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1 **CYTOTOXICITY AND GENOTOXICITY OF LIGHT EMITTED BY**
2 **INCANDESCENT, HALOGEN AND LED BULBS ON RETINAL PIGMENT**
3 **EPITHELIUM CELLS**

4
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27 **ABSTRACT**

28 LED technology has the extraordinary ability to reduce energy consumption, constituting an
29 economic and ecological advantage, so it is planned to replace incandescent, halogen and
30 other inefficient bulbs for public and domestic lighting with LEDs. LEDs present specific
31 spectral and energetic characteristics compared with that of other domestic light sources, so
32 the potential risks for human health of these bulbs need to be explored.

33 The aim of this study was to assess cytotoxicity and genotoxicity of light emitted by different
34 commercial light bulbs: incandescent, halogen and two LED bulbs with different Correlated
35 Colour Temperatures. The evaluation was done on the ARPE-19 as a specific cell model for
36 eye toxicity and on BEAS-2B as a good cell model for toxicology tests.

37 Light induced mainly cytotoxic effects on ARPE-19 and DNA damage on BEAS-2B, so
38 different cell line showed different biological response. Moreover, our findings indicates that,
39 among the four bulbs, cold LED caused the major cytotoxic effect on ARPE-19 and the major
40 genotoxic and oxidative effect on BEAS-2B. Cold LED probably is able to cause more
41 cellular damage because contains more high-energy radiations (blue). These results suggests
42 that LED technology could be a safe alternative to older technologies but the use of warm
43 LED should be preferred to cold LED, which can potentially cause adverse effects on retinal
44 cells.

45

46 **Keywords:** ARPE-19, WST-1 assay, Comet assay, light-emitting diodes, halogen bulb.

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51 **1. INTRODUCTION**

52 In the past century conventional incandescent bulb was almost the only source of electric
53 light used in households. Due to energy saving policy (Commission regulation 244/2009),
54 conventional incandescent bulbs (and other inefficient lighting methods) had to be phased out
55 until September 2012. Incandescent bulbs have to be replaced with energy efficient light
56 sources such as halogen bulbs, compact fluorescent bulbs (CFLs) or light-emitting diode
57 bulbs (LED). All these light sources are extensively used for public and domestic lighting,
58 but for the future it is planned to replace halogen bulbs and CFLs with LEDs (Necz and
59 Bakos 2014).

60 LED technology has the extraordinary ability to reduce energy consumption, constituting an
61 economic and ecological advantage. The importance of this technology has been recognized
62 by giving the 2014 Nobel Prize in Physics to Isamu Akasaki, Hiroshi Amano and Shuji
63 Nakamura “for the invention of efficient blue light emitting diodes (LEDs) which has enabled
64 bright and energy-saving white light sources” (Haim and Zubidat 2015).

65 LEDs are also incorporated in all the screens of electronical devices, such as computers and
66 mobile phones. The development of handheld computer-based technology has provided the
67 opportunity for long-term viewing of illuminated screens. It is recognized that many people
68 are using laptop or tablet computers, or mobile phone technology, for many hours per day
69 (O’Hagan, Khazova and Price 2016).

70 The LED technology is currently being viewed as a huge step in cost-efficient solution for
71 lighting systems and these light sources are extensively used, so it is important assess the
72 potential risks to the environment and human health linked to this new technology.

73 Optical radiation includes ultraviolet light (UV) (100 – 380 nm), visible light (380 – 780 nm)
74 and infrared radiation (IR) (780 – 10 000 nm). Visible light can be divided into blue (short-

75 wavelength radiation), green (medium-wavelength radiation) and red light (long-wavelength
76 radiation) (Youn et al. 2009).

77 Overall, our household light bulbs emit mainly optical radiation but not only in the visible
78 spectrum. There are some other ranges of non-ionizing radiation that are emitted by bulbs and
79 that are possibly hazardous for human health, such as: UV and IR. Also visible light,
80 especially blue light, can impair eyesight (Necz and Bakos 2014). Ultraviolet light and the
81 shorter wavelengths of the visible light pose a potential hazard because they contain more
82 energy (Youn et al. 2009). In particular, the blue light (400 – 500 nm) is likely to be
83 important since it has a relatively high energy (Godley et al. 2005).

84 LEDs present specific spectral and energetic characteristics compared with that of other
85 domestic light sources, so the potential risks of these new light sources need to be explored to
86 answer whether they could be eventually harmful for people (Chamorro et al. 2013).

87 Most white LEDs consist of a short-wavelength emitting diode (blue light mostly) and
88 phosphor emitting at a larger wavelength (mixed white light generation), so they emit many
89 blue radiations (Shen et al. 2016). Blue light, emitted by LED, has been demonstrated to be
90 the most effective frequency for melatonin suppression compared with conventional lighting
91 technologies (Falchi et al. 2011; West et al. 2011). Melatonin strongly regulates numerous
92 vital functions including antioxidant, antiaging and most relevant anti-oncogenic properties
93 (Srinivasan et al. 2011). Reduced levels of melatonin in women exposed to artificial light-at-
94 night during night work and sleep deprivation are associated with an increase in breast cancer
95 risk (Davis, Mirick and Stevens 2001; Schernhammer et al. 2001; Viswanathan, Hankinson,
96 and Schernhammer 2007; Haim and Zubidat 2015).

