# Supplementary information:

# Human amniotic fluid contaminants alter thyroid hormone signalling and early brain development in Xenopus embryos

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# Supplementary Figures:

- **Supporting information for Figure 1**
  - Figure. S1 - (Figure 1 extended data) Thyroid disrupting activity of individual chemicals and mixture assessed with XETA without $T_3$ as a spike and mixture with a $T_4$ spike
- **Supporting information for Figure 2**
  - Figure S2a - NH3 abrogates GFP fluorescence induced by $T_3$ + Mixture 10x
  - Figure S2b - NH3 significantly reduces GFP fluorescence in Mix 10x exposed tadpoles.
  - Figure S2c - Anti-GFP using immunohistochemistry on *Tg(thibz:eGFP)* tadpoles brains is increased by mixture exposure in absence of $T_3$
  - Figure S2d- No change in pH observed with either $T_3$ or mixture

- **Supporting information for Figure 3**
  - Figure S3 (Figure 3 extended data) Mixture exposure modifies thyroid hormone and neuronal development related gene expression in brain when <u>using $T_3$ as a spike</u>
  - Figure S4 (Figure 3 extended data) Mixture exposure modifies neuronal development related gene expression in brain in <u>the absence of a $T_3$ spike</u>.

- **Supporting information for Figure 4**
  - Figure S5 - 3D brain video (CLARITY)
    a) Wild type whole brain
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  - Figure S6 (BEHAVIOUR)
    a) Protocol (schematic) for assessing locomotor activity
    b) Distance covered by tadpoles over 10 minutes (detail minute by minute)
    c) Distances covered by tadpoles with regards to dark and light periods.
    d) Mobility video (Control): Full length video of the 12 well plate subjected to mobility assay
    e) Mobility video ($T_3$): Full length video of the 12 well plate subjected to mobility assay
    f) Mobility video (Mixture 10x): Full length video of the 12 well plate subjected to mobility assay

**Supplementary figure legends**

**Figure. S1**: **Thyroid disrupting activity of individual chemicals and mixture assessed with XETA without $T_3$ as a spike and mixture with a $T_4$ spike (*Figure 1 extended data*):** Screening of thyroid disrupting activity of molecules measured in humans with the Xenopus Embryonic Thyroid Assay (XETA), based on the quantification of fluorescence-using transgenic Thbzip-eGFP animals. Fifteen compounds were tested at different concentrations. Scattered plots are shown with mean+/- SD of three to five independent experiments pooled. The GFP fluorescence was measured and quantified after 72h exposureChemicals tested alone without a $T_3$ spike. **a**, Phenolic compounds: BPA, Triclosan and Benzophenone-3. **b**, Phthalates: DBP and DEHP. **c,** Organochlorine pesticides: HCB and 4’4-DDE. **d,** Perfluorinated compounds: PFOA and PFOS. **e,** Polyaromatic hydrocarbons: 2-Naphtol. **f,** Halogenated compounds: Sodium perchlorate, PCB-153 and BDE-209. **g,** Metals: Methylmercury and Lead chloride. **h,** Exposure to mixed compounds alone and against $T_4$ 10nM spike (**a-g**). Results represent 3 pooled experiments, normalised against $T_3$ or $T_4$ values. Statistics used meta-analysis with Kruskal-Wallis (Mean ± SDs, *$p < 0.05$, ****$p < 0.0001$). Hashes (###) represent $p < 0.001$, $T_4$ vs Control.

**Figure. S2** ***(Figure 2 extended data)***

a) **NH3 abrogates GFP fluorescence induced by T3 + Mixture 10x**. *Tg(thibz:eGFP)* tadpoles were exposed for 3d to Mix 1x or 10x (see table S1 for exact composition), NH3 2µM with or without $T_3$ 5nM for 72h. Quantification of fluorescence was done on whole tadpoles’ head as illustrated.

**b) NH3 significantly reduces GFP fluorescence in Mix 10x exposed tadpoles.** Results of Fig S2a in absence of $T_3$ spike, analyzed with a specific quantification of tadpole brain fluorescence as illustrated.

**c) Anti-GFP using immunohistochemistry on Tg(thibz:eGFP) tadpoles brains is increased by mixture exposure in absence of T3**. *Tg(thibz:eGFP)* tadpoles were exposed for three days to either Mix 1x, 10x or $T_3$ 5nM. Brains were dissected after 3h fixation in PFA 4% at RT and stored in cryoprotectant until being subjected to IHC with the chicken anti eGFP antibody. Left panel represents representative brains in ventral view for each group. Regions of interest (ROI) in brain: Forebrain (FB), Midbrain (MB, Hindbrain (HB) are outlined with hashed lines. Scale bar 200µm. Right panel represents GFP quantification on the whole brain.

**d) No observable change in water pH following addition of either $T_3$ or mixture**. pH stripes (Fisher brand) with either control water (Evian + 1/10000 DMSO), $T_3$ $5.10^{-9}$M (same concentration of DMSO as in control) or mixture at 0.1x, 1x or 10x concentration.

**Figure. S3: (*Figure 3 extended data)* Mixture exposure modifies thyroid hormone and neuronal development related gene expression in brain when using $T_3$ as a spike.** Mixture exposure modifies thyroid hormone and neuronal development related gene expression in brain when using $T_3$ as a spike: RT-qPCR for **a**, *dio1* **b**, *dio2* **c**, *dio3* **d**, *thra* **e**, *thrb* **f**, *sox2* **g**, *tubb2b* **h**, *mbp* and **i**, *bdnf* **j** *thibz* **k**, *klf9,* **l** *mecp2,* **m** *oatp1c1*, **n** *mct8,* **o** *mct10,* **p** *lat1* and **q** *lat2.*

Relative fold change presented using log(2)scale against $T_3$. Statistics used one way non parametric Kruskal-Wallis test (Medians, Whiskers boxes mean and max, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$).

**Figure S4** ***(Figure 3 extended data)*** **Mixture exposure modifies additional thyroid hormone and neuronal development related gene expression in brain: RT-qPCR** for **a,** *klf9* **b**, *cntn4* **c**, *dcx* **d**, *dmnt3a* **e**, *reelin* **f**, *parvalbumin* **g**, *nestin* **h**, *rora* **i**, *pcna* **j**, *olig4* **k**, *mecp2* and **l**, *gfap*, **m** *oatp1c1*, **n** *mct8*, **o** *mct10*, **p** *lat1*, and **q** *lat2* . Relative fold change presented used log2scale with reference done on control groups. Statistics used Kruskal-Wallis tests (Medians, Whisker boxes mean and max_black dots represents values far from the rest but not outliers)), $*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

**Figure S5 (Videos) (related to Figure 4) -** A 360° view of the 3D brain reconstructed after imaging the brains exposed to CLARITY

a) Wild type whole brain (avi file in folder)
b) $T_3$ treated whole brain (avi file in folder)
c) Mix 10x treated whole brain (avi file in folder)

**Figure S6 (related to Figure 4) (BEHAVIOUR)**

a) Protocol (schematic) for assessing locomotor activity. NF48 tadpoles exposed for 3 days are placed one/well in a 12 well plate. Each well contains 4ml of water. Each individual is video tracked for 10 min. Every 30 seconds, light goes on or off in order to stimulate the movements.
b) Distance covered by tadpoles over 10 minutes (detail minute by minute)
c) Distances covered by tadpoles with regards to dark and light periods.
d) Mobility video (Control): Full length video of the 12 well plate subjected to mobility assay
e) Mobility video ($T_3$): Full length video of the 12 well plate subjected to mobility assay
f) Mobility video (Mixture 10x): Full length video of the 12 well plate subjected to mobility assay

