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Toxicity and DNA repair in normal human keratinocytes co-exposed to benzo[a]pyrene and sunlight

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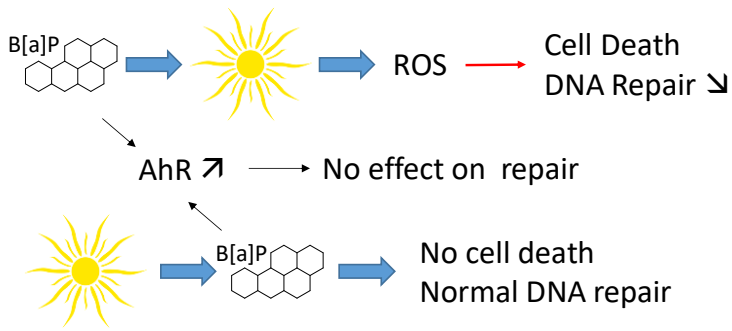
1 **Abstract**

2 Skin has the potential to be exposed to both solar UV radiation and polycyclic aromatic
3 hydrocarbons, especially in occupational environments. In the present work, we investigated
4 how benzo[a]pyrene (B[a]P) modulates cellular phototoxicity and impacts formation and repair
5 of pyrimidine dimers induced by simulated sunlight (SSL) in normal human keratinocytes
6 (NHK). We were especially interested in determining whether the aryl hydrocarbon receptor
7 (AhR) was involved since it was recently shown to negatively impact repair. Addition of 1 μ M
8 B[a]P after exposure to 2 minimal erythemal doses of SSL had little impact on NHK. The
9 inverse protocol involving incubation with B[a]P followed by irradiation led to a strong increase
10 in phototoxicity. Repair of DNA photoproducts was drastically impaired. Using agonists and
11 antagonists of AhR allowed us to conclude that this factor was not involved in these results.
12 Observation of a strong increase in the level of the oxidative marker 8-oxo-7,8-dihydroguanine
13 in the protocol involving B[a]P treatment followed by exposure to SSL strongly suggested that
14 a photosensitized oxidative stress was responsible for cell death and inhibition of DNA repair.
15 Accordingly, both adverse effects were diminished with a lower concentration of B[a]P and a
16 lower SSL dose, leading to less oxidative stress.

17 Keywords: Human skin; sunlight; DNA damage; polycyclic aromatic hydrocarbons; co-
18 exposure; keratinocytes

19

20 **Graphical Abstract**



22 Incubation of human keratinocytes with B[a]P before exposure to simulated sunlight increased
23 cell death and hampered DNA repair. The opposite protocol had no effect on these two
24 endpoints. A significant contribution of AhR activation was ruled out, while photo-oxidative
25 stress was proposed to play a major role under the former experimental conditions.

26

27 **1. Introduction**

28 Skin cancer is a major health concern. A large amount of experimental and epidemiological
29 studies have shown the involvement of overexposure to solar UV radiation (Melnikova and
30 Ananthaswamy, 2005; Molho-Pessach and Lotem, 2007). UV photons, in particular UVB, are
31 absorbed by DNA where they induce the formation of cyclobutane pyrimidine dimers (CPD),
32 pyrimidine (6-4) pyrimidone photoproducts (64PP), and their Dewar valence isomers (Cadet et
33 al., 2005). Bipyrimidine photoproducts are responsible for the mutations found in skin tumors,
34 which are mostly encountered at bipyrimidine sequences (Brash, 2015; Brash et al., 1991;
35 Ziegler et al., 1993). Solar light is not the only genotoxic agent to which skin is exposed. Indeed,
36 chemicals can interact with skin and cause deleterious effects. Polycyclic aromatic
37 hydrocarbons (PAH) are among these toxic compounds to which workers may be cutaneously
38 exposed (Bonzini et al., 2013; Cirila et al., 2007; Herbert et al., 1990; Sellappa et al., 2011;
39 Serdar et al., 2012; Vaananen et al., 2005). PAH are by-products of the combustion of organic
40 matter and are thus found in many products used in industry or released by numerous industrial
41 processes. Among other deleterious health effects, PAH are suspected of exhibiting genotoxic
42 and carcinogenic effects in skin (Boffetta et al., 1997).

43 The toxicity and genotoxicity of PAH in skin has been well investigated, but a complete
44 understanding of the cutaneous impact of these compounds has to include their co-exposure
45 with sunlight, in particular the UV portion of its spectrum. Workers who conduct their
46 occupational activities outdoors (i.e., roofers, road construction crew) can be simultaneously
47 exposed to solar radiation and PAH. Workers exposed to PAH indoors may be exposed to
48 sunlight following the working hours, for instance during recreational activities. In addition to
49 these concerns for occupational safety, the role of co-exposure to PAH and sunlight has been
50 recently pointed out in the general population (Marrot, 2018; Zegarska et al., 2017). Therefore,

51 a precise understanding of biological processes impacted by simultaneous exposure to PAH and
52 sunlight is mandatory to propose an efficient protection strategy.

53 A first possible mechanism is a phototoxicity effect driven by photosensitized reactions and
54 oxidative stress. This aspect has been well studied *in vitro* (Crallan et al., 2005; Mauthe et al.,
55 1995; Soeur et al., 2017; Yu et al., 2006). An additional role of solar UV could be the conversion
56 of parent PAH into more toxic photodegradation products (Teranishi et al., 2010; Toyooka and
57 Ibuki, 2007). An *in vivo* experiment has also shown that co-exposure of mice to UVA and B[a]P
58 increased the level of DNA adducts in skin, possibly as the result of their impaired repair (Saladi
59 et al., 2003). A link with cancer could also be that both B[a]P and UV modulate cell
60 proliferation (Pandey et al., 2018).

61 Another pathway linking PAH and UV exposure involves the aryl hydrocarbon receptor (AhR).
62 This important factor in the cellular response to xenobiotics is essential for the triggering of the
63 metabolism machinery and is activated by binding to compounds, such as dioxins and PAH.
64 Interestingly, UV radiation has also been shown to induce the activation of AhR and trigger a
65 wide variety of biological responses (Esser et al., 2013; Rannug and Fritsche, 2006). Among
66 them is the activation of the xenobiotic metabolism pathway and the overexpression of CYP450
67 enzymes (Fritsche et al., 2007; Nair et al., 2009). This effect was proposed to be mediated by
68 endogenous tryptophan photoproducts and, in particular, 6-formylindolo[3,2-b]carbazole
69 (FICZ), which has been detected in irradiated cells only after incubation with very high, non-
70 physiological concentrations of tryptophan (Fritsche et al., 2007) but not under more
71 biologically relevant conditions (Youssef et al., 2018). The origin and the extent of this
72 stimulation of metabolization remain thus to be assessed. In contrast to what was expected, we
73 recently reported that simulated sunlight (SSL) applied to primary cultures of human
74 keratinocytes or skin explants reduced the B[a]P-induced expression of CYP450 and the level
75 of the B[a]P diol epoxide adducts in DNA (von Koschembahr et al., 2018). Similar results were

76 obtained for the production of metabolites in skin co-exposed to complex PAH mixtures and
77 SSL (Bourgart et al., 2019). The extent of AhR stimulation by sunlight in the presence of PAH
78 is thus an important issue in understanding the toxic and genotoxic effects of co-exposures in
79 skin, particularly since AhR was recently reported to exhibit a negative impact on DNA repair
80 (Pollet et al., 2018).

81 The present work was thus designed to explore the impact of the presence of B[a]P on the
82 deleterious effects of SSL in primary culture of human normal keratinocytes (NHK). We
83 previously studied the modulation of PAH metabolism and DNA damaging properties of B[a]P
84 in the same biological model (von Koschembahr et al., 2018). Here, we focused on cytotoxicity
85 and formation and repair of UV-induced pyrimidine dimers. Emphasis was placed on the
86 possible role played by AhR.

87 **2. Material and methods**

88 *2.1 Culture of primary human keratinocytes*

89 Keratinocyte cultures were prepared from fresh human skin samples, as previously described
90 (Mouret et al., 2006). Human skin samples were obtained according to the French Public Health
91 Code on the use of surgical waste (article L1245-2). Donors were healthy females undergoing
92 elective breast reduction surgery (Centre Hospitalier Universitaire de Grenoble, Grenoble,
93 France) who gave their informed consent. All experiments were performed in accordance with
94 relevant guidelines and regulations. Storage of human skin samples was listed in the
95 CODECOH DC-2019-3391 document validated by the appropriate authorities. Donors were
96 between the ages of 17-58, and their skin were phototypes II or III of the Fitzpatrick
97 classification (Fitzpatrick, 1988). NHK were grown in keratinocyte serum-free medium
98 (KFSM) containing 1% Pen/Strep, 25 µg/mL bovine pituitary extract (BPE), and 0.9 ng/mL of
99 recombinant human epidermal growth factor (EGF). BPE and EGF were removed from the cell

100 culture media 24 hours before B[a]P treatments or irradiations, and throughout the subsequent
101 experiment.

