Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Partitioning into phosphatidylcholine-cholesterol membranes: liposome measurements, coarse-grained simulations, and implications for bioaccumulation

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Chemicals

The following chemicals were all obtained from Sigma-Aldrich (subsidiary of Merck KGaA, Burlington, USA) with purities >95%: 3-nitroaniline, diclofenac, urea, diethyl adipate, L-tryptophan, (R)-(+)-propranolol hydrochloride (propranolol), N,N-dimethyldodecylamine N-oxide (DDAO_C12), 3-(decyldimethylammonio)-propane-sulfonate inner salt (SB3-10), sodium octyl sulfate, sodium decyl sulfate, sodium dodecyl sulfate, hexylamine, dodecylamine, dihexylamine, trihexylamine, cholesterol, 2-oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine (POPC) and phosphate buffer saline (PBS) tablets.

Sodium n-tridecyl sulfate was obtained from Alfa-Aesar (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Chloroform (99.8%) and formic acid (>99%) were from VWR Chemicals (Leuven, Belgium). LC-MS/MS grade water and acetonitrile were from Biosolve Chimie (Dieuze, France).

Details of liposome preparation

Solutions of POPC (and POPC/cholesterol) were prepared in chloroform in a round bottom flask. This flask was placed under a stream of nitrogen gas and gentle agitation until a dry film was formed. This was re-suspended in pH 7.4 phosphate saline buffer, to a concentration of POPC of approximately 13.2 mM, forming a suspension of large multilamellar vesicles followed by 5 freeze-thawed cycles with liquid nitrogen.

This solution was then extruded 11 times through a 100 nM polycarbonate membrane using a LiposoFast basic extruder (both Supplied by Avestin Europe GmbH, Mannheim, Germany) to form unilamellar liposome vesicles.

Details of liposome measurements

The liposome-water partitioning was determined in a 96-well plate Rapid Equilibrium Dialysis (RED) device (by Thermo Fisher scientific). Each well in the RED device contains a donor cell (red cell) and a receptor (white cell) separated by an 8 kDa molecular weight cut-off cellulose membrane, through which the liposomes cannot permeate. The liposome solutions were diluted to approximately 4 mM with PBS solution (pH 7.4) and dosed with the reference chemical 3-nitroaniline and in some cases also diclofenac, plus a mixture of test chemicals according to their suitability for analyses under the same analytical method at 3 different nominal concentrations of 10, 20 and 50 μM. The test materials were grouped as follows: (1) SB3-10, DDAO-C12, and R-propranolol (2) L-tryptophan, urea, and diethyl adipate (3)

hexylamine, dodecylamine, dihexylamine and trihexylamine and (4) octyl sulfate, decyl sulfate, dodecyl sulfate and tridecyl sulfate. The impact of mixing of chemicals in single analyses was evaluated in an initial study comparing log K_{MW} for singly analyzed materials against groups, and no impact was found. Additionally, reference materials were included in the groups throughout, which would have indicated any influence due to mixing. None was observed. In triplicate, 400 µL of the spiked liposome solutions were added to the donor cells of the RED plate and 600 µL of PBS added to the receptor cell. For each test chemical mixture control samples were also prepared where 400 µL solutions of spiked PBS (at 20 µM) were added to the donor cell and 600 µL of PBS added to the receptor cell.

The RED plate was covered and then placed on an orbital shaker at 37°C for a minimum of 4 hours to allow for equilibrium between the donor and receptor cells.

Following incubation 100 µL aliquots were taken from all receptor cells, as well as the donor cells of the control samples and mixed with 100 µL of acetonitrile in HPLC vials. These were stored at 2-8°C for up to 8 days before analyses.

Analysis was carried out by liquid Electrospray Ionization LC-MS/MS using an Agilent 1200 LC system coupled to an Agilent 6460 Triple Quadrupole Mass Spectrometer. In total four separate LC-MSMS external standard quantification methods were developed and used for the analyses of the four mixtures of chemicals mentioned above. All methods used a gradient of 0.1% formic acid in water and 0.1% formic in acetonitrile. For separation of chemical mixtures 1 and 2 a Luna Omega® 1.6um C18 50 × 2.1mm was used. For chemical mixtures 3 and 4 a HiChrom ACE® 3um C18 150 × 2.1mm was used.

