

Supplementary Material for the article:

In vitro toxicity studies of novel solar water disinfection reactors using the E-screen bioassay and the Ames test.

Paloma Ozores Diez^a, M. Inmaculada Polo-López^b, Azahara Martínez-García^b, Monique Waso^{c,d}, Brandon Reyneke^c, Wesaal Khan^c, Kevin G. McGuigan^e, Pilar Fernández-Ibáñez^f, Rosaleen Devery^a, Bríd Quilty^{a*}

*Corresponding Author:

Bríd Quilty

School of Biotechnology, Dublin City University (DCU), Glasnevin, Dublin 9, Ireland.

email brid.quilty@dcu.ie



Figure S1. Prototype I (A) and Prototype II (B) of the polymethyl methacrylate (PMMA) tubular solar reactors at Stellenbosch, South Africa.

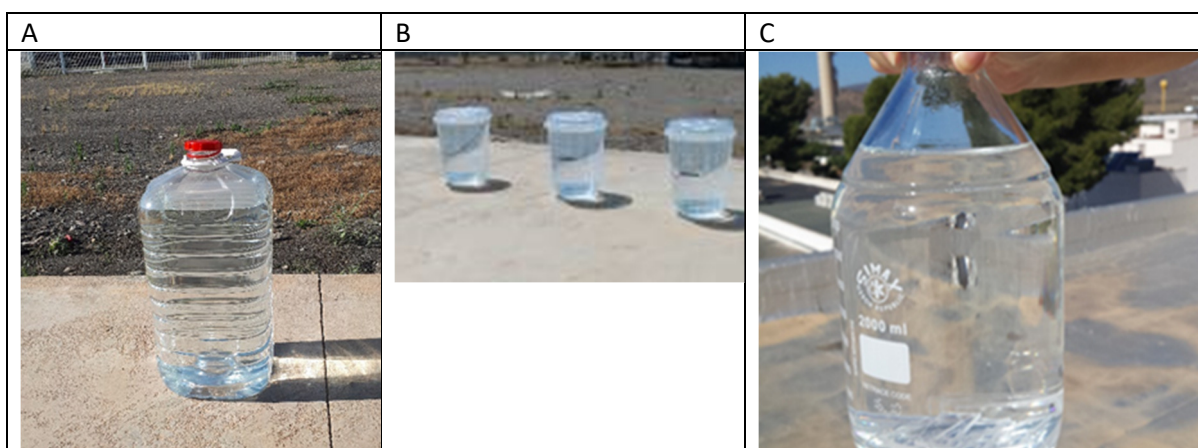


Figure S2. 25 L transparent polyethylene terephthalate (PET) jerrycan (A) 20 L polypropylene (PP) bucket with lid (B) and polymethyl methacrylate (PMMA) plastic pieces submerged in water (C) exposed to natural sunlight at Plataforma Solar de Almería (PSA), Almería, Spain.

| | | | RPE (%) | EEQ (ng/L) |
|-----------------|--------------------------------|---|------------|------------|
| Distilled water | Months of exposure to Sunlight | 3 | -43.8±39.8 | nd |
| | | 6 | -39.2±42 | nd |
| | | 9 | -58.8±17.2 | nd |
| | Months in the Dark | 3 | -50.4±35.1 | nd |
| | | 6 | -44.6±35.7 | nd |
| | | 9 | -33.3±49.4 | nd |
| Well water | Months of exposure to Sunlight | 3 | -14.7±10.7 | nd |
| | | 6 | -21.7±11.3 | nd |
| | | 9 | -36.7±15.8 | nd |
| | Months in the Dark | 3 | 10.7±12.9 | 3.7E-05 |
| | | 6 | 10.1±11 | 2.8E-05 |
| | | 9 | -62.8±27.3 | nd |

Table S1. Relative Proliferative Effects (% RPE) and Estradiol Equivalent (EEQs in ng/L) of water samples from bottles containing pieces of polymethyl methacrylate (PMMA) immersed in distilled water and well water and following exposure to sunlight and darkness for 3, 6 and 9 months at PSA, Almería, Spain. Data refer to mean ± SD of triplicate experiments. nd - not determined.

