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The effects of Bisphenol-A, Zearalenone and Arsenic on specific brain areas

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1. Abbreviations

AR androgen receptor

ARC arcuate nucleus

As arsenic

ATP adenosine triphosphate

AVPV hypothalamic anteroventral periventricular nucleus

BBB blood brain barrier

BPA bisphenol A

CNS central nervous system

DA dopamine

E1 estrone

E2 17β-estradiol

E3 estriol

EAC endocrine active compounds

ERE estrogen responsive elements

ED endocrine disruptors

EDC endocrine disrupting chemicals

ERs estrogen receptors

FSH follicle stimulating hormone

GnRH gonadotropin-releasing hormone

HPG hypothalamic – pituitary - gonadal axis

HPT hypothalamic – pituitary - thyroid axis

LC locus coeruleus

LH luteinizing hormone

NPCs neural progenitor cells

OPCs oligodendrocyte progenitor cells

ROS reactive oxygen species

T3 triiodothyronine

T4 thyroxine

TH thyroid hormone

ZEA zearalenone

2. Introduction

Human and animal health depends on the ability to reproduce and develop normally. This is only possible with a healthy functioning endocrine system. Over 800 chemicals are known to be capable of interfering with hormone receptors, hormone synthesis and hormone conversion. Bergman and colleagues (2013) suggest that only a small fraction of these chemicals have been investigated in tests capable of identifying endocrine effects in organisms. This lack of data leads to uncertainties about the true extent of risks from chemicals that could disrupt the endocrine system. The European Commission describes endocrine disrupting chemical as an "exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function" (Trasande et al., 2015). EDCs were defined by the US Environmental Protection Agency (EPA) as 'an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present and are responsible for homeostasis, reproduction, and developmental process' (Diamanti-Kandarakis et al., 2009). These group of molecules include synthetic chemicals and natural compounds.

In the 70s reproductive problems were observed in wildlife populations and a link was made with exposure to chemicals with hormonal activity. Hormones are important to vertebrates and invertebrates. They are essential for cell differentiation during embryonic development, organ formation, and control of tissue and organ function when the organism reaches adulthood. Estrogen has effects that extend beyond regulation of reproductive functions these include neuroprotective properties. The interference of an exogenous substance with the hormonal system is termed as endocrine disruption and the substances themselves were termed endocrine disruptors. Research of EDs first began on oestrogenic compounds like plastic monomers (bisphenol A), organochlorine pesticides (like DDT), plasticisers like phthalates and constituents/degradation products of detergents (alkyl phenols). It was discovered that hormonal systems other than oestrogen signalling could be disturbed by EDs and multiple mechanisms could be involved. EDs may act via nuclear receptors, non-nuclear steroid hormone receptors (membrane receptors), non-steroid receptors (neurotransmitter receptors), orphan receptors (aryl hydrocarbon receptor), enzymatic pathways involved in the hormone synthesis and metabolism, distribution of hormones within the body, and many other mechanisms that converge upon the endocrine, reproductive and nervous system.

Let’s further review three estrogenic endocrine disruptors and their effect on the brain and other systems.

3. Endocrine Disrupters

Ana Soto was the first to use term endocrine disruptors, she identified several developmental effects of EDCs in wildlife and humans. EDs were thought to perform in one of two ways. One way is that it binds to a steroid receptor mimicking the normal hormone which leads to improper activation of the receptor. The second one is that the ED binds to the receptor and inactivates the ability of the organism’s hormone to active the receptor.

EDCs are environmental agents that are organic, or man-made. Organic EDCs have always been around, they are active in soy, alfalfa, legumes and other plants. Humans have been ingesting these in small amounts for thousands of years. Man-mad EDCs are pesticides, insecticides, industrial chemicals, plasticisers and surfactants. These have been un use for a shorter time. Some EDCs persist in the environment causing them to bioaccumulate through food webs to high concentrations in wildlife and humans. Others with shorter half-lives persist less in the environment which in turn do not remain in humans and wildlife for very long (they are not bio-accumulative). However, exposure to all EDCs can be continual so all represent a concern.

Figure 1 shows the many ways endocrine disruptors can enter the organism from the environment. EDs enters the organism by oral ingestion, mucosal absorption, and skin penetration. The diverse variety of environmental sources as seen in Figure 1 shows the complexity of controlling the source of exposure. This increased exposure has been associated with a host of different ailments include metabolic disorders like obesity, diabetes, and metabolic syndrome.

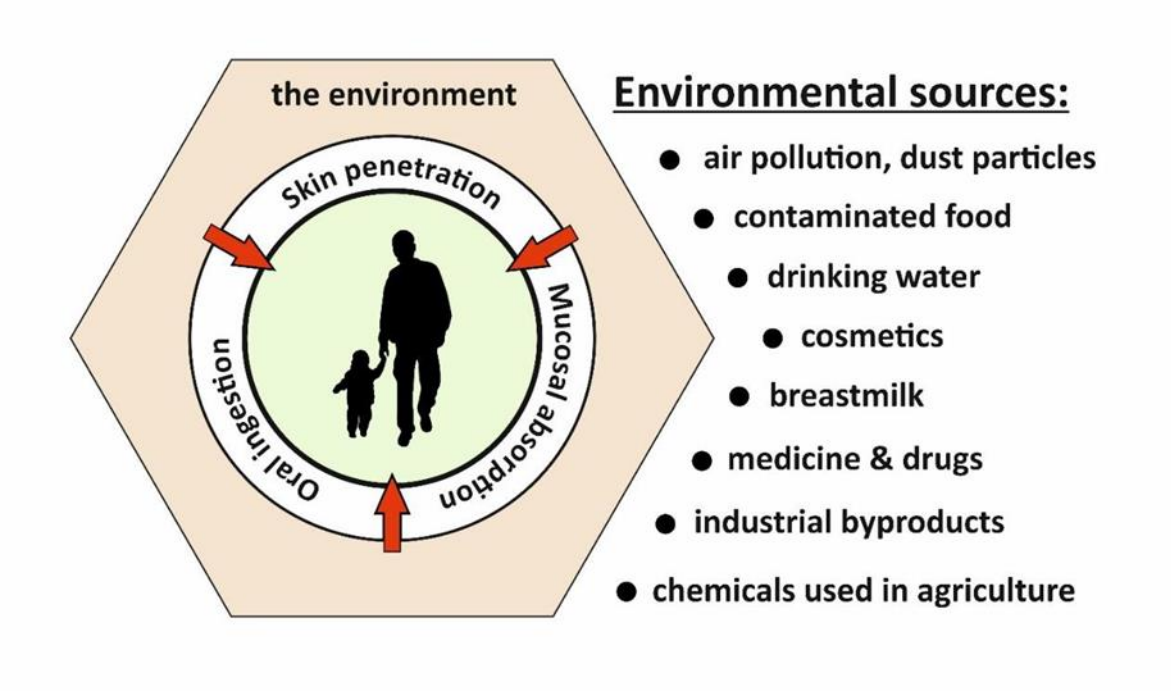


Figure 1: Sources of endocrine disruptor (ED) exposure

EDs usually act through different signalling pathways found in the cell. Estrogen like endocrine disrupting chemicals encompass a group of natural phytoestrogens and manmade compounds that interfere with the development and physiology of estorgen sensitive tissues. With some EDs; with a similar estrogen like structure; a specific part of the intracellular signalling pathway will be either activated or inactivated by the molecules. Arnold and colleagues (2012) suggest that E2 may act on steroid receptors found in the mitochondria which in turn influences the cellular energy balance. The crosstalk between the mitochondrial effects and the genomic and non-genomic pathways will lead to the final response in the cell (Zane et al., 2014). The type of disruption depends on the type of ED however all biochemical processes may be altered between the receptor activation and gene transcription. Lipophilic EDCs can bind to nuclear receptors; through many different mechanisms of action; and can displace the endogenous ligands to modulate hormone responsive pathways.

4. Endocrine system

The endocrine system consists of a network of glands which secret hormones and theses chemical messengers cooperate in growth, development, metabolism, and reproductive functions.

Estrogens exert a vast variety of effects in humans and wildlife. They regulate development and growth by inducing cell proliferation and cell differentiation. The development and physiology of the female reproductive system are susceptible to the effects of environmental chemicals. And reproductive disorders may be from primary disruptions in the development and physiology of key neural networks of hypothalamus rather than the ovaries.

4.1 Role of Estrogen

Estrogens are steroid hormones that include estrone, estriol and 17β- estradiol (E2), the last of which is the most potent endogenous estrogenic compound (Heldring et al., 2007). Estrogens act via three estrogen receptors (ERs) that are Erα, Erβ and the structurally unrelated G protein coupled ER1(GPER1) (Almey et al., 2015). Erα and Erβ are a part of the nuclear receptor superfamily. They form homodimers to bind to DNA. Estrogens (the endogenous steroid 17β estradiol (E2)) enter the cytoplasm and cell nuclear by passive diffusion and then bind to dormant ER causing the shedding of heat shock proteins, acquisition of coactivators and formation of homo or hetero dimers. The then activated ER dimers represent ligand-activated transcription factors and bind to estrogen responsive elements (ERE) of estrogen regulated genes which initiates mRNA synthesis and synthesis of the corresponding gene product. Recent studies indicate that all three types of receptors are localized in the cell membrane allowing rapid non-genomic effects that alter membrane permeability and activates second messenger cascades (Almey et al., 2015). Almey and colleagues (2015) also suggests that estrogen receptors are expressed in the central nervous system (CNS) structures associated with reproductive functions such as the hypothalamus and various other areas in the brain such as the hippocampus, amygdala, cerebellum, and different cortical regions in both sexes.

Estrogens and thyroid hormones (THs) play critical roles in the regulation of neural development. Belcher (1999) found in the postnatal period in rats that estrogen receptors are expressed in the cerebellum form an early age in a developmentally regulated fashion. During the postnatal life the Erβ receptor can be found on the Purkinje cells, stellar cells, basket cells

and Golgi cells. Price and Handa (2000) noted that migrating glial cells and the developing neurites also express the Erβ receptor protein. Thyroid hormones also target all cell types in the cerebellum (Wallis et al., 2010). Abnormalities in the hormonal milieu and in the expression/function of ERs and TRs result in changes in the normal development of certain brain areas. Kuppers (2001) gives evidence that estrogen affects the central dopamine (DA) system and more studies have revealed that some EDCs disrupt the development of DA neurons in the brain. Estrogens are important trophic factors for neurons, enhancing synaptogenesis during ontogenesis but their neuroprotective effects have been demonstrated in adulthood.

