or even from 20 mJ/cm² derives from the fact that the data in fact are not linear in any portion of the curve. In Figure 1, it can be seen that data points at or below 30 mJ/cm² are predominantly below the line, while those between 50 100 mJ/cm² and 100 mJ/cm² are mostly above the line, and beyond 120 mJ/cm², they trend below the line again. A reason that the linear expression does not consistently predict actual data is that these data, as with MS2 datasets from UV disinfection studies including those evaluated in the UVDGM, Appendix A, indicate tailing, or a diminishing increase in inactivation with increasing UV dose. As such, the data points at and above 120 mJ/cm² cause the overall linear expression in Figure 1 to become more horizontal, returning a less conservative UV dose response relationship, where a given inactivation is credited with a higher UV dose than if the data exhibiting tailing had not been incorporated. These also tip the y-intercept to a significantly positive value, such that the standardized curve predicts a 0.55 log inactivation with no UV at all.

Representing the full NWRI dataset with a polynomial equation does indeed give a better fit, as shown in Figure 2. It can be seen here that the slope of the data from 20 to 60 mJ/cm² is steeper than that from 90 to 140 mJ/cm². The NWRI approach of excluding data below 20 mJ/cm² because they are not linear should preclude the use of any MS2 data, as no significant segment of the UV dose response relationship is linear.

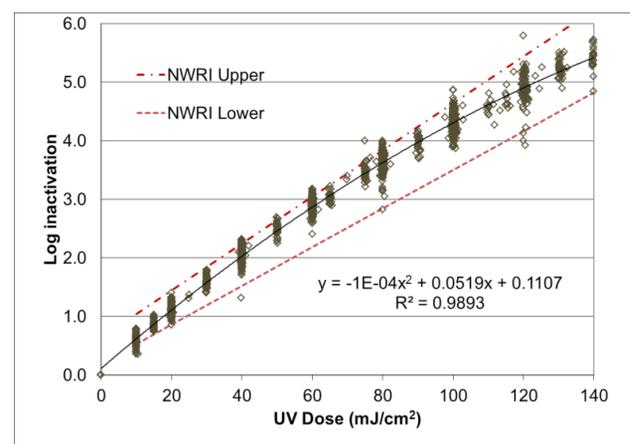


Figure 2. MS2 data used in 3rd Edition standardized UV dose response, and including data points below 20 mJ/cm²

The consequences of using a linear relationship and ignoring the <20 mJ/cm² data manifest in several ways. One

is that the NWRI bounds, while drawn from a different dataset than the standardized curve, also exhibit artificially low slopes and positive y-intercepts. As a result, valid data points, including many of those used to develop the standardized curve, lie outside the bounds. Particularly troublesome are the many above the bounds in the 40 to 100 mJ/cm² range, visible in Figure 2. An MS2 stock with two of these “high” data points could not be used, even though it exhibits a conventional UV dose response. Any bioassay run with a corresponding CB curve that returned two such points would be disqualified. Aside from the cost and inconvenience of re-testing a UV reactor, the disqualification of these higher inactivation data means that conservative results are not used, while less conservative data are readily accommodated by the wide gap between the data and the lower bound line.

As noted above, neither the methods used to generate the raw data for the original (and still current) NWRI bounds, nor the data themselves, are available for review. Until standardized collimated beam procedures became routine in the wake of Bolton and Linden’s 2003 treatise on the subject, laboratories tended to use well practiced methods, but not necessarily incorporating certain factors which affected the total irradiance, such as UV absorbance, reflectance, divergence, and radiant distribution, or Petri factor. These factors are now included in exposure calculations by laboratories for bioassay CBs supporting UVDGM and NWRI bioassays, although NWRI does not require the divergence correction, which varies with distance from the lamp and reduces the measured irradiance (and extends exposure times) by 2% at 25-cm distances. All of these factors adjust the measured center irradiance in the same direction, correcting it downward because of photons lost due to absorbance, reflection, divergence, or poor distribution as quantified by the Petri factor (which typically manifests with less irradiation away from the center). In drinking water and wastewater bioassays, adjustments made for reflection, divergence and distribution typically reduce the measured center irradiance by a total of 5 percent to 10 percent, and UV absorbance would result in an additional reduction. Thus, it might be hypothesized that MS2 data generated in the pre-Bolton and Linden era could have overstated irradiance, and under-calculated exposure times, resulting in a low UV dose response slope. The new, post-UVDGM generated data used in the 2012 Edition 3 standardized curve suggests this is the case, as Figure 2 shows that the recently generated data from three UV validation laboratories consistently bump against and exceed the upper NWRI bound. Evaluating another multi-lab dataset generated since Bolton and Linden, Malley et al, 2004, reported MS2 inactivation frequently fell above the upper NWRI bound, and they suggested a revision of the

