FOLATO - FOLATE

- Abuawad A, Bozack AK, Saxena R, Gamble MV.

Nutrition, One-Carbon Metabolism and Arsenic Methylation. *Toxicology*. 2021 Apr 24:152803. doi: 10.1016/j.tox.2021.152803.

Exposure to arsenic (As) is a major public health concern globally. Inorganic As (InAs) undergoes hepatic methylation to form monomethyl (MMAs)- and dimethyl (DMAs)-arsenical species, facilitating urinary As elimination. MMAsIII is considerably more toxic than either InAsIII or DMAsV, and a higher proportion of MMAs in urine has been associated with risk for a wide range of adverse health outcomes. Efficiency of As methylation differs substantially between species, between individuals, and across populations. One-carbon metabolism (OCM) is a biochemical pathway that provides methyl groups for the methylation of As, and is influenced by folate and other micronutrients, such as vitamin B12, choline, betaine and creatine. A growing body of evidence has demonstrated that OCM-related micronutrients play a critical role in As methylation. This review will summarize observational epidemiological studies, interventions, and relevant experimental evidence examining the role that OCM-related micronutrients have on As methylation, toxicity of As, and risk for associated adverse health-related outcomes. There is fairly robust evidence supporting the impact of folate on As methylation, and some evidence from case-control studies indicating that folate nutritional status influences risk for As-induced skin lesions and bladder cancer. However, the potential for folate to be protective for other As-related health outcomes, and the potential beneficial effects of other OCM-related micronutrients on As methylation and risk for health outcomes are less well studied and warrant additional research.

- Gao X, Zhang C, Zheng P, Dan Q, Luo H, Ma X, Lu C.

Arsenic suppresses GDF1 expression via ROS-dependent downregulation of specificity protein 1. *Environ Pollut*. 2021 Feb 15;271:116302. doi: 10.1016/j.envpol.2020.116302.

Inorganic arsenic, an environmental contaminant, has adverse health outcomes. Our previous studies showed that arsenic causes abnormal cardiac development in zebrafish embryos by downregulating Dvr1/GDF1 expression and that folic acid protects against these effects. However, the mechanism by which arsenic represses Dvr1/GDF1 expression remains unknown. Herein, we demonstrate that specificity protein 1 (Sp1) acts as a transcriptional activator of GDF1. Arsenic treatment downregulated Sp1 at both the mRNA and protein level and its downstream targets GDF1 and SIRT1. Chromatin immunoprecipitation analysis showed that the occupancy of Sp1 on the GDF1 or SIRT1 promoter was significantly reduced in response to arsenite. Further investigation showed that Sp1 overexpression inhibited the arsenic-mediated decrease in GDF1 and SIRT1, while Sp1 knockdown had the opposite effect. We found that expression of the oxidative adaptor p66shc was inversely related to that of SIRT1 and that the binding of SIRT1 to the p66shc promoter was sharply attenuated by arsenite treatment. SIRT1 overexpression attenuated p66shc expression but enhanced GDF1 protein expression, while SIRT1 depletion exerted the opposite effect. Both the antioxidants N-acetylcysteine and folic acid reversed the arsenic-mediated repression of Sp1, GDF1 and SIRT1. Moreover, wild-type

p66shc overexpression enhanced the arsenic-mediated repression of Sp1, GDF1 and SIRT1, which was accompanied by an increase in intracellular reactive oxygen species (ROS) levels, while both overexpression of a dominant negative p66shcSer36Ala mutant and deficiency in p66shc reversed these effects. Taken together, our results revealed that arsenic suppresses GDF1 expression via the ROS-dependent downregulation of the Sp1/SIRT1 axis, which forms a negative feedback loop with p66shc to regulate oxidative stress. Our findings reveal a novel molecular mechanism underlying arsenic toxicity and provide new insight into the protective effect of folic acid in arsenic-mediated toxicity.

- Hu C, Liu M, Wan T, Tang L, Sun B, Zhou B, Lam JCW, Lam PKS, Chen L.

Disturbances in Microbial and Metabolic Communication across the Gut-Liver Axis Induced by a Dioxin-like Pollutant: An Integrated Metagenomics and Metabolomics Analysis. *Environ Sci Technol*. 2021 Jan 5;55(1):529-537. doi: 10.1021/acs.est.0c06884.

To determine how the aryl hydrocarbon receptor (AhR) signaling acts along the gut-liver axis, we employed an integrated metagenomic and metabolomic approach to comprehensively profile the microbial and metabolic networks. Adult zebrafish were exposed to a model agonist of the AhR: polychlorinated biphenyl (PCB) 126. The metagenomic analysis showed that PCB126 suppressed microbial activities related to primary bile acid metabolism in male intestines. Accordingly, a suite of primary bile acids consistently showed higher concentrations, suggesting that bacterial conversion of primary bile acids was blocked. PCB126 also disturbed bacterial metabolism of bile acids in female intestines, as revealed by higher concentrations of primary bile acids (e.g., chenodeoxycholic acid) and activation of the nuclear farnesoid X receptor signaling. In addition, PCB126 exposure impaired the metabolism of various essential vitamins (e.g., retinol, vitamin B6, and folate). Degradation of vitamin B6 by bacterial enzymes was inhibited in male intestines, resulting in its intestinal accumulation. However, PCB126 suppressed the bacterial metabolism of vitamins in female intestines, causing systematic deficiency of essential vitamins. Overall, we found that PCB126 exposure dysregulated gut microbial activities, consequently interrupting bile acid and vitamin metabolism along the gut-liver axis. The findings provided an insight of the AhR action in microbe-host metabolic communication related to PCBs.

