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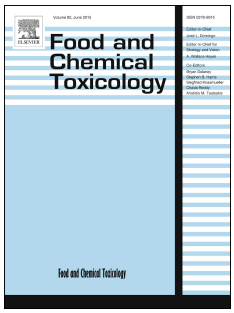

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RENAL AND HEPATIC EFFECTS FOLLOWING NEONATAL EXPOSURE TO LOW DOSES OF BISPHENOL-A AND ^{137}Cs

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ABBREVIATIONS LIST

^{137}Cs	137-Cesium
8-iso-PGF2α	8-iso-prostaglandin F2α
8-OHdG	8-hydroxy-2'-deoxyguanosine
ALP	alkaline phosphatase
BPA	Bisphenol-A
ChE	cholinesterase
CYPs	Cytochrome P450s
GGT	γ-glutamil-transferase
GOT	glutamic oxaloacetic transaminase
GPT	glutamic pyruvic transaminase
IR	ionizing radiation
LDH	lactate dehydrogenase
NAG	N-acetyl-β-D-glucosaminidase
OS	oxidative stress
PND10	postnatal day 10
ROS	reactive oxygen species
RT-PCR	real-time polymerase chain reaction

Introduction

All living organisms are continually exposed to ionizing radiation (IR), which has always existed naturally. In addition, other sources of radiation exposure include human activities that involve the use of radiation and radioactive substances in industrial, military and medical fields (Heredia et al., 2016; Kayser et al., 2014; Verreet et al., 2016). 137-Cesium (^{137}Cs) is one of the most important and widely distributed radionuclides in terrestrial and aquatic environments (Yamaguchi et al., 2014). Because of their chemical structure, similar to potassium, and its solubility in water, ^{137}Cs is easily transported through the environment and food chains. The major sources of ^{137}Cs in the environment are nuclear reactor waste and also the accidental releases from Chernobyl and Fukushima Daiichi Nuclear Power Plants (Ashraf et al., 2014; Ashraf et al., 2013; Buesseler et al., 2012; Jelin et al., 2016). Once the radioactive material has been released to the atmosphere, the resulting fallout, consisting of short and long-lived radionuclides, affects humans, entering the food chain through plants and animals (Franic et al., 2008). The consumption of contaminated drinking water, plants and fish are the main routes of potential human exposure (Belles et al., 2016).

As a result of nuclear accidents, the population living in contaminated areas are daily exposed to low doses of IR. After the Chernobyl disaster, an increase in the incidence of malignant renal tumors was reported in subjects living in Ukraine areas contaminated with ^{137}Cs. In this population, there has also been an association of low doses of IR in renal cell carcinomas (Jargin, 2015; Morell-Quadreny et al., 2011). Furthermore, epidemiological studies carried out among Hiroshima and Nagasaki A-bomb survivors, aged 0-34 years at the time of the bombing, have found a significant increase in the incidence of hepatic and renal cancer (Goto et al., 2012; Preston et al., 2017). Studies with animals have also shown that high doses of IR induce an increase in the structural damage and hepatic toxicity, as well as changes in hepatic functions caused by metabolic enzyme dysregulation (Bakshi et al., 2015).

On the other hand, in the industrialized countries, people is daily co-exposed to a number of environmental pollutants. Thus, it is important to evaluate the health effects of low doses of IR concurrently with toxic agents, such as Bisphenol-A (BPA) (Hernandez and Tsatsakis, 2017). BPA, a known estrogenic chemical, is now a common endocrine-disrupting chemical (Vafeiadi et al., 2016). This synthetic chemical is widely used in the manufacture of polycarbonate plastic employed to manufacture bottles, food packages, and epoxy resins used in the lining metal food and beverage cans and dental sealants (Heredia et al., 2016; Pollock and deCatanzaro, 2014; Willhite et al., 2008). In humans, the main source of exposure is the diet: packaged food and beverages that are contaminated by BPA that migrates from the packaging material, followed by inhalation of household dust, and dermal exposure (EFSA, 2015; Giulivo et al., 2016). BPA, among other endocrine-disrupting chemicals, tend to be stored in human tissues and the effect of its exposure may not become evident until later in life (Tzatzarakis et al., 2015).

Human health concerns arise because the population is constantly exposed to low doses of BPA, particularly during the fetal and postnatal periods. Developing organs are very vulnerable to the abnormal endocrine signal caused by this chemical (Nachman et al., 2014; Perng et al., 2017; Vandenberg et al., 2009). International biomonitoring studies have indicated that a high percentage of the child population (>90%) in Europe, United States and Australia are exposed to BPA. For a same or similar geographical area, the levels of BPA in urine are frequently higher in children than in a general adult population (Healy et al., 2015; Rivas et al., 2016). Hepatic tissue is the primary and main responsible for the BPA metabolism. A number of investigations have shown its adverse health effects on liver function (Korkmaz et al., 2010; Moon et al., 2012; Vahdati Hassani et al., 2017). In addition, exposure to BPA has been associated with albuminuria in adults, as well as in children (Trasande et al., 2013). Moreover, Olea-Herrero et al. (2014) demonstrated that in mice, BPA promotes a podocytopathy with an increase in urinary albumin and glomerular hyperfiltration.

It has been suggested that exposure to IR cause oxidative stress (OS) through the production of free radicals, including reactive oxygen species (ROS) (El-Gazzar et al., 2016; Serra et al., 2015). Animal studies have shown that low doses of IR induce oxidative DNA damage, with an increase in the kidney levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) (Belles et al., 2017; Ozyurt et al., 2014). Furthermore, the induction of the detoxification enzymes by irradiation may represent adaptive responses against OS and protect the cells against subsequent exposure to IR. Cytochrome P450s (CYPs) enzymes are prominently concentrated in the liver and catalyze reactions generally producing the oxidation of endogenous or exogenous substrates (Barouki and Morel, 2001; Haque et al., 2017). On the other hand, growing evidence suggests that BPA-induced damage is also associated with OS. BPA can disturb oxidative homeostasis through direct or indirect pathways, including mitochondrial function, modulation of antioxidant enzymes and increase of thiobarbituric acid-reactive substances (Kabuto et al., 2004; Li et al., 2017; Tiwari et al., 2012). As BPA is primarily metabolized by the liver, this organ is more vulnerable to lower doses of this chemical than other tissues (Moon et al., 2012). Experimental studies demonstrate that BPA inhibits CYPs in the hepatic tissue (Elswefy et al., 2016).

The presence of IR and BPA in the environment may lead to current human exposure to both contaminants. However, accurate mechanisms involved in the tissue damage induced by this co-exposure have not been fully elucidated. Low doses of this co-exposure at very young age could have a negative impact in biological functions. Consequently, in this study we evaluated the toxic effects of low doses of IR and BPA examining alterations in parameters associated with renal and hepatic functions.

2. Material and methods

2.1. Animals

All experiments were performed in C57BL/6J mice (Charles River, CRIFFA, Barcelona, Spain). Mice were housed in standard animal cages in a climate-controlled facility with a constant day–night cycle (light: 08:00am–8:00pm) at a temperature of 22±2ºC, and a relative humidity of 50±10%. Food (Panlab, Barcelona, Spain) and tap water were offered *ad libitum* throughout the study. The use of animals and the experimental protocol were approved by the Animal Care and Use Committee of the Universitat Rovira i Virgili (Catalonia, Spain) and were conducted in accordance with the Spanish Royal Decree 53/2013 on the protection of experimental animals and the European Union Directive (2010/63/EU).