NWRI bounds should be made. Data from that study were in fact evaluated by NWRI for adjustment of the MS2 bounds (R. Sakaji, pers comm, 2003). A draft revision (not publicly distributed), issued in March 2003 for review preceding the May 2003 second edition presented the bounds given in Equations 4 and 5.

$$[4] \quad -\log_{10} (N/N_0) = (0.044) (\text{UV dose, mJ/cm}^2) + 0.7$$

$$[5] \quad -\log_{10} (N/N_0) = (0.036) (\text{UV dose, mJ/cm}^2) + 0.13$$

Comparing the 3rd Edition standardized curve data against these draft bounds (Figure 3) gives a much different picture than seen in Figures 1 and 2, as the draft bounds better accommodate the entire dataset, and do not disqualify bioassays that produced the higher, conservative inactivation points. However, because of a lack of time to allow full review of these data prior to publication of the second edition, the bounds defaulted to the 2000 equations, and were not revisited for the 3rd edition in 2012.

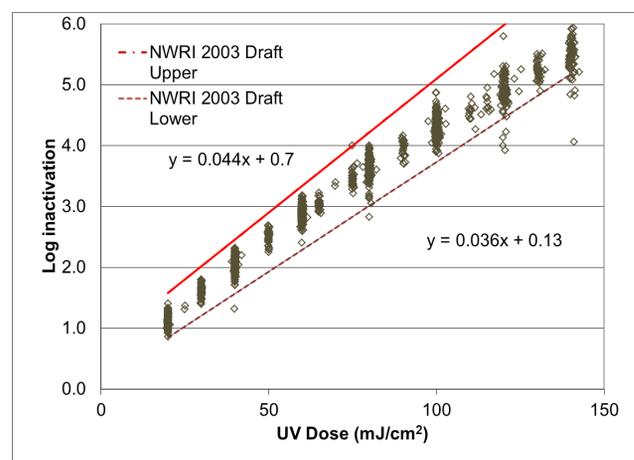


Figure 3. NWRI standardized dataset bounded by limits defined in March 2003 draft 2nd Edition

Finally, aside from the question of whether the NWRI standardized curve adequately represents the dataset used to develop it, one must ask whether the use of a fixed MS2 UV dose response equation is appropriate at all for reactor RED assignment. The underlying assumption justifying its use is that all variability of the MS2 UV dose response is because of experimental artifacts during the collimated beam process and that in all cases, and in all water matrices, MS2 has an exact and constant inactivation kinetics profile. If this is not a valid assumption, reactors may be credited with inaccurate performance ratings, resulting either in validation failure or inadequate public health protection. That a constant UV dose response may not exist across site-drinking water and wastewater qualities is a distinct possibility, as MS2 has been reported to exhibit

increased inactivation kinetics in the presence of certain chemicals or dissolved ions. (Protasowicki and Malley, 2002, Butkus et al 2004, Hargy et al 2007). In wastewater, to which the NWRI guidelines apply, an unlimited array of chemicals could be present which may enhance MS2 inactivation. While a collimated beam exposure with the same water would capture that effect and accurately reflect the site-specific UV dose response at reactor scale, the fixed, standardized curve cannot respond. As a result, the inactivation achieved because of water quality influences would be attributed solely to the efficacy of the UV reactor, and an artificially high RED would be assigned. If the influencing water quality components are intermittent, the enhancement would also be intermittent, but the treatment credit would remain a constant. Finally, as any synergy between UV and water quality components that enhances MS2 disinfection may not be realized in treatment of target pathogens, using a standardized curve and ignoring the true site-specific UV dose response would result in an over-prediction of pathogen disinfection efficacy.

