

Establishment of an *in vitro* photoassay using THP-1 cells and IL-8 to discriminate photoirritants from photoallergens

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## Abstract

At present, there are no *in vivo* or *in vitro* methods developed which has been adopted by regulatory authorities to assess photosensitization induced by chemicals. Recently, we have proposed the use of THP-1 cells and IL-8 release to identify the potential of chemicals to induce skin sensitization. Based on the assumption that sensitization and photosensitization share common mechanisms, the aim of this work was to explore the THP-1 model as an *in vitro* model to identify photoallergenic chemicals.

THP-1 cells were exposed to 7 photoallergens and 3 photoirritants and irradiated with UVA light or kept in dark. Non phototoxic allergens or irritants were also included as negative compounds. Following 24 h of incubation, cytotoxicity and IL-8 release were measured. At subtoxic concentrations, photoallergens produced a dose-related increase in IL-8 release after irradiation. Some photoirritants also produced a slight increase in IL-8 release. However, when the overall stimulation indexes of IL-8 were calculated for each chemical, six out of seven photoallergens tested reached a stimulation index above 2, while the entire set of negative compounds had stimulation indexes below 2. Our data suggest that this assay may become a useful cell-based *in vitro* test for evaluating the photosensitizing potential of chemicals.

Keywords: photosensitization, *in vitro* toxicology, dendritic cells, IL-8



## 1. Introduction

Safety assessment of ingredients is an important part of the development of cosmetics and drugs. For certain medical and consumer products, photosafety testing has become a mandatory regulatory requirement. This may include an assessment of acute phototoxicity (photoirritation), photoallergy, photogenotoxicity and photocarcinogenicity (EMA, 2012). In this sense, differentiation between photoallergenic and phototoxic reactions induced by low molecular weight compounds represents a current problem. Historically, the toxicological evaluation is conducted on animals, however, the 7th Amendment to the Cosmetics Directive (Directive 76/ 768/EEC) aims for the complete replacement of animal testing by 2013. Moreover, the new European Chemicals Legislation (REACH, EU, 2006) favours alternative methods, if validated and appropriate. Therefore, the development of innovative *in vitro* alternatives is needed to comply with the amendment and, in order to replace the currently used *in vivo* methods.

The adverse cutaneous response to phototoxic compounds can be reproduced, *in vitro*, using human skin models. In this sense the 3T3 Neutral Red Uptake phototoxicity test has been adopted by OECD as Test Guideline 432 (OECD, 2004) as a general screen for the phototoxic potential of UV absorbing substances. However, the validated 3T3 NRU PT test has some limitations and may provide false positive results. For this reasons, the Photo RBC and the Human 3-D Skin Model *In Vitro* Phototoxicity test, are two additional assays regarded as useful and important adjunct test to overcome the limitations of the 3T3 NRU PT (Ceridono et al., 2012).

The case of photosensitization and photoallergic reactions is different because there are no *in vivo* methods validated. Various laboratory animal study assays have been proposed, mainly methods that were originally developed for investigating contact allergy and were subsequently adapted for photoallergy, as the guinea-pig maximization test (Jordan, 1982; Magnuson *et al.*, 1969; Maurer *et al.*, 1980), the localized lymph node assay (Ulrich *et al.*, 2001), and the mouse ear swelling model (Gerberick and Ryan, 1990). Although, currently no standardized methodology has been agreed or adopted by regulatory authorities the Photo Local Lymph Nodule Assay protocol is the more promising *in vivo* to identify potential photoallergic from phototoxic compounds. The advantage of the Photo-LLNA is its ability to identify photoirritant and photoallergic compounds by calculation of the Differentiation Index (Neumann et al., 2005), which allow this assay to evaluate photoallergic properties of a supposed photoreactive agent.



Although the exact mechanism of photoallergy is not completely known, it is considered to be a form of delayed type hypersensitivity, as contact dermatitis, which occurs when an exogenous agent (photoallergen) is applied to the skin and subsequently exposed to ultraviolet (UV) and/or visible radiation (Kerr and Ferguson, 2010). In the case of photoallergy, the hapten is a photosensitizer that requires light exposure for its activation (Pendlington and Barratt, 1990). The initial step in the induction of delayed type hypersensitivity is the uptake of the hapten by antigen-presenting cells within the epidermis. The next step implies the migration of such antigen-presenting cells to the lymph node where they stimulate the proliferation of antigen-specific T-lymphocytes (Gerberick *et al.*, 1991a; Gerberick *et al.*, 1991b). Langerhans cells (LCs) are a type of dendritic cells (DCs), which are the main antigen-presenting cells present in the skin. During the induction phase of (photo-)sensitization, they differentiate and mature expressing co-stimulatory and adhesion molecules and secrete various cytokines, including IL-1beta and IL-8 (Banchereau *et al.*, 2000).

In the case of contact sensitization, DC and DC-like cells has been used to develop new *in vitro* methods to discriminate contact sensitizers from irritants including LCs (Krasteva *et al.*, 1996), human peripheral blood mono-nuclear cells (PBMC) (Coutant *et al.*, 1999; Guironnet *et al.*, 2000; Pichowski *et al.*, 2000; Tuschl *et al.*, 2000); CD34+ hematopoietic progenitor cells (HPC) (Boislève *et al.*, 2004), and DC-like cell lines, such as THP-1 (Yoshida *et al.*, 2003), U937 (Python *et al.*, 2007), KG-1 (Hulette *et al.*, 2002), and MUTZ-3 (Azam *et al.*, 2006). Major drawbacks of DCs and peripheral blood derived DCs are their complex and expensive preparation procedures, sourcing issues and their donor-to-donor variability. In this sense, the use of DC-like cell lines is gaining much attention. Notably, two assays MUSST and h-CLAT based on U937 and THP-1 cell lines, respectively, are currently under pre-validation at ECVAM (Aeby *et al.*, 2010).

