Towards Single Egg Toxicity Screening Using Microcoil NMR

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Supporting Information

Experimental section:

A silicone-based caulking tape (item #: SC13121, Home Depot) was utilized to secure the sample holder to the coil and a second silicone-based liquid tape (item #: LTB-400, Liquid Tape, Gardner Bender) was added to ensure a water tight seal. The liquid tape dried in under two minutes and both sealants were easily removed with lukewarm water after completion of experiments.

Figure S1: A water drop, administered via a 2 mL Pasteur pipette is held vertically over a 1000 μm planar microcoil by surface tension.

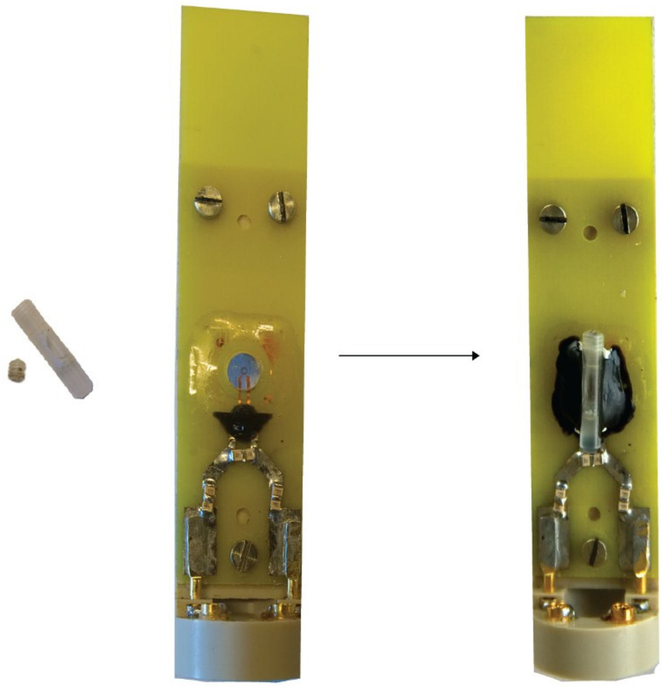

Figure S2: Set-up of the lab-modified sample holder on a 1000 μm coil. The sample holder was secured to the coil via two silicone-based adhesives described above.

