

Supporting information for

Michael Addition in Reactive Extrusion: A facile sustainable route to developing phosphorus based flame retardant materials

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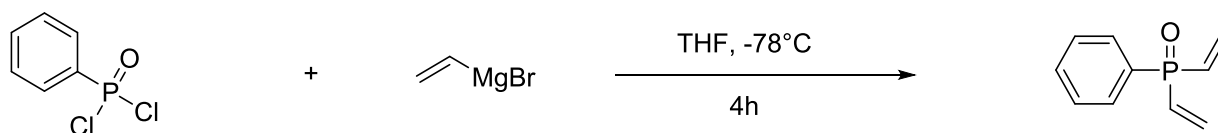
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S1. Synthesis of DVPPPO

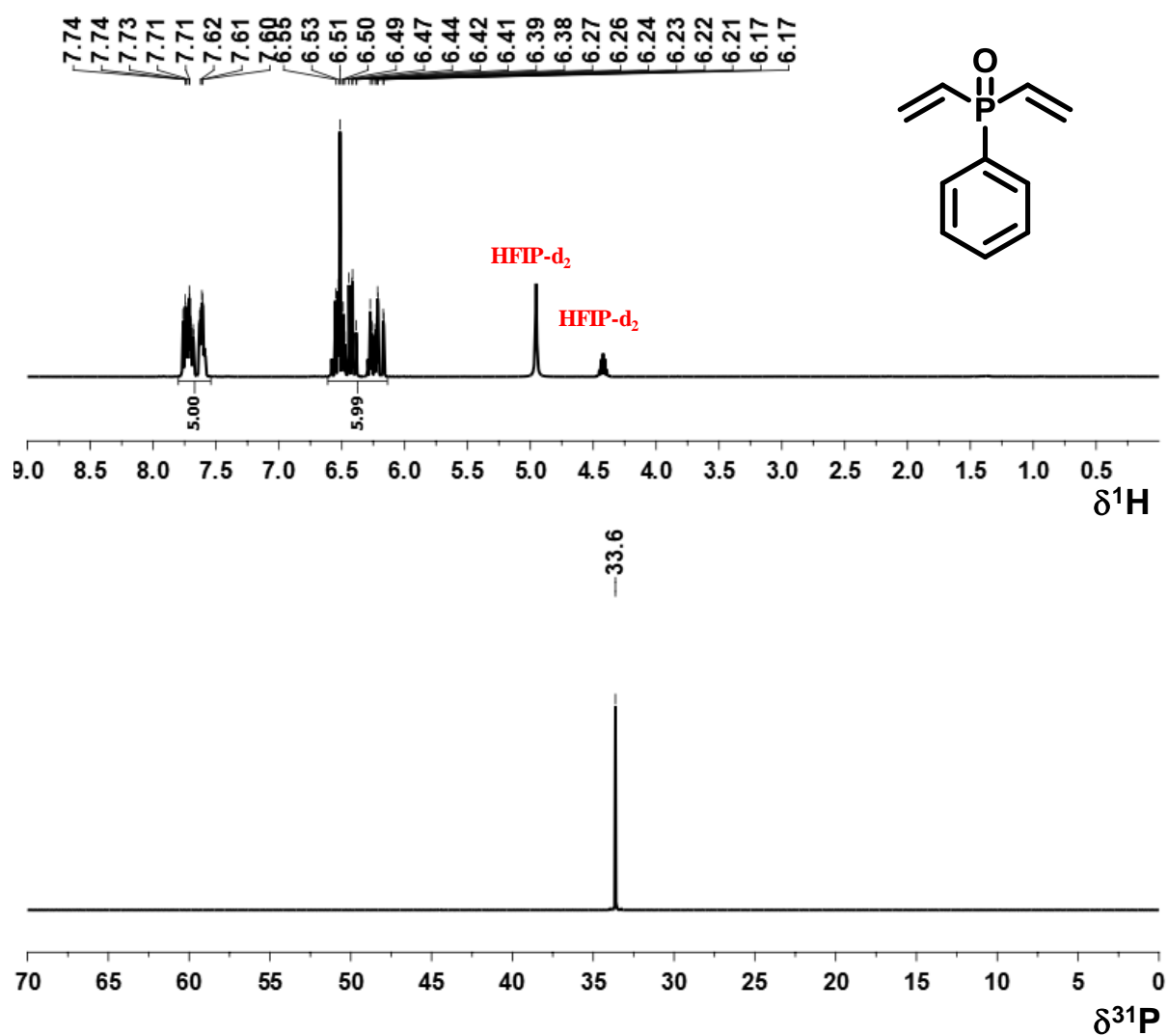


A solution of phenylphosphonic dichloride (70.0 mL, 0.500 mol) dissolved in anhydrous THF (1 L) was cooled to -78°C and mechanically stirred under an atmosphere of dry nitrogen. Vinylmagnesium bromide (1M in THF, 1 L) was slowly added so that the temperature of the reaction mixture never exceeded -70°C . The addition took 4 hours. After an additional 3 hour of stirring at -78°C , in the cold reaction 11 of cold (4°C) ammonium chloride solution ($c(\text{NH}_4\text{Cl}) = 4 \text{ mol/L}$) was added and stirred for 5 min. White precipitate was separated by filtration, affording a solution of two layers. The upper layer (THF layer) was separated and the aqueous layer was extracted with chloroform (2 x 1L). The organic layer was collected and dried