# In utero exposure to carcinogens

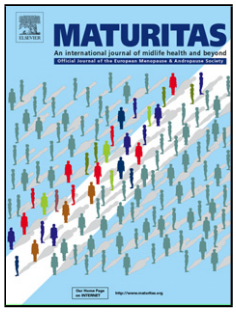
Waring, Rosemary; Harris, R.m.; Mitchell, S.c.

*DOI:*
10.1016/j.maturitas.2016.01.008



*Document Version*
Peer reviewed version

*Citation for published version (Harvard):*
Waring, R, Harris, RM & Mitchell, SC 2016, 'In utero exposure to carcinogens: epigenetics, developmental disruption and consequences in later life', *Maturitas*, vol. 86, pp. 59-63.
https://doi.org/10.1016/j.maturitas.2016.01.008




Title: *In utero* exposure to carcinogens: epigenetics, developmental disruption and consequences in later life

Author: R.H. Waring R.M. Harris S.C. Mitchell

PII: S0378-5122(16)30008-1
DOI: http://dx.doi.org/doi:10.1016/j.maturitas.2016.01.008
Reference: MAT 6541

To appear in: *Maturitas*

Received date: 11-1-2016
Accepted date: 14-1-2016

Please cite this article as: Waring RH, Harris RM, Mitchell S.C.In utero exposure to carcinogens: epigenetics, developmental disruption and consequences in later life.*Maturitas* http://dx.doi.org/10.1016/j.maturitas.2016.01.008

# ***In utero*** **exposure to carcinogens: epigenetics, developmental disruption and consequences in later life**

RH Waring[a*] R.H.Waring@bham.ac.uk, RM Harris[a], SC Mitchell[b]
[a]School of Biosciences, University of Birmingham, Birmingham, UK
[b]Computational and Systems Medicine, Imperial College London, London, UK
[*]Corresponding author.

**Highlights**

- The placental “barrier” is permeable to many environmental chemicals
- Effects of maternal exposure on the fetus may not appear until it reaches adulthood
- Many of these long-term effects are mediated by epigenetic changes
- Humans appear to be less sensitive to these effects than common test organisms

**Abstract**

The uterine environment is often viewed as a relatively safe haven, being guarded by the placenta which acts as a filter, permitting required materials to enter and unwanted products to be removed. However, this defensive barrier is sometimes breached by potential chemical hazards to which the mother may be subjected. Many of these toxins have immediate and recognisable deleterious effects on the embryo, foetus or neonate, but a few are insidious and leave a legacy of health issues that may emerge in later life. Several substances, falling into the categories of metals and metalloids, endocrine disruptors, solvents and other industrial chemicals, have been implicated in the development of long-term health problems in the offspring following maternal and subsequent *in utero* exposure. The mechanisms involved are complex but often involve epigenetic changes which disrupt normal cell processes leading to the development of cancers and also dysregulation of biochemical pathways.

**Abbreviations**

5-mc: 5-methylcytosine
AhR: aryl hydrocarbon receptor
AKT: protein kinase B
BPA: Bisphenol A
CNS: central nervous system
CS: Cigarette smoke
DBP: dibenzo(α1) pyrene
DES: diethylstilboestrol
DNMT: N-methyltransferase
E2: 17-β- oestradiol
ED: endocrine disruptor
ERE: oestrogen-responsive-elements
EZH2: enhancer of Zeste homolog 2 (EZH2)
GSK: glycogen synthase kinase
HAT: histone acetyl transferase
HDAC: histone deacetylase
HOTAIR: (HOX transcript antisense RNA)
IGF-1: insulin-like growth factor-1
miRNA: micro RNA
ncRNA: non-coding RNA
PAH: Polycyclic aromatic hydrocarbons
PCB: polychlorobiphenyl
PCE: Tetrachloroethylene (perchloroethylene)
PI3K: phosphoinositide 3-kinase
TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin
TCE: trichloroethylene

