Supplementary Information

Rapid *in vitro* metabolism of the flame retardant triphenyl phosphate and effects on cytotoxicity and mRNA expression in chicken embryonic hepatocytes

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Table S1. Pathways, RefSeq accession numbers and descriptions of 32 genes on the Avian ToxChip PCR array

Pathway	Gene Symbol	RefSeq Accession	Description
Phase I and II Metabolism	CYP3A37	NM_001001751	Cytochrome P450 A 37
	CYP1A4	NM_205147	Cytochrome P450 1A4
	UGT1A9	XM_001234353	UDP glucuronosyltransferase 1 family, polypeptide A9
	SULT1B1	NM_204545	Sulfotransferase family, cytosolic, 1B, member 1
	SULT1E1	NM_420616	Sulfotransferase family 1E, estrogen-preferring member1
Immune Function	BATF3	XM_419428	Basic leucine zipper transcription factor, ATF-like 3
	IL16	NM_204352	Interleukin 16 (lymphocyte chemoattractant factor)
	HSP90AB1	NM_206959	Heat shock 90kDa protein 1, beta
Glucose and fatty acid metabolism	PDK4	NM_001199909	Pyruvate dehydrogenase kinase, isozyme 4
Oxidative Stress	MT4	NM_205275	Metallothionein 4
	TXN	NM_205453	Thioredoxin
Lipid/Cholesterol Metabolism	ACSL5	NM_001031237	Acyl-CoA synthetase long-chain family member 5
	HMGCR	NM_204485	3-hydroxy-3-methylglutaryl-Coenzyme A reductase
	SLCO1A2	XM_416421	Solute carrier organic anion transporter family, member 1A2
	LBFABP	NM_204634	Fatty acid binding protein 1, liver
	CD36	NM_001030731	CD36 molecule (thrombospondin receptor)
	SCD	NM_204890	Stearoyl-CoA desaturase (delta-9-desaturase)
Thyroid Hormone Pathway	TTR	NM_205335	Transthyretin
	DIO1	NM_001097614	Deiodinase, iodothyronine, type I
	THRSP	NM_213577	Thyroid hormone responsive (SPOT14 homolog, rat)
	IGF1	NM_001004384	Insulin-like growth factor 1 (somatomedin C)
	NCOA3	XM_417385	Nuclear receptor coactivator 3
FXR and LXR	CYP7B1	XM_418276	Cytochrome P450, family 7, subfamily B, polypeptide 1
Cell Death	CASP1	NM_204924	Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)
	LOC100859733	XM_003641931	Cell death activator CIDE-3-like
Steatosis	HSD3B1	NM_205118	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1
Steroid	ALAS1	NM_001018012	Aminolevulinate, delta, synthase1

Metabolism			
	EEF1A1	NM_204157	Eukaryotic translation elongation factor 1 alpha 1
	RPL4	NM_001007479	Ribosomal protein L4
Controls	GGDC	SA_00517	Chicken Genomic DNA Contamination
	RTC	SA_00104	Reverse Transcription Control
	PPC	SA_00103	Positive PCR Control

Table S2. Instrumental parameters, selected reaction monitoring (SRM) mass-to-charge (m/z) transitions, performance evaluation results (instrumental linearity, precision (RSD %), and method limit of quantification (MLOQ) for triphenyl phosphate (TPHP) and diphenyl phosphate (DPHP) using the method based on liquid chromatography-electrospray ionization-triple quadrupole mass spectrometry (LC-ESI-MS/MS) operating in the positive mode.

Compound	Acronym	SRM ion transitions (*m/z*)	Cone voltage(V)	Collision energy (eV)	Linearity, R^2 (0.2-200 ng/mL)[a]	MLOQ (ng/mL, injection concentration)	Precision (RSD %) (20 ng/mL)	Matrix effects (%)[b]
Diphenyl phosphate	DPHP	507.3>243.3	67	28	0.9989	0.19	n/a	n/a
d_{10}-diphenyl phosphate	d_{10}-DPHP	517.4>243.3	42	28	n/a	n/a	5.5	94±4
Triphenyl phosphate	TPHP	327.1>77.1	100	40	0.9986	0.19	n/a	n/a
d_{15}-triphenyl phosphate	d_{15}-TPHP	342.2>82.0	100	40	n/a	n/a	4.2	94±5

[a] Six concentration points included 0.2, 1, 5, 20, 50 and 200 ng/mL, respectively.