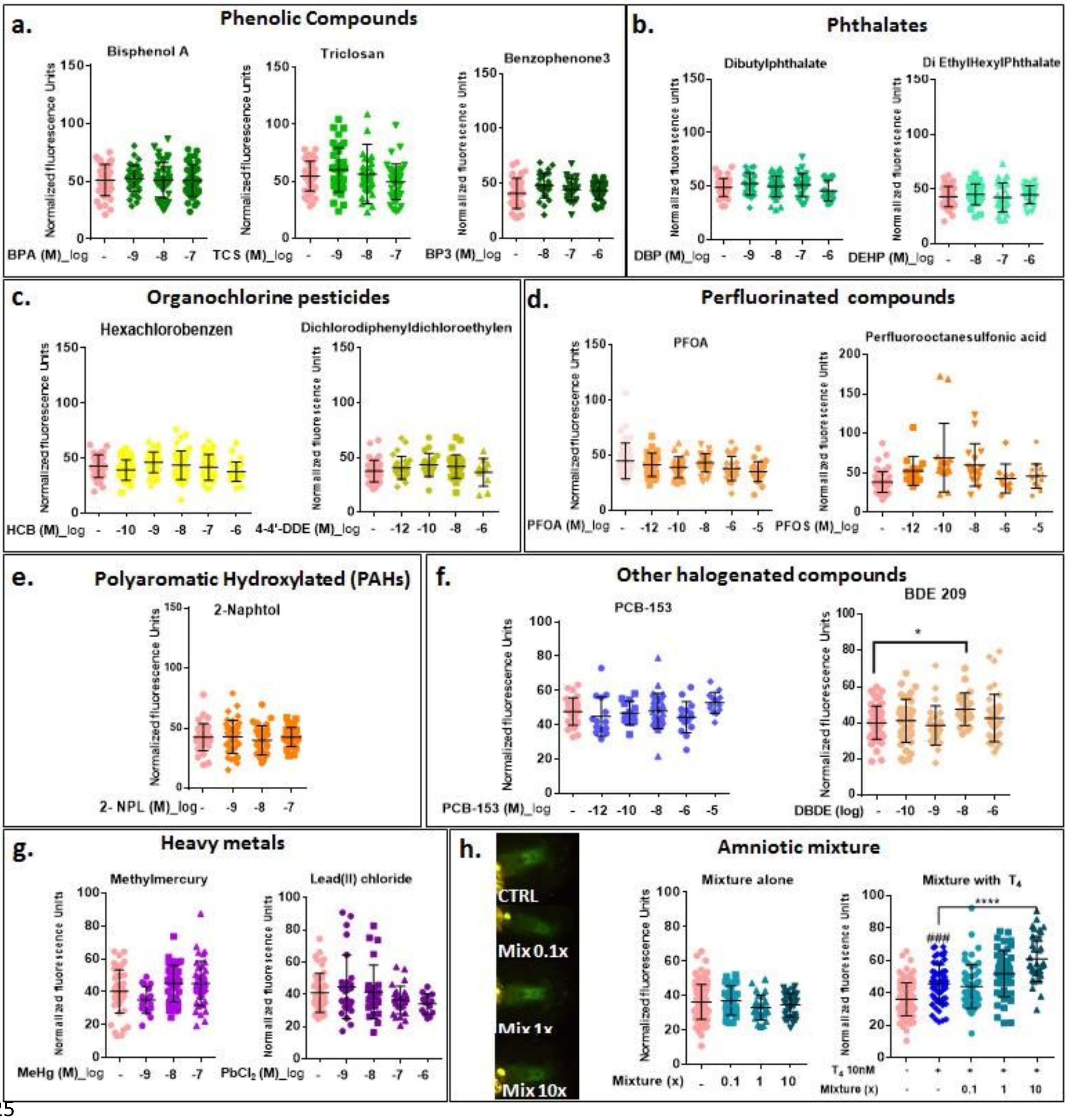
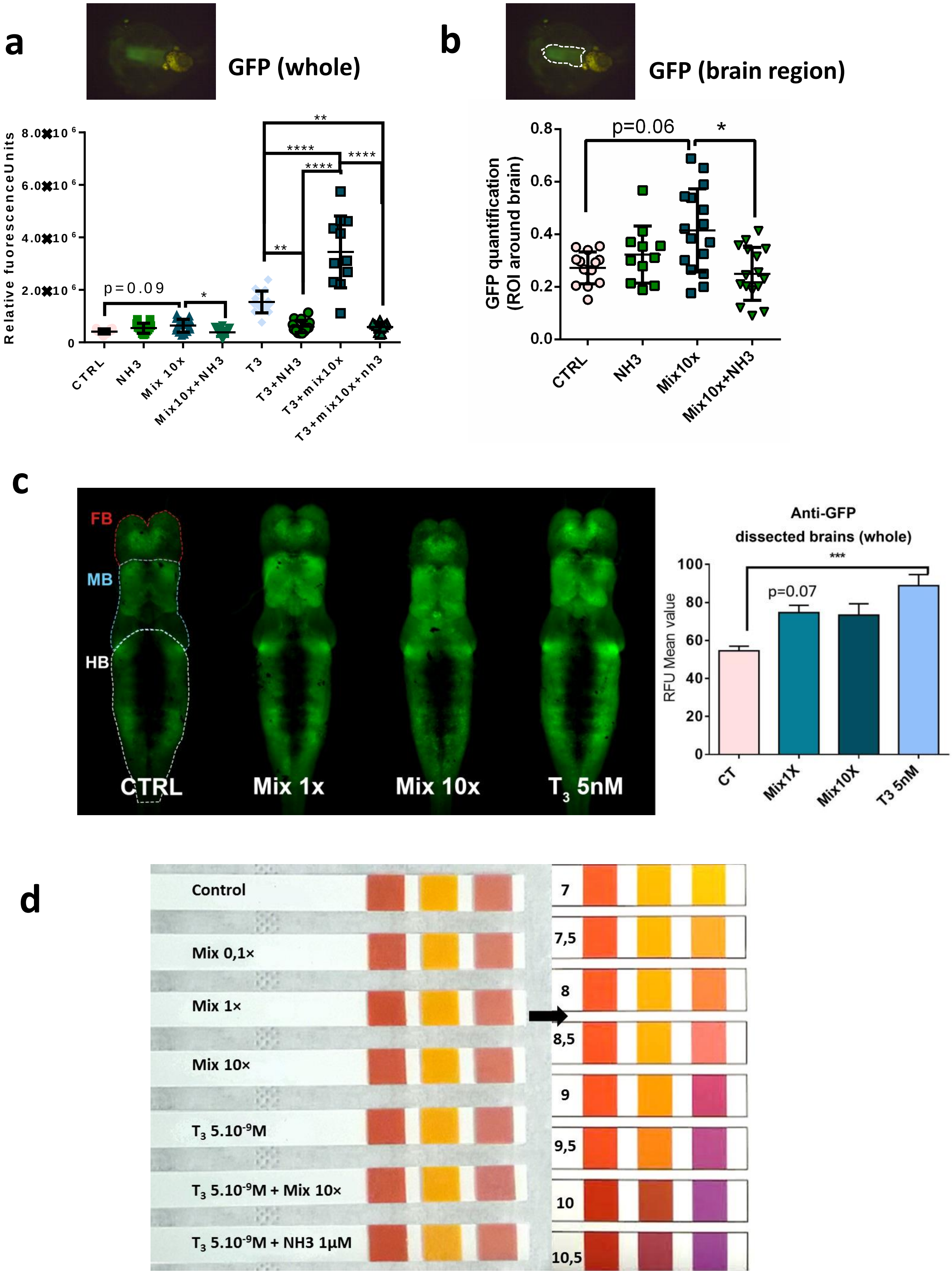
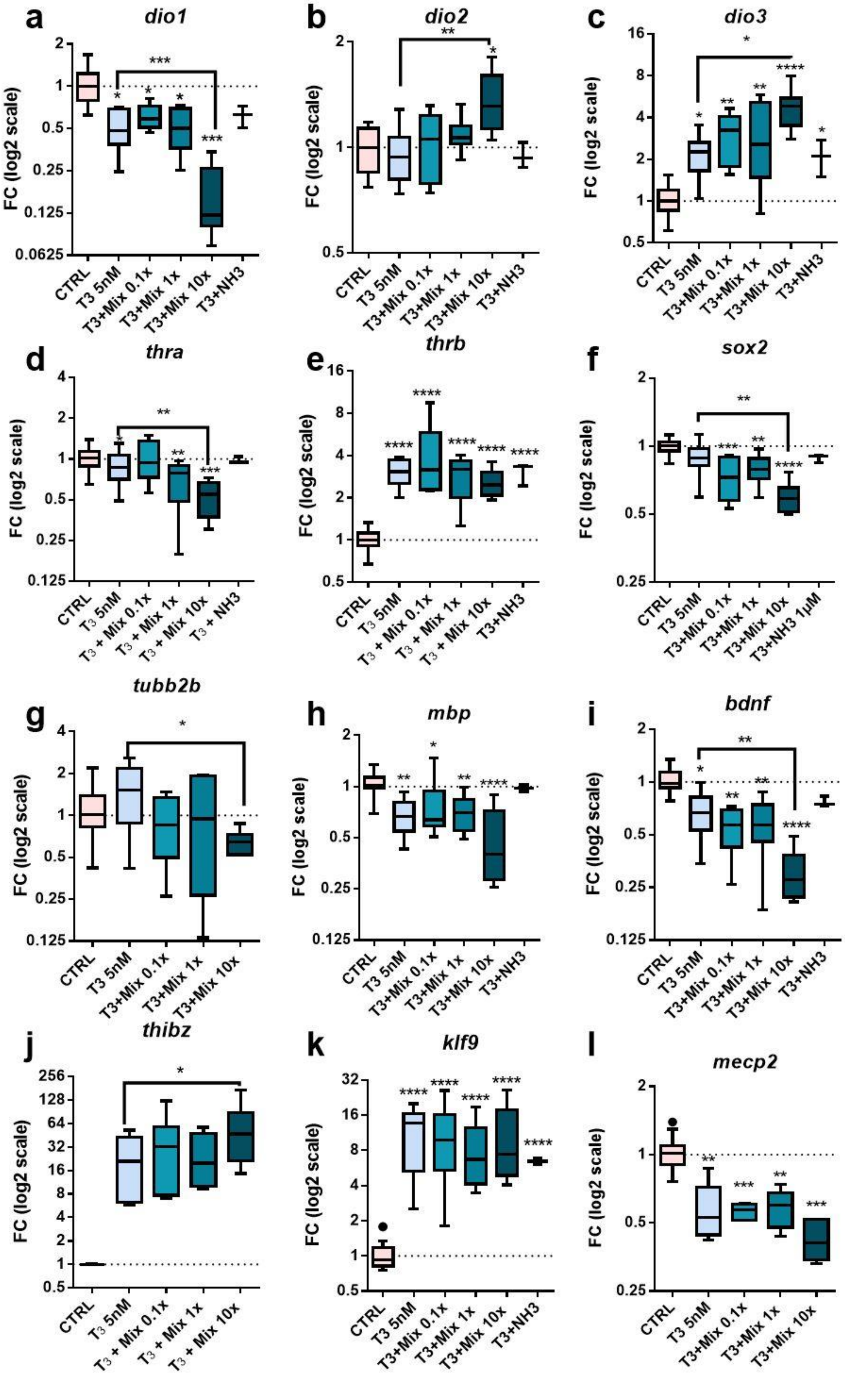