The role of ERs have been demonstrated in cognitive functions and memory processes (Lu et al., 2019). Sheppard and colleagues (2019) describe the effects of E2 and other estrogenic compounds on neuronal excitability and plasticity in the CA1 region of the hippocampus. Zadran et al., (2009) demonstrated that E2 increased glutamate-mediated neuronal excitability in hippocampal rat brain slices and in cultured hippocampal rat neurons in vitro. Erβ plays a critical role in the development of the CNS, neuronal network formation and in establishing the neuroendocrine signalling between the hypothalamus and the reproductive organs and maintenance of a healthy spermatogenesis in males (Hess et al., 1997). So, any disturbance in the balance of ERs will likely lead to cognitive deficiencies, developmental disorders and tumours in the CNS and reproductive system.

Hypothalamus neuroendocrine networks coordinate the regulation of gonadotropin- releasing hormone (GnRH) secretin. Gonadotropin releasing hormone, the principal regulator of reproduction, acts on the anterior pituitary to stimulate the release of the gonadotrophic hormones and gonadal function. In rodents, the specific neuronal populations of hypothalamic anteroventral periventricular (AVPV) nucleus and arcuate (ARC) nucleus exert regulatory control over the reproductive axis. Kisspeptin neural circuit is the master player of the reproductive axis acting upstream in the GnRH neurons. Kisspeptin neurons have been found to express estrogen receptors (ERs) that integrate central and peripheral signals with GnRH release in female rodents and humans.

4.2 Role of Thyroid Hormones

The thyroid gland is the largest endocrine organ in the human body whose function is the systemic metabolic regulation through thyroid hormones (THs) produced by follicular cells and calcitonin produced by parafollicular cells. In both humans and animals, the thyroid hormone is essential for normal brain development. It is becoming clear that mild TH insufficiency can affect cognitive outcome in humans and the developmental timing of transient TH signalling by selectively interfering with TH receptors. The consequences to brain development may be a mosaic of effects on the nervous system because different TRs medicate different actions of TH during development. It has been found that in individuals with hypothyroidism the migration and terminal differentiation of granule cell precursors could be impaired (Faquier et al., 2014).

In animals where the TRα gene is modified (knock-out or knock-in), cardiac functions, thermogenesis, haematopoiesis, and the maturation of specific tissues (e.g. intestines and bones) show pathological alterations.TRβ is required to maintain normal hepatic reactions to THs, it is crucial in regulating the hypothalamic–pituitary–thyroid axis by enabling the TH signals and balancing the hormonal feedback loop, and also essential to sustain the physiology of specific sensory functions (Amma et al., 2001). Environmental chemicals change the steroid hormone- and TH-stimulated gene transcription and translation, different EDs (BPA, ZEA, As) could influence the expression of TRα, TRβ and ERβ mRNA and protein with special emphasis on the potential effects after the co-administration of them with or without treatment of E2, T3 and T4.

4.3 Brain and Endocrine Disruptors

The brain is an essential organ for life and is one of the main parts of the central nervous system.

ERα and ERβ are expressed throughout the rostral–caudal extent of the brain and spinal cord. Brain regions, including the bed nucleus of the stria terminalis (BNST), medial and cortical amygdaloid nuclei, preoptic area (POA), lateral habenula, periaqueductal grey, parabrachial nucleus, locus coeruleus, nucleus of the solitary tract, spinal trigeminal nucleus and superficial laminae of the spinal cord, express both forms of ER (Weiser et al., 2008). Although both receptors are expressed by neurons in the arcuate nucleus and hippocampus, ERα is more

abundant in the arcuate nucleus, and ERβ is more prevalent in the hippocampus (Mitra et al., 2003).

i. Sexual differentiation

The male reproduction in vertebrates is tightly regulated by the brain-pituitary-gonadal axis. The brain secretes gonadotropin-releasing hormone (GnRH) which stimulates the pituitary to produce follicle-stimulating hormone (FSH) and luteinizing hormone (LH) which control Leydig cells to produce and secrete testosterone necessary for spermatogenesis and regulation of the male phenotype.

Hypothalamus is in the diencephalon part of the brain. The hormones secreted from hypothalamus directly and indirectly regulate the pituitary gland, also known as master gland. The hypothalamic-hypophyseal portal system is recruited for this communication. It should be noted that sex differences reside in the hypothalamus. These differences emerge during the end of gestation and shortly after birth and are caused by androgens direct or indirect actions in males. Indirect actions of androgens are mediated by estrogen receptors after testosterone is aromatized to E2 in the hypothalamus.

Prior studies demonstrated that the maintenance of neuronal populations, especially kisspeptin population, in both AVPV and ARC is essential for the maintenance of normal oestrus cyclicity. There are a number of steroid-sensitive neuronal populations located in the AVPV and ARC nuclei, playing critical roles in the driving of LH surge, oestrus cyclicity, and ovulation. Dopaminergic neurons in the AVPV are confirmed to be sexually dimorphic and larger in female rats than in male rats and neonatal testosterone causes masculinization of AVPV dopaminergic neurons. Both AVPV and ARC cellular populations have long been considered the principal site for neuroendocrine control of the reproductive axis and are essential for oestrus cyclicity. It has been generally hypothesized that estrogen and other EEDCs are largely conveyed to GnRH neurons from estrogen sensitive nuclei within the hypothalamus, in particular, the AVPV and ARC. Decreased kisspeptin or the lesion to AVPV both suppress estrogen-induced LH surges and disrupt the oestrus cycles. ARC is the principal original of GnRH pulse generation in rodents and essential for the maintenance of normal oestrus cycle.

ii. Learning and behaviour

The hippocampus controls learning and memory behaviour, and alterations in myelination process as well as neurogenesis in this area have been reported in a wide range of psychiatric illnesses including autism, anxiety, schizophrenia, multiple sclerosis, temporal lobe epilepsy, Alzheimer's disease, depression, and cognitive deficits. Estrogen is a well-known regulator of mood, with reported effects of estradiol treatment ranging from depressant to antidepressant. Several studies have consistently reported that Major Depressive Disorder episodes are twice as common in women as compared to men (Llewellyn et al., 1997).

Oligodendrocytes play a pivotal role in formation of myelin, i.e., myelination process in the CNS during the brain development. Myelin is a multi-layered compacted membrane, which surrounds and electrically insulates the axons, facilitating faster conduction of the nerve impulses. OPCs are the source of immature oligodendrocytes that can undergo a complex and precisely timed program of proliferation, migration, and differentiation into myelin-generating cells. Adult natural stem cells and precursor cells participate in remyelination during adult stages. It is evident that brain functions largely depend on intimate and bidirectional signalling ways between neuron and glia. This bidirectional signalling happens at least in two ways: through direct contact between the cellular elements or in paracrine way. Glial cell count and astrocyte density in specific brain areas may decrease due to many factors. Foreign substances can cause cell cytotoxicity in the glial tissue and even emotional disorders may affect the physiological cell count (Frintrop et al., 2017). Due to the cumulative effects of possible EDs, toxins, neurological and emotional disorders that damage the physiological state of the neuronal tissue, it is not negligible to discuss the results from Glia cultures. Data suggest that EDC might affect monoaminergic neurons in the CNS, possibly leading to behavioural changes.

5. Bisphenol- A

5.1 Introduction

Bisphenol- A (BPA) is a precursor to plastics, polycarbonates, epoxy resins and some polysulfones. BPA-based plastic is clear, tough, and flame resistant. BPA is found in major areas which include electronics, construction materials (windows), automotive (headlight covers) and even data storage (CDs). Food storage containers, baby bottles, water containers and dental fillings are some examples of BPA being commonly used to produce these niche items. It is also used as an additive to PVC and BPAs halogenated derivatives are used as flame retardants. Bisphenol A (BPA) is a high-volume-production monomer (>2.5 × 106 kg year). Because unbound monomers remain after BPA polymerization, BPA molecules can be released from beverage and food containers, for example, from plastic baby bottles or from tin can liners. Human exposure to BPA is thus widespread, and unconjugated BPA molecules are detected in human blood, tissues, and milk.

BPA is a xenoestrogen, exhibiting estrogen-mimicking, hormone-like properties (see Figure 2) (Egan, 2013). It has been known to cause a weak effect however the pervasiveness of BPA containing materials still raises concerns. Bisphenol A (BPA) is known as one of the oldest synthetic compounds with endocrine disrupting activity. The British biochemist Edward Charles Dodds tested BPA as an artificial estrogen in the early 1930s. He found that BPA is 1/37000 as effective as estradiol. This eventually led Dodds to develop a structurally similar compound known as diethylstilbesterol (DES). This was used as a synthetic estrogen drug in woman and animals until the eventual ban due to its cancerous effects.

BPA is associated with various health problems such as obesity, diabetes, chronic respiratory diseases, cardiovascular diseases, renal diseases, behaviour disorders, breast cancer, tooth development disorders, and reproductive disorders. Increasing health concerns have led the industry to seek alternatives to BPA. As BPA is now being excluded from several consumer products, the use of alternative compounds is increasing. However, the chemicals used to replace BPA are also BP analogues and may have similar or higher toxicological effects on organisms. Bisphenol-A was detected in numerous species and, surprisingly, the levels were up to 12-fold higher in juveniles than adults (vom Saal et al., 1998).

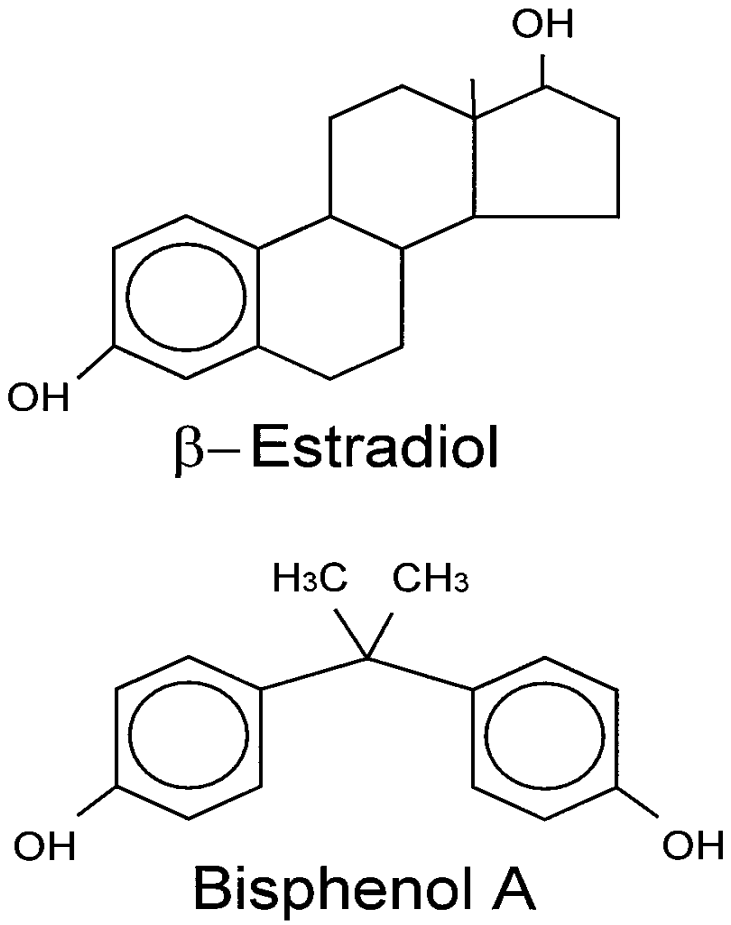


Figure 2: Chemical structure of Estradiol and Bisphenol A; showing the similarities between the molecules.