2.2. Groups and treatment

Sixty male mice were randomly assigned to experimental groups and received a single subcutaneous dose of 0.9% saline solution, ^{137}Cs (CIEMAT, Spain) and/or BPA (239658-50G, Aldrich, Spain) on postnatal day 10 (PND10). Six experimental groups (n=10) were established: control group (0.9% saline solution used as vehicle), BPA group (25µg/kgbw of BPA), Cs4000 group (^{137}Cs with activity of 4000Bq/kgbw), Cs8000 group (^{137}Cs with activity of 8000Bq/kgbw), BPA/Cs4000 group (25µg/kgbw of BPA and ^{137}Cs with activity of 4000Bq/kgbw) and BPA/Cs8000 group (25µg/kgbw of BPA and ^{137}Cs with activity of 8000Bq/kgbw). ^{137}Cs and BPA doses were based on the results of recent investigations (Belles et al., 2016; Heredia et al., 2016; Kass et al., 2015). At the age of two months, mice were individually housed in plastic metabolism cages. Urines (24h) were collected from animals in each group in order to determine biochemical parameters and isoprostane levels. After urine collection, mice were anesthetized by an intraperitoneal injection (100µl) of ketamine (80mg/kg) – xylazine (10mg/kg). Blood samples were collected from the vena cava to determine biochemical serum parameters. Finally, mice were sacrificed by cervical dislocation. Kidneys and liver were removed and stored at -80ºC to quantify DNA damage, as well as to determine CYP1A2 mRNA expression in hepatic tissue.

2.3. Biochemical analysis

In fresh urine collected, the 24h volume, the concentrations of urea, total protein, uric acid and creatinine, as well as the activity of lactate dehydrogenase (LDH), γ-glutamil-transferase (GGT), and N-acetyl-β-D-glucosaminidase (NAG) were measured. Serum samples were analyzed to determine urea, total protein, uric acid and creatinine concentrations, as well as the activities of LDH, GGT, alkaline phosphatase (ALP), cholinesterase (ChE), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT). All these parameters were analyzed using a Cobas Mira automatic analyzer (Roche Pharmaceuticals, Switzerland) (Alonso et al., 2010).

2.4. Quantification of urinary isoprostanes with Enzyme Linked Immunosorbent Assay (ELISA) kit

Urinary 8-iso-PGF2α levels were determined using the Urinary Isoprostane ELISA Kit (EA85, Oxford Biomedical Research, USA). Briefly, urinary samples were diluted 1:5 with Enhanced Dilution Buffer and 100µl of standards. Samples were then loaded to each well of a 96-well plate. After the addition of 100µl of diluted 8-iso-PGF2α Conjugate, samples were incubated for 2h at room temperature. Plate was washed three times adding 300µl of Wash Buffer to each well. 200µl of TMB Substrate was added and the plate was incubated for 20-40min until an appreciable blue hue was observed in blank wells. Finally, 50µl Stop Solution was added and the plate was read at 450nm (Belles et al., 2017).

2.5 Effect of BPA on CYP1A2 mRNA expression

2.5.1. RNA isolation

mRNA was isolated from the liver using PureLink™ RNA Mini kit (12183018A, Ambion by Life technologies, Spain). Briefly, samples were lysed by mechanical disruption in 600µl of lysis solution. After the addition of 600µl of 70% ethanol, samples were mixed and loaded onto a Micro Filter Cartridge Assembly. Samples were centrifuged and the filter was washed three times (using 700µl of Wash Solution I for the first wash and 500µl of Wash Solution II for the rest). The flow was discarded and

a centrifugation at 12000g was performed to dry the filter. Lastly, total RNA was eluted with 50µl of Elution Solution. mRNA concentration was measured using the Qubit RNA Assay Kit (Q32852, Life Technologies, Spain) (Serra et al., 2015).

2.5.2. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

For the reverse transcription to cDNA, 0.5µg of RNA was used with Random Hexamers and SuperScript II Reverse Transcriptase (Life Technologies, Spain). Taqman primers and probes for CYP1A2 and β-actine were obtained from validated and pre-designed Gene Expression Assays (Life Technologies, Spain) and were used in real-time polymerase chain reaction (RT-PCR) amplifications. mRNA expression for each gene and sample was calculated using the recommended 2-δCt method. To normalize the results of the gene of interest, β-actine was used as housekeeping gene (Serra et al., 2015).

2.6. DNA damage quantification

In our study, kidney and liver samples were washed in 0.9% saline and homogenized in 0.2M sodium phosphate pH 6.25 buffer (1:20, w/v) in a Potter-Elvehjem homogenizer with a Teflon pestle (Braun, Germany). Total DNA was extracted using the commercially available DNeasy Blood & Tissue Kit (Qiagen, Germany). The concentration of DNA in each sample was determined using an EPOCHTM 2 Microplate Espectrophotometer (BioTek, USA). For measuring the level of 8-OHdG, we used EpiQuik™ 8-OHdG DNA Damage Quantification Direct Kit (Epigentek, USA) according to manufacturer instruction (Belles et al., 2017).

2.7. Statistics

Data are given as means ± standard deviations (S.D.). To evaluate the homogeneity of variances, the Bartlett's test was used. When the variances were homogeneous, ANOVA was used followed by the Bonferroni's post-hoc test to establish the level of significance among groups. If the variances were not homogeneous, the

Kruskal-Wallis test and subsequently Dunn's post-hoc test was used. All experimental groups were compared with controls and both co-exposed groups were compared with their respective controls. The level of statistical significance for all tests was established at $p<0.05$. All data were analyzed by GraphPad Prism Statistical Analysis software (GraphPad Prism version 5.01 for Windows, USA).

3. Results

The effects in mice of exposure to BPA and ^{137}Cs, alone or in combination, on various urinary parameters are summarized in Table 1. The significant differences between exposed groups and the control group are indicated. For the best understanding of the information provided in tables, significant differences between exposed groups are not shown. However, they are here commented. It was observed that the urinary volume (ml/24h) significantly decreased in all treated groups with respect to the control group. Although urea levels decreased in mice given BPA, alone or combined with ^{137}Cs, no significant differences between groups were noted. In comparison with the control group, Cs4000 group showed the highest activity of LDH. However, the combination of Cs4000 with BPA significantly decreased LDH activity in relation to the control group and BPA-exposed group ($p<0.001$). Similarly, combined administration to BPA and Cs8000 showed lower LDH activity compared to the Cs8000 group ($p<0.05$). Urinary excretion of uric acid and GGT in mice receiving BPA combined with Cs4000 was higher than that in controls and mice exposed only to BPA ($p<0.01$ and $p<0.001$, respectively), or Cs4000 ($p<0.01$ for uric acid). The leftover urinary parameters detailed in the table; total protein, creatinine and creatinine clearance, did not show significant differences between groups.

Urinary excretion of NAG, following individual or combined exposure to BPA and ^{137}Cs, is depicted in Fig. 1. In comparison to the control group, NAG activity increased significantly in mice exposed to BPA, as well as in those exposed to both ^{137}Cs

activities. However, when BPA was administered in combination with Cs4000 or Cs8000, NAG activity was significantly restored to control values.

Mice given BPA and ^{137}Cs-alone or in combination-induced changes in the urinary levels of 8-iso-$PGF_{2\alpha}$ (Fig. 2). With the exception of the Cs4000 group, the remaining groups showed significant higher levels of 8-iso-$PGF_{2\alpha}$ than the control group. In addition, combined exposure to BPA and Cs8000 induced a significant increase in 8-iso-$PGF_{2\alpha}$ excretion in comparison with exposure only to BPA and to Cs8000.