The size distribution of our liposome preparations was measured by dynamic light scattering (Figure S1). A minimum size of ~50 nm (approximately equivalent to 5000 kDa) was measured. This is significantly greater than our dialysis membrane cut-off (8kDa) so no liposome is expected to be present in the receptor cell.

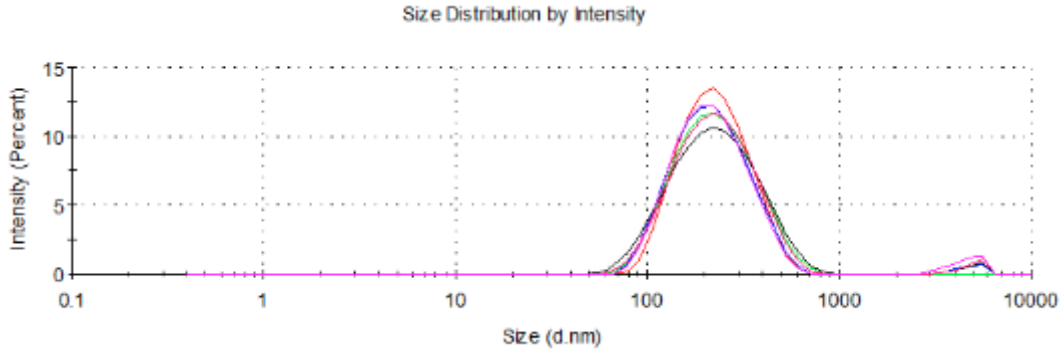

Figure S1: Size distribution of liposomes, as measured by dynamic light scattering

Validation of membrane models

This section provides details of the calculations and resulting plots behind the discussion of membrane model validation in the main article. The POPC/cholesterol model used here is effectively a translation to Martini 3 of the well-characterised Martini 2 model of the same system (Melo et al. 2015).

Condensing effect

The condensing effect of cholesterol on the lipid structure in the simulations was examined by comparing the area per lipid with respect to cholesterol concentration. The area per lipid is defined as

$$A_{\text{lipid}} = \frac{\langle A_{xy} \rangle}{N_{\text{lipid}}}$$

where A_{xy} is the area of the simulation box in the plane of the membrane and N_{lipid} is the total number of POPC and cholesterol molecules in each leaflet of the membrane. The results for both coarse-grained and atomistic systems are plotted in Figure S2.