[b] Minimal ESI(+) matrix effects were demonstrated by the ESI(+) response of 20 ng/mL for the TPHP and DPHP internal standards in treated medium/cell samples as compared to the pure internal standards, with values of 94 ± 4% and 94 ± 5% for d_{15}-TPHP and d_{10}-DPHP, respectively.

Table S3. Compound-dependent operation parameters and the precursor and daughter ions monitored using Waters ACQUITY UPLC® I-Class system coupled to Waters® Xevo™ TQ-S mass spectrometer (UPLC-TQ-S/MS) operated in multiple reaction monitoring (MRM) mode.

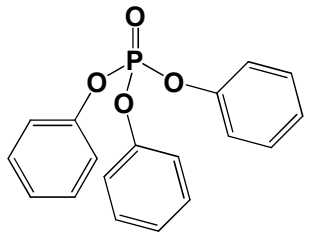
Compounds[a]	Precursor ion (*m/z*)	Daughter ion (*m/z*)	cone voltage (v)	collision energy (eV)
PHP	172.83	78.94	10	20
OH-TPHP	343.07	76.88	10	30
$(OH)_2$-TPHP	359.07	76.88	10	30

[a]The electrospray ionization (ESI) was operated in negative mode for PHP analysis and positive mode for OH-TPHP and $(OH)_2$-TPHP analysis.

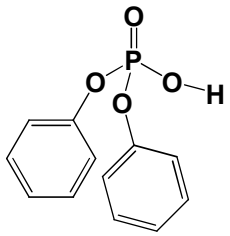
Table S4. Fold changes and *p*-values of 27 target genes on the Avian ToxChip PCR array following exposure of chicken embryonic hepatocytes to 10 μM TPHP or DPHP, respectively. The fold change represents the mean value of three replicates and significant differences in fold change compared to the DMSO vehicle control were determined using a Student's *t*-test. The numbers in red signify that the mRNA levels were significantly altered (fold change ≥ 1.5, $p<0.05$).

Symbol	TPHP		DPHP	
	p value	Fold Change	p value	Fold Change
CYP3A37	0.018	1.26	**0.001**	**-1.68**
CYP1A4	0.166	4.31	0.223	1.78
UGT1A9	0.081	1.46	0.236	1.26
SULT1B1	0.235	1.14	0.266	1.11
BATF3	0.221	1.27	0.868	1.02
PDK4	**0.002**	**-2.46**	**0.001**	**-2.54**
TXN	0.033	1.30	0.237	1.16
ACSL5	0.134	-1.33	**0.005**	**-2.05**
HMGCR	0.001	-1.48	**0.000**	**-2.11**
SLCO1A2	0.201	-1.12	**0.002**	**-1.59**
TTR	0.325	-1.11	0.025	-1.47
DIO1	0.629	-1.04	**0.009**	**-1.86**
THRSP	**0.000**	**-1.83**	**0.002**	**-1.65**
IGF1	**0.022**	**-1.63**	0.275	1.20
NCOA3	0.086	-1.28	0.273	-1.17
SULT1E1	0.002	1.30	0.630	1.02
CYP7B1	0.084	-1.31	**0.001**	**-3.05**
CASP1	0.002	-1.34	0.162	-1.08
LOC100859733	0.074	1.24	0.279	-1.13
HSD3B1	0.755	-1.05	0.749	1.05
ALAS1	0.020	1.24	0.196	1.11
IL16	0.878	-1.01	0.816	1.02
MT4	**0.003**	**2.08**	0.346	1.25
HSP90AB1	0.511	-1.08	0.713	-1.04
CD36	0.281	-1.68	**0.010**	**-2.35**
SCD	0.669	-1.04	0.621	-1.05
LBFABP	0.082	1.18	0.925	1.01