Regarding photosensitization only few works have been published mainly based on DC-like cells (Hoya *et al.*, 2009; Karschuk *et al.*, 2010), and more recently a promising keratinocyte photoassay has been proposed (Galbiati *et al.*, 2013). But to date no *in vitro* accepted alternative is available to identify the photoallergenic potential of new chemicals, although it is expected that chemicals showing photoallergic properties, are likely to give positive reactions in the 3T3 NRU PT test (SCCS/1501/12). On the basis of the evaluation of the photosafety of chemicals in a testing strategy, more efforts should be devoted to develop a reliable *in vitro* method to evaluate the photosensitization potential of new chemicals and to discriminate between photoirritants and photosensitizers.



Recently, we have proposed THP-1 and the release of the chemokine IL-8, along with p38 MAPK activation as a model for screening sensitizers (Mitjans *et al.*, 2008; Mitjans *et al.*, 2010). Based on the assumption that contact photosensitization has the same mechanistic of contact sensitization, the purpose of this work was to explore the suitability of this model to discriminate photosensitizers from photoirritants.

## 2. Materials and methods

### 2.1. Chemicals

Phototoxic and non-phototoxic chemicals were selected based on the information provided by similar published studies and reported to cause allergic contact dermatitis as showed in Table 1.

Chlorpromazine hydrochloride (CPZ, CAS no. 69-09-0) was chosen as known photoallergens and photoirritants. 6-methylcoumarin (6-MC, CAS no. 92-48-8), benzophenone (BZP, CAS no. 119-61-9), ketoprofen (KETO, CAS no. 22071-15-4), avobenzene (1-(4-methoxyphenyl)-3-(4-tertr-butylphenyl)1,3-propendione) (AVO, CAS no. 70356-09-1), 4-aminobenzoic acid (PABA, CAS no. 150-13-0) and 2-ethylhexyl-4-methoxycinnamate (OMC, CAS no. 5466-77-3) as known photoallergens. Ibuprofen (IBU, CAS no. 15687-27-1), retinoic acid (RET, CAS no. 302-79-4) and 8-methoxypsoralen (8-MOP, CAS no. 298-81-7) as known photoirritants. 2-aminophenol (2-AP, CAS no. 95-55-6), nickel sulfate (NiSO<sub>4</sub>, CAS no. 10101-97-0), and diethylmaleate (DEM, CAS no. 141-05-9) as known allergens. Octanoic acid (OCT, CAS no. 124-07-2), and sodium dodecyl sulfate (SDS, CAS no. 151-21-3) were chosen as known irritants. All compounds were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Stock solutions of chemicals were prepared in dimethylsulfoxide immediately before use. The final concentration of dimethylsulfoxide (DMSO) never exceeded 1%.

### 2.2. Cell culture

The human monocytic leukemia cell line THP-1 was cultured in RPMI 1640 medium supplemented with 10% (v/v) of heat inactivated FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 50 µM 2-mercaptoethanol, and incubated at 37 °C, 5% CO<sub>2</sub>.

### 2.3. Chemical treatment



THP-1 cells were seeded into 24-well plates at a density of  $1 \times 10^6$  cell/ml. The final volume in each well was 500  $\mu$ l, the medium used contained 5% FBS and 5  $\mu$ l of different concentrations of the chemicals. Each concentration was tested in triplicate and untreated cells (controls) were exposed to 5  $\mu$ l of the vehicle (DMSO). Two plates were prepared in parallel, one was kept in dark (non-irradiated) and the other was irradiated immediately after applying chemical treatments.

#### *2.4. Light exposure of THP-1*

The irradiation of the samples was carried out in a photostability UV chamber (58 x 34 x 28 cm) equipped with three UVA lamps Actinic BL TL/TL-D/T5 (Philips®, 43 V, 352 nm, 15W). Lamp spectra (Figure 1) show that maximum irradiance is found in the range of UVA irradiation with a peak at 365 nm. Irradiance has been routinely measured through the plate lid before cell exposure with a photoradiometer Delta OHM provided with a UVA probe (HD2302 - Italy) to determine UV dose using the following equation:

$$E \text{ (J/cm}^2\text{)} = t(\text{s}) \times P \text{ (W/cm}^2\text{)}$$

Where E stands for UV dose, t represents the time expressed in seconds and, finally, P is the lamp potency. Cells were irradiated with a dosing of 1.6-2.1 mW/cm<sup>2</sup> to give a final exposure of 1, 2.5, and 5 J/cm<sup>2</sup>.

#### *2.5. Cytotoxicity measured by the MTT assay*

After irradiation cells were incubated 24h (irradiated and non-irradiated plates), then plates were centrifuged, supernatants were collected and kept at -80°C for IL-8 measurement. 500  $\mu$ l of a MTT solution 0.75 mg/ml was then added to each well. Cells were incubated for 3 h at 37 °C, plates were then centrifuged, medium discarded and cells lysed in 250  $\mu$ l/well of a mixture of HCl/isopropanol. 100  $\mu$ l of the resulting solutions was transferred to a 96-well plate and the absorbance was read at 550 nm using a Bio-Rad 550 microplate reader. Cell viability was calculated as the percentage of tetrazolium salt reduction by viable cells on each sample against the untreated cells. Seventy five percent cell viability (CV<sub>75</sub>) was calculated for each chemical by linear regression analysis of data.

#### *2.5. IL-8 release measurement*

IL-8 release was assessed in cell free supernatants using a human ELISA kit from Immuno Tools (Germany). Results are expressed in pg/ml. Limit of detection of 15.6 pg/ml.



