gion of Argentina. Previous studies conducted by our research group showed the presence of flavonoids in the ethanolic extracts and their bioactivities in diverse models. Therefore, the objective of this work was to test the cytotoxic effect of both ethanolic extracts (NaE and CpE) against several human cancer cell lines. The extracts were tested on the following cell lines Caki-2 (kidney clear cell carcinoma), A549 (lung carcinoma), HT-29 (colon adenocarcinoma) and THP-1 (acute monocytic leukemia). Assays were extended to non-cancerous cells lines, the embryonic HEK-293 (kidney) and L-929 (fibroblast). Cells were seeded according to ATCC guidelines and incubated 24 h in humidified atmosphere with 5% CO_2 at 37ºC. Then were treated with NaE or CpE ranging from 10 to 200 mg/mL for 24 h and 48 h. Proliferative rates were assessed with XTT assay compared to untreated cells for determining the IC50. Values were analyzed following the cytotoxicity of the National Cancer Institute, IC50 ≤ 20 μg/mL: high, IC50 ranged from 21 to 200 μg/mL: moderate, IC50 from 201 to 500 μg/mL : weak and IC50 > 501 μg/mL : no cytotoxicity. Both extracts showed a similar bioactivity at 24 h, with a low dose increase in proliferation. However, when analyzed at 48 h, NaE exhibited an IC50 33.37 mg/mL ± 5.4 mg/mL for THP-1, and for non-cancer cell lines an IC50 of 90.10 ± 19.58 μg/mL for L929 fibroblast and 81.11 ± 15.45 μg/mL for HEK-293 embryo cell line. These preliminary results of cytotoxic activity in TPH-1 cell line by NaE encourage us to develop further studies in the identification of its bioactive compounds, as well as, on the underlying anticancer mechanism of action.

571. (269) **THE UV FILTER BENZOPHENONE 3 (BP3) ALTERS THE MIGRATION OF THE EXTRAVILLOUS TROPHOBLAST CELL LINE SWAN 71 VIA ANDROGEN RECEPTOR PATHWAY**

Abud Julián [1,2], Pagotto Romina [3], Zenclussen M. Laura [1,2], Galliani Valentina, Bollati-Fogolín, Mariela [3]; Rodríguez Horacio [1,2].

[1] Instituto de Salud y Ambiente del Litoral (UNL-CONICET), Santa Fe, Argentina. [2] Cátedra de Fisiología Humana (FBCB-UNL), Santa Fe, Argentina. [3] Unidad de Biología Celular (UBC), Institut Pasteur de Montevideo, Uruguay.

BP3 is one of the most commonly substances used in sunscreens and personal care products due to its UV blocking efficacy. Several *in vitro* and *in vivo* studies evidenced the ability of BP3 to act like an endocrine disrupting chemical. The present study focuses on the effect of BP3 on the migration capacity of human trophoblast cells (Swan 71 cell line) and the potential involvement of the androgen receptor (AR) pathway. We analyzed three different BP3 concentrations: a) BP3-2: the predicted no-effect concentration (2 µg/L), b) BP3-20: the concentration detected in the amniotic fluid (20 µg/L) in our previous studies and c) BP3-200: the plasma concentrations reported in humans (200 µg/L). We examined cell migration activity by scratch-wound healing assay, as well as mRNA relative expression levels of molecules of interest such as, AR, matrix metalloproteinase 2 (MMP2), inhibitor of MMP-2 (TIMP2) and laminin a4 (LAMA4). The three doses of BP3 reduced the area of wound closure after 24 h of exposure, evidencing reduced migration of Swan 71 cells when compared to the vehicle. Interestingly, BP3 induced an augmented expression of AR mRNA levels in all concentrations assayed, and of TIMP2 and LAMA4 only in BP3-2. MMP-2 did not show significant changes. In order to confirm whether BP3 acts via an AR-dependent pathway, we then analyzed BP3 effects with and without an AR inhibitor (Flutamide, 1 μM). When the cells were treated with BP3 in the presence of flutamide, the area of wound closure did not change after 24 h, clearly indicating that BP3 acts through a AR-dependent pathway. This was confirmed by the AR mRNA expression restoration in cells exposed to BP3 + flutamide. In conclusion, exposure to relevant doses of BP3 is enough to perturb the migration capability and the expression of AR mRNA levels of the trophoblast cell line Swan 71. These effects were reversed in the presence of an AR inhibitor indicating that BP3 could act via AR-dependent pathway.