**Keywords:** polycyclic aromatic hydrocarbon; cigarette smoke; solvent; endocrine disruptor; metal; metalloid

## 1. Introduction

One of the unanswered questions in toxicology is to what degree carcinogens affect the growing foetus as well as the mother and what the consequences might be. There are suggestions in the literature that cancer in early childhood is primarily caused by some event which occurs *in utero* but as carcinogens dysregulate many biological systems, other health problems could potentially be caused in later life. Such compounds might then also act as 'developmental disruptors' (DDs), causing long-term dysfunction (cancer, chronic disease) in response to an event occurring many years previously. This review summarises key findings regarding potential *in utero* toxins and discusses possible mechanisms for the damage.

### 1.1. Carcinogenesis

Carcinogens act by several mechanisms. They may cause genomic changes, where the DNA sequence is altered, or non-genomic effects where the basic processes of translating DNA into proteins become dysfunctional. More recently, environmental chemicals called 'endocrine disruptors' (EDs) have been described which affect steroid synthesis, degradation and function at the receptors. *In utero* exposure to EDs can lead to a consequent imbalance of androgenic/oestrogenic steroids and this has been linked in rodents with the appearance of tumours of their reproductive systems in later life [1]. The topic has been extensively reviewed and it is still unclear whether similar mechanisms occur in man although pollutants such as plasticisers and flame-retardants have been implicated as potential causes of the increases seen in human breast and prostate tumours. As well as these pathways, epigenetics now seems to play a major role [2].

### 1.2. Epigenetics

Rather than changes in the DNA sequence which alter the genotype, epigenetics affects how cells read the genes and involves the study of how environmental factors/compounds can switch genes on or off, thus altering the phenotype. This can occur by a number of mechanisms (Figure 1). Firstly, there may be alterations in the methylation of the non-coding RNAs (ncRNAs). These are RNA molecules which are transcribed from DNA but their message is not translated into proteins. Instead, they are involved in regulation at the transcriptional and post-transcriptional level. MicroRNAs (miRNAs) can also be altered. These are small nonprotein-coding RNAs that promote the degradation of target messenger RNAs, regulating both transcription and translation. Since methylation changes their conformations it consequently alters their functions. Another major epigenetic mechanism involves re-modelling of chromatin, the complex of DNA and histone proteins, so that the microstructures of DNA or its associated chromatin proteins are modified rather than the code. Post-translational modification of the amino acids making up the histone proteins will alter their shape and function and many substitutions are known, including acetylation (lysine residues), methylation (arginine and lysine residues), phosphorylation (at serine and threonine residues) and ribosylation. The acetylation/deacetylation of lysine by histone acetyl transferase (HAT) and histone deacetylase (HDAC) enzymes has been particularly investigated since the balance between the activities of the two enzymes is linked with cancer progression.

DNA methylation is another well-studied phenomenon [3] where DNA N-methyltransferases (DNMTs) catalyse the addition of a methyl group at the 5-carbon of cytosine to give 5-methylcytosine (5-mC). Usually, this occurs at CpG sites where

a cytidine nucleotide is directly next to a guanidine nucleotide. These 'islands' are mainly located in gene promoter regions and are involved in regulation of gene expression, although in embryonic stem cells 5-mC is also found in non-CpG contexts. Generally, when a CpG site in the promoter region is methylated, gene expression is repressed while gene demethylation by various processes, including hydroxylation, allows expression to occur.

Clearly, carcinogens could act on the developing foetus by any of these mechanisms. Some epimutations appear to be corrected by normal reprogramming and are not transmitted to the offspring while others are not corrected and so can be inherited over several generations. It is not yet clear if the balance between transgenerational and non-transgenerational inheritance is the same in man as in rodent; this ratio may differ between the species [4]. Also, there is evidence that environmental influences and dietary factors which supply or transfer methyl groups for methylation reactions (eg folic acid) and flavonoids can alter degrees of methylation and affect DNA expression [5].