102 *2.2 Treatment of keratinocytes*

103 Protocols involved exposure to SSL and/or treatment by B[a]P. Exposures to SSL were
104 performed with a LS1000 Solar Simulator (Solar Light Company, Glenside, PA), which emitted
105 wavelengths in the 290-400 nm range. The radiation intensity was $22 \text{ J cm}^{-2} \text{ h}^{-1}$ corresponding
106 to 6 minimal erythema doses (MED) per hr. The maximal SSL dose used was 2 MED. B[a]P
107 (Sigma-Aldrich) diluted in DMSO at 10 mM was added to the culture medium to reach the final
108 targeted concentration which ranged between 0.05 and 2 μM depending on the experiments.
109 For the SSL/B[a]P protocol, keratinocytes were rinsed twice with PBS and exposed to 2 MED
110 SSL in PBS. They were then maintained in PBS in the incubator for 1 hour. PBS was removed,
111 and KFSM with or without B[a]P diluted in DMSO was added to reach the final targeted
112 concentration. Samples were collected after increasing periods of time. Controls were incubated
113 with medium containing pure DMSO. For samples treated under the B[a]P/SSL protocol,
114 medium containing 1 μM or less B[a]P was added to the cells. The samples were then
115 maintained in the incubator. Twenty-four hours later, culture medium was removed and the
116 samples rinsed twice with PBS. Similar pre-treatments were performed with medium
117 containing either 1 μM B[a]P and the AhR antagonist CH223191 (concentrations from 2 to 25
118 μM in DMSO) or 2,3,7,8-Tetrachlorodibenzo-p-dioxine (TCDD, concentrations from 2 to 25
119 nM in toluene). SSL irradiations were performed in PBS, and NHK were placed back in culture
120 medium with or without B[a]P. Cell pellets were collected as technical quadruplicates using
121 biological replicates after increasing periods of time and frozen at -20°C . UVB irradiation was
122 performed like exposure to SSL with a 2x15 W UVB lamp (VL-245G, Bioblock) exhibiting an
123 emission spectrum centered at 312 nm.

124 *2.3 Determination of cellular toxicity in NHK*

125 Cytotoxicity of the exposure to SSL and/or B[a]P was assessed from the measurement of
126 mitochondrial activity by using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium
127 bromide (MTT) assay. NHK (20,000 cells per well) were grown in 96-well plates, and exposed
128 to the chosen protocol in terms of B[a]P concentration and dose of SSL. Additionally, NHK
129 were also exposed to CH223191 or TCDD in order to ensure the working concentrations did
130 not have any major cytotoxic effects. After 24 or 48 hours, the exposure media were replaced
131 with 100 μ L of MTT solution and the plates were incubated 1 hour at 37°C. Formazan crystals
132 were dissolved with 100 μ L of DMSO. Absorption was then measured at 560 nm. Assays were
133 reproduced three times with cells from three different donors independently, with n=6, i.e. 6
134 independent replicates in each independent experiment.

135 *2.4 Clonogenicity*

136 Cells were seeded at 20 cells/cm² and grown in 6-well plates (area 9.5 cm²) for 24 hours before
137 being exposed to B[a]P concentration and SSL dose at the chosen values. Irradiations were
138 performed in PBS. Fresh medium was then added, which was changed every fourth day. When
139 colonies in the control samples reached a cell content of approximately 50, namely around the
140 10th day of culture, the medium was removed from all plates and a solution of crystal violet
141 (0.5% in H₂O/EtOH 1:1) was added. After rinsing with water, the colonies were counted in
142 each plate. Results were expressed in % with respect to controls.

143 *2.5 Quantification of DNA damage*

144 DNA was extracted from pellets containing 1 to 2 million cells using a protocol developed to
145 limit spurious oxidation (Ravanat et al., 2002). DNA was recovered in 50 μ l of a deferoxamine
146 solution. DNA was subsequently hydrolyzed by a cocktail of enzymes, all purchased from
147 Sigma-Aldrich. The first incubation was performed using phosphodiesterase II, DNase II, and

148 nuclease P1 (37°C, 2 hours). The second incubation step was then performed using
149 phosphodiesterase I and alkaline phosphatase (37°C, 2 hours).

150 Samples were then injected on an HPLC system (ExionLC, SCIEX) equipped with a reverse-
151 phase HPLC column (150 x 2 mm ID, 3 µm particle size, ODB, Interchim, Montluçon, France).
152 The mobile phase was a gradient of acetonitrile (2 to 25 %) in 2 mM triethylammonium acetate
153 (pH 6). CPDs, 64PPs, and Dewars from TT, TC, CT and CC dinucleotides were quantified on
154 a tandem mass spectrometer (SCIEX QTrap 6500⁺) as previously described (Douki, 2013).
155 8-Oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) was quantified in the same samples in a
156 second injection. The HPLC solvent was then a gradient of methanol in 2 mM ammonium
157 formate and the monitored transition in positive ionization electrospray was m/z 284→168.
158 HPLC-MS/MS was also used to quantify adducts between 2'-deoxyguanosine and B[a]P diol
159 epoxide (BPDE-*N*²-dGuo) using a previously described method (Genies et al., 2013; von
160 Koschembahr et al., 2018). The mass spectrometer was used in the positive ionization mode
161 and the monitored transitions were m/z 570→257 and m/z 570→454. The HPLC solvent was
162 a gradient of acetonitrile in 2 mM ammonium formate. In all types of analyses, unmodified
163 nucleosides were detected with a UV detector. External calibration of the response of the
164 detector was performed for each run of analyses. Authentic standards were injected several
165 times during the run and permitted to control the stability of the sensitivity of the detection and
166 of the retention times. Results were expressed in number of DNA lesions per million normal
167 bases.

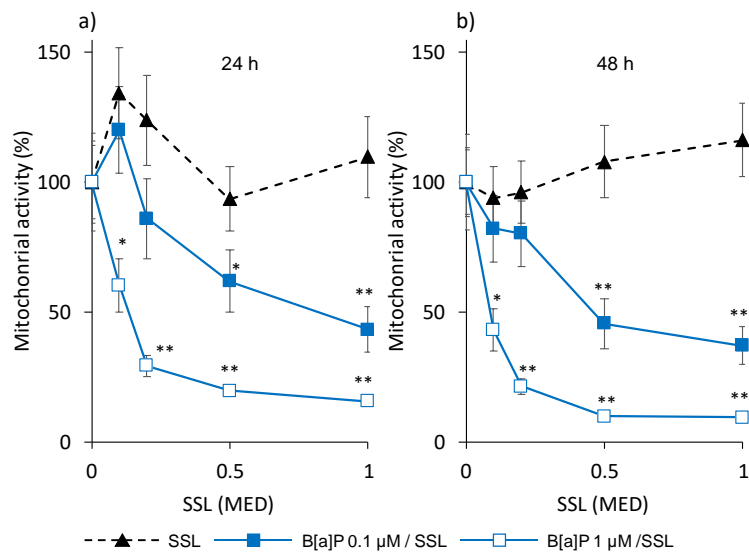
168 *2.6 Statistical analysis*

169 Statistical analyses were performed on pools of replicates originating from different donors.
170 Data were statistically analyzed in GraphPad PRISM using one-way ANOVA followed by
171 application of the non-parametric Dunn's test for multiple comparisons.

172 **3. Results**

173 *3.1 Short-term phototoxicity of co-exposure to B[a]P and SSL*

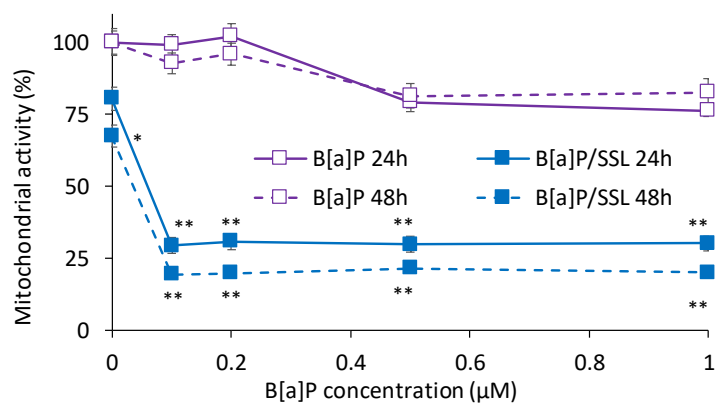
174 Evaluation of the phototoxicity was based on the measurement of the mitochondrial activity,
175 which relied on the MTT assay performed on NHK collected from three different donors.
176 Experiments were repeated in biological triplicates for each of them. We first performed
177 experiments where NHK were incubated with fixed B[a]P concentrations, namely 0.1 and 1
178 μM , and then exposed to increasing doses of SSL (B[a]P/SSL protocol, Fig. 1). The
179 mitochondrial metabolic activity rapidly dropped with increasing SSL dose in NHK treated
180 with 1 μM B[a]P. A similar trend was observed with 0.1 μM B[a]P, but the effect of the SSL
181 dose was lower. An additional experiment was performed with keratinocytes exposed to 2 MED
182 SSL in the presence of increasing concentrations of B[a]P (Supplementary data Fig. S1). B[a]P
183 alone exhibited limited effect while the combined B[a]P/SSL treatment led to increased
184 response already at 0.1 μM B[a]P. It may be added that little difference was observed between
185 the mitochondrial activities of between untreated NHK and those submitted to SSL or B[a]P
186 only (Fig. S1). No effect was observed for the SSL/B[a]P protocol, namely when SSL was
187 applied before treatment with PAH. The values for the MTT assay were 82 ± 9 and 89 ± 12 %
188 for the SSL and SSL/B[a]P protocols, respectively (n=3).