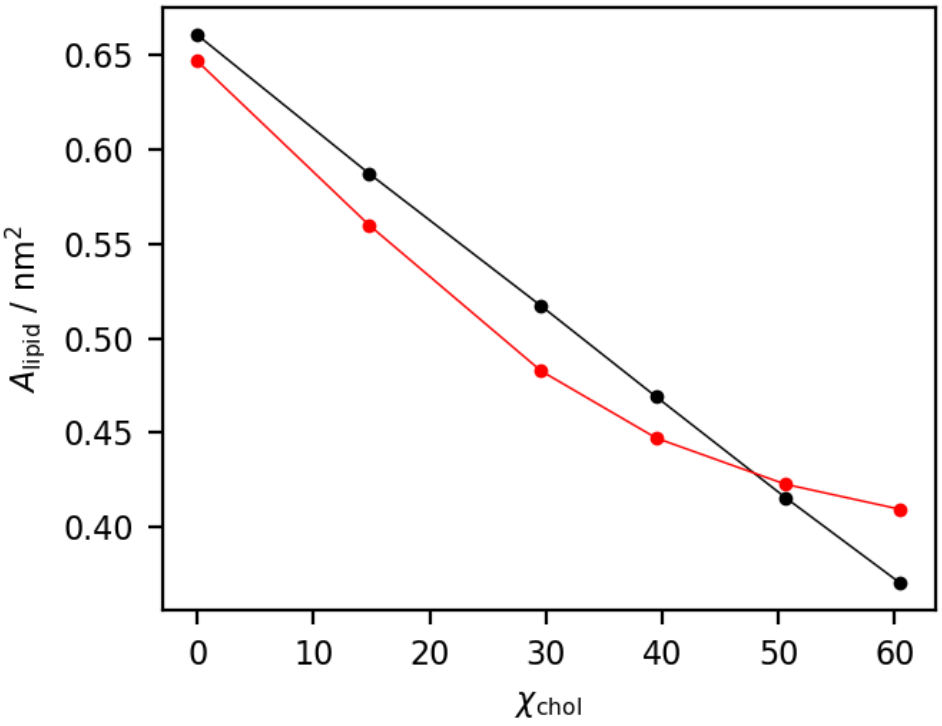

Figure S2: Area per lipid as a function of cholesterol concentration, for the Martini 3 (black) and CHARMM36 (red) models.

Ordering effect

The ordering of the lipid molecules in the membranes was determined by calculating the bond order parameter for each inter-bead bond in the coarse-grained POPC molecule (see Figure S3). The atomistic systems were mapped to a coarse-grained resolution using the *backward* script, (Wassenaar et al. 2015) and the atom-to-bead mappings obtained on the Martini website for POPC and cholesterol (Martini website 2015). The order parameter is defined as

$$P_2 = \frac{3\langle \cos^2\theta \rangle - 1}{2}$$

where θ is the angle between the bond and the bilayer normal (in this case, the z axis). Both models show an increase in P_2 for the alkyl chain bonds as the cholesterol concentration increases. However, the magnitudes of these changes differ between the models, compared in Figure S4. The mapped atomistic results show a large increase in P_2 up to 40% cholesterol, and a much smaller further increase up to 60%. The Martini 3 results show a smaller increase in P_2 for the alkyl chain bonds, and the rate of change is more uniform with respect to cholesterol concentration. Additionally, the difference in ordering between the monounsaturated chain (bonds 6–8) and the fully saturated chain (bonds 9–11) is larger in the Martini 3 results compared to the atomistic. This behaviour of the Martini 3 model is consistent with the established Martini 2 equivalent on which it is based (Melo et al. 2015). It should be noted that the two chains in POPC do show ordering differences, both experimentally and in atomistic simulations, when this is measured using the so-called deuterium order parameter, which relates to the orientations of C-H bonds (Piggot et al. 2017).

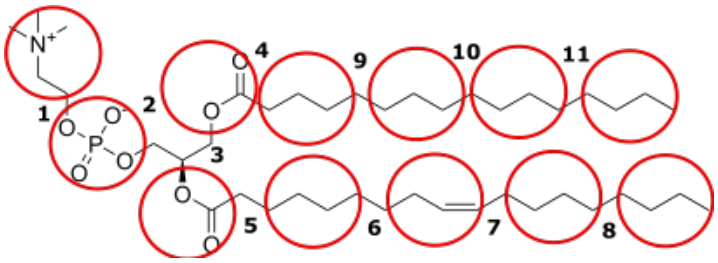

Figure S3: Coarse-grained mapping scheme for POPC, with the numbered bonds used for calculating the order parameter P_2. Note that some atoms are shared between beads in this scheme, so the circles do not correspond exactly to the beads in the coarse-grained representation.