**2. Polycyclic aromatic hydrocarbons and cigarette smoke**

Polycyclic aromatic hydrocarbons (PAH) are known carcinogens and suspected endocrine disruptors. Prenatal exposure is common; a study (NHANES 2001-2006) on 3189 children and adolescents found that total urinary PAH and naphthalene metabolites were associated with obesity in 6-11 year olds [6] but this association was less strong in adolescents (11-19 years). When dibenzo(α1) pyrene (DBP), a common PAH, was administered to pregnant mice, the offspring had increased mortality from T-cell lymphoblastic lymphomas and all those surviving to 10 months had lung tumours while some had liver tumours. Both the foetal and maternal aryl hydrocarbon receptor (AhR) phenotypes seemed to be responsible for the range of effects [7] while short exposure to DBP during late gestation was more toxic than ingestion of DBP in the 3-week postnatal nursing period. Other cell types may be affected; the SETIL study looked at neuroblastoma cases (0-10 years old) in an Italian population and concluded that the risk was increased for children whose mothers had been exposed in pregnancy to hair dyes (0-17 month children, OR= 5.5, 95%Cl: 1.0-29.3) or to aromatic hydrocarbons (OR= 9.2, 95%Cl: 2.4-34.3) [8].

Cigarette smoke (CS) is composed of over 4000 chemicals, some of which are carcinogenic. In mice, exposure to CS *in utero* from gestational day 1 to postnatal day 21 led to low-birth weight offspring with alterations in protein, carbohydrate and lipid metabolism [9] while transplacental CS induced formation of lung adenomas in the offspring 8 months after birth [10]. The authors concluded, in what seems to be a general finding, that the carcinogenic response to CS varied depending on the developmental stage of exposure. Hakonsen *et al* [11] reviewed the extensive literature on this topic and concluded that in humans several aspects of reproductive health were involved. Prenatal exposure to maternal smoking was linked to an increased risk of cryptorchidism but a reduced risk of hypospadias while, in adult life, men had impaired semen quality. Both sexes had a tendency towards accelerated pubertal development.

**3. Solvents**

Prenatal exposure to high levels of alcohol leads to 'foetal alcohol syndrome' where offspring have developmental delay. This condition can be recognised at birth and points to overall toxic effects on a range of cells. However, long-term effects also

occur. In rats, prenatal ethanol renders the offspring more susceptible to reproductive tract tumours when challenged with chemical carcinogens such as N-nitroso-N-methyl urea and to hormones such as testosterone [12]. Exposure to alcohol in foetal life also increases susceptibility to mammary cancer in the adult animals, probably via increases in aromatase and hepatic insulin-like growth factor-1 (IGF-1) mRNA pathways [13]. In females, 17-β- oestradiol (E2) and IGF-1 synergise to regulate formation of terminal end buds and ductal elongation during breast development in puberty and there is cross-talk between the intracellular signalling pathways mediated by the E2 and IGF-1 receptors. Both genomic and non-genomic mechanisms are involved [14]. If alcohol exposure *in utero* induces epigenetic modifications, probably via its known interactions with miRNAs [15], then this may lead to a cascade process where susceptibility to breast cancer is increased in later life

The developing central nervous system (CNS) is very vulnerable to neurotoxicants and childhood brain tumours have been associated with exposure of either parent to solvents, especially benzene (OR=2.72) and other aromatics (OR=1.76) [16]. In what may be a similar example of CNS damage, prenatal exposure to solvents was associated with defective vision in the subsequent children [17]. A toxicological review of trichloroethylene (TCE) and meta analyses of epidemiological studies concluded that TCE is a renal carcinogen in man and probably causes developmental cardiac toxicity [18]. High levels of TCE contamination of groundwater were linked to the development of neural tube defects (OR=2.4) although the confidence intervals (CIs) had a wide range (0.6-9.6) [19].