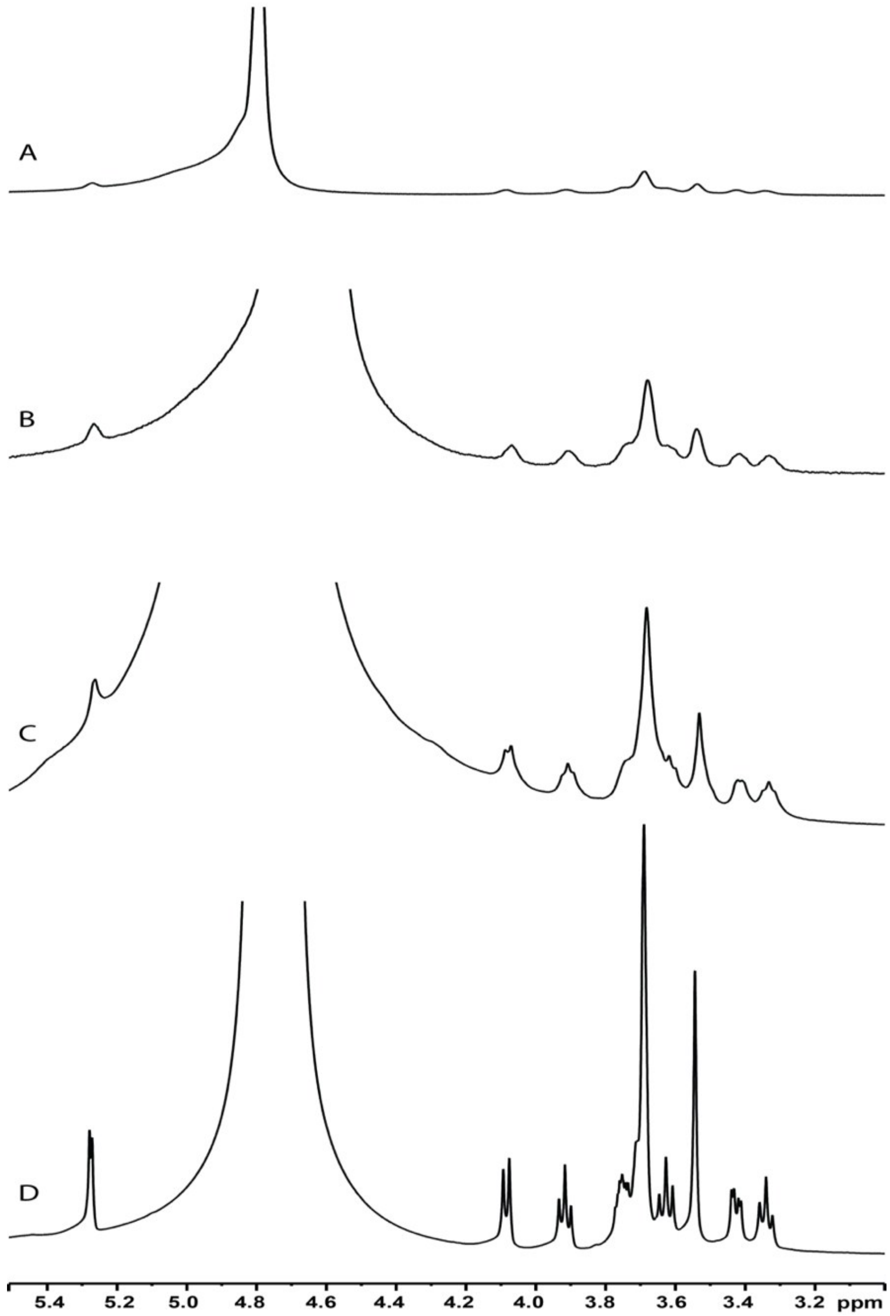

***Figure S3*.** A 1D ^{1}H zg-presaturation experiment was acquired on a 100 mM sucrose analyzed on the A. 50 µm, B. 100 µm, C. 500 µm planar microcoils and on the D. 5-mm saddle microcoil. The line-shape on the saddle coil is superior (in part due to Topshim 3D that could be used on the 5-mm coil) to that on the microcoils, however there is little advantage in using microcoils when the sample is present in excess.