189

190 **Figure 1:** Loss of mitochondrial activity in NHK exposed to increasing doses of SSL with
 191 irradiation performed after 24 hours of treatment with either 0.1 or 1 μM B[a]P. MTT assay
 192 was performed 24- and 48-hours following SSL exposure. Results are mean ± standard error
 193 obtained from three different donors. Statistical significance of the difference with respect to
 194 NHK exposed to SSL only: * $p < 0.05$, ** $p < 0.01$.

195



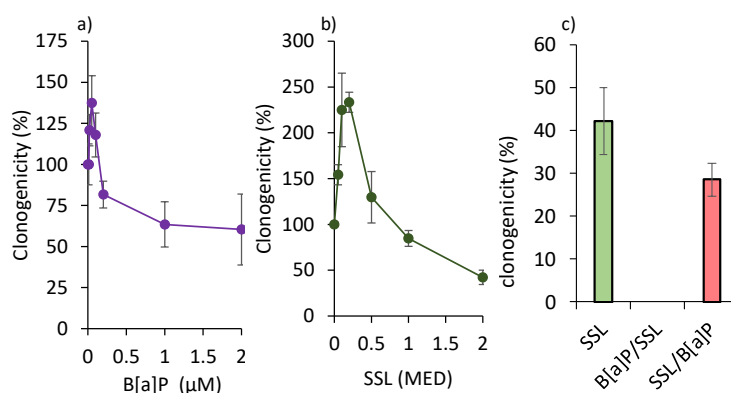
196

197 **Figure S1:** Loss of mitochondrial activity in NHK exposed to increasing concentrations of
 198 B[a]P for 24 hours before being irradiated with 2 MED SSL. MTT assay was performed 24-
 199 and 48-hours following SSL exposure. Results are mean ± standard error obtained from three
 200 different donors. Statistical significance of the difference with respect to NHK exposed to
 201 B[a]P only: * $p < 0.05$, ** $p < 0.01$.

202

203 3.2 Loss of clonogenicity in keratinocytes exposed to B[a]P followed by SSL irradiation

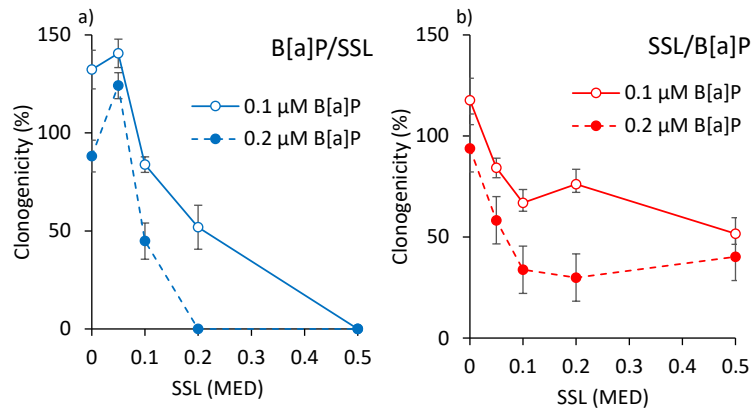
204 In order to better characterize the phototoxicity of co-exposure to SSL and B[a]P, we then
205 performed a series of clonogenicity assays under our various experimental conditions. First, we
206 studied the effect of exposure to pure B[a]P or exposure to SSL only. Under the most drastic
207 conditions (1 μM B[a]P or 2 MED SSL), the clonogenicity was approximately 50% of that
208 observed for untreated cells. It should be stressed that a stimulation effect was observed for the
209 lowest doses of the two agents (Supplementary data Fig. S2). When cells were exposed to 2
210 MED of SSL after 24 hours of incubation with 1 μM of B[a]P, no colonies were detected in the
211 plates. In contrast, when the converse protocol was applied (1 μM B[a]P after 2 MED SSL),
212 the clonogenicity was roughly 70% of that of cells exposed to SSL only. In order to better
213 characterize the impact of co-exposure to B[a]P and SSL, we repeated the experiments on lower
214 ranges of B[a]P concentrations and SSL doses limited between 0.1 and 0.5 MED. As observed
215 above, drastic effects were observed with the B[a]P/SSL protocol since no colonies were
216 observed at 0.2 μM B[a]P with the 2 doses of SSL used, as well as at 0.2 μM with 0.1 MED
217 (Fig. 2). In contrast, more colonies were detected under all conditions in the SSL/B[a]P
218 protocol.



219

220 **Figure S2:** Loss of clonogenicity observed in NHK exposed to a) increasing concentrations of
221 B[a]P, b) increasing doses of simulated sunlight (SSL), and c) co-exposure to 1 μM B[a]P

222 and 2 MED SSL following either the B[a]P/SSL or the SSL/B[a]P protocols. Results from two
223 donors each studied in triplicate were pooled. Results represent the proportion of colonies
224 with respect to vehicle-only treated controls. Reported data represent mean \pm standard error
225 ($n=6$).



226

227 **Figure 2:** Loss of clonogenicity observed in NHK exposed to increasing doses of SSL in
228 combination with 0.1 or 0.2 μM B[a]P. The exposure conditions were either a) the B[a]P/SSL
229 or b) the SSL/B[a]P protocols. Results from two donors each studied in triplicate were
230 pooled. Results represent the proportion of colonies with respect to controls exposed to SSL
231 without B[a]P. Reported data represent mean \pm standard error ($n=6$).

232

233 3.3 Impact of the addition of B[a]P on repair of DNA photoproducts

234 Since formation of DNA damage is a major cause of cell death, we quantified the level of all
235 pyrimidine dimers in the various samples (Supplementary data, Table S1). Initial distribution
236 of the photoproducts was similar under all conditions. CPDs were the most frequent
237 photoproducts with the previously reported sequence effect (TT > TC > CT > CC). 64PPs were
238 present in lower amounts and partly recovered as Dewar valence isomers. The latter observation
239 is explained by the efficient conversion of 64PPs by the UVA component of SSL. A time-course
240 study of our data showed that in samples exposed to SSL only, 64PPs and Dewars were

241 efficiently repaired. A difference was observed for the four CPDs, with TT CPD being the most
242 slowly removed, while CC CPD was the fastest. These results are in line with previously
243 published data (Mouret et al., 2008).

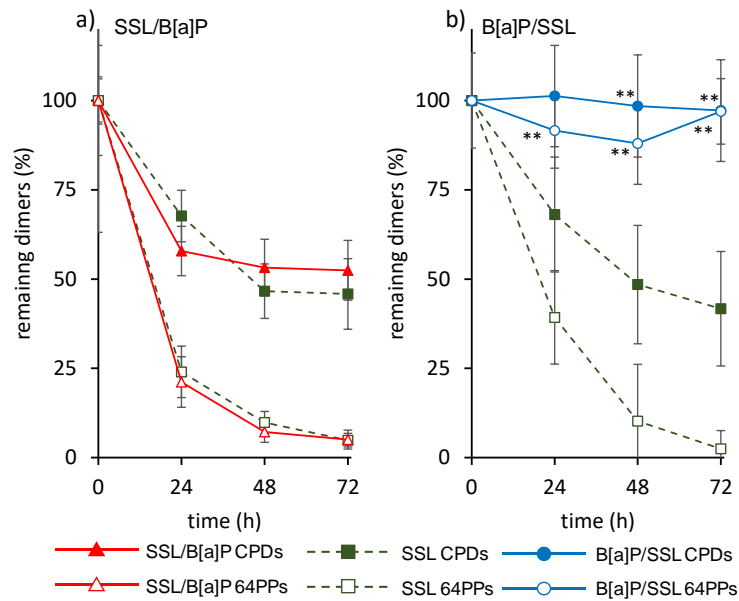
244 The addition of 1 μ M B[a]P 1 hour after exposure to SSL did not modify the repair rate of CPDs
245 (Fig. 3a). To eliminate a possible bias associated with the duration of the delay between
246 irradiation and addition of B[a]P, we repeated the SSL/B[a]P treatment with a delay of 8 hours
247 rather than the 1-hour time period previously used. Under both conditions, addition of B[a]P to
248 the culture medium failed to modify the repair rate of neither CPDs nor 64PPs and Dewars
249 (Supplementary data Fig. S3). In contrast, incubation of keratinocytes for 24 hours with B[a]P
250 prior to exposure to SSL completely inhibited the repair of the three types of photoproducts
251 (Fig. 3b). When UVB was used instead of SSL, no modulation of repair by B[a]P was observed,
252 whether it was added before or after irradiation (Supplementary data Fig. S4).