## 2.6. Data analysis and statistics

Results are expressed as mean  $\pm$  standard error of at least three independent experiments. Statistical analyses were performed using one-way analysis of variance (ANOVA) to determine the differences between datasets, followed by Dunnett's *post-hoc* test for multiple comparisons using the SPSS<sup>®</sup> software (SPSS Inc., Chicago, IL, USA).  $p < 0.05$  and  $p < 0.01$  were considered significant.

## Results

### 3.1. Determination of the appropriate UVA dose

Preliminary experiments were carried out in order to establish the appropriate UVA dose. The aim of those experiments was to find the UVA dose that does not cause cell cytotoxicity or a significant IL-8 release to cell supernatants on control cells (not treated with chemicals) but, at the same time, still provide enough energy to photoactivate the chemicals with photosensitization potential causing a significant response on THP-1 in terms of IL-8 release. To perform this, untreated THP-1 cells were exposed to different UVA doses (1, 2.5 and 5 J/cm<sup>2</sup>) and cell viability and IL-8 release was tested 24 h after light exposure. In parallel, chlorpromazine was selected as a reference compound to test the cell response under UVA conditions. THP-1 cells were treated with different concentrations of chlorpromazine and then were exposed to the different UVA doses. Results are shown in Figure 2. Based on this data, the dose of 2.5 J/cm<sup>2</sup> was selected for further experiments, as it did not cause a decrease on cell viability or a significant release of IL-8 on control cells. This irradiation dose was enough to induce a significant release of IL-8 in presence of chlorpromazine, without a remarkable cell viability decrease.

### 3.2. Effect of the selected chemicals on cell viability

The concentration of the chemical resulting in 75% of cell viability (CV<sub>75</sub>) respect to vehicle treated cells 24 h after treatment was calculated for all chemicals in non-irradiated and irradiated (2.5 J/cm<sup>2</sup>) conditions. Results are presented in Table 2. Photoallergens and photoirritants showed photoreactivity as it is appreciated in the differences between CV<sub>75</sub> values between non-irradiated and irradiated cells. With the exception of 4-aminobenzoic acid and ibuprofen which did not show any cytotoxicity nor phototoxicity in our experimental conditions.



### 3.3. Effect of light and the selected chemicals on IL-8 release

The selection of the concentration range of each chemical was based on the cytotoxicity of the compounds. The use of a sub-toxic concentration of a sensitizer shows to be a key factor for the prediction of sensitization in THP-1 (Ashikaga *et al.*, 2006; Yoshida *et al.*, 2003). For this reason, we selected the CV<sub>75</sub> of each chemical in irradiated conditions as the highest concentration tested and serial dilutions of this concentration were also tested. Results obtained with the selected chemicals are shown in Figures 3 and 4, where the release of IL-8 is represented for irradiated and non-irradiated cells.

The selected photosensitizing chemicals produced a dose-related increase in IL-8 release only under irradiation conditions, with the exception of AV○ and OMC which produced a dose-related increase also without irradiation. The strongest responses were observed with CPZ, 6-MC, BZP and AV○, while KETO, PABA and OMC produced a moderate increase in IL-8 release. The photoirritant 8-MOP failed in producing an increase in IL-8 release, while the photoirritants RET and IBU produced a slight increase. In the case of allergens, NiSO<sub>4</sub>, 2-AP and DEM, the dose-related increase appeared in both irradiated and non-irradiated cells. The irritants OCT and SDS failed to produce a significant IL-8 release under the same experimental conditions.

In order to establish the suitability of the method to discriminate between photoallergens and negative compounds (photoirritants, allergens, and irritants), stimulation indexes (SI) were calculated for irradiated (I-SI) and non-irradiated cells (NI-SI) as the ratio of IL-8 release of treated cells against untreated cells. An overall stimulation index (I-SI/NI-SI) was also calculated as the ratio of the stimulation indexes in irradiated and non-irradiated cells. Those stimulation indexes were represented in Figure 5, with a single concentration for each compound in which cell viability was above the 75%.

Photoallergens showed SI in irradiated conditions that varied between almost 2 to 16, depending on the concentration assayed, while in non-irradiated conditions it could not be detected any stimulation in IL-8 release, with the abovementioned exceptions, AV○ and OMC, which SIs in non-irradiated conditions reached almost 5 and 9 times-folds, respectively. In contrast, in most cases the negative compounds tested (photoirritants, allergens and irritants) exhibited SI in irradiated conditions below 2, with the exceptions of IBU and DEM but they did not exceed the 2.5 times-fold increase.



When the overall SI was calculated, the results were similar to SI in irradiated cells with values between 2 and 17. The exception of OMC persisted with an overall SI of 0.9 mainly due to its high stimulation of IL-8 release on non-irradiated cells. The case of AVO was different to OMC because the SI of irradiated cells was higher than SI of non-irradiated cells; therefore, it exhibited an overall SI of 2.8.

## Discussion

When drugs and chemicals are sold within the marketplace, it is clearly important to know in advance whether a molecule has the potential for photoallergy or not. A missed (photo-) allergen hinders the opportunity of preventive avoidance. Regulatory authorities have historically sought to have clinically relevant predictive information available before chemical approval. The *in vitro* 3T3 NRU PT is currently accepted as a valid screening *in vitro* method to assess phototoxic potential for soluble compounds (OECD, 2004; EMA, 2012). When a potential phototoxic compound is identified using this *in vitro* assay, a photoallergy assessment is generally warranted. However, nor animal-based or *in vitro* method has been accepted for evaluating photosensitization. And over the years, there has been considerable variation in the methodology used for photopatch testing in humans (Kerr and Ferguson, 2010), making even more difficult to predict the photosensitizing potential of a particular chemical.