The results of various serum parameters measured after BPA and/or ^{137}Cs administration are shown in Table 2. As for urine, significant differences between exposed groups and control group are indicated on. However, the significant differences between groups exposed to BPA and/or ^{137}Cs are commented on the text. BPA administration caused a significant increase in urea levels and ALP activity in comparison with the control group. However, when BPA was combined with ^{137}Cs, these parameters were significantly restored to control values, being the values significantly lower than those in the groups exposed only to BPA ($p<0.001$). In contrast, concurrent exposure to BPA and Cs4000 increased significantly the total protein content in comparison with the control, Cs4000 and BPA groups ($p<0.01$ and $p<0.001$, respectively). Mice given ^{137}Cs-alone or combined with BPA showed an increase in uric acid levels with respect to those in the control and BPA ($p<0.01$) groups. The remaining parameters shown in Table 2, creatinine and LDH, did not show significant differences between groups. Exposure to BPA or ^{137}Cs resulted in a decrease in ChE levels compared to those in the control group (Fig. 3). However, combined administration of BPA with ^{137}Cs restored levels to those of the control group. On the other hand, the activity of the transaminases GGT, GOT and GPT were significantly enhanced in all exposed mice compared with the controls (Fig. 4A-C). No differences between mice exposed to BPA-alone or in combination with ^{137}Cs were observed.

Hepatic gene expression of CYP1A2 in mice exposed to BPA and ^{137}Cs, alone or in combination, is depicted in Fig. 5. Following exposure, the mRNA expression of

CYP1A2 in liver decreased in all experimental groups with respect to that in the control group. However, the decline in mRNA expression of CYP1A2 was significant only in mice receiving BPA alone, or combined with Cs8000.

The percentage of 8-OHdG in hepatic and renal tissues of mice treated with BPA and ^{137}Cs, alone or combined, is depicted in Fig. 6A-B. The levels of 8-OHdG in liver were lower in the exposed groups than in the control group. However, the decrease was only significant in the group receiving BPA combined with Cs8000 (Fig. 6A). Moreover, no significant differences were observed in the 8-OHdG renal levels between exposed and control groups (Fig. 6B).

Discussion

The toxic effects of IR occur in various organs. However, taking into account the severity of the damage caused, the kidney is probably the most radiosensitive abdominal organ (Belles et al., 2017; Fuma et al., 2016; Ki et al., 2017). Likewise, the liver is also relatively a radiosensitive organ, which can experience alterations in the metabolic function after irradiation (Barshishat-Kupper et al., 2014). On the other hand, in industrialized countries, people are daily exposed to toxicant pollutants of notable concern, such as BPA (EFSA, 2015). Inside the body, BPA is metabolized by the enzymes that are expressed in the liver (Pollock and deCatanzaro, 2014). Furthermore, BPA has been shown to induce the generation of ROS in kidney, being excreted in urine as BPA-glucuronide (Gonzalez-Parra et al., 2013). However, little is known concerning the toxic effects of combined exposure to low doses of IR and BPA in renal and hepatic tissues, as well as their potential to increase the risk of metabolic and functional disorders, mainly during childhood.

The current results show a decrease in urinary volume (ml/24h) in mice exposed to BPA and ^{137}Cs, alone or in combination. It has been reported that very low levels of urinary output, named oliguria, are considered as markers of renal injury (Prowle et al., 2011). Renal damage is one of the known side effects of IR exposure, and can cause impairment in the glomerular filtration rate (Belles et al., 2017). In relation to

BPA, Yildiz and Barlas (2013) reported that this estrogenic compound induces damage on the kidney of rats. Renal histopathological examination revealed a significant tubular degeneration in the treatment groups when compared to the control group. Furthermore, the current study has shown that combined exposure to BPA and Cs4000 increased urinary excretion of uric acid. During renal dysfunction, the elevated levels of uric acid might be a sensitive indicator of tubular damage, impaired reabsorptive capability of tubular protein, or impaired protein filtration of glomerular barrier (Abdelrahman, 2017). Moreover, uric acid is a powerful non-enzymatic antioxidant agent, which is found elevated under increased OS (Laiakis et al., 2014). In the present investigation, the same effect induced by BPA and ^{137}Cs for uric acid, was also observed for the GGT urinary levels. The combined exposure increased the levels of GGT in comparison with the control group, and the groups individually exposed to BPA or ^{137}Cs. GGT is a membranous enzyme found widely in tissues with secretory activity from various organisms (Kunutsor, 2016). It has been suggested that GGT plays a pro-oxidant role since the extracellular cleavage of GGT induces the production of ROS (Alonso et al., 2010).

Renal damage could be also assessed by measuring LDH activity. LDH is a brush border enzyme originated in the proximal tubule, which is normally present in the urine at small quantities, increasing significantly in the setting of acute kidney injury (Moon et al., 2013). We here observed an increase of LDH activity in mice exposed to Cs4000, while combined with BPA is able to decrease, even lower than control animals. This indicates that after co-exposure to BPA and the lower activity of ^{137}Cs, the kidney shows a reduced metabolic enzyme activity, which is associated with reduced renal function.

The search for new biomarkers to detect renal toxicity during the early phase, in which clinical damage is not prominent, is ongoing. NAG, one of these early biomarkers, is a lysosomal brush border enzyme found in the proximal tubular cells. Due to its relative high molecular weight, it is not filtered through the glomeruli and released into the urine after renal tubular damage. Several studies have demonstrated

that NAG urinary concentrations increase in the injured kidney (Fiseha and Tamir, 2016; Gunes et al., 2016; Tasdemir et al., 2017). Moreover, a relationship between urinary NAG excretion and renal oxidative damage has been reported (Oktem et al., 2006). In the present study, exposure to either BPA or ^{137}Cs induced an increase in NAG urinary excretion. It agrees with the results of a previous investigation conducted in our laboratory, which showed an increase of NAG activity in urine after internal exposure to uranium (Belles et al., 2007). However, it should be noted that, in the current investigation, the combined exposure to both contaminants reduced urinary levels of NAG to values similar to those found in the control group. According to this, it can be suggested that when BPA and ^{137}Cs are administered alone, the renal damage is greater than when they are given together.

Determination of 8-iso-PGF2α, a product of free-radical-catalyzed peroxidation of arachidonic acid, has been proposed as an accurate biomarker of OS in tissue injuries (Zhao and Robbins, 2009). IR-induced OS may produce ROS, which are reported to be the main cause of cytotoxicity, as well as of metabolic and morphologic tissue alterations (El-Gazzar et al., 2016; El-Missiry et al., 2007). Recently, we have investigated the effects of low doses of internal IR in early nephrotoxicity. The results indicated a significant increase in the urinary levels of 8-iso-PGF2α in irradiated mice (Belles et al., 2017). However, only a few studies have examined associations between BPA exposure and OS biomarkers. Maternal BPA exposure at low doses leads to increase levels of lipid peroxidation marker 8-isoprostane (Ferguson et al., 2016; Vahdati Hassani et al., 2017). The present results are consistent with those of these previous studies. Notwithstanding, we have demonstrated that the combined exposure to low doses of IR and BPA produces at long-term, a significant increase in lipid peroxidation.

