FERRO - IRON

- Dai XY, Zhu SY, Li MZ, Talukder M, Zhao Y, Li JL.

Potential Role of Lycopene in the Inhibition of Di(2-ethylhexyl) Phthalate-Induced Ferroptosis in Spleen Via Modulation of Iron Ion Homeostasis. *ACS Pharmacol Transl Sci.* 2021 4(1):386-395.

Di(2-ethylhexyl) phthalate (DEHP) is a synthetic chemical and widely used as a plasticizer. Humans can be exposed to DEHP through direct contact or environmental contamination. Lycopene (Lyc) has been discussed as a potential effector in the prevention and therapy of various diseases. 140 male mice were assigned into control, vehicle control, Lyc (5 mg/kg BW/d), DEHP (500 and 1000 mg/kg BW/d, respectively), and DEHP + Lyc groups and treated with an oral gavage that lasted 28 d. The ultrastructural results showed that DEHP induced pathological changes and mitochondrial injuries. We further revealed that DEHP exposure destroyed the Fe2+ imbalance homeostasis and, consequently, increases of lipid peroxidation and inhibition of cysteine/glutamate antiporter, all of which were involved in the process of ferroptsis. Moreover, the supplementation of Lyc significantly inhibited the ferroptsis changes mentioned above. Altogether, these results indicated that DEHP exposure triggered splenic cell death via ferroptosis; meanwhile, they also shed new evidence on a potential clue for the intervention and prevention of DEHP-related diseases.

- Gao M, Xu Y, Chang X, Song Z.

Fe-Mn oxide modified biochar decreases phthalate uptake and improves grain quality of wheat grown in phthalate-contaminated fluvo-aquic soil. *Chemosphere*. 2021 May;270:129428. doi: 10.1016/j.chemosphere.2020.129428.

We used a pot experiment to investigate the effectiveness of 0.5, 1.0, and 2.0% biochar (BC) or iron-manganese oxide modified biochar (FMBC) additions on the biomass, enzyme activity, and grain quality of wheat plants grown in dibutyl phthalate (DBP) and di-(2-ethylhcxyl) phthalate (DEHP) polluted fluvo-aquic soils, as well as the bioavailability of DBP and DEHP. BC and FMBC applications significantly reduced DBP and DEHP accumulation in grains, which enhanced the content of starch and protein-related enzyme, thereby improving yield, and starch and protein content in wheat grains and increasing the content of minerals including Fe, Mn, K and Ca. Molecular docking assays showed that DBP and DEHP could bind to starch synthase (GBSS) through hydrogen bonds and intermolecular forces, which may have hindered the entry of substrates or occupied the binding sites of the reactants, thus inhibiting the activity of GBSS. In addition, FMBC treatment had a better inhibitory effect on the phytotoxicity of DBP and DEHP on wheat grain than BC treatment. This result might be attributed to the fact that FMBC has more functional groups and porous structure, and larger specific surface area. In summary, these findings contribute to our understanding of the mechanism of phthalate phytotoxicity, which may help us prevent/reduce it in the future.

- Li B, Jiang Y, Wang T, He X, Ma L, Li B, Li Y.

Effect of atrazine on accumulation of iron via the iron transport proteins in the midbrain of SD rats. *Sci Total Environ (Basel).* 2021 Aug 1;780:146666. doi: 10.1016/j.scitotenv.2021.

Atrazine (ATR), a widely used herbicide that belongs to the triazine class, has detrimental effects on several organ systems. It has also been shown that ATR exposure results in dopaminergic neurotoxicity. However, the mechanism of herbicides causing ferroptosis in neurons is less concerned. So, the present study aimed to investigate the effects of long-term oral exposure to ATR

on ferroptosis in adult male rats. In this study, we show that there was a dose-dependent increase in the concentration of iron in the midbrain. Simultaneously, the expression of tyrosine hydroxylase (TH) and Synuclein (α-syn) were altered by the ATR. We carried out miRNA profiling brain tissue in order to identify factors that mediate ferroptosis. We also found that the mRNA and protein expression of the transferrin receptor (TFR), divalent metal transporter 1 (DMT1), hephaestin (HEPH), and ferroportin 1 (Fpn1) in the midbrain were affected by ATR. Based on the current results and previously published data, it is clear that exposure of adult male rats to high doses of ATR leads to iron loading in the midbrain. The long-term adverse effects of ATR on the midbrain have a special relevance after exposure.

