SUPPLEMENTARY DATA

Supplementary Tables

	Experiment 1			Experiment 2			Experiment 3		
[^{3} H]E2 (nM)	Mean pmol	%CV	N	Mean pmol	%CV	N	Mean pmol	%CV	N
1	0.0067	8.0	10	0.0035	28.0	10	0.0060	14.0	10
5	0.0385	7.6	10	0.0416	8.4	10	0.0290	11.8	10
10	0.0856	6.7	10	0.0741	23.3	10	0.0641	19.3	10
36.7	0.3079	15.5	10	0.3187	11.4	10	0.2856	15.0	10

Table S1. Intra-experimental variation in pmol uptake at four different concentrations with one-hour treatment. Mean calculated by averaging the uptake in pmol of each individual embryo from a single experiment. %CV = Coefficient of variation expressed as a percent (standard deviation ÷ mean, x 100). N = number of embryos per well. One well from a single clutch of embryos was tested per concentration per experiment. Note that at 1 nM, mean [3H]E2 uptake was below the limit of detection (0.01 pmol).

	Experiment 1			Experiment 2			Experiment 3		
[^{3} H]E2 (nM)	Mean % uptake	%CV	N	Mean % uptake	%CV	N	Mean % uptake	%CV	N
1	0.2760	14.6	10	0.2910	29.7	10	0.3050	8.9	10
5	0.4640	12.7	10	0.3640	9.0	10	0.3330	8.1	10
10	0.2910	20.5	10	0.2360	25.1	10	0.3460	7.0	10
36.7	0.2990	16.1	10	0.3290	12.4	10	0.2770	15.6	10

Table S2. Intra-experimental variation in percent uptake at four different concentrations with one-hour treatment. Mean calculated by averaging the percent uptake of each individual embryo from a single experiment. %CV = Coefficient of variation expressed as a percent (standard deviation ÷ mean, x 100). N = number of embryos per well. One well from a single clutch of embryos was tested per concentration per experiment.

1-Hour Exposure					
	[^{3}H]E2 pmol uptake				
Treatment Period (hpf)	**mean pmol uptake (SD)**	**compared period (hpf)**	**mean uptake difference from 6 hpf**	**adjusted p-value vs 6 hpf**	**summary**
6 to 7	0.0179 (0.004)	**24 to 25**	0.0195	0.0064	**
		48 to 49	0.0185	0.0093	**
		72 to 73	0.0516	<0.0001	****
		96 to 97	0.1102	<0.0001	****
Treatment Period (hpf)	**mean pmol uptake (SD)**	**compared period (hpf)**	**mean uptake difference from 24 hpf**	**adjusted p-value vs 24 hpf**	**summary**
24 to 25	0.03741 (0.003)	**48 to 49**	-0.0010	0.9994	ns
		72 to 73	0.0321	0.0002	***
		96 to 97	0.0907	<0.0001	****
Treatment Period (hpf)	**mean pmol uptake (SD)**	**compared period (hpf)**	**mean uptake difference from 48 hpf**	**adjusted p-value vs 48 hpf**	**summary**
48 to 49	0.03639 (0.007)	**72 to 73**	0.0331	0.0001	***
		96 to 97	0.0917	<0.0001	****
Treatment Period (hpf)	**mean pmol uptake (SD)**	**compared period (hpf)**	**mean uptake difference from 72 hpf**	**adjusted p-value vs 72 hpf**	**summary**
72 to 73	0.0695 (0.003)	**96 to 97**	0.0586	<0.0001	****
	[^{3}H]E2 % uptake				
Treatment Period (hpf)	**mean % uptake (SD)**	**compared period (hpf)**	**mean uptake difference from 6 hpf**	**adjusted p-value vs 6 hpf**	**summary**
6 to 7	0.1025 (0.025)	**24 to 25**	0.1408	0.0620	ns
		48 to 49	0.2808	0.0005	***
		72 to 73	0.4942	<0.0001	****
		96 to 97	0.7975	<0.0001	****
Treatment Period (hpf)	**mean % uptake (SD)**	**compared period (hpf)**	**mean uptake difference from 24 hpf**	**adjusted p-value vs 24 hpf**	**summary**
24 to 25	0.2433 (0.029)	**48 to 49**	0.1400	0.0880	ns
		72 to 73	0.3533	0.0001	***
		96 to 97	0.6567	<0.0001	****
Treatment Period (hpf)	**mean % uptake (SD)**	**compared period (hpf)**	**mean uptake difference from 48 hpf**	**adjusted p-value vs 48 hpf**	**summary**
48 to 49	0.3833 (0.068)	**72 to 73**	0.2133	0.0076	**
		96 to 97	0.5167	<0.0001	****
Treatment Period (hpf)	**mean % uptake (SD)**	**compared period (hpf)**	**mean uptake difference from 72 hpf**	**adjusted p-value vs 72 hpf**	**summary**
72 to 73	0.5967 (0.083)	**96 to 97**	0.3033	0.0005	***

Table S3. Comparison of uptake at different developmental stages with one-hour exposure. Adjusted p-values calculated with one-way ANOVA with Tukey's multiple comparisons test, comparing pmol or percent uptake between one-hour exposure groups when starting treatment at 6, 24, 48, 72, or 96 hpf. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns not signficant $p \geq 0.05$.