The liver plays a pivotal role in the metabolism of a number of xenobiotics and environmental chemicals, being an important detoxification organ. More than 90% of drugs and xenobiotic compounds are metabolized and detoxified by CYPs and glutathione S-transferase enzymes in the liver (Ma-On et al., 2017). Moreover, a

number of studies have indicated that the expression and metabolic activity of CYPs enzymes is highly associated with OS, due to the high rate of uncoupling of electron transfer and ROS generation (Martinez et al., 2017; Valencia-Olvera et al., 2014). CYP1A2, a member of a number of CYP subfamilies, is predominantly expressed in the hepatic tissue and involved in the metabolism of various exogenous and endogenous compounds (Hussain et al., 2014). In the current study, we have observed that hepatic expression of CYP1A2 decreased in mice exposed to BPA, alone or combined with Cs8000. However, no changes in the CYP1A2 expression has been detected when mice were exposed only to IR. In accordance with our results, data from previous investigations have revealed that BPA interacts with rat hepatic CYP1A, CYP2B-E, and CYP3A *in vitro*. CYP-mediated modifications of BPA may give rise to the formation of unstable reactive intermediates, as well as radical fragments leading to oxidative damage and cellular dysfunction (Shmarakov et al., 2017).

On the other hand, our results did not show significant effects of low radiation doses on the expression of CYP1A2. The complexity of effects of IR on CYPs activity are exemplified in the results obtained after long-term exposure of rats (Souidi et al., 2006). Chronic exposure of rats with post-accidental doses of ^{137}Cs in drinking water for 3 months showed no significant change of CYP27A1 mRNA expression in liver (Souidi et al., 2006). In addition, experiments with rats chronically treated with depleted uranium in drinking water for 9 months showed significant increases in hepatic CYP3A1 mRNA expression, although the expression of CYP1A1 did not change significantly (Rendic and Guengerich, 2012).

DNA is highly susceptible to oxidative damage. Kawanishi et al. (2016) showed that 8-OHdG is a useful biomarker for OS and oxidative DNA. Various studies that have assessed cellular DNA as the primary target for the toxic effects of IR, inducing strand breaks or base deletion (Glebova et al., 2015; Kavanagh et al., 2013; Yoshikawa et al., 2015). In previous investigations, Belles et al. (2017), we found that ^{137}Cs induced significant increases in the percentage of 8-OHdG in adult mice, 10 days after being exposed to ^{137}Cs and 4000Bq/kgbw. In the present study, where PND10

mice have been exposed to ^{137}Cs, BPA or both, neither BPA, nor ^{137}Cs, induced damage in the kidney or liver of mice, two months after exposure. The difference might be due to the age of exposure and the remaining time after analysing the effects.

In conclusion, the current data suggest that, both BPA and ^{137}Cs, induced renal and liver damage as evidenced by various direct or indirect OS parameters. However, when there is a co-exposure, it seems that there are compensatory mechanisms that may reverse the damage induced by each toxic itself. Further studies are needed in order to better understand the synergistic mechanisms behind.

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References

Abdelrahman, R.S., 2017. Protective effect of apocynin against gentamicin-induced nephrotoxicity in rats. Human & experimental toxicology, 960327116689716.

Alonso, V., Linares, V., Belles, M., Albina, M.L., Pujol, A., Domingo, J.L., Sanchez, D.J., 2010. Effects of BDE-99 on hormone homeostasis and biochemical parameters in adult male rats. Food and chemical toxicology 48, 2206-2211.

Ashraf, M.A., Khan, A.M., Ahmad, M., Akib, S., Balkhair, K.S., Bakar, N.K., 2014. Release, deposition and elimination of radiocesium ((137)Cs) in the terrestrial environment. Environmental geochemistry and health 36, 1165-1190.

Ashraf, N., Hoffe, S., Kim, R., 2013. Adjuvant treatment for gastric cancer: chemotherapy versus radiation. The oncologist 18, 1013-1021.

Bakshi, M.V., Azimzadeh, O., Barjaktarovic, Z., Kempf, S.J., Merl-Pham, J., Hauck, S.M., Buratovic, S., Eriksson, P., Atkinson, M.J., Tapio, S., 2015. Total body exposure

to low-dose ionizing radiation induces long-term alterations to the liver proteome of neonatally exposed mice. Journal of proteome research 14, 366-373.

Barouki, R., Morel, Y., 2001. Repression of cytochrome P450 1A1 gene expression by oxidative stress: mechanisms and biological implications. Biochemical pharmacology 61, 511-516.

Barshishat-Kupper, M., Tipton, A.J., McCart, E.A., McCue, J., Mueller, G.P., Day, R.M., 2014. Effect of ionizing radiation on liver protein oxidation and metabolic function in C57BL/6J mice. International journal of radiation biology 90, 1169-1178.

Belles, M., Gonzalo, S., Serra, N., Esplugas, R., Arenas, M., Domingo, J.L., Linares, V., 2017. Environmental exposure to low-doses of ionizing radiation. Effects on early nephrotoxicity in mice. Environmental research 156, 291-296.

Belles, M., Heredia, L., Serra, N., Domingo, J.L., Linares, V., 2016. Exposure to low doses of 137cesium and nicotine during postnatal development modifies anxiety levels, learning, and spatial memory performance in mice. Food and chemical toxicology 97, 82-88.

Belles, M., Linares, V., Luisa Albina, M., Sirvent, J., Sanchez, D.J., Domingo, J.L., 2007. Melatonin reduces uranium-induced nephrotoxicity in rats. Journal of pineal research 43, 87-95.

Buesseler, K.O., Jayne, S.R., Fisher, N.S., Rypina, II, Baumann, H., Baumann, Z., Breier, C.F., Douglass, E.M., George, J., Macdonald, A.M., Miyamoto, H., Nishikawa, J., Pike, S.M., Yoshida, S., 2012. Fukushima-derived radionuclides in the ocean and biota off Japan. Proceedings of the National Academy of Sciences of the United States of America 109, 5984-5988.

EFSA, 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA Journal 13, 3978.

El-Gazzar, M.G., Zaher, N.H., El-Hossary, E.M., Ismail, A.F., 2016. Radio-protective effect of some new curcumin analogues. Journal of photochemistry and photobiology. B, Biology 162, 694-702.

El-Missiry, M.A., Fayed, T.A., El-Sawy, M.R., El-Sayed, A.A., 2007. Ameliorative effect of melatonin against gamma-irradiation-induced oxidative stress and tissue injury. Ecotoxicology and environmental safety 66, 278-286.

Elswefy, S.E., Abdallah, F.R., Atteia, H.H., Wahba, A.S., Hasan, R.A., 2016. Inflammation, oxidative stress and apoptosis cascade implications in bisphenol A-induced liver fibrosis in male rats. International journal of experimental pathology 97, 369-379.

Ferguson, K.K., Cantonwine, D.E., McElrath, T.F., Mukherjee, B., Meeker, J.D., 2016. Repeated measures analysis of associations between urinary bisphenol-A concentrations and biomarkers of inflammation and oxidative stress in pregnancy. Reprod Toxicol 66, 93-98.

Fiseha, T., Tamir, Z., 2016. Urinary Markers of Tubular Injury in Early Diabetic Nephropathy. International journal of nephrology 2016, 4647685.

Franic, Z., Marovic, G., Mestrovic, J., 2008. Radiocaesium contamination of beef in Croatia after the Chernobyl accident. Food and chemical toxicology 46, 2096-2102.

Fuma, S., Kubota, Y., Ihara, S., Takahashi, H., Watanabe, Y., Aono, T., Soeda, H., Yoshida, S., 2016. Radiocaesium contamination of wild boars in Fukushima and surrounding regions after the Fukushima nuclear accident. Journal of environmental radioactivity 164, 60-64.

Giulivo, M., Lopez de Alda, M., Capri, E., Barcelo, D., 2016. Human exposure to endocrine disrupting compounds: Their role in reproductive systems, metabolic syndrome and breast cancer. A review. Environmental research 151, 251-264.