Yin X, Zeb R, Wei H, Cai L.
Acute exposure of di(2-ethylhexyl) phthalate (DEHP) induces immune signal regulation and ferroptosis in oryzias melastigma. *Chemosphere*. 2021 Feb;265:129053. doi: 10.1016/j.chemosphere.2020.129053.

The plasticizer di (2-ethylhexyl) phthalate (DEHP) is becoming increasingly abundant throughout the global environment as plastic pollution becomes highly severe, especially in the ocean. The adverse effects of DEHP have garnered increasing concern as they are recognized as endocrine disruptors. However, information on the effects of DEHP in marine organisms remains limited. In this study, acute toxic effects on marine medaka (Oryzias melastigma) following DEHP exposure were investigated. Transcriptome analysis was performed on the livers of medaka exposed to DEHP for 6 and 24 h. Results showed that 1595 genes were affected in all the analyzed specimens, and several genes expressed variably according to sex. Some pathways associated with immunity, metabolism, and endocrine system were significantly enriched, with the complement system appearing to be the most affected immune pathway. Pathway enrichment indicated that, under acute DEHP exposure, the immune response of females tended to be more sensitive than that of males. In addition, ferroptosis occurred in response to DEHP exposure, which resulted in an enrichment of the ferroptosis pathway along with iron overload, an increase in malondialdehyde (MDA) and lipid peroxidation (LPO) content, and a decrease in glutathione (GSH) levels. These results indicate that a form of cell death characterized by iron-dependence occurred following DEHP exposure, but the underlying mechanism requires further analysis. This study implies that DEHP can alter some molecular regulation patterns within a short period and induce cell death through ferroptosis.

– Xu Y, Song Z, Chang X, Guo Z, Gao M. .
Effects of Fe-Mn oxide-modified biochar composite applications on phthalate esters (PAEs) accumulation in wheat grains and grain quality under PAEs-polluted brown soil. *Ecotoxicol Environ Saf*. 2021 Jan 15;208:111624. doi: 10.1016/j.ecoenv.2020.111624.

Phthalate esters (PAEs), such as dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP), are used extensively as additives and plasticizers, and have become ubiquitous in the environment. PAEs in the soil could have adverse effects on crop plants as well as humans via accumulations in food chain. Thus, it is important to explore strategies to reduce the bioavailability of phthalate esters. We investigated the effects of Fe-Mn oxide-modified biochar composite (FMBC) applications on the quality of wheat grown in DBP- and DEHP-polluted brown soil. The application of FMBC and biochar (BC) increased the wheat grain biomass by 9.71-223.01% and 5.40-120.15% in the DBP-polluted soil, and 10.52-186.21% and 4.50-99.53% in the DEHP-spiked soil in comparison to the controls. All FMBC treatments were better than the BC treatments, in terms of decreasing DBP and DEHP bioavailability for the wheat grains. The activities of the glutamine synthetase and glutamic-pyruvic transaminase in the flag leaves at the filling stage and of granule-bound starch synthase, soluble starch synthase, and adenosine diphosphate-glucose

pyrophosphorylase in the grains at maturity increased significantly with increases in either the BC or FMBC applications. This, in turn, increased the starch, protein, and amino acid content in the wheat grains. Compared with the BC treatment, the FMBC amendment induced only slight increases in the aforementioned factors. This study offers novel insights into potential strategies for decreasing PAEs bioavailability in soil, with potential positive implications for crop quality and environmental health improvements.

- Wei S, Qiu T, Yao X, Wang N, Jiang L, Jia X, Tao Y, Wang Z, Pei P, Zhang J, Zhu Y, Yang G, Liu X, Liu S, Sun X.
Arsenic induces pancreatic dysfunction and ferroptosis via mitochondrial ROS-autophagy-lysosomal pathway. *J Hazard Mater.* 2020 Feb 15;384:121390. doi: 10.1016/j.jhazmat.2019.121390.