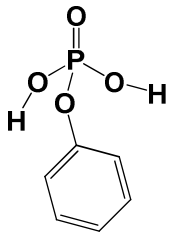
Figure S1. Chemical structures of triphenyl phosphate (TPHP), diphenyl phosphate (DPHP) and phenyl phosphate (PHP). The hydrogen atoms are omitted for clarity.



triphenyl phosphate



diphenyl phosphate



phenyl phosphate

Figure S2. Cytotoxic effects of diphenyl phosphate (DPHP) in chicken embryonic hepatocytes (CEH). CEH were treated with different concentrations (0.01, 0.1, 1, 10, 100 and 1000 μM) of DPHP for 36 h. One-way ANOVA was conducted to compare viability of each treatment group to the DMSO control; no significant differences were observed for any of the treatment groups. (p>0.05)

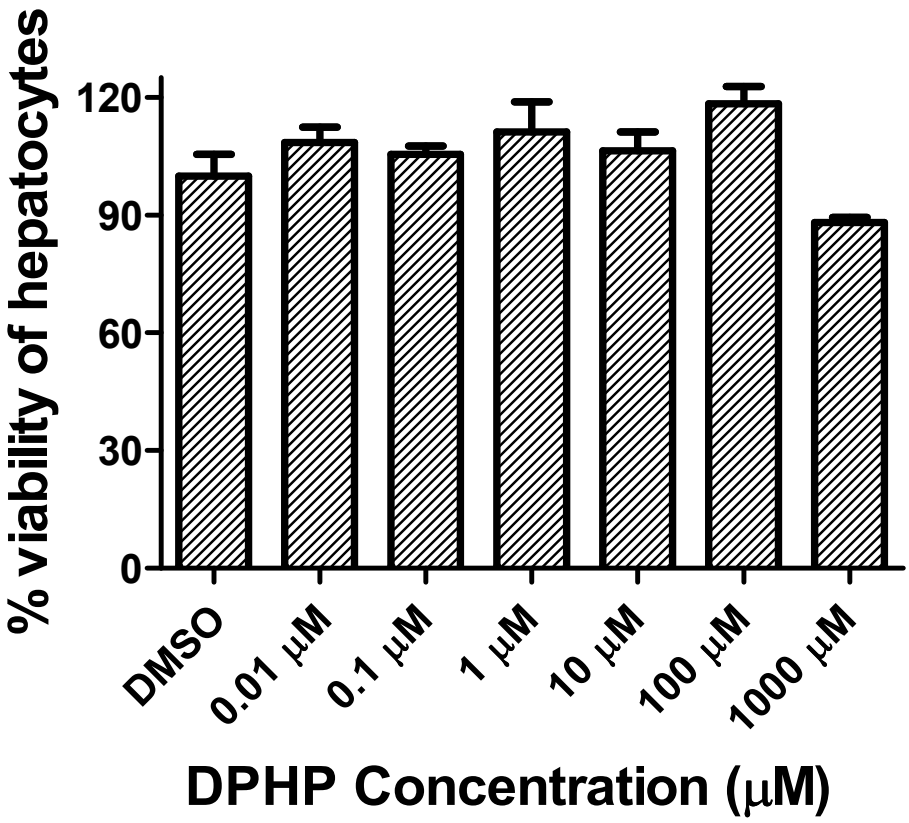


Figure S3. Total ion chromatogram of culture medium of triphenyl phosphate (TPHP)-exposed chicken embryonic hepatocytes using Waters ACQUITY UPLC® I-Class system coupled to Waters® Xevo™ TQ-S mass spectrometer (UPLC-TQ-S/MS) operated in daughters scan mode (daughter ions of m/z 343.07 (upper, A) and m/z 359.07 (lower, B and C), respectively). See the detailed daughter ions of peak A, B, and C in Figure S4.

