

# Influence of trace elements in the epigenetic of mammals

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**Introduction:** Chronic exposure to toxic levels of elements such as arsenic, cadmium, lead, mercury, nickel and others trace elements might cause abnormalities in gene expression affecting metabolic pathways such as those related to trace elements detoxification and the reproductive ability of animals. Rodent species have been the subject of several studies examining the physiological consequences of exposure to toxic levels of arsenic, cadmium and nickel, and how those elements affect their epigenetic mechanisms such as DNA methylation. Results from those studies can be used as an approach of the effects that can potentially occur on small mammals found in sites altered by geochemical or anthropogenic activities (e. g. mining, industrial waste).

**Methods:** An exhaustive literature review was conducted aimed to gain a better understanding of epigenetics, identifying mechanisms involved in the toxicity of trace elements, elucidating the effect of those trace metals in the epigenetics of genes involved in detoxification mechanisms and, finally, determining whether damages caused by exposition to high levels of trace elements are equally evident on any tissue from the same organism.

**Results and Discussion:** Pollutants can influence methylation of DNA patterns, but not all of them follow the same pathway. This varies widely among groups of trace elements or organic compounds. In addition to concentration and time of exposure, a number of other factors affect the toxicity pathway, including age, sex, food sources and, especially, the physiology of the species. Even within the same organism, the methylation patterns associated to a given element vary between tissues. Therefore, selecting the most appropriate tissue for discerning the animal's actual condition is key when assessing the actual health status of wildlife species. Further studies are needed to better characterize the interactions between DNA methylation and trace elements, and elucidate potential mechanisms or interventions that can help to reduce their effects on wildlife health.

**Key words:** ADN methylation, gene, pollution, trace elements, toxicity.

## Resumen

La exposición crónica a niveles tóxicos de elementos como arsénico, cadmio, plomo, mercurio, níquel y otros elementos traza, puede causar alteraciones en la expresión de diversos genes afectando procesos metabólicos como los relacionados con la detoxificación de elementos traza y la capacidad reproductiva de los animales. Las especies de roedores han sido objeto de varios estudios que examinan las consecuencias fisiológicas de la exposición a niveles tóxicos de arsénico, cadmio y níquel y cómo estos elementos afectan sus mecanismos epigenéticos, tales como la metilación del ADN. Los resultados de esos estudios pueden ser utilizados como una aproximación de los efectos que potencialmente pueden ocurrir en pequeños mamíferos que se encuentran en sitios alterados por actividades geoquímicas o antropogénicas (por ejemplo, minería, residuos industriales).

Se llevó a cabo una exhaustiva revisión de la literatura con objeto de lograr una mejor

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comprensión de la epigenética, identificar los elementos traza cuya toxicidad implica mecanismos epigenéticos, dilucidar el efecto de los metales traza en la epigenética de genes implicados en los mecanismos de desintoxicación y, finalmente, determinar si los daños causados por la exposición a niveles altos de elementos traza son igualmente evidentes en cualquier tejido del mismo organismo.

Los contaminantes pueden cambiar los patrones de metilación de ADN, pero no todos ellos siguen la misma ruta; ésta varía ampliamente entre grupos de metales traza o entre compuestos orgánicos. Además de la concentración y el tiempo de exposición, varios otros factores incluyendo la edad, el sexo, las fuentes de alimentos y, especialmente la fisiología de la especie, influyen en la ruta de acción de la toxicidad. Incluso dentro del mismo organismo, los patrones de metilación causados por un elemento dado varían entre los tejidos. Por lo tanto, la selección del tejido más apropiado para discernir la condición real del animal es clave para evaluar el estado real de salud de la vida silvestre. Los mecanismos epigenéticos son una herramienta importante para entender los mecanismos implicados en la toxicidad de los elementos traza, y cómo estos elementos pueden afectar los niveles de metilación del ADN. Se necesitan más estudios para caracterizar mejor las interacciones entre la metilación del ADN y los elementos traza y para dilucidar mecanismos o intervenciones potenciales que pueden ayudar a reducir sus efectos sobre la salud de la vida silvestre.