| Treatment | Exposure conditions | TA 98 | TA 98 | TA mix | TA mix | |
|--------------------------------|-----------------------------|------------------|------------|------------|------------|------------|
| | | (+S9) | (-S9) | (+S9) | (-S9) | |
| | | Baseline | 2.00±1.00 | 3.00±1.00 | 1.91±0.58 | 2.24±0.58 |
| | | Positive control | 22.67±1.53 | 22.83±1.53 | 24.95±0.58 | 21.39±2.00 |
| PMMA pieces in distilled water | Months of Sunlight exposure | 3 | 0.41±2.65 | 0.98±0.58 | 0.42±2.65 | 0.50±0.00 |
| | | 6 | 0.62±1.00 | 1.60±1.73 | 0.21±1.00 | 0.33±0.58 |
| | | 9 | 1.03±1.00 | 1.60±2.65 | 0.21±0.00 | 0.17±0.58 |
| | Months in the Dark | 3 | 0.62±1.00 | 1.24±3.21 | 0.07±0.58 | 0.17±0.58 |
| | | 6 | 0.62±1.00 | 1.78±2.52 | 0.00±0.00 | 0.33±1.15 |
| | | 9 | 0.21±1.00 | 1.16±1.53 | 0.42±1.00 | 0.33±0.58 |
| PMMA pieces in well water | Months of Sunlight exposure | 3 | 0.55±0.58 | 0.87±3.00 | 0.50±1.00 | 1.22±1.53 |
| | | 6 | 1.04±3.46 | 1.86±3.21 | 0.08±0.58 | 0.70±0.58 |
| | | 9 | 0.28±1.53 | 0.76±2.52 | 0.58±2.08 | 0.52±1.00 |
| | Months in the Dark | 3 | 0.48±2.31 | 0.93±1.15 | 0.25±1.00 | 0.35±0.58 |
| | | 6 | 0.35±0.58 | 1.75±1.73 | 0.17±0.58 | 0.52±1.00 |
| | | 9 | 0.69±3.21 | 2.04±2.52 | 0.17±0.58 | 0.35±0.58 |

Table S2. Ames test results of water samples taken from bottles containing pieces of polymethyl methacrylate (PMMA) immersed in well water and in distilled water and following exposure to sunlight for 3, 6 and 9 months at PSA, Almeria, Spain. Controls were incubated in the dark. The test used *S. typhimurium* TA98 and TAMix with (+) and without (-) S9. Results were reported as fold induction over the baseline. A sample that showed a clear fold induction ≥ 2 above the baseline was classified as a mutagen.

| | | Well water | | | | Distilled water | | | |
|-----------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|----------|
| | | Initial | 3 months | 6 months | 9 months | Initial | 3 months | 6 months | 9 months |
| Salinity (mg/L) | Chloride | 315±1.0 | 317±1.0 | 402±5.0 | 312±3.0 | 0.1±0.0 | 0.3±0.2 | 0.1±0.0 | 0.1±0.0 |
| | Nitrite | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| | Nitrate | 5.0±0.0 | 5.0±0.0 | 13±0.0 | 5.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| | Phosphate | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| | Sulfate | 241±1.0 | 241±1.0 | 347±4.0 | 474±33 | 0.2±0.0 | 0.4±0.1 | 0.3±0.0 | 0.3±0.0 |
| | Sodium | 460±2.0 | 466±2.0 | 432±6.0 | 471±5.0 | 0.1±0.0 | 0.6±0.8 | 0.0±0.0 | 0.0±0.0 |
| | Ammonium | 1.0±0.0 | 1.0±0.0 | 1.0±0.0 | 15±2.2 | 0.1±0.0 | 0.1±0.1 | 0.0±0.0 | 0.0±0.0 |
| | Calcium | 7.0±1.0 | 7.0±1.0 | 7.0±1.0 | 7.0±1.0 | 0.0±0.0 | 0.0±0.0 | 0.1±0.1 | 0.1±0.0 |
| | Potassium | 6.0±0.0 | 6.0±0.0 | 5.0±1.0 | 9.0±5.0 | 0.1±0.0 | 0.2±0.1 | 0.2±0.0 | 0.1±0.1 |
| | Magnesium | 65±3.0 | 66±3.0 | 81±1.0 | 65±70 | 0.1±0.0 | 0.2±0.0 | 0.2±0.0 | 0.3±0.0 |
| Conductivity | 2.5±0.0 mS/cm | 2.5±0.0 mS/cm | 2.5±0.1 mS/cm | 2.6±0.0 mS/cm | 4.0±1.9 µS/cm | 5.0±1.9 µS/cm | 4.0±0.0 µS/cm | 4.2±0.2 µS/cm | |