- Saxena R, Liu X, Navas-Acien A, Parvez F, LoIacono NJ, Islam T, Uddin MN, Ilievski V, Slavkovich V, Balac O, Graziano JH, Gamble MV.

Nutrition, one-carbon metabolism and arsenic methylation in Bangladeshi adolescents.. *Environ Res*. 2021 Apr;195:110750. doi: 10.1016/j.envres.2021.

Background: Over 57 million people in Bangladesh are chronically exposed to arsenic-contaminated drinking water. Ingested inorganic arsenic (InAs) undergoes hepatic methylation generating monomethyl- (MMAs) and dimethyl- (DMAs) arsenic species in a process that facilitates urinary As (uAs) elimination. One-carbon metabolism (OCM), a biochemical pathway that is influenced by folate and vitamin B12, facilitates the methylation of As. OCM also supports nucleotide and amino acid synthesis, particularly during periods of rapid growth such as adolescence. While folate supplementation increases As methylation and lowers blood As (bAs) in adults, little data is available for adolescents.

Objectives: To examine the associations between OCM-related micronutrients and As methylation in Bangladeshi adolescents chronically exposed to As-contaminated drinking water.
Methods: We conducted a cross-sectional study of 679 Bangladeshi adolescents, including 320 boys and 359 girls aged 14-16 years. Nutritional status was assessed by red blood cell (RBC) folate, plasma folate, plasma B12 and homocysteine (Hcys). Arsenic-related outcomes included blood arsenic (bAs), urinary arsenic (uAs), and urinary arsenic metabolites expressed as a percentage of total urinary As: %InAs, %MMAs, %DMAs.
Results: Boys had significantly lower B12, higher Hcys, higher bAs, higher uAs, higher %MMAs, and a trend toward lower RBC folate compared to girls. Therefore, regression analyses controlling for water As and BMI were sex stratified. Among girls, RBC folate was inversely associated with bAs, plasma B12 was inversely associated with uAs, and plasma Hcys was inversely associated with %MMA. Among boys, plasma folate was inversely associated with %InAs and positively associated with %DMA, RBC folate was inversely associated with %InAs and positively associated with %MMA, while Hcys was positively associated with %InAs.
Conclusions: These findings suggest that associations between OCM nutritional status, bAs, and distribution of As metabolites in adolescents are similar to previously reported observations in adults and in children. The As methylation findings are statistically significant among boys but not among girls; this may be related to estrogen which more strongly influences OCM in females. The inverse association between Hcys and %MMA in girls is somewhat unexpected given that Hcys is known to be an indicator of impaired OCM and low folate/B12 in adults. Overall, these results indicate that the associations between OCM-related micronutrients and arsenic methylation in adolescents are generally similar to prior findings in adults, though these associations may differ by sex. Additionally, these findings suggest that more investigation into the role of Hcys in adolescent physiology is needed, perhaps particularly for girls. Additional studies are needed to evaluate the impact of OCM and As methylation on As-related adverse health outcomes (such as cancer and cardiovascular disease) in people exposed to As during adolescence.

- Bozack AK, Howe CG, Hall MN, Liu X, Slavkovich V, Ilievski V, Lomax-Luu AM, Parvez F, Siddique AB, Shahriar H, Uddin MN, Islam T, Graziano JH, Gamble MV.

Betaine and choline status modify the effects of folic acid and creatine supplementation on arsenic methylation in a randomized controlled trial of Bangladeshi adults. *Eur J Nutr*. 2020 Sep 11 doi: 10.1007/s00394-020-02377-z.

Purpose: Methylation of ingested inorganic arsenic (InAs) to monomethyl- (MMAs) and dimethyl-arsenical species (DMAs) facilitates urinary arsenic elimination. Folate and creatine supplementation influenced arsenic methylation in a randomized controlled trial. Here, we examine if baseline status of one-carbon metabolism nutrients (folate, choline, betaine, and vitamin B12) modified the effects of FA and creatine supplementation on changes in homocysteine, guanidinoacetate (GAA), total blood arsenic, and urinary arsenic metabolite proportions and indices.
Methods: Study participants (N = 622) received 400 or 800 μg FA, 3 g creatine, 400 μg FA + 3 g creatine, or placebo daily for 12 weeks.
Results: Relative to placebo, FA supplementation was associated with greater mean increases in %DMAs among participants with betaine concentrations below the median than those with levels above the median (FDR < 0.05). 400 μg FA/day was associated with a greater decrease

in homocysteine among participants with plasma folate concentrations below, compared with those above, the median (FDR < 0.03). Creatine treatment was associated with a significant decrease in %MMAs among participants with choline concentrations below the median (P = 0.04), but not among participants above the median (P = 0.94); this effect did not significantly differ between strata (P = 0.10).
Conclusions: Effects of FA and creatine supplementation on arsenic methylation capacity were greater among individuals with low betaine and choline status, respectively. The efficacy of FA and creatine interventions to facilitate arsenic methylation may be modified by choline and betaine nutritional status.
Clinical trial registration: Clinical Trial Registry Identifier: NCT01050556, U.S. National Library of Medicine, https://clinicaltrials.gov ; registered January 15, 2010.

- Desai G, Millen AE, Vahter M, Queirolo EI, Peregalli F, Mañay N, Yu J, Browne RW, Kordas K.

Associations of dietary intakes and serum levels of folate and vitamin B-12 with methylation of inorganic arsenic in Uruguayan children: Comparison of findings and implications for future research. *Environ Res.* 2020 Oct;189:109935. doi: 10.1016/j.envres.2020.109935.