Figure 2 shows the structural similarities between BPA and estradiol which explains why BPA has been found to bind to both nuclear estrogen receptors (ERs), ERα and ERβ. It is 1000- to 2000-fold less potent than estradiol. BPA can both mimic the action of estrogen (see figure 2 on the structural similarities) and antagonize estrogen. This indicates that it can be a selective estrogen receptor modulator (SERM) or a partial agonist of the ER. BPA, at high concentrations, will bind to and act as an antagonist to the androgen receptor (AR) (Teng et al., 2013). BPA has also been found to affect Leydig cell steroidgenesis. This includes affecting 17α-hydroxylase and17,20 lyase and aromatase expression. It also interferes with LH receptor-ligand binding.

BPA affects growth, reproduction, and development in aquatic organisms with fish being the most sensitive. Fish, aquatic invertebrates, amphibians, and reptiles have all been reported to have endocrine effects at environmentally relevant exposure levels lower than those required for acute toxicity. The range varies from1μg/L to 1 mg/L for endocrine-related effects.

BPA has been reported in concentrations of 1–10 ng/ml in serum of pregnant women and cord serum taken at birth. Ikezuki et al. (2002) found BPA to be 5-fold higher in amniotic fluid at 15–18 weeks of gestation, compared with maternal serum, and was found in concentrations of up to 100 ng/g in placenta. It can therefore be hypothesized that the human population is widely exposed to BPA with concentrations accumulating in the foetus.

5.2 Effects of Bisphenol-A on the Brain

It should be noted which further complicates a clear understanding of BPA's effects and mechanism of action is the high degree of variability in the dosing regimen in published reports. It has been hypothesized that BPA's actions are most potent in hormone-sensitive organs and in brain regions when endogenous steroids are particularly low.

There have been several articles examining the effects of BPA in vivo or vitro on the developing and adult brain. Embryonic exposure to BPA has been shown to disrupt normal neocortical development (Nakamura et al., 2006) and adult cortical organization (Nakamura et al., 2007). An in vitro study by Yokosuka et al. (2008) showed that BPA induces changes in both dendritic and synaptic development. They study was on cultured foetal rat hypothalamic cells.

Kobayashi (2020) revealed that BPA induced oxidative stress and changed ROS-induced signal pathways associated with tau-related proteins in the plasma and brain of rats. They found that BPA administration resulted in a reduction in ROS scavenging capacity in plasma, indicating that BPA can decrease the antioxidative capacity (see Figure 3). These results show that a reduction in antioxidant activity correlates with dose-dependent BPA-induced ROS activity and these results are consistent with previous research. Higher doses of BPA (for example, 30 mg/kg/day for six weeks, which is 30,000 times concentration of the dose used in studies) have been shown to induce oxidative damage in the liver and heart of rats. Figure 3 shows that BPA-induced free radicals triggered oxidative stress in the brain/hippocampus and significantly increased oxDJ-1/DJ-1 ratios after eight weeks of treatment. BPA-induced oxidative stress in rat sperm is protected by the administration of the antioxidant N-acetylcysteine (Minamiyama et al., 2010). So, it can be assumed that antioxidants can provide the same protection in the brain.

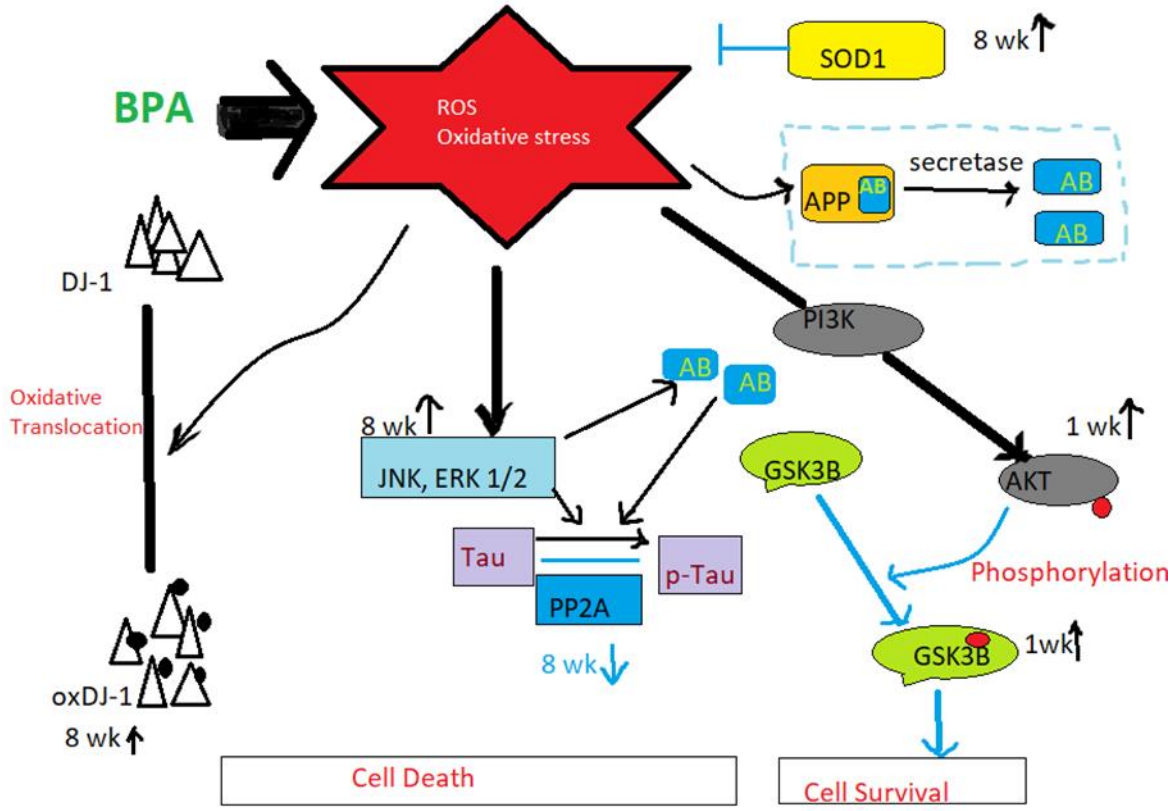


Figure 3: Hypothetical pathways of BPA-induced oxidative stress and tau-related proteins. This figure is based on research done by Kobayashi (2020) showing that BPA-induced free radicals triggered oxidative stress in the brain/hippocampus and significantly increased oxDJ-1/DJ-1 ratios after eight weeks of treatment in rats. Protein phosphatase 2A (PP2A (blue)) in the hippocampus decreased after eight weeks of BPA treatment. The ratio of phosphorylated-GSK3β/GSK3β (green) and phosphorylated-AKT/AKT increased after one week of BPA treatment. The ratio of phosphorylated JNK/JNK and phosphorylated-ERK/ERK (light blue) increased after eight weeks of BPA.

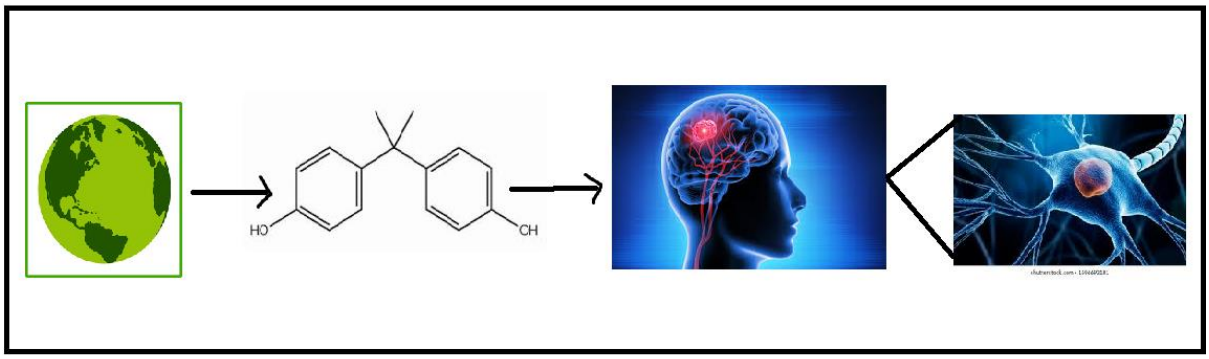
Oka et al. (2003) demonstrated that BPA produced abnormal development and apoptosis specifically in the CNS during early development of *Xenopus laevis* embryos and suggested that these effects were due to non-estrogenic actions on developmental processes.

BPA acts as an estrogen or an anti-estrogen in the hypothalamic region it can be hypothesized that some sexually dimorphic areas could be affected. Sexually dimorphic area is the anteroventral periventricular nucleus of the hypothalamus (AVPV). This area is sexually dimorphic both in the number of total neurons and also the numbers of tyrosine hydroxylase (TH) containing neurons, which, in this area of the brain, are producing dopamine. In rats, injections of BPA for the first 2 days after birth increases AVPV cell numbers in males, making them nearly equal to the numbers of TH cells in the normal female. Estradiol injections have

no effect on TH cell numbers in males. These data suggest an anti-estrogen action of BPA in this neural cell population (Patisaul et al., 2006). The sexually dimorphic nucleus (SDN) is a cluster of neurons that is smaller in females than in males and can be visualized with either general neuronal staining or using antibodies to calbindin A sex difference between normal males and females was found and females treated as neonates with BPA had more calbindin stained cells than control females. The result suggests that BPA has an estrogen-like action in the developing rat SDN.