Glebova, K., Veiko, N., Kostyuk, S., Izhevskaya, V., Baranova, A., 2015. Oxidized extracellular DNA as a stress signal that may modify response to anticancer therapy. Cancer letters 356, 22-33.

Gonzalez-Parra, E., Herrero, J.A., Elewa, U., Bosch, R.J., Arduan, A.O., Egido, J., 2013. Bisphenol a in chronic kidney disease. International journal of nephrology 2013, 437857.

Goto, H., Watanabe, T., Miyao, M., Fukuda, H., Sato, Y., Oshida, Y., 2012. Cancer mortality among atomic bomb survivors exposed as children. Environmental health and preventive medicine 17, 228-234.

Gunes, A., Aktar, F., Tan, I., Soker, M., Uluca, U., Balik, H., Mete, N., 2016. Urinary levels of early kidney injury molecules in children with vitamin B12 deficiency. Archivos argentinos de pediatria 114, 453-457.

Haque, A., Leong, K.H., Lo, Y.L., Awang, K., Nagoor, N.H., 2017. In vitro inhibitory mechanisms and molecular docking of 1'-S-1'-acetoxychavicol acetate on human cytochrome P450 enzymes. Phytomedicine 31, 1-9.

Healy, B.F., English, K.R., Jagals, P., Sly, P.D., 2015. Bisphenol A exposure pathways in early childhood: Reviewing the need for improved risk assessment models. Journal of exposure science & environmental epidemiology 25, 544-556.

Heredia, L., Belles, M., MI, L.L., Domingo, J.L., Linares, V., 2016. Behavioral effects in mice of postnatal exposure to low-doses of 137-cesium and bisphenol A. Toxicology 340, 10-16.

Hernandez, A.F., Tsatsakis, A.M., 2017. Human exposure to chemical mixtures: Challenges for the integration of toxicology with epidemiology data in risk assessment. Food and chemical toxicology 103, 188-193.

Hussain, T., Al-Attas, O.S., Al-Daghri, N.M., Mohammed, A.A., De Rosas, E., Ibrahim, S., Vinodson, B., Ansari, M.G., El-Din, K.I., 2014. Induction of CYP1A1, CYP1A2, CYP1B1, increased oxidative stress and inflammation in the lung and liver tissues of rats exposed to incense smoke. Molecular and cellular biochemistry 391, 127-136.

Jargin, S.V., 2015. Renal cell carcinoma after chernobyl: on the role of radiation vs. late detection. Pathology oncology research 21, 845-846.

Jelin, B.A., Sun, W., Kravets, A., Naboka, M., Stepanova, E.I., Vdovenko, V.Y., Karmaus, W.J., Lichosherstov, A., Svendsen, E.R., 2016. Quantifying annual internal effective 137Cesium dose utilizing direct body-burden measurement and ecological dose modeling. Journal of exposure science & environmental epidemiology 26, 546-553.

Kabuto, H., Amakawa, M., Shishibori, T., 2004. Exposure to bisphenol A during embryonic/fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice. Life sciences 74, 2931-2940.

Kass, L., Durando, M., Altamirano, G.A., Manfroni-Ghibaudo, G.E., Luque, E.H., Munoz-de-Toro, M., 2015. Prenatal Bisphenol A exposure delays the development of the male rat mammary gland. Reprod Toxicol 54, 37-46.

Kavanagh, J.N., Redmond, K.M., Schettino, G., Prise, K.M., 2013. DNA double strand break repair: a radiation perspective. Antioxidants & redox signaling 18, 2458-2472.

Kawanishi, S., Ohnishi, S., Ma, N., Hiraku, Y., Oikawa, S., Murata, M., 2016. Nitrative and oxidative DNA damage in infection-related carcinogenesis in relation to cancer stem cells. Genes and environment : the official journal of the Japanese Environmental Mutagen Society 38, 26.

Kayser, Y., Blachucki, W., Dousse, J.C., Hoszowska, J., Neff, M., Romano, V., 2014. Laboratory-based micro-X-ray fluorescence setup using a von Hamos crystal spectrometer and a focused beam X-ray tube. The Review of scientific instruments 85, 043101.

Ki, Y., Kim, W., Kim, Y.H., Kim, D., Bae, J.S., Park, D., Jeon, H., Lee, J.H., Lee, J., Nam, J., 2017. Effect of Coenzyme Q10 on Radiation Nephropathy in Rats. Journal of Korean medical science 32, 757-763.

Korkmaz, A., Ahbab, M.A., Kolankaya, D., Barlas, N., 2010. Influence of vitamin C on bisphenol A, nonylphenol and octylphenol induced oxidative damages in liver of male rats. Food and chemical toxicology 48, 2865-2871.

Kunutsor, S.K., 2016. Gamma-glutamyltransferase-friend or foe within? Liver international : official journal of the International Association for the Study of the Liver 36, 1723-1734.

Laiakis, E.C., Mak, T.D., Anizan, S., Amundson, S.A., Barker, C.A., Wolden, S.L., Brenner, D.J., Fornace, A.J., Jr., 2014. Development of a metabolomic radiation signature in urine from patients undergoing total body irradiation. Radiation research 181, 350-361.

Li, X., Yin, P., Zhao, L., 2017. Effects of individual and combined toxicity of bisphenol A, dibutyl phthalate and cadmium on oxidative stress and genotoxicity in HepG 2 cells. Food and chemical toxicology 105, 73-81.

Ma-On, C., Sanpavat, A., Whongsiri, P., Suwannasin, S., Hirankarn, N., Tangkijvanich, P., Boonla, C., 2017. Oxidative stress indicated by elevated expression of Nrf2 and 8-OHdG promotes hepatocellular carcinoma progression. Med Oncol 34, 57.

Martinez, C.S., Piagette, J.T., Escobar, A.G., Martin, A., Palacios, R., Pecanha, F.M., Vassallo, D.V., Exley, C., Alonso, M.J., Miguel, M., Salaices, M., Wiggers, G.A., 2017. Aluminum exposure at human dietary levels promotes vascular dysfunction and increases blood pressure in rats: A concerted action of NAD(P)H oxidase and COX-2. Toxicology 390, 10-21.

Moon, M.K., Kim, M.J., Jung, I.K., Koo, Y.D., Ann, H.Y., Lee, K.J., Kim, S.H., Yoon, Y.C., Cho, B.J., Park, K.S., Jang, H.C., Park, Y.J., 2012. Bisphenol A impairs mitochondrial function in the liver at doses below the no observed adverse effect level. Journal of Korean medical science 27, 644-652.

Moon, S.J., Park, H.B., Yoon, S.Y., Lee, S.C., 2013. Urinary biomarkers for early detection of recovery in patients with acute kidney injury. Journal of Korean medical science 28, 1181-1186.

Morell-Quadreny, L., Romanenko, A., Lopez-Guerrero, J.A., Calabuig, S., Vozianov, A., Llombart-Bosch, A., 2011. Alterations of ubiquitylation and sumoylation in conventional renal cell carcinomas after the Chernobyl accident: a comparison with Spanish cases. Virchows Archiv 459, 307-313.

Nachman, R.M., Hartle, J.C., Lees, P.S., Groopman, J.D., 2014. Early Life Metabolism of Bisphenol A: A Systematic Review of the Literature. Current environmental health reports 1, 90-100.

