Cycloheximide and disulfoton are positive in the photoclastogenicity assay but do not absorb UV irradiation: another example of pseudophotoclastogenicity?

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There is considerable concern regarding the biological plausibility of the response of certain chemicals in the in vitro photoclastogenicity assay, suggesting that this assay is oversensitive and lacks specificity. To explore this further, four coded compounds (aminotriazole, propantheline bromide, cycloheximide and disulfoton) were evaluated for their potential response in a photoclastogenicity assay in cultured Chinese hamster ovary (CHO) cells. None of the four compounds were shown to absorb ultraviolet radiation (UVR) or visible light in the 290- to 700-nm region of the electromagnetic spectrum. A fifth coded compound, tetracycline, which absorbs UVR, was also tested as this has previously been shown to be phototoxic in vitro (3T3-NRU assay) and is cytotoxic, but not genotoxic, at high concentrations in standard ‘dark’ genotoxicity assays in mammalian cells. The results showed that cycloheximide, disulfoton and tetracycline were clastogenic in CHO cells following UVR exposure (solar-simulated light at 700 mJ/cm²) but not in the absence of UVR. Aminotriazole and propantheline were negative in the presence and absence of UVR exposure. Follow-up testing showed that neither cycloheximide nor disulfoton was positive in the 3T3-NRU assay, the standard in vitro regulatory test for phototoxicity, a result consistent with their inability to absorb UVR. These data suggest that both cycloheximide and disulfoton are pseudophotoclastogens, like zinc oxide. Together, these data question the specificity of the in vitro photoclastogenicity assay in CHO cells and raises further concern regarding its use for the assessment of chemical photosafety for regulatory purposes. At the very least, a review of the current guidance documents for the photosafety evaluation of pharmaceuticals and cosmetics should be undertaken urgently.

Introduction

It is well known that solar ultraviolet radiation (UVR) induces DNA damage (1) and that UVR exposure is the major etiological cause of skin cancer in humans (1,2). It is also well documented that certain pharmaceuticals and cosmetic ingredients can be activated by UV or visible light when applied to the skin directly or systemically and that this may greatly enhance the carcinogenic effects of UVR (3). For example, it has been known for a long time that certain drug classes, such as fluoroquinolone antibiotics and psoralens, can enhance photocarcinogenesis in animal models (4,5). Moreover, there is now good evidence that patients treated with psoralen plus UVA (PUVA) have a small, but real, increased risk of squamous cell carcinoma (6). As such, photosafety testing has become a regulatory requirement for certain new medicinal products.

In the European Union (EU), pharmaceutical products are regulated under the European Agency for the Evaluation of Medicinal products (EMEA), whilst dermatological and sunscreen products are regulated under the EU Cosmetics Directive. Both types of product require extensive safety dossiers before they can be licensed for human use. The conditions for the photosafety evaluation of pharmaceuticals and cosmetics are described by The EMEA Committee for Proprietary Medicinal Products ‘Notes for Guidance on Phototoxicity Testing’ (7) or the ‘Notes of Guidance of the EU Scientific Committee on Cosmetics and Non-Food Products’ (8), respectively. Both of these documents provide guidance on the strategy and approaches that should be adopted by manufacturers for evaluating the photosafety of products prior to review by regulatory authorities.

Generally, photosafety testing must be considered for compounds that absorb light or UVR between wavelengths 290 and 700 nm of the electromagnetic spectrum and are applied either topically or locally and/or reach the skin or eyes via systemic exposure. Photosafety testing may include an assessment of acute phototoxicity (photoinitiation), photoallergy, ‘photogenotoxicity’ and photocarcinogenicity [for review see (9)]. In terms of photogenotoxicity testing, the main objective is to make an assessment of the potential of a compound to turn into a photochemical carcinogen upon activation with UV or visible (sic solar simulated) radiation. Several in vitro photo-genotoxicity assays, such as the photo-Ames, photo-chromosome aberration (CA) and photo-comet assays have been described in the literature and are based on standard ‘dark’ versions of regulatory assays used for genotoxicity assessment [for reviews see (10,11)].