Table S3. Salinity and conductivity measurements of well water and distilled water from bottles containing submerged pieces of polymethyl methacrylate (PMMA) exposed to sunshine at PSA, Almería, Spain for 3, 6 and 9 months. Data refer to mean ± SD of triplicate experiments.

| | Date of sample | Location | | | |
|--|----------------|------------|------------|------------|------------|
| | | Bonfoi | | Enkanini | |
| | | RPE (%) | EEQ (ng/L) | RPE (%) | EEQ (ng/L) |
| Samples from PMMA SODIS reactor (after treatment) | aug-18 | 6.1±30.6 | 2.4E-06 | -15.5±8.6 | nd |
| | sep-18 | -7.6±22.6 | nd | -12.8±15.3 | nd |
| | oct-18 | 0.2±21.9 | 5.0E-13 | -13.3±16.7 | nd |
| | nov-18 | -6.5±17.3 | 1.3E-08 | -6.8±26.1 | nd |
| | jan-19 | -6.5±17.3 | nd | -7.9±12.1 | nd |
| | feb-19 | 6.5±11.4 | 3.3E-06 | 5.0±17.5 | 9.7E-07 |
| | mar-19 | -18.6±22.7 | nd | - | - |
| | apr-19 | -15.3±23.6 | nd | - | - |
| Samples from harvested rainwater tank (before treatment) | aug-18 | -14.9±20.0 | nd | -11.6±12.5 | nd |
| | sep-18 | -2.5±28.7 | nd | -10.1±17.6 | nd |
| | oct-18 | -8.3±18.6 | nd | -12.8±19.4 | nd |
| | nov-18 | 7.1±30.9 | nd | -16.0±10.2 | nd |
| | jan-19 | -3.3±17.0 | nd | -11.3±13.9 | nd |
| | feb-19 | -19.9±18.4 | nd | -14.3±15.8 | nd |
| | mar-19 | -15.1±22.8 | nd | - | - |
| | apr-19 | -6.5±17.8 | nd | - | - |

Table S4. Relative Proliferative Effects (% RPE) and Estradiol Equivalent (EEQs in ng/L) of water samples taken from harvested rainwater tanks and from tubular polymethyl methacrylate (PMMA) SODIS reactors (before and after SODIS disinfection) at Bonfoi and Enkanini, Stellenbosch, South Africa. Data refer to mean ± SD of triplicate experiments. nd - not determined.

