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# Chlorpyrifos induces endoplasmic reticulum stress in JEG-3 cells

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ABSTRACT

Chlorpyrifos (CPF) is an organophosphorous pesticide widely used in agricultural, industrial, and household applications. We have previously shown that JEG-3 cells are able to attenuate the oxidative stress induced by CPF through the adaptive activation of the Nrf2/ARE pathway. Considering that there is a relationship between oxidative stress and endoplasmic reticulum stress (ER), herein we investigated whether CPF also induces ER stress in JEG-3 cells. Cells were exposed to 50 μM or 100 μM CPF during 24 h in conditions where cell viability was not altered. Western blot and PCR assays were used to explore the protein and mRNA levels of ER stress biomarkers, respectively. CPF induced an increase of the typical ER stress-related proteins, such as GRP78/BiP and IRE1α, a sensor for the unfolded protein response, as well as in phospho-eIF2α and XBP1 mRNA splicing. Additionally, CPF led to a decrease in p53 protein expression. The downregulation of p53 levels induced by CPF was partially blocked when cells were exposed to CPF in the presence of the proteasome inhibitor MG132. Altogether, these findings point out that CPF induces ER stress in JEG-3 cells; however these cells are able to attenuate it downregulating the levels of the pro-apoptotic protein p53.



## 1. Introduction

The endoplasmic reticulum (ER) is the fundamental intracellular organelle responsible for the synthesis, proper folding, localization, and post-translational modifications of proteins in eukaryotic cells. Disturbances in ER function, a process named "ER-stress", trigger the unfolded protein response (UPR) designed to restore protein homeostasis. Three main signaling pathways involving the ER transmembrane sensors: inositol-requiring enzyme 1α (IRE1α), activating transcription factor-6 (ATF6), and protein kinase-like ER kinase (PERK) mediate UPR. The endoribonuclease activity of IRE1α which is controlled by its protein kinase activity splices XBP1 mRNA originating the transcriptional activator (sXBP1) of genes encoding ER chaperones and ER-associated protein degradation (ERAD) components such as glucose regulated protein 78 (GRP78)/immunoglobulin heavy chain-binding protein (BiP), GRP94, and calreticulin. Similarly, ATF6 is transported from the ER to the Golgi where it is cleaved yielding a cytosolic active transcription factor. These pathways lead to ER homeostatic response stress by degrading or refolding misfolded proteins accumulated in the ER lumen. PERK protein phosphorylates and inactivates eIF2α that blocks the entrance of nascent proteins into the ER thus controlling the initiation of mRNA translation globally and reducing protein load on the ER. If the ER stress is prolonged, or the adaptive response fails, apoptotic cell death arises. All three ER-resident proteins sense ER stress through GRP78/BiP binding/release *via* their respective lumenal domains (Lai et al., 2007).

p53 tumor suppressor is a nuclear protein that functions as a regulator of transcription and mediates several biological effects, such as growth arrest, senescence, and apoptosis in response to various forms of stress (Oren, 2003; Ryan et al., 2001). Depending on the experimental conditions, ER stress was reported to either facilitate the reduction (Pluquet et al., 2005; Qu et al., 2004) or the increase in p53 level or activity (Dioufa et al., 2012; Gass et al., 2002; Lin et al., 2012; Lopez et al., 2015). Moreover, it was recently reported that p53 regulates ER function in response to stress (Namba et al., 2015).

Numerous reports indicate that ER stress and the subsequent UPR are involved not only in a diverse range of pathologies such as certain neurodegenerative and metabolic diseases, but also in the cytotoxicity of environmental pollutants, industrial chemicals, and drugs (Kitamura, 2013; Matsuoka and Komoike, 2015). Chlorpyrifos (CPF) is an organophosphorous (OP) pesticide widely used in agricultural, industrial, and household applications. The primary acute toxicological effect of OPs is associated with inhibition of acetylcholinesterase. Additionally, several studies also provide evidence that OP exposure induces oxidative stress by different mechanisms such as an increased formation of reactive oxygen species (ROS) (Abdollahi and Karami-Mohajeri, 2012; Shadnia et al., 2007), depletion of antioxidant defenses, and the impairment of antioxidant enzyme function (Ojha et al., 2011).