BPA's effects on the brain, genome and behaviour are wide-ranging. These include changes in the structural development of the brain, in DNA methylation of the genome, disruption of estrogen regulation at many levels and effects on social behaviour, anxiety, and maternal behaviour. BPA's behavioural effects are related to anxiety, attention and processing of stimuli, the dopaminergic cell population in the midbrain is worth discussion. BPA exposure during prenatal, postnatal, and adulthood periods affects neurotransmitter system, hippocampal neurogenesis, and synaptic transmission, leading to impaired learning and memory and neurobehavior in the rodents. It is also indicated that BPA affects spinogenesis and neurogenesis in the hippocampus, followed by behavioural changes in the rodents and non-human primates.

Figure 4 shows BPA as a low potency estrogenic compound, it could weakly activate ERα, ERβ, ERR-γ and/or inhibit AR, altering the expression of genes responsive to these receptors. It may also act as an agent that affects DNA methylation, changing gene expression patterns during development in primarily hormone-sensitive cells. It may also be a combination of these two pathways where BPA affects the transcription of an estrogen responsive gene and the methylation level of that same gene.



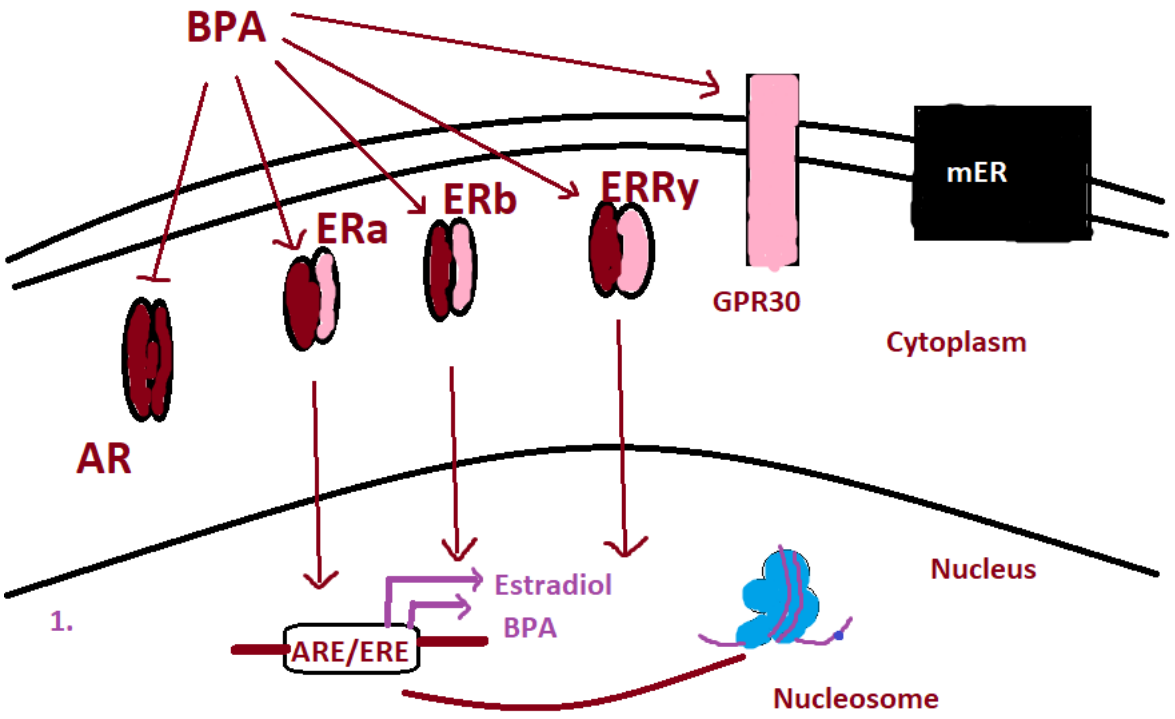


Figure 4: Environmental exposure to BPA affects the developing brain and behaviour. Acting as an endocrine activating compound, BPA can weakly activate several estrogen (ER) and estrogen-related receptors such as ERα, ERβ, ERR-γ, membrane ER (mER) and GPR30 and/or antagonize the androgen receptor (AR). BPA is a low potency activator of these receptors and may change the expression of genes containing estrogen (ERE) or androgen (ARE) response elements in comparison to the endogenous ligand. This gene activation may in turn lead to downstream epigenetic changes like DNA methylation or histone modifications which could further impact transcription.

As shown in Figure 4 BPA is a low potency activator of endocrine receptors and may change the expression of genes containing estrogen (ERE) or androgen (ARE) response elements in comparison to the endogenous ligand. This gene activation may in turn lead to downstream epigenetic changes like DNA methylation or histone modifications which could further impact transcription. At the chromatin level, BPA may act to change DNA methylation at specific loci, although the specificity and exact mechanism has not been determined. A combined action of BPA is also possible where BPA changes the methylation state of specific regions of an estrogen responsive gene or the genes that code for the receptors themselves, ESR1 for example, and alters its expression by changing chromatin structure and allowing the binding of transcription factors (TF).

Tiwari (2014) found that BPA accumulates in the placenta and mother milk; it may transfer to the foetus and infants during gestational and lactational periods. Therefore, children are at higher risk with BPA exposure and associated neurotoxicity. Further, lipophilic nature of BPA permits it to easily penetrate and accumulate into the brain, causing harmful effects on the human and animal health. Numerous studies showed that BPA exerts adverse effects on immune function, reproductive system, and neurological behaviour. Several clinical and experimental studies suggest that exposure of BPA causes cognitive and behavioural alterations and hampers the normal brain development in the human and animals. BPA affects epigenetic changes in promoter associated CpG islands, thus alters the brain development and social behaviour in the rodents. It also causes alterations in synaptic arrangements in the hippocampus that may potentially lead to schizophrenia, depression, and cognitive dysfunctions.

Interestingly, BPA alters proliferation and differentiation of multipotent neural progenitor cells (NPCs) and modulates spinogenesis in the adult hippocampal neurons. It causes developmental neurotoxicity by inhibiting proliferation and differentiation of the rat embryonic midbrain neurons. BPA inhibits differentiation of oligodendrocyte progenitor cells (OPCs), which are involved in the regulation of the myelination and remyelination processes in the brain. There have been studies in rodent and monkey hippocampus using much lower doses of BPA (40–50 μg/kg/day) demonstrated an anti-estrogenic action on synaptogenesis (MacLusky et al., 2005;) In adult female brains E2 enhanced synapse formation, and this effect was blocked by concurrent BPA treatment.

Effects on the Hippocampus

The effects of BPA on hippocampal functions have been investigated, as this region of the brain plays important roles in memory and learning and is particularly sensitive to stressors. MacLusky and colleagues (2005) demonstrated that treatment with BPA in ovariectomized rats concentration-dependently inhibited the estrogen-induced formation of dendritic spine synapses on pyramidal neurons in hippocampal CA1 region.

Data suggests that BPA might interfere with the development and expression of normal gender differences in cognitive function via inhibition of estrogen-dependent hippocampal synapse formation. MacLusky et al. (2005) further noted that BPA might also exacerbate the impairment of hippocampal function during normal aging, as a decrement of endogenous estrogen production. However, Kim and colleagues (2009) observed that treatment with BPA (20 mg⁄kg bw) accelerated the formation of the dentate gyrus in mice at postnatal day 1. Prenatal and postnatal BPA treatment did not affect adult hippocampal neurogenesis in the dentate gyrus. Bisphenol-A attenuated the proliferation of murine-derived multipotent neural progenitor cells and showed cytotoxicity at high concentrations in vitro. Low concentrations of BPA, which possess estrogenic activity, stimulated these cells to differentiate into a neuronal phenotype. Thus, BPA might stimulate neuronal differentiation and disrupt neonatal brain development.

Tiwari (2014) suggests that BPA causes overt neurotoxicity and cognitive and behavioural alterations in the animals and humans. BPA gets accumulated in the different tissues including the brain during lifetime. Maternal BPA exposure reduces body weight and causes developmental abnormalities in the offspring. BPA induces apoptosis in the hippocampal neurons by inducing oxidative stress and altering the MAPK signalling. Previous studies also suggested impairments in learning and memory abilities and motor dysfunctions in the offspring following maternal BPA exposure. These studies underpin a specific role of BPA in the induction of neurotoxicity and behavioural alterations in the human and animals.

However, the effects of BPA on myelination in the hippocampus region during prenatal and postnatal periods are still somewhat unknown. Hippocampus is the major functional unit of the mammalian brain, which is involved in memory, spatial recognition, and navigation processes. The hippocampus contains several myelinated axons that profoundly affect learning and memory via regulation of the information processing in the neural circuits.

It is widely accepted that myelin enhances the speed and efficacy of axon conduction. Myelination is a highly specialized and tightly regulated process, which involved proliferation, migration, and differentiation of OPCs into myelin-generating cells in the CNS during development and throughout the adulthood. Oligodendrocytes are involved in the formation of myelin sheath. Demyelination in the hippocampal axons was reported in several neurodegenerative and neurological. BPA exhibits biphasic response toward proliferation of OPCs. It was found that BPA at concentrations of 50‑500 μM induces apoptosis and cell death, while at lower concentrations (<10 μM) increases neurite extension in neuronal cells and primary neuronal culture BPA treatment decreased differentiation of OPCs, resulting in significant reduction of the number of CNPase+, PDGFR-α+, MBP+, and Olig-1+ cells in culture (Tiwari, 2014).

Considering the cholinergic system in the hippocampus, prenatal and neonatal exposure of mice to BPA was reported to induce memory impairment and marked reduction in choline acetyltransferase-like immunoreactivity in this area (Honma et al., 2006). The memory impairment produced by BPA might therefore be associated with reduction in acetylcholine production in the hippocampus. Thus, EDC potentially attenuate memory function via decrements not only in NMDAR but also in cholinergic neurons.

The offspring of the BPA-exposed group showed no sexual dimorphism in the locus coeruleus (LC) volume. It was concluded that the absence of normal gender differences in LC and behavioural abnormalities in offspring were due to a delayed effect of BPA exposure during the foetal and suckling periods. Overall, BPA exerted similar effects to estrogen on sexual differentiation in LC and on behaviour. The critical period of sexual differentiation of the brain and behaviour in the rat extends from the late foetal stage until the first week after birth (MacLusky 2005). This was also the period when the rats were treated with BPA in the study by Kubo and colleagues (2001).

