Electronic Supplementary Material (ESI) for Soft Matter.

Understanding interactions of plasticisers with a lipid monolayer

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Supplementary Information

Materials and Synthesis

Molecular structures and relevant properties of molecules used in the paper are presented in Figure 1 and Table 1. Abbreviations, the most commonly used name and its IUPAC name is included.

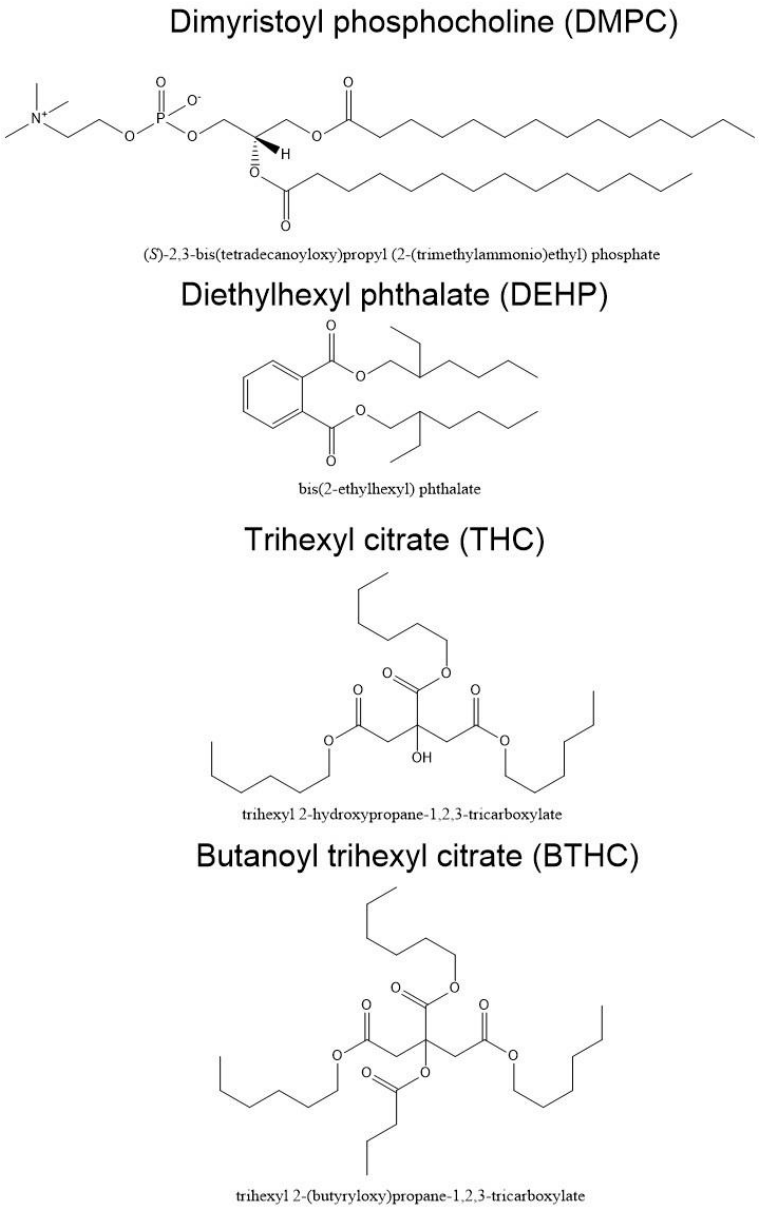

Figure S1. Molecular structures of DMPC, DEHP, THC and BTHC.

Table S1. Relevant abbreviations, data and properties of molecules used.

Compound	CAS-number	Molecular Formula	Molecular Formula	Molecular mass / g mol^{-1}	Molecular mass / g mol^{-1}	Neutron scattering length / fm	Neutron scattering length / fm
	Hydrogenous	Hydrogenous	Deuterated	Hydrogenous	Deuterated	Hydrogenous	Deuterated
DMPC	18,194-24-6	$C_{36}H_{72}NO_8P$	$C_{36}H_{18}D_{54}NO_8P$	677.5	731.8	31.0	593.1
DEHP	117-81-7	$C_{24}H_{38}O_4$	$C_{24}H_4D_{34}O_4$	390.6	424.6	40.6	394.6
BTHC	82,469-79-2	$C_{28}H_{50}O_8$	$C_{28}H_{11}D_{39}O_8$	514.7	553.7	74.0	451.5
BTHC mix	N/A	N/A	N/A	504.2	543.2	69.7	451.5

