INTRODUCTION

Toxicology deals with the study of interaction of chemical substances with living system at different doses for the evaluation of safety exposure limits of chemicals like drugs and man-made and natural substances. The toxicological studies are important for improving the health of humans, animals and their environments. Toxicity testing assist to define flow of events including chemical exposures, distribution, metabolism and importantly its interactions with biologically significant macromolecules, resulting into a toxic end point. The information on chemical substances obtained from toxicological research predicts the safe exposure levels. Thus, research provides understanding of mechanisms of toxicant action and then this information can be used in the prophylaxis and therapeutics. With the identification of specific type of chemical or material hazard toxicological studies can provide scope for proper planning and safety measures to prevent exposure. Further, toxicological data are also important in development of legislation aimed at protecting health and environment by assessing possibility that a particular chemical possess a significant risk to life and environment. Therefore, today the science of toxicology has become one of the most interesting interdisciplinary fields. Biologist, chemist, engineers, ecologist and other scientists have worked together for assessment of environmental effects of chemicals within this discipline.

1. POTENTIAL HEALTH HAZARDS OF XENOBIOTICS

There are numerous toxic chemicals present in the environment and over past century humans have introduced a large number of chemical substances. Some of these chemicals are essential for life and are useful in many ways, but many are toxic and have potential to cause hazard to health and environment. Few chemicals have indirect effect on human health by disturbing balance of environment. Some effects of chemicals are reversible while others are not. The toxic effect of a chemical or its metabolites in a living system are produced only when it reaches to appropriate site of action at a particular concentration for sufficient duration. For comprehensive characterization of the potential hazard of a specific chemical agent, we need

to know type of effects, exposure time and the dose required to produce that effect. In other way the toxicity of a substance can be affected by many different factors such as route of exposure, duration and frequency of exposure, physical form of chemical substance, an individual's overall health *etc.* (Fig. 1). The spectrum of undesired effects of chemicals is broad.

Laboratory experiments on known contaminants determine whether true causal relationship exist between potential causes and effects noted in the field. Comprehensive animal studies have been made on the effect of various contaminants present in the environment (Table 1). Much of the literature on the toxicity of individual substances has detailed the toxic responses at relatively higher dosage of exposure than those actually detected as environmental levels. Many of the substances, such as cadmium, arsenic, chromium, lead, Mercury, nickel, dioxins, polycyclic aromatic hydrocarbon, phenolic compounds, phthalates, and pesticides are considered to be carcinogenic however evidences that these substances cause cancer at detected environmental levels are very less (Ames and Gold, 1990; Mishra *et al.,* 2010; Soto and Sonnenschein, 2010). The potential health hazards of the environmental toxicants are detailed in Table 1.

Epidemiological studies are very important in the assessment of human health to chemicals exposure (Raffaele *et al.,* 2011). Contamination of our environment with huge amount of chemical wastes because of rapid industrialization and urbanization has raised the concerns about the development of specific disease and potential long term impact on the quality of life and the environment. In spite of our suspicions, we have very few clear data, linking human illness to low or moderate environmental exposures of many potentially toxic substances (Duruibe *et al.,* 2007). Few epidemiological studies over a last few decades have described the health hazard by chemical pollution (Table 2). Most of these epidemiological studies have been the outcome of occupation exposures and exposure to the general public through environmental pollution, chemicals escape from the industry or from accidental spill or leakage. This information would be particularly relevant in

situations where one or two specific substances are of concerns but may be less useful for investigating the more general exposures to the toxicants emitted simultaneously in the environment from various industrial processes, which tend to be heterogeneous in nature. Therefore, epidemiology of multiple chemicals exposures is a relatively unexplored field in occupational and environmental health. Combined chemicals exposures are common events therefore studies on potential health hazards from such exposures should be encouraged. The data obtained from chemical mixture toxicity studies will be very useful to relate environmental chemical pollution and causes of increasing health complications. It can help to identify and evaluate health risk, mode of action and establish exposure response relationships form which meaningful permissible levels can be derived.

