Supporting information:

Metabolic and reproductive effects of relatively low concentrations of beclomethasone dipropionate, a synthetic glucocorticoid, on fathead minnows.

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Analytical chemistry for measuring exposure concentrations

Method

Over the course of the experiment, four (days 0,7,14 and 21) water samples (2x50 mL) were taken from each tank, where day 0 was the day fish were transferred into the pre-equilibrated exposure system and day 21 was the terminal fish sampling day.

The Liquid Chromatography-Mass Spectrometry (LC/MS) was performed on a Perkin Elmer (PE Sciex API 305) LC/MS/MS system with an auto sampler (PE series 200) and HPLC Agilent series 1050 (Agilent Technologies, Germany).

Auto sampler parameters: Syringe capacity: 250µl; injection speed: medium; Pre-injection washes: one; Post injection washes: two; washing solvent: methanol; Injection volume: 50µl; advanced inject details: aircushion: 10µl; excess volume: 10µl; needle level:10%; inject delay time : none; partial loop mode; temperature control enabled set point 20.

Column parameters: Symmetry c18, 3.5µm column (Waters, Ireland) 2.1 x 50mm.

Mass Spectrum tune parameters: Source/gas: ion source-turbo spray; nebulizer gas-12; curtain gas-10; ionspray voltage-4500; temperature-350°C. Compound de-clustering potential-20; focussing potential-100; entrance potential-5.5. Detector deflector – 250; CEM-2200. Resolution ion energy -1 and unit Q1 resolution.

Beclomethasone dipropionate (BCMD) standard of a high concentration, 10mg/L, was prepared in 40:60 acetonitrile – milliQ water and was injected using a mobile phase of 60% methanol/ 0.1% formic acid into the LC-MS to obtain the peak retention time and the

corresponding mass spectrum. This resulted in a clear peak at 521.2 m/z with 8.6 minutes retention time. The molecular mass of BCMD is 521g/mol. MS (Q1 – positive polarity) scan of 521.2 cantered widths of 2m/z was run to detect and quantify BCMD. External standards with concentrations, ranging from 100µg/L down to 0.77µg/L in a 2 fold serial dilution were run and used to plot the standard curve. The linearity of the curve was satisfactory with R^2 value of 0.992. Method limit of detection (LOD) and limit of quantification (LOQ) for BCMD were determined as the concentration inducing a signal/noise ratio of 3 and 6 respectively. Spiked samples of 100µg/L, 50µg/L and 10µg/L were run in parallel to the unknowns to confirm the detection and accurate quantification of BCMD in the water samples.

Results

Method LOD and LOQ were1µg/L and 4 µg/L. No detectable peak was found in control, low and medium concentration tank waters. Samples from the high concentration tanks (nominal concentration 10µg/L) were analysed and are presented in Figure 1. From 4 different measurements, concentrations of BCMD on day 0, day 7, day 14 and day 21 were 11.75 ± 2.14µg/L, 8.54 ± 2.99µg/L, 7.82 ± 0.78µg/L and 10.50 ± 4.21µg/L respectively. The overall mean of 16 measurements was 9.65 ± 2.99 µg/L.