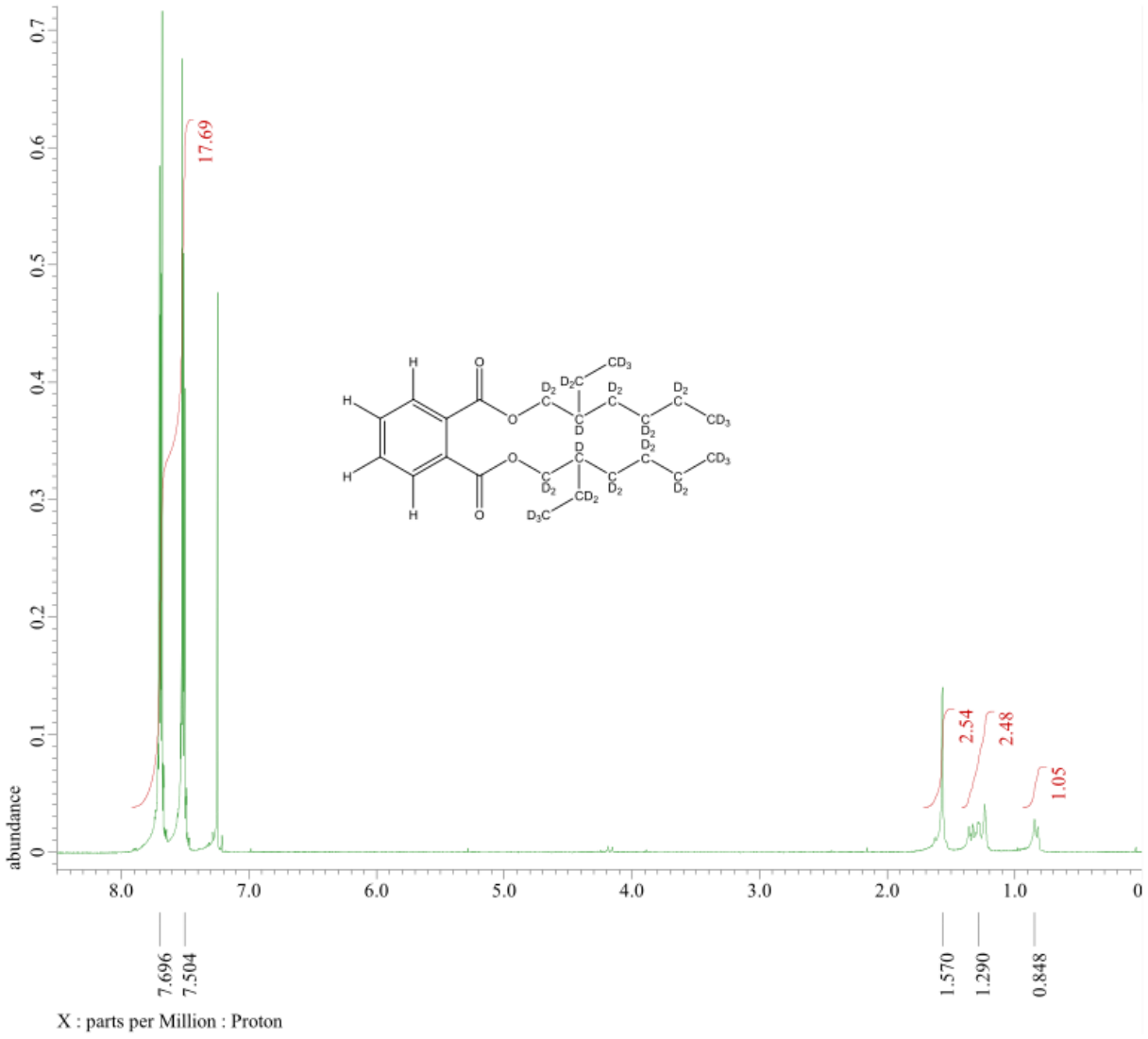

Figure. S2 NMR data from characterisation of deuterated DEHP.

The deuteration level can be calculated from the integrations of the peaks by normalising the integrals to the number of hydrogens each peak contains. There are 4 aromatic hydrogens (that cannot be deuterated) and 34 hydrogens that can be deuterated.

The normalised integral of the 4 aromatic hydrogens is

$$\frac{17.69}{4} = 5.689$$

While the normalised integral for the deuterated hydrogens is

$$\frac{2.54 + 2.48 + 1.05}{34} = 0.179$$

The deuteration level can then be calculated from these values as

$$\frac{5.689}{5.689 + 0.179} = 0.969 = 96.9\%$$

The deuteration level can then be assumed to be ≥96%.