At present, photoallergy testing is mainly conducted using animals, although some *in vitro* test as photoadduct formation and photooxidation can be useful screening tools. Among the different *in vivo* assay the modified LLNA assay or Photo-LLNA is preferred for the reduction in the number of animals required and the lessening of animal pain and distress due to the reduction of the amount of time. However, the 7<sup>th</sup> amendment to the cosmetics Directive (Directive 76/768/EEC) foresees a complete ban on animal testing for cosmetics ingredients from 2013 for all the human-health related effects. On the other hand, the implementation of REACH legislation urgently demands developing feasible and reliable *in vitro* methods

In this sense, the objective of this study was to evaluate the suitability of IL-8 release on THP-1 cells as a method for the detection of chemicals with photosensitizing potential.

Our approach is based on two facts; first, we have recently demonstrated the usefulness of IL-8 release on THP-1 as a method to discriminate between allergens and irritants (Mitjans *et al.*, 2008; Mitjans *et al.*, 2010) second, allergic photocontact dermatitis and allergic contact dermatitis presumably share the same mechanistic basis.



Light source is a crucial factor to study photosensitization being solar simulator lamps generally considered the optimal artificial light source. Nevertheless, other light sources can be considered if the wavelength range emission includes the absorption spectrum of the chemicals studied. In a recent workshop (Ceridono et al., 2012), it was underlined that the waveband concerned is mainly UVA, with some drugs extending to UVB and others to visible. Thus, we have chosen a pure UVA lamp source with an irradiance spectra ranging from 320 nm to 400 nm with a peak at 365 nm similarly to other studies (Galbiati et al., 2013). The use of this irradiation source can underestimate the impact of some light visible photoreactive compounds. However, as the chemicals studied in this work, the majority of phototoxic compounds are predominantly UVA absorbers with significant higher clinical impact than other wavelength absorbers (Ceridono et al., 2012). Moreover, the information obtained with this photoassay will contribute to evaluation of photochemical characteristics using non clinical studies as an integrated process of photosafety assessment.

The first step was to find the appropriate irradiation conditions. We tested several energy doses: 1.0, 2.5, and 5.0 J/cm<sup>2</sup> with an intensity of 1.6-2.1 mW/cm<sup>2</sup>. The higher UVA dose tested, 5 J/cm<sup>2</sup>, produced a significant release of IL-8 in untreated cells, and the lowest, 1 J/cm<sup>2</sup>, was not enough to produce a significant release of IL-8 in presence of chlorpromazine at sub-toxic concentrations. Therefore, the selected light dose in this study was 2.5 J/cm<sup>2</sup> which it did not affect cell viability nor produced a significant IL-8 release. In contrast, in the work of Hoya *et al.* (2009) where they proposed THP-1 and the expression of CD86 and CD54 to assess photoallergenicity, they selected a dose of 5.0 J/cm<sup>2</sup> with an intensity of 1.7 mW/cm<sup>2</sup> as the appropriate test condition to detect the effects of photoallergens (Hoya 2009). However, the dose of 2.5 J/cm<sup>2</sup> with an intensity of 1.7 mW/cm<sup>2</sup> (similar to our experimental conditions) was also enough to induce a dose-dependent induction of CD86 and CD54 expression. These doses (2.5-5 J/cm<sup>2</sup>) are also used in photopatch testing in humans (Bruynzeel *et al.*, 2004, Kerr *et al.*, 2012).

To discriminate photoallergens from photoirritants, we have selected seven known photoallergens, four are UV filters (BZP, AV<sup>O</sup>, PABA, and OMC), one nonsteroidal anti-inflammatory drug (KETO), one antipsychotic drug (CPZ), and a fragrance (6-MC). Under the irradiation conditions, photoallergens induced a dose-response release of IL-8 (Figure 2). We also examined three compounds described as photoirritants using the same experimental conditions, ibuprofen (IBU) a nonsteroidal anti-inflammatory drug, retinoic acid (RET), a topical drug commonly used for treating acne, and 8-methoxypsoralen (8-MOP), a drug used for psoriasis. IBU and RET also produced a slight increase of IL-8 release



statistically significant after irradiation. But when the overall SI was calculated for photoirritants, they did not reach a value above 2. We also included negative compounds, allergens and irritants. In the case of allergens (DEM, 2-AP, and NiSO<sub>4</sub>), we observed significant increases of IL-8 in non-irradiated cells (Figure 3), confirming previous results (Mitjans *et al.*, 2008; Mitjans *et al.*, 2010). The irritants (SDS and OCT) failed to stimulate IL-8 release in irradiated and non-irradiated conditions.

Among photoallergens, CPZ and AVO were the chemicals that produced the strongest reaction in terms of SI after irradiation above 10. In contrast, KETO and PABA were the less potent in our experimental conditions, although, the high incidence of photoallergic reactions reported in human photopatch studies (Diaz *et al.*, 2006; Hindsen *et al.*, 2004; Kerr *et al.*, 2012; Waters *et al.*, 2009; Wennersten *et al.*, 1984). However, it is true that the use of non standardized protocols could mislead the conclusions concerning the potential photoallergenicity of PABA and this product is an stronger absorber of UVB (SCCP/1008/06).

OMC and AVO, two of the photoallergens tested in this study also showed strong responses in non irradiated conditions. It is not surprising that some photoallergens had some sensitization potential. In a recent multicentre study conducted in Europe using photopatch testing on patients with a history of dermatitis of photodermatitis, both compounds produced allergic contact dermatitis and photoallergic contact dermatitis reactions (Kerr *et al.*, 2012). Although Hayden *et al.*, (2005) demonstrated that AVO presents a low skin penetration, there exist some reports of positive photoallergic and allergic contact reactions in front this UV filter (Kerr and Ferguson 2010; Kerr *et al.*, 2012) and it is included in the chemical panel for photopatch test assays (Ibbotson *et al.*, 1997; Bruynzeel *et al.*, 2004). In this sense, it has recently been shown that AVO photodegrades to benzyls and arylglyoxals. The latter compound is a strong skin sensitizer, and photocontact allergy to dibenzoylmethanes may therefore be due to the formation of arylglyoxals in skin (Karlsson *et al.*, 2009).