Tetrachloroethylene (perchloroethylene, PCE) is probably also carcinogenic as exposure appears to increase the risk of bladder cancer, particularly in workers in the dry cleaning industry. Prenatal exposure to PCE may be associated with adult sub-clinical visual dysfunction, where colour discrimination is sub-optimal [20]. In a study on 831 subjects with prenatal exposure to PCE leached from water pipe linings, there was some evidence that risky behaviour in adult life (illicit drug use) was more common in those affected [21], suggesting subtle effects on brain development. Other systems may also be at risk as exposure to organic solvents around the time of conception increased the risk of childhood leukaemia in the offspring [22].

**4. Endocrine disruptors**

These are compounds which interfere with normal endocrine homeostasis. For optimal function, hormone levels must be, as in the story about Goldilocks, ‘not too little, not too much but just right’ and compounds which mimic natural oestrogens (most phenols have some oestrogenic activity because the oestrogen receptor is not very specific) have the potential to be disruptive. Prenatal exposure to both natural and synthetic oestrogens is known to be carcinogenic in man as well as increasing sensitivity to other carcinogens.

Bisphenol A (BPA) is a ubiquitous contaminant of modern life as it is still widely used as a plasticiser and occurs at low levels in human biofluids. It is a known endocrine disruptor (ED). Prenatal exposure in rats to environmentally-relevant levels of BPA induced mammary gland neoplasms by postnatal-day 90 [23] while the mRNA and protein expression of the histone methytransferase Enhancer of Zeste homolog 2 (EZH2) was increased in adult mammary tissue and MCF-7 cells [24]. BPA therefore has epigenetic effects via increased mammary histone trimethylation and this pathway is a mechanism by which EDs can be linked with breast cancer. BPA is also able to bind to oestrogen-responsive-elements (EREs) and modify chromatin (by histone acetylation and methylation) leading to gene activation. This

occurs via a misregulation of the HOTAIR gene (HOX transcript antisense RNA) expression [25].

Diethylstilboestrol (DES), a synthetic nonsteroidal oestrogen, also shows an ability to influence both EZH2 and HOTAIR expression [25]. This compound is infamous for its ability to induce adenocarcinomas of the vagina and cervix in the female offspring of exposed mothers [26]. However, the number of affected individuals was very small considering the enormous amounts of DES that were routinely prescribed to pregnant women. The mechanism of this transplacental carcinogenesis is unclear although various active metabolites, DNA adducts and epigenetic scenarios have been proposed. Recently, evidence has emerged that DES has the ability to alter HOX gene expression [27]. Concern has been expressed over possible transgenerational transmission of DES-related epigenetic alterations. This certainly occurs in rodents but seems uncommon in humans [28]. Menstrual irregularities and possible infertility have been mooted but there appears no high risk of reproductive dysfunction, although further studies are needed for definitive results.

The International Agency for Research on Cancer (IARC) has classified 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as a human multi-site carcinogen and polychlorobiphenyls (PCBs) as probable carcinogens although other dioxins and furan derivatives have not been so categorised owing to lack of evidence. PCBs, furans and dioxins are EDs in animals, influencing thyroid metabolism and also acting via the AhR pathway. Although animal studies have demonstrated that prenatal exposure to dioxins or PCBs can alter the timing of pubertal onset, research in human populations has given equivocal results. Exposure before birth has been associated with an increased risk of infections in infancy, possibly reflecting some thyroid-mediated autoimmune dysfunction [29]. Some reports have suggested an increase in behavioural problems in school-age children [30].

**5. Metal and metalloid carcinogens**

Both nickel and chromium compounds have carcinogenic potential in man and the toxicogenome of nickel (II) shows modifications of transcription factors which underlie the carcinogenic effects and could also lead to long-term damage [31]. Chromium (VI) compounds are known human carcinogens that produce reactive oxygen species (ROS) and primarily target the lungs. The ROS appear to be responsible for inducing the formation of tumours, probably via the activation of PI3K/AKT-dependent GSK-3beta/beta-catenin signalling and inhibition of apoptosis [32]. However, chromium can also induce post-translational modifications of histones and it affects many of the enzymes involved in epigenetic changes such as histone demethylases and methyltransferases. In cell culture, it has been shown to stimulate the formation of benzo[a]pyrene adducts with DNA [33] by cross-linking methyltransferase complexes to chromatin and has co-carcinogenic and co-mutagenic effects probably stemming from its interference with DNA repair processes.