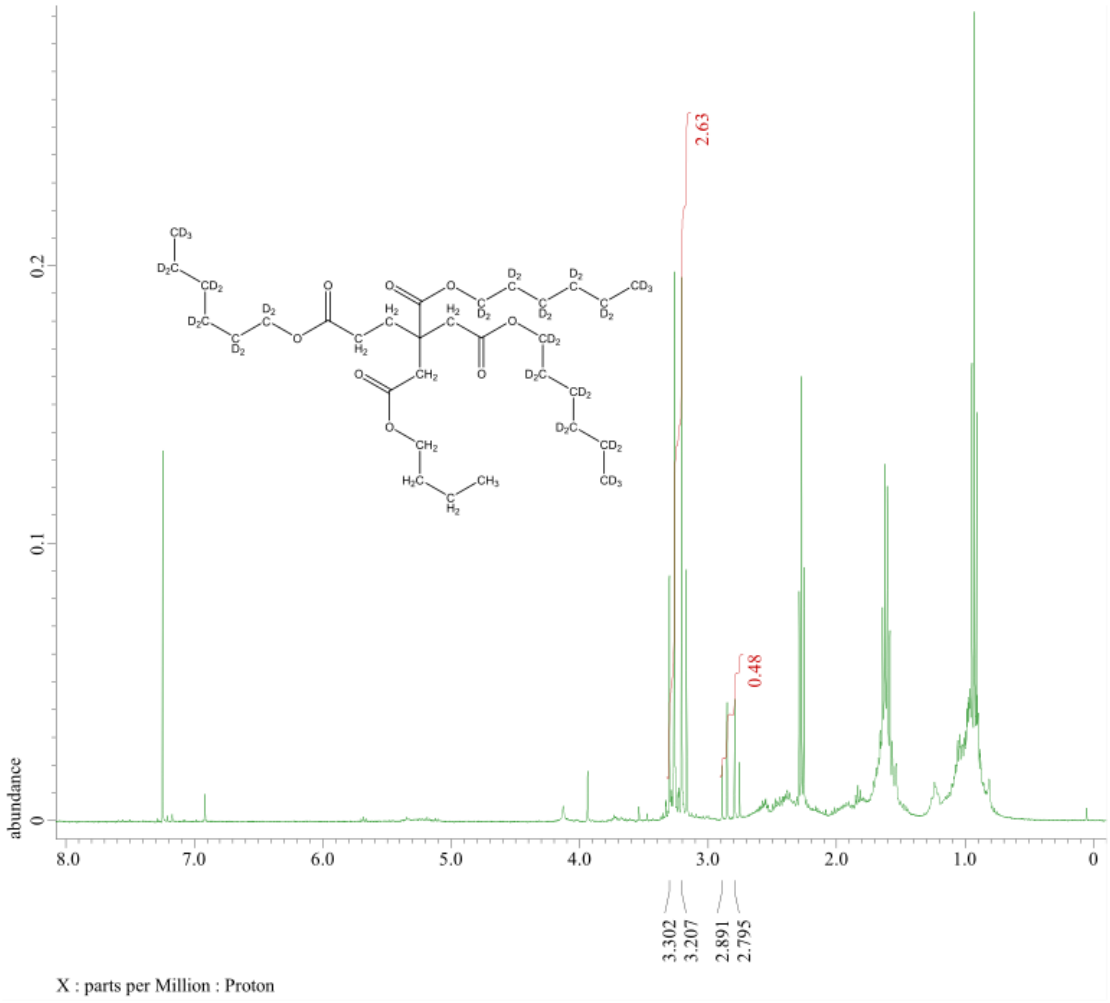

Figure S3. NMR data from characterisation of deuterated BTHC.

Ratio between butyryl functionalised hexyl citrate and the non-functionalised is calculated from the integrals of the two labelled NMR peaks.

$$\frac{0.48}{2.63 + 0.48} = 0.154$$

Sample contains ≈15% of the non-functionalised plasticiser. The level of deuteration cannot be estimated from the sample NMR. However, the deuterated hexanol used in the synthesis is specified to be ≥98% deuterated. The deuteration level is therefore expected to be within 2% of the assumed value.

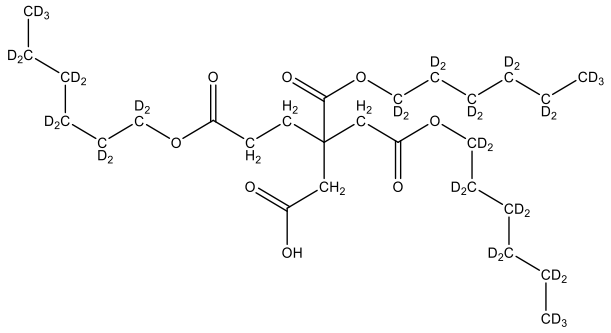

Figure S4. Structure of deuterated non-functionalised tri-hexylcitrate.

