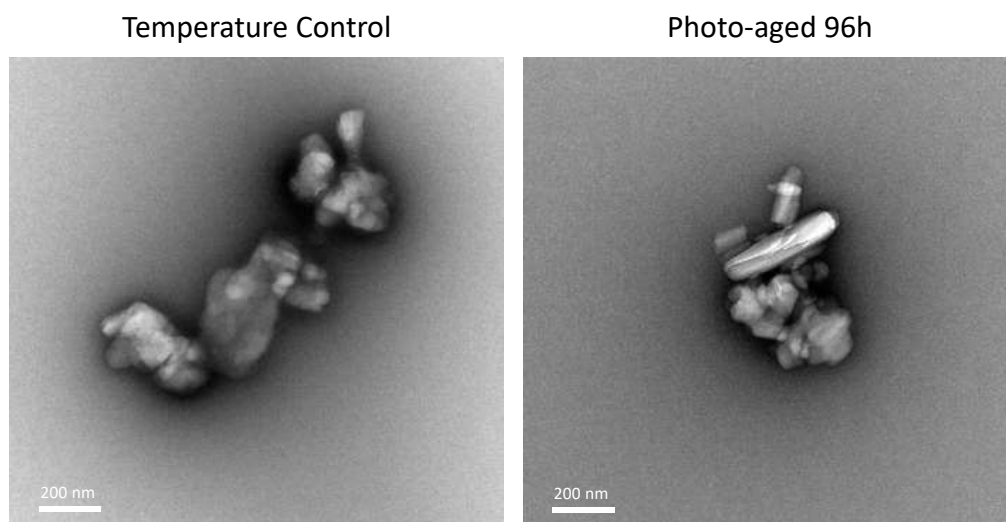


# Accelerated aging modulates the toxicological properties of the diazo tattoo pigment PO13

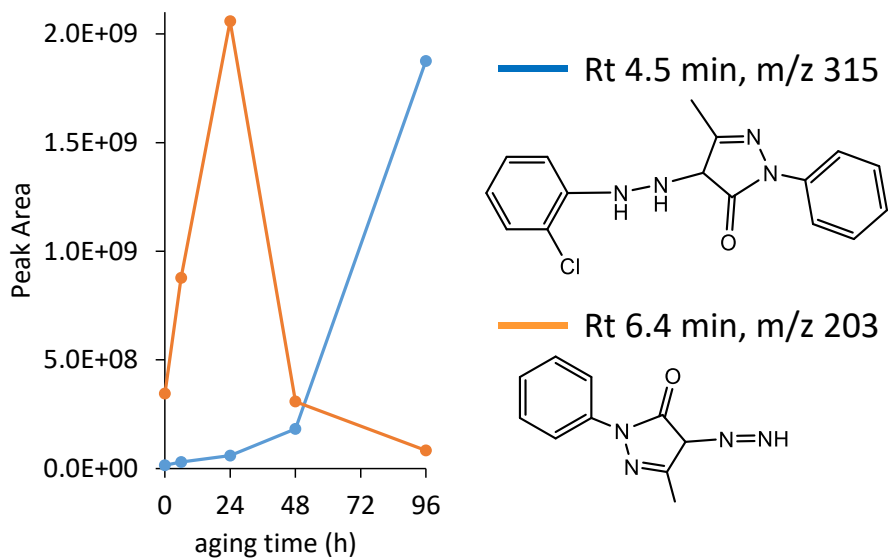
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## Supplementary Information