Background: In the human body, inorganic arsenic (iAs) is methylated via the one-carbon cycle to form monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). Lower proportions of iAs and MMA, and higher proportions of DMA in urine indicate efficient methylation; formation of DMA is thought to detoxify iAs and MMA. Studies on folate, vitamin B-12 and iAs methylation yield mixed findings, depending on whether folate and vitamin B-12 were assessed from diet, supplements, or using a blood biomarker.
Objective: First, to compare the associations of serum concentrations and estimated intake of folate and vitamin B-12 with indicators of iAs methylation. Second, to highlight the implications of these different B-vitamin assessment techniques on the emerging evidence of the impact of dietary modifications on iAs methylation.
Methods: The study was conducted among ~7-year-old children from Montevideo, Uruguay. Serum folate and vitamin B-12 levels were measured on the Horiba ABX Pentra 400 analyzer; urinary arsenic was measured using High-Performance Liquid Chromatography on-line with Inductively Coupled Plasma Mass Spectrometry. Dietary intakes were assessed using the average of two 24-h dietary recalls. Linear regressions assessed the associations of serum levels, and dietary intakes of folate (n = 237) and vitamin B-12 (n = 217) with indicators of iAs methylation. Models were adjusted for age, sex, body mass index, total urinary arsenic, and rice intake.
Results: Serum folate and vitamin B-12 levels were above the adequacy threshold for 99% of the participants. No associations were observed between serum folate, serum vitamin B-12, or vitamin B-12 intake and iAs methylation. Folate intake was inversely associated with urinary %MMA [β (95% confidence interval): -1.04 (-1.89, -0.18)].
Conclusion: Additional studies on the role of B-vitamins in iAs methylation are needed to develop a deeper understanding of the implications of assessing folate and vitamin B-12 intake compared to the use of biomarkers. Where possible, both methods should be employed because they reflect different exposure windows and inherent measurement error, and if used individually, will likely continue to contribute to lack of consensus.

- Hsueh YM, Lin YC, Chung CJ, Huang YL, Hsieh RL, Huang PT, Wu MY, Shiue HS, Chien SN, Lee CY, Lin MI, Mu SC, Su CT.

Combined effect of polymorphisms of MTHFR and MTR and arsenic methylation capacity on developmental delay in preschool children in Taiwan. *Arch Toxicol.* 2020 Jun;94(6):2027-2038.

Polymorphisms of methylenetetrahydrofolate reductase (MTHFR) and methionine synthase (MTR) are related to cognitive dysfunction and mental disability. These genes, along with folate and vitamin B12 levels, are regulators of one-carbon metabolism, which synthesizes S-adenosylmethionine (SAM) as a methyl donor for arsenic methylation. The aim of this study was to explore whether polymorphisms of MTHFR and MTR influence arsenic methylation capacity and plasma folate and vitamin B12 levels and if these influences cause developmental delay in preschool children. A total of 178 children with developmental delay and 88 without developmental delay were recruited from August 2010 to March 2014. A high-performance liquid chromatography-hydride generator and atomic absorption spectrometer were used to determine urinary arsenic species. Plasma folate and vitamin B12 concentrations were measured by SimulTRAC-SNB radioassay. Polymorphisms of MTHFR C677T, MTHFR A1298C, and MTR A2756G were examined by polymerase chain reaction and restriction fragment length variation. The results show that MTHFR C677T C/T and T/T genotypes had a lower risk of developmental delay than the C/C genotype (odds ratio [OR] = 0.47; 95% confidence interval, 0.26-0.85). Subjects with the MTHFR C677T C/C genotype had significantly lower plasma folate and vitamin B12 levels than those with the MTHFR C677T C/T and T/T genotype. The MTHFR C677T C/C genotype combined with high total urinary arsenic and poor arsenic methylation capacity indices significantly increased the OR of developmental delay in a dose-response manner. This is the first study to show the combined effect of MTHFR C677T genotype and poor arsenic methylation capacity on developmental delay.

- Kim DJ, Venkataraman A, Jain PC, Wiesler EP, DeBlasio M, Klein J, Tu SS, Lee S, Medzhitov R, Iwasaki A.

Vitamin B12 and folic acid alleviate symptoms of nutritional deficiency by antagonizing aryl hydrocarbon receptor. *Proc Natl Acad Sci U S A.* 2020 Jul 7;117(27):15837-15845. doi: 10.1073/pnas.2006949117

Despite broad appreciation of their clinical utility, it has been unclear how vitamin B12 and folic acid (FA) function at the molecular level to directly prevent their hallmark symptoms of deficiency like anemia or birth defects. To this point, B12 and FA have largely been studied as cofactors for enzymes in the one-carbon (1C) cycle in facilitating the de novo generation of nucleotides and methylation of DNA and protein. Here, we report that B12 and FA function as natural antagonists of aryl hydrocarbon receptor (AhR). Our studies indicate that B12 and FA bind AhR directly as competitive antagonists, blocking AhR nuclear localization, XRE binding, and target gene induction mediated by AhR agonists like 2,3,7,8-tetrachlorodibenzodioxin (TCDD) and 6-formylindolo[3,2-b]carbazole (FICZ). In mice, TCDD treatment replicated many of the hallmark symptoms of B12/FA deficiency and cotreatment with aryl hydrocarbon portions of B12/FA rescued mice from these toxic effects. Moreover, we found that B12/FA deficiency in mice induces AhR transcriptional activity and accumulation of erythroid progenitors and that it may do so in an AhR-dependent fashion. Consistent with these results,

we observed that human cancer samples with deficient B12/FA uptake demonstrated higher transcription of AhR target genes and lower transcription of pathways implicated in birth defects. In contrast, there was no significant difference observed between samples with mutated and intact 1C cycle proteins. Thus, we propose a model in which B12 and FA blunt the effect of natural AhR agonists at baseline to prevent the symptoms that arise with AhR overactivation.

