Journal of Hazardous Materials 197 (2011) 137–143

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Induction of micronuclei by 2-hydroxypyridine in water and elimination of solution genotoxicity by UVC (254 nm) photolysis

Charalambos G. Skoutelis, Dimitris Vlastos*, Marianna C. Kortsinidou, Ioannis T. Theodoridis, Maria I. Papadaki

Department of Environmental and Natural Resources Management, University of Ioannina, Seferi 2, Agrinio 30100, Greece

ARTICLE INFO

Article history:
Received 24 February 2011
Received in revised form
16 September 2011
Accepted 17 September 2011
Available online 22 September 2011

Keywords:
Genotoxicity
Micronucleus assay
2-Pyridinol mineralisation
2-Hydroxypyridine genotoxicity
Pyridines

ABSTRACT

2-Hydroxypyridine (2-HPY) is a major first-stage product formed upon the photolytic destruction of 2-halogenated pyridines. Genotoxicity of 2-HPY in water was studied as a function of concentration. Aqueous solutions of 2-HPY were irradiated by ultraviolet (UV) at 254 nm. 2-HPY concentration, solution total organic carbon (TOC) concentration and solution genotoxicity were measured as a function of treatment time and their profile as a function of time is presented in this work. 2-HPY was found to be genotoxic at all concentrations in the range of 5–400 μg ml^{-1}. 2-HPY mineralises completely upon prolonged UV irradiation. All untreated and irradiated solution samples, taken at different photo-treatment times, were tested in cultured human lymphocytes applying the cytokinesis block micronucleus (CBMN) assay. The genotoxicity of the solution was reduced near to the control level after prolonged UV irradiation.

1. Introduction

Pyridine and pyridine derivatives (PPDs) are an important class of environmental pollutants, usually and mostly of anthropogenic origin, with potential environmental and health threats [1–3]. They are extensively encountered in the chemical industry as solvents, starting compounds or intermediates during the synthesis of pesticides, dyes and pharmaceuticals [4]. Once the pyridine compounds enter the environment, they can persist for long periods [5]. As reported by Vieno et al. [6] who studied the occurrence of eight pharmaceuticals in the raw and treated sewage of 12 sewage treatment plants in Finland, carbamazepine, a compound containing the pyridinic moiety, was not eliminated during treatment, but in fact, even higher concentrations were frequently found in the treated than in the raw sewages. According to the authors, the increase in concentration was shown to be most likely due to enzymatic cleavage of the glucuronic conjugate of carbamazepine and release of the parent compound in the treatment plant. The destruction of pyridinic compounds and their reactive behaviour is not adequately researched. 2-Chloropyridine (2-CPY), in particular, as reported in our previous work [7], appears to be formed as a secondary pollutant during the biotransformation pathways of specific insecticides, such as imidacloprid. The main imidacloprid metabolites identified in mammals and plants, all contain the 2-CPY moiety [8].

In our previous work [7], we studied the photolytic (UV-254 nm) degradation of 2 g l^{-1} aqueous solutions of 2-CPY. The changes of solution genotoxicity were measured as a function of phototreatment time. Via the photolytic treatment complete genotoxicity removal of the solution was achieved. 2-CPY aqueous solutions were found to become genotoxic in the concentration of 100 μg ml^{-1} [7]. Photolytic degradation of 2-CPY, results primarily in 2-hydroxypyridine (2-HPY), (C_5H_4NOH), production. 2-HPY, is also the major intermediate formed upon 2-bromopyridine and 2-iodopyridine photolytic degradation [9]. 2-HPY formed during photolysis of 2 g l^{-1}, 2-CPY aqueous solutions, reached a maximum concentration of approximately 860 mg l^{-1}. Solution genotoxicity peaked near 2-HPY maximum concentration, indicating that 2-HPY was most likely responsible for the maximum observed in solution genotoxicity. In an effort to elucidate whether the increased solution genotoxicity was owing to 2-HPY, to other compounds formed in lower concentrations, or to potential synergistic effects, it was considered imperative to examine the genotoxicity profile as a function of time of aqueous solutions with 2-HPY as the original substrate. This paper reports our findings from this study.