over Na_2SO_4 . After filtration, all the volatiles were removed in vacuum and purification done by vacuum distillation (Bp. 123°C at 0.02 mbar). White oil was obtained upon standing turn into solid with 76 % yield.

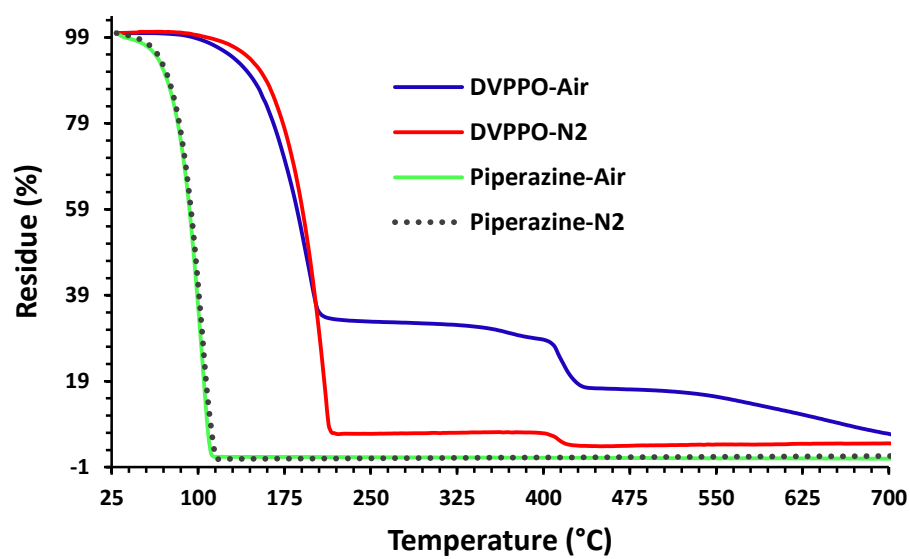
S2. NMR analysis of DVPPPO

The ^1H , ^{13}C and ^{31}P NMR spectra were recorded using the Bruker standard pulse programs on a 5 mm CryoProbe™ Prodigy probe equipped with z-gradient applying 90° pulse lengths of 11.4 μs (^1H), 10.0 μs (^{13}C) and 16.0 μs (^{31}P) on a Bruker AV-III 400 NMR spectrometer (Bruker Biospin AG, Fällanden, Switzerland). ^1H NMR data are reported as follows: chemical shift, numbers of protons contributing to the signal intensity, multiplicity (s = singlet, d = doublet, t = triplet, q = quartets, m = multiplet, br = broad) and coupling constants (J in Hz). The ^{31}P NMR chemical shifts were referenced to an external sample with neat H_3PO_4 at 0.0 ppm. ^1H NMR chemical shifts were referenced to the residual solvent signals at 4.41, 4.86 for ^1H and 68.0, 120.6 for 1,1,1,3,3,3-Hexafluoro-2-propanol- d_2 (HFIP- d_2) respectively.