253

254 **Table S1:** Level of pyrimidine dimers in NHK exposed to 2 MED SSL only or in combination
 255 with 1 μ M B[a]P treatment. Results are expressed in number of photoproducts per millions
 256 bases and represent mean \pm standard error.

time	TT CPD	TT 64PP	TT Dewar	TC CPD	TC 64PP	TC Dewar	CT CPD	CC CPD
SSL/B[a]P experiment								
SSL only								
0h	133.2 \pm 10.4	3.0 \pm 0.3	1.7 \pm 0.1	57.4 \pm 4.8	7.6 \pm 1.4	2.2 \pm 0.4	34.1 \pm 1.6	20.9 \pm 4.9
24h	95.4 \pm 10.1	0.7 \pm 0.2	0.0 \pm 0.0	39.3 \pm 7.2	2.8 \pm 0.9	0.0 \pm 0.0	12.2 \pm 2.6	19.3 \pm 6.2
48h	70.3 \pm 8.2	0.6 \pm 0.3	0.0 \pm 0.0	19.4 \pm 5.1	0.7 \pm 0.5	0.0 \pm 0.0	9.5 \pm 2.5	15.3 \pm 6.0
72h	76.3 \pm 9.2	0.2 \pm 0.1	0.0 \pm 0.0	20.1 \pm 6.6	0.4 \pm 0.2	0.0 \pm 0.0	6.4 \pm 1.5	9.8 \pm 5.2
SSL / B[a]P								
0h	131.9 \pm 8.1	2.1 \pm 0.6	1.3 \pm 0.3	55.5 \pm 5.3	5.9 \pm 2.3	1.3 \pm 0.3	34.4 \pm 1.5	24.9 \pm 2.4
24h	89.4 \pm 8.8	0.6 \pm 0.2	0.0 \pm 0.0	30.4 \pm 3.1	1.7 \pm 0.6	0.0 \pm 0.0	11.9 \pm 1.9	11.1 \pm 3.2
48h	89.2 \pm 12.9	0.3 \pm 0.2	0.0 \pm 0.0	21.3 \pm 4.5	0.5 \pm 0.0.3	0.0 \pm 0.0	8.5 \pm 1.6	12.3 \pm 4.6
72h	91.3 \pm 13.1	0.1 \pm 0.1	0.0 \pm 0.0	19.6 \pm 5.6	0.3 \pm 0.2	0.0 \pm 0.0	6.7 \pm 1.6	11.8 \pm 4.0
B[a]P/SSL experiment								
SSL only								
0h	111.0 \pm 14.8	2.5 \pm 0.3	1.1 \pm 0.1	71.2 \pm 17.4	10.9 \pm 1.2	2.6 \pm 0.7	30.4 \pm 3.5	13.8 \pm 2.0
24h	73.0 \pm 12.5	0.4 \pm 0.1	0.7 \pm 0.3	59.1 \pm 20.2	4.1 \pm 1.7	1.5 \pm 0.6	11.5 \pm 4.1	10.5 \pm 4.0
48h	48.5 \pm 12.8	0.7 \pm 0.4	0.1 \pm 0.0	43.9 \pm 20.0	0.3 \pm 0.1	0.6 \pm 0.3	8.4 \pm 3.8	8.9 \pm 4.2
72h	41.5 \pm 10.4	0.4 \pm 0.2	0.0 \pm 0.0	44.3 \pm 17.8	0.0 \pm 0.0	0.0 \pm 0.0	3.7 \pm 1.6	4.8 \pm 1.8
B[a]P / SSL								
0h	119.9 \pm 14.4	3.1 \pm 0.4	1.4 \pm 0.2	78.5 \pm 17.2	15.5 \pm 1.7	3.0 \pm 0.7	29.9 \pm 3.4	12.4 \pm 1.7
24h	119.6 \pm 15.4	2.5 \pm 0.3	1.2 \pm 0.2	76.9 \pm 19.3	13.8 \pm 1.7	3.6 \pm 1.0	33.6 \pm 3.9	13.7 \pm 2.3
48h	115.9 \pm 14.4	2.8 \pm 0.3	1.4 \pm 0.2	78.0 \pm 17.5	13.8 \pm 1.4	2.3 \pm 0.4	31.1 \pm 2.9	12.0 \pm 2.0
72h	107.9 \pm 14.8	2.6 \pm 0.3	1.4 \pm 0.3	79.2 \pm 16.7	13.8 \pm 1.6	4.5 \pm 1.6	31.6 \pm 3.2	15.3 \pm 2.2

257



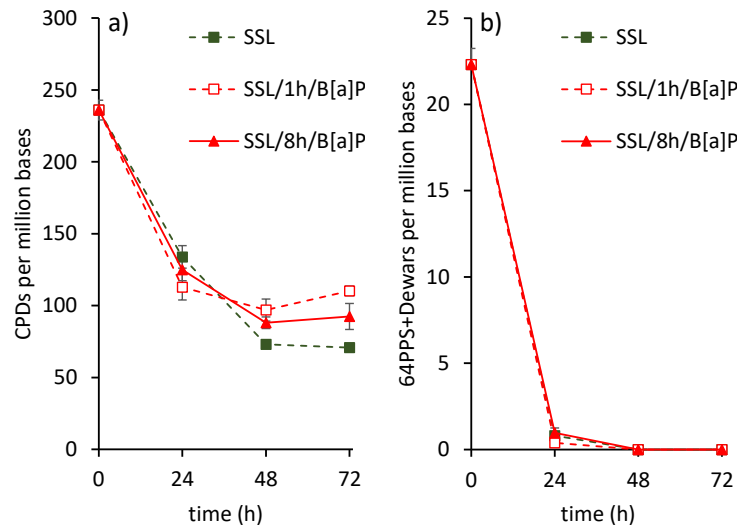
258

259 **Figure 3:** Repair of pyrimidine dimers in NHK exposed to 2 MED SSL only or a) with a
 260 subsequent 1 μ M B[a]P treatment performed 1 hour after irradiation, b) after a 24-hour
 261 preliminary incubation with 1 μ M B[a]P. Results are the sum of either the level of the four
 262 CPDs or of TT and TC 64PPs and Dewars. Results were obtained from three donors each
 263 studied in three independent cultures. Data were normalized to 100 % for each donor and
 264 pooled. They are expressed in percentage of remaining lesions and represent mean \pm
 265 standard error (n=9). Statistical significance with respects to SSL only *: p<0.05; **: p<0.01.

266

p<0.01.

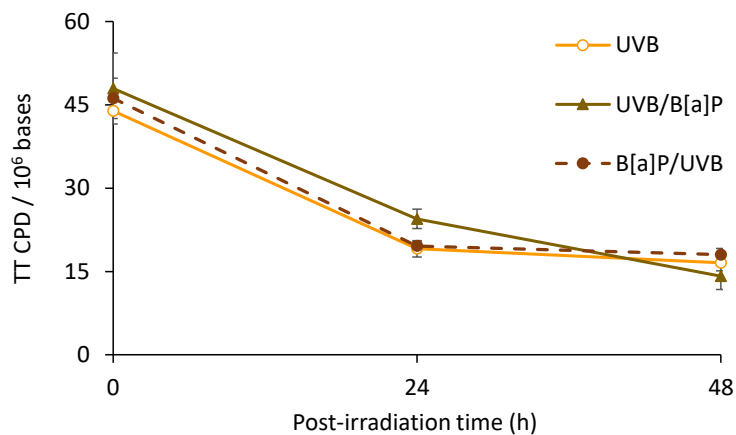
267



268

269 **Figure S3:** Repair of pyrimidine dimers in NHK exposed to 2 MED SSL only or with a
 270 subsequent 1 μM B[a]P treatment performed 1 or 8 hours after irradiation. Data are shown
 271 for a) all 4 CPDs and, b) the TT and TC 64PPs and Dewars. Results from two donors each
 272 studied in triplicate were pooled. They are expressed in number of photoproducts per millions
 273 bases and represent mean \pm standard error (n=6).