Photochemical advanced oxidation processes (PAOPs) are gaining importance in water treatment and among others, UV-driven photochemical treatment and TiO_2 based heterogeneous photocatalysis have been extensively used to effectively degrade many organic pollutants including PPDs [e.g. 2,5,7,10–12]. As we reported

* Corresponding author. Tel.: +30 26410 74148; fax: +30 26410 74150.
E-mail address: dvlastos@cc.uoi.gr (D. Vlastos).

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doi:10.1016/j.jhazmat.2011.09.065

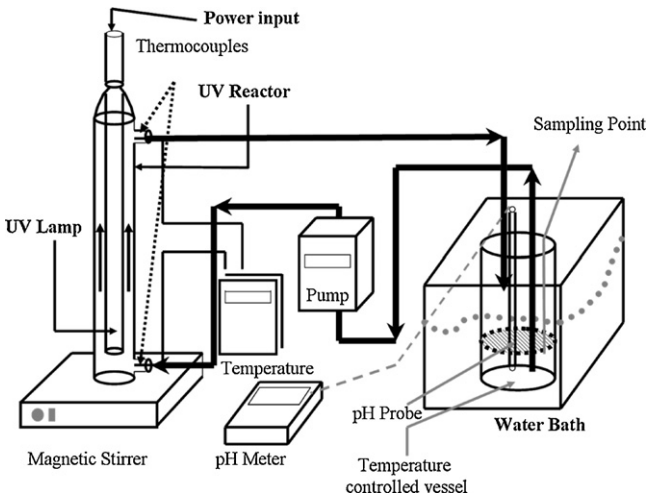

Fig. 1. Experimental set-up for solution phototreatment.

previously [7], chemical photodegradation of organic compounds is being extensively studied, but the elimination of their genotoxicity is marginally researched. Only few investigations have been carried out with the aim to evaluate the *in vitro* genotoxicity of degradation products of organic pollutants in human lymphocytes. Genotoxic substances may represent a health hazard to humans but also may affect organisms in the environment. Thus, there is an obvious scope while studying the chemical degradation of organic pollutants to measure the accompanying solution genotoxicity changes.

In the present work, the genotoxicity study employed the cytokinesis block micronucleous (CBMN) assay for the detection of micronuclei (MN) in the cytoplasm of interphase cells. Micronuclei may originate from acentric chromosome fragments or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division. The simplicity, rapidity and sensitivity of the CBMN assay make it a valuable tool for genotoxicity screening. The assay detects the potential clastogenic and aneugenic activity of chemicals in cells that have undergone cell division after exposure to the test chemical [13,14]. The use of cytochalasin-B, an inhibitor of actin polymerization, which prevents cytokinesis while permitting nuclear division [15,16] leads to formation of binucleated (BN) cells which are scored for the presence of MN [17–19].

The focus of this work is to present the history of the genotoxic effects in human lymphocytes *in vitro* which accompany the chemical degradation of 2-HPY aqueous solutions treated by means of direct photolysis (254 nm). The photolytic degradation of 2-HPY under different conditions has been presented elsewhere [2].

2. Materials and methods

2.1. Chemicals and photo-treatment

2-HPY (C_5H_4NOH) was supplied by Sigma–Aldrich (product code H56800, CAS number 142-08-5, purity ≥97%). It was used without any further purification. Milli-Q purity water was used in all dilutions.

2.2. Measurements employed in photolytic and genotoxicity studies

Two identical series of experiments were performed. 210 ml of an aqueous solution containing 860 μg ml^{-1} of 2-HPY was irradiated isothermally at around 35 °C until complete TOC removal. The experimental set-up employed for the phototreatment of the solution is shown in Fig. 1. It consists of a glass reactor where the solution is illuminated by means of a low-pressure, 110 W, mercury lamp (90-0049-07) with 90% emittance at 254 nm, as described in detail in [20,21]. The experiments were performed in a closed but not air tight system under vigorous agitation and at solution natural pH, which was continuously measured by means of a Mettler Toledo FG 2 portable pH meter.