- Linnenkamp BDW, Raskin S, Esposito SE, Herai RH.

A comprehensive analysis of AHRR gene as a candidate for cleft lip with or without cleft palate. *Mutat Res*. 2020 Jul-Sep;785:108319. doi: 10.1016/j.mrrev.2020.108319.

Cleft lip and palate (CL/P) is among the most common congenital malformations and affects 1 in 700 newborns. CL/P is caused by genetic and environmental factors (maternal smoking, alcohol or drug use and others). Many genes and loci were associated with cleft lip/palate but the amount of heterogeneity justifies identifying new causal genes and variants. AHRR (Aryl-Hydrocarbon Receptor Repressor) gene has recently been related to CL/P however, few functional studies analyze the genotypephenotype interaction of AHRR with CL/P. Several studies associate the molecular pathway of AHRR to CL/P which indicates this gene as a functional candidate in CL/P etiology.
Methods: Systematic Literature Review was performed using PUBMED database with the keywords cleft lip, cleft palate, orofacial cleft, AHRR and synonyms. SLR resulted in 37 included articles.
Results: AHRR is a positional and functional candidate gene for CL/P. In silico analysis detected interactions with other genes previously associated to CL/P like ARNT and CYP1A1. AHRR protein regulates cellular toxicity through TCDD mediated AHR pathway. Exposure to TCDD in animal embryos is AHR mediated and lead to cleft palate due to palate fusion failure and post fusion rupture. AHRR regulates cellular growth and differentiation, fundamental to lip and palatogenesis. AHRR decreases carcinogenesis and recently a higher tumor risk has been described in CL/P patients and families. AHRR is also a smoking biomarker due to changed methylation sites found in smokers DNA although folate intake may partially revert these methylation alterations. This corroborates the role of maternal smoking and lack of folate supplementation as risk factors for CL/P.
Conclusion: This research identified the importance of AHRR in dioxin response and demonstrated an example of genetic and environmental interaction, indispensable in the development of many complex diseases.

- Na L, Q B, Xiumei Z, Lingzi Z, Deqin H, Xuanxuan Z, Huanhuan G, Yuan L, Xiujuan C.

Research into the intervention effect of folic acid on arsenic-induced heart abnormalities in fetal rats during the periconception period. *BMC Cardiovasc Disord*. 2020 Mar 17;20(1):139. doi: 10.1186/s12872-020-01418-z.

Background: The incidence of CHD is the highest among birth defects and is increasing year to year. CHD seriously harms the health of infants and young children and presents a large economic burden to families and society. The pathogenesis of CHD and preventive measures are the focus of current research. Our research aimed to explore the intervention effect of folic acid on heart abnormalities resulting from sodium arsenic ($NaAsO_2$) exposure during the periconception period.

Methods: Sixty 35-day-old female SD rats were randomly divided into 5 groups with 12 rats in each group. Group A was the control group. The rats were given distilled water and ordinary chow. The rats in group B were given distilled water containing 75 mg/L NaAsO2 and ordinary chow. The rats in groups C, D, and E were given distilled water containing 75 mg/L NaAsO2 and chow containing 0.53 mg/kg, 5.3 mg/kg, and 10.6 mg/kg folic acid, respectively. The general condition of the embryos and the histopathology of the embryonic hearts were examined. The acetylation levels of histone H3K9 in heart tissues and the expression levels of Mef2C (which is related to heart development) were observed.
Results: The embryo weight and placental weight of groups B-E were significantly lower than those of group A ($P < 0.05$). The heart malformation rate of the fetal rats in groups B-E was significantly higher than that of the fetal rats in group A ($P < 0.05$). We found that the level of H3K9 acetylation in fetal rat cardiomyocytes in groups B-E was significantly higher than that in group A ($P < 0.05$) and that the level of H3K9 acetylation in groups C-E was lower than that in group B ($P < 0.05$). The mRNA level of Mef2C in fetal rat cardiomyocytes in group B-E was significantly higher than that in group A ($P < 0.05$), and the mRNA level of Mef2C in groups C-E was significantly lower than that in group B ($P < 0.05$).
Conclusion: Supplementation with folic acid during the periconception period can interfere with the toxic effects of arsenic on the heart. The mechanism may be that lowering the acetylation levels of histone H3K9 in heart tissues leads to decreased expression levels of Mef2C, which may play a protective role in heart development in fetal rats.

- Oulhote Y, Lanphear B, Braun JM, Webster GM, Arbuckle TE, Etzel T, Forget-Dubois N, Seguin JR, Bouchard MF, MacFarlane A, Ouellet E, Fraser W, Muckle G.

Gestational Exposures to Phthalates and Folic Acid, and Autistic Traits in Canadian Children. *Environ Health Perspect*. 2020; 128(2):27004. doi: 10.1289/EHP5621.