When we analyzed the results of all the compounds, we found that six out of seven photoallergens reached an overall SI above 2.5. While the entire set of negative compounds (photoirritants, allergens, and irritants) had overall SI below 2.5. Therefore, we propose a tentative cut-off value of 2.5 in the overall SI to identify chemicals with photosensitizing potential. Based on these data, sensitivity and accuracy of the method was calculated obtaining 85.7% and 93.3%, respectively, with a positive predictivity of 100% and a negative predictivity of 88.8%.



Although we need to further confirm the applicability of the test evaluating a wider battery of chemicals, we consider that this assay may become a useful cell-based *in vitro* test for evaluating the photosensitizing potential of chemicals.



## Conflict of interest and Funding information

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## Legend to figures

Figure 1: Spectral power distribution of Actinic BL TL/TL-D/T5 lamp. Energy was measured at various distances from irradiation source.

Figure 2. IL-8 release and cell viability induced by increasing amounts of UVA irradiation (0-5 J/cm<sup>2</sup>) in untreated cells and cells treated with 0.5  $\mu$ M of chlorpromazine. Results are presented as mean  $\pm$  ES of 3 independent experiments. Statistical analysis was performed with Dunnett's multiple comparison test (\*p <0.05, \*\*p <0.01).

Figure 3. IL-8 release induced by increasing concentrations of the selected photoallergens (CPZ, 6-MC, BZP, KETO, AVO, PABA, OMC) and photoirritants (8-MOP, RET, IBU) in non-irradiated (open circles) and irradiated cells (black squares). SI calculated for each concentration tested is also shown (black triangles). Cell viability was found to be above the 75% in all the concentrations tested as measured by the MTT assay. Results are presented as mean  $\pm$  SE of at least 3 independent experiments, and statistical analysis was performed with Dunnett's multiple comparison test (\* p <0.05, \*\*p <0.01).

Figure 4. IL-8 release induced by increasing concentrations of the negative compounds (NiSO<sub>4</sub>, 2-AP, DEM, OCT, SDS) in non-irradiated (open circles) and irradiated cells (black squares). SI calculated for each concentration tested is also shown (black triangles). Cell viability was found to be above the 75% in all the concentrations tested as measured by the MTT assay. Results are presented as mean  $\pm$  SE of at least 3 independent experiments, and statistical analysis was performed with Dunnett's multiple comparison test (\* p <0.05, \*\*p <0.01).

Figure 5. The increases of IL-8 release are expressed as stimulation indexes for non-irradiated (NI-SI) and irradiated cells (I-SI). An overall stimulation index (I-SI/NI-SI) was calculated as the ratio of the stimulation indexes in irradiated and non-irradiated cells. The concentrations assayed were: 0.63  $\mu$ M (CPZ), 80  $\mu$ M (6-MC), 50  $\mu$ M (BZP), 62.5  $\mu$ M (KETO), 100  $\mu$ M (AVO), 7.5 mM (PABA), 500  $\mu$ M (OMC), 25  $\mu$ M (RET), 0.015  $\mu$ M (8-MOP), 1000  $\mu$ M (IBU), 12.5  $\mu$ M (2-AP), 100  $\mu$ M (DEM), 5.0 mM (OCT), 125  $\mu$ M (SDS), 500  $\mu$ M (NiSO<sub>4</sub>).



Table 1. Chemicals selected according to their classifications as photoirritants and/or photoallergens.

<b>Compound</b>	<b>Category</b>	<b>Reference</b>
Chlorpromazine	Photoallergen/photoirritant	Ibbotson et al., 1997; Lankerani and Baron, 2004; Hoya et al., 2009; Karschuk et al., 2010
6-methylcoumarin	Photoallergen	Ibbotson et al., 1997; Lankerani and Baron, 2004; Hoya et al., 2009; Cardoso et al., 2009; Karschuk et al., 2010
Benzophenone	Photoallergen	Lankerani and Baron, 2004; Hoya et al., 2009; Kerr and Ferguson, 2010; Kerr et al., 2012;
Ketoprofen	Photoallergen	Lankerani and Baron, 2004; Hoya et al., 2009; Kerr and Ferguson, 2010; Kerr et al., 2012;
Avobenzone	Photoallergen	Ibbotson et al., 1997; Kerr and Ferguson, 2010; Kerr et al., 2012
4-aminobenzoic acid	Photoallergen	Ibbotson et al., 1997; Lankerani and Baron, 2004; SCCP, 2006; Water et al., 2009; Kerr and Ferguson, 2010;
2-ethylhexyl-4-methoxycinnamate	Photoallergen	Ibbotson et al., 2001; Kerr and Ferguson, 2010; Kerr et al., 2012;
Retinoic acid	Photoirritant	Lankerani and Baron, 2004
8-methoxypsoralen	Photoirritant	Lankerani and Baron, 2004
Ibuprofen	Photoirritant	Lankerani and Baron, 2004
2-aminophenol	Allergen	Mitjans et al., 2010
Diethylmaleate	Allergen	Mitjans et al., 2010
Nickel sulfate	Allergen	Mitjans et al., 2008; Karschuk et al., 2010
Octanoic acid	Irritant	Robinson MK, 2002
Sodium dodecyl sulfate	Irritant	Mitjans et al, 2008; Hoya et al., 2009



Table 2. Concentration ( $\mu\text{M}$ ) of the chemical that induced 75% viability ( $\text{CV}_{75}$ ) in non-irradiated and irradiated conditions ( $2.5 \text{ J/cm}^2$ ).