572. (338) **AIRBORNE PARTICULATE MATTER EXPOSURE IMPAIRS LUNG REDOX METABOLISM INVOLVED IN TISSUE DAMAGE REPAIR MECHANISMS**

Sofía Reynoso[1], Timoteo Marchini[1], Mariana Garcés[1], Lourdes Cáceres[1], Agustina Freire[1], Valeria Calabró[1], Alejandro Berra[2], Pablo Evelson[1], Natalia Magnani[1]

[1]Universidad de Buenos Aires. CONICET. Instituto de Bioquímica y Medicina Molecular (IBIMOL), Facultad de Farmacia y Bioquímica. Ciudad de Buenos Aires, Argentina. [2]Universidad de Buenos Aires. Facultad de Medicina. Departamento de Patología

Is estimated that 91% of the world's population breathes polluted air leading to more than 7 million premature deaths per year. Airborne pollutants such as particulate matter (PM) are associated with enhanced health risk as they can trigger or aggravate several pulmonary diseases. Our aim was to assess if alterations in the lung oxidative metabolism initiated by toxicological mechanisms triggered after PM inhalation were associated with a delayed tissue injury repair. To characterize our model, BALB/c mice were exposed to filtered air (FA) or urban air (UA) from Buenos Aires City, in whole-body exposure chambers. Results showed that after 8 weeks of UA exposure, mice developed lung redox alterations and local inflammation without histological damage, therefore that was the time point selected to further evaluate the oxidative metabolism after a moderate lung injury induced by intratracheal instillation of 0.1 N hydrochloric acid (HCl). Pulmonary tissue was evaluated 5 days after HCl treatment. Tissue oxygen consumption was assessed as a whole lung metabolism marker, and the increase observed in mice breathing FA by HCl treatment, was not detected in HCl-mice exposed to UA (p<0.05). Interestingly, SOD activity showed the same trend (p<0.05), even though transcription factor Nrf2 expression was higher after the injury in the UA group (p<0.05). While no edema was observed in any group, local inflammation measured as total cell count in bronchoalveolar lavage (BAL), was significantly increased only after UA exposure and HCl instillation (p<0.05) compared to control values. Hence, mice breathing UA might not be able to modulate the redox metabolism involved in lung damage repair mechanisms. Our results highlight the importance of tissue healing mechanisms evaluation as valuable knowledge for developing adequate therapeutic approaches aiming to ensure restoration of normal alveolar architecture required for proper lung function.

573. (352) **CHRONIC EXPOSURE TO URBAN AIR POLLUTION IN BUENOS AIRES CITY INDUCES OXIDATIVE STRESS AND INFLAMMATION IN MICE OLFACTORY BULB**

Freire, Agustina[1]; Garcés, Mariana[1]; Cáceres, Lourdes[1]; Magnani, Natalia[1]; Marchini, Timoteo[1]; Martinefski, Manuela[2]; Tripodi, Valeria[2]; Berra, Alejandro[3]; Alvarez, Silvia[1]; Calabró, Valeria[1]; Evelson, Pablo[1].

[1] Universidad de Buenos Aires. CONICET. Instituto de Bioquímica y Medicina Molecular (IBIMOL), Facultad de Farmacia y Bioquímica.Ciudad de Buenos Aires, Argentina.

[2] Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de tecnología farmacéutica.

[3] Universidad de Buenos Aires.Facultad de Medicina. Departamento de patología.

Previous reports indicate that central nervous system (CNS) is a target of air pollution, causing tissue damage and functional alterations. Oxidative stress and neuroinflammation have been pointed out as possible mechanisms mediating these effects. The aim of this work was to study the chronic effects of urban air pollution on mice olfactory bulb (OB), focusing on oxidative stress and inflammation markers. Male 8-week-old BALB/c mice were exposed to filtered air (FA, control) or urban air (UA) inside whole-body chambers, located in a highly polluted area of Buenos Aires city, for up to 4 weeks. Reduced glutathione levels (GSH) were decreased by 75% after 4 w of exposure to UA (p<0.05). In accordance with these results, an increase in glutathione reductase activity was found at the same time point (p<0.05 vs. FA). Total superoxide dismutase (SOD) activity, including a differential analysis of its cytosolic and the mitochondrial isoforms, Cu/Zn-SOD and Mn-SOD respectively, were determined. Cu/Zn-SOD activity showed an initial decrease after 1 w of UA exposure compared to FA, and a subsequent increase of 50% at week 4 (p<0.05), while no changes were observed for Mn-SOD activity.