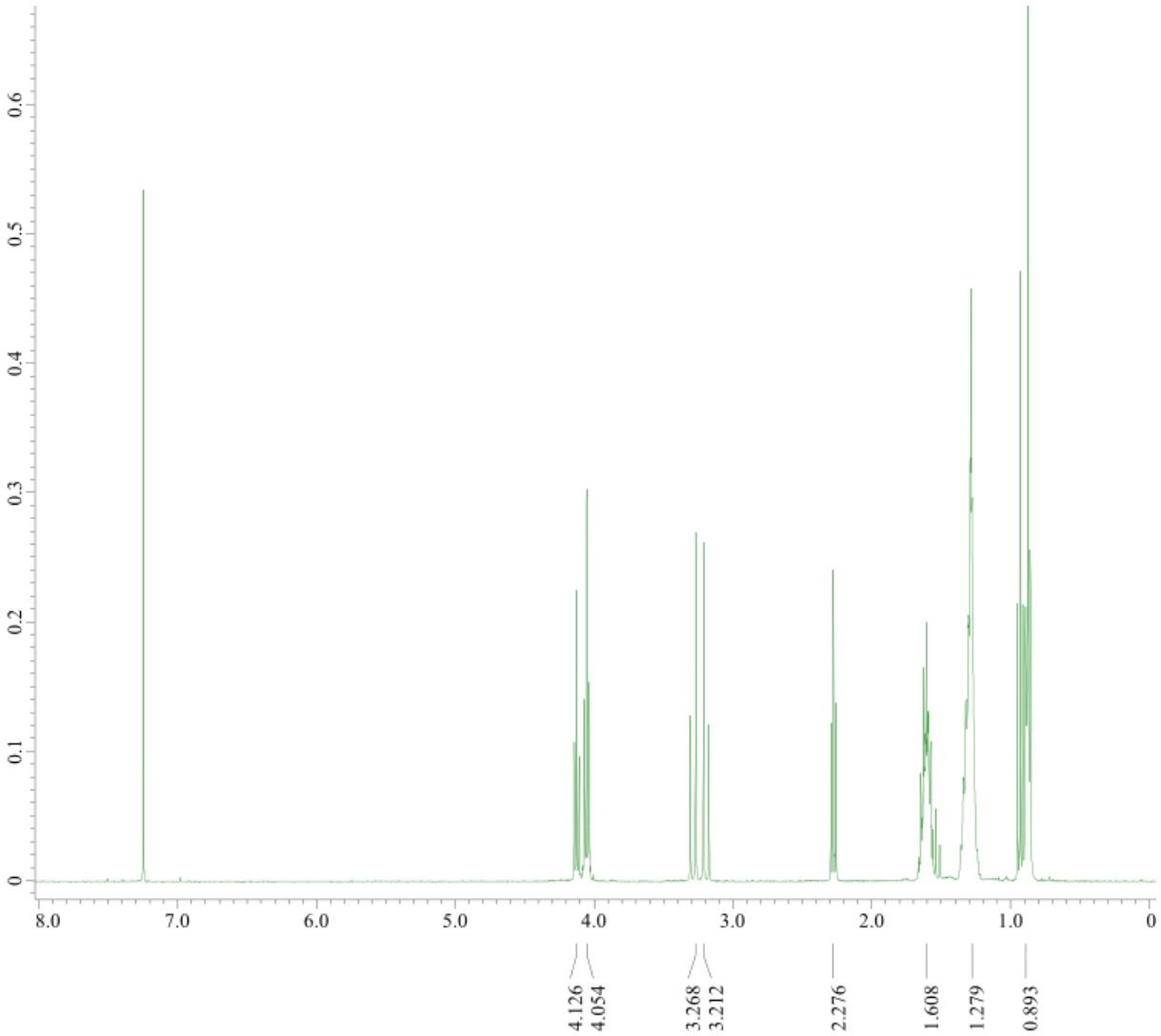

Figure S5. NMR data for hydrogenous BTHC as obtained from Vertellus.