a.
Phenolic Compounds
Bisphenol A
Triclosan
Benzophenone3
Normalized fluorescence Units
BPA (M)_log
TCS (M)_log
BP3 (M)_log
b.
Phthalates
Dibutylphthalate
Di EthylHexylPhthalate
DBP (M)_log
DEHP (M)_log
c.
Organochlorine pesticides
Hexachlorobenzen
Dichlorodiphenyldichloroethylen
HCB (M)_log
4-4'-DDE (M)_log
d.
Perfluorinated compounds
PFOA
Perfluorooctanesulfonic acid
PFOA (M)_log
PFOS (M)_log
e.
Polyaromatic Hydroxylated (PAHs)
2-Naphtol
2- NPL (M)_log
f.
Other halogenated compounds
PCB-153
BDE 209
PCB-153 (M)_log
DBDE (log)
g.
Heavy metals
Methylmercury
Lead(II) chloride
MeHg (M)_log
PbCl2 (M)_log
h.
Amniotic mixture
CTRL
Mix 0.1x
Mix 1x
Mix 10x
Mixture alone
Mixture with T4
Mixture (x)
T4 10nM
Mixture (x)

**Figure S1**



a
GFP (whole)
Relative fuorescenceUnits
8.0×10 6
6.0×10 6
4.0×10 6
2.0×10 6
0
p=0.09
*
**
****
****
****
**
CTRL
NH3
Mix 10x
Mix10x+NH3
T3
T3+NH3
T3+mix10x
T3+mix10x+nh3
b
GFP (brain region)
GFP quantification
(ROI around brain)
0.8
0.6
0.4
0.2
0.0
p=0.06
*
CTRL
NH3
Mix10x
Mix10x+NH3
c
FB
MB
HB
CTRL
Mix 1x
Mix 10x
T3 5nM
Anti-GFP
dissected brains (whole)
***
p=0.07
RFU Mean value
100
80
60
40
20
0
CT
Mix1X
Mix10X
T3 5nM
d
Control
Mix 0,1×
Mix 1×
Mix 10×
T3 5.10-9M
T3 5.10-9M + Mix 10×
T3 5.10-9M + NH3 1µM
7
7,5
8
8,5
9
9,5
10
10,5


**Figure S2**



a
dio1
b
dio2
c
dio3
d
thra
e
thrb
f
sox2
g
tubb2b
h
mbp
i
bdnf
j
thibz
k
klf9
l
mecp2
FC (log2 scale)
CTRL
T3 5nM
T3+Mix 0.1x
T3+Mix 1x
T3+Mix 10x
T3+NH3
T3+NH3 1µM