| Location | Date of sample ↓ | TA 98 (+S9) | TA 98 (-S9) | TA mix (+S9) | TA mix (-S9) | |
|----------|---------------------------------------|------------------|----------------|-----------------|-----------------|------------|
| | | Baseline | 2.82±1.15 | 2.00±1.00 | 2.86±1.53 | 2.82±1.15 |
| | | Positive control | 15.24±2.65 | 24.00±1.00 | 16.31±1.15 | 23.33±1.53 |
| Bonfoi | Samples from PMMA SODIS reactor | aug-18 | 0.59±2.08 | 0.59±2.08 | 0.73±0.58 | 0.17±0.58 |
| | | sep-18 | 1.18±3.21 | 1.89±1.53 | 1.46±1.15 | 0.35±1.15 |
| | | oct-18 | 1.06±3.00 | 1.77±1.73 | 0.37±0.58 | 0.09±0.58 |
| | | nov-18 | 0.95±0.58 | 1.65±3.79 | 0.00±0.00 | 0.78±0.00 |
| | | jan-19 | 0.95±1.53 | 1.06±2.00 | 0.00±0.00 | 0.69±2.08 |
| | | feb-19 | 1.18±0.58 | 0.95±0.58 | 0.00±0.00 | 0.60±1.53 |
| | | mar-19 | 0.59±0.58 | 1.54±4.16 | 0.00±0.00 | 1.04±2.65 |
| | | apr-19 | 0.59±0.58 | 1.54±1.15 | 0.73±1.15 | 0.78±1.73 |
| | Samples from harvested rainwater tank | aug-18 | 0.35±1.73 | 1.18±3.51 | 1.46±1.15 | 0.35±1.15 |
| | | sep-18 | 1.18±1.53 | 1.77±2.00 | 1.83±1.15 | 0.43±1.15 |
| | | oct-18 | 0.47±1.53 | 1.89±2.08 | 1.10±0.00 | 0.26±0.00 |
| | | nov-18 | 0.95±0.58 | 0.47±1.53 | 0.37±0.58 | 0.52±1.00 |
| | | jan-19 | 0.95±0.58 | 1.54±3.51 | 0.37±0.58 | 0.78±1.00 |
| | | feb-19 | 0.95±0.58 | 1.30±1.53 | 0.37±0.58 | 1.04±1.00 |
| mar-19 | | 0.47±0.58 | 1.30±1.53 | 0.37±0.58 | 1.12±2.08 | |
| apr-19 | | 0.83±0.58 | 0.83±2.31 | 0.37±0.58 | 0.78±1.00 | |
| Enkanini | Samples from PMMA SODIS reactor | aug-18 | 1.17±0.58 | 1.12±1.53 | 0.47±1.15 | 0.24±1.15 |
| | | sep-18 | 0.93±1.53 | 0.95±2.52 | 0.24±0.58 | 0.12±0.58 |
| | | oct-18 | 1.17±1.15 | 1.12±0.58 | 0.47±0.58 | 0.24±0.58 |
| | | nov-18 | 0.35±1.00 | 0.86±0.58 | 0.00±0.00 | 0.77±2.52 |
| | | jan-19 | 0.93±0.58 | 0.52±1.73 | 0.12±0.58 | 0.71±2.00 |
| | | feb-19 | 0.70±1.00 | 0.52±1.00 | 0.47±0.58 | 0.47±2.08 |
| | | feb-19 | 0.70±1.00 | 0.52±1.00 | 0.47±0.58 | 0.47±2.08 |
| | Samples from harvested rainwater tank | aug-18 | 0.70±2.00 | 0.95±1.53 | 0.59±1.15 | 0.30±1.15 |
| | | sep-18 | 0.93±2.31 | 0.86±1.15 | 0.71±1.00 | 0.59±1.53 |
| | | oct-18 | 0.58±1.15 | 0.43±1.15 | 0.12±0.58 | 0.06±0.58 |
| | | nov-18 | 1.05±1.73 | 0.43±1.15 | 0.24±0.58 | 0.59±1.15 |
| | | jan-19 | 0.82±2.31 | 0.69±1.53 | 0.00±0.00 | 0.53±0.00 |
| | | feb-19 | 0.70±2.00 | 0.78±3.00 | 0.12±0.58 | 0.89±0.00 |
| | | feb-19 | 0.70±2.00 | 0.78±3.00 | 0.12±0.58 | 0.89±0.00 |

Table S5. Ames test results of water samples from polymethyl methacrylate (PMMA) tubular SODIS reactors and their associated harvested rainwater tanks at Bonfoi and Enkanini, Stellenbosch, South Africa. The test used *S. typhimurium* TA98 and TAMix with (+) and without (-) S9. Results were reported as fold induction over the baseline. A sample that showed a clear fold induction ≥ 2 above the baseline was classified as a mutagen.

