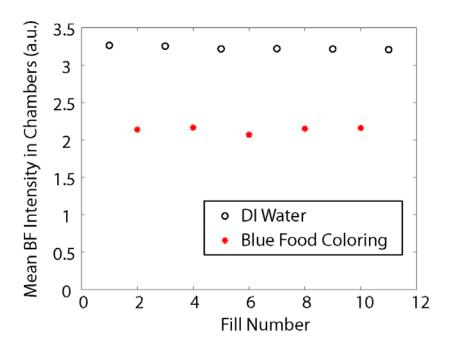
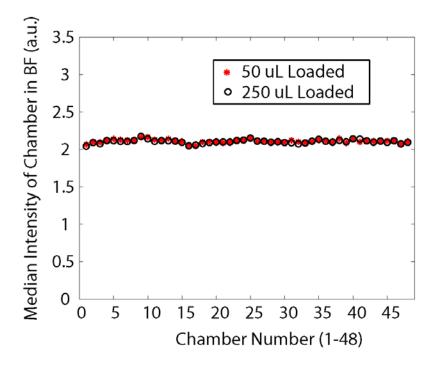
## Supplementary Materials for "Microfluidic chamber arrays for whole-organism behavior-based chemical screening" by Chung, Zhan et al.



**Figure S1.** Repeated loading of DI water and blue food coloring into the device using a syringe pump operated at 0.3 ml/min. 100  $\mu$ L of solution was injected for each loading cycle. We observed that the chambers reach steady state concentration after 50  $\mu$ L injections.

To gauge uniformity over all 48 chambers, we compare chamber 1 (the first chamber in the serpentine channel flow path) and chamber 48 (the last chamber in the serpentine chamber flow path) over all of the repeated loadings. Using a paired t-test between the dataset for chamber 1 and chamber 48, we find no statistical difference (p>.6).



**Figure S2.** Loading of blue food coloring into a device primed with DI water using a syringe pump injecting at 0.3 ml/min. Red asterisks indicate the chamber intensities for each chamber after 50 μl of the dye had been injected through the device. Black circles indicate the chamber intensities for each chamber after 250 μl of the dye had been injected through the device. The mean intensity over all chambers for 50 μl injection was 2.1107e+004 (a.u.); the mean intensity over all chambers for 250 μl injection was 2.1077e+004 (a.u.). There is no statistical difference between the data sets (p>0.3), indicating 50 μl is sufficiently large for exchanging out the existing solution in all chambers.

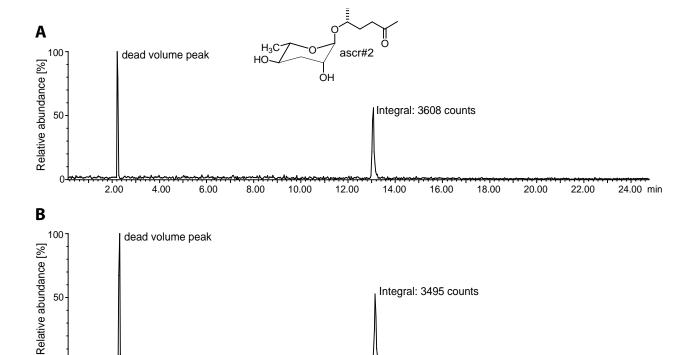
2.00

4.00

6.00

8.00

10.00



**Figure S3.** HPLC-MS ion chromatograms for the ion m/z = 269, corresponding to [M+Na]<sup>+</sup> of ascaroside ascr#2. **A**: HPLC-MS ion chromatogram obtained with injection of 1  $\mu$ L of a 10  $\mu$ M solution of ascr#2 in water. **B**: HPLC-MS ion chromatogram obtained with injection of 1  $\mu$ L of the same solution after pass through microfluidic device. Integration of the peaks representing ascr#2 using MassLynx software (Waters) indicated less than 5% loss.

12.00

14.00

16.00

20.00

22.00

24.00 min

18.00

## Supplementary Method for HPLC-MS characterization of compound adsorption:

Device was equilibrated with water. Subsequently  $100~\mu L$  of a  $10~\mu M$  solution of ascr#2 in water was pumped through the device via syringe pump. Collection was started after  $50~\mu L$  of solution had passed through the device. HPLC-MS analysis was performed using an Agilent 1100~Series~HPLC system equipped with an Agilent Eclipse XDB-C18 column (9.4 x 250 mm, 5  $\mu m$  particle diameter). A 0.1% acetic acid – acetonitrile solvent gradient was used, starting with an acetonitrile content of 5% for 5 min which was increased to 100% over a period of 40 min. The HPLC system was connected to a Quattro II mass spectrometer (Micromass/Waters), which was operated in positive-ion electrospray ionization mode.