Aliquots of approximately 0.5 ml were periodically drawn from the reactor and they were split into three (not equal) parts to be used for the HPLC, TOC and genotoxicity analyses, respectively. The two parts used for the chemical analysis were filtered through 0.22 μm membrane filters. A filtered sample of 20 μl was analysed by means of high performance liquid chromatography (HPLC) in a Dionex P680 system with a Dionex 1024 dio-array equipped with an Acclaim C18 5 μm 120 Å, 4.6 mm × 250 mm column using a gradient mobile phase starting at 98:2 water:acetonitrile and ending at 50:50 water:acetonitrile, at 1 ml min^{-1} flow rate and at 23 °C. UV detection was set at 226, 217, 211, 204 and 200 nm. A calibration curve was established, using external standards at various concentrations and was used for quantification. Samples analyses in HPLC were run in duplicates and mean values of two separate measurements were used as result. The same parent samples were used for the genotoxicity study of 2-HPY as described in Section 2.2.

Total organic carbon (TOC) was analysed by means of combustion catalytic oxidation/NDIR method on a Shimadzu TOC-V CSH analyser.

2.3. CBMN assay in human lymphocytes in vitro

Blood samples were obtained from two non-smokers, healthy individuals (28 years old) not undergoing any drug treatment, who did not have any viral infection or X-ray exposure for over a year.

Blood samples were kept under sterile conditions in heparinized tubes. Whole blood (0.5 ml) was added to 6.5 ml Ham's F-10 medium (Gibco), 1.5 ml foetal bovine serum (Gibco) and 0.3 ml phytohaemagglutinin (Gibco) to stimulate cell division. 2-HPY untreated solution was added to final concentrations of 5, 10, 50, 100, 200 and 400 μg ml^{-1}. Two identical sets of two independent experiments were conducted for all aforementioned concentrations as well as for positive and negative controls. The reported results represent the pooled data from the two donors' replicated cultures.

In the case of 2-HPY phototreated solutions the appropriate solution volume was added 24 h after culture initiation. For each 2-HPY phototreated solution, as well as for positive and negative controls, two independent cultures were carried out. The same solution volume was added in all cultures. Consequently, the treated samples had been identically diluted by the culture fluids as the untreated samples containing 50 μg ml^{-1} of 2-HPY. This ensured concentration consistency with each other and with our previous work [7]. For photolytically treated solutions the reference to 50 μg ml^{-1} concentration was such as to indicate the initial untreated solution value and not the actual 2-HPY concentration at the time the sample was withdrawn.

Mitomycin-C (MMC) (Sigma) at final concentration of 0.5 μg ml^{-1} served as positive control in all respective experiments [22].

Subsequently, 6 μg ml^{-1} cytochalasin-B (Cyt-B) (Sigma) was added to the culture medium 44 h after its initiation and 20 h after the addition of the appropriate chemical agent, i.e. 2-HPY, solution.

This concentration of Cyt-B was selected so as to obtain a higher percentage of binucleated (BN) cells and a lower baseline micronuclei (MN) frequency [23].

Cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 72 h. 72 h after the initiation of culture, cells were harvested and collected by centrifugation. A mild hypotonic treatment with 3:1 solution of Ham's medium and Milli-Q H_2O was left for

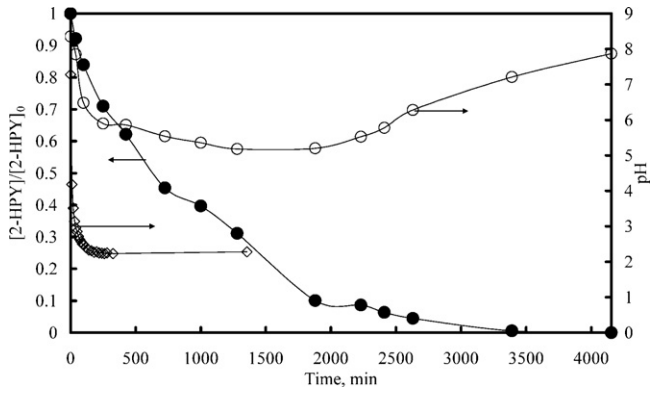

Fig. 2. Profiles of normalised concentration: 2-HPY (●), solution natural pH (○), at 35 °C. For comparison purposes, 2 g l^{-1}, aqueous 2-CPY solution pH profile (◊).

