Supporting Information

In Vitro Metabolism of the Flame Retardant Triphenyl Phosphate in Chicken Embryonic Hepatocytes and the Importance of the Hydroxylation Pathway

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Supporting Material I:
Synthesis of meta- and para-hydroxytriphenyl phosphates

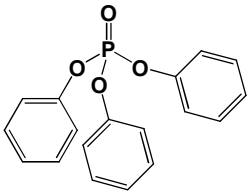
Meta- and *para*-hydroxytriphenyl phosphates were synthesized in house. Pyridine (730 µL, 9.1 mmol, 2.5 eq.) was added at once to a stirred suspension of diphenylphosphoryl chloride (750 µL, 3.6 mmol), and 1.0 g (9.1 mmol, 2.5 eq.) either benzene-1,4-diol (for parahydroxytriphenylphosphate) or benzene-1,3-diol (for metahydroxytriphenylphosphate) in anhydrous THF (1 mL) (Caution: fairly significant but brief exotherm!). The mixture was stirred overnight and partitioned between CH2Cl2 (15 mL) and brine (15 mL). The aqueous phase was extracted with CH2Cl2 (15 mL). The combined extracts were washed with brine (2 x 15 mL) and dried with Na_2SO_4. The drying agent was removed by filtration. Silica gel (~2 g) was added and the filtrate was concentrated to dryness under reduced pressure. Flash column chromatography (RediSepRf SiO_2 (24g), 100% CH_2Cl_2→ 30% EtOAc in CH2Cl2) gave the desired phosphate. Parahydroxytriphenylphosphate formed as a colorless solid (0.603 g, 49, and metahydroxytriphenylphosphate formed as a colorless oil that slowly crystallized upon standing at room temperature (0.800 g, 65%) EIMS m/z: 343 ([M+H]+).

Table S1. Selected reaction monitoring (SRM) ion transitions (*m/z*), cone voltage (V) and collision energy (eV) for diphenyl hydroxyphenyl phosphate (OH-TPHP) and diphenyl methoxyphenyl phosphate (MeO-TPHP) using a Waters ACQUITY UPLC® I-Class system (UPLC) coupled to Waters® Xevo™ TQ-S mass spectrometer (TQ-S/MS) operated in the electrospray ionization (ESI; positive) mode.

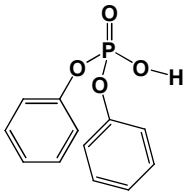
Compounds	SRM ion transitions (*m/z*)	Cone voltage(V)	Collision energy (eV)
Diphenyl hydroxyphenyl phosphates (OH-TPHPs)	343.07>76.88[a]	10	30
	343.07>141[b]	10	30
Diphenyl methoxyphenyl phosphates (MeO-TPHPs)	357.09>76.88	10	30

[a] quantifier transition; [b] qualifier transition.

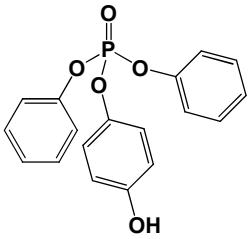
Figure S1. Chemical structures of triphenyl phosphate (TPHP) and the possible metabolites, and glucuronide and sulfate conjugates formed in chicken embryonic hepatocytes (The hydrogen atoms on the phenyl rings are omitted for clarity).



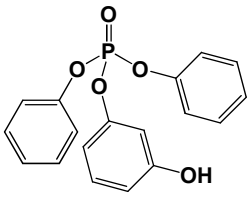
Triphenyl phosphate (TPHP)



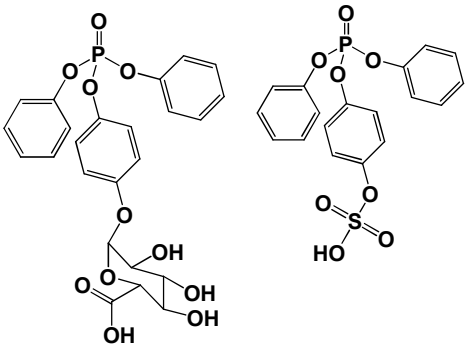
Diphenyl phosphate (DPHP)