97 The eye is constantly exposed to radiations. Light in excess (high energy or long-time
98 exposure) may cause eye injury when focused onto retina. Although the eye has developed
99 very precise mechanism of light adaptation and has several protective mechanism against

100 light exposure, prolonged or intense exposure may affect the human vision (Contin et al.
101 2016).

102 European Standard EN 62471:2008 (European Standard 2008) gives guidance for evaluating
103 the photobiological safety of broad band lighting sources (including LED) and systems, it
104 specifies the spectral blue-light hazard function $B(\lambda)$, and states the limiting values,
105 measuring quantities useful to evaluate the potential photobiological hazard of light exposure.

106 Constant exposure to light in excess can produce retinal degeneration as a consequence of
107 photoreceptor or retinal pigment epithelium (RPE) cells death (Contin et al. 2016). Moreover,
108 light in excess may damage the human vision promoting retinal degeneration or accelerating
109 some genetic diseases, such as retinitis pigmentosa or age-related macular degeneration
110 (Contin et al. 2016).

111 Visible light affects mitochondrial respiration and decreases mitochondrial homeostasis
112 (Osborne et al. 2010; Li, Fan, and Ma 2011) and it can also directly cause nuclear DNA
113 damage in retinal ganglion cells (Li, Fan, and Ma 2011).

114 It has been hypothesized that in particular blue light can damage the retina causing
115 photoretinitis (Necz and Bakos 2014) and the development of age-related macular
116 degeneration (Youn et al. 2009). Studies *in vivo* show that retinal exposure at elevated levels
117 of blue light leads to photochemical damage on the photoreceptors and retinal pigment
118 epithelial cells (Youn et al. 2009). It has been reported that blue light induced retinal damage
119 is mainly caused by the production of reactive oxygen species (ROS) (Moon et al. 2017).

120 Excessive oxidative stress can cause dysfunction in retinal cells by the oxidation of proteins,
121 lipids and DNA and eventually results in cell death by apoptosis (Moon et al. 2017). Studies
122 *in vitro* have shown that irradiation of mammalian cells (human primary epithelial cells) with
123 blue light induces both mitochondrial and DNA damage via reactive oxygen species (ROS)
124 (Godley et al. 2005). Also low intensity of blue light can induce ROS production and

125 apoptosis in RPE cells (A2E-loaded ARPE-19) (Moon et al. 2017). Moreover, the study of
126 Nakanishi-Ueda and collaborators (2013), showed that blue light emitted by LED causes an
127 increase of ROS, lipid peroxidation and subsequent cellular injuries in cultured bovine RPE
128 cells. Others authors (Kuse et al. 2014) demonstrated that also the cone photoreceptor-derived
129 cells (661 W) can be damaged via ROS by blue light emitted by LED. The harmful blue light
130 effect was also confirmed *in vivo* (Wu et al. 1999; Narimatsu et al. 2015; Ham, Mueller and
131 Sliney 1976; Gorgels and Norren 1995; Moon et al. 2017). Blue light induced retinal damage
132 in rats, whereas green light did not (Wu et al. 1999). The retinal damage was mediated by
133 apoptosis, and the damage in the rat retina increased with the use of shorter wavelength of
134 blue light (Gorgels and Norren 1995). Moreover similar results were confirmed in a previous
135 study using a rhesus monkey (Ham, Mueller and Sliney 1976). Recently, it was demonstrated
136 that blue light exacerbated the increase in the ROS level and inflammatory cytokine
137 expression as well as macrophage recruitment in the RPE-choroid of mice exposed to light
138 (Narimatsu et al. 2015).

139 The mechanisms by which light can cause damage to the retina have not been completely
140 understood and properties of light that induce this damage have not been precisely related to
141 simple photometric characteristics like peak wavelength and Correlated Colour Temperature
142 (CCT). Few studies evaluated genotoxicity induced by light and little is known about the
143 biological effects induced by different types of LED bulbs.

144 The aim of this study was to evaluate cytotoxicity and genotoxicity of light emitted by
145 different commercial light bulbs that have the same amount of luminous flux emitted. In
146 particular, the tested bulbs were a halogen lamp bulb, two LED bulbs with different
147 Correlated Colour Temperatures (CCT) (warm white and cold white) and, in comparison, an
148 old incandescent bulb, which is currently no commercially available because it does not
149 comply with energy requirements.

150 The human RPE cells (ARPE-19) were used as a specific cell model for eye toxicity and the
151 human bronchial epithelial cells (BEAS-2B) were used as a good cell model for *in vitro*
152 toxicology tests.

153 **2. METHODS AND MATERIALS**

154 **2.1 Cell culture**

155 The spontaneously immortal human RPE cells (ARPE-19) and the virus transformed human
156 bronchial epithelial cells (BEAS-2B) were obtained from the American Type Culture
157 Collection.

158 ARPE-19 were grown as a monolayer, maintained in Dulbecco's modified Eagle's medium
159 (DMEM) supplemented with 10% (v/v) fetal calf serum, 2% L-glutamine 200 mM, 1%
160 sodium pyruvate 100 mM and 1% penicillin 10 000 U/ml - streptomycin 10 000 µg/ml, at
161 37°C in an humidified atmosphere containing 5% CO₂.

162 BEAS-2B were grown as a monolayer, maintained in RPMI 1640 supplemented with 10%
163 (v/v) FCS, 2% L-glutamine 200 mM and 1% penicillin 10 000 U/ml - streptomycin 10 000
164 µg/ml, at 37°C in an humidified atmosphere containing 5% CO₂.