**Palabras clave:** contaminación, elementos traza, gen, metilación ADN, toxicidad.

## Introduction

Environmental pollution is a growing concern for its potential consequences on wildlife health (Shorter *et al.* 2012). It is common knowledge that pollution, either from anthropogenic or geochemical origin, affects wildlife; however, the specific mechanisms of action, how pollution issues should be addressed and what studies should be conducted are aspects still generally unknown. Trace elements are included among chemical contaminants. These elements are named as trace because they are commonly found in concentrations lower than 0.1 % in the environment (Pais and Jones 1997) and lower than 0.01 % of bodyweight in animal and plant tissues (Adriano 2001). Some trace elements (*e. g.* mercury) have no known biological function, while others are especially important for certain groups of organisms (*e. g.* boron for plants) and most are considered essential for the life of plants and animals, but might become toxic at levels above those required for each species (Templeton *et al.* 2000).

High levels of trace elements can be found in different parts of the world, mostly in relation with anthropogenic or geochemical activities (*e. g.* volcanic activity, hydrothermal sources; Amaral *et al.* 2007). The toxicity of trace elements will depend on the chemical form present in the environment. For example, hexavalent chromium ( $\text{Cr}^{6+}$ ) is more toxic than the trivalent species ( $\text{Cr}^{3+}$ ). The organic presentation of the mercury, the methylmercury is more toxic than its inorganic form (Templeton *et al.* 2000). Variations in toxicity of trace elements are related to the chemical form that is more easily taken up by organisms. Environmental factors such as acidification (*e. g.* acid rain) can increase the possibility of assimilation of metals and metalloids and, thus, their toxicity (Nordberg *et al.* 1985).

Toxic compounds ingested by organisms are metabolized through various detoxification mechanisms, through which they are usually biotransformed to less toxic chemical

presentation to be excreted subsequently. Sometimes, however, depending on factors such as dose and length of exposure, biotransformation mechanisms can be exceeded, resulting in the impairment of the specimen's health (Ghoshal *et al.* 2000; Amaral *et al.* 2007). Although the early effects of pollutants in the environment can go unnoticed, these can lead to serious ecological disturbances in the long term (Baccarelli and Bollati 2009). In addition to the potential toxicity of an element at high concentrations, the effect of its interaction with other pollutants derived from anthropogenic activities such as agricultural or industrial discharges should also be taken into account.

The impact on organisms can be expressed in several ways, including chromosomal abnormalities or reduced reproductive capacity. Over time, this can undermine populations, even to the point of causing their gradual disappearance and the local extinction of one or more species (Hook and Fisher 2001; Matzuk and Lamb 2002; Choe *et al.* 2003; Amaral *et al.* 2007).

The effects of pollution on wild communities, particularly on the epigenetics of different species, such as those caused by trace metals, has lately drawn growing attention (Salnikow and Zhitkovich 2008; Arita and Costa *et al.* 2009; Shorter *et al.* 2012). However, although many of the effects in different species are well known, the specific mechanisms at work remain unknown. This is why the chronic bioaccumulation of high levels of trace elements may lead to carcinogenic (Silbergeld 2003; Waalkes 2003), teratogenic (Calevro *et al.* 1998) or mutagenic (Stearns *et al.* 2002) effects, and modify the activity of enzymes and transport proteins (Jacob *et al.* 2002; Amaral *et al.* 2007; Salnikow and Zhitkovich 2008), depending on the particular element involved. Epigenetics allows discerning how these effects take place in mammals and assess the extent of direct damage produced (Guillette and Gunderson 2001; Pilsner *et al.* 2010).

Rodents have been the subject of several studies examining the physiological consequences of exposure to trace elements as pollutants (Dewey and Dawson 2001; Shorter *et al.* 2012), including epigenetic alterations (Baccarelli and Bollati 2009; Arita and Costa 2009). These mammals have a cosmopolitan distribution and can be found in many places that are polluted as a result of anthropogenic activities, so that studies on rodents can be used as an approach of what may happen to wildlife exposed to high concentrations of one or several trace elements.

Trace elements are among the environmental factors that can affect wildlife epigenetics and thereby alter their phenotypic traits. The epigenetics concept followed hereby is the one related to meiotically and mitotically heritable changes in gene expression that are not coded in the DNA sequence itself (Levenson and Sweatt 2005). Altered patterns of gene expression can occur through a variety of mechanisms based on RNA, DNA or proteins (Egger *et al.* 2004).