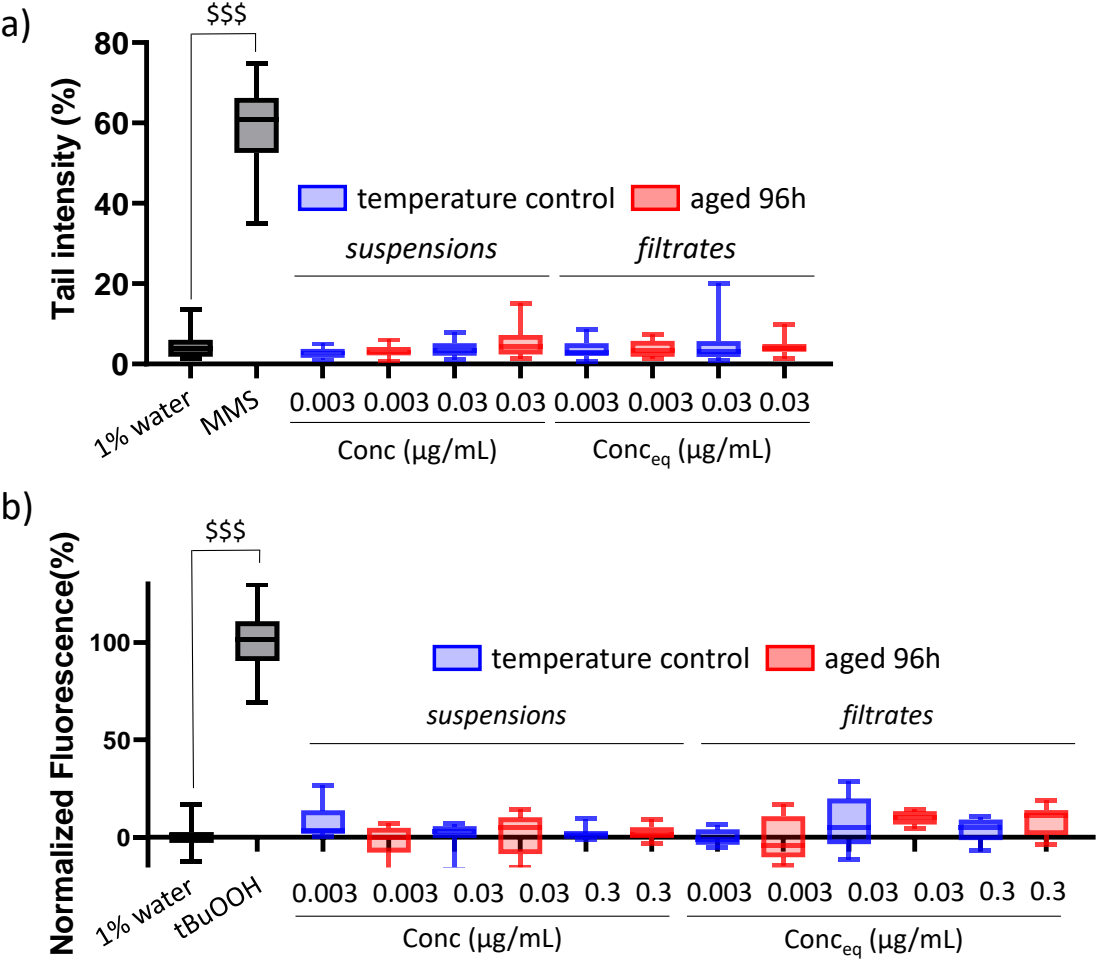
**Figure S1:** Transmission electron microscopy analysis of Pigment Orange 13 particles. Particles were taken from samples of either temperature control or photo-aged suspensions.



**Figure S2:** Time-course study of formation of two PO13 photoproducts in aqueous suspension. The two photoproducts ( $m/z = 315$  and  $m/z = 203$ ) were eluted at 4.5 and 6.4 min, respectively. They were quantified by integration of the MS2 chromatograms at different times of aging. The putative structure were inferred from the molecular weight and MS2 features.