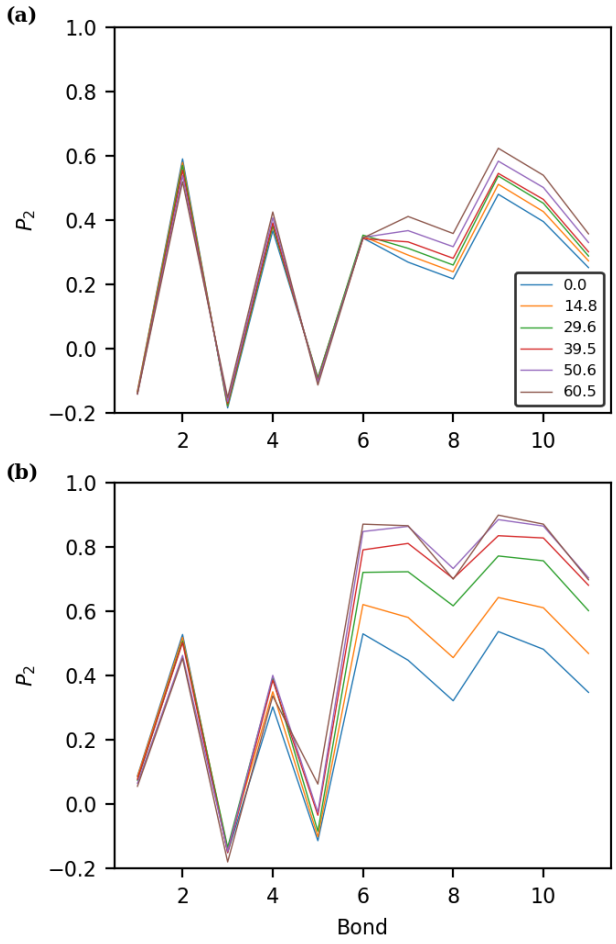

Figure S4: Acyl chain order parameters for all coarse-grained bonds in POPC, averaged over all POPC molecules in a bilayer, for (a) Martini 3 and (b) CHARMM36 mapped to the Martini resolution. Results are shown for 0.0 to 60.5 mol% cholesterol, as shown in the legend. Bond numbers are defined in Figure S3.

Liquid structure

The liquid structure of the lipid bilayers was investigated by calculating 2D radial distribution functions (RDFs) of the lipid tails of POPC, as shown for the monounsaturated tail in Figure S5. For the pure POPC system, the peak heights and positions are very similar for the Martini and CHARMM36 simulations. As cholesterol is added, the increase in the peak heights is greater for CHARMM36. At high cholesterol concentrations, the atomistic model also shows a significant increase in the long-range ordering, which is not seen to the same extent in the coarse-grained model. These results indicate a greater increase in ordering for the atomistic systems, consistent with the P_2 order parameter results presented above.

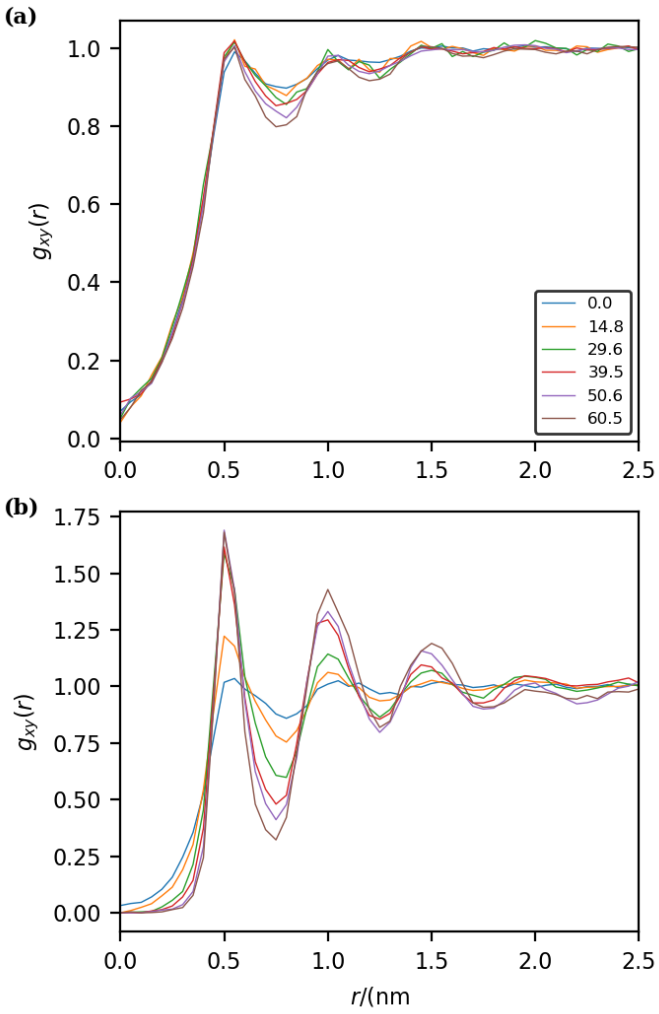

Fig. S5: 2D radial distribution functions for the monounsaturated lipid tail (center of geometry), for (a) Martini 3 and (b) CHARMM36 mapped to the Martini resolution. Results are shown for 0.0 to 60.5 mol% cholesterol, as shown in the legend.