Chronic arsenic exposure is a significantly risk factor for pancreatic dysfunction and type 2 diabetes (T2D). Ferroptosis is a newly identified iron-dependent form of oxidative cell death that relies on lipid peroxidation. Previous data have indicated that ferroptosis is involved in various diseases, including cancers, neurodegenerative diseases, and T2D. However, the concrete effect and mechanism of ferroptosis on pancreatic dysfunction triggered by arsenic remains unknown. In this study, we verified that ferroptosis occurred in animal models of arsenic-induced pancreatic dysfunction through assessing proferroptotic markers and morphological changes in mitochondria. In vitro, arsenic caused execution of ferroptosis in a dose-dependent manner, which could be significantly reduced by ferrostatin-1. Additionally, arsenic damaged mitochondria manifested as diminishing of mitochondrial membrane potential, reduced cytochrome c level and production of mitochondrial reactive oxygen species (MtROS) in MIN6 cells. Using the Mito-TEMPO, we found the autophagy level and subsequent ferroptotic cell death induced by arsenic were both alleviated. With autophagy inhibitor chloroquine, we further revealed that ferritin regulated ferroptosis through the MtROS-autophagy pathway. Collectively, NaAsO2-induced ferroptotic cell death is relied on the MtROS-dependent autophagy by regulating the iron homeostasis. Ferroptosis is involved in pancreatic dysfunction triggered by arsenic, and arsenic-induced ferroptosis involves MtROS, autophagy, ferritin.

- Abarikwu SO, Wokoma AFS, Mgbudom-Okah CJ, Omeodu SI, Ohanador R.
Effect of Fe and Cd Co-Exposure on Testicular Steroid Metabolism, Morphometry, and Spermatogenesis in Mice. *Biol Trace Elem Res*. 2019 Jul;190(1):109-123.

The mechanism of testicular toxicity of simultaneous multiple exposures to metals is poorly understood. Previous studies reported that the toxic effect of cadmium (Cd) is modified by tissue concentration of iron (Fe). Using the mice (Mus musculus) model in the present study, we demonstrated that combined Cd (25 mg kg-1 bw) and Fe (100 mg kg-1 bw) treatment increased both Cd and Fe testicular concentrations much more than separate exposures to either of the metals. Intratesticular Cd and Fe concentrations were inversely correlated ($r = - 0.731$, $p < 0.05$) on administration of Fe but not on combined exposure to both metals when they were positively correlated (versus Cd; $r = 0.793$, versus Fe; $r = 0.779$, $p < 0.05$). Additionally, Cd + Fe treatment increased testicular lipid peroxidation and depleted intratestesticular testosterone, cholesterol and glutathione concentrations much more than their separate treatment. This was also associated with decreased activity of the germ cell marker, testicular lactate dehydrogenase, and increased testicular myeloperoxidase activity. These changes resulted in decreased seminiferous epithelial height, tubular diameter, germ cell (spermatogonia, spermatocytes, and spermatids) numbers, and severe tissue damage. In conclusion, Cd + Fe intake have synergistic toxic effects on testicular steroid formation and spermatogenesis due to the high testicular concentrations of both metals.

- Abarikwu SO, Oruitemeka S, Uwadileke IA, Omeodu SI, Okoye NF, Mgbudom-Okah CJ, Ohanador R.
Oral administration of cadmium depletes intratesticular and epididymal iron levels and inhibits lipid peroxidation in the testis and epididymis of adult rats. *J Trace Elem Med Biol.* 2018 Jul;48:213-223.

Cadmium (Cd)-induced tissue injury depends on the accumulated Cd which differentially affects endogenous iron (Fe). To investigate this, adult rats were treated by oral gavage with Cd (50 mg/kg body wt.) once a week for 15, 30 and 60 days and sacrificed a day after last administration. After the 15th and 30th day of treatment, Cd had no effect on thiobarbituric acid reactive substances (TBARS) and endogenous Fe levels but exhibited anti-androgenic effects ($p < 0.05$) and caused histological damages. At day 60, Cd was accumulated by 156.30% and 364.77% above control values at concentrations that decreased endogenous Fe levels by 46.41% and 50.31% in the testis and epididymis respectively. The histological damages were characterized by decreased tubular diameter, damage to the epithelium leading to loss of tubular germ cells and absent of spermatozoa in the epididymal lumen. Although myeloperoxidase activities were increased, TBARS levels were found to decrease significantly at day 60 in the serum, testis and epididymis suggesting that the histological damages were not caused by lipid peroxidation. Furthermore, TBARS correlated negatively with Cd in the testis ($r = -0.251$, $p < 0.05$) and epididymis ($r = -0.286$, $p < 0.05$); Fe correlated positively with TBARS in the testis ($r = +0.217$, $p < 0.05$) and Cd correlated negatively with Fe in the testis ($r = -0.461$, $p < 0.05$) and epididymis ($r = -0.109$, $p < 0.05$). The antioxidant enzymes, superoxide dismutase and glutathione peroxidase were also decreased in the gonads after 60 days Cd treatment. Overall, anti-androgenic effects and histo-pathological changes are early indicators of direct effects of Cd and occur before decrease in TBARS which is secondarily related to the modifying of Fe contents.