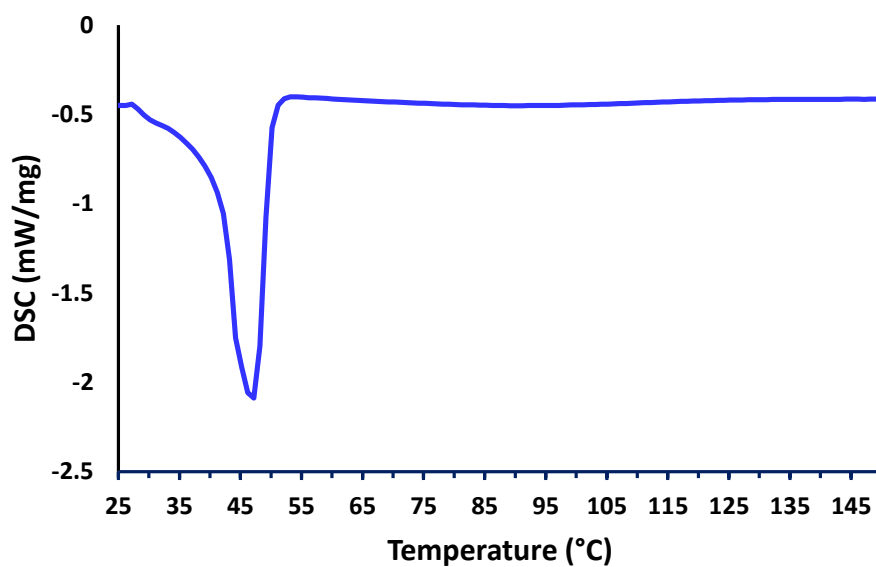
^1H NMR (400.2 MHz, HFIP- d_2) δ (ppm): 7.74-7.60 (m, 5H), 6.5-6.17 (m, 6H), **^{31}P NMR (162.0 MHz, HFIP- d_2)** δ (ppm): 33.6.



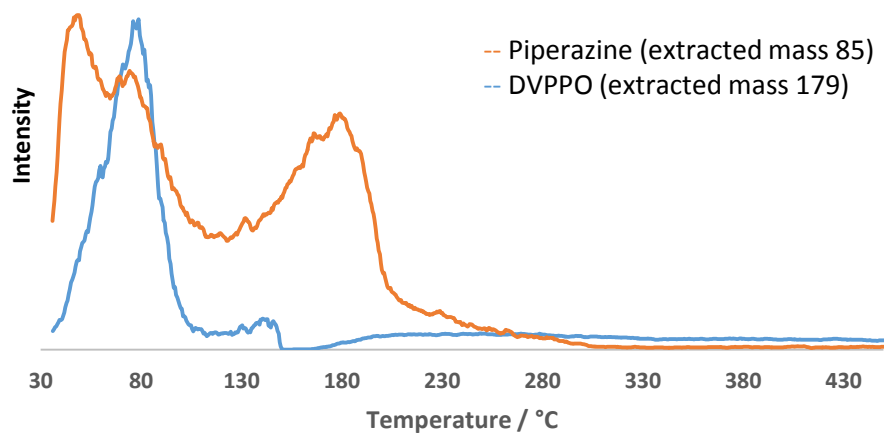
S2. ¹H and ³¹P{¹H} spectra of **DVPPPO** (HFIP-d₂).



S3A. TGA data of DVPPPO

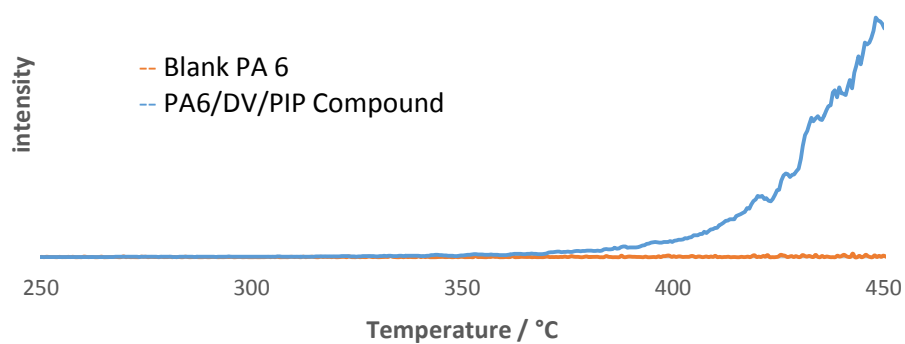


S3B. DSC data of DVPPPO

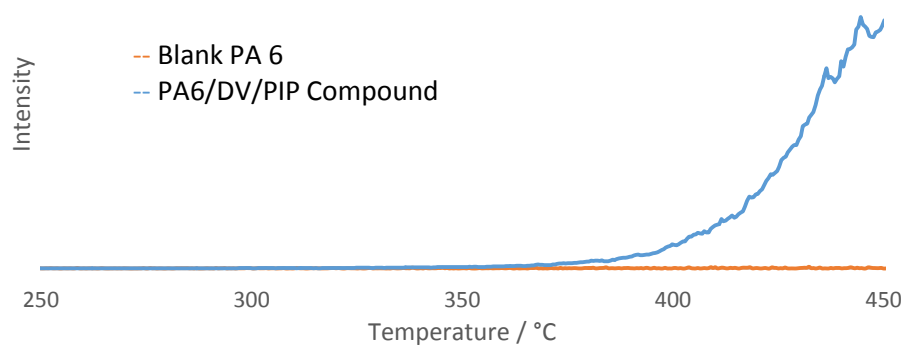
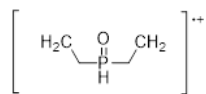