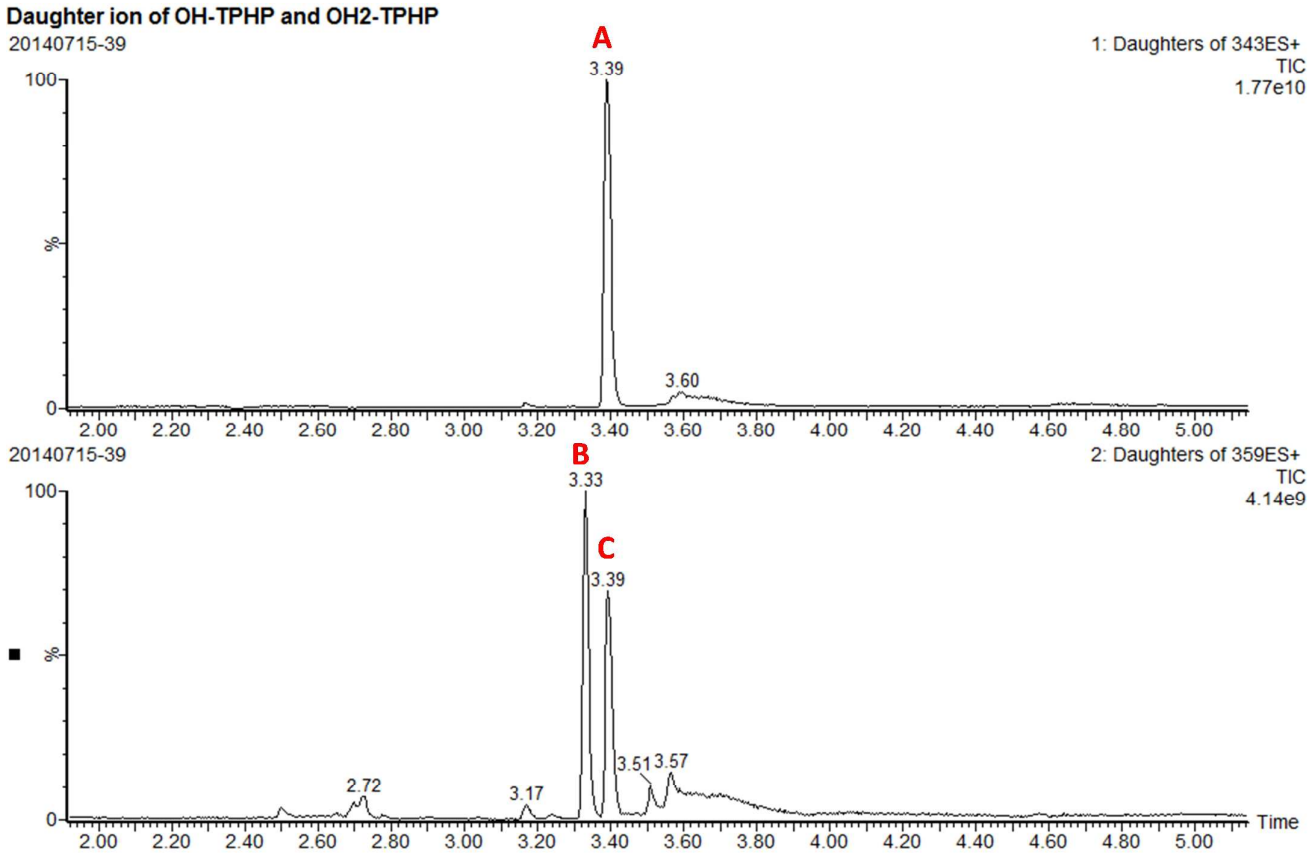


Figure S4. Daughter ions of m/z 343.07 (A) and m/z 359.07 (B and C) using Waters ACQUITY UPLC® I-Class system coupled to Waters® Xevo™ TQ-S mass spectrometer (UPLC-TQ-S/MS) operated in daughters scan mode. Daughter ion 'A' was assigned to be OH-TPHP; 'B' and 'C' were assigned to two $(OH)_2$-TPHP isomers. See the total ion chromatogram of peak A, B and C in the Figure S3.