165 **2.2 Lamps and exposure**

166 Experimental illuminating system included commercial warm LED bulb (provided by
167 KADELED-light line S.r.l.), commercial cold LED bulb (provided by SI S.r.l.) and halogen
168 bulb (provided by GREENPLUX S.r.l.). In comparison, an old incandescent bulb was tested,
169 this type of lamp is currently no commercially available because it does not comply with the
170 energy requirements. The characteristics of the four bulbs are reported in Table 1: bulbs
171 differ for power, Correlated Colour Temperature and energy efficiency class but produce an
172 equivalent luminous flux (lumen). The spectral intensity distribution of all sources was
173 measured with a Minolta CL500A illuminance spectrophotometer in three different
174 conditions: in air in a dark room with the sensitive area of the meter toward the lamp (Cond.

175 A) and in two different positions inside the cell culture incubator, one with the meter on the
176 bottom of the incubator (Cond. B) to evaluate the changes induced by selective wavelengths
177 reflections of the incubator walls, and one to assess the spectral distribution of the incident
178 light on cells with the meter inside the incubator with the sensitive area toward the lamp and
179 a plate between the sensitive area and the lamp (Cond. C). The results are shown in Fig. 1.

180 The experimental illuminating system was installed into the cell culture incubator, which
181 maintained a temperature of 37°C. In order to reduce the interference of medium, each
182 illuminating system irradiated the basal surface of culture plates (Shen et al. 2016), which
183 were positioned 14 cm above the light sources directly. The distance of the light from the cell
184 cultures was based on the distance used by other recent studies (Shen et al. 2016; Xie et al.
185 2014). In addition, during light exposure, the culture medium was changed to DMEM or
186 RPMI 1640 without phenol red containing 2% HEPES buffer and without fetal calf serum to
187 reduce the chromophores present in the culture medium (Xie et al. 2014).

188 The cultured cells were irradiated for 1h and 4h. The spectral irradiance and the illuminance
189 on the cells cultures on plates were measured inside the incubator with the CL500A in
190 measurement condition (Cond C). From the measured values of spectral irradiance, the total
191 blue-light weighted exposure, calculated as the integral of irradiance weighted against the
192 spectral blue-light hazard function $B(\lambda)$ for the exposure time (European Standard 2008), was
193 calculated for the two exposure times (1h and 4h). Illuminance and total blue-light weighted
194 exposure are shown in Table 2 (the measurement uncertainty is 5%), while the blue-light
195 weighted exposure is shown in Figure 2.

196 The discrepancies in the illuminance and total blue-light weighted exposure values among the
197 lamps, are due to the different luminous spatial intensity distributions of the lamps:
198 incandescent lamp and cold LED have a strong light emission in the vertical direction (i.e.

199 toward the cell plates), while warm LED has a horizontal emission, due to the geometrical
200 arrangement of the emitting surface.

201 Cells kept in the dark, incubated in the same incubator of the exposed cells, are considered as
202 control group. The exposure times were chosen in order to evaluate short-time effects
203 induced by light. The exposure times are comparable with previous studies (Nankanishi-Ueda
204 et al. 2013; Godley et al. 2005; Roechelecke et al. 2009; Youn et al. 2009).

205 To ensure that assays were not influenced by an eventually system temperature increase
206 caused by bulbs, during the incubation the temperature was measured at 10, 15, 20, 30, 60,
207 90, 120, 150, 180, 210, 240 minutes after incubation using RS Digital Thermometer 1319, K-
208 Type. Figure 3 shows the temperature variation induced by different types of light inside the
209 irradiated and control wells. After inserting the plates into the incubator, the temperature
210 inside wells rises, is stabilized during 20 minutes and remains almost unchanged for the
211 whole incubation time. Comparing the temperature among wells exposed to different light
212 bulbs, the incandescent bulb and the halogen bulb determined the highest temperature values,
213 probably because these bulbs (less energetically efficient) dissipate part of the energy as heat.
214 The slight difference in temperature variation (1° C between the minimum and maximum
215 recorded temperature) reasonably did not influence the results of biological assays performed
216 on the cells.

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222 **2.3 WST-1**

223 The cell viability was evaluated using the Cell Proliferation Reagent WST-1 (Roche). This
224 assay is based on the cleavage of tetrazolium salts to soluble formazan dye by mitochondrial
225 succinate-tetrazolium reductase which exists in the mitochondrial respiratory chain and is
226 active only in viable cells. The quantity of formazan dye in the medium is directly
227 proportional to the number of viable metabolically active cells.

228 Briefly, cells were seeded in 24-well plates at a density of 5×10^4 cells/well and, after
229 exposure, 50 μ l of Cell Proliferation Reagent WST-1 (Roche) were added to each cell culture
230 well and incubated for 3 h at 37°C, protecting the plate from the light.

231 To avoid any interference in light absorption owing to the cells and cell debris, at the end of
232 incubation, contents of each well were transferred in an optically clear 96-well flat bottom
233 plate. Formazan dye formed by metabolically active cells was quantified by measuring its
234 absorbance (440nm) using a microtiter plate reader (Tecan Infinite Reader M200 Pro).

235 Negative control were obtained by absorbance measurement of culture cell medium of
236 control cells. Data from exposed cells were expressed as a percent of viable cells. All
237 experiments were performed in quadruplicate and the data were represented as the mean \pm
238 standard deviation.