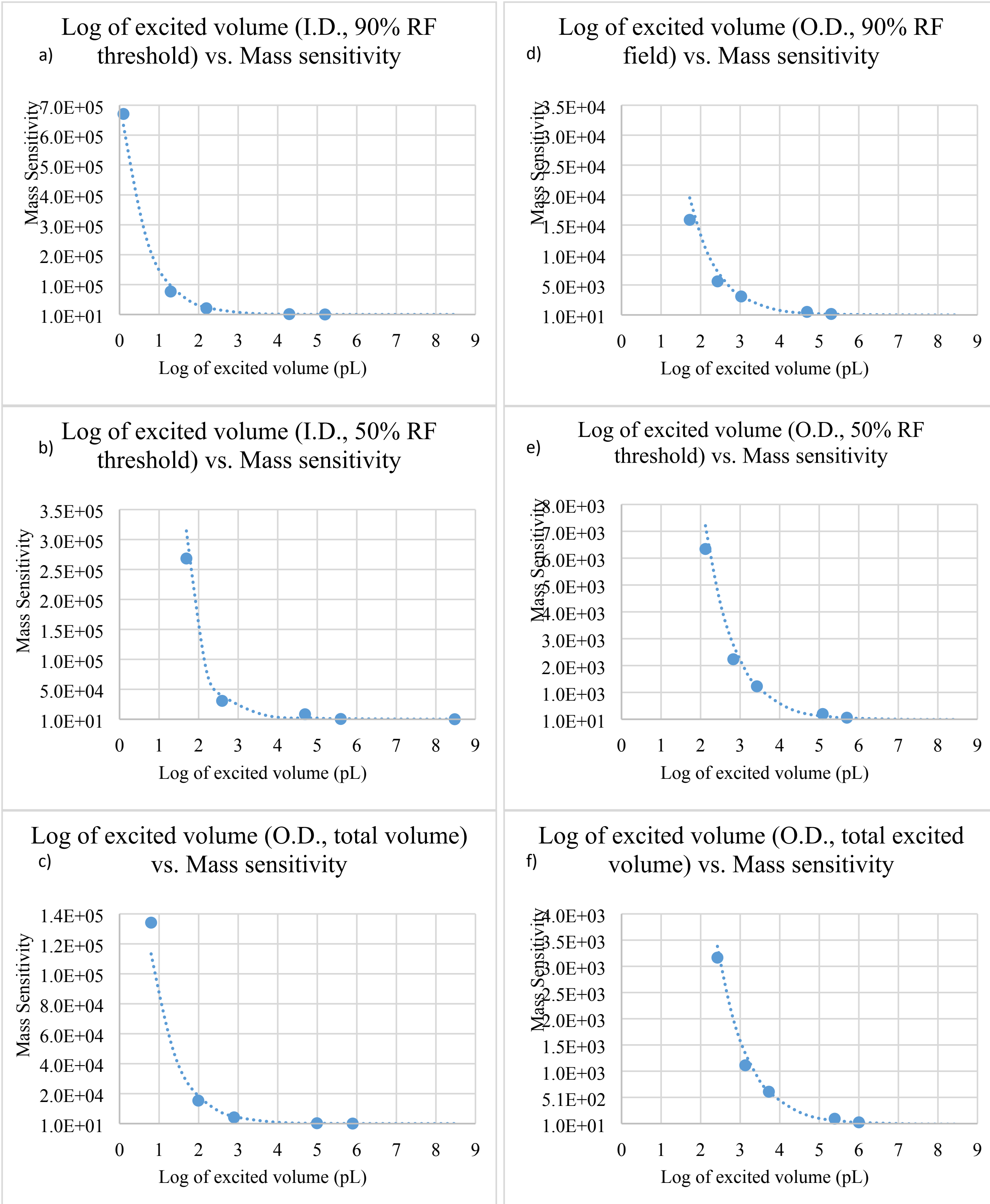

Figure S4. The mass sensitivity (SNR/V_{sample} in pL) from 256 scans of a pre-saturation experiment was plotted against the log of the excited volume based on the coil inner diameter (I.D.) and outer diameter (O.D.) at 90% RF threshold, 50% RF threshold and total volume. The relationship based on the I.D. are displayed in a), b) and c, while the O.D. are observed in d), e) and f).

Table S1A. SNR for 100 mM sucrose using various microcoils with different I.D's. (20-1000 μm) and a 5-mm saddle coil. Calculations are based on 90%, 50%, and total RF field lines previously reported by Massin et al.[1]

Coil size I.D.	SNR (256 scans)	Mass sensitivity vs 5mm using volume at 90% RF field (detectable volume in nL)	Mass sensitivity vs 5mm using volume at 50% RF field (detectable volume in nL)	Mass sensitivity vs 5mm using total volume the RF penetrates (detectable volume in nL)
20 μm	76 ± 4	670 867 (0.00130)	268 348 (0.00310)	134 174 (0.00630)
50	136 ± 6	76 832 (0.0196)	30 733 (0.0491)	15 366 (0.0982)
100	296 ± 16	20 902 (0.157)	8 361 (0.393)	4 181 (0.785)
500	2281± 9	1 289 (19.6)	515 (49.1)	258 (98.2)
1000	3110 ± 17	220 (157)	88 (393)	44 (785)
5 mm	27 045 ± 12	1 (300 000)	1 (300 000)	1 (300 000)

Table S1B. SNR for 100 mM sucrose using various microcoils with different O.D's. (130-1130 μm) and a 5-mm saddle coil. Calculations are based on 90%, 50%, and total RF field lines previously reported by Massin et al.[1]

Coil size O.D. (I.D.)	SNR (256 scans)	Mass sensitivity vs 5mm using volume at 90% RF field (detectable volume in nL)	Mass sensitivity vs 5mm using volume at 50% RF field (detectable volume in nL)	Mass sensitivity vs 5mm using total volume the RF penetrates (detectable volume in nL)
130 μm (20)	76 ± 4	15 879 (0.0531)	6 351 (0.133)	3 176 (0.266)
185 (50)	136 ± 6	5 612 (0.269)	2 245 (0.672)	1 122 (1.34)
260 (100)	296 ± 16	3 092 (1.06)	1 237 (2.66)	618 (5.31)
790 (500)	2281± 9	516 (49.0)	207 (122)	103 (245)
1130 (1000)	3110 ± 17	172 (200)	69 (501)	34 (1 003)
5 mm	27 045 ± 12	1 (300 000)	1 (300 000)	1 (300 000)