- Rashid H, Sharma S, Beigh S, Ahmad F, Raisuddin S.
Bisphenol A-Induced Endocrine Toxicity and Male Reprotoxicopathy are Modulated by the Dietary Iron Deficiency. *Endocr Metab Immune Disord Drug Targets*. 2018;18(6):626-636.

Introduction: Bisphenol A (BPA) is suspected to cause hormonal imbalance in humans. Dietary factors are known to bring changes in hormonal profile. In order to study chemico-biological interaction of iron deficiency on toxicity outcome of BPA exposure, we studied the modulatory effects of iron deficiency on the hormone levels in rats chronically-exposed to BPA.
Methods: Weanling rats maintained on normal and iron-deficient diets were exposed to low level of BPA at 0, 1, 5 and 10 ppm for six months through drinking water. The serum levels of thyroidstimulating hormone (TSH), testosterone, progesterone and estradiol were measured in the animals by enzyme-linked immunosorbent assay kit. Histopathology was performed to check the pathological changes in gonads.
Results: No significant change was observed in TSH, progesterone and estradiol levels at 1 and 5 ppm BPA. However, at 10 ppm BPA a significant increase in TSH level was observed in the animals maintained on an iron-deficient diet of either sex. BPA caused a significant change in testosterone level even at 5 and 10 ppm doses in animals of either sex. However, in male rats 1 ppm dose also showed a significant effect in the animals maintained on iron deficient diet. Changes in the histoarchitecture of the testes at high dose of BPA (10 ppm) were more remarkable in anemic rats.
Conclusion: These results suggest that iron deficiency has no generalized effect on hormonal levels in BPA-treated animals and trends indicate a more remarkable effect in male animals at hormonal and tissue levels.

- Kordas K, Roy A, López P, García-Vargas G, Cebrián ME, Vera-Aguilar E, Rosado JL. **Iron and Zinc Supplementation Does Not Impact Urinary Arsenic Excretion in Mexican School Children**. *J Pediatr*. 2017 Jun;185:205-210

Objective: To examine the role of iron and zinc in arsenic excretion and metabolism in children.
Study design: An analysis of urinary arsenic (UAs) concentrations from a double-blind randomized trial originally testing the efficacy of iron and zinc for lowering blood lead levels in children. A 2 × 2 factorial design was used, with children randomized individually, stratified by sex and classroom, to receive 30?mg ferrous fumarate (n?=?148), 30?mg zinc oxide (n?=?144), iron and zinc together (n?=?148), or placebo (n?=?151). Of the 602 children enrolled, 527 completed the 6-month treatment, and 485 had both baseline and final UAs values. The baseline total UAs concentration ranged from 3.2 to 215.9?μg/L.
Results: At baseline, children in the highest tertile of serum ferritin concentration had higher excretion of dimethylarsinic acid (DMA; 1.93?±?0.86%; P?<?.05), but lower excretion of monomethylarsonic acid (-0.91?±?0.39%; P?<?.05), compared with children in the lowest tertile. In an intention-to-treat analysis, iron had no effect on arsenic methylation or UAs excretion, but children receiving zinc had lower %DMA in urine (-1.7?±?0.8; P?<?.05).
Conclusions: Iron and zinc status are not related to arsenic metabolism in children, and supplementation with these minerals has limited application in lowering arsenic concentrations.

- Rotter I, Kosik-Bogacka DI, Dołęgowska B, Safranow K, Kuczyńska M, Laszczyńska M. **Analysis of the relationship between the blood concentration of several metals, macro- and micronutrients and endocrine disorders associated with male aging.** *Environ Geochem Health.* 2016 Jun;38(3):749-61.