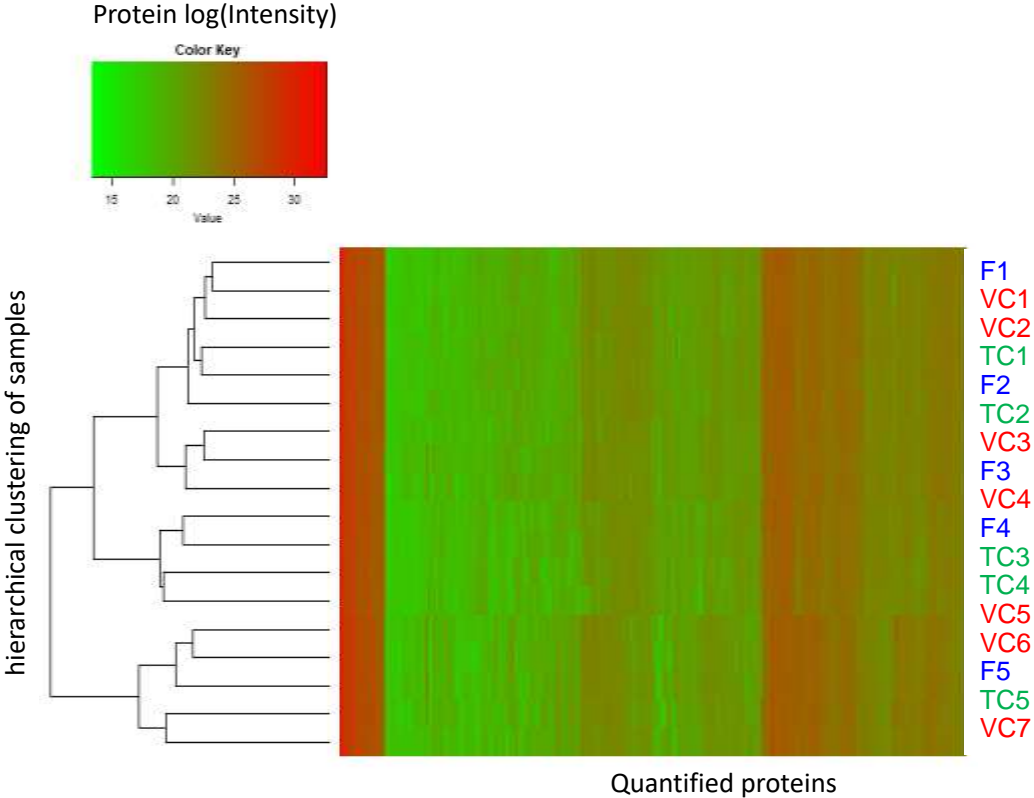
**Figure S3:** Detection of a) induction of DNA strand breaks and b) production of ROS in HaCaT keratinocytes exposed to PO13 suspensions or filtrates of increasing concentration. Samples were either photo-aged or temperature controls. Water (1%) was used as vehicle control. MMS and tBuOOH were used as positive controls for the comet assay and the H<sub>2</sub>-DCFDA test, respectively. Results were expressed as the median with the first and third quartiles and the minimum and maximum values. \$ indicates significant differences from vehicle control (Kruskal-Wallis test followed by Dunn's multiple comparisons, \$\$\$ p<0.001).



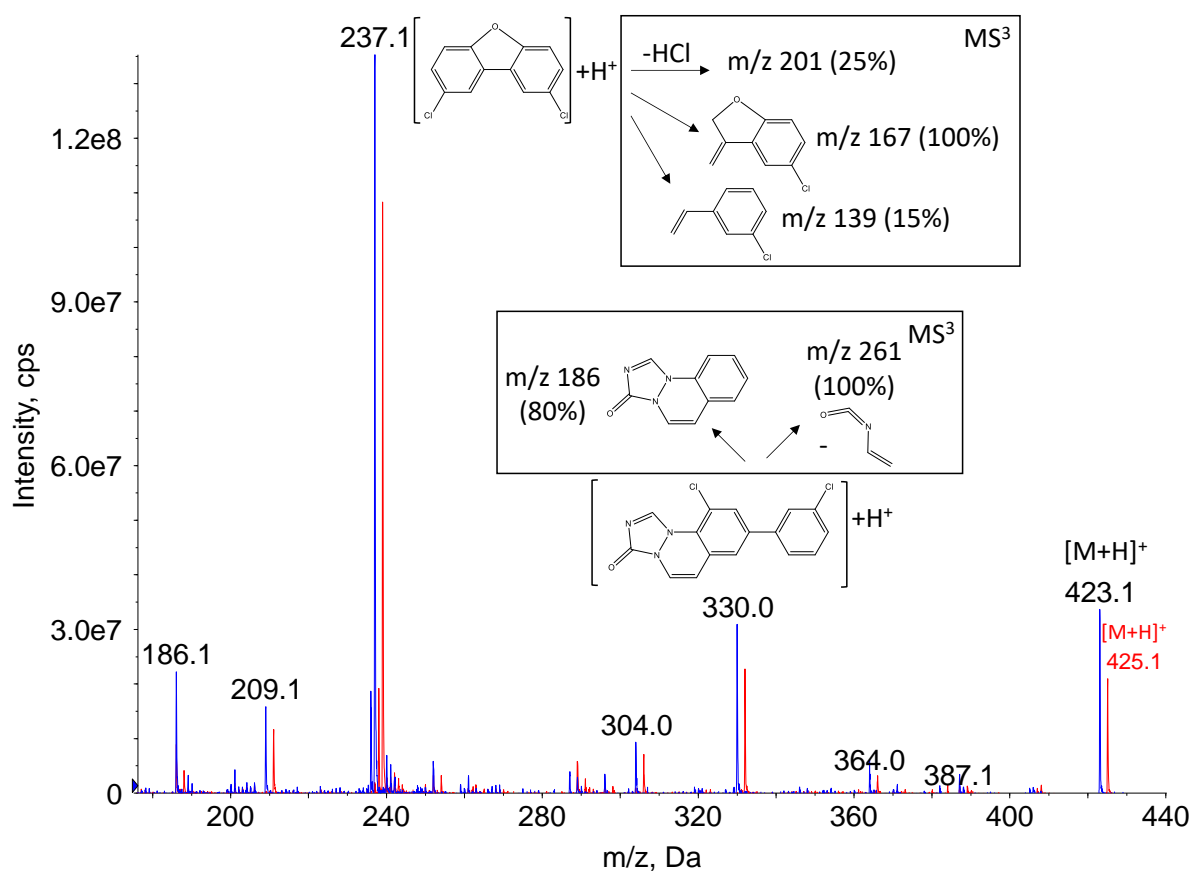
**Table S1.** Modulation of protein expression involved in redundant biological pathways after exposure of HaCaT cells to a photo-aged suspension of Pigment Orange 13 or its filtrate, compared to temperature control. Biological pathways common to at least two modulated proteins are indicated. Red and green indicate overexpressed and underexpressed proteins, respectively.