239 ***2.4 Comet assay***

240 DNA damage has been evaluated by alkaline single cell gel electrophoresis (Comet assay),
241 according to the recommended procedure (Tice et al. 2000). Cells were cultured for 12 h in 6-
242 well plates at a density of 3×10^5 cells/well before exposure to light. The proportion of living
243 cells was determined by trypan blue staining. Cells were treated with different light bulbs for
244 1h or 4h. After exposure, cell viability was checked again. Cells (3×10^5) were mixed with
245 140 μ l of 0.7% low melting point agarose (LMA) and 20 μ l were placed on the slides coated
246 with 1% of normal melting agarose (NMA), with LMA added as the top layer. Cells were
247 lysed at 4°C in the dark overnight (8 mM Tris-HCl, 2.5 M NaCl, 100 mM EDTA disodium

248 salt dihydrate, 1% TRITON X-100 and 10% DMSO, pH 10). DNA was allowed to unwind
249 for 20 min in alkaline electrophoresis buffer (1 mM EDTA tetrasodium salt dihydrate, 300
250 mM NaOH, 10% DMSO, pH >13) and subjected to electrophoresis in the same buffer for 20
251 min (1 V/cm and 300 mA). The slides were then soaked with neutralization buffer (0.4 M
252 Tris-HCl, pH 7.5, 4 °C, 3 min), fixed with ethanol 70% (-20 °C, 5 min) and air dried.

253 All steps for slide preparation were performed under yellow light to prevent additional DNA
254 damage.

255 DNA was stained with ethidium bromide (20 µg/ml) and analyzed using a fluorescence
256 microscope (Axioskop HBO 50, Zeiss). A hundred randomly selected cells per sample (2
257 spot) were analyzed using an image analysis system (Comet Assay IV) (Perceptive
258 Instruments Ltd, Stone, Staffordshire, UK). The % tail DNA was selected as the parameter to
259 estimate DNA damage (Tice et al. 2000; Collins 2004).

260 ***2.5 Fpg-Comet***

261 The formamidopyrimidine glycosylase (Fpg)-modified Comet assay was used to evaluate
262 oxidative DNA damage. The test was carried out as described above with the exception that,
263 after lysis, the slides were washed three times for 5 min with Fpg Buffer (40 mM Hepes,
264 0.1M KCl, 0.5 mM EDTA disodium salt dihydrate, 0.2 mg/ml bovine serum albumin, pH 8).

265 Then, the slides were incubated with 0.5 unit of Fpg enzyme (Escherichia coli Fpg Enzyme
266 and Buffer- TREVIGEN) at 37°C for 30 min. Control slides were incubated with buffer only.

267 A hundred randomly selected cells per sample (2 spot) were analyzed using an image
268 analysis system (Comet Assay IV) (Perceptive Instruments Ltd, Stone, Staffordshire, UK).

269 For each experimental point, the mean % tail DNA from enzyme untreated cells (direct DNA
270 damage) and mean % tail DNA for Fpg-enzyme treated cells (direct and indirect DNA
271 damage) were calculated.

272 ***2.6 Statistical analyses***

273 Statistical analyses were performed using IBM SPSS software (ver. 24.0). The results of
274 WST-1 and Comet assay are presented as the mean of quadruplicate (WST-1) and duplicate
275 (Comet assay) \pm standard deviation. Differences between exposed and control cells for each
276 time of exposure (1h and 4h) were tested by T-test Student. Differences of cytotoxicity and
277 genotoxicity induced by different bulbs after the same time of exposure were tested by one-
278 way analysis of variance (ANOVA) followed by Tukey's test procedure. Significance was
279 accepted at $p < 0.05$.

280

281 **3. RESULTS**

282 **3.1 Cytotoxicity – WST-1**

283 The results of the effects of different light on cell viability (WST-1 assay) on ARPE-19 are
284 presented in figure 4. The incandescent bulb induced a decrease in viability after only 4h
285 ($p < 0.05$) (fig. 4a), while halogen bulb caused a significant cytotoxic effect both after 1h and
286 4h ($p < 0.001$) and the effect increased with the increase of exposure time (fig. 4b).
287 Considering effects induced by LEDs, warm LED induced a decrease in viability after 4h that
288 was not significant (fig. 4c), on the contrary cold LED, similar to halogen bulb, showed a
289 significant cytotoxic effect both after 1h and 4h ($p < 0.001$) and the effect increased with the
290 increase of exposure time (fig. 4d). The ANOVA analysis, performed assuming cytotoxicity
291 induced after exposure (1h) as dependent variables and the different bulbs as independent
292 variables, showed the general significance of the model ($F = 28.422$, $p < 0.001$). Post hoc
293 Tukey's test emphasised the cytotoxicity induced by cold LED that was the highest compared
294 to other light bulbs (cold LED vs incandescent bulb $p < 0.001$, cold LED vs halogen bulb
295 $p < 0.05$, cold LED vs warm LED $p < 0.001$).