Also, protein expression of NOX4 and NOX2 (p47^{phox} subunit), both NOX isoforms commonly found increased in inflammatory processes, were augmented in UA group, after 1 and 4 w, respectively ($p<0.05$). Moreover, inducible nitric oxide synthase (iNOS) protein expression levels were found augmented for all exposure times evaluated, in UA compared to FA ($p<0.05$), suggesting an inflammatory process in OB, which is in accordance with previous results, where astrocyte activation was found in this tissue. Taken together, UA exposure showed an increase in oxidants production and inflammation markers in OB that might lead to tissue oxidative stress and an inflammatory response in this tissue. These data indicate that oxidative stress may play a key role in CNS damage mechanisms triggered by air pollution.

574. (353) **NEONATAL EXPOSURE TO A GLYPHOSATE-BASED HERBICIDE AND ITS EFFECTS ON THE OVIDUCT OF EWE LAMBS**

Lourdes Lovera[1], Ramiro Alarcón[1], Paola Inés Ingaramo[1], Ana Laura Alegre[1], Oscar Edgardo Rivera[2], Gisela Haydee Dioguardi[2], Mónica Muñoz-de-Toro[1], Enrique Hugo Luque[1]

[1]Instituto de Salud y Ambiente del Litoral (ISAL-CONICET), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. [2]Instituto de Investigación Agropecuaria, Ambiente y Salud (IIPAAS), Facultad de Ciencias Agrarias, Universidad Nacional de Lomas de Zamora.

Glyphosate-based herbicides (GBH) are the most widely used agrochemicals raising concern about its effects on animal and human health. We have previously reported that neonatal exposure of ewe lambs to a low dose of GBH induced permanent changes in the uterus and ovaries. Moreover, neonatal exposure of female rats to GBH decreased the fertility rate. Here, our aim was to assess the long-term effects of neonatal oral exposure to GBH in the oviduct of ewe lambs. To achieve this, ewe lambs were orally exposed from postnatal day (PND) 1 to PND14 to vehicle (n=6) or the reference dose of a GBH (1 mg glyphosate/Kg/day) (n=4). Since oviduct is target of estrogen, we also tested the effect of a gonadotrophic stimulus (pFSH, Follitropin 50 mg/day from PND41 to PND43) in GBH treated ewe lambs: vehicle+pFSH (n=6) and GBH+pFSH (n=4). At PND45, the isthmus and ampulla of oviducts were collected, paraffin embedded or stored at -80°C until mRNA extraction. The thickness of the myosalpinx was determined by digital analysis of picrosirius-hematoxylin-stained oviduct in transversal sections using FIJI software. The expression of Ki67 (as cell proliferation marker), and of α-alpha smooth muscle actin (α-SMA) were evaluated by immunohistochemistry. The gene expression of steroid receptors (ERα and PR) was evaluated by RT-PCR. The thickness of the myosalpinx and cell proliferation showed no differences between the experimental groups. Moreover, no alterations in the expression of α-SMA or steroid receptors mRNA was found. These results demonstrate that neonatal exposure to a low dose of GBH alone or with a gonadotrophic treatment does not alter the development of the oviduct myosalpinx, which allows us to assume that function of oviduct (gametes transport and fertilization of the oocyte) might not be affected following GBH exposure. Nevertheless, more studies are needed to conclude that the decrease in female fertility due to GBH treatment is not due to oviduct alterations.

575. (361) **EFFECT OF HEXACHLOROBENZENE (HCB) AND PROTEIN X OF THE HEPATITIS B VIRUS (HBV) ON LIVER CELL GROWTH DYSREGULATION**

Lucia Coli[1], Verónica Mathet[2], Ezequiel Ridruejo[1], Emiliano Gentile[2], Melisa Kurtz[3], Noelia Miret[1], José Oubiña[2] y Laura Alvarez[1]

[1]Laboratorio de Efectos Biológicos de Contaminantes Ambientales (LEBCA), Departamento de Bioquímica Humana, Facultad de Medicina, UBA, [2] Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPAM), UBA-CONICET, [3]Laboratorio de Bio-Toxicología Ambiental, Escuela de Ciencia y Tecnología, Universidad Nacional de San Martín.

Chronic hepatitis B and exposure to persistent organic pollutants (POPs) can lead to cellular hepatocarcinoma (HCC), the most common liver tumor. HBV DNA encodes transactivator X (HBx). HCB is a COP promoter of hepatic preneoplastic foci. We have shown that HCB deregulates cell growth in rat liver and HepG2 cells, involving TGF-β1, a reversed effect with an Rβ1 inhibitor.