Proteins	Fold change	p-value	Biological pathway involving several proteins whose expression is modulated
Photoaged suspension			
ASAH1	3.42	0.039	Lipid metabolism
SPTC2	0.54	0.027	
PGES2	5.64	0.005	
AGK	4.41	0.046	
UMPS	2.32	0.048	Metabolism of pyrimidines (UMPS, HPRT) and purines (APT)
HPRT	3.64	0.034	
APT	2.01	0.032	
CUL2	2.29	0.022	Protein modification (ubiquitination)
UBR4	2.49	0.009	
Filtrate of photoaged suspension			
PGH1	10.31	0.019	Lipid metabolism
ACOT8	2.45	0.009	
ECI2	2.89	0.036	
CP1B1	0.24	0.000	
AL4A1	2.30	0.003	Metabolism of pyrimidines (UMPS, HPRT) and purines (APT)
FAAA	2.46	0.011	
UMPS	3.40	0.025	Protein modification (ubiquitination)
PGH1	10.31	0.019	

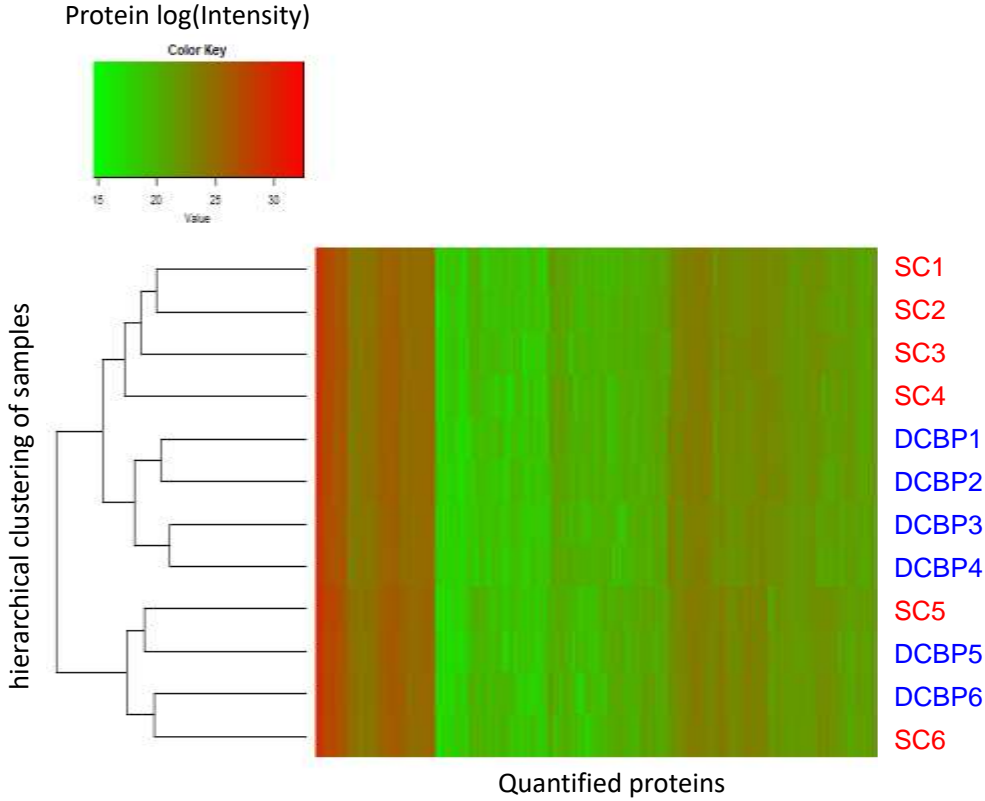
**Figure S4:** Heatmap and dendrogram for the study of the effect of Pigment orange 13 filtrate on the proteome. Cells were exposed to the filtrate of a PO13 suspension photo-aged for 96h (F), its temperature control (TC) and the vehicle control (VC). The *heatmap* represents the intensity of protein expression after the filtration, normalization and imputation steps. The scale is indicated at the top left of the figure. The dendrogram indicates the hierarchical clustering of the samples by statistical treatment.