S4A. DIP-MS data of piperazine and DVPPPO

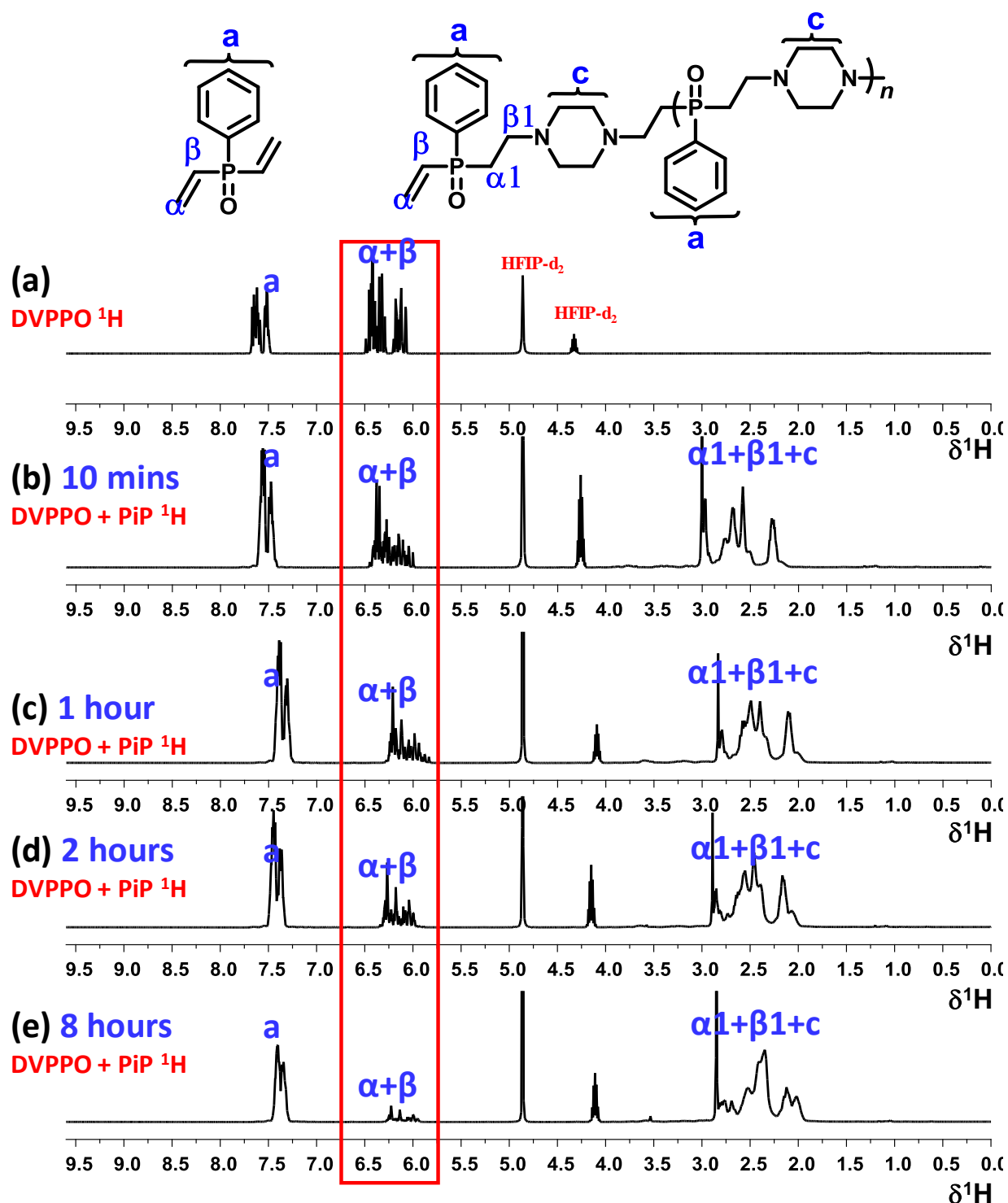
PO(extracted mass 47)



Phosphorous species (extracted mass 104)