Compound	$\text{CV}_{75}$ Non-Irradiated	$\text{CV}_{75}$ Irradiated
Chlorpromazine	4.7	1.0
6-methylcoumarin	> 1000*	75.5
Benzophenone	514.8	48.6
Ketoprofen	> 1000*	49.7
Avobenzene	> 200*	100,0
4-aminobenzoic acid	> 1000*	> 1000*
2-ethylhexyl-4-methoxycinnamate	652.7	500.0
Retinoic acid	123.2	29.9
8-methoxypsoralen	702.2	0.2
Ibuprofen	> 1000*	> 1000*
2-aminophenol	>1000*	60.2
Diethylmaleate	394.2	346.2
Nickel sulfate	958.8	510.1
Octanoic acid	> 1000*	> 1000*
Sodium dodecyl sulfate	105.8	91.1

$1 \times 10^6/\text{ml}$  cells were treated with increasing concentration of the selected chemicals or vehicle control in both irradiated and non-irradiated conditions. Cell viability was assessed by MTT reduction as described in Materials and Methods section.  $\text{CV}_{75}$  was calculated by linear regression analysis of data of at least 3 independent experiments. \*Maximal concentration assayed with no effects on cell viability.



Figure1

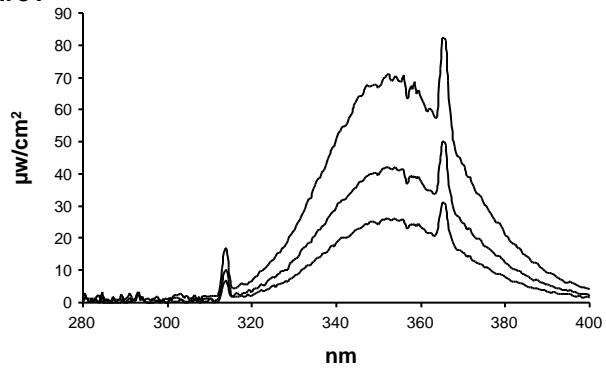
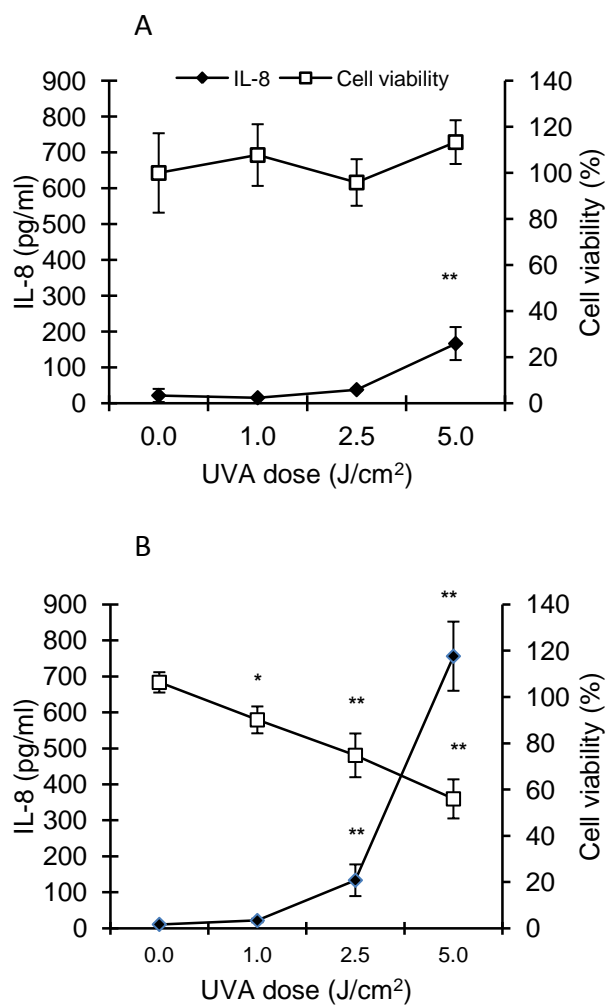