Oktem, F., Ozguner, F., Yilmaz, H.R., Uz, E., Dundar, B., 2006. Melatonin reduces urinary excretion of N-acetyl-beta-D-glucosaminidase, albumin and renal oxidative markers in diabetic rats. Clinical and experimental pharmacology & physiology 33, 95-101.

Olea-Herrero, N., Arenas, M.I., Munoz-Moreno, C., Moreno-Gomez-Toledano, R., Gonzalez-Santander, M., Arribas, I., Bosch, R.J., 2014. Bisphenol-A induces podocytopathy with proteinuria in mice. Journal of cellular physiology 229, 2057-2066.

Ozyurt, H., Cevik, O., Ozgen, Z., Ozden, A.S., Cadirci, S., Elmas, M.A., Ercan, F., Goren, M.Z., Sener, G., 2014. Quercetin protects radiation-induced DNA damage and apoptosis in kidney and bladder tissues of rats. Free radical research 48, 1247-1255.

Perng, W., Watkins, D.J., Cantoral, A., Mercado-Garcia, A., Meeker, J.D., Tellez-Rojo, M.M., Peterson, K.E., 2017. Exposure to phthalates is associated with lipid profile in peripubertal Mexican youth. Environmental research 154, 311-317.

Pollock, T., deCatanzaro, D., 2014. Presence and bioavailability of bisphenol A in the uterus of rats and mice following single and repeated dietary administration at low doses. Reprod Toxicol 49, 145-154.

Preston, D.L., Sokolnikov, M.E., Krestinina, L.Y., Stram, D.O., 2017. Estimates of Radiation Effects on Cancer Risks in the Mayak Worker, Techa River and Atomic Bomb Survivor Studies. Radiation protection dosimetry 173, 26-31.

Prowle, J.R., Liu, Y.L., Licari, E., Bagshaw, S.M., Egi, M., Haase, M., Haase-Fielitz, A., Kellum, J.A., Cruz, D., Ronco, C., Tsutsui, K., Uchino, S., Bellomo, R., 2011. Oliguria as predictive biomarker of acute kidney injury in critically ill patients. Crit Care 15, R172.

Rendic, S., Guengerich, F.P., 2012. Summary of information on the effects of ionizing and non-ionizing radiation on cytochrome P450 and other drug metabolizing enzymes and transporters. Current drug metabolism 13, 787-814.

Rivas, A., Monteagudo, C., Heras-Gonzalez, L., Mariscal-Arcas, M., Lorenzo-Tovar, M.L., Olea-Serrano, F., 2016. Association of bisphenol A exposure with dietary quality indices in Spanish schoolchildren. Food and chemical toxicology 94, 25-30.

Serra, N., Rosales, R., Masana, L., Vallve, J.C., 2015. Simvastatin Increases Fibulin-2 Expression in Human Coronary Artery Smooth Muscle Cells via RhoA/Rho-Kinase Signaling Pathway Inhibition. PloS one 10, e0133875.

Shmarakov, I.O., Borschovetska, V.L., Blaner, W.S., 2017. Hepatic Detoxification of Bisphenol A is Retinoid-Dependent. Toxicological sciences 157, 141-155.

Souidi, M., Tissandie, E., Grandcolas, L., Grison, S., Paquet, F., Voisin, P., Aigueperse, J., Gourmelon, P., Gueguen, Y., 2006. Chronic contamination with 137cesium in rat: effect on liver cholesterol metabolism. International journal of toxicology 25, 493-497.

Tasdemir, M., Fucucuoglu, D., Ozman, O., Sever, L., Onal, B., Bilge, I., 2017. Silicate calculi, a rare cause of kidney stones in children. Pediatr Nephrol 32, 371-374.

Tiwari, D., Kamble, J., Chilgunde, S., Patil, P., Maru, G., Kawle, D., Bhartiya, U., Joseph, L., Vanage, G., 2012. Clastogenic and mutagenic effects of bisphenol A: an endocrine disruptor. Mutation research 743, 83-90.

Trasande, L., Attina, T.M., Trachtman, H., 2013. Bisphenol A exposure is associated with low-grade urinary albumin excretion in children of the United States. Kidney international 83, 741-748.

Tzatzarakis, M.N., Vakonaki, E., Kavvalakis, M.P., Barmpas, M., Kokkinakis, E.N., Xenos, K., Tsatsakis, A.M., 2015. Biomonitoring of bisphenol A in hair of Greek population. Chemosphere 118, 336-341.

Vafeiadi, M., Roumeliotaki, T., Myridakis, A., Chalkiadaki, G., Fthenou, E., Dermitzaki, E., Karachaliou, M., Sarri, K., Vassilaki, M., Stephanou, E.G., Kogevinas, M., Chatzi, L., 2016. Association of early life exposure to bisphenol A with obesity and cardiometabolic traits in childhood. Environmental research 146, 379-387.

Vahdati Hassani, F., Mehri, S., Abnous, K., Birner-Gruenberger, R., Hosseinzadeh, H., 2017. Protective effect of crocin on BPA-induced liver toxicity in rats through inhibition of oxidative stress and downregulation of MAPK and MAPKAP signaling pathway and miRNA-122 expression. Food and chemical toxicology 107, 395-405.

Valencia-Olvera, A.C., Moran, J., Camacho-Carranza, R., Prospero-Garcia, O., Espinosa-Aguirre, J.J., 2014. CYP2E1 induction leads to oxidative stress and cytotoxicity in glutathione-depleted cerebellar granule neurons. Toxicology in vitro 28, 1206-1214.

Vandenberg, L.N., Maffini, M.V., Sonnenschein, C., Rubin, B.S., Soto, A.M., 2009. Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. Endocrine reviews 30, 75-95.

Verreet, T., Verslegers, M., Quintens, R., Baatout, S., Benotmane, M.A., 2016. Current Evidence for Developmental, Structural, and Functional Brain Defects following Prenatal Radiation Exposure. Neural plasticity 2016, 1243527.

Willhite, C.C., Ball, G.L., McLellan, C.J., 2008. Derivation of a bisphenol A oral reference dose (RfD) and drinking-water equivalent concentration. Journal of toxicology and environmental health. Part B, Critical reviews 11, 69-146.

Yamaguchi, M., Kitamura, A., Oda, Y., Onishi, Y., 2014. Predicting the long-term (137)Cs distribution in Fukushima after the Fukushima Dai-ichi nuclear power plant accident: a parameter sensitivity analysis. Journal of environmental radioactivity 135, 135-146.

Yildiz, N., Barlas, N., 2013. Hepatic and renal functions in growing male rats after bisphenol A and octylphenol exposure. Human & experimental toxicology 32, 675-686.

Yoshikawa, Y., Yamasaki, A., Takatori, K., Suzuki, M., Kobayashi, J., Takao, M., Zhang-Akiyama, Q.M., 2015. Excess processing of oxidative damaged bases causes hypersensitivity to oxidative stress and low dose rate irradiation. Free radical research 49, 1239-1248.

Zhao, W., Robbins, M.E., 2009. Inflammation and chronic oxidative stress in radiation-induced late normal tissue injury: therapeutic implications. Current medicinal chemistry 16, 130-143.