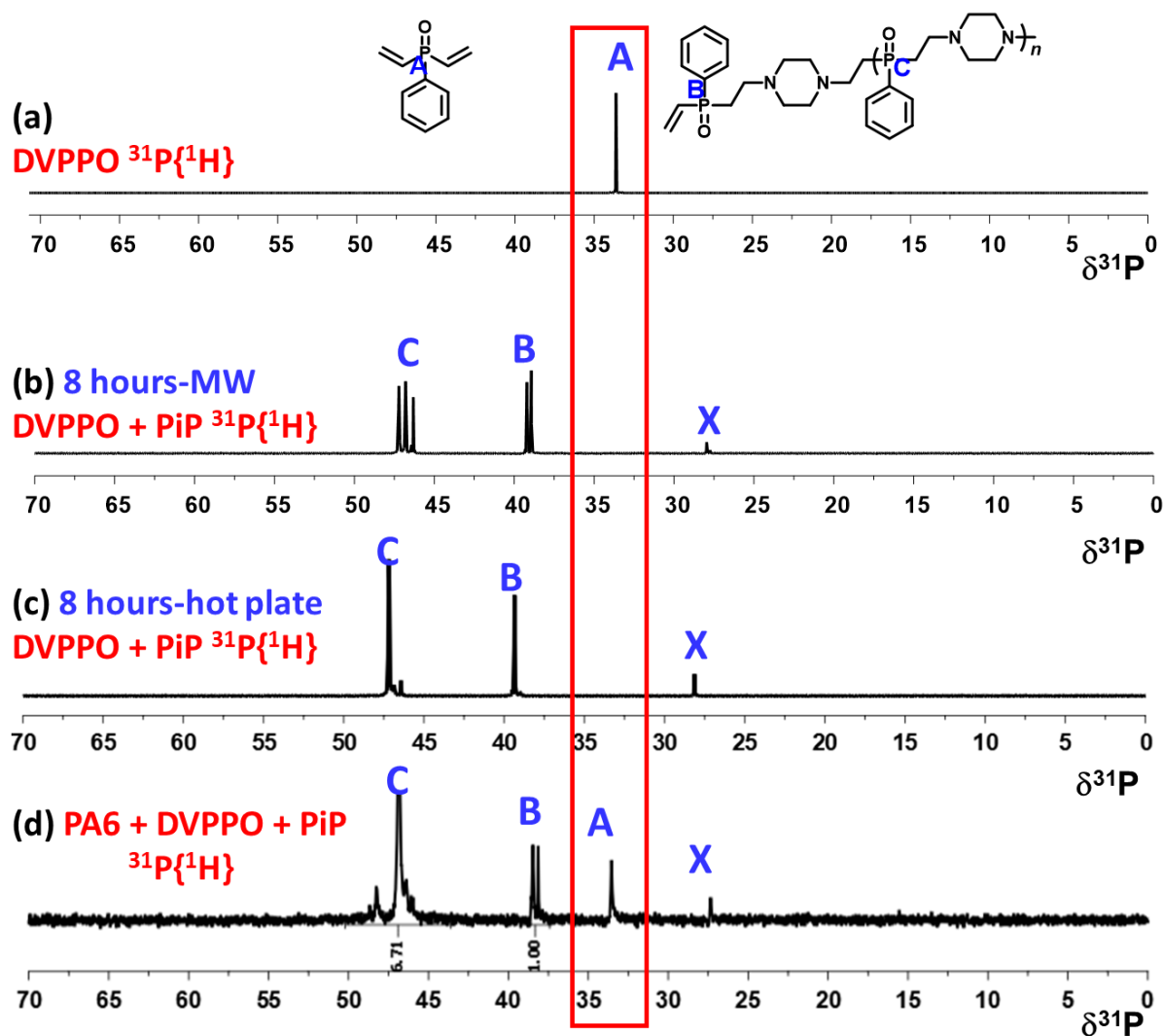


S4B. DIP-MS data of PA6 and PA6/DV/PIP Compound



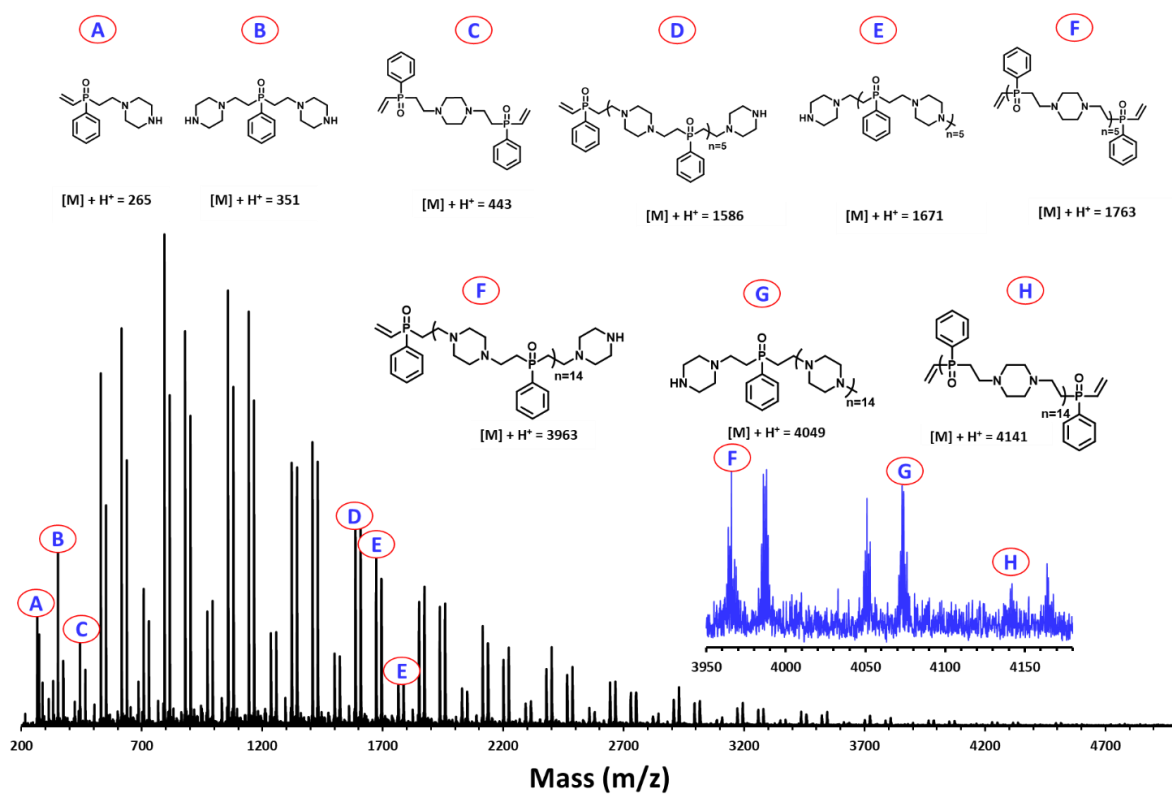
S5A. Michael Addition Kinetic Study(^1H NMR data)

a) ^1H spectra of DVPPO b) ^1H spectra of DVPPO with piperazine after 10 minutes c) ^1H spectra of DVPPO with piperazine after 1-hour d) ^1H spectra of DVPPO with piperazine after 2 hours e) ^1H spectra of DVPPO with piperazine after 8 hours (HFIP- d_2).





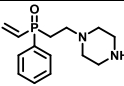
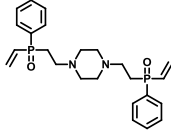
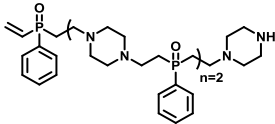
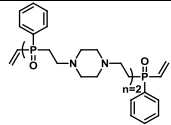
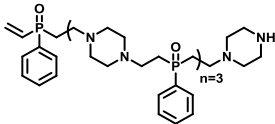
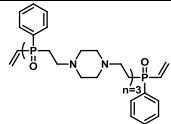
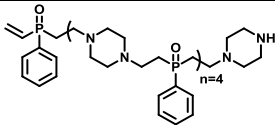
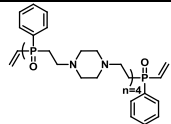
S5B. Michael addition study with different methods (^{31}P NMR data)

a) $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of DVPPO, b) $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum DVPPO with piperazine after 8 hours in microwave, c) $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of DVPPO with piperazine after 8 hour on hot plate, d) $^{31}\text{P}\{^1\text{H}\}$ spectra of PA6/DV/PIP fiber (HFIP- d_2).