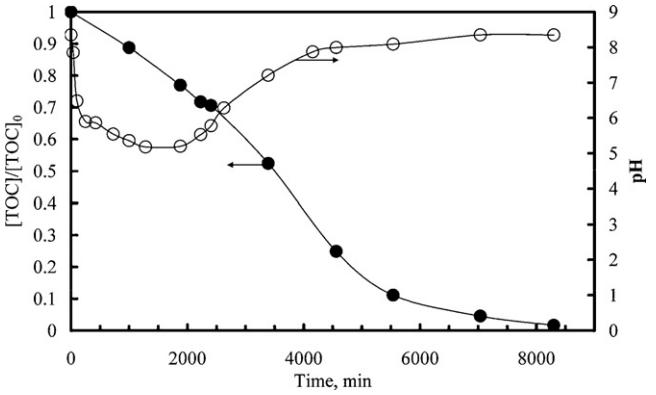

Fig. 3. Normalised TOC (●), removal profile as a function of time during photolytic treatment of 860 mg l^{-1}, 210 ml solution of 2-HPY in a closed but not airtight system at 35 °C, at solution natural pH. Solution pH (○).

3 min at room temperature which was followed by 10 min fixation (for at least 3 times) with a fresh 5:1 solution of methanol/acetic acid. Cells were stained with 7% Giemsa [22,24,25].

Standard criteria were used for scoring MN [18,26]. The scoring of micronuclei was performed manually and by (at least) two, independently working, experienced researchers. In order to determine possible cytotoxic effects, the Cytokinesis Block Proliferation Index (CBPI) was calculated by counting at least 2000 cells for each experimental point. CBPI is given by the equation: CBPI = $M_1 + 2M_2 + 3(M_3 + M_4)/N$ where M_1, M_2, M_3 and M_4 correspond to the numbers of cells with one, two, three and four nuclei and N is the total number of cells [27].

2.4. Statistical analysis

All results are expressed as the mean frequency ± standard error (MF ± se). The statistical analysis of the MN data was conducted using the G-test for independence on 2 × 2 tables. The chi-square test (χ^2 test) was used for the analysis of CBPI among each treatment. Statistical decisions were based on a significance level of 0.05. The statistical software used for data analysis were, the Origin 7.0 (OriginLab Corporation, Northampton, USA), the Minitab statistical software (Minitab Inc., PA, USA) and the Statistical Package for Social Sciences (SPSS) for Windows, version 17.0.

3. Results and discussion

3.1. Photolytic treatment of 2-HPY

2-HPY concentration of the photolytic experiments was measured as a function of time. The time-profile of 2-HPY concentration and the solution pH of one of the measurements used for the genotoxicity study are shown in Fig. 2. The TOC removal and the solution natural pH during the entire measurement are shown in Fig. 3. For comparison purposes, Fig. 2 also shows the pH profile of measurements employing 2-CPY as original substrate, in an initial concentration of 2 g l^{-1} of 2-CPY in water.

As can be seen in Fig. 2, the concentration of the studied substance (2-HPY) was halved within 725 min of phototreatment, although TOC concentration was reduced by approximately 5% during the same period (Fig. 3). After 4000 min of phototreatment, 2-HPY was completely removed, while TOC removal had reached 75%. Nearly complete TOC removal was achieved after 8000 min. Moreover, as can be seen in Fig. 3, the phototreated solution pH drops rapidly at the early stages of irradiation to approximately 6.0 and it reduces slowly to reach a minimum of 5.2. After approximately 2000 min of treatment, when 90% of 2-HPY has been removed it starts rising again to reach its initial value (≈8.0) when 80% of TOC has been removed.

In our previous work [7], 2-HPY removal was faster (50% removal within 250 min of irradiation), owing to the solution lower pH. For the readers' convenience, the pH during 2-CPY phototreatment is shown in Fig. 2. As can be seen in this case, the pH drops rapidly at a level below 3.0 due to the rapid Cl^- removal from the aromatic ring. As mentioned earlier, the maximum 2-HPY concentration reached in that measurement was the same as the initial 2-HPY concentration in the experiments of the present work. However, in the present work where 2-HPY was the original substrate, the minimum pH value reached, was 5.2 and after 250 min of initiation, less than 30% of the original substrate had been removed. The higher pH values are responsible for this slower removal, because, as reported in [2], 2-HPY photolytic removal is faster at lower pH.