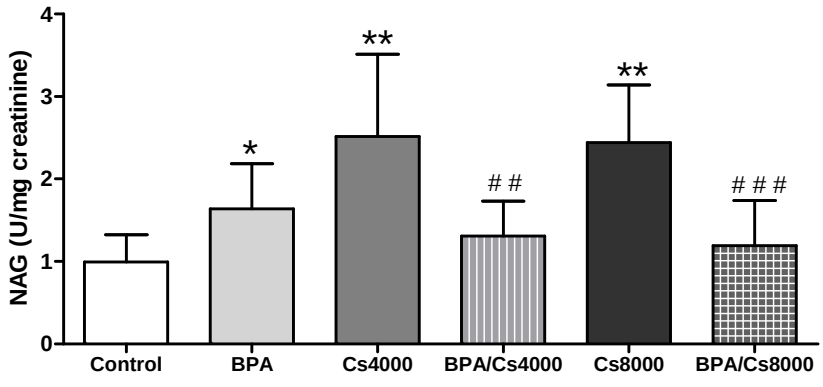

Figure 1. Urinary N-acetyl-β-D-glucosaminidase (NAG) levels in mice exposed to Bisphenol-A (BPA), Cs4000 or Cs8000, alone or in combination. Results are expressed as means ± S.D. Statistics: ANOVA and Bonferroni's test. *p<0.05 and **p<0.01 indicate significant differences versus control group. $^{\#\#}$p<0.01 and $^{\#\#\#}$p<0.001 indicate significant differences between combined exposure to BPA/^{137}Cs and exposure to ^{137}Cs alone.

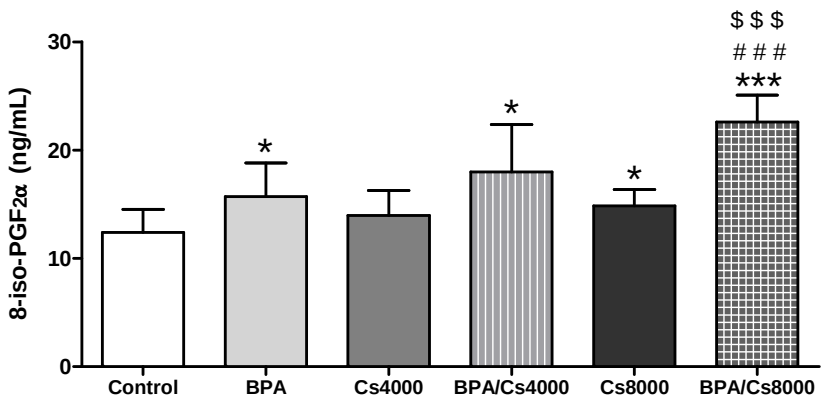

Figure 2. Urinary 8-iso-prostaglandin F2α (8-iso-$PGF_{2\alpha}$) levels in mice exposed to Bisphenol-A (BPA), Cs4000 or Cs8000, alone or in combination. Results are expressed as means ± S.D. Statistics: ANOVA and Bonferroni's test. *p<0.05 and ***p<0.001 indicate significant differences versus control group. $^{###}$p<0.001 indicates significant differences between BPA/Cs8000 and Cs8000 groups. $^{\$\$\$}$p<0.001 indicates significant differences between BPA/Cs8000 and BPA groups.

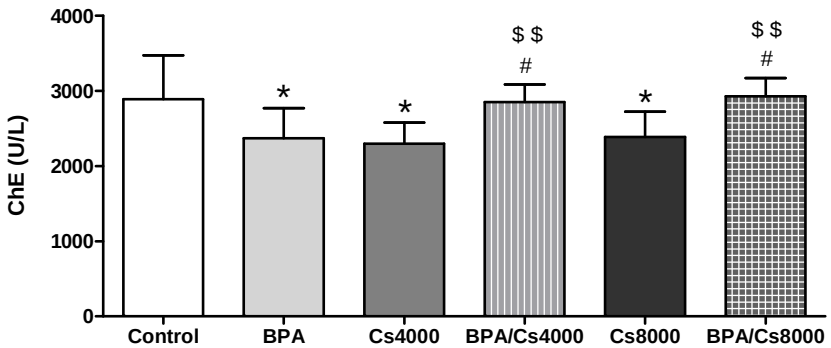

Figure 3. Serum Cholinesterase (ChE) levels in mice exposed to Bisphenol-A (BPA), Cs4000 or Cs8000, alone or in combination. Results are expressed as means ± S.D. Statistics: ANOVA and Bonferroni's test. *p<0.05 indicates significant differences versus control group. $^{\$\$}$p<0.01 indicates significant differences between combined exposure to BPA/^{137}Cs and exposure to BPA alone. $^{\#}$p<0.05 indicates significant differences between combined exposure to BPA/^{137}Cs and exposure to Cs4000 or Cs8000.

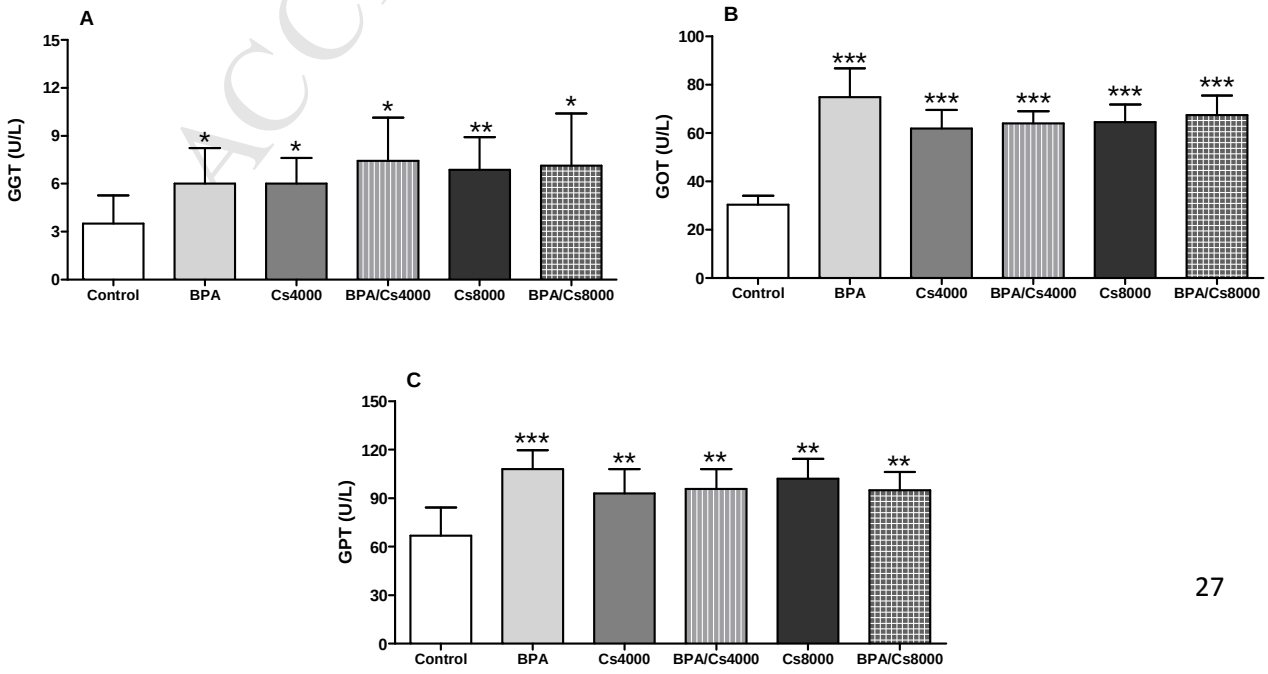

Figure 4 (A-C). Serum γ-glutamil-transferase (GGT) (A), glutamic oxaloacetic transaminase (GOT) (B) and glutamic pyruvic transaminase (GPT) (C) levels in mice exposed to Bisphenol-A (BPA), Cs4000 or Cs8000, alone or in combination. Results are expressed as means ± S.D. Statistics: ANOVA and Bonferroni's test (A-B), and Kruskal-Wallis and Dunn's test (C). *p<0.05, **p<0.01 and ***p<0.001 indicate significant differences versus controls.