296 The results of the effects of different light on cell viability (WST-1 assay) on BEAS-2B are
297 presented in figure 5. As reported on ARPE-19, the incandescent bulb induced slight decrease

298 in viability that was significant after only 4h ($p<0.001$) (fig. 5a). Differently from ARPE-19,
299 halogen bulb was not cytotoxic on BEAS-2B (fig. 5b). Considering effects induced by LEDs,
300 contrary to ARPE-19 a low cytotoxic effect was observed for warm LED (1h and 4h,
301 $p<0.001$) (fig. 5c), while no cytotoxicity was observed for cold LED (fig. 5d). The ANOVA
302 analysis, performed assuming cytotoxicity induced after exposure (4h) as dependent
303 variables and the different bulbs as independent variables, showed the general significance of
304 the model ($F= 116.753$, $p<0.001$). Post hoc Tukey's test confirmed the major cytotoxic effect
305 induced by incandescent bulb and warm LED than halogen bulb and cold LED (incandescent
306 bulb *vs* halogen bulb $p<0.001$, incandescent bulb *vs* cold LED $p<0.001$, warm LED *vs*
307 halogen bulb $p<0.001$, warm LED *vs* cold LED $p<0.001$).

308 **3.2 Genotoxicity – Comet and Fpg-Comet assays**

309 The alkaline version of the Comet assay (sensitive to DNA strand breaks, direct oxidative
310 DNA lesions and alkali-labile sites) was used to evaluate the genotoxic effects of light, while
311 the Fpg- modified Comet assay was used to assess the oxidative (direct and indirect) DNA
312 damage.

313 The results of genotoxic effect induced by different lamp bulbs on ARPE-19 are presented in
314 figure 6. Considering the exposure with incandescent light, no genotoxic effect was showed
315 in enzyme untreated cells (direct DNA damage) (fig. 6a). On the contrary, halogen lamp
316 exposure caused a significant DNA damage after 4 hours ($p<0.05$) (fig. 6b). Similar to
317 halogen lamp, also LEDs (warm and cold) induced genotoxicity after 4 hours exposure
318 ($p<0.05$) (fig. 6c and 6d). The ANOVA analysis showed no significance of the model, so the
319 comparison of genotoxicity induced after 4h by different bulbs on ARPE-19 was not
320 statistically significant.

321 On the ARPE-19 cells, it was not possible to perform the Fpg-Comet assay because the
322 oxidized sites were high in the control cells (results not shown).

323 The results of genotoxic effect induced by different lamp bulbs on BEAS-2B are presented in
324 figure 7. On BEAS-2B, incandescent light caused a significant DNA damage after 4 hours
325 ($p < 0.05$) (fig. 7a). As reported on ARPE-19, halogen lamp, warm LED and cold LED were
326 genotoxic after 4 hours on BEAS-2B ($p < 0.05$) (fig. 7b, 7c, 7d). The damage induced by cold
327 LED was higher than damage caused by exposure to incandescent, halogen and warm LED
328 lamps (fig. 7d). The ANOVA analysis, performed assuming genotoxicity induced after
329 exposure (4h) as dependent variables and the different bulbs as independent variables,
330 showed the general significance of the model ($F = 27.730$, $p < 0.05$). Post hoc Tukey's test
331 emphasised major genotoxicity induced by cold LED than the others bulbs (cold LED vs
332 incandescent bulb, halogen bulb and warm LED; $p < 0.05$). The genotoxic effect induced on
333 BEAS-2B was higher than the effect induced on ARPE-19, although the observed
334 genotoxicity was overall low.

335 On the BEAS-2B cells, the Fpg modified Comet assay was performed successfully. The
336 results of genotoxic effect (direct and indirect DNA damage) induced by different lamp bulbs
337 on BEAS-2B are reported in figure 8. Incandescent, halogen and warm LED bulbs induced a
338 significant DNA damage with respect to the control cells in enzyme treated cells ($p < 0.05$)
339 (fig. 8a, 8b and 8c respectively). However, there were no differences between the DNA
340 damage in enzyme treated cells and the DNA damage induced in enzyme untreated cells,
341 resulting in no oxidative damage induced by these bulbs. On the contrary, a major statistically
342 significant increase of DNA damage was observed in enzyme treated cells (direct and indirect
343 DNA damage) with respect to the control cells after 4 hours exposure with cold LED
344 ($p < 0.05$) (fig. 8d). Considering that, the subtraction of the mean % tail DNA in enzyme
345 treated cells from the relative mean % tail DNA in enzyme untreated cells, compared with
346 unexposed cells at each experimental point, provides the intensity of the oxidative damage, a
347 significant oxidative damage was observed for cold LED ($p < 0.05$). The ANOVA analysis,

348 performed assuming genotoxic effect (direct and indirect DNA damage) induced after
349 exposure (4h) as dependent variables and the different bulbs as independent variables,
350 showed the general significance of the model ($F= 126.643$, $p<0.001$). Post hoc Tukey's test
351 emphasised major genotoxicity induced by cold LED than the others bulbs (cold LED vs
352 incandescent bulb, halogen bulb and warm LED; $p<0.001$).

353

354 **4. DISCUSSION**

355 *In vitro* assays provide rapid and effective means of screening and ranking chemicals and
356 physical agents for a number of toxicological endpoints. They allow targeted investigations
357 on issues that can not be adequately addressed by other methods, such as analysis of
358 mechanisms of toxicity at both the molecular and cellular level (Eisenbrand et al. 2002).
359 Toxicity testing can be refined by considering the target organ of the test compound *in vivo*
360 and selecting a cell system that is appropriate on the basis of metabolic competence and of
361 organ/tissue specific toxicity (Eisenbrand et al. 2002). However, it should be kept in mind
362 that some tissues/cell lines may be more susceptible to cytotoxicity or other biological effects
363 than others (Vinken and Blaauboer 2017), so it is useful testing chemical or physical agent on
364 different cell types.