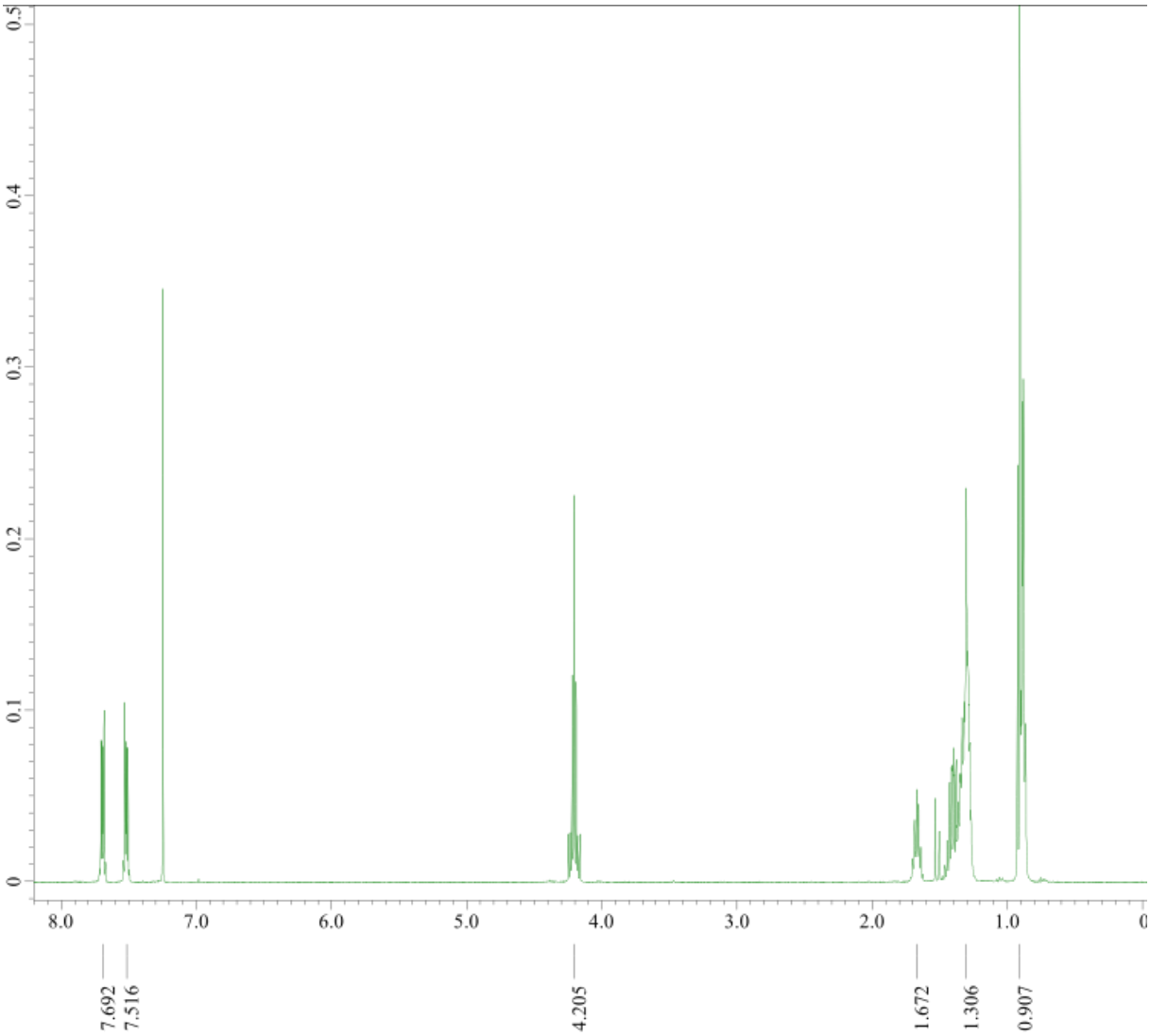

Figure S6. NMR data for hydrogenous DEHP as obtained from Sigma-Aldrich.

Modelling Neutron Reflectivity Data

It is advantageous to model data in terms of molecular composition and structure rather than simply in terms of scattering length density and thickness of arbitrary layers. This can incorporate readily constraints such as the different scattering lengths for deuterium labelled components. It is necessary to calculate the scattering length density from the composition for each sample in order to calculate the reflectivity when fitting multiple data sets for different contrasts simultaneously.

For a mixture of two molecules, the scattering length density for a layer, $\rho_{l,}$ is calculated as:

$$\rho_l = (b_1 \Gamma_1 + b_2 \Gamma_2) / t \qquad \text{Equation S1.}$$

where b_1 and b_2 are the scattering lengths (note: not the scattering length density) calculated from the isotopic composition of molecules 1 and 2 (Table S1) that have areal densities (number of molecules per unit area) Γ_1 and Γ_2 respectively. The thickness of the layer is *t*. In a fit with multiple contrasts, the values of Γ_1 and Γ_2 as well as *t* are constrained to be the same for each data set measured at a given surface pressure and are the parameters that are optimised in a least-squares fit procedure, while b_1 and b_2 are set to known values corresponding to the isotopic composition.

Additional neutron data and fits

Monolayer thicknesses as determined by fitting neutron reflectivity data for all the relevant data sets are shown here. Fits of all reflection measurements and parameters for the fits are also included. No significant trends are observed for the thicknesses. Data are included for completeness.