Objectives: To analyze in vitro 2 models of HCC generation -associated to HCB or to the expression of HBx- and in vivo the hepatic and angiogenic promoter HCB effect in Balb/c nude mice inoculated with HepG2. M&M: the HCB effect on PCNA (Western blot)/TGF-β1 (RT-PCR) was studied in vitro in: 1.1) Huh-7: HCB at various doses (0.005, 0.05, 0.5 and 5 μM) and times (15, 30, 60, 90 and 120 min); 1.2) Huh-7 transiently transfected with HBx; 2) HepG2.2.15 (with stable expression of HBV) and 3) EA-hy926 (endothelial). In 1.2, 2 and 3 5 μM HCB, 24h was used. Mice: HCB i.p. (0.3 and 3 mg/kg), and were inoculated with HepG2. Were evaluated: a) PCNA, b) TGF-β1, c) N°. of tumor areas, d) histology (H&E) and e) N°. of vessels/mm^2. Results: In Huh-7, TGF-β1 increased (20%, $p<0.05$; 69% and 78%, $p<0.01$, with 0.05, 0.5 and 5 μM HCB, respectively) and PCNA (45% and 60%, $p<0.01$, with 0.5 and 5 μM HCB, respectively). In Huh-7 / HBx, PCNA and TGF-β1 increased 66% and 71% ($p<0.01$). In Huh-7 / HBx and 5 μM HCB, PCNA increased 120% ($p<0.001$). In HepG2.2.15 PCNA was overexpressed 76% ($p<0.001$). In EA-hy926, PCNA (29%, $p<0.05$) and TGF-β1 (43%, $p<0.01$) increased. In mice, PCNA (39%; $p<0.01$), TGF-β1 (48%; $p<0.01$), preneoplastic areas (320%; $p<0.001$) and vascularization (35%; $p<0.01$).

Conclusions: HCB promotes preneoplastic cell proliferation and angiogenesis in nude mice inoculated with HepG2, while HCB and HBx induce in vitro cell proliferation associated with the increase of TGF-β1 in the examined lines, a proliferative effect increased to that promoted by the HCB being able to participate in the induction of HCC.

576. (368) **EXPOSURE TO THE ENDOCRINE DISRUPTOR HEXACHLOROBENZENE PROMOTES BREAST TUMOR GROWTH AND METASTASIS IN A HER2-POSITIVE BREAST CANCER MODEL**

Lorena Vanesa Zárate[1], Jorge Armando Peña Agudelo[2], Noelia Victoria Miret[1], Alejandro Javier Nicola Candia[2], Florencia Ana Chiappini[1], Carolina Andrea Pontillo[1], Marianela Candolfi[2], Andrea Silvana Randi[1].

[1]Universidad de Buenos Aires, Facultad de Medicina, Departamento de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Paraguay 2155, Piso 5, (CP 1121), Buenos Aires, Argentina. [2]Universidad de Buenos Aires, Facultad de Medicina, Instituto de Investigaciones Biomédicas, Laboratorio de Inmunoterapia Antitumoral, Paraguay 2155, Piso 10, (CP1121), Buenos Aires, Argentina.

The death rate from breast cancer has increased significantly in the last 25 years. Exposure to endocrine-disrupting chemicals, such as pesticides, has been postulated as a risk factor in this disease. We have previously demonstrated that the organochlorine Hexachlorobenzene (HCB) has stimulated breast tumor progression, favoring cell migration, invasion and angiogenesis. HCB is a weak ligand of the Aromatic Hydrocarbon Receptor (AhR), a transcription factor related to tumor and vascular development. Furthermore, we have reported that HCB acts as an endocrine disruptor in the mammary gland and uterus in different animal models. Estrogens play a fundamental role in the etiology of breast cancer, whose actions are mediated by their α and β isoform receptors. However, the role of ER-β is not entirely clear, with evidence of a reduction in proliferation and angiogenesis in ERα-positive breast cancer cell lines, while in triple-negative cells (RE-/RP-/HER2-) performs the opposite function. G-protein-coupled ER (GPR30) mediates the action of estrogens in breast tumors and cancer-associated fibroblasts, leading to tumor progression. In the present study, we have examined the action of HCB (0.03, 0.3 and 3 mg/kg body weight, bw) in a LM3 syngeneic breast cancer mouse model (ER-/PR-/HER2+). Our results indicated that HCB induces an increase in tumor weight and volume at 3 mg/kg bw ($p<0.05$) and promotes a rise in the metastasis number in the lung at 0.3 mg/kg bw ($p<0.001$). In the tumor tissue, HCB enhances the protein expression of AhR and Vascular Endothelial Growth Factor (VEGF) at all doses tested (Western Blot;