24-Hour Exposure					
	[^{3}H]E2 pmol uptake				
Treatment Period (hpf)	**mean pmol uptake (SD)**	**compared period (hpf)**	**mean uptake difference from 6 hpf**	**adjusted p-value vs 6 hpf**	**summary**
6 to 30	0.1303 (0.013)	**24 to 48**	0.0888	0.1682	ns
		48 to 72	0.2241	0.0005	***
		72 to 96	0.7235	<0.0001	****
		96 to 120	0.4758	<0.0001	****
Treatment Period (hpf)	**mean pmol uptake (SD)**	**compared period (hpf)**	**mean uptake difference from 24 hpf**	**adjusted p-value vs 24 hpf**	**summary**
24 to 48	0.2192 (0.015)	**48 to 72**	0.1352	0.0407	*
		72 to 96	0.6346	<0.0001	****
		96 to 120	0.3870	<0.0001	****
Treatment Period (hpf)	**mean pmol uptake (SD)**	**compared period (hpf)**	**mean uptake difference from 48 hpf**	**adjusted p-value vs 48 hpf**	**summary**
48 to 72	0.3544 (0.057)	**72 to 96**	0.4994	<0.0001	****
		96 to 120	0.2517	0.0003	***
Treatment Period (hpf)	**mean pmol uptake (SD)**	**compared period (hpf)**	**mean uptake difference from 72 hpf**	**adjusted p-value vs 72 hpf**	**summary**
72 to 96	0.8538 (0.071)	**96 to 120**	-0.2477	0.0003	***
	[^{3}H]E2 % uptake				
Treatment Period (hpf)	**mean % uptake (SD)**	**compared period (hpf)**	**mean uptake difference from 6 hpf**	**adjusted p-value vs 6 hpf**	**summary**
6 to 30	0.832 (0.100)	**24 to 48**	1.031	0.4151	ns
		48 to 72	2.988	0.0023	**
		72 to 96	6.548	<0.0001	****
		96 to 120	4.628	<0.0001	****
Treatment Period (hpf)	**mean % uptake (SD)**	**compared period (hpf)**	**mean uptake difference from 24 hpf**	**adjusted p-value vs 24 hpf**	**summary**
24 to 48	1.863 (0.155)	**48 to 72**	1.958	0.0627	ns
		72 to 96	5.518	<0.0001	****
		96 to 120	3.598	0.0003	***
Treatment Period (hpf)	**mean % uptake (SD)**	**compared period (hpf)**	**mean uptake difference from 48 hpf**	**adjusted p-value vs 48 hpf**	**summary**
48 to 72	3.82 (0.541)	**72 to 96**	3.560	0.0013	**
		96 to 120	1.640	0.1450	ns
Treatment Period (hpf)	**mean % uptake (SD)**	**compared period (hpf)**	**mean uptake difference from 72 hpf**	**adjusted p-value vs 72 hpf**	**summary**
72 to 96	7.38 (1.370)	**96 to 120**	-1.920	0.0694	ns

Table S4. Comparison of uptake at different developmental stages with 24-hour exposure. Adjusted p-values calculated with one-way ANOVA with Tukey's multiple comparisons test, comparing pmol or percent uptake between 24-hour exposure groups when starting treatment at 6, 24, 48, 72, or 96 hpf. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns not signficant $p \geq 0.05$.