274

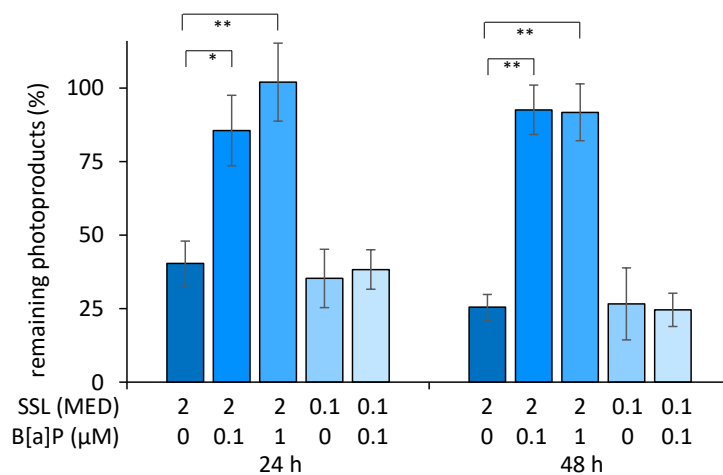


275

276 **Figure S4:** Repair of TT CPD in NHK exposed to either UVB only (UVB), UVB with a
 277 subsequent B[a]P treatment performed 1 hour after irradiation (UVB/B[a]P), or UVB after a
 278 24-hour preliminary incubation with B[a]P (B[a]P/UVB). The B[a]P concentration was 1
 279 μM and the UVB dose 0.1 J/cm². Results are the mean \pm standard deviation for three
 280 independent cell cultures from the same donor.

281

282 To further characterize the effects of incubation of B[a]P prior to SSL irradiation, we performed
 283 a time-course study of the level of bipyrimidine dimers in keratinocytes from additional donors.
 284 We applied the B[a]P/SSL protocol and B[a]P was used at concentrations of both 0.1 and 1 μ M,
 285 with 2 MED SSL. The impact of preliminary incubation with 0.1 μ M B[a]P on repair of CPDs
 286 induced by 2 MED SSL was as significant as with 1 μ M (Fig. 4). When much milder conditions
 287 were used (0.1 MED SSL and 0.1 μ M B[a]P), no impact of B[a]P was observed on the repair
 288 of photoproducts (Fig. 4)



289

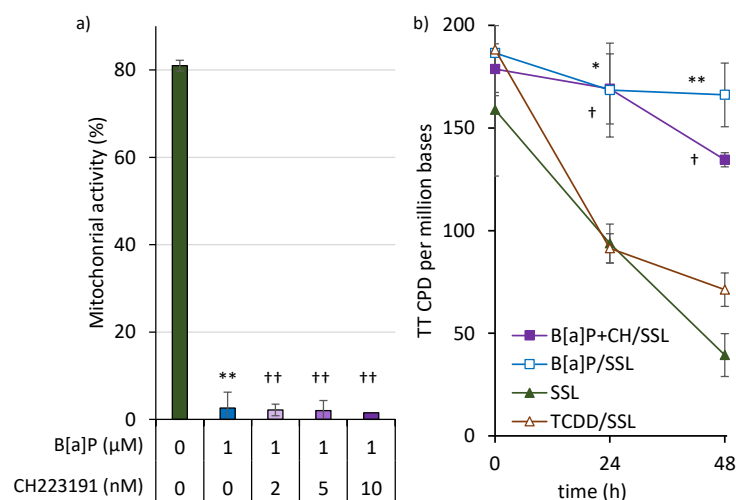
290 **Figure 4:** Repair of CPDs in NHK exposed to SSL only or with a preliminary B[a]P
 291 treatment. Cells from a first series of 3 donors exposed to 2 MED SSL after 24 hour
 292 incubation with B[a]P at 0.1 or 1.0 μ M. Cells from 2 other donors were exposed to 0.1 MED
 293 SSL with or without preliminary treatment with 0.1 μ M B[a]P. Results are expressed in
 294 number of photoproducts per millions bases and represent mean \pm standard error. Statistical
 295 significance with respects to SSL only *: $p < 0.05$; ** $p < 0.01$

296

297 *3.4 Effect of modulation of AhR on the impact of B[a]P on phototoxicity and repair of DNA*
298 *photoproducts*

299 A series of experiments were performed with the dioxin TCDD and the AhR antagonist
300 CH223191. TCDD led to a moderate loss of viability with an average 80% MTT score
301 (Supplementary data, Table S2), with respect to the toluene (vehicle solvent) control over the
302 range of concentrations investigated (Fig. 5a). Toluene controls were performed together with
303 DMSO controls. No difference in viability was detected. CH223191 exhibited no detectable
304 effect on cell viability (Supplementary data, Table S2). The efficiency of the antagonist effect
305 of CH223191 at the concentration used in the subsequent experiments (5 μ M) was checked by
306 measuring by HPLC-MS/MS the level of DNA adducts to the diol epoxide of B[a]P (BPDE-
307 N^2 -dGuo). As expected, the observed value was lower after 24-hour incubation in samples
308 containing CH223191 (23.2 ± 1.2 compared to 8.9 ± 1.7 BPDE- N^2 -dGuo per 10^6 bases with B[a]P
309 and B[a]P+CH223191, respectively). Addition of CH223191 to B[a]P during the pre-treatment
310 step before exposure to SSL improved neither the phototoxicity of the B[a]P/SSL protocol (Fig.
311 5a) nor the inhibition of DNA repair (Fig. 5b). Pre-incubation of NHK with TCDD alone did
312 not affect the repair of photoproducts (Fig. 5b)

313



314

315 **Figure 5:** Effects of the AhR agonist TCDD and AhR-antagonist CH223191 in NHK. a)

316 Decrease in the mitochondrial activity was measured at 48 hours by performing the MTT

317 assay in NHK exposed to SSL (2 MED) after incubation in medium containing B[a]P (1 μM)

318 and increasing concentrations of CH223191 (CH 2, 5, and 10 μM). b) Repair of TT CPD in

319 NHK exposed to SSL (2 MED) with or without a preliminary 24-hour incubation with either

320 B[a]P 1 μM, B[a]P 1 μM + 5 μM CH223191 (CH), or 10 nM TCDD. Results are mean ±

321 SEM from two donors studied in three independent cultures. Statistical significance:

322 B[a]P/SSL vs SSL *: $p < 0.05$; **: $p < 0.01$; B[a]P+CH223191/SSL vs SSL †: $p < 0.05$; ††:

323 $p < 0.01$

324

325 **Table S2:** Mitochondrial activity measured at 48 hours by the MTT assay in NHK exposed to

326 increased concentrations of CH223191 or TCDD. Concentrations are expressed in μM and nM,

327 respectively. Results are expressed in % and represent the mean ± standard deviation for 3

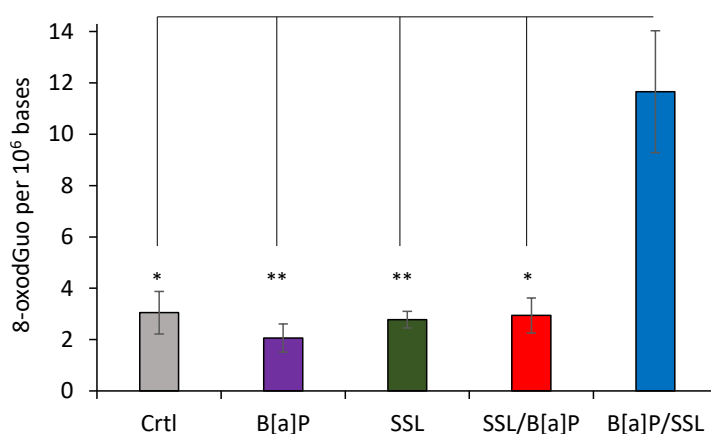
328 donors (n=3) compared to vehicle-only treated controls.

concentration	2	5	10	25
CH223191 (μM)	114±10	89±24	94±13	100±12
TCDD (nM)	68±1	85±6	75±28	84±13

329

330 3.5 Formation of 8-oxodGuo in NHK exposed to B[a]P and SSL

331 The level of 8-oxodGuo was quantified by HPLC-MS/MS in the DNA of NHK exposed either
332 to B[a]P, SSL, the SSL/B[a]P protocol or the B[a]P/SSL protocol. Increase in the level of
333 8-oxodGuo was observed only under the latter conditions when the B[a]P concentration was 1
334 μM and the SSL dose 2 MED with a 4-fold larger value than in control NHK (Fig. 6). In
335 contrast, no significant increase was observed when the latter values were decreased to 0.1 μM
336 and 0.1 MED (2.6 ± 0.5 and 2.9 ± 0.6 8-oxodGuo per 10^7 bases in control and cells exposed to the
337 B[a]P/SSL protocol).



338

339 **Figure 6:** Level of 8-oxodGuo in NHK exposed to 2 MED SSL, B[a]P, SSL/B[a]P and
340 B[a]P/SSL with or without preliminary incubation with 1 μM B[a]P for 24 hours. Controls
341 are non-irradiated, DMSO-treated cells. Samples were collected immediately after
342 irradiation. Data were obtained from two donors each studied in three independent cultures.
343 Results are mean \pm SEM. Statistical differences with respect to B[a]P/SSL: *: $p < 0.05$; **:
344 $p < 0.01$.