Recently, an internal GlaxoSmithKline (GSK) review of compounds which had triggered a requirement for photosafety consideration, based on current EMEA guidelines, showed that more than half of all development compounds fell within the criteria described by EMEA, namely that they ‘absorb light between 290 and 700 nm…and reach the skin or eyes via systemic exposure’. Of these compounds, approximately 60% were classified as positive or equivocal in standard in vitro photosafety (phototoxicity/photogenotoxicity) assays. Furthermore, ~40% of these ‘positive’ compounds gave contradictory results between the phototoxicity and/or photoclastogenicity assays. Indeed, 18% of compounds were determined to be photoclastogenic in Chinese hamster ovary (CHO) cells despite being negative in the 3T3-NRU phototoxicity assay. The

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standard regulatory test for phototoxicity assessment in vitro. Clearly, this level of discordance is somewhat surprising given that the mechanisms underlying both phototoxicity and photogenotoxicity are believed to be the same, i.e. generation of reactive oxygen species and/or reactive intermediates due to chemical photoinstability.

Based on the above results and on GSK’s clinical experience, we believe that the in vitro photosafety assays are substantially overpredicting human hazard. Moreover, the biological plausibility of the response of certain chemicals suggests to us that the in vitro photosafety (phototoxicity/photogenotoxicity) assays are oversensitive and that the photogenotoxicity assay in particular, may also lack specificity. The experience of other industrial companies appears to mirror that of GSK (European Federation of Pharmaceutical Industries and Associations, personal communication). Furthermore, there have been published reports of reproducibility issues with photogenotoxicity assays between laboratories, e.g. with the photo-micronucleus test and photo-comet assay (12), and recently, the identification of a new phenomenon, pseudophotoclastogenicity, was described by Dufour et al. (13).

Based on our own experience with in vitro photosafety assays and, in particular, our growing concern regarding the sensitivity and specificity of the photoclastogenicity assay in CHO cells, we decided to further investigate this assay with a battery of four coded compounds (aminotriazole, propantheline bromide, cycloheximide and disulfoton). These were selected because all have been described as cytotoxic at high concentrations but not genotoxic (14), and importantly, none of the compounds absorbed UV light. All the compounds were investigated for their potential to induce structural CAs in a standard screening version of the photoclastogenicity assay in cultured CHO cells. A fifth coded compound, tetracycline, which absorbs UV light, was also investigated as this had previously been shown to be phototoxic in vitro (15).

Materials and methods

Chemicals

Aminotriazole (CAS 61-82-5, batch 025K0577), propantheline bromide (CAS 50-34-0, batch 018H0726), cycloheximide (CAS 66-81-9, batch 065K1226), disulfoton (CAS 298-04-4, batch 4131X), tetracycline (CAS 60-54-8, batch 431039/1) and 8-methoxypsoralen (8-MOP) were purchased from Sigma Aldrich Co. (Poole, UK) and 4-nitroquinoline-1-oxide (4-NQO) was purchased from Aldrich Chemical Co. (Gillingham, UK). All chemicals were of the highest purity available.

UV absorbance

The UV-Vis absorbance spectrum for each test compound was acquired on an Agilent 8453 UV-Vis spectrometer. Approximately 1 mg of test substance was diluted until all bands >280 nm registered an absorbance <1.

Cell cultures

CHO-Wbl cells (supplied to Covance by Dr S. Galloway, West Point, PA, USA) were used in the photoclastogenicity assay and Balb/c 3T3 mouse fibroblast cells (American Type Culture Collection, Rockville, MD, USA; clone A31) were used in the phototoxicity assay. The CHO-Wbl cells were subcultured regularly to maintain a stable karyotype. Both cell types were screened for mycoplasma contamination.

Photoclastogenicity

The photoclastogenicity assays described in this study were conducted at Covance Laboratories (Harrogate, UK) and were based on current recommendations for photogenotoxicity testing (7). The protocol and light source have been described in detail elsewhere (13).