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In humans, prenatal OP exposure is associated with decreased birth weight and length (Whyatt et al., 2004; Windham and Fenster, 2008), alterations in developmental and in psychomotor indices (Eskenazi et al., 2007) and immunological abnormalities (Thrasher et al., 2002). Although most studies have focused on the direct effect of OPs on the fetus, few reports have assessed their effects on the placenta. It has been reported that placental function may be interfered by OP compounds at many levels. For example, altered placental acetylcholinesterase, catalase, and carboxylesterase activity has been associated with prenatal exposure to OP pesticides (Rovedatti et al., 2012; Souza et al., 2005; Vera et al., 2012). Azinphos-methyl, phosmet and CPF modified placental phosphoinositide metabolism and phosphoinositide-4 kinase activity (Souza et al., 2004), and CPF induced apoptosis in the trophoblastic-derived JAR cell line, through a signaling mechanism not dependent on FAS/Tumor Necrosis Factor, activation of caspases or inhibition of cholinesterase (Saulsbury et al., 2008). Changes in the cytokine pattern towards an inflammatory profile have been described in JEG-3 exposed to phosmet and CPF (Guinazu et al., 2012). Additionally, we have reported that JEG-3 ·cells exposed to CPF alter the expression of human chorionic gonadotropin β-subunit, glial cell missing-1 and ATP-binding cassette sub-family G member 2 molecules which are relevant for the maintenance of a healthy pregnancy (Ridano et al., 2012). More recently, we have shown that JEG-3 cells were able to attenuate the oxidative stress induced by CPF through the adaptive activation of the Nrf2/ARE pathway that includes increases in the glutathione reductase and hemeoxygenase-1 mRNA levels (Chiapella et al., 2013).

This study was aimed to investigate whether the adaptive response of JEG-3 cells to CPF exposure also involves ER stress. Moreover, since ER stress and the p53 tumor suppressor are interrelated, modulation of any of these processes could contribute to ameliorate cell toxicity (Pluquet et al., 2005; Qu et al., 2004). We therefore monitored changes in proteins related to ER stress and p53 expression levels in JEG-3 cells in response to CPF exposure. We demonstrated for the first time that CPF causes ER stress leading to a destabilization of the p53 tumor suppressor in trophoblast cells. These findings extend our knowledge about the signaling pathways and biochemical processes possibly involved in the placenta response to CPF exposure. Furthermore, they suggest that the modulation of ER stress signaling pathways is an important issue for protection against cellular damage induced by xenotoxicants.

## 2. Materials and methods

### 2.1. Reagents and antibodies

CPF (*O*,*O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) with purity of 99.5% and all other reagents (analytical grade) were purchased from the Sigma Chemical Company (St. Louis, MO, USA).

Goat polyclonal anti-p53 (FL-393-G) was obtained from Santa Cruz Biotechnology. Rabbit monoclonal anti-p-eIF2α (Ser51) (3398) and rabbit polyclonal anti-total-eIF2α (9722) were from Cell Signaling Technology. Mouse monoclonal anti-β-actin antibodies were obtained from Sigma Chemical Co.

All antibodies against ER stress markers (ER Stress Antibody Sampler Kit #9956) were from Cell Signaling Technology. Rabbit anti-goat IgG antibody was from Zymed. IRDye 680RD donkey anti-goat IgG (P/N 096-68074), IRDye 800CW donkey anti-rabbit IgG (P/N 096-32213) and IRDye 680RD donkey anti-mouse IgG (P/N 92668072) were obtained from Li-Cor Biosciences.

### 2.2. Cell culture and CPF treatment

The choriocarcinoma-derived JEG-3 cell line (ATCC, HTB-36) was purchased from the American Type Culture Collection (ATCC, Rockville, USA) and grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 0.1 mg/mL streptomycin. CPF was prepared as a 0.25 M stock solution in dimethylsulfoxide (DMSO). Twenty four hours before CPF treatment, $5.5 \times 10^5$ cells were plated on each well of 6-well plates in 2 mL of culture medium and treated with CPF (0, 50, 100 μM) for 24 h. Final DMSO concentrations did not exceed 0.04%. Under these conditions cell viability, measured by 3-(4, 5-dimethlthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) assay, was not affected (Ridano et al., 2012). Treatments were performed under 1% of FBS to avoid the interaction of the toxic with serum proteins (Saulsbury et al., 2008).