## Results and Discussion

*Epigenetic Mechanisms.* DNA methylation is one of the main epigenetic mechanisms that affect chromatin structure (a highly compressed structure present in the cell nucleus composed of DNA and protein), without changing the DNA nucleotide sequence (Levenson and Sweatt 2005). This epigenetic mechanism involves the addition of a methyl group to the DNA chain, thus affecting the DNA structure and replication ability. Methylation in mammals is related to the action of a set of enzymes collectively called DNA methyltransferases

(Okano *et al.* 1998). The function of these enzymes is to incorporate a methyl group at the 5 position of the pyrimidine ring of cytosine. The product is 5-methylcytosine (Chen *et al.* 1991), which is found in about 4 % of the mammalian genomic DNA, mainly in cytosine-guanine dinucleotides (CpG; Baylin 2005). Between 60 and 90 % of such dinucleotides are methylated (Bird 1986); the rest of them are unmethylated and form small clusters known as cytosine-guanine islands (Levenson and Sweatt 2005). Such islands are located at the beginning or near the promoter regions in about 50 % of all mammalian genes, where DNA transcription starts (Bird 1986; Antequera and Bird 1993). Under normal conditions, the vast majority of these islands are unmethylated (Antequera and Bird 1999). Methylation in the promoter region of a gene silences its expression (Jacob *et al.* 2002). An imbalance in methylation patterns can cause either hypo- or hypermethylation relative to the species' normal DNA methylation level.

*Factors influencing methylation patterns.* Methylation patterns and levels vary both between organisms and between tissues within the same individual. In the animal kingdom, species range from a) those whose genome lacks any detectable level of methylated cytosine (*e. g.* the worm *Caenorhabditis elegans*; Bird 2002) because it does not encode DNA methyltransferase; b) those containing very low methylation levels, basically constrained to dinucleotides formed by cytosine and thymine instead of cytosine and guanine (the fruit fly *Drosophila melanogaster*; Gowher *et al.* 2000; Bird 2002); and c) those having an extensively methylated genome, as is the case of vertebrates. Birds and mammals have similar methylation levels, which are lower than those found in fish and amphibians. This has been related to body temperature and, thus, to evolutionary processes (Varriale 2014).

The individual's age is an important variable in epigenetic processes. In mammalian somatic cells, the levels of 5-methylcytosine may show temporal and spatial variations. During the early development stages, rodents show a significant reduction in methylation levels, decreasing to around 30 % of their typical somatic level of methylation in adults (Monk *et al.* 1987; Kafri *et al.* 1992; Bird 2002). This implies that virtually no gene is silenced and all of them can be expressed. It is in the early stages of development when the regulation occurs as to which genes are expressed and which are not. The silencing process is carried out through methylation, by means of the methyltransferase family of enzymes (DNMT). This family of enzymes includes isoforms DNMT1, DNMT3a and DNMT3b. During rodent development, DNMT1 appears to be responsible for maintaining the species-specific DNA methylation patterns, whereas DNMT3a and DNMT3b are involved in the establishment of new embryonic methylation patterns (Okano *et al.* 1999; Bestor 2000; Baylin 2005). Therefore, the latter two isoforms will be responsible for achieving the level of silenced genes observed in the adult and which existed at the time of implantation (Okano *et al.* 1998, 1999; Bird 2002). However, this process does not take place in all animal species; for example, in the zebrafish (*Danio rerio*; MacLeod *et al.* 1999) no decrease in methylation levels is observed during embryonic development. These variations between species reflect the role that DNA methylation plays in each organism. In mammals, the demethylation cycle followed by *de novo* methylation during the early embryonic stages is critical in determining somatic DNA methylation patterns, which will be stable and inheritable as a clonal methylation pattern (Mesa-Cornejo *et al.* 2006).

Another factor to consider is the organism's sex, as it has been observed that pollutants

can differentially affect methylation patterns in males and females of the same species. In male polar bears (*Ursus maritimus*), an inverse relationship between mercury exposure and DNA methylation levels in the lower brainstem has been recorded. These changes are related to DNA hypomethylation and, consequently, lead to changes in long-term chromosomal stability, thus underlying the development of autoimmune diseases and affecting reproduction, which has not been observed in females (Pilsner *et al.* 2010).