Noradrenaline (NA) containing neurons are densely distributed in the LC, and affect the limbic system, which plays important roles in both open-field behaviour and passive avoidance learning (Heybach et al., 1978). The morphological changes in the LC induced by BPA (Kubo et al., 2001) might therefore be associated with behavioural changes. One group of investigators undertook to investigate how bisphenol A might alter neuro- and synaptogenesis in adult brains, a choice derived from its estrogenic properties. They have now shown that bisphenol A interferes with this process at dose levels presumed free from adverse effects. Overall, it

inhibits hippocampal synaptogenesis induced by estradiol in ovariectomized rats; It prevents synaptogenesis induced by testosterone in both control and gonadectomized adult male rats in the hippocampus and prefrontal cortex; It prevents synaptogenesis, induced by estradiol, in the hippocampus and prefrontal cortex of ovariectomized female monkeys.

BPA might produce alterations not only in DA elements but also in G-protein-coupled receptors involved in the neurotransmission of various peptides (Ishido et al., 2005). The notion that exposure to BPA during the foetal period disrupts the development of DA neurons is supported by other studies. In vitro exposure to BPA appears to influence the function of DA neurons (Yoneda et al., 2003). In mice, the DA system appears to be most susceptible to the effects of BPA during organogenesis and lactation periods (Narita et al., 2007). Tian et al. (2010) demonstrated that prenatal and postnatal treatment with BPA (100 μg/kg body weight [bw]/d) increased motor behaviour in the central zone in an open field test. Autoradiographic receptor-binding assays for the DA D1 and DA D2 receptors, and the DA transporter (DAT), revealed that BPA treatment increased the density of D2 receptor binding in the striatum while decreasing DAT binding. This suggests that chronic exposure to BPA might induce alterations in the DA system. Considering neurotransmitters and their metabolites, Honma et al. (2006) demonstrated that prenatal and postnatal exposure to BPA altered levels of DA, NA, 3,4-dihydroxyphenylacetic acid, homovanillic acid, serotonin, its metabolite 5-hydroxyindoleacetic acid, acetylcholine, and choline in 4 regions of the brain in 3-wk-old rats, and in 8 regions in 6-wk-old rats and rat dams. Data suggested that these changes are related to the estrogenic activity of BPA.

Nakamura et al. (2010) studied the concentrations of neurotransmitters in the brain after the subcutaneous injection of BPA (20 μg/kg bw/d) in pregnant mice, from embryonic day 0 until postnatal day 21. DA, 5-HT, and its metabolites in the striatum, dorsal raphe, thalamus, and substantia nigra were assessed at postnatal week 3 and/or 14–15. Prenatal and lactational exposures to BPA were found to perturb neurotransmitter systems in adulthood.

An electrophysiological study by Zhou et al. (2009) provided functional evidence that prenatal and neonatal exposure to low-dose BPA (20 μg/kg bw/d) disrupted the development of long-term potentiation (LTP) and long-term depression (LTD) in the dorsolateral striatum by altering the function of DA receptors in male rats. It is thus possible that transient exposure to EDC might exert significant effects on the CNS.

Moriyama et al. (2002) reported that the estrogenic compound BPA (4,4'-isopropylidenediphenol) binds to the thyroid receptor (TR). BPA is the first environmental chemical known to bind to the TR and affect TH signalling in vitro. BPA binds to and antagonizes T3 activation of the TR. BPA reduced T3-mediated gene expression in culture by enhancing the interaction with the nuclear receptor corepressor (N-CoR). Maternal exposure to BPA caused an increase in serum T4 of both male and female pups but simultaneously increased the expression of RC3/ neurogranin in the hippocampus.

6. Zearalenone

6.1 Introduction

Mycotoxins are highly toxic filamentous fungi secondary metabolites that contaminate agricultural commodities used as animal feed (Hussein and Brasel, 2001). It is estimated that there is 25% of the worlds crop is contaminated with mycotoxins (Cast, 2003).

Zearalenone (ZEA) is a mycotoxin produced by *Fusarium* species, it can be detected in cereals, feed and processed food products worldwide. Zearalenone (ZEA), 6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcylic acid l-lactone; is produced as a secondary metabolite by a number of *Fusarium* species including *F. culmorum*, *F. graminearum* (Caldwell et al., 1970). 32% of the crop samples were contaminated with ZEN according to the European Union. This signifies the importance of ZEN effects in domestic animals and humans (Kiss et al., 2018). Mycotoxin contaminations cause a number of economic impacts, including the destruction of highly contaminated crops unsuitable for human or animal consumption, costly mycotoxin screening programs, reduced production efficiency and impaired livestock health due to contaminated feeds (CAST, 2003).

ZEA is rapidly absorbed after oral administration and the oral bioavailability has been estimated to reach 0.80–0.85 of the ingested dose (Kuiper-Goodman et al., 1987). Studies in pigs indicate that in the gastrointestinal tract ZEA is metabolized in enterocytes to its major metabolites α and β zearalenol (See Figure 5). These hydroxylated metabolites can be glucuronidated and directly excreted, completing the pre-systemic elimination (Biehl et al., 1993).

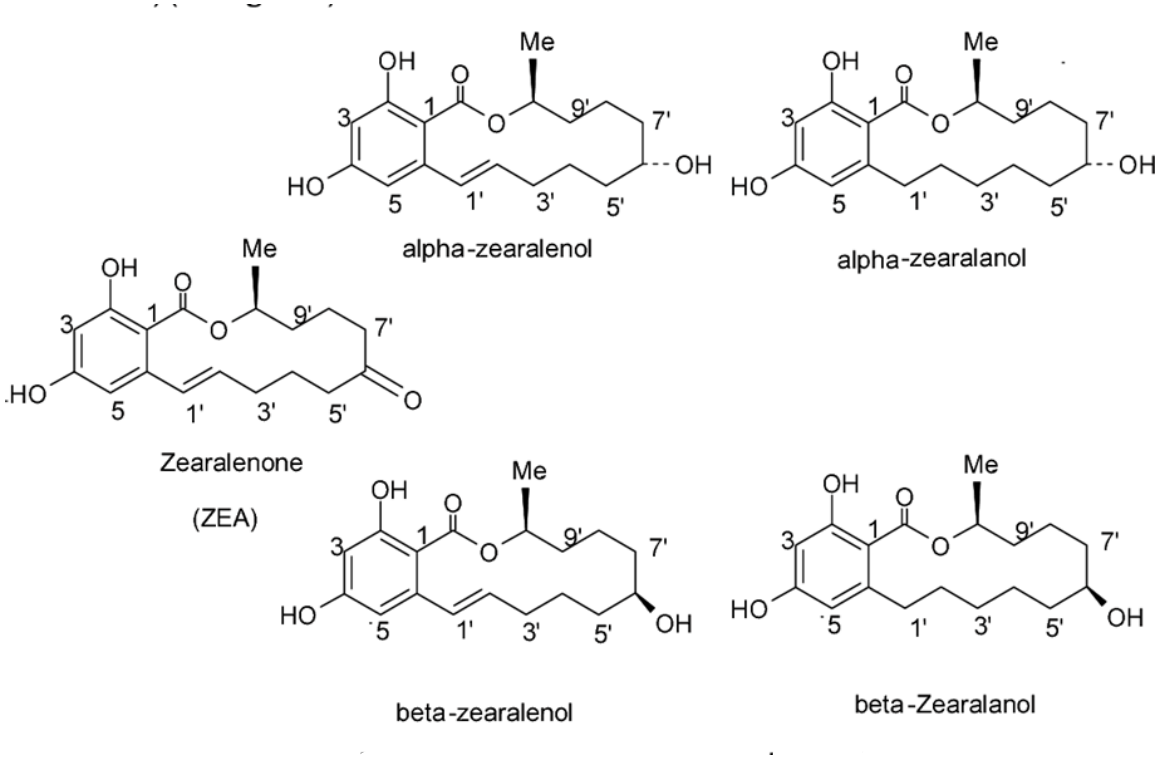


Figure 5: Chemical structure of Zearalenone and its derivatives

ZEA activates estrogen receptors resulting in function and morphological changes in the reproductive organs despite its non-steroidal structure. Pigs are the most sensitive species, and clinical signs of exposure include ovarian atrophy, decreased fertility, persistent corpus lutea, prolonged oestrus intervals, and stillbirth. Based on the binding affinity data, ZEN and its metabolites are the most potent natural estrogens. Contamination of foods and feed materials has been reported from almost all continents. The concentration of ZEN in food and feed materials can vary from a few micrograms up to 276 mg/kg in animal feed materials and between 0.001 and 175 mg/kg in cereal grains intended for human consumption, depending on plant variety, geographic region, and climatic conditions in the production year.

It has a similar structure to estrogen and competes with 17 β-estradiol for binding to the estrogen receptor, resulting in fertility and reproductive problems (Takemura et al., 2007). ZEA, and its metabolites α-ZOL and β-ZOL (see Figure 5), have an estrogenic effect associated with their structural similarity to estrogen. More-over, incubation with ZEA, α-ZOL and β-ZOL have been found to induce the production of progesterone, estradiol, testosterone and cortisol in an H295R cell line, indicating that not only ZEA, but also its metabolites, might act

as endocrine-disrupting agents (Frizzell et al., 2011). Following ingestion and absorption, zearalenone is metabolized mainly by enterocytes and hepatocytes (Molina-Molina et al., 2014). Two major biotransformation pathways of ZEA are known in animals. The first one is hydroxylation of ZEA to two stereoisomers: α-zearalenol (α-ZOL) by the 3α- form of hydroxyl steroid dehydrogenase (HSD) and β-zearalenol (β-ZOL) by the 3 β-form (Ediage et al., 2012). It has also been suggested that two metabolites, α-ZOL and β-ZOL, might be present in bile and urine as products of ZEA metabolism which are probably converted by intestinal mucosa or erythrocytes (Metzler et al., 2010).

ZEA and its metabolites are transferred to the foetus by the placenta, and associations have been identified between maternal ZEN exposure and adverse pregnancy outcomes, such as impaired development and reduced litter size (Zhang et al., 2014). It is also suggested that foetal malformation can be caused by excessive ZEA administration to the mother.

Sex hormone binding globulin (SHBG) is the major transport protein for the lipophilic endogenous oestrogens and androgens in blood serum and binds these steroid hormones with high affinity. ZEA proved to poorly bind to the SHBG from humans (Martin et al., 1978) implying that most of the ZEA present in blood is available for cellular uptake and activation of ERs. The most important and well researched targets of ZEA are the neuroendocrine and reproductive organs. severe anatomical and physiological disorders can be found after the exposure, leading to increased chance of still birth, pseudopregnancy, anoestrus, and developmental disorders in the foetus of the pregnant animal. Studies have shown that neonatal or prepubertal administration of ZEN can cause adverse effects such as advanced onset of vaginal opening (VO), prolonged oestrus, and decreased fertility in female rodents. Additionally, the mycotoxin has a carcinogenic effect. ZEA stimulates the cell proliferation in tumours of the female reproductive tract and breast malignancies in humans (Withanage et al., 2001). The mycotoxin changes the expression of substances in the nerve fibres of the gastrointestinal such as vasoactive intestinal peptide (Gonkowski et al., 2015).