S6. MALDI-TOF analysis of model phopshine oxide macromolecules

DVPPO/Pipearzine model reaction in microwave at 100 °C , 30 bar , 1 hour

Mass (m/z), [M+H] ⁺	Structures
87	
179	
265	
443	
529	
707	
793	
971	
1057	
1235	

S7. UPLC-MS data of the extracts from PA6/DVPPPO/Piperazine fibers

S8. Sublethal toxicity assessment methods

Detection of the pro-inflammatory cytokine tumor necrosis factor alpha (TNF- α) using the enzyme-linked immunosorbent assay (ELISA)

Detection of TNF- α was performed as previously described.[1] In brief, THP-1 cells were seeded into 96-well cell culture plates at a density of 4×10^4 cells per well in 200 μ L complete cell culture medium containing 200 nM PMA and were allowed to differentiate for 72 h. After two washing steps in pre-warmed (37 °C) PBS, cells were treated for 8 h with increasing concentrations of DVPPO in 100 μ L complete cell culture medium per well. Lipopolysaccharide (LPS; 10 and 100 ng/mL) served as a positive control. The amount of TNF- α was quantified in the cell culture supernatant (diluted 1:10) by sandwich-ELISA, according to the manufacturer's instructions (eBioscience). Data shown represent the mean and corresponding standard deviations of three independent experiments.