Background: The etiology of autism spectrum disorder is poorly understood. Few studies have investigated the link between endocrine-disrupting chemicals and autistic traits. We examined the relationship between gestational phthalates and autistic traits in 3- to 4-y-old Canadian children. We also investigated potential effect modification by sex and folic acid supplementation.
Methods: We enrolled 2,001 women>18yearswomen>18years of age during the first trimester of pregnancy between 2008 and 2011 from 10 cities in Canada. At 3-4 years of age, 610 children underwent neuropsychological assessments including the Social Responsiveness Scale-II (SRS-2) as a measure of autistic traits and social impairment. We measured 11 phthalate metabolites in maternal first trimester urine samples and assessed folic acid supplementation from reported intakes. We estimated covariate-adjusted differences in SRS-2 *T*-scores with a doubling in phthalate concentrations in 510 children with complete data.
Results: Mean total SRS *T*-score was 45.3 (SD=6.1SD=6.1). Children with higher gestational exposure to mono-*n*-butyl (MBP) and mono-3-carboxypropyl (MCPP) concentrations exhibited significantly higher total SRS *T*-scores, indicating greater overall social impairment, as well as higher scores on subdomains, indicating deficits in social cognition, social communication, social motivation, and restricted interests/repetitive behaviors. A doubling in MBP or MCPP concentrations was associated with 0.6 (95% CI: 0.1, 1.0) and 0.5 (95% CI: 0.1, 0.8) higher total SRS *T*-scores. Associations were consistently and significantly stronger in boys (β_{MBP}=1.0βMBP=1.0; 95% CI: 0.4, 1.6; n=252n=252) compared with girls (β_{MBP}=0.1βMBP=0.1; 95% CI: −0.6-0.6, 0.7; n=258n=258) and among children who had

lower prenatal folic acid supplementation (<400μg/d<400μg/d) (βMBP=1.3βMBP=1.3; 95% CI: 0.4, 2.3; n=59n=59) compared with those who had adequate folic acid supplementation (≥400μg/d≥400μg/d) (βMBP=0.4βMBP=0.4; 95% CI: −0.1-0.1, 0.8; n=451n=451).
Conclusions: Higher gestational concentrations of some phthalate metabolites were associated with higher scores of autistic traits as measured by the SRS-2 in boys, but not girls; these small size effects were mitigated by first trimester-of-pregnancy folic acid supplementation.

- Pi X, Qiao Y, Wang C, Li Z, Liu J, Wang L, Jin L, Ren A.

Concentrations of organochlorine pesticides in placental tissue are not associated with risk for fetal orofacial clefts. *Reprod Toxicol*. 2020 98: 99-106.

Previous epidemiological studies have shown that prenatal exposure to organochlorine pesticides (OCPs) entails a variety of adverse impacts on fetal health, but it is not yet known whether it is associated with risk for orofacial clefts (OFCs). This study of 103 fetuses or newborns with a diagnosis of OFCs (cases) and 103 healthy newborns without malformations (controls) examined whether prenatal exposure to OCPs, as indicated by their concentrations in placental tissue, is a risk factor for OFCs. No differences were found in the median concentrations of OCPs between cases and controls, with exception of o,p'-dichlorodiphenyldichloroethylene, o,p'-dichlorodiphenyldichloroethane, and total o,p'-dichlorodiphenyltrichloroethane (DDTs), whose concentrations were higher in controls than in cases (Ps < 0.05). Although higher concentrations of placental δhexachlorocyclohexane and isodrin were found to be associated with decreased risk for OFCs in logistic regression, no association was observed in the Bayesian kernel machine regression, a novel statistical model in analyzing exposure mixtures. Women who reported periconceptional folic acid supplementation had lower placental concentrations of DDTs than women who did not. In conclusion, no association between levels of OCPs in placental tissue and risk for OFCs was observed in this population. Supplementation with folic acid may help decrease the levels of DDTs in placental tissue, but further studies are needed to confirm this unexpected finding.

- Yin J, Hong X, Ma L, Liu R, Bu Y.

Non-targeted metabolomic profiling of atrazine in Caenorhabditis elegans using UHPLC-QE Orbitrap/MS. *Ecotoxicol Environ Saf*. 2020 Dec 15; 206:111170. doi: 10.1016/j.ecoenv.2020.111170.

The widespread use of the herbicides Atrazine (ATR) has been raised attention due to its ubiquitous occurrence in the environment. As an endocrine disruptor, ATR causes reproductive, immune, nervous system toxicity in biota. In this study, we aimed to investigate metabolic profile characteristics and potential metabolic biomarker that reflects specific damage in toxic effect after ATR exposure. Hence, a metabolomics study was performed to determine the significantly affected metabolites and the reproduction and locomotion of C. elegans were investigated. Mediation analysis was used to evaluate the mediating effect of metabolites on association between ATR exposure and toxic effect. ATR (≥0.04 mg/L) caused the significant dose dependent reduction of brood size and locomotion behavior, however, the body length and width were significantly decreased only in 40 mg/L group. These results

suggesting that brood size, head thrashes and body bends are more sensitive indictor to assessment ATR toxicity in C. elegans. Meanwhile, metabolomics analysis revealed that ATR exposure can induce metabolic profiles significant alterations in C. elegans. We found that 9 metabolites significantly increased and 18 metabolites significantly decreased, such as phosphatidylcholine, GMP, CDP-choline, neopterin etc. Those alteration of metabolites were mainly involved in the pathways: glycerophospholipid metabolism, glycolysis/gluconeogenesis, folate biosynthesis, glycine, serine and threoninemetabolism, pyrimidine and purine metabolism. Overall, these changes are signs of possible oxidative stress and ATP synthesis disruption modification. Mediation analysis showed a significant indirect effect of ATR exposure on brood size, via 7,8-dihydroneopterin 2',3'-cyclic-p, and phosphatidylcholine might mediate association between ATR exposure and body bends, suggesting that 7,8-dihydroneopterin 2',3'-cyclic-p and phosphatidylcholine might be potentially specificity marker for brood size and body bend respectively. This preliminary analysis investigates metabolic characteristics in C. elegans after ATR exposure, helping to understand the pathways involved in the response to ATR exposure and provide potential biomarkers for the safety evaluation of ATR.