Briefly, CHO-Wbl cells were cultured in McCoy’s 5A medium containing 10% (v/v) foetal calf serum and 100 μg/ml gentamycin. Prior to treatment, single-cell suspensions were obtained and cell counts were taken to provide a baseline for the calculation of cytotoxicity (see below). Individual cultures were established for test compound treatment and maintained at 37°C in a humidified atmosphere with 5% (v/v) CO2 in air, until 30–50% confluent. Cell cultures were treated with vehicle (negative control) and a range of concentrations of test compound. All treatments consisted of a UV irradiation (700 ml/cm²) and non-irradiated arm. Positive control treatments were 8-MOP (2.0 μg/ml in the presence of UV irradiation) or 4-NQO (0.25 μg/ml in the absence of UV irradiation). Following treatment with the vehicle or with the appropriate test compound, cultures were incubated at 37°C in the dark for at least 15 min and then exposed to the required dose of UV irradiation. Following irradiation, cell cultures were further incubated for a total of 3 h prior to removal of the test material. The cells were then washed and allowed to recover for 1 h prior to harvest. Non-irradiated cultures were treated for 3 h at 37°C in the dark, washed and allowed to recover for 17 h prior to harvest. Colchicine (~1 μg/ml) was added to all cultures ~1.5 h prior to cell harvest, to arrest dividing cells in metaphase. The cells were harvested and cell counts were determined for each culture using a Coulter Counter to provide an assessment of population doubling (PD). The remaining cells were used to prepare slides for CA analysis using standard methodology.

Cytotoxicity was determined by the decrease in PDs relative to controls. PDs were calculated for each treatment culture as follows:

\[ PD = \frac{\log(N/X_0)}{\log 2} \]

where \( N \) = mean final cell count/culture at each concentration; \( X_0 \) = starting (baseline) count.

A UV dose of 700 ml/cm² has been shown to cause a small increase in the incidence of CHO-Wbl cells with chromosomal aberrations.

Cytogenetic analysis

All slides were coded prior to analysis. Where possible 100 metaphases were analysed from each concentration tested, except for the exception of the vehicle controls from which 200 metaphases were analysed. The modal chromosome number of CHO-Wbl cells was 21 and only cells with 19–23 chromosomes were considered acceptable for analysis. Any cell with >23 chromosomes was recorded separately as containing numerical aberrations and classified as being either polyploid, hyperdiploid or endoweduplicated. Structural aberrations were classified according to the International System for Human Cytogenetic Nomenclature scheme (16).

Phototoxicity

The phototoxicity assay described in this study was conducted at MB Research Laboratories (Spinnerstown, PA, USA) and was based on current recommendations (17).

Briefly, Balb/c 3T3 cells were cultured in Dulbecco’s modified Eagle medium containing 10% (v/v) newborn calf serum and 4 mM glutamine and penicillin/streptomycin. Cells were seeded into 96-well microtitre plates and treated with a range of concentrations of cycloheximide or disulfoton or the positive control, chlorpromazine (0.1–90 μg/ml). Vehicle (purified water) and untreated controls were also included on each plate. The highest concentration tested (1000 μg/ml) was the maximum as recommended for this assay, according to current guidelines. The cultures were treated for ~1 h at 37°C prior to irradiation. One set of plates was exposed to 5 J/cm² UVA and a second set of plates was kept in the dark for the same period. After irradiation, the medium was changed from each well and the cells were washed with phosphate-buffered saline. Finally, 0.2 ml medium was added to each well and the plates were incubated further for ~2 h at 37°C in a humidified atmosphere of 5% CO2 in air. At the end of the incubation period, cell viability was assessed by the measurement of Neutral Red Uptake for 3 h (18).

Analysis of data

Photogenotoxicity assay. The frequency of cells with structural CAs in treated CHO cultures were compared with concurrent vehicle controls using the Fisher’s exact test in both UV-irradiated and non-irradiated treatment arms of the study.

3T3-NRU phototoxicity assay. The cell viability obtained from each of the concentrations of the test article was compared with that from the vehicle control and the percent inhibition (of viability) calculated. For prediction of phototoxicity, the concentration responses obtained in the presence and absence of UVA irradiation were compared at the concentration inhibiting cell viability by 50% of the vehicle control (i.e. EC50) to provide a photoirritancy factor (PIF) value. A test article was considered phototoxic if the PIF value was ≥5.
Photoclastogenicity

Results

Absorbance spectra

The UV-Vis absorbance spectrum for each test compound is shown in Figure 1. Tetracycline, as expected, showed a small increase in absorbance between 230 and 420 nm, the other compounds, aminotriazole, propantheline bromide, cycloheximide and disulfoton, showed no evidence of absorbance between 290 and 700 nm.