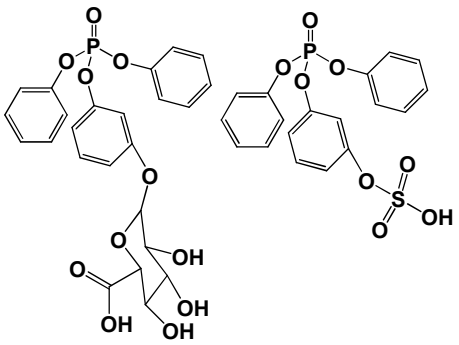
para-OH-TPHP



meta-OH-TPHP



para-OH-TPHP conjugates



meta-OH-TPHP conjugates

Figure S2. Daughter ions and retention times of the detected m/z 343.0730 ion in triphenyl phosphate (TPHP)-exposed chicken embryonic hepatocytes (A), *para*-OH-TPHP standard (B), and *meta*-OH-TPHP standard(C), using Waters ACQUITY UPLC® I-Class system coupled to Waters® Xevo™ TQ-S mass spectrometer (UPLC-ESI(+)-TQ-S/MS) operated in daughter scan mode.

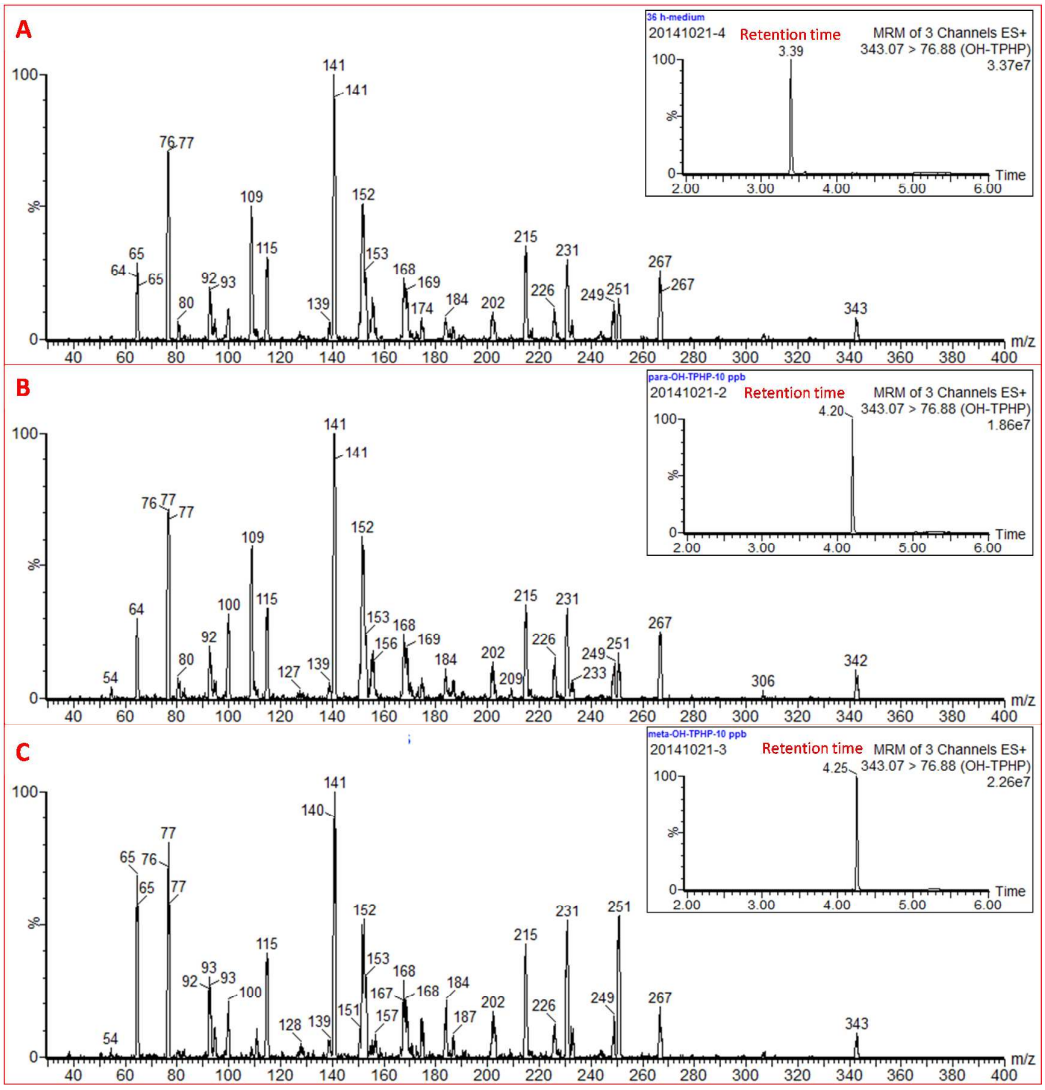


Figure S3. Retention times of *para*-OH-TPHP (A), *meta*-OH-TPHP (B) and the detected *m/z* 343.0730 ion in TPHP-exposed CEH medium (C) after methylation using diazomethane.

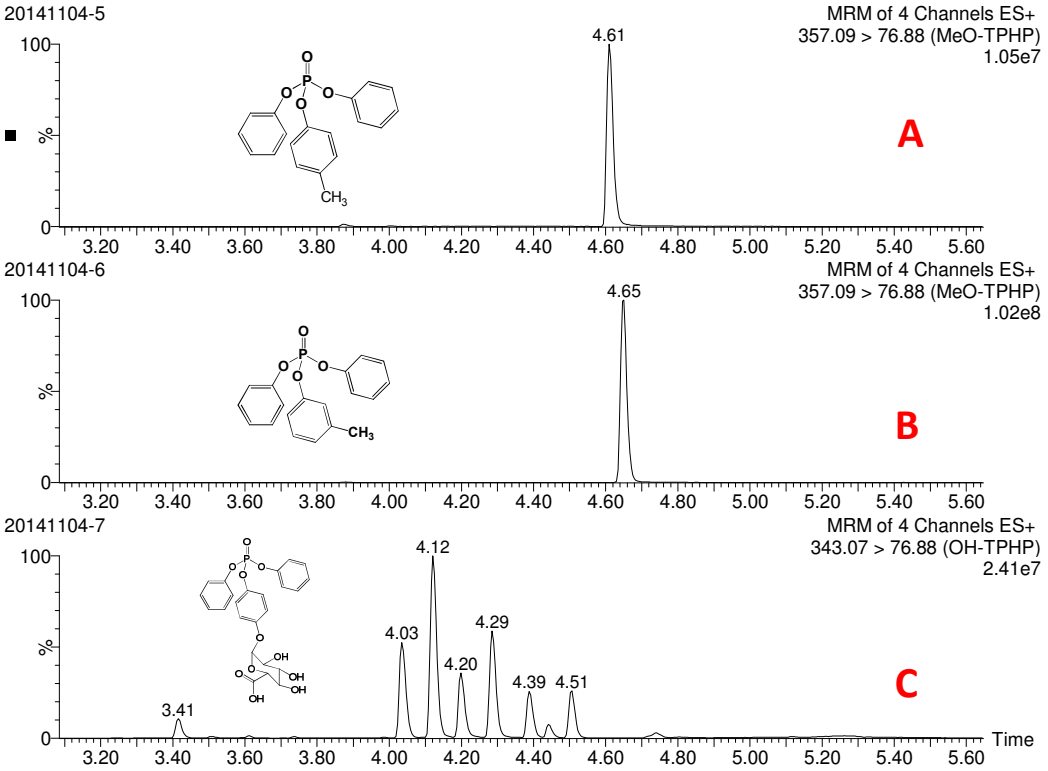


Figure S4. Time-dependent UPLC-ESI(+)--TQ-S/MS response of *para*-OH-TPHP conjugates in *para*-OH-TPHP-exposed chicken embryonic hepatocyte medium.