Supplementary Figures

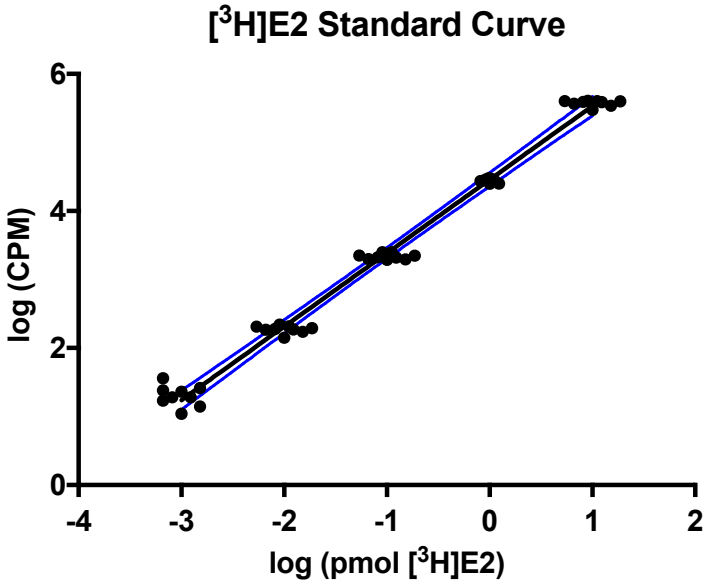

Figure S1. Standard curve for pmol [^{3}H]E2 uptake calculations. The radioactivity of 10 µL of a known concentration of [^{3}H]E2 was measured on a scintillation counter in triplicate on three different days for a total of 9 measurements per concentration (black circles). A linear relationship was found when performing a power analysis of the mean of each concentration, Y = 1.073*X + 4.459 where X = log(pmol [^{3}H]E2) and Y = log(CPM). r^2 = 0.9992 (unweighted, best-fit linear regression line). Outer blue lines represent 95% CI.

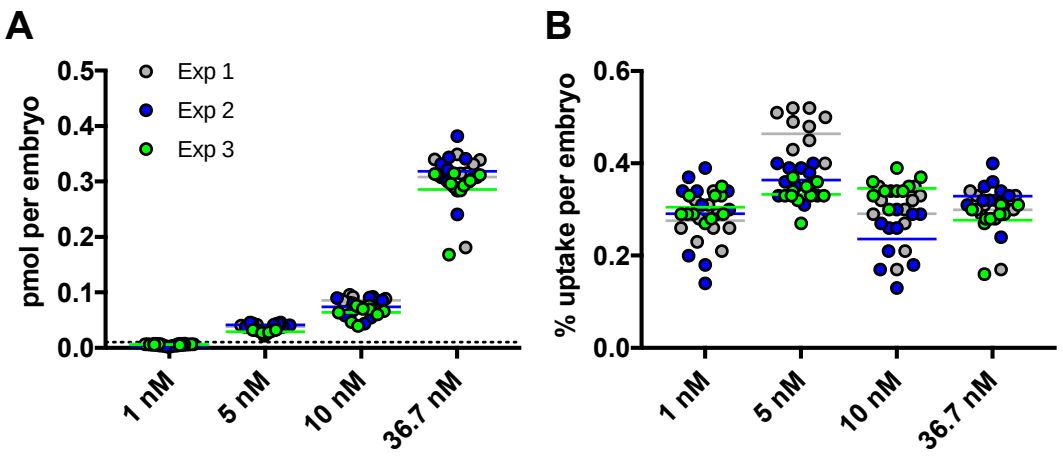

Figure S2. Individual embryo variability within experiments. (A-B) Experiments from Figure 1 (1-hour treatment starting at 48 hpf) displayed where each dot represents the pmol **(A)** or percent **(B)** [^{3}H]E2 uptake from a single embryo. Experimental replicates (n=10 embryos per experiment) are represented as different colored dots (grey, blue, or green) in the graph. Horizontal lines (grey, blue or green) are the mean from each experiment.