Table S2A.a. Data acquired for a *Cypselurus poecilopterus* egg on planar microcoils (50, 500 µm I.D.) and 5-mm saddle coil. Calculations are based on the volume within the 90% RF field lines.[1]

Coil size I.D.	Actual SNR (16 scans)	SNR normalized to 256 scans	Volume of sample observed (V_{sample}, nL)[I]	Filling factor of the coil[II]	% of the sample observed[III]	Mass sensitivity vs 5mm
50	178 ± 3	712 ± 3	0.0196	100% of the coil is filled	0.00047%	158 222
500	1831± 21	7324 ± 21	19.6	100	0.47	1628
5 mm	240 ± 8	960 ± 8	4 190	1.40	100	1

[I] Example =For the 500 µm coil the 90% RF field lines penetrate only 100 µm which translates to observing 19.6 nL of the fish egg.
[II] Example= 500 µm coil has on total observable volume of 19.6 nL when the coil is overfilled, however because the fish egg has a volume of 4 190 nL the filling factor is 100%.
[III] Example = 500 µm coil observes only 19.6 nL of the egg, however the egg's total volume is 4 190 nL, as a result only 0.47% of the egg is observed.

Table S2A.b. Data acquired for a *C. poecilopterus* egg on planar microcoils (185, 790 µm O.D.) and 5-mm saddle coil. Calculations are based on the volume within the 90% RF field lines.[1]

Coil size O.D. (I.D.)	Actual SNR (16 scans)	SNR normalized to 256 scans	Volume of sample observed (V_{sample}, nL)[I]	Filling factor of the coil[II]	% of the sample observed[III]	Mass sensitivity vs 5mm
185 (50)	178 ± 3	712 ± 3	0.269	100% of the coil is filled	0.0064%	11 558
790 (500)	1831± 21	7324 ± 21	49.0	100	1.17	653
5 mm	240 ± 8	960 ± 8	4 190	1.40	100	1

[I] Example =For the 500 µm (790 µm O.D.) coil the 90% RF field lines penetrate only 100 µm which translates to observing 49.0 nL of the fish egg.
[II] Example= 500 µm coil has on total observable volume of 49.0 nL when the coil is overfilled, however because the fish egg has a volume of 4 190 nL the filling factor is 100%.
[III] Example = 500 µm coil observes only 49.0 nL of the egg, however the egg's total volume is 4 190 nL, as a result only 1.17% of the egg is observed.

Table S2B.a. Data acquired for a *C. poecilopterus* egg on planar microcoils (50, 500 µm I.D.) and 5-mm saddle coil. Calculations are based on the volume within the 50% RF field line and the coil I.D.[1]

Coil size I.D.	Actual SNR (16 scans)	SNR normalized to 256 scans	Volume of sample observed (V_{sample}, nL)[I]	Filling factor of the coil[II]	% of the sample observed[III]	Mass sensitivity vs 5mm
50	178 ± 3	712 ± 3	0.0491	100% of the coil is filled	0.0012%	63 289
500	1831± 21	7324 ± 21	49.1	100	1.20	651
5 mm	240 ± 8	960 ± 8	4 190	1.40	100	1

[I] Example =For the 500 µm coil the 50% RF field lines penetrate only 250 µm which translates to observing 49.1 nL of the fish egg.
[II] Example= 500 µm coil has on total observable volume of 49.1 nL when the coil is overfilled, however because the fish egg has a volume of 4 190 nL the filling factor is 100%.
[III] Example = 500 µm coil observes only 49.1 nL of the egg, however the egg's total volume is 4 190 nL, as a result only 1.18% of the egg is observed.