**Figure S3**

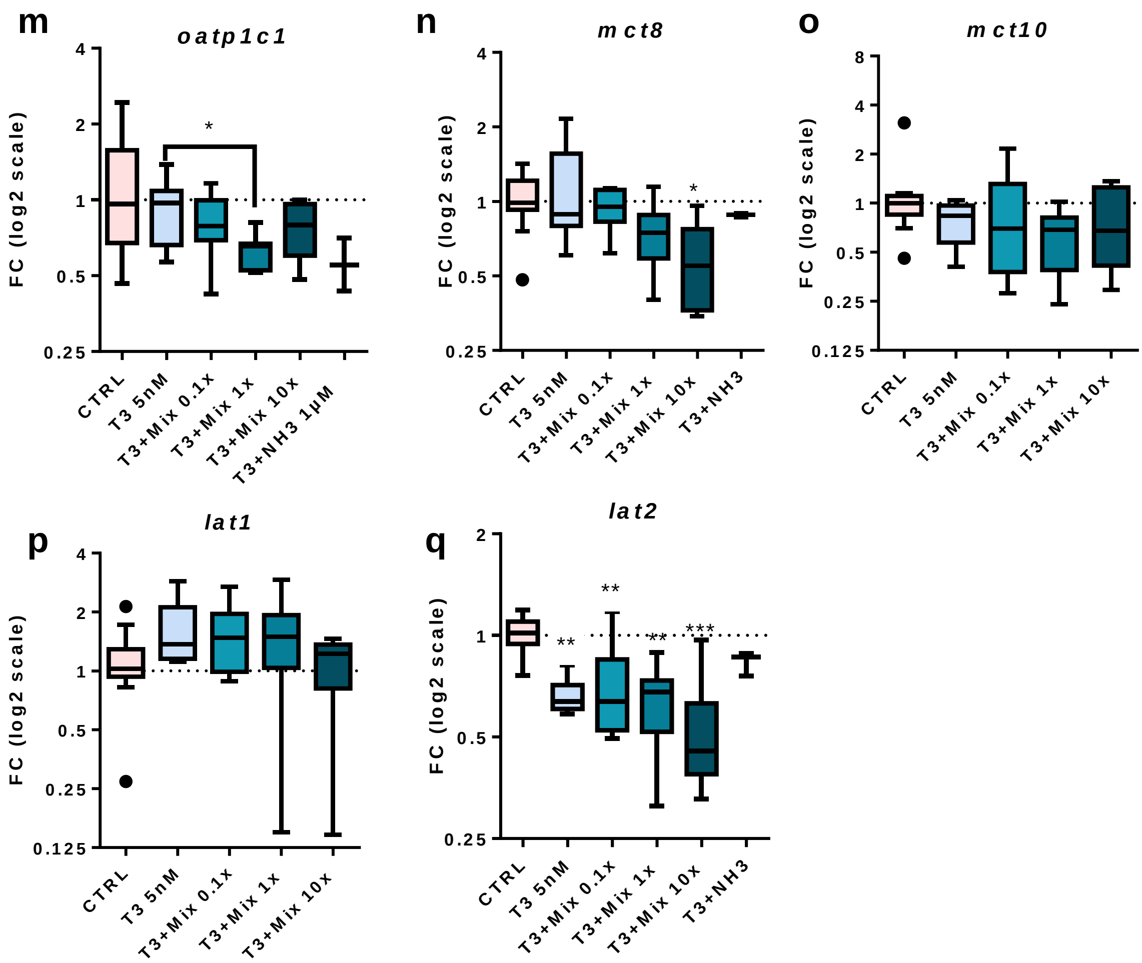



**Figure S3 (continued)**

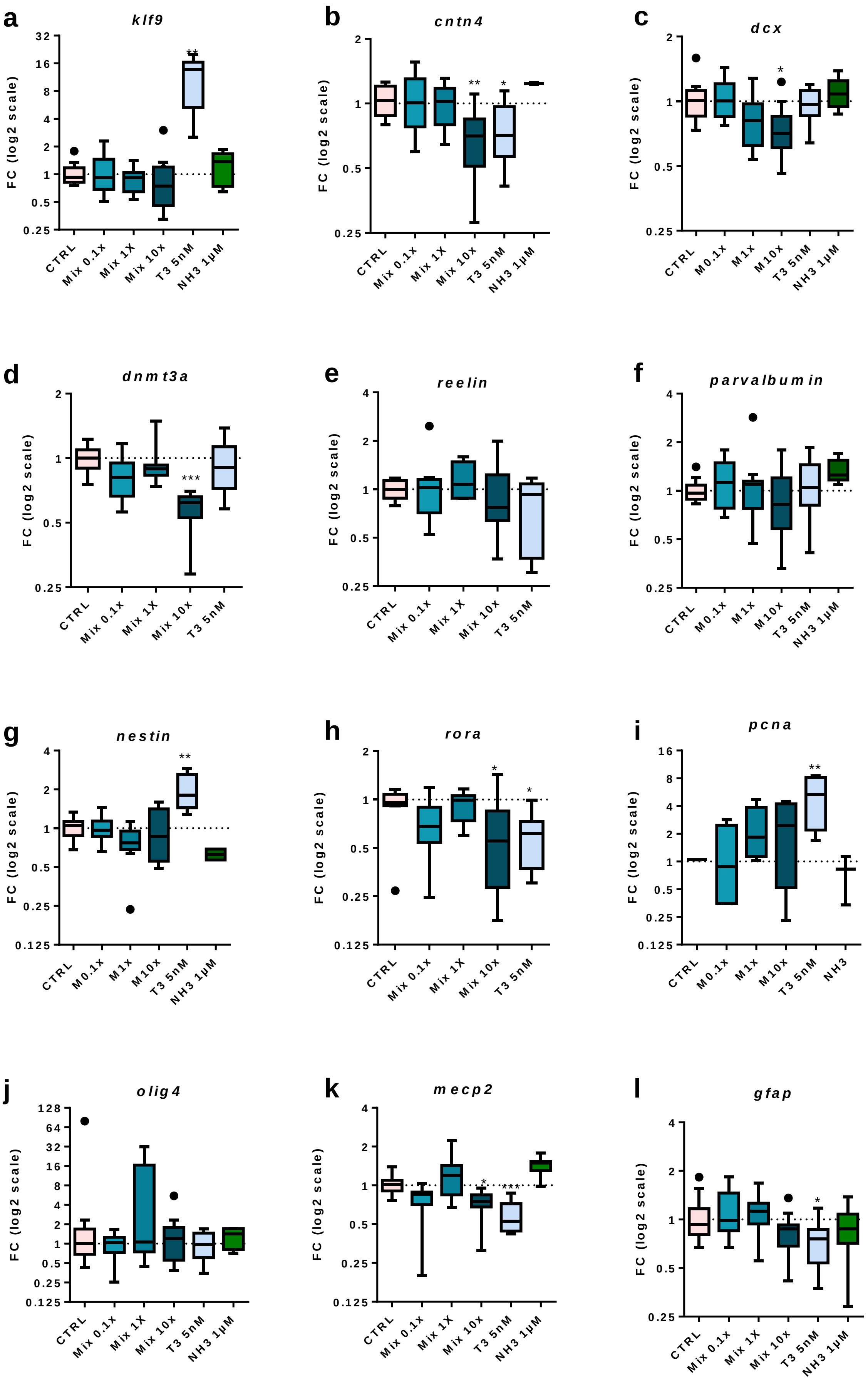

a
klf9
b
cntn4
c
dcx
d
dnmt3a
e
reelin
f
parvalbumin
g
nestin
h
rora
i
pcna
j
olig4
k
mecp2
l
gfap
FC (log2 scale)
0.125
0.25
0.5
1
2
4
8
16
32
64
128
*
**
***
CTRL
Mix 0.1x
Mix 1X
Mix 10x
T3 5nM
NH3 1µM
M0.1x
M1x
M10x
NH3