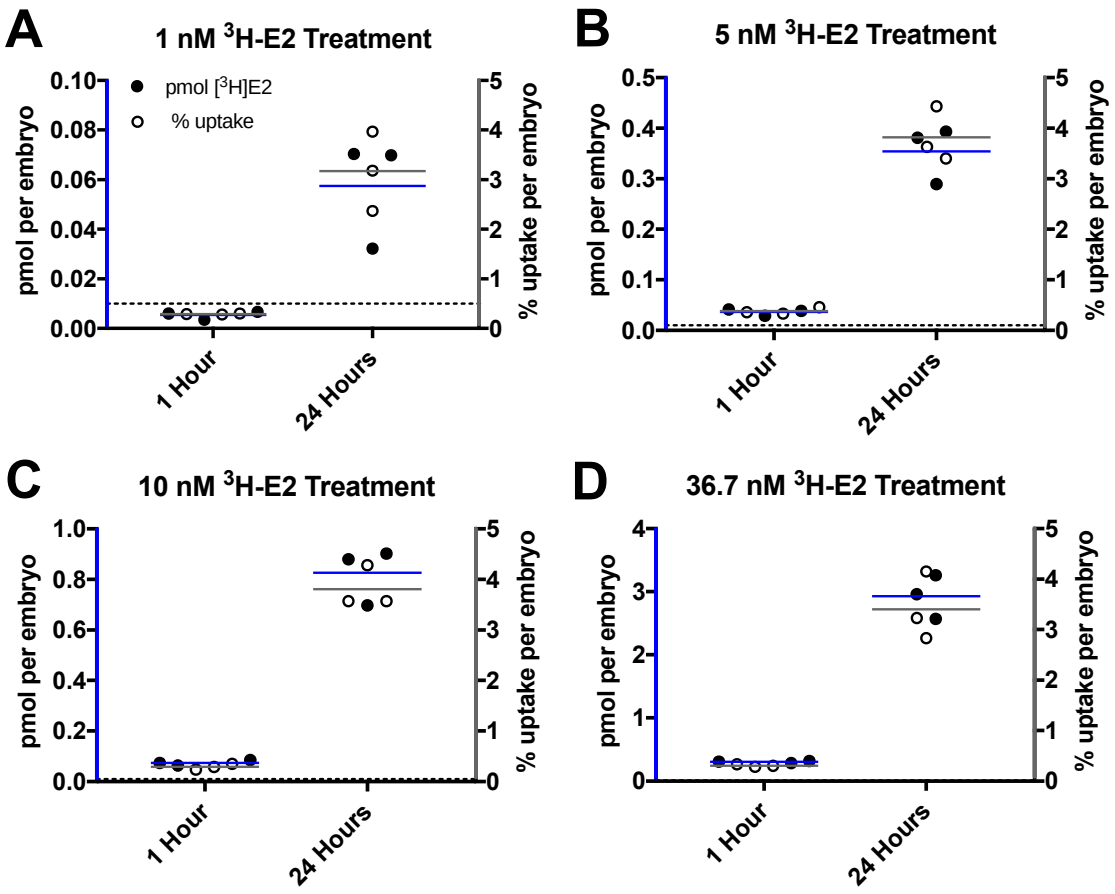

Figure S3. Estradiol uptake increases with increasing exposure duration. (A-D) Data from Figure 1 displayed to highlight the change in absolute (black circles) or percent (white circles) uptake with increasing exposure duration at **(A)** 1 nM, **(B)** 5 nM, **(C)** 10 nM, and **(D)** 36.7 nM [^{3}H]E2 treatment. There was an increase of approximately 10-fold with each concentration when increasing treatment duration from 1 hour to 24 hours.

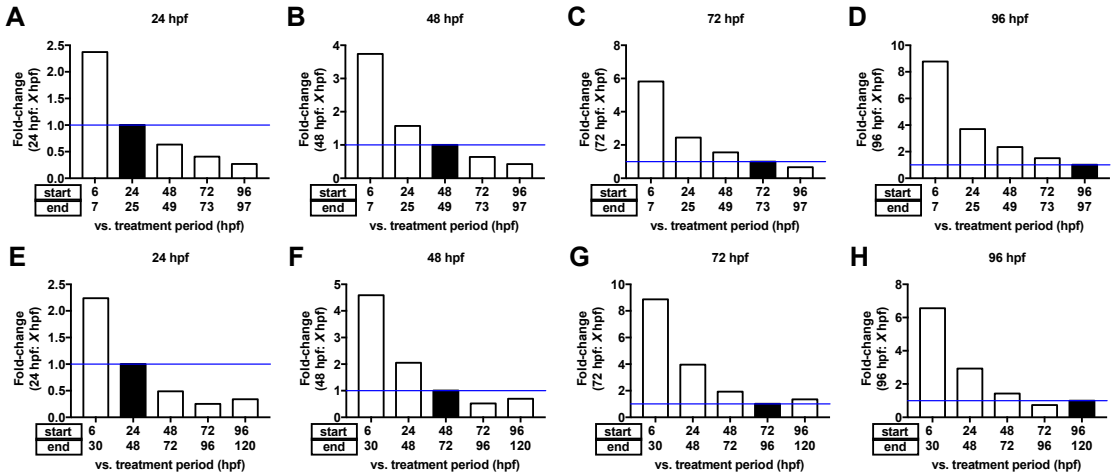

Figure S4. Estradiol uptake is age-dependent. Fold-change in percent uptake between embryos of developmental stages tested in Figure 2A, 2B at 1- and 24-hour exposures. Labels on x-axis describe the treatment group as compared to all other treatment groups. Fold-change was calculated by dividing the mean percent uptake of each developmental stage by the mean percent uptake of every other developmental stage (n=3-5 experiments). The blue line at y=1 represents no change between groups. Below this line represents lower uptake, above this line represents higher uptake. The black bar in each graph represents comparison to self. **(A-D)** 1-hour exposure to 5 nM [^{3}H]E2 starting at **(A)** 24 hpf, **(B)** 48 hpf, **(C)** 72 hpf, **(D)** 96 hpf. **(E-H)** 24-hour exposure to 5 nM [^{3}H]E2 starting at **(E)** 24 hpf, **(F)** 48 hpf, **(G)** 72 hpf, **(H)** 96 hpf. Percent uptake increased with increasing developmental stage up to 96 hpf with 1-hour exposure, while percent uptake increased up to the 72 hpf developmental stage and then plateaued with 24-hour exposure.