Table S2B.b. Data acquired for a *C. poecilopterus* egg on planar microcoils (185, 790 µm O.D.) and 5-mm saddle coil. Calculations are based on the volume within the 50% RF field line and the coil O.D.[1]

Coil size O.D. (I.D.)	Actual SNR (16 scans)	SNR normalized to 256 scans	Volume of sample observed (V_{sample}, nL)[I]	Filling factor of the coil[II]	% of the sample observed[III]	Mass sensitivity vs 5mm
185 (50)	178 ± 3	712 ± 3	0.672	100% of the coil is filled	0.0160%	4 627
790 (500)	1831± 21	7324 ± 21	122	100	2.91	262
5 mm	240 ± 8	960 ± 8	4 190	1.40	100	1

[I] Example =For the 500 µm (790 µm O.D.) coil the 50% RF field lines penetrate only 250 µm which translates to observing 122 nL of the fish egg.
[II] Example= 500 µm coil has on total observable volume of 122 nL when the coil is overfilled, however because the fish egg has a volume of 4 190 nL the filling factor of the coil is 100%.
[III] Example = 500 µm coil observes only 122 nL of the egg, however the egg's total volume is 4 190 nL, as a result only 2.91 % of the egg is observed.

Table S2C.a. Data acquired for a *C. poecilopterus* egg on planar microcoils (50, 500 µm I.D.) and 5-mm saddle coil. Calculations are based on a total volume.[1]

Coil size I.D.	Actual SNR (16 scans)	SNR normalized to 256 scans	Volume of sample observed (V_{sample}, nL)[I]	Filling factor of the coil[II]	% of the sample observed[III]	Mass sensitivity vs 5mm
50	178 ± 3	712 ± 3	0.0982	100% of the coil is filled	0.0023%	31 644
500	1831± 21	7324 ± 21	98.2	100	2.34	326
5 mm	240 ± 8	960 ± 8	4 190	1.40	100	1

[I] Example =For the 500 µm coil (the total volume) penetrates ~500 µm which translates to an excitation volume of 98.2 nL.

[II] Example= 500 µm coil has on total observable volume of 98.2 nL when the coil is overfilled, however since the fish egg volume is 4 190 nL, the filling factor is 100%.

[III] Example = 500 µm coil observes 98.2 nL of the fish egg which translates to 2.34% of the egg.

Table S2C.b. Data acquired for a *C. poecilopterus* egg on planar microcoils (185, 790 µm O.D.) and 5-mm saddle coil. Calculations are based on a total volume.[1]

Coil size O.D. (I.D.)	Actual SNR (16 scans)	SNR normalized to 256 scans	Volume of sample observed (V_{sample}, nL)[I]	Filling factor of the coil[II]	% of the sample observed[III]	Mass sensitivity vs 5mm
185 (50)	178 ± 3	712 ± 3	1.34	100% of the coil is filled	0.032%	2 320
790 (500)	1831± 21	7324 ± 21	245	100	5.85	131
5 mm	240 ± 8	960 ± 8	4 190	1.40	100	1

[I] Example =For the 500 µm (790 µm O.D.) coil (the total volume) penetrates ~500 µm which translates to an excitation volume of 245 nL.

[II] Example= 500 µm coil has on total observable volume of 245 nL when the coil is overfilled, however since the fish egg volume is 4 190 nL, the filling factor is 100%.

[III] Example = 500 µm coil observes 245 nL of the fish egg which translates to 5.85% of the egg.

Table S3A.a. Data acquired for a *Daphnia magna* egg on planar microcoils (50, 500 µm I.D.) and 5-mm saddle coil. Calculations are based on the volume within the 90% RF field line.[1]

Coil size I.D.	Actual SNR (512 scans)	SNR normalized to 256 scans and W5 losses	Volume of sample observed[I] (V_{sample}, nL)	Filling factor of the coil[II]	% of the sample observed[III]	Mass sensitivity vs 5mm (single pulse)[IV]	Mass sensitivity vs 5mm (multiple pulse)[V]
50	385 ± 5	1361 ± 5	0.0196	100% of the coil is filled	0.47	205 333	41 066
500	355 ± 4	1255 ± 4	2.09	Only 10% of the coil is filled	50	1775	335
5 mm	2 ± 0.3	1.41 ± 0.3	4.19	0.0014% observed	100	1	1

[I] Example =For the 500 µm coil the 90% RF field lines penetrate only 100 µm which translates to observing only half of the egg or 2.09 nL.

[II] Example= 500 µm coil has on total observable volume of 19.6 nL when the coil is overfilled, however since only 2.09 nL is filled with egg leading to a 10% filling factor.

[III] Example = 500 µm coil observes only 2.09 nL of the egg, however the egg's total volume is 4.19 nL, as a result only 50% of the egg is observed.

[IV] Mass sensitivity for a single pulse is calculated after correcting for W5 losses and allows comparison to the fish egg and sucrose samples.