### 2.3. Quantitative reverse transcription PCR (qRT-PCR)

Total RNA was extracted from cultured cells using TRI Reagent® (Sigma), according to the manufacturer's instructions. Single-stranded cDNAs were synthesized with random primers (Invitrogen) in 20 μL final volume. Briefly, 1 μg of total RNA was incubated with random primers (6.25 ng/μL, Invitrogen) and 200 U of M-MLV reverse transcriptase (Invitrogen). The reaction was performed as previously described (Durand et al., 2004).

For qPCR, cDNA was mixed with Power SYBR® Green PCR Master Mix (Applied Biosystems) and the following primers: p53 forward 5′-CTA GCT CGC TAG TGG GTT GC-3′ and p53 reverse 5′-GAA GAC GGC AGC AAA GAA AC-3′ (300 nM each one); cyclophilin A forward 5′-GTC AAC CCC ACC GTG TTC TT-3′ and cyclophilin A reverse 5′-CTG CTG TCT TTG GGA CCT TGT-3′ (100 nM each one), were added to a final volume of 15 μL. qPCR was carried out on an Applied Biosystems 7500 Real-Time PCR System with Sequence Detection Software v1.4. The cycling conditions included a hot start at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. Specificity was verified by melting curve analysis and agarose gel electrophoresis. Each sample was analyzed in triplicate. Transcript levels were normalized to those of cyclophilin A and relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Litvak et al., 2002). Amplification efficiency for each set of primers was near 98%. RNA samples incubated without reverse transcriptase during cDNA synthesis, as well as PCR reactions using water instead of template showed no amplification.

### 2.4. Analysis of XBP1 mRNA splicing

To evaluate XBP1 activation, the splicing of XBP1 mRNA was examined by RT-PCR. Briefly, PCR was performed with the forward: 5′-AAG AAC ACG CTT GGG AAT CG-3′ and reverse: 5′-ACT CCC CTT GGC CTC CAC-3′ primers using Taq DNA polymerase (Life Technologies) according to the manufacturer's instructions. The PCR procedure was carried out with denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. The final extension was set at 72 °C for 10 min. Fragments of unspliced (uXBP1, 219-bp) and spliced (sXBP1, 193-bp) were separated by electrophoresis on 10% PA gels and visualized by ethidium bromide staining.

### 2.5. SDS-PAGE and western blotting

Protein samples were resolved by 10% SDS-PAGE gels. After migration, proteins were electrotransferred to nitrocellulose (Amersham Bioscience). The membrane was blocked in Tris-buffered saline (TBS) (25 mM Tris, 150 mM NaCl, 2 mM KCl, pH 7.4) containing 0.2% v/v Tween-20 and 5% w/v non-fat dry milk, washed and incubated with each one of the following primary antibodies: mouse monoclonal anti-β-actin (1:3000), rabbit polyclonal anti-p-eIF2α (1:500), rabbit polyclonal anti-total eIF2α (1:1000), goat polyclonal anti-p53 (1:1000), for 1 h at room temperature or overnight 4 °C with shaking, as indicated by the manufactures. The blots for ER stress markers (IRE1α, GRP78/BiP, calnexin, PDI) were analyzed with the ER Stress Antibody Sampler Kit (Cell Signaling Technology) according to the manufacture instructions.

After washing, the blots were incubated with rabbit anti-goat secondary antibody (1:5000) in TBS at room temperature for 1 h. Protein-antibody complexes were visualized by an enhanced chemiluminescence detection system (SuperSignal West Pico; Pierce). Blots were quantified by densitometry using Gel-Pro Analyzer. Alternatively, the blots were incubated with IRDye 800CW donkey anti-rabbit IgG, IRDye 680RD donkey anti-goat IgG or IRDye 680RD donkey anti-mouse IgG antibodies (1:15,000) in TBS at room temperature for 1 h, protected from light. After washing with TBS plus 0.2% Tween-20, the membranes were visualized and quantified using the Odyssey Infrared Imaging System (LI-COR, Inc., Lincoln, NE, USA). Protein expression was normalized to β-actin expression.

### 2.6. Statistical analysis

Significant differences were identified using the nonparametric paired Wilcoxon test or one sample *t*-test. Statistically significant differences were considered for $p < 0.05$.