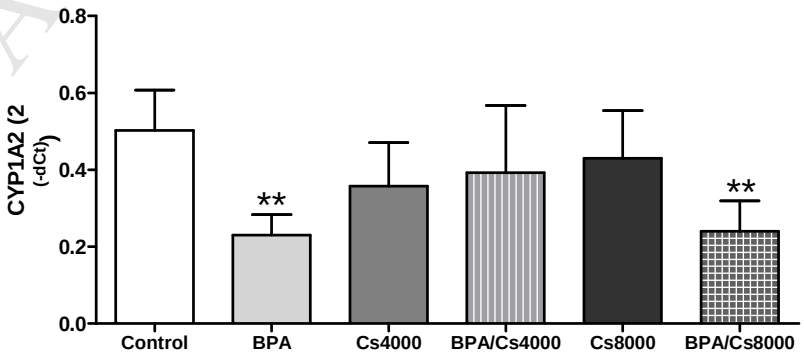

Figure 5. Hepatic CYP1A2 gene expression in mice treated with Bisphenol-A (BPA), Cs4000 or Cs8000, alone or in combination. Results are expressed as means ± S.D. Statistics: ANOVA and Bonferroni's test. **p<0.01 indicates significant differences versus control group.

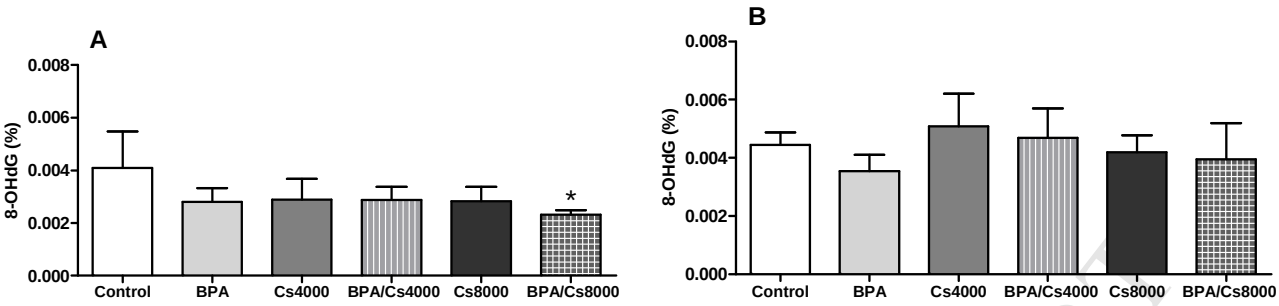

Fig. 6 (A-B). Percentages of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in hepatic (A) and renal (B) tissue in mice treated with Bisphenol-A (BPA), Cs4000 or Cs8000, alone or in combination. Results are expressed as means ± S.D. Statistics: ANOVA and Bonferroni's test. *$p<0.05$ indicates significant differences versus control group.

Table 1. Urinary parameters in mice exposed to Bisphenol-A (BPA), Cs4000 or Cs8000, alone or in combination

	Control	BPA	Cs4000	BPA/Cs4000	Cs8000	BPA/Cs8000
Urinary Volume (mL/24h) [1]	0,80 ± 0,28	0,40 ± 0,14 ***	0,43 ± 0,15 **	0,40 ± 0,18 **	0,39 ± 0,10 **	0,50 ± 0,14 **
Urea (mg/24h) [2]	0,37 ± 0,06	0,26 ± 0,11	0,44 ± 0,22	0,27 ± 0,14	0,46 ± 0,22	0,27 ± 0,09
Total protein (mg/24h) [1]	4,31 ± 1,52	5,37 ± 2,30	5,13 ± 2,09	4,34 ± 1,80	5,54 ± 2,23	3,83 ± 1,22
Uric acid (mg/24h) [2]	0,043 ± 0,007	0,042 ± 0,025	0,034 ± 0,019	0,125 ± 0,072 *	0,033 ± 0,017	0,050 ± 0,020
Lactate dehydrogenase (LDH) (U/24h/Kg) [2]	0,34 ± 0,11	0,25 ± 0,11	1,26 ± 0,44 ***	0,19 ± 0,08 *	0,53 ± 0,17	0,24 ± 0,13
γ-glutamil-transferase (GGT) (U/24h/Kg) [2]	3,15 ± 0,87	2,10 ± 1,04	4,64 ± 2,50	6,60 ± 1,79 *	3,14 ± 1,67	3,16 ± 1,12
Creatinine (mg/24h) [2]	0,41 ± 0,15	0,44± 0,27	0,27 ± 0,007	0,45 ± 0,24	0,37 ± 0,11	0,50 ± 0,14
Creatinine Clearance (mL/min) [1]	0,066 ± 0,029	0,058 ±0,020	0,048 ±0,023	0,072 ± 0,045	0,068 ± 0,034	0,080 ± 0,033

Results are expressed as means ± S.D.

Statistics:

[1] ANOVA and Bonferroni's test.

[2] Kruskal-Wallis and Dunn's test.

* Significantly different at $p<0.05$ with respect to the control group

** Significantly different at $p<0.01$ with respect to the control group

*** Significantly different at $p<0.001$ with respect to the control group

Table 2. Serum parameters in mice exposed to Bisphenol-A (BPA), Cs4000 or Cs8000, alone or in combination

	Control	BPA	Cs4000	BPA/Cs4000	Cs8000	BPA/Cs8000
Creatinine (mg/dL) [2]	0,41 ± 0,09	0,34 ± 0,05	0,52 ± 0,16	0,46 ± 0,05	0,49 ± 0,16	0,51 ± 0,10
Urea (mg/dL) [1]	55,00 ± 7,38	73,43 ± 8,06 ***	54,25 ± 6,99	43,13 ± 7,98 *	57,38 ± 7,69	47,00 ± 8,23 *
Total protein (g/L) [2]	73,44 ± 12,69	57,28 ± 3,06	68,65 ± 7,74	94,81 ± 8,93 *	80,53 ± 9,80	81,97 ± 7,66
Uric acid (mg/dL) [2]	4,88 ± 1,39	2,71 ± 0,54	8,19 ± 1,94 *	10,91 ± 1,85 **	8,66 ± 1,78 **	8,43 ± 1,09 *
Lactate dehydrogenase (LDH) (U/L) [1]	496,50 ± 125,70	365,50 ± 125,20	534,20 ± 141,30	418,90 ± 184,30	410,30 ± 105,10	513,10 ± 86,16
Alkaline Phosfatase (ALP) (U/L) [1]	155,10 ± 45,27	239,70 ± 16,06 ***	178,80 ± 23,01	131,70 ± 26,80	157,00 ± 35,96	128,90 ± 35,09

Results are expressed as means ± S.D.

Statistics:

[1] ANOVA and Bonferroni's test.

[2] Kruskal-Wallis and Dunn's test.

* Significantly different at $p<0.05$ with respect to the control group

** Significantly different at $p<0.01$ with respect to the control group

*** Significantly different at $p<0.001$ with respect to the control group

Highlights – Bullet points

- BPA and ^{137}Cs, induce renal and liver damage throughout direct or indirect oxidative stress parameters.
- When there is a BPA and ^{137}Cs co-exposure, it seems that there are compensatory mechanisms that may reverse the damage induced by each toxic itself.
- Hepatic expression of CYP1A2 decrease in mice exposed to BPA. However, no changes in the CYP1A2 expression has been detected when mice were exposed only to ionizing radiation.