**Figure S4**

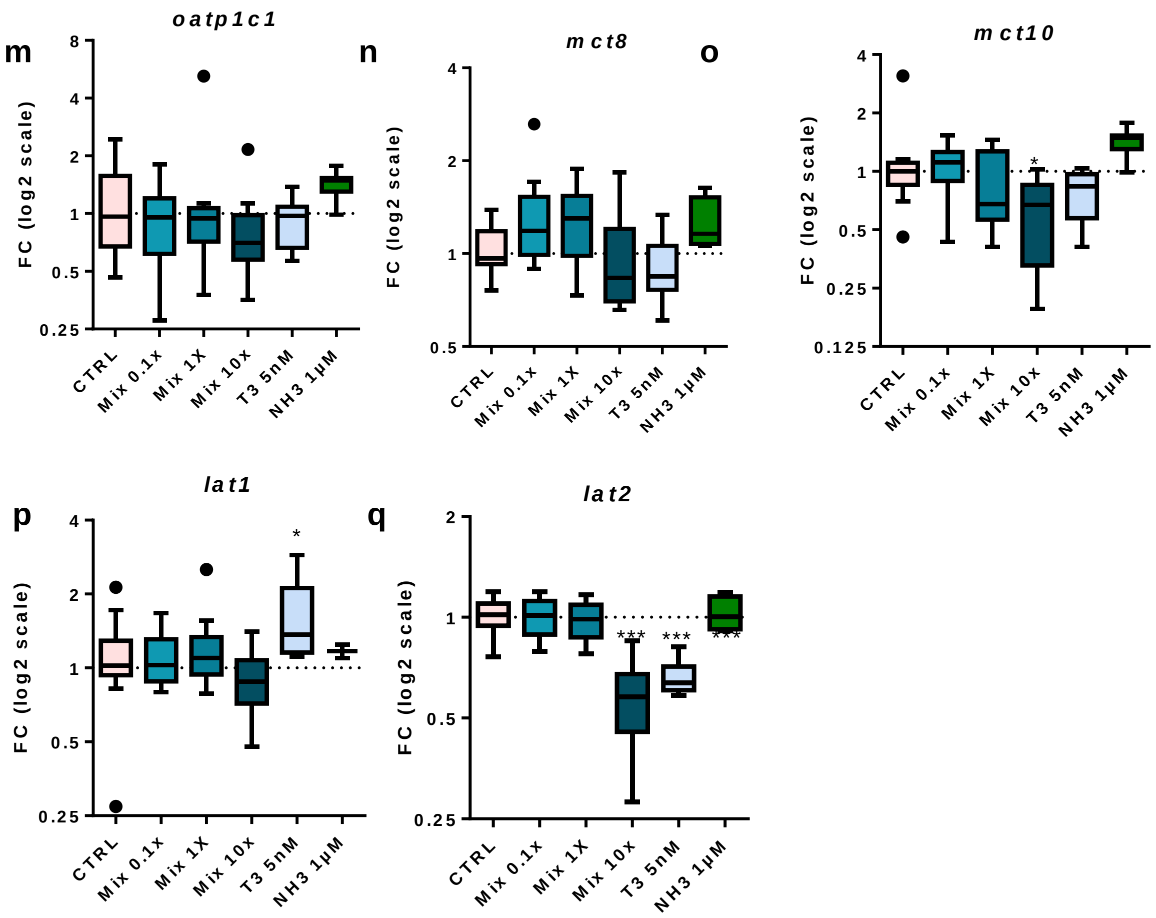



**Figure S4 (continued)**

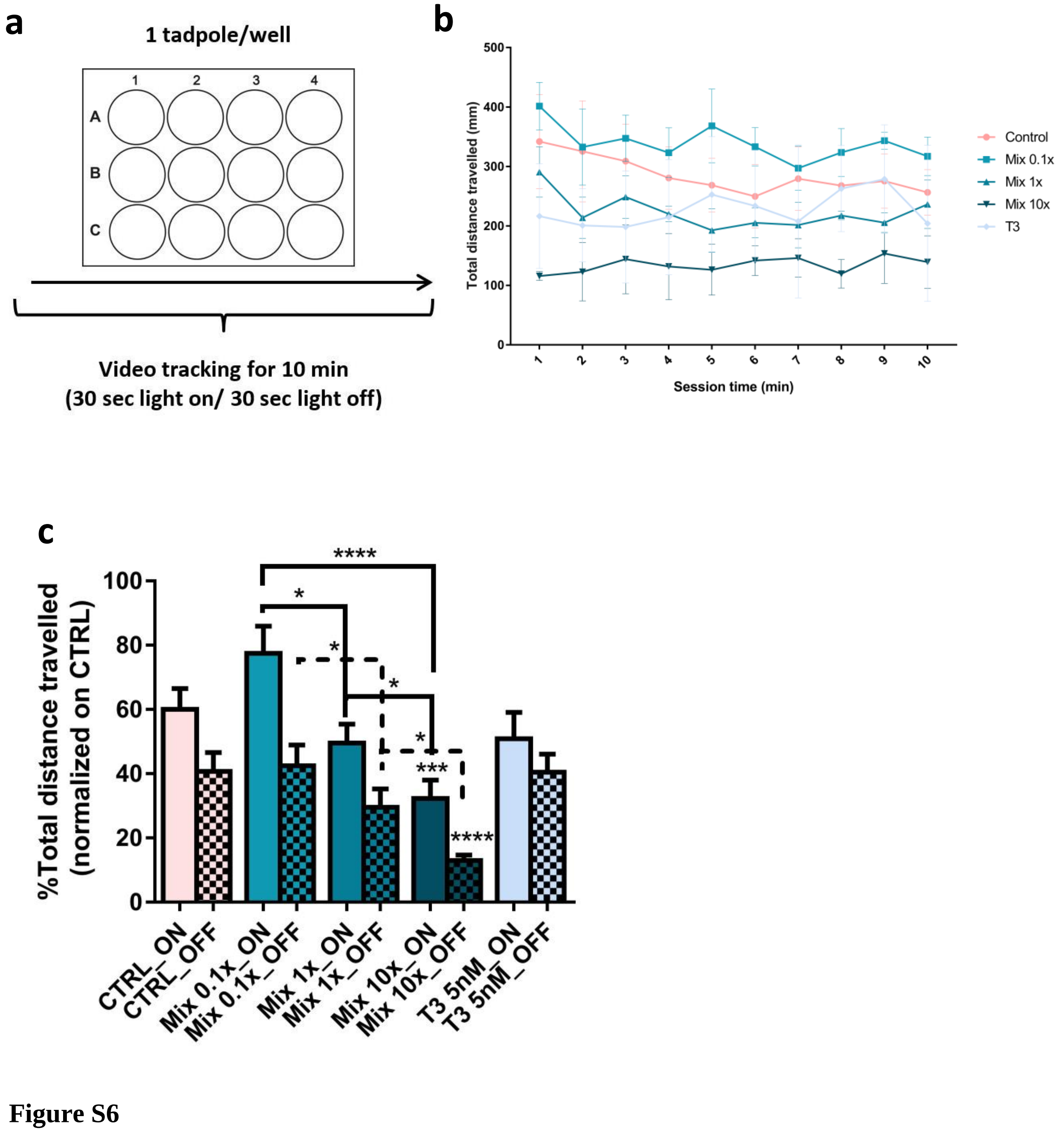

a
1 tadpole/well
1
2
3
4
A
B
C
Video tracking for 10 min
(30 sec light on/ 30 sec light off)
b
Total distance travelled (mm)
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
Session time (min)
Control
Mix 0.1x
Mix 1x
Mix 10x
T3
c
%Total distance travelled
(normalized on CTRL)
100
80
60
40
20
0
****
*
*
*
*
***
****
CTRL_ON
CTRL_OFF
Mix 0.1x_ON
Mix 0.1x_OFF
Mix 1x_ON
Mix 1x_OFF
Mix 10x_ON
Mix 10x_OFF
T3 5nM_ON
T3 5nM_OFF