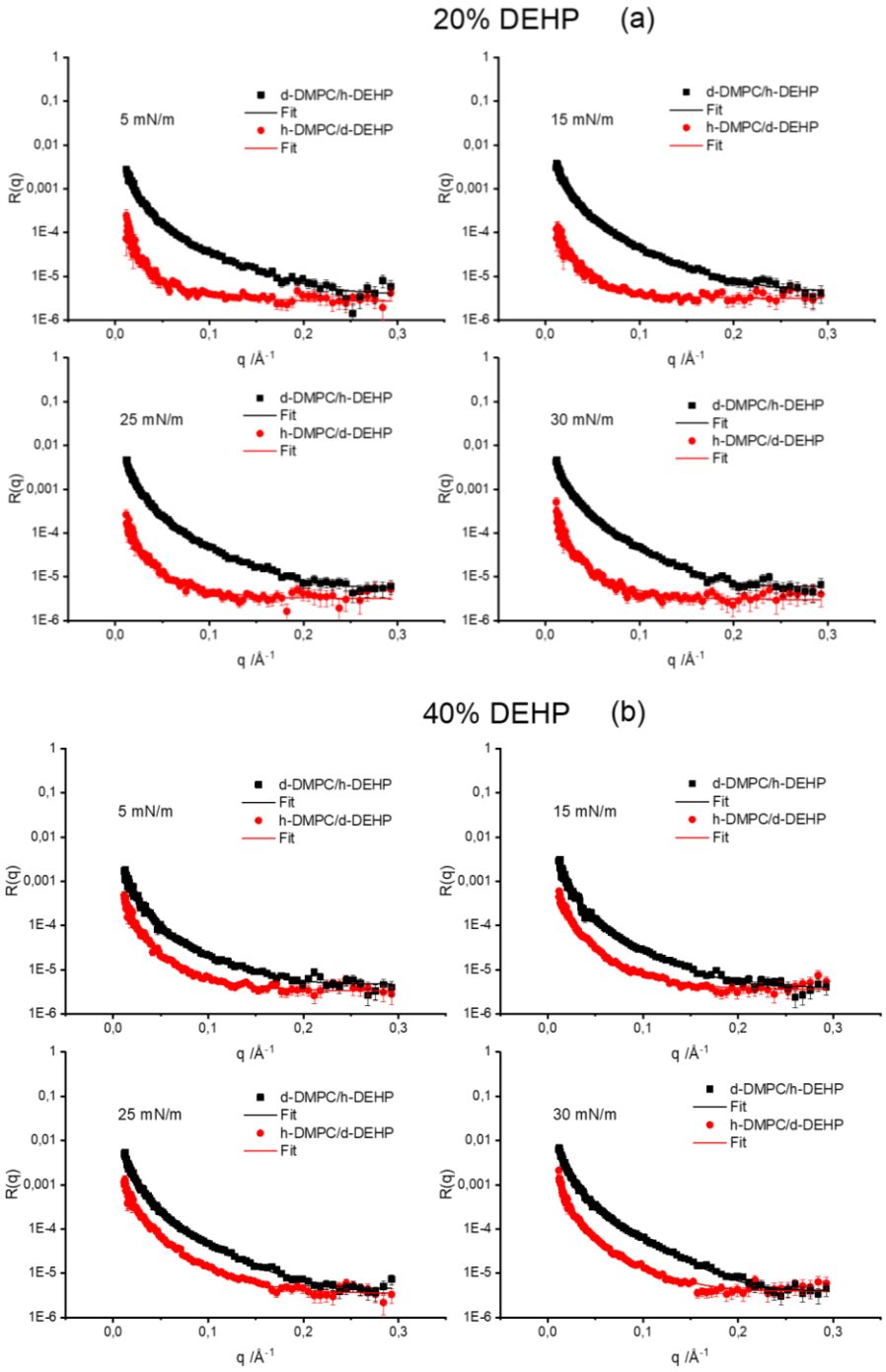

Figure S7. Reflectivity data and associated fits for monolayers containing a nominal and initial concentration of a) 20% and b) 40% DEHP by mol.

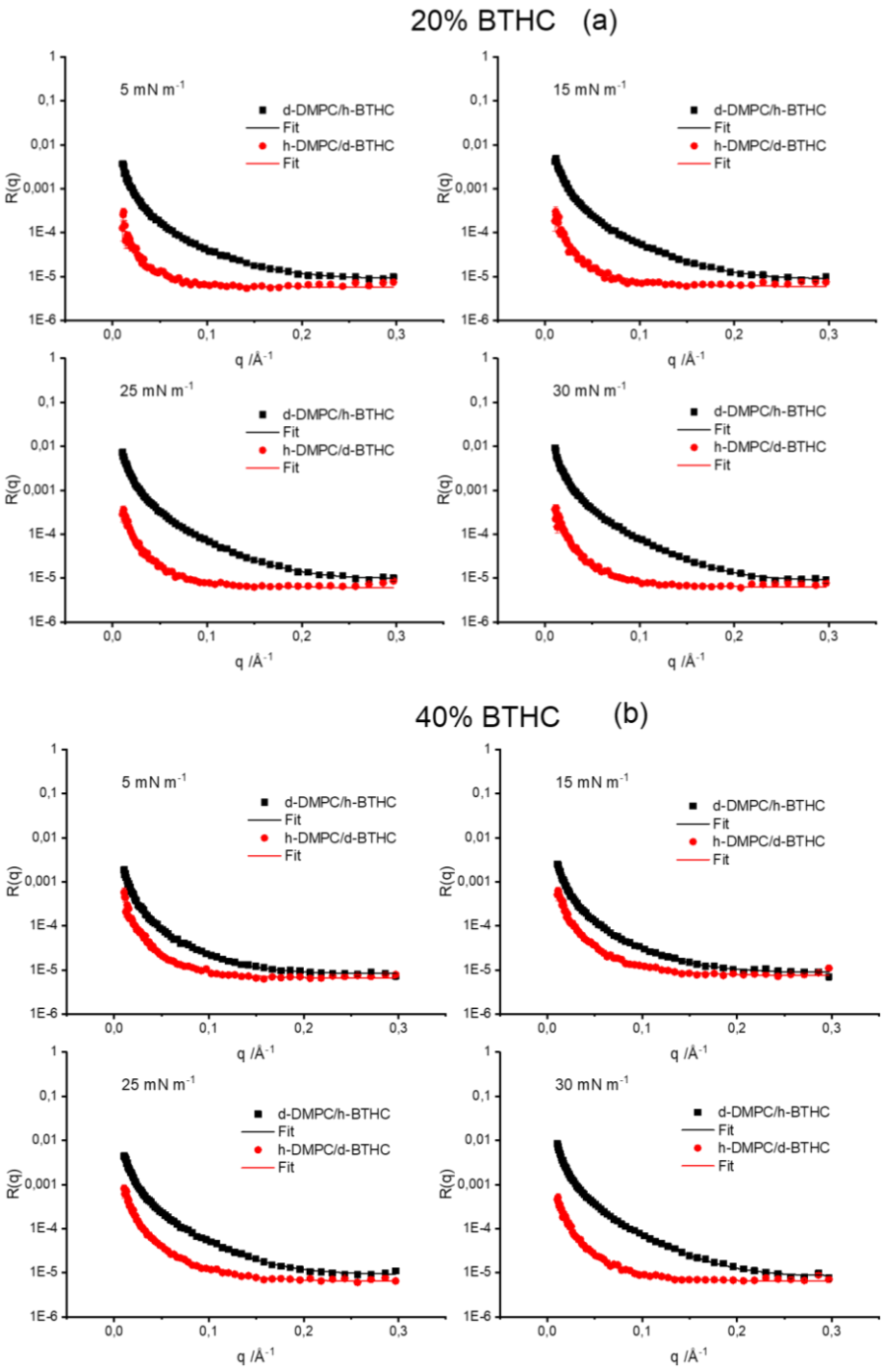

Figure S8. Reflectivity data and associated fits for monolayers containing a nominal and initial concentration of a) 20% and b) 40% BTHC by mol.