ZEA and its metabolites exert their estrogenic effects through binding to estrogen receptors (ERs). It is assumed that the receptor–zearalenone complex is transferred to the nucleus where it binds to estrogen-responsive elements thereby activating gene transcription (Riley,1998). Boyd and Wittliff (1978) various studies confirmed that ZEA and its metabolites act as competitive agonists/antagonist on both types of estrogen receptors. Experiments with cells, transfected either with the human estrogen receptors demonstrated that ZEA is a full agonist

for the ER receptor, whereas it acts as partial agonist on the ER (Kuiper et al., 1987). Binding studies with subcellular fractions of various organs, including the hypothalamus, liver, uterus, and the mammary gland from different species, indicated that the relative binding affinities of ZEA and its metabolites varies between <0.01 and 0.10 of that of 17β-oestradiol (E2), with α-ZOL having a higher binding activity. ZEA is about as oestrogenic as the strongest phytoestrogen, the α-stereoisomers of the reductive metabolites ZOL and ZAL are about 10-fold more active than ZEA and the β-isomers, and the affinity of ZEA and its reductive metabolites to ERα and ERβ is about the same, whereas phytoestrogens bind preferentially to ERβ.

6.2 Effects of Zearalenone on the Nervous System

It is known that estrogen receptors are present in the brain, and phytoestrogens cross the blood–brain barrier. Previous studies show that ZEA can exhibit the neurotoxicity in the neurons by the decrease of brain calcium binding protein level (Lephart et al., 2000) and involvement in oxidative stress mechanisms (Venkataramana et al., 2014). ZEA binds to estrogen receptors, may have deleterious effects on brain cells, such as induction of neuron apoptosis (Doi and Uetsuka 2011) or changes in gene expression and influence on the level of brain-derived neurotropic factor (BDNF) one of the key regulators of brain function (Pan et al., 1999). Zen could also affect the nerves in the gastrointestinal system. The gastrointestinal (GI) tract is innervated by both the enteric nervous system (ENS) located within intestinal wall and sympathetic, parasympathetic, and sensory extrinsic innervation.

ZEA treatment of immature female rats caused precocious puberty and increased expression of the neuropeptide kisspeptin in the hypothalamus (Kriszt et al., 2015). It has been shown that α-zearalanol (α-ZAL), a metabolite of ZEA ameliorates memory impairment in ovariectomized mice (Dong et al., 2013). α-ZAL has been tested also *in vitro,* on rat hippocampal neuronal cultures, and its neuroprotective effect against amyloid-β induced neurotoxicity has been demonstrated. Bodi et al. (2021) research focused on brain areas which are not traditionally linked to reproductive functions, the somatosensory cortex and the CA1 region of the hippocampus. Brain slices of untreated rats were incubated in ZEA-containing solution, and cortical and hippocampal field potentials were studied. Bodis (2021) *in vitro* findings indicated that in case of high-concentration direct, acute exposure of brain slices, ZEA can alter the excitability and synaptic plasticity phenomena in these brain areas.

Although the effect of ZEA and estrogenic compounds is stronger on female reproductive functions, estrogenic compounds have significant effects on the brain functions of both sexes, and in general, no sex-related expression pattern differences were found for ERs in the brain areas examined in Bodi et al. (2021) study.

Faber and Hughes have demonstrated that neonatal exposure to ZEA alters post pubertal pituitary response to GnRH and androgenizes the preoptic area (SDN-POA) in rats. Taken together, humans and animals (especially livestock) are at a risk of exposure to ZEA through consumption of contaminated food. The influence of ZEA on the brain as AVPV and ARC hypothalamic nuclei are also important targets for estrogens and EEDCs, especially in neonatal critical periods. It is plausible that AVPV and ARC neuronal populations are sensitive to the disrupting actions of exogenous compounds with sex steroid-like activity that may pose long-lasting consequences in terms of reproductive health. Results from our study demonstrate that administration of ZEA to neonatal female mice advances puberty and disrupts the function of the reproductive axis. Observed alterations are also accompanied by decreased expression of kisspeptin mRNA levels and decrease in neuronal density in the AVPV and ARC hypothalamic nuclei. Evidence exists that kisspeptin is critical for the timing of puberty, as mutations to Kiss1r or kisspeptin gene function result in hypogonadism it is plausible to imagine that ZEA-induced early puberty could result from advanced maturation of the kisspeptin system, either in the AVPV or in the ARC or both. the compounds altering neuroendocrine control and steroid production or binding to ERs are able to disturb the onset of puberty.

Endocrine disruptors can disturb HPG axis, and it adversely affects the function of the reproductive system (Xu et al., 2017). It is found that ZEA causes adverse effects on growth indices of GSI and caspase-3 activity by altering the hypothalamic–pituitary–gonadal (HPG) axis. Reproduction is mainly regulated by the HPG axis in zebrafish (Ma et al., 2012). The estrogenic compound has the potency to bind with the nuclear ER throughout the tissues of the HPG axis and impairs the reproductive function by altering steroid hormone synthesis, which can lead to reproductive toxicity in adult female zebrafish.

On human neuroblastoma cells, cytotoxic effects were observed at 24 h ZEA incubation above 25 μM (Venkataramana et al., 2014). ZEA mycotoxin is extremely toxic to rapidly dividing cells resulting in immunosuppressing effects in the hosts (Bennett and Klich, 2003). The toxin also exerted its effects on astrocytes by regulating the plasma membrane receptors responsible for glutamate uptake.

The neuronal biomarkers TH, BDNF and AADC play pivotal role in the survival as well as differentiation of dopaminergic neurons (Chen et al., 2013). The gene expression level of these neuronal markers (BDNF, TH and AADC) was monitored by quantitative real time RT-PCR after ZEA treatment in SH-SY5Y cells. Venkataramana et al. (2014) found that ZEA altered the gene expression profile of neuronal biomarkers confirming the neurotoxic potential of ZEA.

One endocrine-disrupting effect of ZEA might be associated with disruption of hormone production by the pituitary gland. There has been reports that both ZEA and its metabolites might modulate LH production in bovine cells. They postulate that this effect may be caused by estradiol receptor GPR30 modulation (Nakamura and Kadokawa, 2015). Prepubertal exposure to dietary ZEA might cause premature activation of the hypothalamic kisspeptin-GPR54-GnRH signalling pathway, which leads to the advancement of vaginal opening and enlargement of the uterus at the periphery in rats (Yang et al., 2016).

ZEA and its metabolites were reported to have a beneficial modulatory effect on neurogenesis where it improved memory in mice (Dong et al., 2014, 2013) and rats (Dong et al., 2015). The authors suggest that this effect may be associated with the estrogenic properties of ZEA mediated by ER alpha (Dong et al., 2015). Turcotte et al. 2005 showed in his study the complexity in determination of whether or not dietary estrogens are likely to have overall positive or negative effects in neuronal function.

In the EU, maximum levels of ZEA are 20 μg/kg for food for babies and infants, 50 μg/kg for maize-based snacks and breakfast cereals, and 200 μg/kg for unprocessed maize and certain maize products (Gromadzka et al., 2009). Currently, the mean dietary intake of ZEA in various European countries is estimated to range from 1 ng/kg bw/day to 420 ng/kg bw/day (EFSA, 2004).

7. Arsenic

7.1 Introduction

Arsenic (As) is a crystalline metalloid with characteristics between metals and non-metals. As is ranked as the 20th most occurring trace element in the earths crust, 14th in seawater, and 12th in the human body (Mandal et al, 2004). Arsenic a potent toxicant which is widely distributed though drinking water and food is considered a serious worldwide environmental health threat.

As noted by Hughes et al., (2011) Arsenic is poisoning millions of people globally and is a natural contaminant of groundwater in certain parts of the world and is also leaching into aquifers from surrounding arsenic-rich geological formation (World Health Organisation (WHO, 2001)). Exposure to the metalloid may occur as a result of natural or anthropogenic activities. It majorly finds its way anthropogenically its way into the environment as a result of metal smelting operations. The enrichment and pollution of surface sediments and groundwater by Arsenic (As) several countries including West Bengal in India, Bangladesh, Italy, China and Nigeria have been reported. The elevated levels of As in sediments and groundwater have been attributed to the intense activities in agriculture and unregulated discharge of industrial waste into rivers.

There has been many reports detailing early developmental and adult exposure to arsenic being associated with health problems including cognitive, cardiovascular, metabolic, and metastatic disorders that are manifested across the lifespan. Kitchin (2001) noted that arsenite and arsenate are recognised to cause acute and chronic toxicity to a wide variety of organisms including humans. Organs that are most affected by arsenic are those involved in absorption, accumulation, and excretion (Duker et al., 2005). It has been shown that toxicity of arsenic depends on its chemical form as well as oxidative stress (Mandal et al., 2004). Chronic inorganic arsenic exposure has been linked to loss of body weight, cancer of the skin and internal sites, burning sensations of eyes, solid swelling of legs, liver fibrosis, chronic lung disease, gangrene of toes, metabolic disorders (diabetes) and dysfunction of endocrine system, nervous system and reproductive system (Nandi et al., 2005).

7.2 Effects of Arsenic on the Nervous System

Blood-brain barriers (BBB) is composed of there main cellular components; endothelial cell, astrocyte end feet and pericytes. In between the cerebral endothelial cells there are tight junctions present as diffusion barriers where diffusion of water, gases and hydrophobic molecules takes entry passively and elective transport for glucose and amino acids is permissible for neural function. Increasing arsenic exposure with time can alter tight junctions (TJs) proteins (see Figure 6) which further increases the blood brain barrier permeability. Figure 6 shows how Arsenic can efficiently down-regulate TJs proteins and mTOR protein expression with an increase in Beclin 1, LC3 and Atg12 level (Yoon et al, 2006). Due to more permeability Arsenic can easily cross the blood-brain barriers and can directly affect the CNS. Its crossing capability through the blood-brain barrier and accumulating in different regions of the brain tissue could possibly lead to neurological disorders. Neurons are unable to divide so the damage in the neurons leads to permanent functional abnormalities of the total nervous system. This damage in the nervous system impairs proper impulse propagation. To understand the importance of Figure 6 one needs to understand autophagy. Autophagy plays a key role in maintaining intracellular homeostasis by degrading intracellular macromolecules and damaged organelles, and both excessive and repressed autophagy levels are pernicious in cells. Autophagy is the formation of double membrane bound vesicles called autophagosomes, which engulf the cargo and transport it to the vacuole/lysosome for breakdown and recycling. The mRNA and protein expression levels of autophagic markers LC3, Beclin1, Atg13 and P62 were significantly increased by As. These consequences revealed that arsenic exposure induced dysfunctional autophagy in the brain (Figure 6).