Photoclastogenicity

The results of the photoclastogenicity assay are shown in Table I and summarized in Figure 2. The aberrant cell frequencies observed in non-irradiated vehicle control cultures were reported to be consistent with the historical vehicle control range for CHO cells observed at Covance Laboratories. Following UV irradiation (700 mJ/cm²), there was a 2- to 3-fold increase in the frequency of cells with structural CAs (% CAs) compared with the non-irradiated vehicle control cultures, as expected. Treatment with both positive control compounds, 4-NQO and 8-MOP, resulted in statistically significant (P ≤ 0.01) increases in the frequency of CHO cells with CA in the non-irradiated and irradiated treatment arms, respectively.

In the absence of UVR, none of the test compounds (aminotriazole, propantheline bromide, cycloheximide, disulfoton and tetracycline) resulted in an increase in % CA compared with concurrent vehicle controls, when treated up to concentrations resulting in either an ~50% decrease in PD or in the case of aminotriazole, when treated up to the recommended regulatory guideline limit concentration of 10 mM.

In the presence of UVR, aminotriazole and propantheline bromide were negative for clastogenicity. However, cycloheximide treatment resulted in a statistically significant (P ≤ 0.01) increase in the frequency of cells with CA (10%) compared with the concurrent vehicle control (2.5%), but only at the lowest concentration analysed (0.16 µg/ml) which was associated with a 15% decrease in PD. Although there were concentration-dependent increases in cytotoxicity at higher concentrations (i.e. 30 and 45% reduction in PD at 0.9 and 1.3 µg/ml, respectively), these were only accompanied by small (2-fold or less) and non-significant increases in % CA. In contrast, treatment with disulfoton resulted in a statistically significant (P ≤ 0.01) increase in the frequency of cells with CA (10%) compared with the concurrent vehicle control (3%) at the highest concentration tested (88 µg/ml). This was associated with a 58% decrease in PD. No increases in structural CAs were observed at any of the lower concentrations (50 and 67 µg/ml) tested.

Tetracycline treatment resulted in a statistically significant (P ≤ 0.01) increase in the frequency of cells with CA compared with the concurrent vehicle control, both at the lowest (48 µg/ml) and highest (113 µg/ml) concentrations tested, which induced 30 and 44% cytotoxicity, respectively. The intermediate treatment concentration resulted in only a small, non-significant,
increase in % CA; therefore, it is difficult to conclude whether there was a concentration-dependent increase in clastogenicity. However, large increases (~5- to 13-fold) in numerical aberrations, primarily as a result of endoreduplication, were also observed at all concentrations tested (Table II).

**Phototoxicity**

The results of the phototoxicity assay with cycloheximide and disulfoton are presented in Figure 3 and show that neither compound was phototoxic in the 3T3-NRU assay (the PIF for disulfoton could not be calculated and was only 0.48 for cycloheximide; MPE's were 0.032 and 0.029, respectively). The positive control, chlorpromazine, induced a clear positive response with a PIF of 18.49 (MPE 0.355). In the untreated controls, optical density values were greater than 0.4, thus indicating that the cells were capable of neutral red uptake and therefore viable. Irradiated solvent controls had 80% viability compared to non-irradiated controls. The assay was therefore considered valid.

**Discussion**

Four compounds (aminotriazole, propantheline bromide, cycloheximide and disulfoton) were selected based on their cytotoxicity/genotoxicity profiles and evaluated for their potential to induce structural CAs in cultured CHO cells in the presence of UV light (solar-simulated light at 700 mJ/cm²), i.e. photoclastogenicity. This was despite the fact that none of the compounds absorb UV or visible light in the region of 290 and 700 nm of the electromagnetic spectrum. A fifth compound, tetracycline, which does absorb UV light between 230 and 420 nm, was also tested as this compound has previously been shown to be positive in a phototoxicity assay using Balb/c 3T3 cells (15) and, therefore, served as a positive comparator.

The results of the photoclastogenicity assays showed that cycloheximide, disulfoton and tetracycline were clastogenic to CHO cells following UV irradiation but were not clastogenic to non-irradiated cultures. Tetracycline treatment in the presence of UV light also caused large increases in numerical CAs in CHO cells, predominantly via endoreduplication. This result is in contrast to those observed with two structurally related compounds, doxycycline and oxytetracycline, which have previously been shown to be photoclastogenic in a photocomet assay in Chinese hamster V79 cells (19), but were reported to be negative in a photo-micronucleus assay (20). In contrast, the remaining two test compounds in the current study, aminotriazole and propantheline, were negative for clastogenicity and numerical CAs, both in the presence and absence of UV exposure.