*Effects on reproduction.* The environment also affects the reproductive success of animal species. The area inhabited by females live prior to conception and during early pregnancy affects oocyte development and the early embryonic stages. Nutrition is one of the most critical environmental factors in this stage. Alterations in food quantity and composition affect oocyte maturation, gastrulation, and even embryo survival. In this regard, one aspect worth noting is the influence of diet on the methylation patterns of numerous genes (Ashworth *et al.* 2009). The diet provides the methyl groups that will be used in the process and will be associated with the imprint or “methylation marks” involved in silencing a gene. Examples are all the mechanisms involved in the inactivation of one of the mother’s two X chromosomes and the parentally imprinted genes. Genomic imprinting is the process by which one of the two parental genes is silenced, leaving only one active. Most genes in diploid organisms are expressed regardless of their parental origin. However, a few genes (approximately 100) are regulated and transcribed in a monoallelic fashion. For these genes, called imprinted (or marked) genes, the expression and regulation pattern depends on their parental origin. That is, they behave differently if they are of paternal or maternal origin (Reik 2007; Reig and Concha 2012). Any error during this delicate process may lead to either the two parental genes being expressed or none of them, thereby altering the phenotype and resulting in various diseases, some of which might be lethal. Although methylation patterns are stable and inheritable at the genomic level, there are at least two embryonic development stages where the global methylation pattern is erased and reset: the generation of germ cells and the embryonic implantation period (Reik *et al.* 2001). This is one of the major processes in which methylation is involved, enabling or silencing gene expression. For this process to take place, mammals must get methyl groups from food since these cannot be synthesized. Consequently, the diet should contain methionine, choline, and cofactors such as folic acid and pyridoxines, all being substances that supply methyl groups (Kaminker 2007).

*Effect of trace elements on DNA methylation patterns.* The presence of pollutants in the diet, particularly trace metals, can disrupt DNA methylation patterns. Trace elements such as cadmium, chromium, nickel, arsenic and mercury have been found to cause neurodegenerative disorders, and can also act as endocrine disruptors. That is, they interfere with the biosynthesis of hormones, as well as with their metabolism and activity, leading to the loss of homeostasis of the endocrine-reproductive system (Baccarelli and Bollati 2009; Diamanti-Kandarakis *et al.* 2009).

Pollutant concentration and time of exposure have been generally related, among other things, to the hypomethylation of a number of genes. This effect directly results in a low survival rate and prematurity, as well as on post-natal growth problems (Ashworth *et al.* 2009; Shutoh *et al.* 2009).

DNA hypomethylation and hypermethylation are attributed to several factors, including various environmental agents such as exposure to above-normal concentrations of trace elements such as copper, chromium, lead, nickel, cadmium and arsenic (Tkeshelashvili *et al.* 1991; Silbergeld 2003; Salnikow and Zhitkovich 2008; Arita and Costa 2009). Except for chromium, which forms stable adducts with DNA, the other elements are considered as very weak mutagens (Takiguchi *et al.* 2003; Arita and Costa 2009). These elements can directly act as genotoxic agents, but *in-vitro* tests have shown that elements such as cadmium require such high doses that impair cell growth (Misra *et al.* 1998); therefore, it is still unclear how this could happen *in vivo* (Takiguchi *et al.* 2003). Another possibility is the indirect effect of trace elements on the accuracy of the DNA replication process and impairment of apoptosis (Pulido and Parrish, 2003; Arita and Costa 2009).

It is known that cadmium toxicity partially derives from its being a common inhibitor of enzymes, including DNA-methyltransferase. At the concentrations needed for this metal to inhibit up to 84 % of this enzyme activity in rat liver cells, other elements such as zinc, nickel, lead and arsenic do not affect the activity of this enzyme in the same type of cell lines (Takiguchi *et al.* 2003). The complexity of cadmium toxicity is further evidenced by the observation that at the beginning of the inhibition of the DNA methyltransferase activity, DNA hypomethylation occurs. However, under chronic cadmium exposure, cells undergo a morphological transformation that promotes cell proliferation and a significant increase in the activity of DNA methyltransferase, which leads to DNA hypermethylation (Takiguchi *et al.* 2003). Out of 1,629 genes exposed to cadmium, 675 (41.44 %) showed hypermethylation, 899 (55.19 %) hypomethylation, and 55 (3.38 %) a mixture of hyper- and hypomethylation in the cytosine - guanine islands of their promoter regions (Wang *et al.* 2012b). An overall hyper- or hypomethylation will reflect a pool of genes with different methylation levels. This explains why cells exposed for short periods of time show hypomethylation by not having accumulated sufficient methylated genes, whereas cells exposed to cadmium for longer periods of time show DNA hypermethylation due to the larger number of methylated genes (Wang *et al.* 2012a).