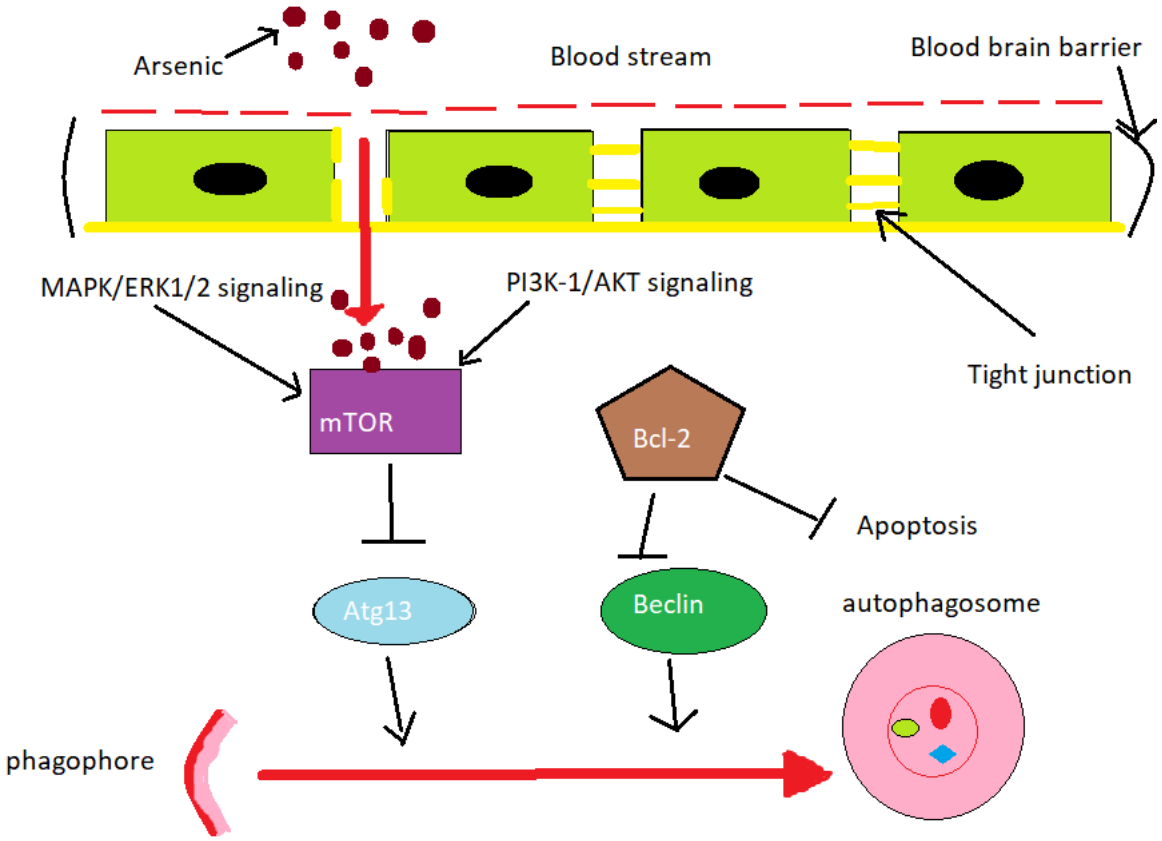


Figure 6: Arsenic can cross the blood-brain barrier and induce autophagy. Arsenic can down-regulate Tight Junction proteins and mTOR protein expression with an increase in Beclin 1, LC3 and Atg12 level. Which induces dysfunctional autophagy in the brain leading to damage in the nervous system/brain.

Lipid content is high in brain tissue than other parts of the body with about 30% of the weight of the brain containing lipids. Lipid molecules are very susceptible to damage by reactive oxygen species. It has been shown that arsenite induced production of Reactive Oxygen Species (ROS) results in damage to mitochondrial membrane and cell death. This process is a central step in neuronal injury. Arsenic, a toxic metalloid, degenerates the ROS scavenging capability of antioxidant enzymes. When the ROS generation starts due to arsenic toxicity the antioxidant enzymes increase their level for boosting purposes. However, when ROS level increases too much it inactivates the antioxidant enzymes by inactivation the catalytic site. This results in the antioxidant enzymes level decreasing and cell becomes toxic. Arsenic causes damage to biological system because of its ability to generate oxidative stress in the cells. Arsenic is known to generate ROS and free radicals like hydrogen peroxide, hydroxyl radical, nitric oxide ,superoxide anion, dimethyl arsenic peroxy radical and dimethyl arsinic radical in living systems (Flora et al, 2005). In the brain of arsenic exposed animals, the inhibition of antioxidant enzymes, glutathione peroxidase and superoxide dismutase suggest an imbalance antioxidant/prooxidant ratio resulting in oxidative stress.

Haider and Najar (2008) study suggest that arsenic neurotoxicity in rats initiated peroxidative reactions in membrane lipids of the brain. The study concluded that the losses of lipid classes, glutathione and ascorbic acid are attributed to peroxidative damage and the binding of arsenic with a sulfhydryl group of enzymes. It is then hypothesised that repeated exposure of humans to arsenic may result in disturbing cell signalling, apoptosis and mutagenesis. Due to the arsenic toxicity, the enzyme responsible for re-oxidation reaction of this complex is blocked. As a result, pyruvate production, a pivotal component of energy production, is reduced. When acetyl co-A level critically decreases, cell enters in death pathway. The impairment of energy metabolism in neuronal cells creates severe damages to these cells and neuronal control over the multiple organ and whole body diminished drastically. Arsenic also inhibits the production of succinyl co-A and hampers ATP production in cell and blocks the energy supply entirely as the succinyl co-A present in complex II of electron transport chain. So, the ATP synthesis is also prevented.

Growing evidence for animal and human studies indicated that arsenic might have deleterious effects on the central nervous systems. Arsenic exposure could produce developmental abnormalities, including malformations, decreased growth rate, mortality, neuronal tube defects and an array of behavioural changes through effects on the developing brain directly since arsenic freely crosses the placenta and blood‑brain barrier (BBB) in humans and animals. Neural tube defects, spontaneous abortion, stillbirth, and neonatal deaths were reported in arsenic exposed pregnant women who are consuming drinking water contaminated with high arsenic levels. Arsenic can alter the motor and cognitive functions by the dysfunctions of multiple neurobiological processes including those of dopaminergic, cholinergic, glutamatergic, and monoaminergic signalling pathways. Arsenic can delay and inhibit the process of neurodevelopment of the central nervous system during the foetal and early life of the human.

Ingestion of toxic doses of arsenic may develop neurological symptoms, such as drowsiness, confusion, fever, convulsions, and coma. The most common arsenic-induced neurological lesion is a peripheral neuropathy with a “stocking glove” distribution of dysesthesia. Occupational exposure to arsenic fumes displayed the symptoms such as cognitive impairment and dysfunctions of learning.

Adedara et al. (2017) found a decrease in the plasma levels of pituitary hormones (LH and FSH) and the gonadal hormone, testosterone, following administration of As alone, Mn alone

and their mixture to rate. This indicates the adverse effects of separate and combined exposure to these metals on the hypothalamic-pituitary-testicular axis.

Arsenic consumption in brain can damage hypothalamus. As an outcome, the function of pituitary gland hampers and creates a hormonal imbalance throughout the whole body. Recent studies demonstrate that arsenic is a potent endocrine disrupter especially it alters steroid hormone receptor-mediated gene regulation at very low level. As mentioned in the beginning endocrine disruptors usually act in 2 ways however arsenic acts in a different way. It is suggested that in the presence of arsenic, the activated receptor is unable to stimulate the appropriate cascade of signals. Arsenic-induced neurotoxicity has impact on neurotransmitters which are responsible for communicating between cell to cell within the brain. Arsenic inversely regulates norepinephrine level and helps in inducing dopamine and serotonin level. Inorganic. Arsenic induction in dose-dependent manner shows reduction of the brain and total body weight in several experiments. Calderon et al. (1999) found that environmental exposure to As has an influence on CNS function. They suggest that verbal comprehension, long-term memory, and attention are higher brain functions that could be affected in exposed children to As. In the brain, organic chemicals or hormone of catecholamine family does not act as neurotransmitter such as nor do epinephrine (NE), dopamine (DA), and serotonin (5-HT). These neurotransmitters have a crucial role in learning and memory. The dopaminergic system is a target of arsenic induction.

Arsenic consumption by rat decreases acetylcholinesterase (AChE) activity which helps in metabolism of another neurotransmitter, acetylcholine. Mitochondria are the primary target in arsenic-induced genotoxicity Arsenic shows its mutagenic response through the mitochondrial damage. Mitochondrial membrane potential decreases along with arsenic toxicity by generating reactive oxygen species and DNA fragmentation. Arsenic has been shown to alter developmental myelination and remyelination potential in the CNS of the human and animals. Arsenic can disrupt cell-cell communication via neurotransmitter alteration. Sometimes arsenic induction in neurons may increase the level of inhibitory neurotransmitters and decrease the level of excitatory neurotransmitter. As a result, total integrated system of body is affected.

The glucocorticoid signalling pathway has been demonstrated to be programmed in response to prenatal environments. The GR signalling system has been linked with cognitive deficit. Several studies have demonstrated that arsenic produces specific disruptions in glucocorticoid transcriptional activity and steroid receptor function (Ahir et al., 2013). Foetal levels of

glucocorticoid are regulated by both the foetal and the placental tissues through the developmentally regulated isozymes, 11β-hydroxysteroid dehydrogenases (11β-HSD) interconvert active and inactive cortisol (corticosterone). The developmental expression and levels of these isozymes are seen as being a critical regulation point for hypothalamic–pituitary–adrenal (HPA) axis negative feedback (Brunton and Russell, 2011). Maternal exposure to arsenic may alter the timing of 11β-HSD isozyme expression levels in an attempt to adapt to the influence of arsenic on GR signalling. Additionally, arsenic readily crosses the placenta and might directly alter expression and levels of the GR signalling complex within the developing foetal brain. The GR pathway sits at a critical interaction and integration point to play a role in all of the key arsenic associated effects from: inflammation to cognition, and from immunity to neoplasia (Caldwell et al., 2015) found lower brain levels of GR protein and mRNA in the arsenic-exposed mice. Additionally, arsenic exposure altered the developmentally expressed ratio of the 11βHSD isozymes. Proper levels and temporal expression of the 11β-HSD1 is required for development of negative feedback regulation.