[V] Mass sensitivity for a multiple pulse includes W5 losses and demonstrates the performance loss for multiple pulse experiments on the present coils.

Table S3A.b. Data acquired for a *D. magna* egg on planar microcoils (185, 790 µm O.D.) and 5-mm saddle coil, however calculations for the 790 µm coil are omitted as the *D. magna* egg does not cover the entire coil region (only 1/3). Calculations are based on the volume within the 90% RF field line.[1]

Coil size O.D. (I.D.)	Actual SNR (512 scans)	SNR normalized to 256 scans and W5 losses	Volume of sample observed[I] (V_{sample}, nL)	Filling factor of the coil[II]	% of the sample observed[III]	Mass sensitivity vs 5mm (single pulse)[IV]	Mass sensitivity vs 5mm (multiple pulse)[V]
185 (50)	385 ± 5	1361 ± 5	0.269	100% of the coil is filled	6.4%	15 013	3 003
5 mm	2 ± 0.3	1.41 ± 0.3	4.19	0.0014% observed	100	1	1

[I] Example =For the 50 µm (185 µm O.D.) coil at 90% RF field lines penetrate out 10 µm which translates to observing 0.269 nL of the *D. magna* egg.

[II] Example= 50 µm coil has on total observable volume of 0.269 nL when the coil is overfilled, however since the egg's total volume is 4.19 nL, 100% of the coil is filled with sample.

[III] Example = 50 µm coil observes only 0.269 nL of the egg, however the egg's total volume is 4.19 nL, as a result only 6.4% of the egg is observed.

IV Mass sensitivity for a single pulse is calculated after correcting for W5 losses and allows comparison to the fish egg and sucrose samples.
V Mass sensitivity for a multiple pulse includes W5 losses and demonstrates the performance loss for multiple pulse experiments on the present coils.

Table S3B.a. Data acquired for a *D. magna* egg on planar microcoils (50, 500 µm I.D.) and 5-mm saddle coil. Calculations are based on the volume within the 50% RF field line.[1]

Coil size I.D.	Actual SNR (512 scans)	SNR normalized to 256 scans and W5 losses	Volume of sample observed[I] (V_{sample}, nL)	Filling factor of the coil[II]	% of the sample observed[III]	Mass sensitivity vs 5mm (single pulse)[IV]	Mass sensitivity vs 5mm (multiple pulse)[V]
50	385 ± 5	1361 ± 5	0.0491	100% of the coil is filled	1.17	92 420	18 484
500	355 ± 4	1255 ± 4	4.19	Only 9% of the coil is filled	100	1 000	200
5 mm	2 ± 0.3	1.41 ± 0.3	4.19	0.0014% observed	100	1	1

I Example =For the 500 µm coil the 50% RF field lines penetrate 250 µm out which translates to observing the egg in its entirety (4.19 nL). Since this RF field line extended out past the 200 µm diameter of the egg, the observed volume was assumed to contain the entire egg.
II Example= 500 µm coil has on total observable volume of 49.1 nL when the coil is overfilled, however since only 4.19 nL is filled with egg leading to a 9% filling factor.
III Example = 500 µm coil observes the entire egg.
IV Mass sensitivity for a single pulse is calculated after correcting for W5 losses and allows comparison to the fish egg and sucrose samples.
V Mass sensitivity for a multiple pulse includes W5 losses and demonstrates the performance loss for multiple pulse experiments on the present coils.

Table S3B.b. Data acquired for a *D. magna* egg on planar microcoils (185, 790 µm O.D.) and 5-mm saddle coil. Calculations for the 790 µm coil are omitted as the *D. magna* egg fully sits within the 500 µm I.D. as such the O.D is not relevant in this case. Calculations are based on the volume within the 50% RF field line.[1]

Coil size O.D. (I.D.)	Actual SNR (512 scans)	SNR normalized to 256 scans and W5 losses	Volume of sample observed[I] (V_{sample}, nL)	Filling factor of the coil[II]	% of the sample observed[III]	Mass sensitivity vs 5mm (single pulse)[IV]	Mass sensitivity vs 5mm (multiple pulse)[V]
185 (50)	385 ± 5	1361 ± 5	0.672	100% of the coil is filled	16.0%	6 010	1 202
5 mm	2 ± 0.3	1.41 ± 0.3	4.19	0.0014% observed	100	1	1

I Example = For the 50 µm (185 µm O.D.) coil at 50% RF field lines penetrate out 25 µm which translates to observing 0.672 nL of the *D. magna* egg.