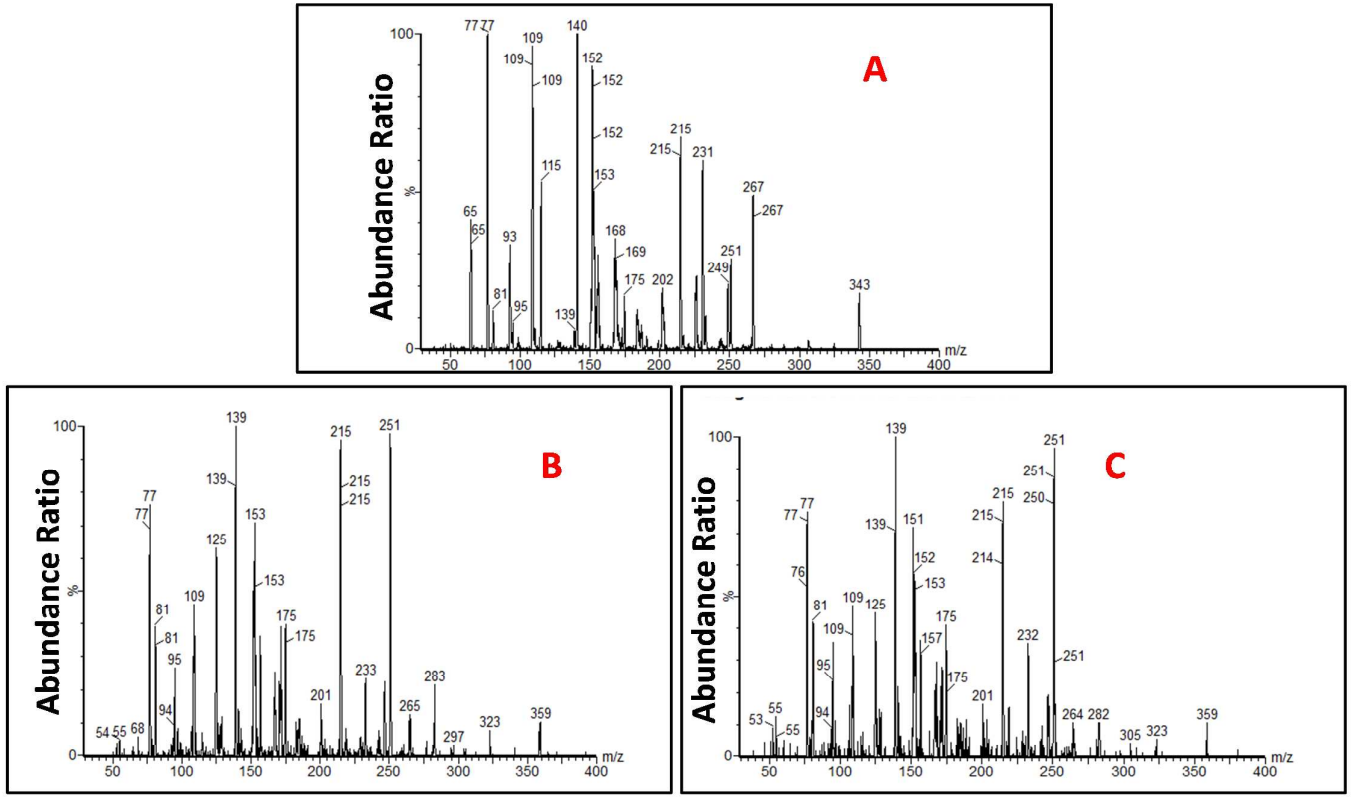


Figure S5. Response comparison between the initial TPHP concentration (retention time (RT) = 4.56) and formed OH-TPHP (RT = 3.37) in chicken embryonic hepatocyte (CEH) samples. This total ion chromatogram was generated by injecting 1:1 CEH medium (collected at 0 and 36 h, respectively) mixture into Waters ACQUITY UPLC® I-Class system coupled to a Waters® Xevo™ TQ-S mass spectrometer (UPLC-TQ-S/MS) operated in multiple reaction monitoring (MRM) mode.