## 3. Results

### 3.1. CPF increased the phosphorylation of eIF2α

ER stress triggers the unfolded protein response (UPR) which has distinctive adaptive pathways intended to restore protein homeostasis (Lai et al., 2007). The present work was performed to investigate whether CPF induces ER stress in JEG-3 cells. Consequently, we examined the major mediators of these pathways. For the first series of experiments, JEG-3 cells were treated with 50 or 100 μM CPF for 24 h, and changes in the phosphorylation of eIF2α were measured by western blot analysis. Under these conditions no change in cell viability, as assessed by the MTT assay, was observed (data not shown). As shown in Fig. 1, a slight but significant increase in eIF2α phosphorylation was detected at both concentrations of CPF exposure.

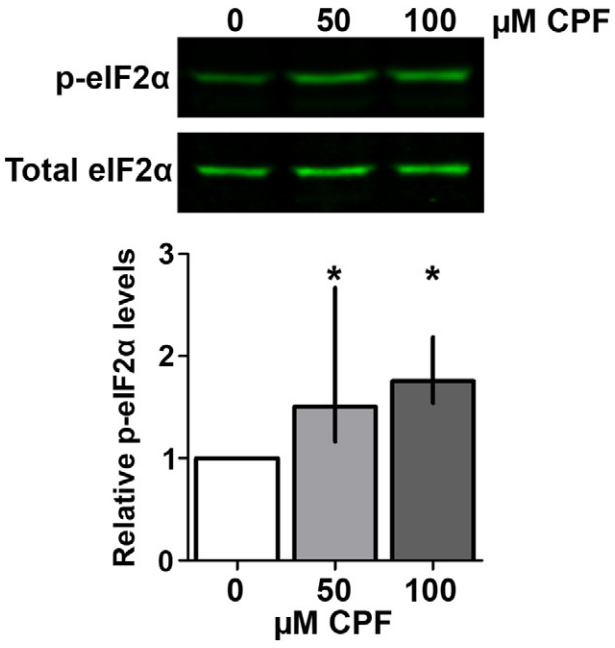


**Fig. 1.** CPF increases the phosphorylation of eIF2α. Western blot analysis of p-eIF2α and total eIF2α in protein extracts from JEG-3 cells exposed to 0, 50 or 100 μM CPF during 24 h. The graph represents the ratio p-eIF2α/total eIF2α expression level normalized to the control (0 μM CPF) condition defined as 1. Values are median and 25th–75th percentiles of six independent experiments. *Statistically significant difference from control ($p < 0.01$).

This result indicates that eIF2α protein was phosphorylated and inactivated by PERK that led to shut off protein translation globally and to reduce protein load on the ER.

### 3.2. CPF induced the activation of IRE1α-XBP1 pathway

Next, we analyzed the effect of CPF exposure on the IRE1α–XBP1 pathway that provides an adaptive response to ER stress by degrading or refolding misfolded proteins accumulated in the ER lumen. Upon ER stress, XBP1 is spliced by IRE1α, thereby generating functional spliced XBP1 (sXBP1) (Lai et al., 2007). As shown in Fig. 2A, a tendency to increase was observed in the IRE1α protein level. Even though no significant increase in total IRE1α was detected, 50 or 100 μM CPF led to a clear increase in the sXBP1 mRNA fragment (193 nt), that was accompanied by a significant decrease in the levels of the unspliced pre-mRNA (uXBP1) (219 nt) (Fig. 2B). These data indicate that JEG-3 cells exposed to CPF results in the activation of IRE1α-XBP1 pathway.

### 3.3. CPF induced the expression of GRP78 protein

The final phase of UPR is the induction of ER chaperone protein expression, which is an attempt to restore ER function and increase the protein folding capacity of the cell. To examine whether CPF exposure triggers in JEG-3 cells such adaptation, protein expression levels of GRP78, calnexin, and PDI were examined. CPF induced the expression of GRP78 in a concentration-dependent manner at 24 h of CPF exposure, whereas calnexin and PDI protein amounts were not modified in these experimental conditions (Fig. 3).