Figure 1: Spectral power distribution of Actinic BL TL/TL-D/T5 lamp



Figure2





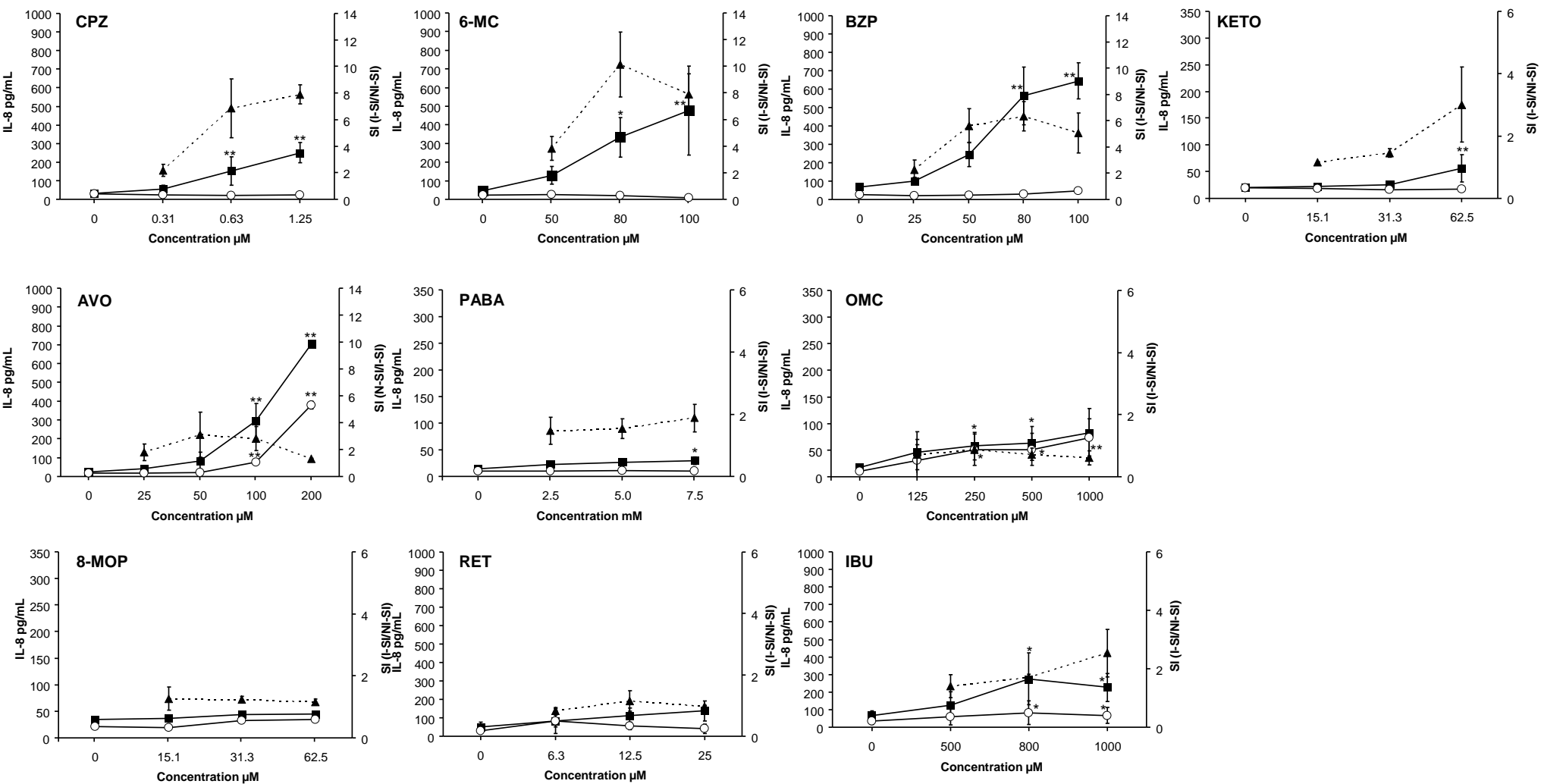
**Figure3**

Figure 3. IL-8 release induced by increasing concentrations of the selected photoallergens (CPZ, 6-MC, BZP, KETO, AVO, PABA and OMC) and photoirritants (8-MOP, RET and IBU) in non-irradiated (open circles) and irradiated cells (black squares). SI calculated for each concentration tested is also shown (black triangles). Cell viability was found to be above the 75% in all the concentrations tested as measured by the MTT assay. Results are presented as mean  $\pm$  SE of at least 3 independent experiments, and statistical analysis was performed with Dunnett's multiple comparison test (\* p < 0.05, \*\*p < 0.01).