Unlike cadmium, nickel can simultaneously inhibit the activity of DNA methyltransferase and cause DNA hypermethylation (Lee *et al.* 1998). A mechanism proposed to explain this phenomenon includes the ability of this element to replace magnesium in the phosphate backbone of DNA in heterochromatin. Magnesium replacement takes place in heterochromatin because of the higher magnesium concentration therein relative to euchromatin. The replacement of magnesium by nickel increases heterochromatin condensation, which in turn will trigger the *de-novo* methylation of DNA in critical genes, such as those associated with tumour suppression or senescent genes (Lee *et al.* 1995; Arita and Costa 2009).

Another mechanism by which trace elements may also alter methylation patterns is the reduction of S-adenosylmethionine levels. This coenzyme is the main donor of methyl groups in living organisms and is therefore required for all cellular methylation reactions. The deficiency of methyl donor compounds, such as folic acid, in the diet may also impair the synthesis of S-adenosylmethionine. As levels of this metabolite decrease in cells, various methylation processes become compromised. One example is the arsenic detoxification process that takes place to eliminate this metal from the organism. In the environment, arsenic commonly occurs as either arsenate ( $\text{As}^{+5}$ ) or arsenite ( $\text{As}^{+3}$ ), the latter being the

more toxic form, often associated with cancer risk (Bertolero *et al.* 1987). Arsenic enters the body primarily as arsenite by simple diffusion. By contrast, only a small portion of arsenate will be able to cross cell membranes, as this requires an energy-dependent active transport system. Arsenite accumulates within cells more readily than arsenate which, in any case, will also be converted to arsenite by a reduction reaction in which glutathione plays a key role (Salnikow and Zhitkovich 2008).

The elimination of arsenite from the organism requires its methylation through a series of reactions catalyzed by the cytosolic arsenic (+3) methyltransferase. This arsenic-methylation reaction, that uses S-adenosylmethionine as a methyl donor (Thomas *et al.* 2007) reduces the amount of S-adenosylmethionine that is available in the organism, thus impairing the natural gene-silencing methylation process, hence leading to the expression of genes that would otherwise be silenced.

In rodents, it has been found that the consumption of high folate levels helps reduce total arsenic content in the liver of pregnant females, but does not prevent the effects of exposure to inorganic arsenic on foetuses weight or hepatic S-adenosylmethionine concentrations. Foetuses exposed only to arsenic showed a slight effect on the DNA methylation pattern in their liver, in contrast to those exposed to both arsenic and folate, which showed changes in the methylation patterns of the cytosine-guanine islands of 2,931 genes, several of the which are associated with neurological development, cancer, cell cycles and signaling networks; the biological pathways worst affected include the one regulating foetal development (Tsang *et al.* 2012).

However, the presence of tumours associated with prolonged arsenic exposure has also been related to other mechanisms. One of those is the modification of histones of the promoter region of the p16 tumour-suppressor gene, which suppresses the expression of this gene and leads to a higher incidence of tumours, as observed in mice supplied with water containing inorganic arsenate for 6 months (Suzuki and Nohara 2012).

Another likely mechanism is the effect of arsenic on the expression of genes involved in lipid metabolism in the liver, which leads to an increase in lipogenesis and the intracellular accumulation of triglycerides in this tissue. This process is believed to play a key role in tumorigenesis, as it favours the increase of oxidative stress that can cause DNA damage and, thus, genetic mutations that are commonly expressed as tumour formation (Nohara *et al.* 2012).

*Effects of trace elements on the methylation patterns of genes involved in detoxification systems.* The elimination of trace elements from the body may be impaired when the activity of any of the enzymes involved in the process, such as the DNA methyltransferase in the case of arsenic elimination, is affected. Metallothioneins are another family of proteins that play a key role in detoxification processes of elements such as cadmium. These, low-molecular weight cysteine-rich proteins, play an important role in the homeostasis of divalent metals, in addition to being responsible for reducing their toxicity. This is achieved by binding metals via sulfhydryl groups (Heuchel *et al.* 1994). In most mammals, metallothioneins occur in four isoforms: MT-I, MT-II, MT-III and MT-IV. The first two isoforms are expressed in all tissues, MT-III is expressed only in the brain and MT-IV is only present in the stratified squamous epithelium of the skin, the tongue and the upper gut (Aschner *et al.* 1997). In most tissues, MT-I and MT-II levels are deemed negligible, but can rise dramatically by a variety of agents,