### 3.4. CPF promoted the destabilization of the p53 tumor suppressor protein

Considering that p53 is a tumor suppressor that plays a major role regulating cellular response to different stressors we examined whether CPF modifies p53 mRNA and protein levels. Specifically, qRT-PCR indicated that p53 mRNA levels were not affected by CPF exposure for 24 h (Fig. 4A). In contrast p53 protein amount was significantly decreased in CPF-treated cells (Fig. 4B). To address whether this decrease could be due to changes in p53 protein stability, cells were exposed to 100 μM CPF and p53 protein levels were measured at different time

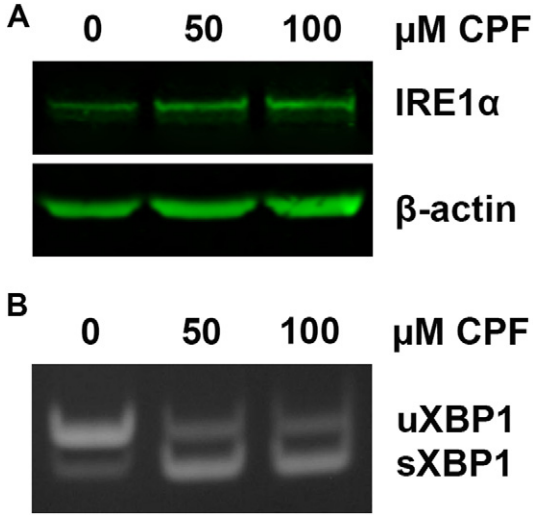


**Fig. 2.** CPF induces activation of IRE1α-XBP1 pathway. (A) Western blot analysis of IRE1α (top panel) in protein extracts from JEG-3 cells exposed to 0, 50 or 100 μM CPF during 24 h. β-Actin was used as a loading control (middle panel). (B) sXBP1 and uXBP1 mRNA expression levels (bottom panel) assayed by semiquantitative RT-PCR from JEG-3 cells exposed to 0, 50 or 100 μM CPF during 24 h. The images are representatives of at least three independent experiments.

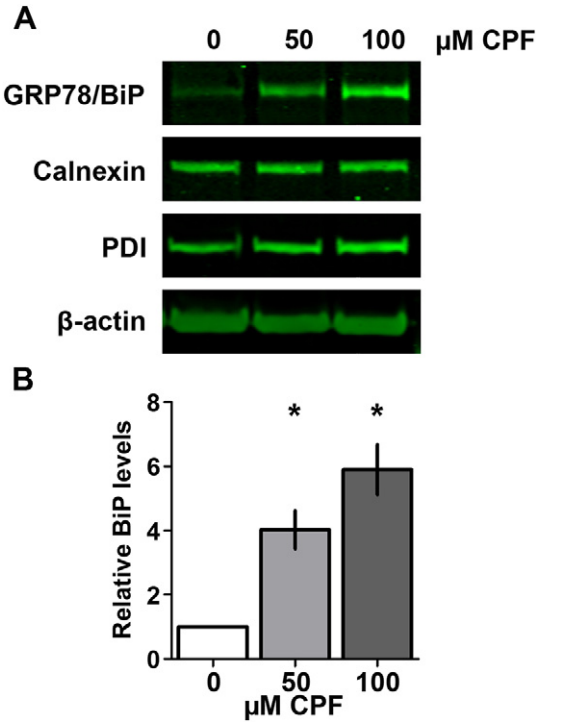


**Fig. 3.** CPF induces the expression of the GRP78/BiP protein. (A) Western blot analysis of GRP78/BiP, calnexin, and PDI in protein extracts from JEG-3 cells exposed to 0, 50 or 100 μM CPF during 24 h. β-Actin was used as a loading control. (B) The graph represents the GRP78/BiP expression levels normalized to β-actin in CPF-exposed cells during 24 h, and expressed relative to the control (0 μM CPF) condition defined as 1. Values are mean and SEM of five independent experiments. *Statistically significant difference from control ($p < 0.01$).