- Yin S, Wei J, Wei Y, Jin L, Wang L, Zhang X, Jia X, Ren A.

Organochlorine pesticides exposure may disturb homocysteine metabolism in pregnant women. *Sci Total Environ*. 2020 Mar 15;708:135146. doi: 10.1016/j.scitotenv.2019.135146.

Maternal exposure to organochlorine pesticides (OCPs) has an adverse impact on maternal and fetal health, and excessive homocysteine is related to a variety of adverse pregnancy outcomes. Biomimetic studies suggest that OCPs interfere with folate-dependent pathways, but little evidence is available from studies with human subjects. This study explored whether exposure to OCPs interferes with the metabolism of homocysteine, which is folate dependent. A total of 313 pregnant women at 12-20 weeks gestation were recruited in Shanxi province, China, from 2014 to 2015. Plasma concentrations of 20 OCPs, including dichlorodiphenyltrichloroethane and metabolites (DDTs), hexachlorobenzene (HCB), and hexachlorocyclohexanes (HCHs), were analyzed by gas chromatography-mass spectrometry. Blood folate concentrations were analyzed by microbiological assay, and plasma homocysteine concentrations were determined by enzyme-linked immunosorbent assay. Information on demographics, lifestyle behaviors, and folic acid supplementation was collected by in-person interview. Of the women, 99% reported having taken folic acid supplements. Results of a logistic regression analysis showed that higher plasma levels of OCPs were associated with increased odds of higher plasma homocysteine after adjustment for potential confounding factors. Positive correlations were observed between plasma OCPs and plasma homocysteine concentrations: HCB ($r = 0.176$, $p = 0.002$), β-HCH ($r = 0.172$, $p = 0.002$), ρ,ρ'-DDE ($r = 0.132$, $p = 0.020$), ρ,ρ'-DDD ($r = 0.161$, $p = 0.004$), and o,ρ'-DDT ($r = 0.144$, $p = 0.011$). Plasma concentrations of OCPs were negatively correlated with red blood cell (RBC) folate in the low-RBC-folate subgroup, but the correlations were not statistically significant. A positive correlation was observed between OCPs and homocysteine in the low-RBC-folate subgroup. These findings suggest that OCPs may disturb the folate-dependent homocysteine metabolism pathway.

- Bommarito PA, Xu X, González-Horta C, Sánchez-Ramirez B, Ballinas-Casarrubias L, Luna RS, Pérez SR, Ávila JEH, García-Vargas GG, Del Razo LM, Stýblo M, Mendez MA,

Fry RC.
One-carbon metabolism nutrient intake and the association between body mass index and urinary arsenic metabolites in adults in the Chihuahua cohort. *Environ Int*. 2019 123: 292-300.

Background: Exposure to inorganic arsenic (iAs) via drinking water is a serious global health threat. Various factors influence susceptibility to iAs-associated health outcomes, including differences in iAs metabolism. Previous studies have shown that obesity is associated with iAs metabolism. It has been hypothesized that this association can be explained by confounding from nutritional factors involved in one-carbon metabolism, such as folate or other B vitamins, whose intake may differ across BMI categories and is known be associated with iAs metabolism. However, no studies have explored whether this association is confounded by nutritional factors.
Methods: We investigated the relationship between body mass index (BMI) and the distribution of urinary arsenic species in a cross-sectional cohort of 1166 adults living in Chihuahua, Mexico from 2008 to 2013. Nutrient intake related to one-carbon metabolism, including folate, vitamin B2, and vitamin B12, was assessed using a food frequency questionnaire developed for Mexican populations. Multivariable linear regression was used to estimate the association between BMI and the distribution of urinary arsenic metabolites. Effect modification by drinking water iAs level and sex was also examined.
Results: After adjusting for potential confounders, including age, educational attainment, smoking, alcohol consumption, seafood consumption, water iAs, and sex, BMI was negatively associated with the proportion of urinary inorganic arsenic (%U-iAs) and urinary monomethylated arsenic (%U-MMAs) and positively associated with urinary dimethylated arsenic (%U-DMAs). This relationship was not influenced by additional adjustment for folate, vitamin B2, or vitamin B12 intake. Additionally, there was significant effect modification by both drinking water iAs level and sex.
Conclusions: This study provides further evidence for an association between BMI and arsenic metabolism. However, contrary to previous hypotheses, these results suggest that this association is not confounded by the intake of micronutrients involved in one-carbon metabolism.

- Bozack AK, Hall MN, Liu X, Ilievski V, Lomax-Luu AM, Parvez F, Siddique AB, Shahriar H, Uddin MN, Islam T, Graziano JH, Gamble MV.

Folic acid supplementation enhances arsenic methylation: results from a folic acid and creatine supplementation randomized controlled trial in Bangladesh. *Am J Clin Nutr*. 2019 Feb 1;109(2):380-391.