345 4. Discussion

346 The present study aimed at assessing whether co-exposure of normal human keratinocytes to
347 B[a]P and simulated sunlight could modulate the phototoxicity and genotoxicity of UV

348 radiation. We also wanted to get insights into the underlying mechanisms, specifically by
349 determining if AhR was involved. It has been shown that, possibly under the influence of
350 tryptophan photoproducts (Diani-Moore et al., 2006; Fritsche et al., 2007; Nair et al., 2009;
351 Rannug et al., 1995), exposure to UV radiation increased, by a factor of approximately 5, the
352 expression of CYP450 genes due to AhR activation (Katiyar et al., 2000; Nair et al., 2009;
353 Rannug and Fritsche, 2006; Wei et al., 1999). Interestingly, it was recently reported that AhR
354 exhibited a negative effect on cellular DNA nucleotide excision repair capacities (Pollet et al.,
355 2018). Another AhR-mediated pathway for the decreased repair efficiency could be the ability
356 to hinder mitochondrial functions and limit the production of ATP, which is essential for repair
357 (Aly and Domenech, 2009; Chen et al., 2010; Hwang et al., 2016; Kennedy et al., 2013).

358 We first studied cell death either with the short-term MTT assay or on a longer time-scale by
359 quantification of the clonogenicity of NHK. In these first experiments, the SSL dose was set at
360 2 MED, which corresponds to less than 1 hour of exposure on a summer day in mid-latitude in
361 Europe with a UV index of 6. The B[a]P concentration was 1 μ M which may be considered as
362 relevant to some occupational exposures. Values as high as 100 pmol/cm² of B[a]P have been
363 reported for workers in the aluminum industry (VanRooij et al., 1992). We have recently
364 reported that the B[a]P content in skin typically exposed to complex mixtures is around 40% of
365 the applied dose after 48h. If a penetration depth of a few hundred of micrometers, namely the
366 epidermis and a part of the dermis, is considered, the corresponding local concentration is then
367 the micromolar range. Cytotoxicity measured by these two complementary approaches showed
368 that under the range of doses used, pure B[a]P and SSL only exhibited limited toxicity. When
369 NHK were exposed to the largest SSL dose studied (2 MED) and then exposed to 1 μ M B[a]P,
370 namely the upper limit of the applied concentrations, phototoxicity was only slightly increased
371 with respect to irradiation only. In addition, the DNA repair kinetics observed with the
372 SSL/B[a]P protocol were similar to those obtained in cells exposed to SSL only, consistent with

373 the limited impact of this treatment on cell survival. These results were the same whether a
374 delay of 1 or 8 hours was applied between exposure to SSL and B[a]P. Altogether, these
375 observations suggest a limited impact of B[a]P on the cellular responses triggered by exposure
376 to UV alone, and therefore a limited effect of the activation of the AhR pathway on DNA repair.

377 In drastic contrast to data obtained with the SSL/B[a]P treatment, the reverse protocol with
378 incubation for 24 hours with 1 μ M B[a]P followed by exposure to 2 MED SSL led to complete
379 cellular toxicity. The same observation was made at 0.5 MED for B[a]P concentrations of 0.2
380 μ M. At 0.1 μ M, cells survived a subsequent exposure to SSL when the dose was 0.1 MED at
381 the most. The bulk of these results shows that the incubation with B[a]P prior to irradiation
382 strongly sensitizes NHK, unlike the reverse protocol. Similar results of a drastically increased
383 phototoxicity by low concentrations of PAH were recently reported for fibroblasts exposed to
384 UVA1 (Soeur et al., 2017). Our results thus show that biologically relevant doses of SSL
385 applied after incubation with B[a]P concentration possibly found upon occupational
386 contamination are much more toxic than the two toxic agents applied separately. It could be
387 tempting to propose that incubation with B[a]P led to activation of the AhR pathway, which
388 resulted in decreased DNA repair (Pollet et al., 2018) and subsequently increased cell death as
389 the result of apoptosis triggered by high levels of DNA damage.

390 However, this hypothesis was the opposite of the conclusion made on the basis of the results of
391 SSL/B[a]P protocol, which shows no effect of the co-exposure. We therefore performed a series
392 of additional experiments to unravel the origin of the phototoxicity and the decreased DNA
393 repair in the B[a]P/SSL experiments. We first investigated the dose effects. A low concentration
394 of 0.1 μ M B[a]P in the latter protocol induced the same effect on DNA repair as 1 μ M when 2
395 MED SSL was applied. This result shows that the decrease in repair activity observed in the
396 B[a]P/SSL protocol is not explained by the sole presence of B[a]P. Normal repair activity and
397 reduced cell toxicity were observed only when both the B[a]P concentration and the SSL dose

398 were lowered to 0.1 μ M and 0.1 MED, respectively. This result further shows that presence of
399 both B[a]P and SSL are required for the observed effects.

400 In order to test the hypothesis of the involvement of AhR in the B[a]P/SSL-mediated negative
401 impact on DNA repair, we repeated the experiments involving SSL irradiation following a 24-
402 hour B[a]P treatment in the presence of CH223191, an AhR antagonist that would be expected
403 to prevent repair inhibition should AhR be involved. We could not see any influence of
404 CH223191 on the effects of the B[a]P/SSL protocol on either the mitochondrial impairment or
405 the repair of DNA photoproducts. We performed the converse experiment by treating
406 keratinocytes with TCDD, a dioxin that is a strong AhR agonist and should hypothetically
407 mimic the effects of B[a]P. Results were compared with those of SSL and B[a]P/SSL protocols.
408 TCDD did not decrease the repair rate of pyrimidine dimers after exposure to SSL. These two
409 series of results allow us to rule out a significant contribution of AhR in the B[a]P-mediated
410 inhibition of DNA repair. This conclusion is in agreement with the limited modulation of DNA
411 repair gene expression and the lack of modification at the repair protein level in mouse
412 hepatoma cell lines exposed to TCDD (Schreck et al., 2009). The fact that addition of B[a]P
413 after irradiation, which efficiently induces AhR in NHK as shown by increased CYP450
414 expression and formation of DNA adducts (von Koschembahr et al., 2018), did not affect repair
415 even 48 hours after exposure to SSL, thus further supporting a very limited role of AhR.

416 A more likely alternative explanation to the impact of B[a]P on UV effects could be a role of
417 photosensitized induction of oxidative stress. This effect has been reported in several *in vitro*
418 studies (Crallan et al., 2005; Mauthe et al., 1995; Ors and Gulce Iz, 2018; Soeur et al., 2017;
419 Vaz Batista Filgueira et al., 2007; Xia et al., 2015; Yu et al., 2006). Accordingly, we observed
420 a larger induction of 8-oxodGuo, a typical DNA oxidation marker, when the B[a]P/SSL
421 protocol was applied under the most cytotoxic conditions. The decreased DNA repair activity
422 could thus be explained by the fact that most cells are dying and metabolically inactive. The

423 strong phototoxicity of B[a]P has been recently documented in human fibroblasts exposed to
424 UVA1 at concentrations of 5 and 50 nM (Soeur et al., 2017). Although these data cannot be
425 directly compared to ours because of the difference in cell type and UV source, they illustrate
426 the strong photosensitizing properties of B[a]P. It should be mentioned that another role of
427 B[a]P-mediated photosensitization could be the increasingly documented negative impact of
428 oxidative stress induced by UVA on nucleotide excision repair (Courdavault et al., 2005; Guven
429 et al., 2015; Karran and Brem, 2016; Kimeswenger et al., 2018; Montaner et al., 2007; Mouret
430 et al., 2006). However, the contribution of the strong loss of cell viability in NHK exposed to 1
431 μ M B[a]P and then to 2 MED SSL is likely to be much more predominant.

432 A major contribution of photosensitization in our present observations would explain why the
433 effect of B[a]P is observed only when it is added to the NHK before irradiation and not after.
434 Involvement of a photosensitization process could also explain our observations made at 0.1
435 μ M B[a]P. Increased phototoxicity and decreased DNA repair activity was observed when, SSL
436 was applied at 2 MED but not 0.1 MED. If B[a]P alone was responsible for these effects, the
437 same observations should have been made, irrespective of the SSL dose. The underlying
438 mechanism thus clearly requires interaction between B[a]P and SSL, like in photosensitization
439 reactions. The observation that UVB did not exhibit the same effect as SSL is another clue for
440 the major involvement of photosensitization. Indeed, UVB is applied in a much lower physical
441 dose than SSL and is more weakly absorbed by B[a]P, the maximum of absorption of which is
442 in the UVA range. Therefore, UVB is expected to less efficiently induce B[a]P-mediated
443 photosensitized processes. Our observations are reminiscent of those made in fibroblasts, where
444 the UVA1 portion of the UV spectrum was found to be responsible for the largest part of the
445 phototoxic and photo-oxidative effects of irradiation in the presence of PAH (Soeur et al.,
446 2017).