Coarse-grained mappings

The results of the mapping and bead assignment process for the solutes, as described in the main article, are listed in Table S1.

Table S1 - Mappings and assignment to Martini 3 bead types (Souza, et al. 2021) from the procedure described in the main article for the solutes tested in this work. For each solute, each colour represents a coarse-grained bead.

Molecule	SMILES	Martini 3 bead mappings
3-nitroaniline	c1ccc([N+]([O-])=O)cc1N	TC5 TC5 SN3 SN3
diclofenac	Clc1cccc(Cl)c1[NH+]c2ccccc2CC(=O)[O-]	SC4 TC5 N2 TC5 TC5 TC5 SQ5n-
propranolol	CC(C)[NH2+]CC(O)COc1cccc2c1cccc2	Q2p+ P4 TC5 TC5 TC5 TC5 TC5 TC5

hexylamine	CCCCCC[NH3+]	C2 SQ4p+
dihexylamine	CCCCCC[NH2+]CCCCCC	SC2 TC2 SQ3p+ TC2 SC2
SB3-10	CCCCCCCCCC[N+](C)(C)CCCS(=O)(=O)[O-]	TC2 C2 SC2 Q2+ TC2 SQ4p-
DDAO-C12	CCCCCCCCCCCC[N+](C)(C)[O-]	C2 C2 TC2 P6
Octyl sulfate	CCCCCCCCOS(=O)(=O)[O-]	C2 C2 Q2-
Decyl sulfate	CCCCCCCCCCOS(=O)(=O)[O-]	C2 C2 TC2 Q2
Dodecyl sulfate	CCCCCCCCCCCCOS(=O)(=O)[O-]	C2 C2 C2 Q2-

Experimental data

Data for reference compounds

The measured log K_{MW} values for both 3-nitroaniline and diclofenac are given in Table S2. The measured values for each solute were averaged for each cholesterol mol% and dose concentration in order to calculate the final values for log K_{MW} given in the main text.

Table S2 - log K_{MW} measurements for the two reference compounds, 3-nitroaniline and diclofenac. The group numbers refer to the groups of test compounds listed in the section "Details of liposome measurements" above, with which the reference compound was a co-solute.

	3-nitroaniline			diclofenac		
Test material group	1	2	3	4	1	2
10 µM, 0%	2.78	2.23	2.70	2.32	3.19	2.66
	2.81	2.06	2.62	2.25	3.20	2.59
	2.87	2.18	2.55	2.33	3.26	2.59
20 µM, 0%	2.33	2.14	2.38	2.18		2.75
	2.75	1.78	2.32	2.23	2.16	2.68
	2.57	1.85	2.46	2.09	2.44	2.64

50 μM, 0%	2.15	1.81	2.67	2.51	3.53	2.46
	2.42	1.81	2.71	2.13	3.67	2.50
	2.46	1.88	2.65	2.01	3.68	2.51
10 μM, 15%	2.33	1.69	2.56	2.46	2.91	2.69
	2.19	1.67	2.50	2.28	2.88	2.67
	2.32	1.79	2.55	2.21	2.95	2.61
20 μM, 15%	1.65	2.08	2.43	2.26	2.66	2.71
	2.34	1.85	2.55		2.89	2.66
	2.16	1.90	2.53		2.88	2.61
50 μM, 15%	2.67	2.02	2.15	1.50	2.65	2.56
	2.74	1.59	2.06		2.64	2.44
	2.61	1.63	1.80	1.60	2.79	2.42
10 μM, 30%	1.91	1.93	1.83	2.74	2.74	2.44
	1.96	1.84	2.42	2.93	2.96	2.34
	1.74	2.37	2.37	2.60	2.68	2.61
20 μM, 30%		1.73	2.19	2.45	2.57	2.51
		1.91		2.65	2.73	2.56
		1.96		2.30	2.45	2.55
50 μM, 30%	2.28	1.44	1.84	1.74	2.44	2.26