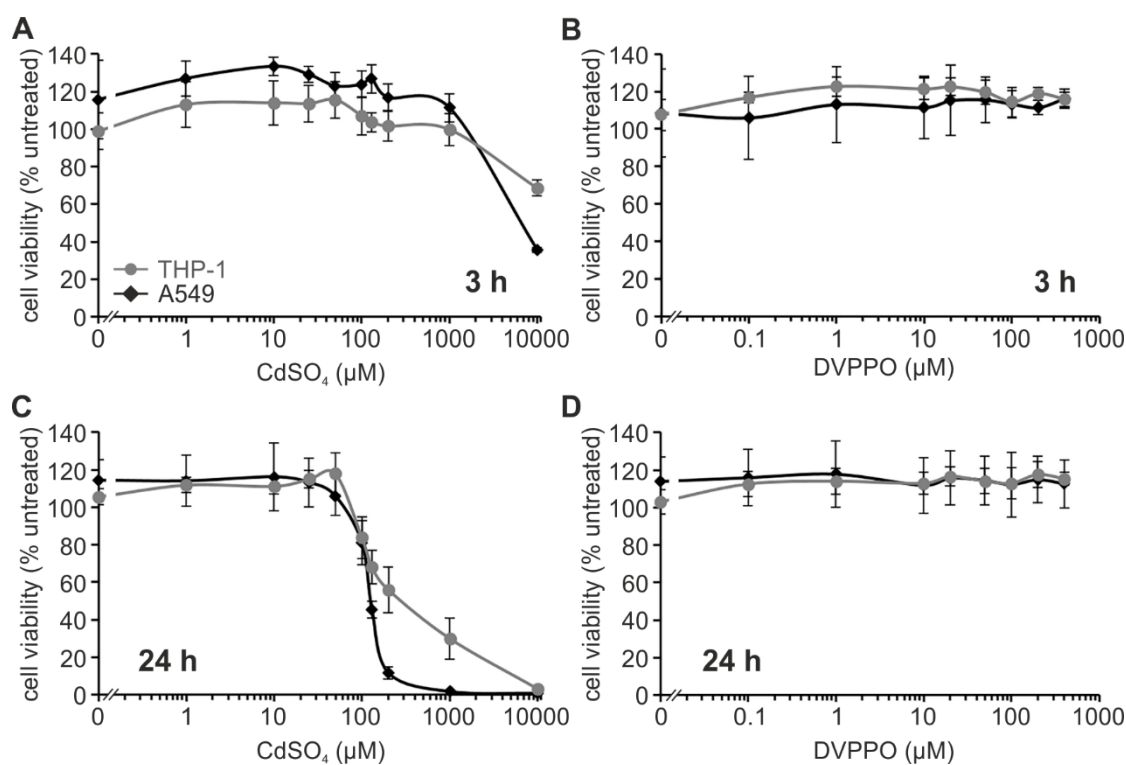
Genotoxicity assessment (alkaline comet assay)

To assess the genotoxicity of DVPPO the alkaline comet assay was performed as described previously [1] . The method is recapitulated only briefly and adaptations are described.

THP-1 cells were seeded into 12-well cell culture plates at a density of 2×10^5 cells per well in 1 mL complete cell culture medium containing 200 nM PMA and were allowed to differentiate for 72 h. After two washing steps with pre-warmed (37 °C) PBS, cells were treated for 30 min or 3 h with increasing concentrations of DVPPO in 1 mL complete cell culture medium per well. Ethyl methanesulfonate (EMS, 15 mM, 30 min; Sigma) served as the positive control. After treatment cells were harvested using 80 μ L 0.5% trypsin-EDTA and 120 μ L complete cell culture medium per well. 40 μ L of the resulting cell suspension were carefully mixed with 160 μ L 0.5% (w/v) low melting agarose (LMA; Sigma) in PBS (pre-warmed to 37 °C). 70 μ L of this mixture were immediately applied to microscope slides, pre-coated with 1.5% (w/v) agarose in PBS and overlaid with a coverslip. LMA was allowed to solidify for at least 10 min at 4 °C before coverslips were removed and cells lysed in pre-cooled (4 °C) lysis