Background: Arsenic exposure through drinking water persists in many regions. Inorganic As (InAs) is methylated to monomethyl-arsenical species (MMAs) and dimethyl-arsenical species (DMAs), facilitating urinary excretion. Arsenic methylation is dependent on one-carbon metabolism, which is influenced by nutritional factors such as folate and creatine.
Objective: This study investigated the effects of folic acid (FA) and/or creatine supplementation on the proportion of As metabolites in urine.
Design: In a 24-wk randomized, double-blinded, placebo-controlled trial, 622 participants were assigned to receive FA (400 or 800 μg per day), 3 g creatine per day, 400 μg FA + 3 g creatine per day, or placebo. The majority of participants were folate sufficient; all received As-removal

water filters. From wk 12-24, half of the participants receiving FA received placebo.
Results: Among groups receiving FA, the mean decrease in ln(%InAs) and %MMAs and increase in %DMAs exceeded those of the placebo group at wk 6 and 12 ($P < 0.05$). In the creatine group, the mean decrease in %MMAs exceeded that of the placebo group at wk 6 and 12 ($P < 0.05$); creatine supplementation did not affect change in %InAs or %DMAs. The decrease in %MMAs at wk 6 and 12 was larger in the 800 μg FA than in the 400 μg FA group ($P = 0.034$). There were no differences in treatment effects between the 400 μg FA and creatine + FA groups. Data suggest a rebound in As metabolite proportions after FA cessation; at wk 24, log(%InAs) and %DMAs were not significantly different than baseline levels among participants who discontinued FA supplementation.
Conclusions: The results of this study confirm that FA supplementation rapidly and significantly increases methylation of InAs to DMAs. Further research is needed to understand the strong cross-sectional associations between urinary creatinine and As methylation in previous studies.
This trial was registered at https://clinicaltrials.gov as NCT01050556.

- Desai G, Barg G, Vahter M, Queirolo EI, Peregalli F, Mañay N, Millen AE, Yu J, Browne RW, Kordas K.

Low level arsenic exposure, B-vitamins, and achievement among Uruguayan school children. *Int J Hyg Environ Health*. 2020 223:124-131.

Objectives: Millions of children globally, including the U.S., are exposed to low levels of arsenic from water and food. Arsenic is a known neurotoxicant at high levels but its effects at lower exposure levels are understudied. Arsenic methylation capacity, influenced by B-vitamin intake and status, potentially influences arsenic toxicity. In a cross-secitonal study of 5-8 year-old children from Montevideo, we assessed the relationship between urinary arsenic (U-As) and academic achievement, and tested for effect modification by B-vitamin intake, status, and arsenic methylation capacity.
Methods: Broad math and reading scores were calculated based on six subtests (calculation, math facts fluency, applied problems, sentence reading fluency, letter word identification, passage comprehension) from the Woodcock-Muñoz Achievement Battery. B-vitamin intake was assessed from two non-consecutive 24-h dietary recalls, serum folate and vitamin B-12 levels were measured in a subset of participants. Arsenic methylation capacity was measured as the proportion of urinary monomethylarsonic acid (%MMA). Multiple imputation using chained equations was conducted to account for missing covariate and exposure data. Ordinal regressions assessed associations between U-As and achievement score tertiles in the complete case and imputed samples. A "B-vitamin index" was calculated using principal component analysis. Interactions by urinary %MMA and the B-vitamin index were assessed.
Results: Median specific gravity adjusted U-As was 11.7 μg/L (range: 2.6, 50.1). We found no association between U-As and broad math and reading scores, nor effect modification by %MMA or B-vitamins.
Conclusion: At low-levels of exposure, U-As does not appear to affect children's academic achievement.

- Gules O, Yildiz M, Naseer Z, Tatar M.

Effects of folic acid on testicular toxicity induced by bisphenol-A in male Wistar

rats. *Biotech Histochem*. 2019 Jan;94(1):26-35.
Erratum in: *Biotech Histochem*. 2019 Jan;94(1):ei. PMID: 30079777.

We investigated the protective effect of the folic acid (FA) against bisphenol-A (BPA) induced toxicity in rat testis. We used four groups of seven adult male Wistar albino rats. The control group was fed corn oil, the BPA group was given BPA, the FA group was given FA and the FA + BPA group was given FA initially followed by BPA 1 h later. The BPA, FA and corn oil were administered by oral gavage for 14 days. At the end of the experiment, testis sections were examined for histological and histomorphometric characteristics. The TUNEL method was used to detect apoptosis and immunohistochemistry was used to examine the distribution of spermatogonial stem cells. Levels of serum testosterone were measured, and sperm viability and morphology were determined. The histological structure of the testis was normal in the control and FA groups. Although the number of TUNEL positive cells/tubule increased, the seminiferous epithelium height (SEH) at stages VII-VIII decreased in the BPA group compared to the control, FA and FA + BPA groups. The number of TUNEL positive cells/tubule decreased and the SEH at stages VII-VIII increased in the FA + BPA group compared to the BPA group. No significant difference in spermatogonial stem cells was found among groups. The level of serum testosterone and percentage of viable sperm was significantly lower, while the head, midpiece and total sperm abnormalities were significantly higher in the BPA treated group compared to control, FA, FA + BPA groups. It appears that the toxic effects of BPA on testis might be minimized by FA treatment.

- Lin YC, Chung CJ, Huang YL, Hsieh RL, Huang PT, Wu MY, Ao PL, Shiue HS, Huang SR, Su CT, Lin MI, Mu SC, Hsueh YM.

Association of plasma folate, vitamin B12 levels, and arsenic methylation capacity with developmental delay in preschool children in Taiwan. *Arch Toxicol*. 2019 93: 2535-2544.