**Figure S6**

| Gene name | Forward Sequence (5' - 3') | Reverse Sequence (5' - 3') | Gene Accession/Xenbase id |
|---|---|---|---|
| | | | |
| **dio1** | 5'-CAGCAGATGAATGGGCATTGA-3' | 5'-TGTCTAACACTACTGGGCAAGAAGGT-3' | |
| NM_001095667.1 | | | NM_001095667.1 |
| Long Copy | | | XB-GENE-979948 |
| Short copy | | | XB-GENE-17345940 |
| | | | |
| **dio2** | 5'-CAAATCCAGCCAGAGAGTCAATGG-3' | 5'-TGACCTGCTTGTAGGCATCCA-3' | |
| AF354707.1 | | | AF354707.1 |
| Long Copy | | | XB-GENE-1017482 |
| Short copy | | | XB-GENE-17339189 |
| | | | |
| **dio3** | 5'-CACAAAAAGTGCGACCAAACG-3' | 5'-GCCTTGTTGCAGTTTACT-3' | |
| NM_001087863.1 | | | NM_001087863.1 |
| Long Copy | | | XB-GENE-865707 |
| Short Copy | | | XB-GENE-17336015 |
| | | | |
| **thra** | 5'-CGCCTTGGTCTCTTCGGAT-3' | 5'-CCAGAAGTGGGGAATGTTGTGTT-3' | |
| NM_001088126.1 | | | NM_001088126.1 |
| Long Copy | | | XB-GENE-5966552 |
| Short Copy | | | XB-GENE-17340389 |
| | | | |
| **thrb** | 5'-AAGAGTGGTTGATTTTGCCAAAA-3' | 5'-AGGGACATGATCTCCATACAACAG-3' | |
| NM_001097064.1 | | | NM_001097064.1 |
| Long Copy | | | XB-GENE-6070716 |
| Short Copy | | | XB-GENE-6252616 |
| | | | |
| sox2 | 5'-CCAGTCCACCTGTAGTCACCTCT-3' | 5'-CACTTCTGCCCCCAGGTAGGTAC-3' | |
| NM_001088222.1 | | | NM_001088222.1 |
| AF022928.1 | | | AF022928.1 |
| Long Copy | | | XB-GENE-865099 |
| Short Copy | | | XB-GENE-17341038 |
| | | | |
| tubb2b (ntub) | 5'-ACACGGCATTGATCCTACAG-3' | 5'-AGCTCCTTCGGTGTAATGAC-3' | |
| NM_001086064.1 | | | NM_001086064.1 |
| Long Copy | | | XB-GENE-866032 |
| Short Copy | | | XB-GENE-17340462 |

| | | | |
|---|---|---|---|
| mpb | 5'-GATCAAAGCAAATGGCAACA-3' | 5'-AACCTGCCCAGTGAGTCAAG-3' | |
| NM_001090291.1 | | | NM_001090291.1 |
| Long Copy | | | XB-GENE-959622 |
| Short Copy | | | XB-GENE-17333093 |
| | | | |
| bdnf | 5'-CCCCCATGAAAGAAGCCAGT-3' | 5'-ACTGGGACCACCTATGCTCT-3' | |
| NM_001085482.1 | | | NM_001085482.1 |
| Long Copy | | | XB-GENE-6252642 |
| Short Copy | | | XB-GENE-955559 |
| | | | |
| thibz | 5'-ACCTCCACAGAATCAGCAGC-3' | 5'-GCAGAGAACGAGCAAGGAGT-3' | |
| NM_001085805.1 | | | NM_001085805.1 |
| Long Copy | | | XB-GENE-6252652 |
| Short Copy | | | XB-GENE-865112 |
| | | | |
| klf9 | 5'-TGTGGCAAAGTTTATGGGAAGTCT-3' | 5'-GGCGTTCACCTGTATGGACTCT-3' | |
| NM_001085597.1 | | | NM_001085597.1 |
| U35409.1 | | | U35409.1 |
| Long Copy | | | XB-GENE-865026 |
| Short Copy | | | XB-GENE-6252653 |
| | | | |
| mecp2 | 5'-CCATGTACGAAGACCCCACTCT-3' | 5'-GACCAGATTTTCTTTGCTTGAGTTT-3' | |
| NM_001088385.1 | | | NM_001088385.1 |
| Long Copy | | | XB-GENE-17346751 |
| Short Copy | | | XB-GENE-494744 |
| | | | |
| cntn4 | 5'-CAGAGCAAAGGACGCCTACA-3' | 5'-AGCACTGATACATGCCGGAG-3' | |
| NM_001091581.1 | | | NM_001091581.1 |
| Long Copy | | | XB-GENE-17337426 |
| Short Copy | | | XB-GENE-866211 |
| | | | |
| dcx | 5'-ACACCCAAATCCAAGCAGTC-3' | 5'-TCAGAGTCATCCAGAGACAGG-3' | |
| No NCBI | | | |
| Long Copy | | | XB-GENE-17344744 |
| Short Copy | | | XB-GENE-17344745 |

| | | | |
|---|---|---|---|
| dnmt3a | 5'-CCAGAAAGGTTTTCCCATCA-3' | 5'-CGATCATCTGCTTGTTCTGG-3' | |
| No NCBI | | | |
| Long Copy | | | XB-GENE-6488573 |
| Short Copy | | | XB-GENE-17330625 |
| | | | |
| reelin | 5'-TTGTGAATATTCTTTTGCCTGCTAGT-3' | 5'-GCCACCAGCGGAAACG-3' | |
| AF427525.1 | | | AF427525.1 |
| Long Copy | | | XB-GENE-982846 |
| Short Copy | | | XB-GENE-17331849 |
| | | | |
| parvalbumin | 5'-AGCCGCTGACTCATTTAACC-3' | 5'-ACTTCGATCCTGGTCGAGAA-3' | Sequence in the box |
| No NCBI | | | |
| Long Copy | | | Xelaev18023736m |
| Short Copy | | | Xelaev18025727m |
| | | | |
| nestin | 5'-GAGGTGGCCACATACAGGTC-3' | 5'-TGCAGTTCATTTTTGTCACCA-3' | |
| NM_001087857.1 | | | NM_001087857.1 |
| Long Copy | | | XB-GENE-17340193 |
| Short Copy | | | XB-GENE-865974 |
| | | | |
| Rora | 5'-TGTAAGGGTTTCTTCCGACGAA-3' | 5'-TCTGGCGTGGACATGAGTAAGT-3' | Sequence in the box |
| No NCBI | | | |
| Long Copy | | | XB-GENE-17343726 |
| Short Copy | | | XB-GENE-17343727 |
| | | | |
| pcna | 5'-CGTCGCGGTAATCCCTTACA-3' | 5'-CCAACACCTTCTTCAGGATGGA-3' | |
| NM_001087542.1 | | | NM_001087542.1 |
| Long Copy | | | XB-GENE-972527 |
| Short Copy | | | XB-GENE-17342178 |
| | | | |
| olig4 | 5'-GCGGGAAGTGATGCCATACT-3' | 5'-TGGAAATTTTGGAGAGCTTTCG-3' | |
| No NCBI | | | |
| Long Copy | | | XB-GENE-6488436 |
| Short Copy | | | XB-GENE-17335240 |