Fitting parameters

Table S2. Parameters obtained for the combined fit of the different contrasts for each mixture and surface pressure shown in the Figures. Estimated error in thicknesses obtained is ≈15% while the estimated error in surface excess is ≈5%. Background was fitted but is not included in the listed parameters.

20% DEHP

Surface pressure / mN m^{-1}	Thickness / Å	Surface excess / $Å^{-2}$	Molar Ratio, x_{mol} DEHP:DMPC
5	16	8.6×10^{-3}	0.23
15	17	9.98×10^{-3}	0.19
25	20	1.07×10^{-2}	0.23
30	23	1.06×10^{-2}	0.26

40% DEHP

Surface pressure / mN m^{-1}	Thickness / Å	Surface excess / $Å^{-2}$	Molar Ratio, x_{mol} DEHP:DMPC
5	21	6.5×10^{-3}	0.65
15	20	7.9×10^{-3}	0.61
25	21	1.08×10^{-2}	0.69
30	21	1.24×10^{-2}	0.58

60% DEHP

Surface pressure / mN m^{-1}	Thickness / Å	Surface excess / $Å^{-2}$	Molar Ratio, x_{mol} DEHP:DMPC
5	22	4.5×10^{-3}	1.20
15	22	5.7×10^{-3}	1.22
25	23	9.7×10^{-3}	0.58
30	21	1.17×10^{-2}	0.33

20% BTHC

Surface pressure / mN m^{-1}	Thickness / Å	Surface excess / $Å^{-2}$	Molar Ratio, x_{mol} BTHC:DMPC
5	15	8.5×10^{-3}	0.23
15	16	1.03×10^{-2}	0.24
25	16	1.19×10^{-2}	0.23
30	18	1.29×10^{-2}	0.22
25	17	1.21×10^{-2}	0.23
15	16	1.05×10^{-2}	0.24

40% BTHC

Surface pressure / mN m^{-1}	Thickness / Å	Surface excess / $Å^{-2}$	Molar Ratio, x_{mol} BTHC:DMPC
5	17	5.7×10^{-3}	0.61
15	14	6.9×10^{-3}	0.63
25	16.	9.768×10^{-3}	0.49
30	18	1.24×10^{-2}	0.28
25	17	1.00×10^{-2}	0.44
15	16	7.1×10^{-3}	0.63
5	18	5.6×10^{-3}	0.61

60% BTHC

Surface pressure / mN m^{-1}	Thickness / Å	Surface excess / $Å^{-2}$	Molar Ratio, x_{mol} BTHC:DMPC
5	19	3.1×10^{-3}	1.46
15	22	3.9×10^{-3}	1.54
25	17	7.8×10^{-3}	0.60
30	17	9.8×10^{-3}	0.46
25	17.	8.2×10^{-3}	0.53
15	18	4.0×10^{-3}	1.53

DMPC

Surface pressure / mN m^{-1}	Thickness / Å	Surface excess / $Å^{-2}$
5	14	$1.11E \times 10^{-2}$
15	15	1.34×10^{-2}
25	17	1.50×10^{-2}
30	17	1.56×10^{-2}
25	16	1.51×10^{-2}
15	15	1.34×10^{-2}
5	14	1.11×10^{-2}