365 This study investigated the cytotoxicity and genotoxicity of different commercially available
366 light bulbs: incandescent bulb, halogen bulb, warm LED and cold LED. The effect was
367 evaluated *in vitro* on two different human cell lines deriving from the RPE (ARPE-19) and
368 bronchial epithelium (BEAS-2B). These cell lines were chosen as a specific cell model for
369 eye toxicity (ARPE-19) and a good cell model for *in vitro* toxicology tests (BEAS-2B) in
370 order to evaluate possible different biological response. To support comparison, we chose cell
371 lines derived from epithelium and non-tumoral, because cell lines originates from cancer
372 might perform aberrant functionality (Vinken and Blaauboer 2017).

373 As a specific cell model for eye, we chose ARPE-19 because the RPE cells have vital support
374 functions for retina (e.g. maintain ionic composition, filter nutrients and provide
375 photoprotection) and are important for the physiology and pathology of the retina. It has been
376 documented that RPE cell cultures and also immortalized cell lines may adopt a variety of
377 morphological and biochemical phenotype, more or less resembling the equivalent RPE
378 tissue (Pfeffer and Philp 2014).

379 In comparison, we chose to use a human bronchial cell line, which is extensively used to
380 study the impact of toxicants on lung, the BEAS-2B cells. According to a recent study on
381 human lung cell model (Courcot et al. 2012), BEAS-2B exhibited the highest similarities
382 with primary cells and the lowest number of dysregulated genes compared with non-tumoral
383 lung tissues, so they are a good model for toxicology studies.

384 The WST-1 assay was performed to assess cytotoxicity on the two cell lines. On ARPE-19
385 different bulbs induced different cytotoxic effect: halogen bulb and cold LED caused the
386 major cytotoxic effect. Comparing the characteristics of the two tested LEDs, they differ in
387 CCT and spectrum (Fig. 1 and Table 1). The CCT is a characteristic that identifies the
388 perceived tonality of light distribution of the radiation in the spectral band of the visible. If
389 the dominant colour of the light tends to red, the light emitted will have a warm perceived
390 tone (low CCT values); if the dominant colour of the light tends to blue, the light emitted will
391 have a more cold perceived tone (high CCT values). Therefore, the CCT reflects the optical
392 and spectral characteristics of a specific white LED light to some extent (Xie et al. 2014). Our
393 LED, warm and cold, have respectively low and high CCT values, so they emit light
394 composed by radiations that tends respectively to red and blue colours. We hypothesize that
395 our cold LED was more cytotoxic than warm LED because it emits more blue radiations and
396 in the region were $B(\lambda)$ (European Standard 2008) is close to its maximum value (Fig. 1(b)).
397 According with our results, other studies demonstrated that the blue component of the visible

398 spectrum (400-500 nm) is associated with retinal damage and the development of age-related
399 macular degeneration (Necz and Bakos 2014; Nakanishi-Ueda et al. 2013; Kuse et al. 2014;
400 Shaban and Richter 2002). Negative effect of blue light exposure on ARPE-19 cells was
401 previous demonstrated by others. Youn and collaborators (2009), testing lights with different
402 wavelengths (400, 420 and 435.8 nm), found that only the 400 nm light can cause significant
403 dose-dependent decreases in ARPE-19 cell viability. Moon et al. (2017) recently obtained
404 similar results testing the blue light effect on ARPE-19 containing A2E (a fluorophore):
405 shorter wavelength blue light resulted in an increased production of ROS and induced
406 reduction in viability and activation of caspase-3/7. Also King and collaborators (2004) and
407 Roehlecke and collaborators (2009), after exposure to blue light, demonstrated increase of
408 ROS production, induction of cytotoxicity through mitochondrial-dependent mechanism and
409 mitochondrial damage on ARPE-19. Therefore, as shown by other studies, we can
410 hypothesized that the CCT is an important parameter that could induce different biological
411 effects: with the increase of CCT there is a major cytotoxic effect. This is a direct implication
412 of the physical principle that shorter Electromagnetic wavelengths (like blue light
413 wavelengths) have higher energy, but it is expressed through a more simple parameter of easy
414 understanding.

415 Considering our results on ARPE-19, not only cold LED but also halogen bulb was highly
416 cytotoxic, although the tested halogen light has a low CCT. This result is in agreement with
417 the study of Yoshida and collaborators (2013). They found that blue light irradiation by
418 quartz tungsten halogen lamp and LED decreased cell proliferation of human gingival
419 fibroblasts (HGF) in a time-dependent manner and caused morphological changes especially
420 in the mitochondria. Moreover, according to our results they found that cytotoxicity was
421 significant higher after LED irradiation than after quartz tungsten halogen irradiation.

422 Therefore our study and the study of Yoshida and collaborators (2013) suggests that also
423 halogen bulbs with a low CCT can induce cytotoxic effect.