3.2. CBMN assay in human lymphocytes in vitro

The results obtained from human peripheral blood lymphocyte cultures treated with different concentrations of 2-HPY and MMC are shown in Table 1A. Statistically significant differences ($p < 0.01$ and $p < 0.001$) in comparison with the control in the BNMN as well as in the MN frequencies were seen at all tested 2-HPY concentrations. The reported control and positive control frequencies of MN are in accordance with the published values in the used cytogenetic end-points [28,29].

No statistically significant differences of the cytotoxic index were observed between control and 2-HPY treated cultures. This indicates that the lowest CBPI index value which was measured during 2-CPY phototreatment [7] at the peak of 2-HPY concentration, cannot be exclusively attributed to 2-HPY. The increased cytotoxicity of the samples collected during 2-CPY phototreatment at the time of the high 2-HPY concentration, could be caused by different 2-CPY degradation products, by synergistic effects between different compounds present in the solution at that time, where 2-HPY may have played a key role, or probably due to a shift of the equilibrium of 2-HPY keto–enol forms, caused by the presence of other products or moieties.

Our results showed more than three-fold increase in MN frequencies at the lower tested concentration (5 μg ml^{-1}), more than four-fold increase in the next three tested concentrations (10, 50 and 100 μg ml^{-1} respectively) and six-fold increase in the highest tested concentrations (200 and 400 μg ml^{-1} respectively) of 2-HPY, compared with the control value. The recorded frequencies show a plateau in the range of concentrations 10–100 μg ml^{-1}. This is probably because 2-HPY in (aqueous) solution exists in two tautomeric

Table 1
Induction of BNMN, total MN and CBPI values in human lymphocytes treated with 2-HPY.

	BNMN MF (‰) ± se	MN MF (‰) ± se	CBPI MF ± se
A. Effect of 2-HPY concentration (μg ml^{-1})			
0	2.5 ± 0.29	2.5 ± 0.29	1.87 ± 0.03
5	8.0 ± 0.41**	8.5 ± 0.29**	1.89 ± 0.05
10	10.5 ± 0.65***	11.25 ± 0.63***	1.90 ± 0.05
50	9.75 ± 1.03***	11.25 ± 1.49***	1.96 ± 0.05
100	11.0 ± 1.35***	11.0 ± 1.35***	1.92 ± 0.07
200	13.25 ± 1.80***	14.75 ± 1.70***	1.97 ± 0.8
400	13.25 ± 2.78***	15.25 ± 3.57***	1.89 ± 0.07
MMC (0.5 μg ml^{-1})	67.5 ± 2.22***	88.5 ± 3.71***	1.28 ± 0.08***
B. Effect of phototreatment time (initial concentration of 2-HPY: 50 μg ml^{-1})			
Control	3.5 ± 0.5	4.0 ± 0.0	1.99 ± 0.07
MMC (0.5 μg ml^{-1})	67.0 ± 5.0***	83.5 ± 5.5***	1.36 ± 0.17***
Time (min)			
0	15.5 ± 0.5***	16.5 ± 0.5***	1.91 ± 0.01***
40	13.0 ± 0.0***	14.5 ± 0.5***	1.90 ± 0.02***
100	8.0 ± 1.0*	9.0 ± 0.0*	1.91 ± 0.04***
250	10.5 ± 0.5**	11.0 ± 1.0**	1.89 ± 0.07***
425	8.5 ± 0.5*	8.5 ± 0.5*	1.88 ± 0.02***
725	7.5 ± 0.5	7.5 ± 0.5	1.85 ± 0.03***
1000	5.5 ± 1.5	6.0 ± 1.0	1.71 ± 0.05***
1280	6.0 ± 0.0	6.5 ± 0.5	1.75 ± 0.01***
1880	5.5 ± 0.5	6.0 ± 0.0	1.79 ± 0.05***
2230	5.5 ± 0.5	6.0 ± 0.0	1.78 ± 0.0***
2410	6.0 ± 1.0	7.0 ± 0.0	1.72 ± 0.03***
2630	5.5 ± 0.5	6.0 ± 0.0	1.74 ± 0.01***
3390	4.0 ± 1.0	5.0 ± 0.0	1.84 ± 0.02***
4155	5.0 ± 0.0	5.0 ± 0.0	1.89 ± 0.01***
4560	5.0 ± 0.0	5.5 ± 0.5	1.91 ± 0.02***