The use of a standardized MS2 UV dose response does provide a hedge against variability in the collimated beam process, but at the cost of having the reactor RED assigned by a UV dose response curve that may not represent the actual inactivation kinetics. A fixed, standard curve does not use the true UV dose response determined in a well-controlled CB process, and it does not account for changes in MS2 UV dose response caused by water quality influences, or any real laboratory-specific inactivation kinetics variation arising from the host/phage interaction. The disconnect between actual and standardized UV dose responses is exaggerated in the NWRI third edition protocol by the fact that the 'curve' is expressed as a linear relationship, which it decidedly is not.

A better means of characterizing a bioassay microbes UV dose response is to ensure that thorough quality control measures are used by the microbiology laboratories undertaking validation bioassays. The use of a second radiometer, as required in the UVDGM, would verify the incident irradiance measurement. Because the radiometer measurements made for the bioassay CB set the irradiance for all exposure calculations, they directly affect the resulting UV dose response curve and ultimately the RED assignment for that reactor for all present and future operation based on that bioassay. Radiometer readings tend not to be randomly variable, with one measurement being high and the next low, but rather, if not perfectly accurate, will be consistently high or low off the true value, or will drift in a given direction over time. Neither sudden divergence from true calibration nor slow drift of the laboratory radiometer will be readily apparent unless a reference radiom-

eter or chemical actinometer is used to confirm readings.

The requirement that MS2 UV dose responses meet linear statistical bounds that were developed from a population of data not representative of well-measured MS2 inactivation kinetics can cause good phage stock and well-run bioassays to be disqualified. Re-defining the NWRI bounds equations as polynomial expressions based on the more recent dataset and including data below 20 mJ/cm² (and preferably excluding data well above the UV dose range relevant to the bioassay) could be undertaken simply by editing the Excel trendline format.

Statistically sound UV dose response curves developed in a well-controlled manner, using seeded feed water, and analyzed in the same manner as the reactor inlet and outlet samples, will capture the impact of any site- or lab-specific inactivation kinetic nuances, and offer a more accurate RED assignment than one based on a pre-established MS2 UV dose relationship.

REFERENCES

- Bolton, J.R. and Linden, K.G. 2003. Standardization of Methods for Fluence (UV Dose) Determination in Bench-scale UV Experiments. *ASCE Jour. Env.*, 129(3):209-216.
- Butkus, M.A., et al, 2004. Use of Aqueous Silver to Enhance Inactivation of Coliphage MS-2 by UV Disinfection. *Appl. Env. Micro.* 70:2848-2853.
- Hargy, T.M., et al, 2007. Synergistic Effect of UV Irradiation and Silver Ions in the Disinfection of Coliphage MS2 and *Bacillus subtilis* Spores. *Proceedings, Disinfection 2007*, Water Environment Federation
- Malley, J.P., et al. 2004. Inactivation of Pathogens with Innovative UV Technologies. #91024. AWWARE, Denver, Colo.
- National Water Research Institute. 2000, 2003, 2012. *Ultraviolet Disinfection Guidelines for Drinking Water and Water Reuse*, editions 1, 2, 3. NWRI, Fountain Valley, Calif.
- Protasowicki, R.G., and Malley, J.P. 2002. UV Treatment of a Groundwater Supply – From Piloting to Start-up Experience in the Town of Norfolk, Mass. *IUVA News* 4(3): 4-7.
- USEPA. (2006) *Ultraviolet Disinfection Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule*. Office of Water (4601) EPA 815-R-06-007.