| 20 L PP SODIS Reactors | | RPE (%) | EEQ (ng/L) |
|--------------------------------|---|------------|------------|
| Months of exposure to Sunlight | 3 | 5.0±36.5 | 9.8E-06 |
| | 6 | 5.6±7.8 | 1.7E-05 |
| | 9 | -16.1±11.2 | nd |
| Months in the Dark | 3 | 1.6±28.4 | 3.8E-08 |
| | 6 | -10.2±20.2 | nd |
| | 9 | -12.0±21.9 | nd |

Table S6. Relative Proliferative Effects (% RPE) and Estradiol Equivalent (EEQs in ng/L) of water samples from 20 L PP (polypropylene) buckets following 3-, 6- and 9-months' exposure to sunlight and darkness at PSA, Almeria, Spain. Data - mean ± SD of triplicate experiments. nd - not determined.

| Reactor | Exposure conditions | | TA 98 (+S9) | TA 98 (-S9) | TA mix (+S9) | TA mix (-S9) |
|----------------|-----------------------------|---|-------------|-------------|--------------|--------------|
| | Baseline | | 2.00±0.00 | 2.24±0.58 | 2.24±0.58 | 1.91±0.58 |
| | Positive control | | 22.83±1.53 | 20.50±1.00 | 21.39±2.00 | 24.42±1.15 |
| 20 L PP bucket | Months of Sunlight exposure | 3 | 0.67±0.58 | 0.45±1.00 | 1.34±2.00 | 0.52±1.00 |
| | | 6 | 2.67±2.52 | 0.74±1.15 | 0.74±1.53 | 0.52±1.73 |
| | | 9 | 1.33±2.89 | 0.74±0.58 | 1.34±0.00 | 0.52±1.00 |
| | Months in the Dark | 3 | 2.17±1.15 | 0.89±1.00 | 1.34±1.00 | 0.35±0.58 |
| | | 6 | 1.33±0.58 | 0.74±2.08 | 0.45±1.00 | 0.52±1.00 |
| | | 9 | 0.67±0.58 | 0.89±1.00 | 0.45±1.00 | 0.70±1.15 |

Table S7. Ames test results for water samples taken from 20 L polypropylene (PP) bucket SODIS reactors following 3-, 6- and 9-months' exposure to sunlight or darkness at PSA, Almería, Spain. The test used *S. typhimurium* TA98 and TAMix with (+) and without (-) S9. Results were reported as fold induction over the baseline. A sample that showed a clear fold induction ≥2 above the baseline was classified as a mutagen.

| Reactor | Exposure conditions | | TA 98 (+S9) | TA 98 (-S9) | TA mix (+S9) | TA mix (-S9) |
|-------------------------------|-----------------------------|------------------|----------------|----------------|-----------------|-----------------|
| | | Baseline | 3.00±1.00 | 1.82±1.15 | 2.00±1.00 | 1.24±0.58 |
| | | Positive control | 22.83±1.53 | 25.62±1.53 | 22.50±4.36 | 36.71±0.58 |
| 25 L transparent PET jerrycan | Months of Sunlight exposure | 3 | 0.40±2.65 | 1.44±1.00 | 0.00±0.00 | 0.27±0.58 |
| | | 6 | 0.87±1.53 | 1.58±3.79 | 0.10±0.58 | 0.27±0.58 |
| | | 9 | 0.73±1.53 | 1.99±0.58 | 0.48±0.58 | 0.80±1.00 |
| | Months in the Dark | 3 | 0.33±1.15 | 1.37±4.51 | 0.29±1.00 | 0.54±0.58 |
| | | 6 | 0.73±1.15 | 1.10±2.08 | 0.38±1.15 | 0.80±0.00 |
| | | 9 | 0.27±0.58 | 1.86±1.00 | 0.00±0.00 | 0.27±0.58 |
| 2 L PET bottle | Months of Sunlight exposure | 3 | 0.68±1.53 | 1.08±4.00 | 0.38±1.15 | 0.37±1.15 |
| | | 6 | 0.43±1.15 | 1.08±1.00 | 0.19±1.15 | 0.55±1.00 |
| | | 9 | 0.94±1.53 | 1.44±0.58 | 0.38±1.53 | 0.18±0.58 |
| | Months in the Dark | 3 | 0.34±0.58 | 1.51±1.00 | 0.29±1.00 | 1.10±1.00 |
| | | 6 | 0.94±0.58 | 1.22±1.53 | 0.48±0.58 | 0.18±0.58 |
| | | 9 | 0.85±3.21 | 1.51±2.65 | 0.57±1.73 | 0.55±1.00 |