2-HPY, 2-hydroxypyridine; BNMN, micronucleated binucleated cells; MN, micronuclei; CBPI, Cytokinesis Block Proliferation Index; MMC, Mitomycin-C; MF (‰) ± se, mean frequencies (‰) ± standard error; MN were scored in 4000 (A) and 2000 (B) binucleated lymphocytes per experimental point [*G*-test for BNMN and MN; χ^2 for CBPI].
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

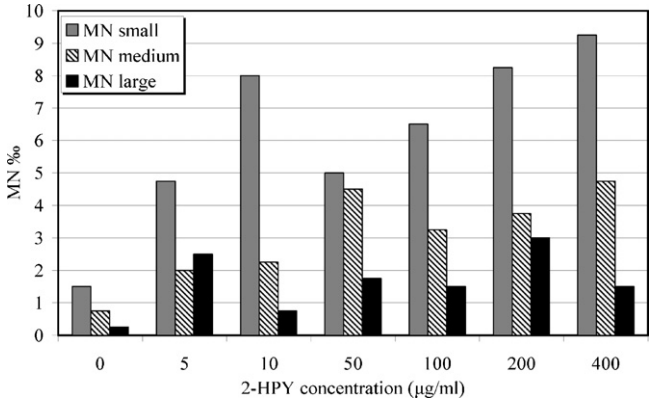

Fig. 4. Mean frequencies (‰) of MN per size, of untreated 2-HPY solutions.

forms, the enol form and the keto form, which are generated due to intramolecular and intermolecular proton-transfer reactions [30]. Such non-covalent interactions like hydrogen bonding, with a wide range of molecular components of blood serum, may affect the rate of biotransportation, distribution and absorption of 2-HPY in lymphocytes and therefore its availability to interact with the genetic material.

The size ratio of MN in the *in vitro* CBMN assay is an alerting index as effective as the fluorescence *in situ* hybridization (FISH) analysis for the discrimination of clastogenic and aneugenic effects [24,31].

Data on the size ratio of MN (‰) induced by pretreated 2-HPY are presented in Fig. 4 and they are compared to the control. Compared to the control, a three to six-fold increase in small size MN frequency was observed in all tested concentrations of 2-HPY, while the observed increase in large MN frequency was three to twelve-fold.

An increase in both, small and large size MN frequencies were observed, indicating a possible clastogenic and aneugenic effect. As explained later, these observations are consistent with our previous study [7].

Table 1B, shows the results obtained from peripheral blood lymphocytes cultures treated with 2-HPY before and after treatment by UV light in different photo-treatment times.

2-HPY induced a statistically significant increase ($p < 0.001$) in the frequency of binucleated micronucleated (BNMN) cells (15.5 ± 0.5) and total MN (16.5 ± 0.5) at the concentration of 50 μg ml^{-1}, before UV light treatment, compared to the control (4.0 ± 0.0). In the case of treatments by UV light at 40, 100, 250 and 425 min, the frequencies of BNMN and MN were still significant ($p < 0.001$, $p < 0.01$, $p < 0.05$). However, in the case of treatments by UV light at 725 up to 4560 min our data indicated no significant statistical differences in both BNMN and MN frequencies compared to the control value.

The 2-HPY cytotoxic effect before and after UV irradiation was evaluated by the CBPI index. Regarding this index, statistically significant differences ($p < 0.001$) on CBPI were detected between control and untreated or treated by UV irradiation 2-HPY cultures. As can be seen in Table 1B, solution irradiation caused a drop of CBPI value at approximately 1000 min, thus indicating the formation of cytotoxic intermediates. Subsequently CBPI value was gradually increasing and then decreasing again at approximately 2410 min. Finally, it increased again, to approach the control value after 4560 min of irradiation. The lowest CBPI values are observed at 1000 and 2410 min.