A GUM taskforce recently defined photochemical genotoxicity or photogenotoxicity as the property of a compound to induce genotoxic effects when irradiated with UV and/or visible (vis) (sic solar simulated) light (11) and stated a ‘prima facia’ requirement for the compound to absorb (UV and/or visible) light. Given the absence of UV absorbance by cycloheximide and disulfoton, the positive photoclastogenicity data observed in the present study suggest that both compounds are pseudophotoclastogens in CHO cells. This conclusion is supported by the results of follow-up testing using the 3T3-NRU test, i.e. the regulatory standard for in vitro phototoxicity assessment. The results showed that neither cycloheximide nor disulfoton was phototoxic in vitro which is consistent with their inability to absorb light between the wavelengths of 290 and 700 nm.

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**Table I. Summary of structural CA data in CHO-Wbl cells**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (µg/ml)</th>
<th>Cytoxicitya</th>
<th>% CAb</th>
<th>UV exposure, 700 mJ/cm²</th>
<th>Cytoxicity</th>
<th>% CA</th>
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<td>—</td>
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<td>—</td>
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<td></td>
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<td>3.0</td>
<td>24</td>
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<tr>
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<td>—</td>
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*a Cytotoxicity was based on % reduction in PD.

*b% of cells with at least one aberration but excluding those with gaps only.

*c P < 0.05 determined by Fisher’s exact test.
wavelengths of 290 and 700 nm in the electromagnetic spectrum and the inability to generate photo-induced reactive chemical species.

Zinc oxide, an ingredient widely used in dermatological preparations and sunscreens, which has been tested in accordance with the EU Cosmetics Directive was shown to be photostable, non-photoreactive, non-photo-(cyto)toxic (i.e. negative in the 3T3-NRU test in vitro), non-photoirritant or non-photoclastic in humans and negative in the Ames or photo-Ames tests (8). Although considered to be a weak clastogen in vitro, but negative in vivo, zinc oxide was reported to induce an ~4-fold increase in structural CAs in the presence of UV light compared with non-irradiated cultures in CHO and V79 cells in vitro. As such and in the absence of guidelines on the interpretation of photogenotoxicity test results, it could be interpreted that zinc oxide was a putative photoclastogen, albeit a weak one. This is despite the fact that any genuine photogenotoxic activity would appear to have little or no biological plausibility (i.e. the generation of photo-induced reactive chemical species). Further investigation demonstrated that zinc oxide induced similar levels of structural CAs in CHO cells pre-irradiated with UV light prior to treatment and also in cultures treated with zinc oxide and simultaneously irradiated with UV light (13). Based on these findings, it has since been concluded that the increased clastogenicity was not a result of the photoactivation of zinc oxide, but instead a consequence of an increased susceptibility of CHO cells to zinc oxide-mediated clastogenic effects following UV irradiation of cells per se, i.e. zinc oxide was a pseudophotoclastogen. However, since neither cycloheximide nor disulfoton were clastogenic in non-irradiated CHO cultures when treated up to cytotoxic concentrations (i.e. 50% decrease in PD), then the susceptibility of UV-irradiated CHO cells to intrinsic clastogenicity per se does not provide an adequate explanation for the observation of pseudophotoclastogenicity in the present study.

The observation that tetracycline treatment was also associated with photo-induced endoreduplication in CHO cells is also worthy of comment. Endoreduplication is a process that involves two successive rounds of DNA replication without an intervening mitosis and leads to the formation of diplochromosomes. It therefore constitutes a special form of polyploidy [for review see (21)]. Although its physiological significance is poorly understood, some consider endoreduplication to be a cell cycle of terminal differentiation since endoreduplicated cells generally do not divide (22). In addition to arising spontaneously, endoreduplication can be induced in mammalian cells in vitro by physical treatments and a great variety of chemicals with different mechanisms of action (21). The safety concern for endoreduplication is the potential of genetic instability in polyploid cells to provide a pathway to genomic alterations.