Table S8. Ames test results for water samples taken from polyethylene terephthalate (PET) reactors following 3-, 6- and 9-months' exposure to sunlight or darkness at PSA, Almería, Spain. The test used *S. typhimurium* TA98 and TAMix with (+) and without (-) S9. Results were reported as fold induction over the baseline. A sample that showed a clear fold induction ≥ 2 above the baseline was classified as a mutagen.

| | | 20 L PP Bucket SODIS reactor | | |
|--------------------|-----------|------------------------------|----------|----------|
| | | 3 months | 6 months | 9 months |
| Salinity (mg/L) | Chloride | 33.34 | 40.94 | 29.72 |
| | Nitrite | 0.13 | 0.12 | 0.00 |
| | Nitrate | 0.58 | 1.65 | 0.44 |
| | Phosphate | 0.00 | 0.00 | 0.00 |
| | Sulphate | 25.51 | 35.00 | 21.59 |
| | Sodium | 48.72 | 44.64 | 46.13 |
| | Ammonium | 0.03 | 0.00 | 0.00 |
| | Calcium | 0.75 | 0.67 | 0.73 |
| | Potassium | 0.48 | 0.67 | 0.25 |
| | Magnesium | 7.08 | 8.62 | 6.90 |
| Conductivity mS/cm | | 2.62 | 2.52 | 2.41 |

Table S9. Salinity and conductivity measurements of the well water exposed to sunshine in 20 L polypropylene (PP) buckets for 3, 6 and 9 months at PSA, Almería, Spain. Only single values available due to lack of water sample.

| | | 25 L PET Jerrycan SODIS reactor | | | 2 L PET Bottle SODIS reactor | | |
|-----------------------|-----------|---------------------------------|----------|----------|------------------------------|----------|----------|
| | | 3 months | 6 months | 9 months | 3 months | 6 months | 9 months |
| Salinity (mg/L) | Chloride | 31.6±0.4 | 31.0±0.1 | 29.7±0.7 | 33.7±0.2 | 41.4±0.6 | 29.7±1.6 |
| | Nitrite | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| | Nitrate | 0.6±0.0 | 0.5±0.0 | 0.5±0.0 | 0.5±0.0 | 1.4±0.0 | 0.5±0.0 |
| | Phosphate | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| | Sulfate | 24.0±0.4 | 22.9±0.4 | 21.3±1.1 | 25.6±0.1 | 35.4±0.5 | 21.9±1.4 |
| | Sodium | 46.6±0.6 | 47.3±0.2 | 48.0±0.6 | 49.6±0.2 | 44.6±0.5 | 46.7±1.8 |
| | Ammonium | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 |
| | Calcium | 0.7±0.1 | 0.9±0.1 | 0.9±0.2 | 0.9±0.0 | 0.7±0.1 | 0.7±0.1 |
| | Potassium | 0.5±0.2 | 0.3±0.2 | 0.2±0.1 | 0.4±0.1 | 0.8±0.0 | 0.3±0.3 |
| | Magnesium | 6.9±0.2 | 6.8±0.1 | 6.5±0.3 | 6.8±0.1 | 8.4±0.1 | 6.3±0.4 |
| Conductivity mS/cm | | 2.4±0.0 | 2.4±0.1 | 2.4±0.0 | 2.6±0.0 | 2.6±0.0 | 2.5±0.0 |

Table S10. Salinity and conductivity measurements of the well water exposed to sunshine in 25 L polyethylene terephthalate (PET) transparent jerrycans and 2 L polyethylene terephthalate (PET) bottles for 3, 6 and 9 months at PSA, Almería, Spain. Data refer to mean ± SD of triplicate experiments.