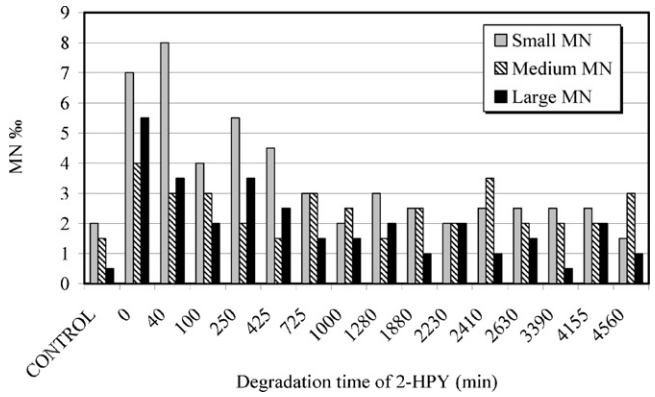

Fig. 5. Mean frequencies (‰) of MN per size, of UV phototreated 2-HPY aqueous solutions.

As in the case of untreated samples, the reported control and positive control frequencies of MN in this case are consistent with the published values in the used cytogenetic end-points [28,29].

As can be seen in Table 1B, the genotoxic activity of UV-treated 2-HPY decreased immediately 40 min after solution irradiation where the mean frequency of induced MN was equal to 14.5 instead of 16.5 for untreated 2-HPY sample ($t = 0$). From that point onwards, the genotoxic effect of 2-HPY gradually decreases eventually approaching, but not reaching, the control level. The above results are consistent with our previous findings [7]. As reported in [7], 2-HPY is the primary product formed during 2-CPY phototreatment. The genotoxicity of phototreated 2-CPY samples was increasing even after 2-CPY removal, reaching its maximum at the maximum 2-HPY concentration (approximately 50 $\mu g\,ml^{-1}$).

Data on the size ratio of MN (‰) induced by 2-HPY before and after irradiation compared to the control, are presented in Fig. 5. The size ratio of MN of the phototreated 2-HPY samples shows a gradual decrease in both small and large size MN frequencies, compared with those of the untreated 2-HPY solution.

The large MN values and CBPI of the phototreated solution are shown in Fig. 6. As can be seen in Fig. 6, the frequency of large MN drops during photo-treatment. On the other hand, the mixture cytotoxicity is significant at 1000 min of treatment, when over 60% of 2-HPY has been removed. This indicates that the mixture cytotoxicity at this point is very likely to be due to a degradation product formed later on, or due to synergistic effects of different compounds. The cytotoxicity approaches again the control value after 4560 min of treatment, when 75% of solution TOC has been removed and if cytotoxicity is due to a specific compound, the cytotoxic product has most likely been photo-destructed.

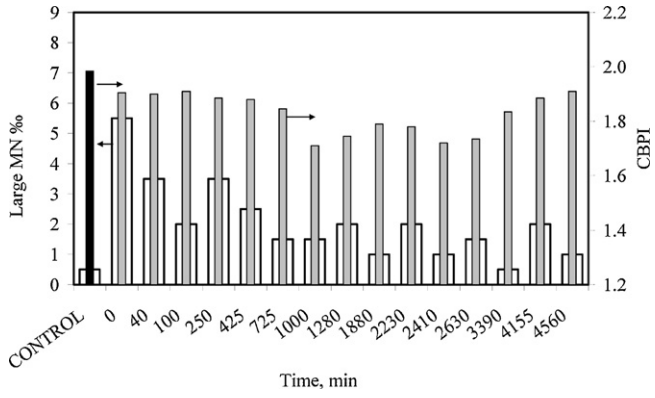

Fig. 6. Mean frequencies (‰) of large MN found in 2000 BN cells (left-hand axis, wide bars) and mean CBPI value (right hand axis, narrow grey bars) of diluted phototreated samples.

There are no studies reporting on the genotoxic activity of 2-HPY and its effects in human lymphocytes. Similarly, there are very few studies in the literature on the mutagenicity and/or genotoxicity of the parent compound, 2-CPY [7,21,32–35].

The findings reported in a comparative study between pyridine and five of its metabolites (including 2-HPY) in *in vivo* induction of CYP1A1 expression in the lung, kidney and liver in the male Sprague-Dawley rats revealed that 2-HPY caused tissue-specific induction or repression of CYP1A1 [36].

Another study, which investigated the inducibility of cytochrome P4501A1 gene (CYP1A1) expression in human lung samples, reported that pyridine metabolites, particularly 2-HPY, are better inducers than the parent compound [37].