Although cadmium is classed as a renal toxicant, it is also a carcinogen of the gastrointestinal tract. It is known to cross the placental barrier and is linked to damage in neonates [34]. Exposure to cadmium was associated with distinct and specific patterns of DNA hyper- and hypo-methylation in both foetal and maternal DNA [35]. Cadmium and also arsenic have been shown to affect the glucocorticoid receptor signal transduction pathway by epigenetic modifications, correlating with the increased susceptibility to infections seen in human populations exposed prenatally to these metals [36].

Arsenic is of particular concern as contaminated food and drinking water expose many millions of people to levels above those considered safe. Epidemiological studies have shown an association between arsenic exposure *in utero* and the future development of cancer as well as cardiovascular and respiratory diseases [37,38] and there are suggestions that humans are particularly susceptible [39]. In mice, arsenic is able to act as a complete carcinogen but, like cadmium, may also play a role as a co-carcinogen or co-promotor following *in utero* exposure [40]. During malignant transformations, arsenic appears to block cell differentiation thereby enhancing the survival of cancerous stem cells and creating an excess that may precipitate later oncogenic events and increase susceptibility to cancer [41]. Examination of umbilical cord blood from human neonates exposed to arsenic *in utero* showed increased expression of several miRNAs associated with signalling pathways related to cancer development and diabetes mellitus while there was a decrease in those involved with immune surveillance [42]. When methylation of neonatal cord blood-derived DNA was compared with maternal arsenic levels, loci in CpG islands had higher methylation levels in the highest exposed group. Some loci showed a linear dose-dependent relationship between methylation and arsenic exposure [43]. Reduction of histone acetylation (H4 at lysine 16) by direct binding to histone acetyltransferase (hMOF) has also been demonstrated [44].

## 6. Conclusions

It is still not certain how far the results from rodents are applicable to humans. Particularly in the case of epigenetics, rodents seem to be quite susceptible although this may not necessarily be true for man [45]. The whole situation is made more complex because there are indications from both animal studies and some human findings that the timing of action of the carcinogen is critical to the results occurring in later life. This must of course reflect the precise mechanisms which are involved at a time when the biological processes of development are changing very rapidly both qualitatively and quantitatively. Further, there is some evidence in human populations and in animal work that epigenetic changes may be modified by external factors. Hence, whether any individual responds to *in utero* exposure to carcinogens by developing dysfunction in later life must represent a combination of genetic susceptibility, time of exposure, extent of exposure and dietary and environmental influences [46,47,48]. This important aspect of human health has received relatively little attention. However, increasing our understanding of the mechanisms underlying developmental disruption should lead to better therapeutic approaches for individuals exposed *in utero* to carcinogens.

## Author contributions

Dr Rosemary Waring researched and wrote about 70% of the manuscript.
Dr Stephen Mitchell researched and wrote about 20% of the manuscript.
Dr Robert Harris researched and wrote about 10% of the manuscript and designed and drew figure 1.

## Funding

The authors have received no funding for this article.

## Conflict of interest

The authors declare no conflict of interest.