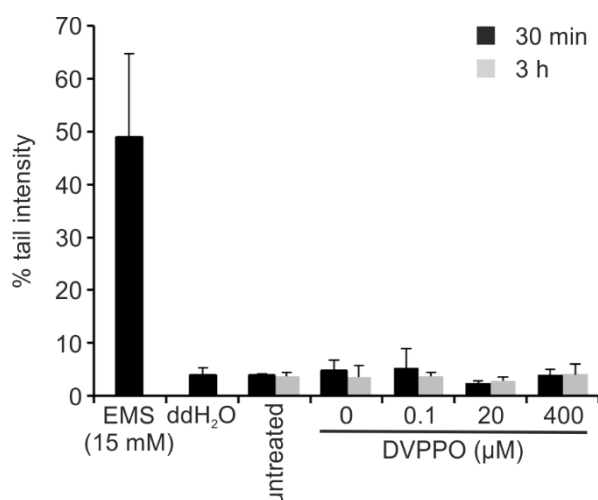
solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% (w/v) Na-Sarcosinate, 10% (v/v) DMSO, 1% (v/v) Triton-X-100; pH10) for 1 h at 4 °C in the dark. Slides were placed into a horizontal electrophoresis tray (PHERO-Comet 20-E, Biotec-Fischer) containing pre-cooled (4 °C) electrophoresis solution (0.33 M NaOH, 1 mM EDTA) and DNA was allowed to unwind for 20 min without current. Electrophoresis was performed for additional 20 min at a constant voltage of 24 V, resulting in a current of 300 mA. Thereafter, samples were neutralized in 0.4 M TRIS (pH 7.5) for 5 min, rinsed with ddH₂O, dehydrated in absolute ethanol for 5 min, and air-dried overnight. DNA was stained with 1 x SYBR® Gold nucleic acid stain (Molecular Probes) solution in ddH₂O for 20 min at room temperature (RT) in the dark. After two washing steps in ddH₂O (10 min each) samples were air-dried overnight at RT. Samples were re-hydrated with 20 µL ddH₂O per gel, covered with a cover slip and imaged using a Zeiss Axio Imager.Z1 microscope, a 10x objective (EC Plan-Neofluar) and ZEN software (Carl Zeiss Microscopy). Images were analyzed using Comet Assay IV software (Perceptive Instruments) and DNA damage was quantified using the retrieved % tail intensity values.

Data shown represent the mean and corresponding standard deviations of three independent experiments. For each experiment and treatment condition 100 individual cells (=comets) were analyzed.



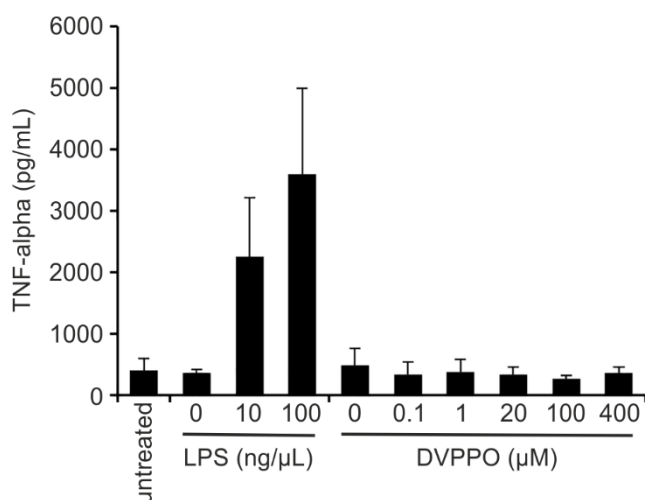
S 9. Cell viability

Assessment of cell viability 3 and 24 h after DVPPO treatment. A549 cells (black rhombs) as well as THP-1 macrophages (grey dots) were treated with increasing concentrations of DVPPO for 3 h (B) or 24 h (D). Increasing concentrations of CdSO_4 (A and C) served as the positive control. Values are normalized to untreated control samples and represent the mean and standard deviations of three independent experiments with three technical replicates each. The legend shown in (A) applies to all graphs (A to D).



S10. Genotoxicity

DVPPO does not induce DNA damage in THP-1 macrophages. THP-1 macrophages were treated for 30 min (black bars) or 3 h (grey bars) with the indicated concentrations of DVPPO or EMS as a positive control. DNA damage levels were determined using the alkaline comet assay and are given in % tail intensity. Data represent the mean and standard deviations of three independent experiments.



S11. Inflammatory response

DVPPO does not induce TNF-α release in THP-1 macrophages. THP-1 macrophages were treated for 8 h with the indicated concentrations of DVPPO or LPS as a positive control, and the amount of TNF-α in the supernatant was determined. Data represent the mean and standard deviations of three independent experiments.

References

- [1] May S, Hirsch C, Rippl A, Bohmer N, Kaiser J-P, Diener L, et al. Transient DNA damage following exposure to gold nanoparticles. *Nanoscale*. 2018;10(33):15723-35.