**Figure S5:** Fragmentation mass spectra of DCBP, the PO13 photoproduct, isolated from photodegradation in isopropanol. The sample was analyzed on a 6500 QTrap spectrometer with the third quadrupole used as a linear ion trap. The reported spectrum was obtained either upon fragmentation of the pseudo molecular ion at  $m/z$  423 (2  $^{35}\text{Cl}$  atoms, blue trace) or  $m/z$  425 (1  $^{35}\text{Cl}$  and 1  $^{37}\text{Cl}$ , red trace). The fragments of the two spectra differed by 2 mass units, showing the presence of 2 chlorine atoms. One exception was the fragment at  $m/z$  186 which was similar for both ions. The insets report some MS3 information. The percentages correspond to the relative intensity of the ions in the MS3 spectra.



**Figure S6:** Heatmap and dendrogram for the study of the impact of DCBP on the proteome. All DCBP samples and solvent control (SC) are shown. The heatmap represents the intensity of protein expression after filtration, normalization, and imputation steps. The scale is indicated at the top left of the figure. The dendrogram indicates the hierarchical clustering of samples by statistical treatment.



**Table S2:** Modulation of protein expression involved in redundant biological pathways after exposure of HaCaT cells to DCBP. Biological pathways common to at least two modulated proteins are indicated in this table. Red and green indicate overexpressed and underexpressed proteins, respectively.

Proteins	<i>Fold change</i>	<i>p-value</i>	Biological pathway involving several proteins whose expression is modulated
CP1B1	138.80	4.99E-11	Biosynthesis of steroid hormones; lipid metabolism; vitamin A metabolism
CP1A1	646.78	5.89E-09	
CP4F3	0.44	0.014	Lipid metabolism
ECI2	0.37	7.95E-03	
OHCHR	0.48	0.045	
TECR	0.45	0.032	
AGK	0.22	4.54E-03	
ASAH1	0.17	9.13E-04	
SGPL1	0.71	0.040	
CDC23	0.43	5.19E-03	Protein modification (ubiquitination)
PRP19	0.55	0.024	
TMTC3	0.34	0.019	Protein modification (glycosylation)
ALG2	0.40	0.037	
DOPP1	3.26	0.015	
GALT5	1.64	0.043	
3HIDH	0.51	6.21E-03	Biosynthesis or degradation of amino acids
OAT	0.64	0.033	
PCKGM	0.45	8.01E-04	Gluconeogenesis
G6PC3	2.96	0.049	
UMPS	0.31	7.11E-03	Metabolism of pyrimidines or purines
PURA2	3.42	0.013	



**Figure S7:** Comparison of the cytotoxicity of a series of pristine pigments in HaCaT cells (concentration  $0.3 \text{ mg mL}^{-1}$ ). PG7: Pigment Green 7, PB15:3: Pigment Blue 15:3, PV23: Pigment Violet 23, PY74: Pigment Yellow 74, PR254: Pigment Red 254, PR122: Pigment Red 122, PO13: Pigment Orange 13. Viability was assessed by the MTT assay. Results, obtained from three independent biological replicates performed in technical triplicates, are expressed as the median, the first and third quartiles and the minimum and maximum values.