447

448 **5. Conclusion**

449 Our work provides additional evidence of the possible cutaneous impacts that PAH, even in
450 low quantities, can have when exposed to sunlight. Comparison of different protocols and
451 specific experiments allowed us to rule out a major contribution of the AhR factor. It rather
452 seems that the observed increase in toxicity is a classical phototoxicity reaction mediated by
453 photosensitized-oxidative stress. In terms of risk management, this implies that extensive
454 cleaning of skin before exposure to sunlight is an efficient safety procedure, at least as far as
455 phototoxicity is concerned. However, it should be kept in mind that we also provided evidence
456 that exposure to UV, applied either before or after contamination, modulated the metabolism
457 of PAH (Bourgart et al., 2019; von Koschembahr et al., 2018). The consequences of these
458 observations in risk management have to be further explored.

459

460 **Conflict of Interest**

461 The authors state no conflict of interest.

462

463 **Acknowledgements**

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467

468 **References**

469 Aly, H.A., Domenech, O., 2009. Cytotoxicity and mitochondrial dysfunction of 2,3,7,8-
470 tetrachlorodibenzo-p-dioxin (TCDD) in isolated rat hepatocytes. *Toxicology Letters* 191, 79-
471 87.

472 Boffetta, P., Jourenkova, N., Gustavsson, P., 1997. Cancer risk from occupational and
473 environmental exposure to polycyclic aromatic hydrocarbons. *Cancer Causes Control* 8, 444-
474 472.

475 Bonzini, M., Facchinetti, N., Motolese, A., Casa, M., Parassoni, D., Lega, M., Lombardo, M.,
476 Borchini, R., Ferrario, M.M., 2013. Looking for "lost occupational cancers": a systematic
477 evaluation of occupational exposure in a case series of cutaneous squamous cell carcinomas in
478 Italy. *Medicina Del Lavoro* 104, 224-235.

479 Bourgart, E., Persoons, R., Marques, M., Rivier, A., Balducci, F., von Koschembahr, A., Beal,
480 D., Leccia, M.T., Douki, T., Maitre, A., 2019. Influence of exposure dose, complex mixture
481 and ultraviolet radiation on skin absorption and bioactivation of polycyclic aromatic
482 hydrocarbons *ex vivo*. *Archives of Toxicology* 93, 2165-2184.

483 Brash, D.E., 2015. UV signature mutations. *Photochemistry and Photobiology* 91, 15-26.

484 Brash, D.E., Rudolph, J.A., Simon, J.A., Lin, A., McKenna, G.J., Baden, H.P., Halperin, A.J.,
485 Ponten, J., 1991. A role for sunlight in skin cancer: UV-induced p53 mutations in squamous
486 cell carcinoma. *Proceedings of the National Academy of Sciences of the United States of*
487 *America* 88, 10124-10128.

488 Cadet, J., Sage, E., Douki, T., 2005. Ultraviolet radiation-mediated damage to cellular DNA.
489 *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 571, 3-17.

490 Chen, S.C., Liao, T.L., Wei, Y.H., Tzeng, C.R., Kao, S.H., 2010. Endocrine disruptor, dioxin
491 (TCDD)-induced mitochondrial dysfunction and apoptosis in human trophoblast-like JAR
492 cells. *Molecular Human Reproduction* 16, 361-372.

493 Cirila, P.E., Martinotti, I., Buratti, M., Fustinoni, S., Campo, L., Zito, E., Prandi, E., Longhi, O.,
494 Cavallo, D., Foa, V., 2007. Assessment of exposure to Polycyclic Aromatic Hydrocarbons
495 (PAH) in Italian asphalt workers. *Journal of Occupational and Environmental Hygiene* 4, 87-
496 99.

497 Courdavault, S., Baudouin, C., Charveron, M., Canguilhem, B., Favier, A., Cadet, J., Douki,
498 T., 2005. Repair of the three main types of bipyrimidine DNA photoproducts in human
499 keratinocytes exposed to UVB and UVA radiations. *DNA Repair (Amst)* 4, 836-844.

500 Crallan, R.A., Ingham, E., Routledge, M.N., 2005. Wavelength dependent responses of primary
501 human keratinocytes to combined treatment with benzo[a]pyrene and UV light. *Mutagenesis*
502 20, 305-310.

503 Diani-Moore, S., Labitzke, E., Brown, R., Garvin, A., Wong, L., Rifkind, A.B., 2006. Sunlight
504 generates multiple tryptophan photoproducts eliciting high efficacy CYP1A induction in chick
505 hepatocytes and in vivo. *Toxicological Sciences* 90, 96-110.

506 Douki, T., 2013. The variety of UV-induced pyrimidine dimeric photoproducts in DNA as
507 shown by chromatographic quantification methods. *Photochemical and Photobiological*
508 *Sciences* 12, 1286-1302.

509 Esser, C., Barga, I., Weighardt, H., Haarmann-Stemann, T., Krutmann, J., 2013. Functions
510 of the aryl hydrocarbon receptor in the skin. *Seminars in immunopathology* 35, 677-691.

511 Fitzpatrick, T.B., 1988. The validity and practicality of sun-reactive skin types I through VI.
512 *Archives in Dermatology* 124, 869-871.

513 Fritsche, E., Schafer, C., Calles, C., Bernsmann, T., Bernshausen, T., Wurm, M., Hubenthal,
514 U., Cline, J.E., Hajimiragha, H., Schroeder, P., Klotz, L.O., Rannug, A., Furst, P., Hanenberg,
515 H., Abel, J., Krutmann, J., 2007. Lightening up the UV response by identification of the

516 arylhydrocarbon receptor as a cytoplasmatic target for ultraviolet B radiation. *Proceedings of*
517 *the National Academy of Sciences of the United States of America* 104, 8851-8856.

518 Genies, C., Maitre, A., Lefebvre, E., Jullien, A., Chopard-Lallier, M., Douki, T., 2013. The
519 extreme variety of genotoxic response to benzo[a]pyrene in three different human cell lines
520 from three different organs. *PLoS One* 8, e78356.

521 Guven, M., Brem, R., Macpherson, P., Peacock, M., Karran, P., 2015. Oxidative Damage to
522 RPA Limits the Nucleotide Excision Repair Capacity of Human Cells. *Journal of Investigative*
523 *Dermatology* 135, 2834-2841.

524 Herbert, R., Marcus, M., Wolff, M.S., Perera, F.P., Andrews, L., Godbold, J.H., Rivera, M.,
525 Stefanidis, M., Lu, X.Q., Landrigan, P.J., et al., 1990. Detection of adducts of deoxyribonucleic
526 acid in white blood cells of roofers by ³²P-postlabeling. Relationship of adduct levels to
527 measures of exposure to polycyclic aromatic hydrocarbons. *Scandinavian Journal of Work,*
528 *Environment & Health* 16, 135-143.

529 Hwang, H.J., Dornbos, P., Steidemann, M., Dunivin, T.K., Rizzo, M., LaPres, J.J., 2016.
530 Mitochondrial-targeted aryl hydrocarbon receptor and the impact of 2,3,7,8-tetrachlorodibenzo-
531 p-dioxin on cellular respiration and the mitochondrial proteome. *Toxicology and Applied*
532 *Pharmacology* 304, 121-132.

533 Karran, P., Brem, R., 2016. Protein oxidation, UVA and human DNA repair. *DNA Repair*
534 (Amst) 44, 178-185.

535 Katiyar, S.K., Matsui, M.S., Mukhtar, H., 2000. Ultraviolet-B exposure of human skin induces
536 cytochromes P450 1A1 and 1B1. *Journal of Investigative Dermatology* 114, 328-333.

537 Kennedy, L.H., Sutter, C.H., Leon Carrion, S., Tran, Q.T., Bodreddigari, S., Kensicki, E.,
538 Mohny, R.P., Sutter, T.R., 2013. 2,3,7,8-Tetrachlorodibenzo-p-dioxin-mediated production of
539 reactive oxygen species is an essential step in the mechanism of action to accelerate human
540 keratinocyte differentiation. *Toxicological Sciences* 132, 235-249.

541 Kimeswenger, S., Dingelmaier-Hovorka, R., Foedinger, D., Jantschitsch, C., 2018. UVA1
542 impairs the repair of UVB-induced DNA damage in normal human melanocytes. *Experimental*
543 *Dermatology* 27, 276-279.

544 Marrot, L., 2018. Pollution and Sun Exposure: A Dele serious Synergy Mechanisms and
545 Opportunities for Skin Protection. *Current Medicinal Chemistry* 25, 5469-5486.

546 Mauthe, R.J., Cook, V.M., Coffing, S.L., Baird, W.M., 1995. Exposure of mammalian cell
547 cultures to benzo[a]pyrene and light results in oxidative DNA damage as measured by 8-
548 hydroxydeoxyguanosine formation. *Carcinogenesis* 16, 133-137.

549 Melnikova, V.O., Ananthaswamy, H.N., 2005. Cellular and molecular events leading to the
550 development of skin cancer. *Mutation Research* 571, 91-106.