[II] Example= 50 μm coil has on total observable volume of 0.672 nL when the coil is overfilled, however since the egg's total volume is 4.19 nL, 100% of the coil is filled with sample.
[III] Example = 50 μm coil observes only 0.672 nL of the egg, however the egg's total volume is 4.19 nL, as a result only 16.0% of the egg is observed.
[IV] Mass sensitivity for a single pulse is calculated after correcting for W5 losses and allows comparison to the fish egg and sucrose samples.
[V] Mass sensitivity for a multiple pulse includes W5 losses and demonstrates the performance loss for multiple pulse experiments on the present coils.

Table S3C.a. Data acquired for a *D. magna* egg on planar microcoils (50, 500 μm I.D.) and 5-mm saddle coil. Calculations are based on the total volume.[1]

Coil size I.D.	Actual SNR (512 scans)	SNR normalized to 256 scans and W5 losses	Volume of sample observed[I] (V_{sample}, nL)	Filling factor of the coil[II]	% of the sample observed[III]	Mass sensitivity vs 5mm (single pulse)[IV]	Mass sensitivity vs 5mm (multiple pulse)[V]
50	385 ± 5	1361 ± 5	0.0982	100% of the coil is filled	2.34	46 210	9 242
500	355 ± 4	1255 ± 4	4.19	Only 4% of the coil is filled	100	1 000	200
5 mm	2 ± 0.3	1.41 ± 0.3	4.19	0.0014% observed	100	1	1

[I] Example =For the 500 μm coil (the total volume) penetrates 500 μm out which translates to observing the egg in its entirety (4.19 nL). Since this RF field line extended out past the 200 μm diameter of the egg, the observed volume was assumed to contain the entire egg.
[II] Example= 500 μm coil has on total observable volume of 98.2 nL when the coil is overfilled, however since only 4.19 nL is filled with egg leading to a 4% filling factor.
[III] Example = 500 μm coil observes the entire egg.
[IV] Mass sensitivity for a single pulse is calculated after correcting for W5 losses and allows comparison to the fish egg and sucrose samples.
[V] Mass sensitivity for a multiple pulse includes W5 losses and demonstrates the performance loss for multiple pulse experiments on the present coils.

Table S3C.b. Data acquired for a *D. magna* egg on planar microcoils (185, 790 μm O.D.) and 5-mm saddle coil, however calculations for the 790 μm coil are omitted as the *D. magna* egg does not cover the entire coil region (only 1/3). Calculations are based on the total volume.[1]

Coil size O.D. (I.D.)	Actual SNR (512 scans)	SNR normalized to 256 scans and W5 losses	Volume of sample observed[I] (V_{sample}, nL)	Filling factor of the coil[II]	% of the sample observed[III]	Mass sensitivity vs 5mm (single pulse)[IV]	Mass sensitivity vs 5mm (multiple pulse)[V]
185 (50)	385 ± 5	1361 ± 5	1.34	100% of the coil is filled	32.0%	3 014	603
5 mm	2 ± 0.3	1.41 ± 0.3	4.19	0.0014% observed	100	1	1

[I] Example = For the 50 µm (185 µm O.D.) coil (the total volume) penetrates ~50 µm which translates to an excitation volume of 1.34 nL.
[II] Example= 50 µm coil has on total observable volume of 1.34 nL when the coil is overfilled, however since the egg's total volume is 4.19 nL, 100% of the coil is filled with sample.
[III] Example = 50 µm coil observes only 1.34 nL of the egg, however the egg's total volume is 4.19 nL, as a result only 32.0% of the egg is observed.
[IV] Mass sensitivity for a single pulse is calculated after correcting for W5 losses and allows comparison to the fish egg and sucrose samples.
[V] Mass sensitivity for a multiple pulse includes W5 losses and demonstrates the performance loss for multiple pulse experiments on the present coils.

References:

1 C. Massin, G. Boero, F. Vincent, J. Abenhaim, P. A. Besse and R. S. Popovic, in *Sensors and Actuators, A: Physical*, 2002, **97**, 280–288.