Beyond 30 years of age, men experience a decline in the production of testosterone, yet only a few develop late-onset hypogonadism. This study was designed to determine the relationship between blood concentrations of metals, macro- and micronutrients and age-related testosterone deficiency and associated hormonal changes in aging men. The research involved 313 men aged 50-75 years. We used ELISA to determine the concentrations of total testosterone (TT), free testosterone (FT), estradiol (E2), dehydroepiandrosterone sulfate (DHEAS) and sex hormone-binding globulin (SHBG). We calculated free androgen index (FAI). With the use of emission spectrometry in inductively coupled argon plasma, we determined the whole-blood concentrations of lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As) and tungsten (W), as well as serum concentrations of magnesium (Mg), iron (Fe), calcium (Ca), copper (Cu), zinc (Zn), selenium (Se), chromium (Cr), manganese (Mn) and molybdenum (Mo). The study showed no relationship between TT and FT and the concentrations of metals. Men with TT deficiency had significantly lower concentrations of Mg and Fe and increased Mn. Men with FT deficiency had higher W and Cr levels and lower Fe. Assessing the correlation between the concentrations of hormones, SHBG and FAI, and the concentration of metals and macro- and microelements in the blood of the men, we found positive correlations between the concentrations of TT-Mg, TT-Fe, TT-Mo, FT-Fe, E2-As, SHBG-Mn, FAI-W, FAI-As, FAI-Zn and FAI-Ca, and negative correlations between the concentrations of TT-Mn, FT-Cd, FT-Cr, E2-Hg, E2-Cr, SHBG-W, SHBG-As, SHBG-Zn, SHBG-Ca, FAI-Pb and FAI-Mn. Positive correlations between As and E2 and between As and FAI may suggest a lack of association between this metal and hypogonadism in people not exposed to excess As levels. Our research indicates a positive relationship between the concentrations of Mg, Fe and Zn and endocrine system in aging men, in contrast to Mn and Cr. Toxic metals (Cd, Pb) seemed to negatively affect the level of bioavailable testosterone. In persons not exposed to As, As does not contribute late-onset hypogonadism. Heavy metals (Pb, Cd, Hg and W) may contribute to a lower concentration of DHEAS. The role of W in men with LOH was found to be ambiguous, as on the one hand its concentration was higher in men with FT deficiency, and on the other hand it positively correlated

with FAI, which in turn indirectly indicates testosterone availability. Copper and selenium do not seem to play any significant role in the occurrence of TT deficiency in aging men.

- Erkekoglu P, Arnaud J, Rachidi W, Kocer-Gumusel B, Favier A, Hincal F.
The effects of di(2-ethylhexyl) phthalate and/or selenium on trace element levels in different organs of rats. J *Trace Elem Med Biol.* 2015 Jan;29:296-302.

Di(2-ethylhexyl)phthalate (DEHP), a widely used plasticizer for synthetic polymers, is known to have endocrine disruptive potential, reproductive toxicity, and induces hepatic carcinogenesis in rodents. Selenium (Se) is a component of several selenoenzymes which are essential for cellular antioxidant defense and for the functions of mammalian reproductive system. The present study was designed to investigate the effects of DEHP exposure on trace element distribution in liver, testis, and kidney tissues and plasma of Se-deficient and Se-supplemented rats. Se deficiency was produced by feeding 3-week old Sprague-Dawley rats with ≤0.05mg Se/kg diet for 5 weeks, and supplementation group were on 1mg Se/kg diet. DEHP treated groups received 1000mg/kg dose by gavage during the last 10 days of feeding period. Se, zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) levels were measured by inductively coupled plasma mass spectrometry (ICP-MS). Se supplementation caused significant increases in hepatic, renal, and testicular Se levels. With DEHP exposure, plasma Se and Zn, kidney Se, Cu and Mn levels were significantly decreased. Besides, liver Fe decreased markedly in all the DEHP-treated groups. Liver and kidney Mn levels decreased significantly in DEHP/SeD group compared to both DEHP and SeD groups. These results showed the potential of DEHP exposure and/or different Se status to modify the distribution pattern of essential trace elements in various tissues, the importance of which needs to be further evaluate