| | | | |
|---|---|---|---|
| mct8 | 5'-CATTGCAGGCCTCTTACGTG-3' | 5'-TCTCTCATGGACCAGCGGTA-3' | |
| XM_018232946.1 | | | XM_018232946.1 |
| Long Copy | | | XB-GENE-17342332 |
| Short Copy | | | XB-GENE-17342333 |
| oatp1c1 | 5'-GTCCGTACTGCTGCTCGATGT-3' | 5'-TGGGAGCGTCTTCCAGTACAA-3' | |
| No NCBI | | | |
| Long Copy | | | XB-GENE-17343436 |
| Short Copy | | | XB-GENE-17343437 |
| mct10 | 5'-TTGGTCCACCTATTGCAGGTATT-3' | 5'-TGGATCCATGGTATCAGGCATA-3' | |
| No NCBI | | | |
| Long Copy | | | XB-GENE-6487093 |
| Short Copy | | | XB-GENE-17345674 |
| lat1 | 5'-GGCCTATCAAGGTAAACATTTTGC-3' | 5'-CACACTCAACAGGTGTCATGTAGAAT-3' | |
| NM_001090065.1 | | | NM_001090065.1/NM_001096373.1 |
| Long Copy | | | XB-GENE-6254908 |
| Short Copy | | | XB-GENE-865334 |