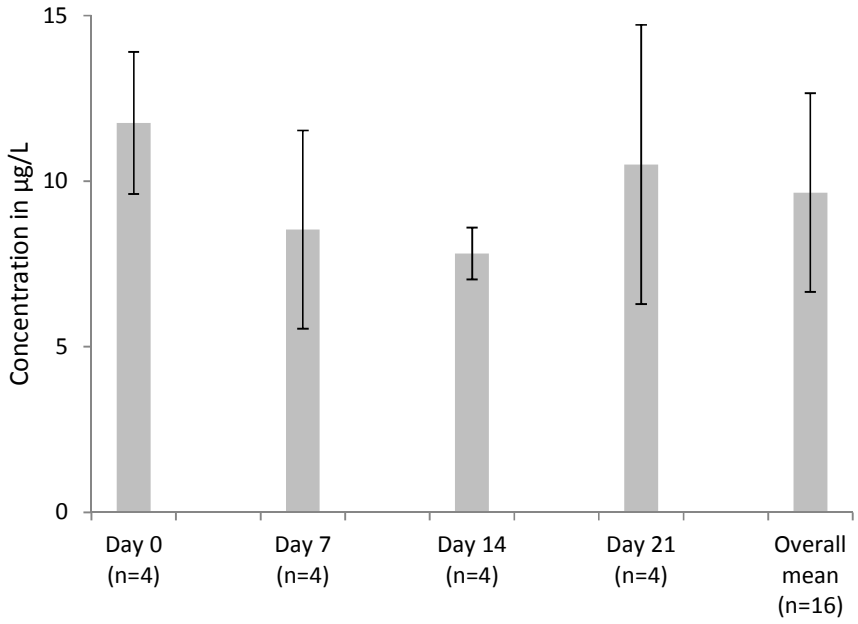

Figure s1: Measured concentrations of BCMD from the high exposure concentration (nominal of 10µg/L) tanks on four different sampling days and the overall mean concentration. Error bars represent standard deviations from the mean.

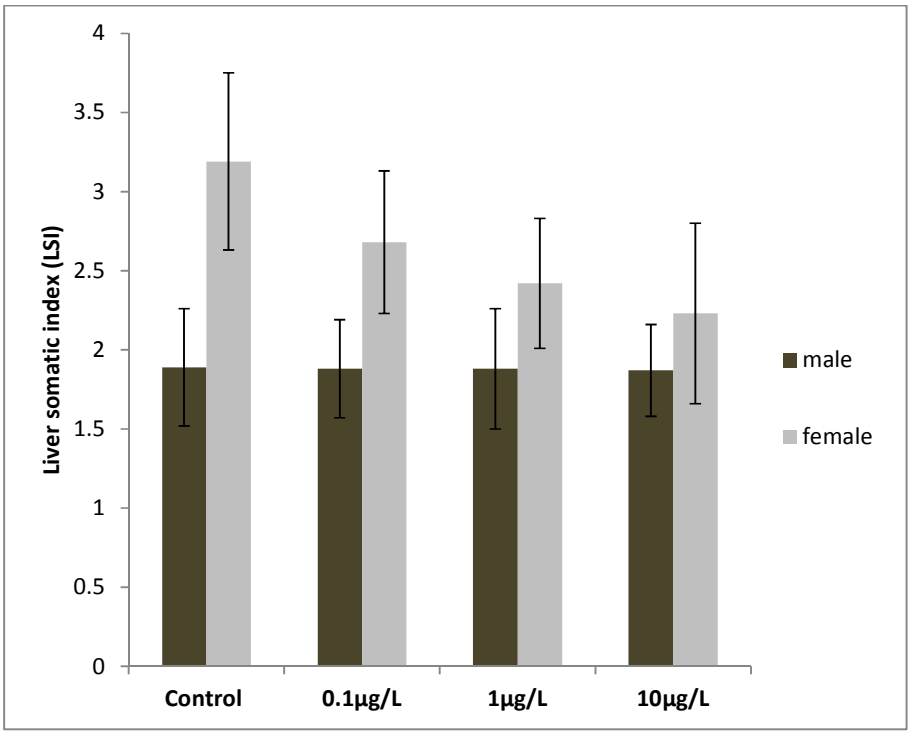

Figure s2. The liver-somatic index (LSI) of fish from controls and BCMD treated fish. Trend analysis revealed that the LSI of female fish had a significant decreasing trend with increasing concentration of BCMD (JT test; $p < 0.001$).

Table s1. Table showing forward (F) and reverse (R) primer sequence information for five genes studied in the BCMD exposure experiment. Annealing temperature (Tm), GC percentage, product length and the sources of the sequences used to design the primers.