Considering that the neuronal connections established during development and even in adulthood arise from the chemical communication between nerve cells, altered signaling may either reflect or give rise to structural modifications, and hence to changes of behavior. Microglial cells are a specialised population of macrophages that are found in the central nervous system (CNS). They remove damaged neurons and infections and are important for maintaining the health of the CNS. They are also involved in trophic neuronal support during development, synaptic organisation, myelin turnover, phagocytosis of apoptotic cells in the developing brain, control of neuronal excitability, phagocytic debris removal, and most importantly brain protection and repair. Neuroinflammation is a tightly controlled phenomenon and kept in check by different inhibitory signals which otherwise can lead to different disease state in the central nervous system. Contact-dependent cell-cell interaction between microglia and neurons (figure 7) through CD200R and CD200 is one of the major inhibitory signals. So CD200 plays a key role in the regulation of neuroinflammation. Figure 7 shows arsenic-induced neuroinflammation, it activates microglial cells. Reports suggest that downregulation of CD200 acts as a trigger for several neurodegenerative diseases like Alzheimer's. Interestingly, arsenic has been predicted to induce Alzheimer's like neurodegenerative disease in human as well as reported to induce pre-onset of Alzheimer's like symptoms in rats, which is very much associated with neuroinflammation (Gharibzadeh et al 2008).

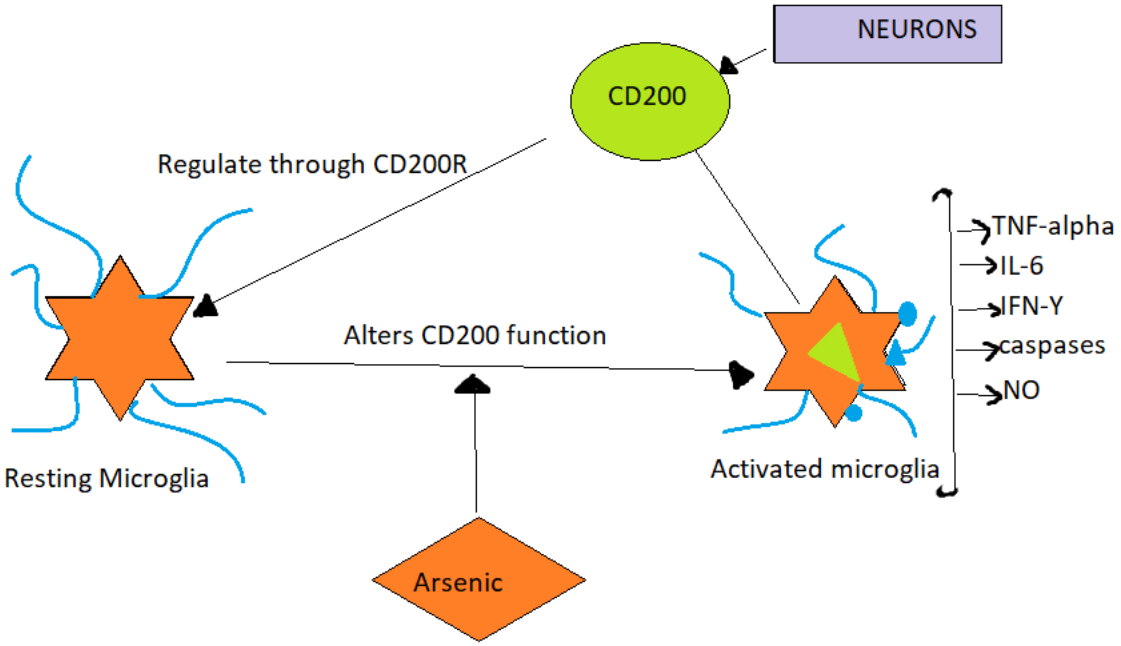


Figure 7: Arsenic-mediated activation of microglial cells and induction of inflammatory mediators and signalling molecules. CD200 is a neural surface glycoprotein expressed in neurons and can give protection by binding to the microglial CD200 receptor (CD200R) CD200 transmits receptor mediated signals for regulating pro-inflammatory activity of microglial cells. Production of the reactive oxygen species in microglial initiates both pro- and anti-inflammatory cytokine actions in microglia. The pro-inflammatory responses initiated by arsenic trioxide in the microglial cells generate apoptotic signaling via IL-1β. The inflammatory markers are IL-1β, TNFα, and INFγ.

7.3. Harmful effects of Arsenic on other Organs

Previous investigations showed that chronic exposure to either As induced oxidative stress and impaired testicular function of spermatogenesis and steroidogenesis in laboratory animals. This metalloid exposure has been linked to increased incidences of adverse health effects including cancer. Studies have shown that exposure to arsenic at high levels can lead to development of skin, bladder, liver and lung cancers (Hughes et al., 2011, Smeester et al., 2011). Severe metabolic disorders such as diabetes and other non-cancer effects such as, cardiovascular disorders, goitre, hepatomegaly, bronchitis have also been associated with arsenic exposure. Arsenic produces toxic effects on female reproductive system in rodent models also (Chattopadhyay et al., 1999, 2001). Reproductive tract function in the female is controlled primarily by the interaction of the ovarian sex steroids estradiol and progesterone. In the uterus, estradiol-17b (E2) initiates a series of biochemical responses in uterine cells in preparation for the possibility of pregnancy, including cell hypertrophy and hyperplasia (Nephew et al., 2000). Chattopadhyay et al. (2001, 2003) exposed adult rats to 0.4ppm of sodium arsenite for 28 days, and found loss in uterine weight, reduction in uterine peroxidase activity along with diminished levels of estradiol, LH and FSH.

8. Conclusions

A plethora of evidence supports the active role of estrogen in the CNS as a developmental and functional regulator as well as a neuroprotective molecule. These three endocrine disruptors (BPA, ZEA, As) clearly cause long term damage to the brain/nervous system and other systems. The hormonal imbalance caused by these EDs is due to their ability to bind to estrogen receptors and TH receptors causing dysregulated feedback loops and disturbed cellular signalling pathways.

Exposure to EDs early in life (intrauterine or even postnatally) leads to developmental and physiological disorders later in adolescence. The disruption might be intracellular, changing the physiology of specific cells or it may have a more generalized, tissue or organ physiology changing effect. specific substances may disrupt specific intracellular pathways, resulting in defective gene transcription. The extracellular effect results in a global change in the neuroendocrine system of the animal, influencing the physiology of the neural tissue. E.g., disrupting the hypothalamic–pituitary–thyroid axis may lead to hypothyroidism cause a variety of abnormalities in the CNS (Pasquini and Adamo, 1994). Given the ubiquitous presence of BPA in our environment the potential transgenerational impacts of this ED are large and important (Wolstenholme et al, 2011).

What remains to be seen is how data can be generated in an attempt to produce an "end all, be all" study on BPA, ZEA and As will be used by regulatory bodies for making human health decisions. Collectively, the data should reveal the plausibility of non-linear dose effects, multiple modes of action, and the pressing need to incorporate more endocrine-sensitive organs (such as the brain) and endpoints in guideline studies. Regulators remain reluctant to limit BPA and other EDCs from consumer products and struggle to synthesise relevant data for EDCs. (Patisaul et al 2019).

In a 2014 literature review considering the use of BPA in food packaging, the Food and Drug Administration (FDA) determined that, out of 36 published neurotoxicity studies, seven were relevant to hazard characterisation but only one had utility for risk assessment. The report upheld the conclusion that BPA poses no significant health risks at current exposure levels. However, limits and difficulties in testing EDCs has been described for a long time, while considering that we need to test low doses to see relevant endocrine modulations and high doses (or more preferably very long-term treatment period) to identify adverse effects. This is in complete contradiction with the pressure around reducing animal testing (3Rs).

Since the 1970s, there have been dramatic increases in previously rare neurodevelopmental disorders. As previously described; endocrine disrupting chemicals can interfere with neurodevelopment affecting cognition and sexual behaviour in both wildlife and humans. Neurobehavioral disorders have increased in prevalence in human populations. The reasons for this are multiple and not understood. Wildlife and humans are exposed to a wide variety of EDCs that differ greatly in their physical-chemical properties. Further, these compounds are generally present at very low levels and in complex matrices requiring highly selective and sensitive methods.

To conclude research findings on these three endocrine disruptors put together a new perspective on how exposure effects the pathogenesis of children, adults, and wildlife. The underlying mechanisms of these shared changes may provide an avenue for novel treatments and address preventative strategies for neurodevelopment disorders in children and adults.

The continuing need for rigorous human risk assessment of all new chemicals is therefore obvious but nevertheless worth restating for those who have not had first-hand experience of global disasters like that caused by tributyltin. The future of EDCs monitoring should focus on a variety of mechanisms including government actions to reduce exposures. This has been effective in the bans and restrictions of certain EDCs such as lead, tributyltin which has contributed to the decrease in frequency of disorders in humans and wildlife. An integrated international effort is needed to define the role of EDCs in human and wildlife health/populations.

9. Summary

Endocrine Disruptors (EDs) have been a cause for concern throughout the 20th and 21st century. EDs are environmental agents and can be either organic or man-made. They are found throughout the world. The amount of manufactured chemicals is increasing yearly. All these endocrine disruptors have the potency to be toxic, so it is important to monitor our environment and identify them. They have a similar chemical structure to what endocrine organs produce in animals produce so they can bind to steroid hormone receptors and enhance or inhibit the actions of endogenous hormones. Its acts as a steroid receptor agonists or antagonists or both. Which interferes with normal hormonal regulation and functioning of the endocrine and neuroendocrine systems leading to harmful short- and long-term effects on the organism.

To understand more about the harmful effects of EDs we need to review on an individual and systemic level. The main topics of this thesis, three EDs with different origins and structures, were chosen to show to similar effects on the organism. These three EDs have similar estrogenic effects on humans and animals. Bisphenol A (BPA) is an industrial by-product during plastic synthesis; Zearalenone (ZEA), a mycotoxin well-known for its adverse effects in the agricultural industry, causing major losses in both animal counts and agricultural profit in Europe; Arsenic (As), a very strong substance with an ED characteristic, occurring naturally or after contamination in groundwater and drinking water.

There has been extensive research on the reproductive effects of EDs. However, the focus of this thesis is a more in-depth view on the long-term effects on the brain and nervous system. Damage to the brain effects all other systems. Research on all systems in the body affected by EDs will further help understand and monitor the effects, which in turn will lead to more robust government regulations globally.

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And all my love to my Mum for her support and strength.

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