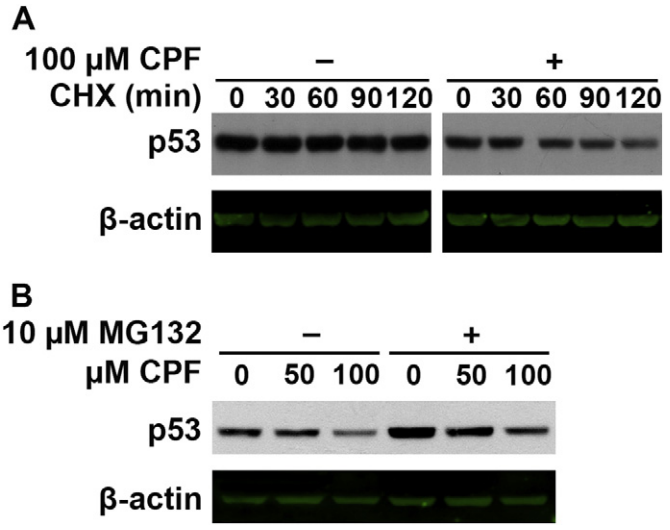


**Fig. 5.** CPF promotes destabilization of p53 tumor suppressor protein. JEG-3 cells were exposed or not to the indicated CPF concentrations during 24 h and then treated with cycloheximide (CHX, 20 μg/mL) for the indicated time (A) or incubated in the presence/absence of MG132 (10 μM) during 4 h (B). p53 expression levels were assayed by western blot. β-Actin expression was used as a loading control. The images are representatives of at least three independent experiments.

points after treatment with 20 μg/mL cycloheximide, a known inhibitor of protein biosynthesis. Western blot assays revealed that p53 levels dropped earlier in JEG-3 cells exposed to CPF than in control cells (Fig. 5A). We further investigated this by measuring p53 levels in 100 μM CPF-treated cells in the presence/absence of the proteasome inhibitor MG132. We noticed that downregulation of p53 protein levels in CPF-treated JEG-3 cells was partially blocked when cells were treated with 10 μM MG132 during 4 h (Fig. 5B). Thus, CPF exposure decreases p53 protein half-life in ER-stressed JEG-3 cells through the proteasome degradation machinery.

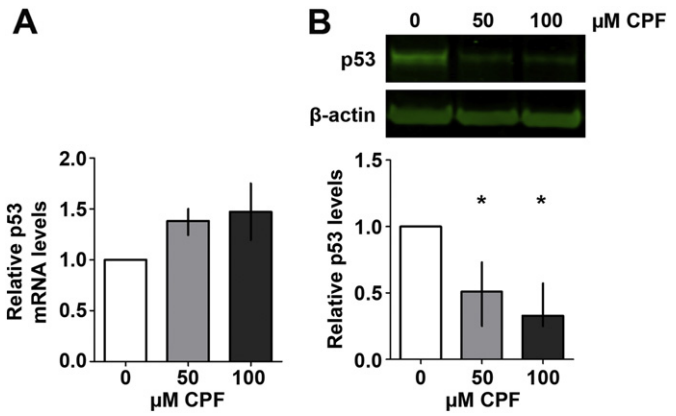


**Fig. 4.** CPF exposure reduces p53 protein expression. JEG-3 cells were exposed to 0, 50 or 100 μM CPF during 24 h. (A) p53 mRNA expression was determined by qRT-PCR. Results are expressed as fold change in p53 expression levels after normalizing to cyclophilin A relative to the corresponding normalized mRNA levels in control (0 μM CPF) cells. The values represent the median and 25th–75th percentiles of at least three independent experiments performed by triplicate. (B) p53 protein levels from JEG-3 cells exposed to CPF were analyzed by western blot. The graph represents the p53 levels normalized to β-actin value, and expressed relative to the corresponding normalized level in control (0 μM CPF) condition defined as 1. Values are mean and SEM of seven independent experiments. *Statistically significant difference from control ($p < 0.01$).

## 4. Discussion

Even though several reports have highlighted that ER stress and the subsequent UPR are involved in the cytotoxicity of environmental pollutants, industrial chemicals, and drugs (Duan et al., 2014; Kitamura, 2013; Matsuoka and Komoike, 2015), only a few studies have documented placental ER stress mediated by environmental factors. For example, it was reported that maternal exposure to pollutants such as cadmium or nicotine induces UPR in rat placenta (Wang et al., 2012; Wong et al., 2015), and nicotine and ethanol treatment induces ER stress in BeWo-trophoblast cell line (Repo et al., 2014).

In the present study, we demonstrated for the first time that CPF exposure promotes ER stress in JEG-3 cells. This response was evidenced by the activation of IRE1α-XBP1 pathway as well as through an increase in eIF2α phosphorylation. Additionally, increased levels of BiP/GRP78 suggest that CPF triggers the adaptive phases of the cellular response. Although not all mediators of ER stress response were examined, our results indicate that CPF activates at least two of these branches: IRE1α-XBP1 and PERK-eIF2α pathways. These pathways are known to lead to the self-protective pathways (Kadowaki and Nishitoh, 2013).