424 Our study showed that light induced more cytotoxicity on ARPE-19 than on BEAS-2B cells.
425 ARPE-19 are retinal cells and the most common mechanism by which light is thought to
426 cause damage to retina is the photochemical. Considering our results, we hypothesized that
427 ARPE-19 cells were particularly susceptible to blue radiations because, as RPE cells, have
428 many mitochondria, so they have many cytochromes. The cytochromes are chromophores, so
429 they interact with wavelengths in the high-energy portion of the visible spectrum and cause
430 the generation of free radicals (King et al. 2004; Youssef, Sheibani and Albert 2011). This
431 hypothesis is confirmed by an experimental study attesting that mitochondria are an
432 important source of toxic oxygen radicals in the short wavelength light-exposed RPE cells
433 (King et al. 2004; Youn et al. 2009). Moreover RPE cells are especially susceptible to
434 oxidative stress, induced by light, because of their high membrane lipid levels (e.g.
435 polyunsaturated fatty acids) (Youn et al. 2009).

436 The Comet assay was used to assess the genotoxicity induced by light on ARPE-19 and
437 BEAS-2B cells. Considering results on ARPE-19, halogen, warm LED and cold LED bulbs
438 caused a significant slight genotoxic damage after 4h exposure. During Comet assay, after 4h
439 exposure, the wells treated with light presented cells detached from the bottom, confirming
440 the major cytotoxicity observed on ARPE-19 than on BEAS-2B. The detached cells probably
441 was lost after washing with PBS increasing the proportion of living cells detected by trypan
442 blue staining, which was not lower than 70% (incandescent: 87.5%, halogen: 96.7%, warm
443 LED: 86.2%, cold LED: 84.7%). Moreover, during Comet assay scoring, many hedgehogs
444 (comets with almost all DNA in the tail) were scored after treatment with light, especially
445 after 4h exposure. In agreement with the major cytotoxicity observed in our study on ARPE-
446 19 than on BEAS-2B, in literature, it was largely suggested that these comets come from

447 heavily damaged cells and represent cells engaging in apoptosis. However, some authors do
448 not agree with this interpretation claiming that hedgehogs can correspond to one level on a
449 continuum of genotoxic damage and are not diagnostic of apoptosis (Lorenzo et al. 2013).
450 We hypothesized that the high cytotoxicity detected on ARPE-19, probably concealed the
451 detection of a high genotoxic effect.

452 On the ARPE-19 cells, it was not possible to perform the Fpg modified Comet assay because
453 the oxidized sites were high in the control. The same evidence was found by Sparrow, Zhou,
454 and Cai (2003). They evaluated DNA damage induced by blue light (430 nm) on ARPE-19
455 cells loaded with A2E and the ability of cells to repair DNA. They found high oxidized site in
456 the control and hypothesized the presence of pre-existing base changes. According to our
457 results, the same study demonstrated that the light caused a time-dependent DNA damage.
458 The DNA damage induced by light exposure on ARPE-19 was studied also by Youn and
459 collaborators (2009), using confocal laser scanner microscopy. Their results showed that only
460 radiations with lower wavelength caused the increased degradation of DNA/RNA (especially
461 RNA) in comparison with the control cells. Previously also Hafezi and collaborators (1997)
462 and Seko and collaborators (2001) showed that light induced apoptosis in the retinal cells,
463 especially revealing DNA fragmentation and nucleic chromatin alteration (Youn et al. 2009).
464 The results of Youn and collaborators, Hafezi and collaborators and Seko and collaborators
465 correspond to our results on cytotoxicity and to the presence of hedgehog during the scoring
466 of Comet assay.

467 Considering our genotoxicity results on BEAS-2B cells, the Comet assay performed without
468 Fpg showed that all type of bulbs caused a significant direct DNA damage after 4h exposure.
469 Cold LED caused the major genotoxic effect. Similar results were obtained by Chamorro and
470 collaborators (2013). They investigated the effects of LED radiations (blue-468nm, green-
471 525nm, red-616nm and white light) on human RPE cells (HREpiC). They found that all types

472 of light induced a significant DNA damage and the greatest damage was observed for the
473 blue LED light (468 nm). A major genotoxic effect induced by cold light was found also by
474 Xie and collaborators (2014) using Comet assay on lens epithelial cells (hLECs).
475 Our study investigated the oxidative DNA damage using Fpg modified Comet assay.
476 Contrary to ARPE-19, Fpg modified Comet assay was performed successfully on BEAS-2B
477 cells. Incandescent, halogen and warm LED bulbs induced no oxidative DNA damage. On
478 the contrary, a statistically significant increase of oxidative DNA damage was observed after
479 4 hours exposure with cold LED. Our results using Fpg enzyme confirmed the major
480 biological effects of cold LED and suggested that blue light could induced reactive oxygen
481 species and oxidative stress leading to oxidative DNA damage, as supposed above and
482 confirmed by other studies (Sparrow, Zhou, and Cai 2003; King et al. 2004; Roehlecke et al.
483 2009; Moon et al. 2017).