**References**

[1] S.M. Choi, S.D. Yoo, B.M. Lee, Toxicological characteristics of endocrine-disrupting chemicals: developmental toxicity, carcinogenicity and mutagenicity, J. Toxicol. Environ. Health B Crit. Rev. 7 (1) (2004) 1–24.
[2] P. Kaur, L.E. Shorey, E. Ho, R.H. Dashwood, D.E. Williams, The epigenome as a potential mediator of cancer and disease prevention in prenatal development, Nutr. Rev. 71 (7) (2013) 441–57.
[3] G. Ficz, New insights into mechanisms that regulate DNA methylation patterning, J. Exp. Biol. 218 (1) (2015) 14–20.
[4] J.R. McCarrey, Distinctions between transgenerational and non-transgenerational Epimutations, Mol. Cell. Endocrinol. 398 (1–2) (2014) 13–23.
[5] K. Vanhees, I.G.C Vonhogen, F.J. van Schooten, R.W. Godschalk, You are what you eat and so are your children: the impact of micronutrients on the epigenetic programming of offspring, Cell. Mol. Life Sci. 71 (2) (2014) 271–85.
[6] F. Scinicariello, M.C. Buser, Urinary polycyclic aromatic hydrocarbons and childhood obesity: NHANES (2001-2006), Environ. Health Perspect. 122 (3) (2014) 299–303.
[7] Z. Yu, C.V. Loehr, K.A. Fischer, M.A. Louderback, S.K. Krueger, R.H. Dashwood, et al., In utero exposure of mice to dibenzo[a,l] pyrene produces lymphoma in the offspring: role of the aryl hydrocarbon receptor, Cancer Res. 66 (2) (2006) 755–62.
[8] S. Parodi, D.F. Merlo, A. Ranucci, L. Miligi, A. Benvenuti, R. Rondelli, et al., Risk of neuroblastoma, maternal characteristics and perinatal exposures: the SETIL study, Cancer Epidemiol. 38 (6) (2014) 686–94.
[9] L. Canales, J. Chen, E. Kelty, S. Musah, C. Webb, M.M. Pisano, et al., Developmental cigarette smoke exposure: liver proteome profile alterations in low birth weight pups, Toxicology 300 (1–2) (2012) 1–11.
[10] R. Balansky, G. Ganchev, M. Iltcheva, M. Nikolov, V.E. Steele, S. De Flora, Differential carcinogenicity of cigarette smoke in mice exposed either transplacentally, early in life or in adulthood, Int. J. Cancer 130 (5) (2012) 1001–10.
[11] L.B. Håkonsen, A. Ernst, C.H. Ramlau-Hansen, Maternal cigarette smoking during pregnancy and reproductive health in children: a review of epidemiological studies, Asian J. Androl. 16 (1) (2014) 39–49.
[12] S. Murugan, C. Zhang, S. Mojtahedzadeh, D.K. Sarkar, Alcohol exposure in utero increases susceptibility to prostate tumorigenesis in rat offspring, Alcohol. Clin. Exp. Res. 37 (11) (2013) 1901–9.
[13] T.A. Polanco, C. Crismale-Gann, W.S. Cohick, Alcohol exposure in utero leads to enhanced prepubertal mammary development and alterations in mammary IGF and estradiol systems, Horm. Cancer 2 (4) (2011) 239–48.
[14] W.S. Cohick, C. Crismale-Gann, H. Stires, T.A. Katz, Fetal alcohol exposure and mammary tumorigenesis in offspring: role of the estrogen and insulin-like growth factor systems, Adv. Exp. Med. Biol. 815 (2015) 403–24.
[15] R.C. Miranda, MicroRNAs and ethanol toxicity, Int. Rev. Neurobiol. 115 (2014) 245–84.
[16] S. Peters, D.C. Glass, K.R. Greenop, B.K. Armstrong, M. Kirby, E. Milne, et al., Childhood brain tumours: associations with parental occupational exposure to solvents, Br. J. Cancer 111 (5) (2014) 998–1003.
[17] C. Till, C.A. Westall, G. Koren, I. Nulman, J.F. Rovet, Vision abnormalities in young children exposed prenatally to organic solvents, Neurotoxicology 26 (4) (2005) 599–613.