	2.26	1.25	1.85	2.01	2.54	2.39
	2.37	1.65	2.12	1.82	2.32	2.32

Calculated log K_{MW} for 3-nitroaniline in this work is typically in the range (1.78–2.62), mean 2.20 ± 0.42 (SD) n = 100. Partitioning for the reference compound 3-nitroaniline in Set 2 is generally low, suggesting that recovery for all solutes in this set is unreliable. For this reason, regardless of individual solute recovery, no results from this set are reported.

Data for test compounds

Control experiments were run with an identical setup as outlined above, but without liposome. Recovery from the donor and receptor cells in these controls for the seven test compounds are given in Table S3.

Table S3- Recovery from the donor and receptor cells for the seven test compounds

	Propranolol	ddao-c12	sb3-10	Dihexylamine	octyl sulfate	decyl sulfate	dodecyl sulfate
Control donor recovery (%)	67.5	82.5	80.1	78.7	85.6	77.8	54.0
Control receptor recovery (%)	69.2	77.8	69.9	79.0	106	97.5	70.3

The measured log K_{MW} values for the seven test compounds are given in Table S4. The values for each cholesterol mol% and dose concentration for each solute were averaged in order to calculate the final values for log K_{MW} given in the main text.

Table S4- Individual log K_{MW} measurements for the seven test compounds.

	Propranolol	ddao-c12	sb3-10	Dihexylamine	octyl sulfate	decyl sulfate	dodecyl sulfate
10 μM, 0%	3.25	3.65	2.54	2.15	2.54	3.70	4.18

	3.29	3.67	2.60	1.61	2.59	3.76	4.23
	3.36	3.83	2.77		2.62	3.75	4.16
20 μM, 0%	2.93	3.06	2.04	2.46	2.33	3.64	5.21
	3.16	3.28	2.69	2.38	2.11	3.49	4.27
	3.12	3.25	2.57	2.45	1.90	3.46	
50 μM, 0%	2.89	3.21	1.59	2.83	2.32	3.26	4.28
	2.99	3.28	2.16	2.83	1.86	3.21	4.53
	3.06	3.34	2.35	2.82	1.67	3.23	
10 μM, 15%	2.94	3.15	2.76	2.47	2.68	3.76	4.03
	2.91	3.15	2.69	2.39	2.32	3.56	4.04
	2.97	3.20	2.75	2.45	2.59	3.79	4.06
20 μM, 15%	2.63	2.84	1.32	2.39	2.13	3.41	4.20
	2.84	3.04	2.33	2.47	0.85	3.33	4.24
	2.84	3.04	2.26	2.44		3.40	
50 μM, 15%	2.60	2.99	1.13	2.83	2.03	3.19	3.34
	2.61	3.00	0.90	2.81	2.09	3.19	
	2.73	3.06	2.06	2.77	1.40	2.86	
10 μM, 30%	2.97	3.12	2.85		2.46	3.58	3.98
	3.13	3.27	3.08		2.85	3.83	4.18

	2.91	3.03	2.82		2.57	3.60	3.94
20 μM, 30%	2.74	2.79	2.39	1.60	2.15	3.39	4.38
	2.88	2.84	2.70				4.59
	2.67	2.73	2.31				
50 μM, 30%	2.61	2.93	2.11	2.61	1.20	3.05	4.24
	2.71	2.96	2.29	2.58	1.76	3.09	
	2.56	2.89	1.83	2.65		3.18	

Other supporting files

- GROMACS topology files (itp) for cholesterol in Martini 3 and the solutes tested in this work.
- Spreadsheets (xlsx) of liposome measurement results.
- Version 1.0 of the cg_param_m3 script was used for this work and is available at https://github.com/cgkmw-durham/cg_param_m3/tree/martini3_v1

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