484

485 **5. CONCLUSION**

486 Our results indicates that light induced mainly cytotoxic effects on ARPE-19 and DNA
487 damage on BEAS-2B, so different cell line models showed different biological response. The
488 difference is probably due to a different susceptibility between the two cell lines. In
489 particular, ARPE-19 cells seemed to be more susceptible to light exposure. On ARPE-19
490 cells light induced a cytotoxic effect which probably concealed the detection of a high
491 genotoxic effect. The use of different cell models was important because only on BEAS-2B
492 cells (which are more resistant) it was possible the detection of oxidative DNA damage
493 induced by blue light. This kind of damage leaded to the hypothesis that light induced effects
494 are mediated by oxidative stress, confirming hypothesis made before by other authors.
495 Moreover, in our experimental conditions, among the four (incandescent, halogen, warm
496 LED, cold LED) commercial bulbs, cold LED caused the major cytotoxic effect on ARPE-19

497 and the major genotoxic and oxidative effect on BEAS-2B. Commercial cold LED is able to
498 cause more cellular damage probably because contains more high-energy radiations (blue)
499 than the other bulbs. While further evaluations are be needed to assess biological effects of
500 light emitted by different sources for the same amount of total exposure, the different Total
501 Blue-light Exposure among sources gives also relevance to the findings on cytotoxic effects
502 of halogen bulb and highlights the need of more investigations on the topics. Ultimately our
503 results indicates that LED technology could be a safe alternative to older technologies but the
504 use of warm LED should be preferred because the light emitted by cold LED can potentially
505 cause adverse effects on retinal cells.

506

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643 **TABLES**

644 **Table 1.** Characteristics of the four tested bulbs.

	Incandescent bulb	Halogen bulb	Warm LED	Cold LED
Power	25 W – 230 V	18 W – 220/240 V	3 W	3.5 W – 230 V
Declared luminous flux	≈ 200 Lumen	210 Lumen	250 Lumen	300 Lumen
Measured Correlated Colour Temperature	warm white 2589 ± 5 K	warm white 2652 ± 5 K	warm white 2700 ± 5 K	cold white 6500 ± 14 K
Energy efficiency class	≈E	C	A+	A+

645

646 **Table 2.** Illuminance and Total Exposure of the four tested bulbs measured and calculated for

647 Cond. C

	Incandescent bulb	Halogen bulb	Warm LED	Cold LED
Measured Illuminance, Cond.C	602 ± 27 lx	215 ± 9 lx	434 ± 20 lx	1126 ± 50 lx
Calculated Total blue-light weighted exposure 1 h Cond.C.	0,144 ± 0,007 W/m ² h	0,049 ± 0,002 W/m ² h	0,091 ± 0,004 W/m ² h	0,878 ± 0,044 W/m ² h
Calculated Total blue-light weighted exposure 4 h Cond.C.	0,577 ± 0,028 W/m ² h	0,196 ± 0,009 W/m ² h	0,364 ± 0,018 W/m ² h	3,512 ± 0,018 W/m ² h

648

649 **LEGEND TO FIGURES**

650 **Table 1.** Characteristics of the four tested bulbs.

651 **Table 2.** Illuminance and Total Exposure of the four tested bulbs measured and calculated for

652 Cond. C.

653 **Figure 1.** Normalized Spectral intensity distribution of the four lamps as measured in Cond.

654 A, Cond. B, Cond.C (a) and Normalized Spectral intensity distribution of the four lamps in

655 Cond. A, and Cond.C with Blue-light hazard weighting function $B(\lambda)$ (b), the purple lines
656 identifies the Blue-light range.

657 **Figure 2.** Blue light weighted spectral exposure measured in Cond C.

658 **Figure 3.** Temperature variations induced by the four different (incandescent, halogen, warm
659 LED, cold LED) bulbs inside irradiated and control wells.

660 **Figure 4.** Cytotoxicity of ARPE-19 cells exposed for 1h or 4h to light emitted by the
661 different bulbs: incandescent (a), halogen (b), warm LED (c), cold LED (d). Bars represent
662 the mean % cell viability (quadruplicate), error bars represent standard deviation of mean.

663 Asterisks indicate statistically significant differences vs control cells (C- cell viability 100%)

664 * $p < 0.05$; ** $p < 0.001$ (T- test Student).

665

666 **Figure 5.** Cytotoxicity of BEAS-2B cells exposed for 1h or 4h to light emitted by the
667 different bulbs: incandescent bulb (a), halogen bulb (b), warm LED (c), cold LED (d). Bars
668 represent the mean % cell viability (quadruplicate), error bars represent standard deviation of
669 mean. Asterisks indicate statistically significant differences vs control cells (C- cell viability
670 100%) * $p < 0.05$; ** $p < 0.001$ (T- test Student).

671

672 **Figure 6.** Genotoxic effect, evaluated by the Comet assay, of ARPE-19 cells exposure (1h or
673 4h) to light emitted by the different bulbs: incandescent bulb (a), halogen bulb (b), warm
674 LED (c), cold LED (d). Bars represent the mean % tail intensity value from two spots, error
675 bars represent standard deviation of mean. Asterisks indicate statistically significant
676 differences vs control cells (C-) * $p < 0.05$ (T- test Student).

677

678 **Figure 7.** Genotoxic effect, evaluated by the Comet assay, of BEAS-2B cells exposure (1h or
679 4h) to light emitted by the different bulbs: incandescent bulb (a), halogen bulb (b), warm

680 LED (c), cold LED (d). Bars represent the mean % tail intensity value from two spots, error
681 bars represent standard deviation of mean. Asterisks indicate statistically significant
682 differences vs control cells (C-) *p< 0.05 (T- test Student).

683

684 **Figure 8.** Genotoxic effect, evaluated by the Fpg-Comet assay, of BEAS-2B cells exposure
685 (1h or 4h) to light emitted by the different bulbs: incandescent bulb (a), halogen bulb (b),
686 warm LED (c), cold LED (d). Bars represent the mean % tail intensity value from two spots,
687 error bars represent standard deviation of mean. Asterisks indicate statistically significant
688 differences vs control cells (C-) *p< 0.05 (T- test Student).