Primer name	Position in its sequence and length (bp)	Primer sequence: 5' -3'	Exon/intron boundary	Tm	GC%	Secondary structure	Product length	Source
β-actin F	824-842(19)	CCTTCCTTCCTGGGTATGG	836,838	63.2	57.8	None	158	Gene Bank:BC165331
β-actin R	981-962(20)	TCCTTCTGCATACGGTCAGC	NO	65.3	55	Week		
VTG F	1278-1298(21)	TGGCCTCTGCAGCAATATCAT	NO	70.9	57.1	Week	128	Brian *et al*., 2008 Gene Bank: AF130354
VTG R	1405-1385(21)	TGGCCTCTGCAGCAATATCAT	NO	66.7	47.6	None		
PEPCK F	1508-1529(22)	GCTGCTGAACACAAAGGTAAAGTG	1522,26	65.7	45.8	Week	153	*Danio rerio* (Gene Bank: GI31418741), *Cyprinus carpio* (partial sequence; Gene Bank: GI24637091) and *Oncorhynchus mykiss* (Gene Bank: GI13506885)
PEPCK R	1660-1640(21)	GAACCAGTTGACGTGGAAGAT	NO	62.9	47.6	Week		
GR F	1737-1756(20)	CCATGCCTCAGCTGGTGCCC	NO	74.6	70	Week	134	Gene Bank: AY533141
GR R	1870-1851(20)	GCCGCCCAGCCTGTTCAGAG	NO	73.6	70	None		
18s rRNA F	19-40(22)	AATGTCTGCCCTATCAACTTTC	NO	60.9	40.9	None	117	Filby and Tyler, 2007 GenBank: AY855349
18s rRNA R	135-117(19)	TGGATGTGGTAGCCGTTTC	NO	63.6	52.6	None		

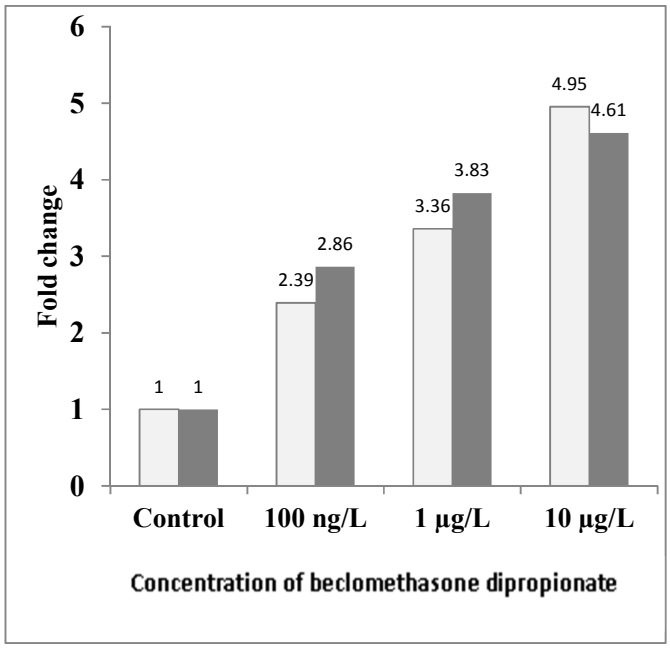

Figure s3. Differences in PEPCK (light coloured bars) and GR (dark coloured bars) mRNA expression after exposure to different concentrations of BCMD. Mean values shown are fold change, plotted relative to the control group.

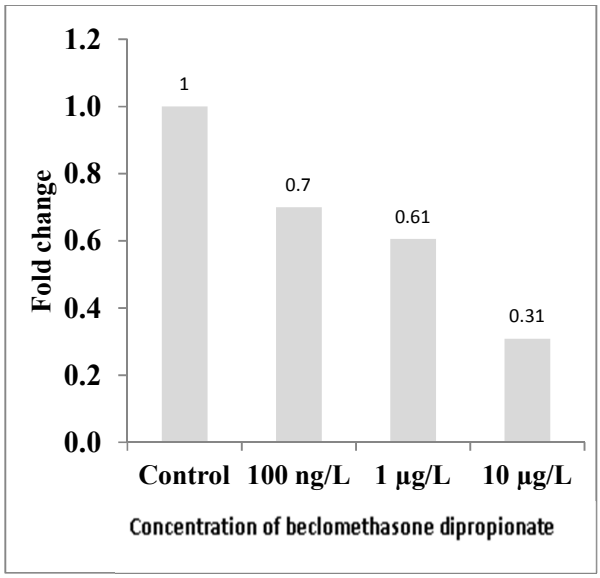

Figure s4. Differences in Vtg mRNA expression after exposure to different concentrations of BCMD. Mean values shown are fold change, plotted relative to the control group.