Developmental delay has been associated with inefficient arsenic methylation capacity in preschool children. Folate and vitamin B12 are important nutrients that produce s-adenosylmethionine during single-carbon metabolism and provide methyl groups for arsenic methylation. The aim of the present study was to explore whether plasma folate and vitamin B12 levels influence arsenic methylation capacity and in turn are related to developmental delay in preschool children. A case-control study was conducted in 178 children with developmental delay and 88 normal children, who were recruited from Shin Kong Wu Ho-Su Memorial Teaching Hospital from August 2010 to March 2014. Arsenite (AsIII), arsenate (AsV), monomethylarsonic acid (MMAV), and dimethylarsinic acid (DMAV) in the urine was determined by high-performance liquid chromatography-linked hydride generator and atomic absorption spectrometry. Plasma folate and vitamin B12 levels were measured using a SimulTRAC-SNB radioassay. The results show that the combination of high plasma folate and high vitamin B12 levels were correlated with efficient arsenic methylation capacity (low MMAV %, low InAs %, and high DMAV %). High MMAV % significantly increased and high DMAV % and secondary methylation index decreased the odds ratio (OR) of developmental delay in a dose-dependent manner in both low plasma folate and low vitamin B12 (low/low) groups; the multivariate OR and 95% confidence interval were 5.01 (0.83-30.06), 0.21 (0.04-1.23), and 0.20 (0.03-1.20), respectively. This is the first study to show that the combination of high plasma folate and high vitamin B12 levels increases arsenic methylation capacity and indirectly

decreases the OR of developmental delay in preschool children.

- Huang MC, Douillet C, Dover EN, Zhang C, Beck R, Tejan-Sie A, Krupenko SA, Stýblo M.

Metabolic Phenotype of Wild-Type and As3mt-Knockout C57BL/6J Mice Exposed to Inorganic Arsenic: The Role of Dietary Fat and Folate Intake. *Environ Health Perspect*. 2018 Dec;126(12):127003. doi: 10.1289/EHP3951.

Background: Inorganic arsenic (iAs) is a diabetogen. Interindividual differences in iAs metabolism have been linked to susceptibility to diabetes in iAs-exposed populations. Dietary folate intake has been shown to influence iAs metabolism, but to our knowledge its role in iAs-associated diabetes has not been studied.
Objective: The goal of this study was to assess how folate intake, combined with low-fat (LFD) and high-fat diets (HFD), affects the metabolism and diabetogenic effects of iAs in wild-type (WT) mice and in As3mt-knockout (KO) mice that have limited capacity for iAs detoxification.
Methods: Male and female WT and KO mice were exposed to 0 or [Formula: see text] iAs in drinking water. Mice were fed the LFD containing [Formula: see text] or [Formula: see text] folate for 24 weeks, followed by the HFD with the same folate levels for 13 weeks. Metabolic phenotype and iAs metabolism were examined before and after switching to the HFD.
Results: iAs exposure had little effect on the phenotype of mice fed LFD regardless of folate intake. High folate intake stimulated iAs metabolism, but only in WT females. KO mice accumulated more fat than WT mice and were insulin resistant, with males more insulin resistant than females despite similar %fat mass. Feeding the HFD increased adiposity and insulin resistance in all mice. However, iAs-exposed male and female WT mice with low folate intake were more insulin resistant than unexposed controls. High folate intake alleviated insulin resistance in both sexes, but stimulated iAs metabolism only in female mice.
Conclusions: Exposure to [Formula: see text] iAs in drinking water resulted in insulin resistance in WT mice only when combined with a HFD and low folate intake. The protective effect of high folate intake may be independent of iAs metabolism, at least in male mice. KO mice were more prone to developing insulin resistance, possibly due to the accumulation of iAs in tissues.

- Jalali LM, Koski KG.

Amniotic fluid minerals, trace elements, and prenatal supplement use in humans emerge as determinants of fetal growth. *J Trace Elem Med Biol*. 2018 50:139-145.

Amniotic fluid (AF), which is swallowed by the developing fetus, contains minerals and trace elements, but their association with fetal growth has not been explored. Our objectives were to assess (1) whether concentrations of AF minerals and trace elements were associated with changes in 5 fetal ultrasound measurements (estimated weight, bi-parietal diameter, head circumference, abdominal circumference, femur length) between 16-20 and 32-36 wks gestation and (2) whether a prenatal supplement was associated with concentrations of AF minerals and trace elements or the 5 fetal ultrasound measurements. We measured, using inductively coupled plasma-mass spectrometry (ICP-MS), 15 minerals and trace elements (aluminum, arsenic, calcium, chromium, copper, iron, lead, magnesium, nickel, potassium, rubidium, selenium, silver strontium, zinc) in amniotic fluid collected from 176 pregnant women undergoing age-related amniocentesis for genetic testing (15.7 ± 1.1 wks). AF mineral

concentrations, prenatal supplement use, and determinants of ultrasound measurements during early and late pregnancy were used in models to assess their impact on change in fetal ultrasound measurements. Positive associations were identified for change in bi-parietal diameter with AF calcium, for change in head circumference with AF copper and nickel, and for change in femur length with AF selenium. Arsenic was negatively associated with estimated fetal weight, and this relationship was modified by prenatal supplement use. Additionally, AF chromium concentrations were lower in women taking prenatal supplements. In conclusion, AF minerals were associated with fetal ultrasound indices, supporting a biological role for calcium, copper, nickel and selenium in promoting in-utero fetal growth. Evidence of a mineral-vitamin interaction between arsenic and folic acid in prenatal supplements and mineral-mineral interaction between iron and chromium would suggest that attention be paid to mineral and trace element formulation of prenatal supplements.