[18] W.A. Chiu, J. Jinot, C.S. Scott, S.L. Makris , G.S. Cooper, R.C. Dzubow, et al., Human health effects of trichloroethylene: key findings and scientific issues. Environ. Health Perspect. 121 (3) (2013) 303–11.
[19] P.Z. Ruckart, F.J. Bove, M. Maslia, Evaluation of exposure to contaminated drinking water and specific birth defects and childhood cancers at Marine Corps Base Camp Lejeune, North Carolina: a case control study, Environ. Health 12 (2013) 104.
[20] K.D. Getz, P.A. Janulewicz, S. Rowe, J.M. Weinberg, M.R. Winter, B.R. Martin, et al., Prenatal and early childhood exposure to tetrachloroethylene and adult vision, Environ. Health Perspect. 120 (9) (2012) 1327–32.
[21] A. Aschengrau, J.M. Weinberg, P.A. Janulwicz, M.E. Romano, L.G. Gallagher, M.R. Winter, et al., Affinity for risky behaviors following prenatal and early childhood exposure to tetrachloroethylene (PCE)-contaminated drinking water: a retrospective cohort study, Environ. Health 10 (2011) 102.
[22] T.I. Sung, J.D. Wang, P.C. Chen, Increased risk of cancer in the offspring of female electronics workers, Reprod. Toxicol. 25 (1) (2008) 115–9.
[23] N. Acevado, B. Davis, C.M. Schaeberle, C. Sonnenschein, A.M. Soto, Perinatally administered bisphenol a as a potential mammary gland carcinogen in rats, Environ. Health Perspect. 121 (9) (2013) 1040–6.
[24] L. F. Doherty, L.F. Bromer, J.G. Zhou, T.S. Aldad, H.S. Taylor, In utero exposure to diethylstilbestrol (DES) or bisphenol-A (BPA) increases EZH2 expression in the mammary gland: an epigenetic mechanism linking endocrine disruptors to breast cancer, Horm. Cancer 1 (3) (2010) 146–155.
[25] H. Du, H.S. Taylor, The role of HOX genes in female reproductive tract development, adult function and fertility, Cold Spring Harb. Perspect. Med. 6 (1) (2015) pii: a023002. doi: 10.1101/cshperspect.a023002. [Epub ahead of print]
[26] A.L. Herbst, H. Ulfelder, D.C.Poskanzer, Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women, N. Engl. J. Med 284 (15) (1971) 878–881.
[27] A.Bhan, I.Hussain, K.I.Ansari, S.A. Bobzean, L.I. Perrotti, S.S. Mandal, Bisphenol-A and diethylstilbestrol exposure induces the expression of breast cancer associated long noncoding RNA HOTAIR in vitro and in vivo, J. Steroid Biochem. Mol. Biol.141 (2014) 160–170.
[28] R.M. Harris, R.H. Waring, Diethylstilboestrol—a long term legacy? Maturitas, 72 (2) (2012) 108–112.
[29] C. Miyashita, S. Sasaki, Y. Saijo, N. Washino, E. Okada, S. Kobayashi, et al., Effects of prenatal exposure to dioxin-like compounds on allergies and infections during pregnancy, Environ. Res. 111 (4) (2010) 551–8.
[30] G.W. ten Tusscher, M. Leijs, L.C. de Boer, J. Legler, K. Olie, H. Spekreijse et al., Neurodevelopmental retardation, as assessed clinically and with magnetoencephalography and electro-encephalography, associated with perinatal dioxin exposure, Sci. Total Environ. 491–2 (2014) 235–9.
[31] Y. Chervona, A. Arita, M. Costa, Carcinogenic metals and the epigenome: understanding the effects of nickel, arsenic and chromium, Metallomics 4 (7) (2012) 619–27.
[32] Y.O. Son, P. Pratheeshkumar, L. Wang, X. Wang, J. Fan, D.H. Kim, et al., Reactive oxygen species mediate Cr(VI)-induced carcinogenesis through P13K/AKT-dependent activation of GSK-3-β/β-catenin signaling, Toxicol. Appl. Pharmacol. 271 (2) (2013) 239–48.
[33] M. Schnekenburger, G. Talaska, A Puga, Chromium cross-links histone deacetylase 1-DNA methyltransferase 1 complexes to chromatin, inhibiting histone-