There is increasing recognition of an association between ER stress and p53; and both death-promoting and protective functions have been reported for p53 in ER stress (Bourougaa et al., 2010; Pluquet et al., 2005; Qu et al., 2004). Depending on the experimental conditions, ER stress can facilitate the reduction or the increase in p53 level or activity. In line with this, an increase in the expression of the pro-apoptotic protein p53 was induced by malathion in murine L929 fibroblasts (Masoud et al., 2003), whereas human granulosa cells showed dose-dependent morphological changes and reduced p53 expression levels after exposure to mancozeb (Paro et al., 2012). Interestingly, it has been proposed that ER stress prevents the pro-apoptotic function of p53 by enhancing its nucleo-cytoplasmic export and degradation as a mechanism of cell adaptation to ER stress induced by pharmacological or physiological means (Pluquet et al., 2005; Qu et al., 2004).

Consistently, the decreased stability of the p53 protein observed in JEG-3 cells after 24 h of CPF exposure could contribute to maintain cell viability.

Multiple evidences have established a close interaction between oxidative stress- and ER stress-induced by pollutants. For example, 2,4,6-trichlorophenol induced oxidative stress, ER stress, and apoptosis in mouse embryonic fibroblasts (Zhang et al., 2014); whereas Huang and collaborators demonstrated that cigarette smoking extracts induce ER stress associated with oxidative stress and that enhancing Nrf2 and XBP1 activity help to reduce oxidative and ER stress and protect retinal pigment epithelium cells from cigarette smoke-induced damage (Huang et al., 2015). In addition, it was reported that exogenous carbon monoxide activated Nrf2 through the phosphorylation of PERK, resulting in HO-1 expression (Kim et al., 2007). In line with the last two reports, in a previous study, we have shown that CPF disturbs redox balance and triggers antioxidant defense mechanisms in JEG-3 cells (Chiapella et al., 2013). In response to CPF exposure, glutathione reductase and HO-1 mRNA levels were increased with no changes in SOD1 mRNA in JEG-3 cells. Moreover, these cells were able to attenuate the oxidative stress induced by CPF through the adaptive activation of the Nrf2/ARE pathway (Chiapella et al., 2013). These data and present results indicate that CPF promotes oxidative and ER stress in JEG-3 cells, nevertheless these stresses lead to an adaptive protective response mediated by an increased degradation of the pro-apoptotic protein p53 and the activation of the Nrf2/ARE pathway.

As we have previously reported, several limitations do not allow us to extrapolate present findings to the actual *in vivo* effects of CPF on placenta during pregnancy (Ridano et al., 2012). Among them, the CPF concentrations used in our experiments should be considered. Several *in vitro* studies have employed concentrations in the micromolar range to analyze CPF effects on cell models (Amani et al., 2016; Patnaik and Padhy, 2016; Zarei et al., 2016). The problems associated with the translation of OP induced effects in *in vitro* culture conditions to *in vivo* exposure doses, as well as the inherent difficulties associated with exposure assessment to non-persistent OP pesticides, as CPF, are largely recognized (Bradman and Whyatt, 2005; Damalas and Eleftherohorinos, 2011; Needham, 2005). An important longitudinal cohort study performed in an agricultural community (the Salinas Valley in Monterey County, CA) found extreme CPF levels in blood of women and newborns of about 1500 ng/mL (*i.e.* around 4 μM) (Huen et al., 2012). Moreover, meconium OP values, which yield a longer-term dosimeter of prenatal exposure (Barr et al., 2005), suggest that the fetus and placenta may be exposed to higher CPF doses in agricultural communities (Ostrea et al., 2002). In addition, it is well-known that CPF is metabolically activated to its more toxic oxon-derivative, responsible of cholinesterase inhibition. Consistently, we have previously demonstrated that in the same experimental conditions used here, JEG-3 cell's acetylcholinesterase activity decreased suggesting that CPF-oxon was indeed produced (Chiapella et al., 2013), thus we cannot rule out that oxon could also trigger the ER stress. Despite the limitations discussed above, present findings and our previous report (Chiapella et al., 2013) are important because they highlight the molecular mechanisms through which trophoblast cells respond to the presence of CPF. They suggest that placental cells are capable to control the oxidative and ER stress insults generated by OPs like CPF, thus contributing to preserve placental functions.