Iba et al. [38] reported that 2-HPY significantly induce the CYP1A1 mRNA expression and CYP1A1-preferential activity ethoxyresorufin O-deethylase in wild-type HepG2 cells. Similarly, only 2-HPY induced the expression of a xenobiotic response element directed reporter gene in transfected HepG2 cells. Cytochrome P4501A1 (CYP1A1) is a member of the cytochrome P450 (CYP) supergene family of enzymes which catalyze the biotransformation of xenobiotics and endogenous compounds to either biologically active or inert products, depending on the substrate and CYP form involved [39]. Prominent catalytic activities of CYP1A1 include bioactivation of promutagens, notably certain polyaromatic hydrocarbons (PAHs) and heterocyclic amines, to derivatives capable of initiating oncogenic mutations in DNA [40–42].

In addition, Carlson [43] reported an *in vivo* hepatotoxicity of 2-HPY in rats which were administered i.p. daily for 5 days.

2-HPY exhibits two stable tautomeric forms: the enol form (2-HPY) and the keto form 2-pyridone (2-PY). The keto–enol tautomerization between 2-HPY and 2-PY has been extensively investigated experimentally in various phases, in matrices, and also in theoretical studies as one of the simplest systems of intramolecular and intermolecular proton-transfer reactions [30].

Three planaraminepalladium(II) complexes of the form: trans-$PdCl_2L_2$, code named TH5, TH6 and TH7 where L = 3-hydroxypyridine, 2-hydroxypyridine and 4-hydroxypyridine respectively had been investigated for antitumour activity against ovarian cancer cell lines by Huq et al. [44]. They found that the compounds possess significant antitumour activity. They state that the variations in activity of TH5, TH6 and TH7 indicate that non-covalent interactions such as hydrogen bonding may be playing a significant role in activity. They also report that the presence of 2-HPY ligand confers greatest activity, indicating that hydroxyl group in 2-HPY ligand is better able to form hydrogen bonds with DNA. However, they say that in the absence of supporting information the idea remains speculative [44].

The results reported in untreated 2-HPY tested samples indicate a possible photoinduced tautomerization reaction, which probably correlated with a proton transfer between tautomeric forms of 2-HPY and DNA bases. The above mentioned hypothesis may play a key role in the observed *in vitro* genotoxic effects on human lymphocytes genetic material.

The presented results in this paper and the findings of our recent study [7] reveal that 2-CPY and its major metabolite 2-HPY, each separately or in combination after physicochemical and/or biological transformation may elicit isolated or synergistic genotoxicity and induce damage to DNA.

Various combinations of chemicals are being detected in the environment with increasing frequency. This has raised awareness that the genotoxicity of individual chemicals may not correspond to the genotoxic effects resulting from exposure to chemicals in combination. From that point of view, the genotoxicity peak reported

in our previous work [7], which was observed when 2-HPY concentration was maximum, may indeed originate from 2-HPY, as the present findings do not contradict any of those reported in [7]. At the same time, the research presented here, showed that the increased cytotoxicity of 2-CPY photo-treated solutions at the time of 2-HPY peak concentration cannot be exclusively attributed to 2-HPY. However, as the photo-treated solutions of 2-CPY or 2-HPY result in the formation of a cocktail of chemicals in solution, the possibility that the measured genotoxicity, including cytotoxicity, originates from synergistic effects, cannot be ruled out. Nevertheless, the most important finding of the present research, is that solution genotoxicity, whichever its origin, can be completely removed following adequate UV phototreatment. Genotoxicity was eliminated before complete TOC removal and substantially later than 2-HPY destruction.

4. Conclusions

The genotoxicity and cytotoxicity of 2-HPY was studied using untreated and phototreated solutions. 2-HPY was found to be genotoxic. Untreated 2-HPY solutions were not found cytotoxic. Cytotoxicity of photo-treated solutions increased with phototreatment and subsequently decreased after 70% of 2-HPY was removed. It was subsequently removed, to increase again when 60% of TOC was removed, thus indicating possible formation of cytotoxic intermediates. Following adequate photo-treatment however, solution genotoxicity reached the control value, thus proving that phototreatment of aqueous solutions is an effective way of elimination of their genotoxicity.

Conflict of interest

The authors declare that they have no competing financial or conflicts of interest with regard to this work.

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