Figure4

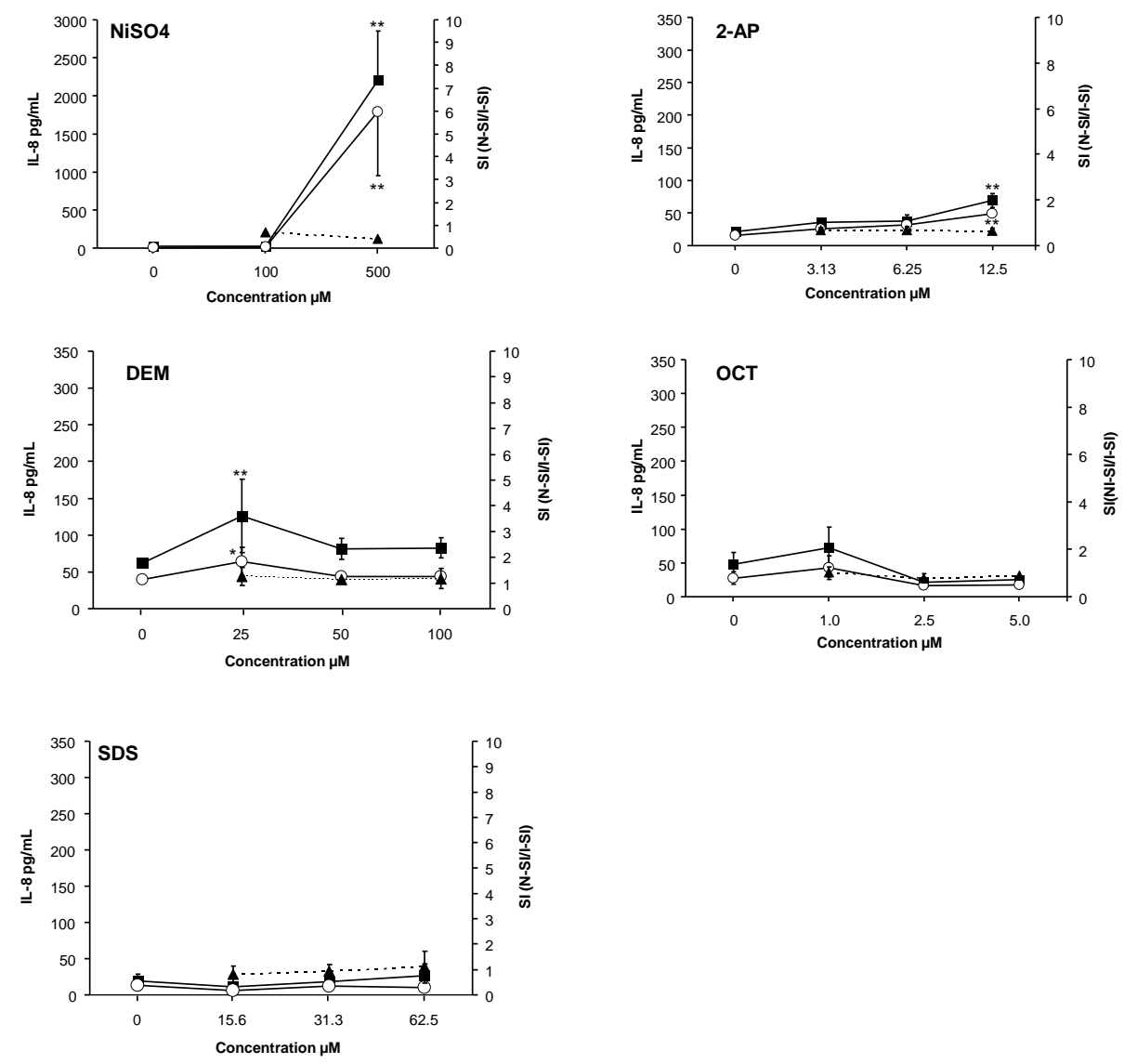


Figure 4. IL-8 release induced by increasing concentrations of the negative compounds in non-irradiated (open circles) and irradiated cells (black squares). SI calculated for each concentration tested is also shown (black triangles). Cell viability was found to be above the 75% in all the concentrations tested as measured by the MTT assay. Results are presented as mean  $\pm$  SE of at least 3 independent experiments, and statistical analysis was performed with Dunnett's multiple comparison test (\* p < 0.05, \*\*p < 0.01).



Figure5

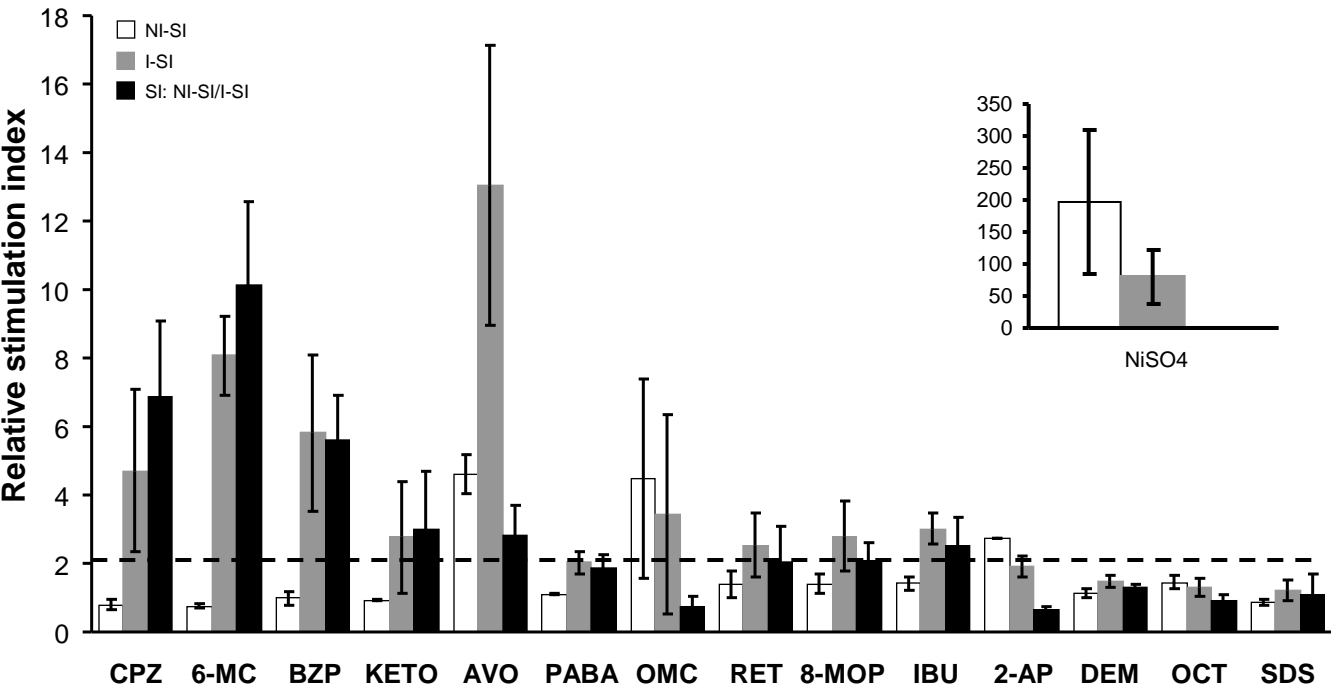


Figure 5. The increases of IL-8 release are expressed as stimulation indexes for non-irradiated (NI-SI) and irradiated cells (I-SI). An overall stimulation index (I-SI/NI-SI) was calculated as the ratio of the stimulation indexes in irradiated and non-irradiated cells. The concentrations assayed were: 0.63  $\mu$ M (CPZ), 80  $\mu$ M (6-MC), 50  $\mu$ M (BZP), 62.5  $\mu$ M (KETO), 100  $\mu$ M (AVO), 7.5 mM (PABA), 500  $\mu$ M (OMC), 25  $\mu$ M (RET), 0.015  $\mu$ M (8-MOP), 1000  $\mu$ M (IBU), 12.5  $\mu$ M (2-AP), 100  $\mu$ M (DEM), 5.0 mM (OCT), 125  $\mu$ M (SDS), 500  $\mu$ M (NiSO4).



### Highlights

- No *in vitro* methods have been adopted to assess photosensitization
- We explore the use of IL-8 release by THP-1 cells to identify photosensitizers
- Photoallergens induced a dose-related increase of IL-8 after irradiation
- A tentative cut-off is proposed to discriminate photoallergens from photoirritants
- The assay may become a useful cell-based *in vitro* test to assess photosensitization