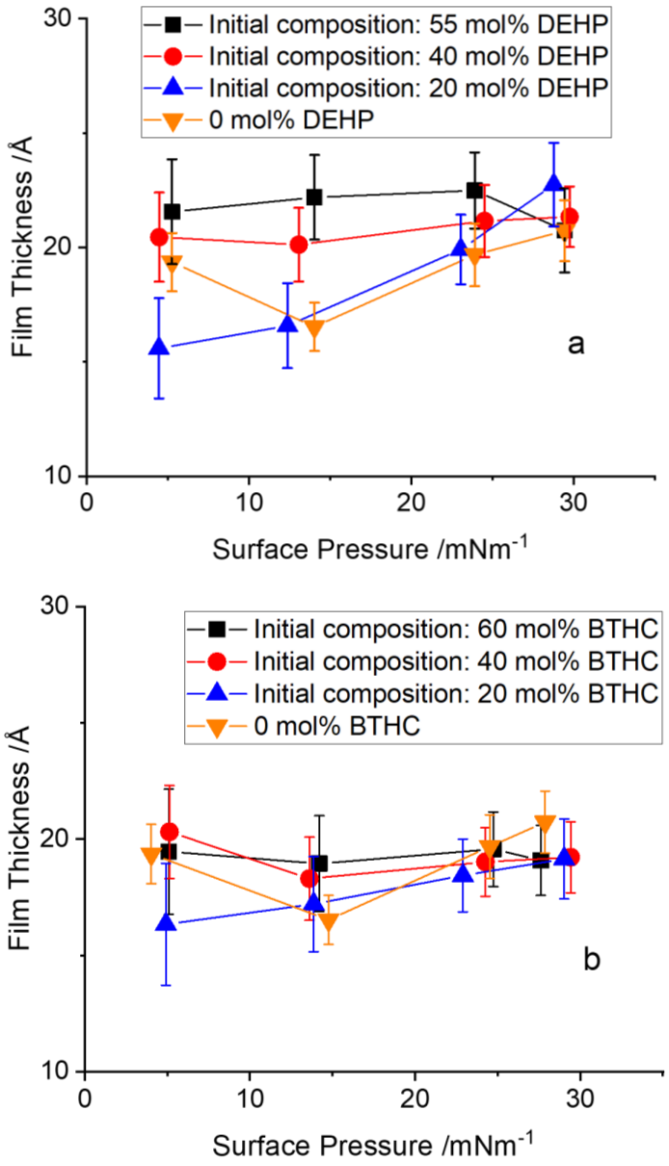

Figure S9. Layer thickness as obtained from neutron reflectivity plotted against measured surface pressure for a) DEHP and DMPC and b) BTHC and DMPC. In each case 4 compositions were measured (0 – Inverted triangle, 20 - Triangle, 40 - Circle and 55/60 mol % - Square respectively). No significant changes in thickness are observed with respect to changes in composition for either plasticiser.

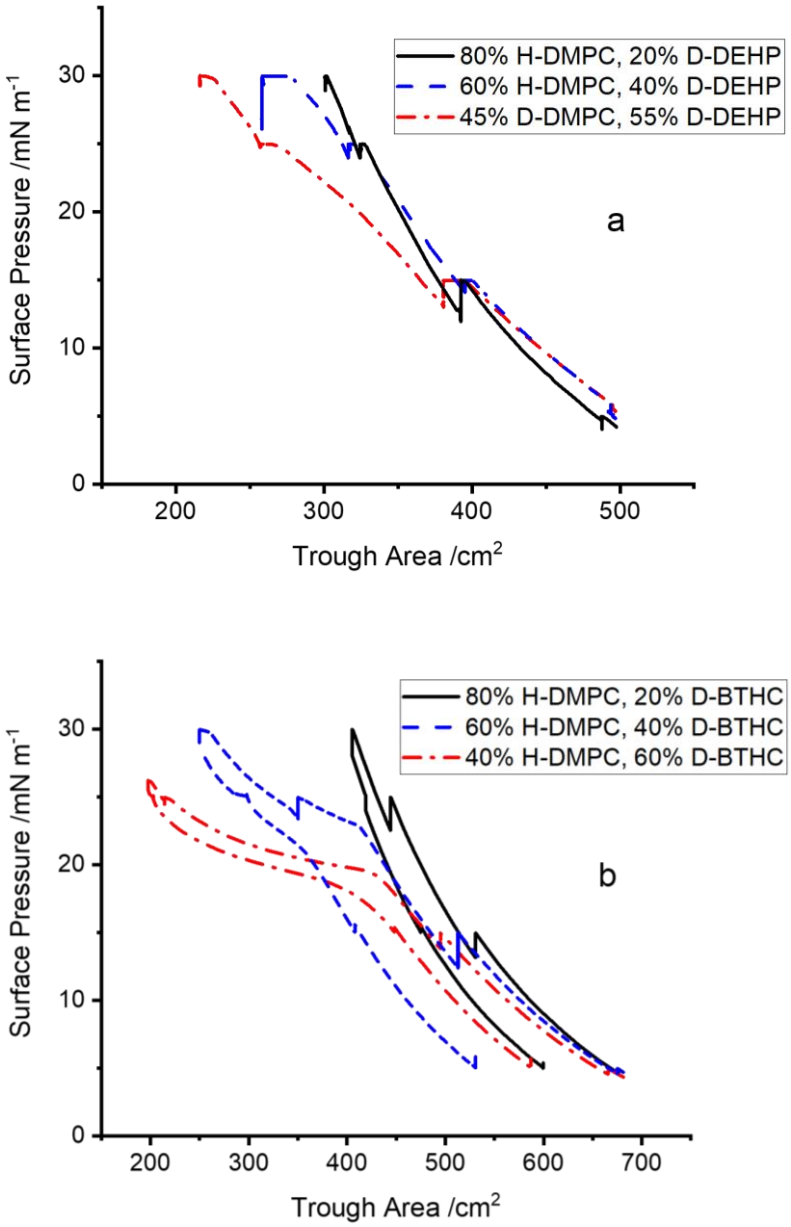

Figure S10. Pressure-area isotherms recorded during neutron reflectivity measurements for comparison of a) DMPC/DEHP mixtures b) and DMPC/BTHC mixtures. Drops in measured surface pressure correspond to the neutron measurements where the trough area was held fixed for extended periods until sufficient neutron data were acquired.