Fig. 2. Photoclastogenicity data for (a) aminotriazole, (b) propantheline bromide, (c) cycloheximide, (d) disulfoton and (e) tetracycline. Blue line indicates % CAs in the absence of UV irradiation and pink line indicates % CAs in the presence of UV (700 mJ/cm²) irradiation.
aneuploidy (chromosome loss) thereby pointing to a possible link between endoreduplication and tumourigenesis. However, it is important to note that cell cycle checkpoints exist in normal cells which greatly reduce the potential for endoreduplication, either via mitotic catastrophe [reviewed by Mansilla et al. (23)] or through cell cycle arrest resulting in the elimination of polyploid cells by apoptosis (24).

The CHO-Wbl cell line used in the photoclastogenicity assay carries a mutated p53 gene, expresses abnormal protein and lacks the G1 checkpoint (25) and, moreover, this cell line has a significantly less attenuated response to UV-induced damage compared with CHO clone ECCAC No. 85050302 (data not shown). As such, it is likely that the cell cycle checkpoints that normally prevent the progression of polyploid cells through the cell cycle are abrogated in CHO-Wbl cells. Consequently, the CHO clone may tolerate genetic damage, including changes in the haploid complement which would be lethal to normal cells. In other words, the CHO-Wbl cell line, like other rodent cell lines, appears to be relatively insensitive to the consequences of genomic instability, i.e. they may tolerate genetic damage which may be lethal to normal cells and this in turn may lead to biologically irrelevant results for risk assessment purposes consistent with the pseudophotoclastogenicity observation by Dufour et al. (13) and in this article. As such, we also consider that the photo-associated endoreduplication data should be interpreted with caution. It is already well documented that primary human lymphocytes yield fewer positive results (and by implication fewer irrelevant positive results) compared with rodent cell lines commonly used in Genetic Toxicology (25, 26). Indeed, recent research to investigate the practical consequence of polyploidy in human peripheral blood lymphocytes in vitro showed that of 20 tests showing increased polyploidy, none produced evidence of aneugenicity (27). These authors concluded that monitoring polyploidy (and therefore by implication, endoreduplication) is not a reliable marker of aneuploidy induction.

Photogenotoxicity testing may represent a potential hurdle for many clinical development compounds since those considered positive or equivocal in the in vitro assays require additional evaluation in vivo. This extended evaluation may include photocarcinogenicity assessment in animals and/or an assessment of photosensitivity in humans. Both investigations are time consuming and costly (both in terms of animal use and financial) and may represent an impractical hurdle, particularly early in development, when termination may be viewed as the easiest option. As such, it is imperative that the assays used to identify any potential photogenotoxic hazard are robust (i.e. reproducible), selective (i.e. predictive of in vivo/human risk) and not have inappropriate sensitivity and positive predictivity. The observation that cycloheximide and disulfoton were positive in the photoclastogenicity assay in CHO cells despite being incapable of absorbing UVR/light is therefore of considerable concern. Both compounds, like zinc oxide, are likely pseudophotoclastogens. More importantly, however, is the question of how many other compounds have been deemed positive in the photoclastogenicity assay in CHO cells because of some hitherto, undefined, pseudophotoclastogenic response and not because of photoactivation per se? At the very least, there must be a question mark over those compounds reported positive for the induction of structural CAs in CHO cells following UV irradiation but negative in the 3T3-NRU assay.

The basis for clastogenicity in the presence of UV light seen with pseudophotoclastogens remains unclear. This may due to the compromised ability of CHO-Wbl cells to handle UV-induced DNA damage in treated cultures and it would be interesting to follow up these studies with additional investigations, e.g. to...
see if pre-irradiation also produces a photoclastogenic response with cycloheximide and disulfoton. Nevertheless, the biological significance of photoclastogenicity data for hazard identification and risk assessment remains highly questionable. If false positives are generated from non-UV absorbers, then surely it is also producing false positives amongst UV-absorbing compounds. In vitro clastogenicity tests are already notorious for high rates of false-positive results (28, 29). As such, the results of the current studies substantiate our concern regarding the use of the in vitro photoclastogenicity assay for the assessment of chemical photosafety for regulatory purposes and must surely question whether the assay is fit for purpose. Moreover, it is vital to ascertain whether our concerns should be extended to other photogenotoxicity assays, including the new three-dimensional human skin models now being evaluated in a number of laboratories. At the very least, a review of the current guidance documents for the photosafety evaluation of pharmaceuticals and cosmetics should be undertaken urgently.

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References


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