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Cytotoxicity and genotoxicity of light emitted by incandescent, halogen, and LED bulbs on ARPE-19 and BEAS-2B cell lines

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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% CO<sub>2</sub>.

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% CO<sub>2</sub>.

### 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  $5 \times 10^4$  cells/well and, after  
229 exposure, 50  $\mu$ 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  $3 \times 10^5$  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 ( $3 \times 10^5$ ) were mixed with  
245 140  $\mu$ l of 0.7% low melting point agarose (LMA) and 20  $\mu$ 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
<b>Power</b>	25 W – 230 V	18 W – 220/240 V	3 W	3.5 W – 230 V
<b>Declared luminous flux</b>	≈ 200 Lumen	210 Lumen	250 Lumen	300 Lumen
<b>Measured Correlated Colour Temperature</b>	warm white 2589 ± 5 K	warm white 2652 ± 5 K	warm white 2700 ± 5 K	cold white 6500 ± 14 K
<b>Energy efficiency class</b>	≈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
<b>Measured Illuminance, Cond.C</b>	602 ± 27 lx	215 ± 9 lx	434 ± 20 lx	1126 ± 50 lx
<b>Calculated Total blue-light weighted exposure 1 h Cond.C.</b>	0,144 ± 0,007 W/m <sup>2</sup> h	0,049 ± 0,002 W/m <sup>2</sup> h	0,091 ± 0,004 W/m <sup>2</sup> h	0,878 ± 0,044 W/m <sup>2</sup> h
<b>Calculated Total blue-light weighted exposure 4 h Cond.C.</b>	0,577 ± 0,028 W/m <sup>2</sup> h	0,196 ± 0,009 W/m <sup>2</sup> h	0,364 ± 0,018 W/m <sup>2</sup> h	3,512 ± 0,018 W/m <sup>2</sup> 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).