Fig. 6 summarizes present findings. Collectively, they provide strong evidence supporting the notion that CPF treatment induces ER stress, which in turn leads to the activation of the major pathways of UPR concomitant with an increased degradation of p53 protein.

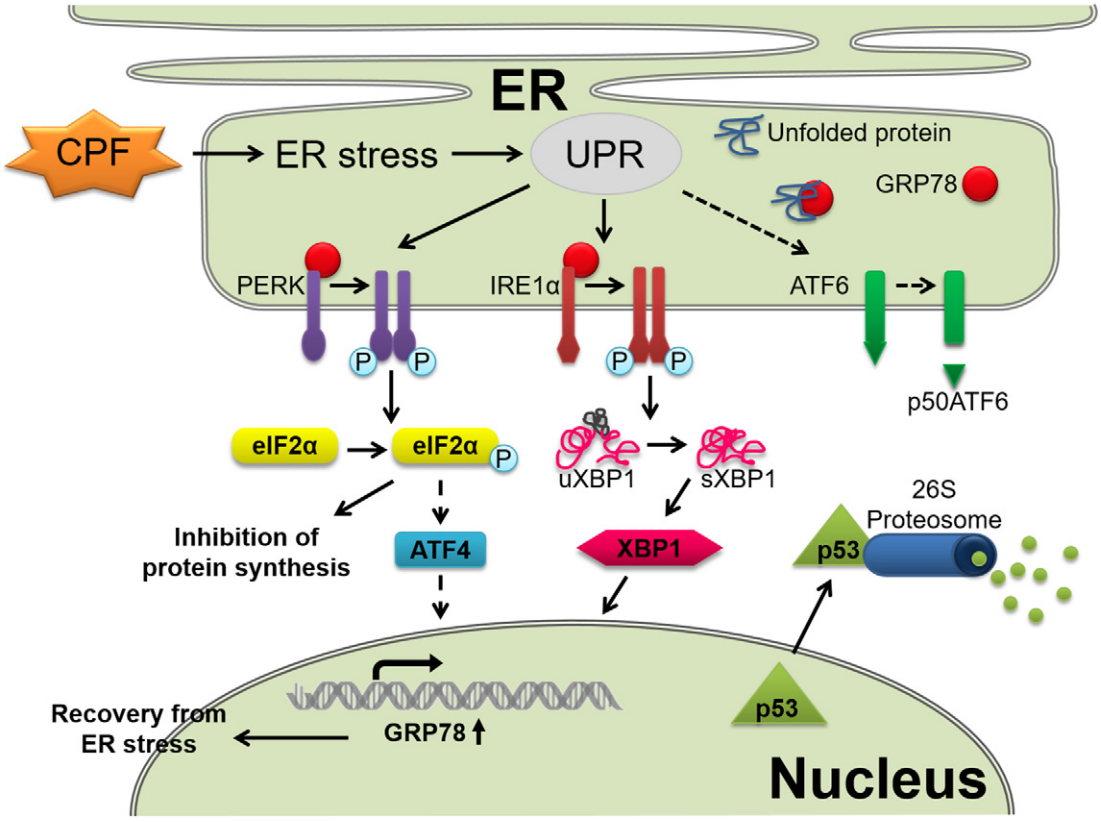


**Fig. 6.** Proposed molecular mechanisms of UPR triggered by CPF in JEG-3 cells. CPF treatment disturbed ER homeostasis and induced the accumulation of unfolded proteins in the ER triggering the UPR. CPF exposure induces PERK phosphorylation; phosphorylated PERK initiates eIF2α phosphorylation, which suppresses global mRNA translation and inhibits protein synthesis to protect cells against ER stress. GRP78 plays a crucial role in regulating dynamic ER homeostasis and contributes to cell recovery from ER stress. The PERK-eIF2α and IRE1α-XBP1 pathways measured in this study are represented by solid arrows whereas those not measured are represented by dashed arrows. In the presence of CPF, the ER stress induces p53 protein degradation.

## Transparency document

The Transparency document associated with this article can be found in the online version.

## Acknowledgements

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