remodeling marks critical for transcriptional activation, Mol. Cell. Biol. 27 (20) (2007) 7089–101.
[34] A.P. Sanders, L. Smeester, D. Rojas, T. DeBussycher, M.C. Wu, F.A. Wright, et al., Cadmium exposure and the epigenome: exposure-associated patterns of DNA methylation in leukocytes from mother-baby pairs, Epigenetics 9 (2) (2014) 212–21.
[35]. A.F.Mohanty, F.M. Farin, T.K. Bammler, J.W. MacDonald, Z. Afsharinejad, T.M. Burbacher, et al., Infant sex-specific placental cadmium and DNA methylation associations, Environ. Res. 138 (2015) 74–81.
[36] J.E. Rager, A.Yosim, R.C. Fry, Prenatal exposure to arsenic and cadmium impacts infectious disease-related genes within the glucocorticoid receptor signal transduction pathway, Int. J. Mol. Sci. 15 (12) (2014) 22374–91.
[37] E.J. Tokar, W. Qu, M.P. Waalkes, Arsenic, stem cells, and the developmental basis of adult cancer, Toxicol. Sci. 120 Suppl 1 (2011) S192–203.
[38] P. Koedrith, H. Kim, J.I. Weon, Y.R. Seo, Toxicogenomic approaches for understanding molecular mechanisms of heavy metal mutagenicity and carcinogenicity, Int. J. Hyg. Environ. Health 216 (5) (2013) 587–98.
[39] C. Steinmaus, C. Ferreccio, J. Acevedo, Y. Yuan, V. Durán, S. Cuevas, et al., Increased lung and bladder cancer incidence in adults after in utero and early-life arsenic exposure, Cancer Epidemiol. Biomarkers Prev. 23 (8) (2014) 1529–38.
[40] M.P.Waalkes, J. Lui, J.M.Ward, B.A. Diwan, Mechanisms underlying arsenic carcinogenesis: hypersensitivity of mice exposed to inorganic arsenic during gestation, Toxicology 198 (1–3) (2004) 31–38.
[41] K. Salnikow, A. Zhitkovich, Genetic and epigenetic mechanisms in metal carcinogenesis and cocarcinogenesis: nickel, arsenic, and chromium, Chem. Res. Toxicol. 21 (1) (2008) 28–44.
[42] J.E. Rager, K.A. Bailey, L. Smeester, S.K. Miller, J.S. Parker, J.E. Laine, et al., Prenatal arsenic exposure and the epigenome: altered microRNAs associated with innate and adaptive immune signaling in newborn cord blood, Environ. Mol. Mutagen. 55 (3) (2014) 196–208.
[43] D.C. Koestler, M. Avissar-Whiting, E.A. Houseman, M.R. Karagas, C.J. Marsit, Differential DNA methylation in umbilical cord blood of infants exposed to low levels of arsenic in utero, Environ. Health Perspect. 121 (8) (2013) 971–7.
[44] D.Liu, D.Wu, L. Zhao, Y. Yang, J. Ding, L. Dong, et al., Arsenic trioxide reduces global histone H4 acetylation at lysine 16 through direct binding to histone acetyltransferase hMOF in human cells, PLoS One 10 (10) (2015) e0141014. doi: 10.1371/journal.pone.0141014.
[45] W.W. Tang, S. Dietmann, N. Irie, H.G. Leitch, V.I. Floros, C.R. Bradshaw, et al., A unique gene regulatory network resets the human germ line epigenome for development, Cell 161 (6) (2015) 1453–67.
[46] U. Lim, M.A. Song, Dietary and lifestyle factors of DNA methylation, Methods Mol. Biol. 863 (2012) 359–76.
[47] A.L. Teh, H. Pan, L. Chen, M.L. Ong, S. Dogra, J. Wong, et al., The effect of genotype and in utero environment on interindividual variation in neonate DNA methylomes, Genome Res. 24 (7) (2014) 1064–74.
[48] C.M. Guerrero-Bosagna, M.K. Skinner, Environmental epigenetics and phytoestrogen/phytochemical exposures, J Steroid Biochem. Mol. Biol. 139 (2014) 270–6.

**Figure Captions**

Figure 1 Pathways in epigenetics