551 Molho-Pessach, V., Lotem, M., 2007. Ultraviolet radiation and cutaneous carcinogenesis.
552 *Current problems in dermatology* 35, 14-27.

553 Montaner, B., O'Donovan, P., Reelfs, O., Perrett, C.M., Zhang, X., Xu, Y.Z., Ren, X.,
554 Macpherson, P., Frith, D., Karran, P., 2007. Reactive oxygen-mediated damage to a human
555 DNA replication and repair protein. *EMBO Reports* 8, 1074-1079.

556 Mouret, S., Baudouin, C., Charveron, M., Favier, A., Cadet, J., Douki, T., 2006. Cyclobutane
557 pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA
558 radiation. *Proceedings of the National Academy of Sciences of the United States of America*
559 103, 13765-13770.

560 Mouret, S., Charveron, M., Favier, A., Cadet, J., Douki, T., 2008. Differential repair of UVB-
561 induced cyclobutane pyrimidine dimers in cultured human skin cells and whole human skin.
562 DNA Repair (Amst) 7, 704-712.

563 Nair, S., Kekatpure, V.D., Judson, B.L., Rifkind, A.B., Granstein, R.D., Boyle, J.O.,
564 Subbaramaiah, K., Guttenplan, J.B., Dannenberg, A.J., 2009. UVR exposure sensitizes
565 keratinocytes to DNA adduct formation. Cancer prevention research (Philadelphia, Pa.) 2, 895-
566 902.

567 Ors, G., Gulce Iz, S., 2018. Cytoprotective effect of a functional antipollutant blend through
568 reducing B a P-induced intracellular oxidative stress and UVA exposure. Turkish Journal of
569 Biology 42, 453-462.

570 Pandey, H., Talukdar, A., Gangte, J.S., Gupta, S.D., Chandra, N.C., 2018. Cholesterol
571 homeostasis and cell proliferation by mitogenic homologs: insulin, benzo-alpha-pyrene and UV
572 radiation. Cell Biology and Toxicology 34, 305-319.

573 Pollet, M., Shaik, S., Mescher, M., Frauenstein, K., Tigges, J., Braun, S.A., Sondenheimer, K.,
574 Kaveh, M., Bruhs, A., Meller, S., Homey, B., Schwarz, A., Esser, C., Douki, T., Vogel, C.F.A.,
575 Krutmann, J., Haarmann-Stemmann, T., 2018. The AHR represses nucleotide excision repair
576 and apoptosis and contributes to UV-induced skin carcinogenesis. Cell Death and
577 Differentiation.

578 Rannug, A., Fritsche, E., 2006. The aryl hydrocarbon receptor and light. Biological Chemistry
579 387, 1149-1157.

580 Rannug, U., Rannug, A., Sjoberg, U., Li, H., Westerholm, R., Bergman, J., 1995. Structure
581 elucidation of two tryptophan-derived, high affinity Ah receptor ligands. Chemistry & Biology
582 2, 841-845.

583 Ravanat, J.L., Douki, T., Duez, P., Gremaud, E., Herbert, K., Hofer, T., Lasserre, L., Saint-
584 Pierre, C., Favier, A., Cadet, J., 2002. Cellular background level of 8-oxo-7,8-dihydro-2'-
585 deoxyguanosine: an isotope based method to evaluate artefactual oxidation of DNA during its
586 extraction and subsequent work-up. Carcinogenesis 23, 1911-1918.

587 Saladi, R., Austin, L., Gao, D., Lu, Y., Phelps, R., Lebwohl, M., Wei, H., 2003. The
588 combination of benzo[a]pyrene and ultraviolet A causes an in vivo time-related accumulation
589 of DNA damage in mouse skin. Photochemistry and Photobiology 77, 413-419.

590 Schreck, I., Chudziak, D., Schneider, S., Seidel, A., Platt, K.L., Oesch, F., Weiss, C., 2009.
591 Influence of aryl hydrocarbon- (Ah) receptor and genotoxins on DNA repair gene expression
592 and cell survival of mouse hepatoma cells. Toxicology 259, 91-96.

593 Sellappa, S., Mani, B., Keyan, K.S., 2011. Cytogenetic Biomonitoring of Road Paving Workers
594 Occupationally Exposed to Polycyclic Aromatic Hydrocarbons. Asian Pacific Journal of
595 Cancer Prevention 12, 713-717.

596 Serdar, B., Lee, D., Dou, Z.H., 2012. Biomarkers of exposure to polycyclic aromatic
597 hydrocarbons (PAHs) and DNA damage: a cross-sectional pilot study among roofers in South
598 Florida. BMJ Open 2.

599 Soeur, J., Belaidi, J.P., Chollet, C., Denat, L., Dimitrov, A., Jones, C., Perez, P., Zanini, M.,
600 Zobiri, O., Mezzache, S., Erdmann, D., Lereaux, G., Eilstein, J., Marrot, L., 2017. Photo-
601 pollution stress in skin: Traces of pollutants (PAH and particulate matter) impair redox
602 homeostasis in keratinocytes exposed to UVA1. Journal of Dermatological Science 86, 162-
603 169.

604 Teranishi, M., Toyooka, T., Ohura, T., Masuda, S., Ibuki, Y., 2010. Benzo[a]pyrene exposed
605 to solar-simulated light inhibits apoptosis and augments carcinogenicity. *Chemico-Biological*
606 *Interactions* 185, 4-11.

607 Toyooka, T., Ibuki, Y., 2007. DNA damage induced by coexposure to PAHs and light.
608 *Environmental Toxicology and Pharmacology* 23, 256-263.

609 Vaananen, V., Hameila, M., Kalliokoski, P., Nykyri, E., Heikkila, P., 2005. Dermal exposure
610 to polycyclic aromatic hydrocarbons among road pavers. *Annals of Occupational Hygiene* 49,
611 167-178.

612 VanRooij, J.G., Bodelier-Bade, M.M., De Loeff, A.J., Dijkmans, A.P., Jongeneelen, F.J., 1992.
613 Dermal exposure to polycyclic aromatic hydrocarbons among primary aluminium workers. *La*
614 *Medicina del lavoro* 83, 519-529.

615 Vaz Batista Filgueira, D.D.M., Salomao de Freitas, D.P., de Souza Vottol, A.P., Fillmann, G.,
616 Monserrat, J., Geracitano, L.A., Trindade, G.S., 2007. Photodynamic action of benzo a pyrene
617 in K562 cells. *Photochemistry and Photobiology* 83, 1358-1363.

618 von Koschembahr, A., Youssef, A., Beal, D., Calissi, C., Bourgart, E., Marques, M., Leccia,
619 M.T., Giot, J.P., Maitre, A., Douki, T., 2018. Solar simulated light exposure alters
620 metabolism and genotoxicity induced by benzo[a] pyrene in human skin. *Scientific Reports*
621 8, 10.1038/s41598-41018-33031-41598.

622 Wei, Y.D., Rannug, U., Rannug, A., 1999. UV-induced CYP1A1 gene expression in human
623 cells is mediated by tryptophan. *Chemico-Biological Interactions* 118, 127-140.

624 Xia, Q.S., Chiang, H.M., Yin, J.J., Chen, S.J., Cai, L.N., Yu, H.T., Fu, P.P., 2015. UVA
625 photoirradiation of benzo a pyrene metabolites: induction of cytotoxicity, reactive oxygen
626 species, and lipid peroxidation. *Toxicology and Industrial Health* 31, 898-910.

627 Youssef, A., von Koschembahr, A., Caillat, S., Corre, S., Galibert, M.D., Douki, T., 2018. 6-
628 Formylindolo[3,2-b]carbazole (FICZ) is a Very Minor Photoproduct of Tryptophan at
629 Biologically Relevant Doses of UVB and Simulated Sunlight. *Photochem Photobiol* 95, 237-
630 243.

631 Yu, H., Xia, Q., Yan, J., Herreno-Saenz, D., Wu, Y.S., Tang, I.W., Fu, P.P., 2006.
632 Photoirradiation of Polycyclic Aromatic Hydrocarbons with UVA Light – A Pathway Leading
633 to the Generation of Reactive Oxygen Species, Lipid Peroxidation, and DNA Damage.
634 *International Journal of Environmental Research and Public Health* 3, 348-354.

635 Zegarska, B., Pietkun, K., Zegarski, W., Bolibok, P., Wisniewski, M., Roszek, K., Czarnecka,
636 J., Nowacki, M., 2017. Air pollution, UV irradiation and skin carcinogenesis: what we know,
637 where we stand and what is likely to happen in the future? *Postepy Dermatologii I Alergologii*
638 34, 6-14.

639 Ziegler, A., Leffel, D.J., Kunala, S., Sharma, H.W., Shapiro, P.E., Bale, A.E., Brash, D.E.,
640 1993. Mutation hotspots due to sunlight in the p53 gene of nonmelanoma skin cancers.
641 *Proceedings of the National Academy of Sciences of the United States of America* 90, 4216-
642 4220.

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