| | Family/ complet name | Molecule (abbreviation)_CAS n° | Use | DETECTION RATE in pregnant women (non pregnant) WOODRUFF 2011 | Tolerable daily intake | Concentrations measured in urine during pregnancy | Concentrations measured in human serum | Median concentration found in umbilical cord blood serum | Median concentration measured in amniotic fluid | Molecular Weight | Molarity/ mass | Final Concentration in the mixture 0.1x | Final concentration in the mixture 1X | Final concentraion in the mixture 10X | Mixture mother solution concentrations (100 000x in DMSO) | Volume of each compound at 10-1M concentration (µl) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Environmental phenols | Bisphenol A (BPA)_80-05-7 | plasticizer | 96% (96%) | 4 µg/kg bw/day | 1.63 µg/L (Woodruff et al., 2011)_1,36µg/l (Aris 2014)_0.4µg/L (Jiménez-Díaz et al, 2016) | 0,49+/-2,47 ng/ml (Burstyn et al, 2013) (pregnant mother)_7.43 µg/L (Shekhar et al, 2016) | 1,23 ng/ml (Aris 2014)_0,16ng/ml (Gerona et al 2013) | [0-0,46] (Eldow et al;, 2012)_second trimester: [nd-0.75ng/ml) median 0.47 ng/ml (Chen et al;, 2011)_ ND (Philippat et al;, 2013)_ 7.75 µg/L (Shekhar et al, 2016) | 228.29 g/mol | 0.2 10-8M ( 0.5 µg/L | 0.2 10-9M | 0.2 10-8M | 0.2 10-7M | 0.2 10-3M | 2 |
| 2 | Environmental phenols | Triclosan (TCS), dichlorophenoxyphenol _3380-34-5 | antibacterian concervator (cosmetic) | 87%(81%) | Substances for which an Acceptable Daily Intake or Tolerable Daily Intake could not be established, but where the present use could be accepted.5 mg/kg | 23.81µg/l (Woodruff et al., 2011) | | | 19.4µg/l (amniotioc high quartile) Philippat et al., 2013_7.04 µg/L (Shekhar et al, 2016) | 289.54 g/mol | 0.7 10-7M (19.4µg/l) | 0.7 10-8M | 0.7 10-7M | 0.7 10-6M | 0.7 10-2M | 70 |
| 3 | Environmental phenols | benzophenone-3 (BP-3), oxybenzone_131-57-7 | UV filter (cosmetics)_ | 100%(98%) | 0.03mg/kg b.w./day | 38.09 µg/l (Woodruff et al., 2011) _4.7µg/L Median/ Max : 3200µg/L (Hines et al, 2015)__77µg/L Median (Phillipat et al, 2013) | | | 0-15.7µg/l (amniotic) median 0,8 µg/l Philippat et al., 2013 | 182.22 g/mol | 0.86 10-7M (15.7 µg/l) | 0.9 10-8M | 0.86 10-7M | 0.86 10-6M | 0.86 10-2M | 86 |
| 8 | Phthalates | Dibutyl phthalate (DBP)_ 84-74-2 | softener (plastic, shampoo) | 99% (99% )_MBP | 0.01 mg/kg body weight per day | 18.83 µg/l (geometric mean in pregnant women 50th percentile 17.1 µg/l 95th percentile 143.8 µg/l (Woodruff et al;, 2011) data for MBP _____metabolite MBP in mother urine 1st trimester [8,9;26,9;78;30,9] max 541 mg/l (females group) _ [19,4;28,1;79,6;232,6] max=524 mg/l (males group) Huang e al *; 2009 | | 68.14 µg/l (cord blood) (Huang et al. 2014)__cord blood serum 6µg/l ( Zhang et al., 2009)._ Meconium 6mg/g (Zhang et al., 2009) | around 80µg/ of MBP in amniotic fluid (50th percentile male et females) [39.3;45.6;85.5;134.6] max =192 µg/l (female groupe for MBP) Huang et al 2009 [28,4;44,3;81,3;127,8] max=145 µ/L ml male group Huang et al 2009 | 278.34 g/mol MBP 222.24g/mol | 0.24 10-6M (53.4 µg/l) | 0.24 10-7M | 0.24 10-6M | 0.24 10-5M | 0.24 10-1M | 240 |
| 7 | Phthalates | di-2-éthylhexylphthalate (DEHP)_117-81-7 | plastic softener | 100% (100%)_MEP | 0.05 mg/kg body weight per day | 226.53 µg/l (geometric mean) (Woodrufff at al 2011) | | 187.16 µg/l (cord blood ) Huang et al., 2014__cord blood serum 1µg/l ( Zhang et al., 2009)_ Meconium 3mg/g (Zhang et al., 2009)_mothermilk 10µg/l (Main et al 2006) | DEHP metabolite at 0.27µg/l (median Jensen et al 2012)_ 22.1 µg/l 50 percentile (amniotic fluid MEHP)[0;5;24;91,1] (max 148µg/l ) for females and [0;2,6;22,1;100,6] max 110 µg/l males (amnitoic fluid MEHP) Huang et al., 2009_1.7-2.6 µg/l Silva et al. 2004 (metab)__ | 390.56 g/mol MEHP: 278.15 | 0.08 10-6M (22.1 µg/l) | 0.1 10-7M | 0.1 10-6M | 0.1 10-5M | 0.11 10-1M | 110 |
| 5 | Organochlorine pesticides | Hexachlorobenzen (HCB)_118-74-1 | fungicide, pesticide and precursor of synthetic caoutchouc | 100% (99%) | 0.16 mg/kg body weight per day | | 13.74 ng/g lipid (Woodruff et al., 2011) (pregnant mother) | | 0.023µg/l (median levels ) or 0.097µg/l (mean) in amniotic fluids 100 women tenerife ( Luzardo et al, 2009)__0.24 ng/l (Germany Van der Ven et al. 1992)__0.02 ng/l (mean in Tanzania Van der Ven et al. 1992)__0.032 µg/l (mean in Meeker et al 2009)__6.6µg/l (mean in Daglioglu et al. 2013) | 284.78 g/mol | 0.8 10-11M (0.0023µg/l) | 0.8 10-12M | 0.8 10-11M | 0.8 10-10M | 0.8 10-6M | 0.8 |
| 4 | Organochlorine pesticides | Dichlorodiphenyldichloroethylene (4-4' DDE, p-p'DDE metabolite DDT), _72-55-9 | insecticide (DDT metabolite) | 100% (99%) | PTDI of 0.01 mg/kg b.w (provisional TDI) | | 198.34 ng/g lipid (Woodruff et al., 2011) (pregnant mother) | | 0.21 µg/l mean (Foster et al., 2000)__ 0.030µg/l (mean in Luzardo et al. 2009)__0.384µg/l (mean Meeker at l 2009)__5.4µg/l mean (Daglioglu et al. 2013) | 318.02 g/mol | 0.66 10-9M (0.21 µg/l) | 0.7 10-10M | 0.66 10-9M | 0.66 10-8M | 0.66 10-4M | 0.66 |
| 13 | Perfluorinated compounds | Perfluorooctanoic acid (PFOA)_ 335-67-1 | fluorosurfactant | 99% (99%) | 150 ng/kg b.w/day | | 50th percentile: 2.6µg/l; 95th percentile 5.6µg/l (Woodruff et al., 2011) _[0.309-7.31] median 1.045 µg/l (Cariou et al., 2015) (pregnant mother) | [0.311-7.06] µg/l median 0.860 µg/l (Cariou et al. 2015) | 0.1-1.8 µg/l mean 0.3µg/l (amniotic fluid) Stei et al., 2012 | 414.07 g/mol | 0.43 10-8M (1.8µg/l) | 0.43 10-9M | 0.43 10-8M | 0.43 10-7M | 0.43 10-3M | 4.3 |
| 12 | Perfluorinated compounds | Perfluorrooctanyl sulfonate (PFOS)_ 1763-23-1 | fluorosurfactant | 99% (100%) | 0.3 µg/kg bw/day | | 50th percentile: 12µg/l; 95th percentile 21.8µg/l (Woodruff et al., 2011) __[0.316-24..5] median 3.065 µg/l (Cariou et al; 2015) (pregnant mother) | [nd-8.04]µg/l median 1.115µg/l (Cariou et al., 2015) | 1.1-4.5 µg/L (amniotic fluid) Jensen et al;, 2012 | 538.23 g/mol | 0.8 10-8M (4.5µg/l) | 0.8 10-9M | 0.8 10-8M | 0.8 10-7M | 0.8 10-3M | 8 |
| 11 | Poly aromaric Hydroxylated compounds (PAHs) | 2-Napthol_ 135-19-3 | fluorescent colorless crystallin solid (naphtalen derivative) | 100%(100%) | none | 2.49µg/l in pregnant women (geometric mean) (50th percentile: 2.4µg/l; 95th percentile 14.7µg/l (Woodruff et al., 2011) | | | 0.72µg/L median (amniotic fluid) Bradman et al;, 2003 | 144.17 g/mol | 0.5 10-8M (0.72µg/l) | 0.5 10-9M | 0.5 10-8M | 0.5 10-7M | 0.5 10-3M | 5 |
| 6 | Poly chlorinated | Sodium perchlorate Monohydrate (NaClO4-)_ 7791-07-3 | explosive properties used in aeronautics | 100%(100%) | Perchlorate : 0.3 µg/kg b.w | 4.3 µg/l (50th percentile:; 95th percentile 34µg/l (Woodruff et al., 2011) 3.35µg/L (maternal urine), | [-; 0,893] mean 0,417 µg/l (Blount et al., 2009) (pregnant mother) | [-; 0,480] mean 0,246 µg/l (Blount et al., 2009) _mean 0,440 µg/l (Amitai et al., 2007) | 0.057–0.38 µg/l (amniotic fluid) Blount et al., 2009 | 122.44 g/mol | 0.3 10-8M (0.38µg/l) | 0.3 10-9M | 0.3 10-8M | 0.310-7M | 0.3 10-3M | 7.5 µl (à partir de 1g/L |
| 9 | Polybrominated compounds (PBDEs) | Decabromodiphenylether (BDE 209 )_ 1163-19-5 | flame retardant | 87% (68%) | 1,7µg/kg b.w | 0.08–106.49µg/g creatinine (Ho & al, 2015) | 1.59 ng/g lipid weight (Ho & al, 2015) | 15.8 ± 9.88 ng/g– 1 lipid in placenta, 13.2 ± 7.64 ng/g– 1 lipid in breast milk, 16.5 ± 19.5 ng/g– 1 lipid in fetal cord blood, and 1.80 ± 1.99 ng/ml– 1 in neonatal urine. (Chen & al, 2014) | 3795 pg/ml (all PBDE) 16% de BDE209 so: 607.2pg/ml ou ng/L Miller et al 2012 | 959.22 g/mol | 0.63 10-9M (607.2 ng/l) | 0.63 10-10M | 0.63 10-9M | 0.63 10-8M | 0.63 10-4M | 0.63 |
| 10 | Polychlorinated compounds (PCBs) | 2,2',4,4',5,5'-Hexachlorbiphenyl (PCB-153)_35065-27-1 | flame retardant found in Arochlor (PCB mixture) (banned) | 100% (100%) | 20 ng/kg b.w/day | | 50th percentile: 8.8µg/g lipid; 95th percentile 22.5 µg/g lipid (Woodruff et al., 2011) Danish mother : 1,303µg/l (median) or 152 ng/g lipid in serum (Kristensen et al, 2016) | 0.97 µg/l Portugal (cord blood) Lopes et al 2014 _0.04ng/ml (wet) cord blood 0.21µg/l German (Bucholski et al., 1996) 0.38µg/l Slovakia (Park et al., 2008) 0.21µg/l Spain (Grimalt et al., 2010) | 0.009µg/l (Luzardo & al, 2009) __0.064µg/l (Meeker et al., 2009)__0.16µg/l (in Germany Van der Ven et al., 1992__0.02µg/l (in Tanzania Van der Ven et al., 1992) __ 3.8µ/l (Daglioglu et al., 2013) | 360.88g/mol | 0.2 10-8M (0.72µg/) | 0.2 10-9M | 0.2 10-8M | 0.2 10-7M | 0.2 10-3M | 2 |
| 14 | Heavy metals | Methyl mercury (MeHg cL) _115-09-3 | | 89% (92%) | 1.3 µg/kg b.w/week (TWI not per day) 0.035 to 0.08µg/kg/day | | 63µg/L (Obiri et al, 2016) -Ghana | | 0.2 µg/ l max 11µg/l (amniotic fluid) mercury Kozikowska et al., 2013__0.37 µg/l (Pier Franca Luglie et a 2005) | 215.62 g/mol | 0.5 10-7M (11 µg/l) | 0.5 10-8M | 0.5 10-7M | 0.5 10-6M | 0.5 10-2M | 50 |
| 15 | Heavy metals | Lead (II) chloride (PbCl2)_ 7758-95-4 | pigments, fuels | 94% (99%) | ADI (acceptable daily intake) 7µg/kg/day | Median : 0.5µg/L (Christensen, 2012) | 1.290 µg/dL ±0.578 (Baranowska-Bosiack et al, 2016)( Pregnant mothers)<br><br>5th-95th percentile(Nunes et al, 2010) en µg/L<br>8.0–47.0 German<br>5.0–132.0 UK<br>39.7–275.7 Italian<br>11.4–62.8 French<br>3.5–170.0 Swedish<br>5.1–163.0 Brazil<br>58µg/L (Obiri&al, 2016) -Ghana | 30µg/l (Semczuk et al.,1998)__24µg/l (Senczuk et al 1994)__in breast milk : 0.174 µg/dL ±1.15 (Baranowska-Bosiack et al, 2016) | 59.6 µg/l Korpela et al., 1986 | 278.108 g/mol | 0.21 10-6M (59.6µg/l) | 0.21 10-9M | 0.21 10-8M | 0.21 10-7M | 0.21 10-3M | 2.1 |

# Supplementary References related to Supplementary Table 1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