including exposure to metals, ultraviolet radiation and agents producing reactive oxygen species. Basal levels of MT-I and MT-II, as well as levels induced by the presence of metals, are mediated by the Metal-Responsive Transcription Factor MTF-1 (Heuchel *et al.* 1994; Giedroc *et al.* 2001; Wimmer *et al.* 2005). MTF-1 has been maintained throughout evolution in humans (Brugnera *et al.* 1994), mice (Radtke *et al.* 1993), fish (Maur *et al.* 1999) and even in the fruit fly (Zhang *et al.* 2001). In both lymphosarcoma cells and rodent hepatomas, the cytosine-guanine islands in the promoter region of the MT-1 gene have been found to be completely methylated and, thus, the transcription of this metallothionein is silenced; the reason of this remains unknown. However, also observed is that when the promoter region of the MT-1 gene is demethylated—and is, therefore, overexpressed—, MT-1, either alone or in combination with other factors, may suppress the growth of some tumours (Ghoshal *et al.* 2002). The Metal-Responsive Transcription Factor is also essential for liver development in embryos, as well as for the completion of pregnancy. By contrast, during adulthood it is not essential and its deficiency does not lead to death (Wimmer *et al.* 2005).

No induction of the gene encoding MT-1 occurs in rat hepatomas and mouse lymphosarcomas. This is because most cytosines are methylated in CpG dinucleotides present in the promoter region of the gene. In hepatomas, the maintenance activity as *de-novo* DNA methyltransferases 3a and 3b is three to six times as high as in the liver of animals where the MT-1 gene has no methylated cytosines. The same pattern was observed in the liver and thymus of animals with hepatomas and lymphosarcoma (Jacob *et al.* 2002). The fact the same gene is not induced in one tissue (*e. g.* tumour) while it is in the tissue of an adjacent organ (*e. g.* liver), has been attributed to the intervention of methylation promoters in the diseased tissue (Waalkes *et al.* 1996; Jacob *et al.* 2002).

A given pollutant preventing the expression of a gene associated with a detoxification mechanism does not mean that the same happens to other genes also involved in the elimination of toxic substances from the body. In the cellular detoxification process, there are protein families other than metallothioneins that also participate in the removal of pollutants or noxious substances from cells. These include Glutathione S-Transferases (GSTs), which are a group of enzymes involved in the elimination of endogenous compounds, as well as of environmental pollutants and drugs. GST levels increase in the presence of products derived from oxidative stress and inflammatory processes caused by trace elements in above-normal concentrations in the organism, among other reasons. This is why the gene encoding Glutathione S-Transferase (GSTP1) has been proposed as a “caregiver” gene (Nakayama *et al.* 2004). Hypermethylation of the GSTP1 gene—and, therefore, its inactivation—has been associated with various forms of cancer, and occurs in over 70 % of high-grade intraepithelial neoplasias.

In rodent hepatoma and lymphosarcoma, it has been observed that when GSTP1 is activated, MT-1 is not. Conversely, MT-1 was induced by cadmium in tumours in which GSTP1 is suppressed due to methylation of the promoter. The mechanism involved is highly complex, as the lack of MT-1 is due to two reasons: the methylation of the transcription factor (MTF-1, as previously mentioned), without which the gene cannot be expressed; and an increased activity of the repressors of this gene, including the Nuclear Factor I (NFI; Majumder *et al.* 2001; Jacob *et al.* 2002). Also important is that, paradoxically, tumour-suppressor genes such as p53 and p16 are expressed in these same cancer cells (Jacob *et al.* 2002), which would be expected to be suppressed in these cells. Among all these



mechanisms, the potential importance of MT-1 as tumour suppressors is worth noting, in addition to their important role in metal homeostasis.

The evidence summarized above shows that exposure to contamination levels of trace elements can alter the overall DNA methylation patterns, depending on the number of genes that are affected and become either over- or under-expressed, or even silenced. However, it should be stressed that, in most cases, the effect of trace elements is not associated with mutations but with epigenetic processes. In rodents, over 200 genes have been associated with reproductive impairment, including chromosomal aberrations and infertility (Matzuk and Lamb 2002), when these are damaged by some factor, including exposure to pollutants. This highlights the importance of considering changes in DNA methylation patterns as a result of the toxic effects of trace elements, and of including this approach in environmental studies.

## Acknowledgements

This article is one of the outcomes of the author's stay at the University of California, Berkeley, made possible by a grant from CONACyT (203952, 151189). The authors are grateful to Maria Elena Sanchez Salazar whom provided valuable editorial services.

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*Sometido: 11 de septiembre de 2014*

*Revisado: 1 de octubre de 2014*

*Aceptado: 14 de noviembre de 2014*

*Editor asociado: Consuelo Lorenzo*