2. HETEROGENEOUS CHEMICAL MIXTURE

Human exposure to pollutants, ambient and/or occupational, is almost always heterogeneous. The toxicological profile of individual chemical may describe its pharmacological properties and toxicological responses however, does not necessarily explain the actual toxic potentials to humans when the exposure is heterogeneous. The research carried out during past several decades has identified potential hazardous chemicals, their toxic manifestations and permissible limits for release of pollutant into the environment. In past for chemical risk assessment the toxicological studies evaluated the effect of single chemical in biological system.

These chemicals in our bodies are a necessary evil without any toxicological importance, particularly at such low levels and represent the toxicological *Unknown Unknowns* (Mumtaj, 2010). Exposure to persistent chemicals could lead to their accumulation and to increasingly high tissue concentrations (Hangberg, 1996; El-Shahawi *et al.,* 2010). The potentials to accumulate, to biodegrade and to excrete the chemicals are variable among individuals and hence the toxic responses are also different. Such chemical body burdens and their possible variations from person to person, together

with the unlimited combinations of chemical mixtures that may be inherent in human populations, are difficult to be assessed by routine toxicity testing.

The Department of Health and Human Services Centers for Disease Control and Prevention, CDC (2009) sampled large human population for survey of chemical body burden (sample size ranged from a low of 1854 samples for PCB and a high of 8945 for cadmium or lead. It was alarming to note that 219 chemicals were present in the blood and/or urine of the population and 75 of these compounds were recorded for the first time in USA population. These analytical surveys have been repeated during the past decade and the number of detected chemicals is increasing in each survey which suggests that probably due to inadequacy of analytical methods or very low concentrations of the chemicals all the chemicals are not detected in the blood or urine samples. It is suspected that total number of environmental pollutants and other chemicals as body burden in the USA population could be much higher than 219. It is stated in the report (2009) that the presence of chemical at very low levels in human blood or urine sample does not necessarily indicate that it is biologically available at toxic levels or it is the causative agent of a particular toxic response. However, mere presence of such several chemicals does magnify the potential risk to the individual. Unfortunately we do not know how many such chemicals are present at very low or undetecTable levels in our body. The human health risk remains unassessed for such unknown heterogeneous chemical mixtures. The use of different combination drugs in therapeutics has also increased in past two decades (Atangwho *et al.,* 2010; Roy *et al.,* 2011). An individual, who suffers from more than one ailment simultaneously, like diabetes and hypertension, will be taking combinations of variety of medicines (Rosenthal *et al.,* 2010). The therapeutic doses of drugs, if not cleared out from body completely may result into low level body burden with great potentials to interact with already existing toxicant burden (Mumtaz *et al.,* 2010). This is bound to lead to a very challenging situation where the toxicants and drugs form much different mixture of chemicals in the body than one could expect.

For the toxicological evaluation, it is essential to understand the basic concepts of combined action and interaction of chemicals and to be able to distinguish between the whole mixture analysis (top-down approach) and component interaction analysis (bottom-up approach) (Feron *et al.,* 1998; Groten *et al.,* 2001). Generally the mixtures are grouped as simple and complex mixtures depending on their composition (Fig. 2 and 3). As per one of the classification, the simple mixture has much less number of component chemicals (e. g. up to 8-10 only), while the complex mixture has many more chemical components (e. g. up to 100 or more). The other system refers to the type and combinations of chemicals. Components of the same chemical class, like metals or organochlorine pesticides are simple mixtures where the number of chemical components is not important. The complex mixtures, therefore, would be composed of components of different chemical classes; like a mixture of hazardous waste site or that of effluent arising from different industries. If the qualitative and quantitative composition of any complex mixture is known it becomes easier to evaluate the mixture toxicity or at least it is possible to categorize it, however, if the compositions largely unknown or undeterminable it is difficult to speculate the chemical interactions and thus the ultimate toxicity. The toxicological evaluation would, however, describe relevant toxicity; these data can be used to characterize the hazard following exposure to the entire mixture and for the risk assessment. Although, information on dose-response relationship can be generated, in such cases it is difficult to determine the interactions of component chemicals and to define the mode of action of the mixture.

The literature is piled up with the reports on experimental and epidemiological evidences of adverse effects of chemicals on different systems (Table 1 and 2). Although, toxicity studies of single substances are important for obtaining basic toxicological information like LD_{50} values, threshold doses, accepTable daily intake, No observed adverse effect levels etc. however, there is strong evidence that chemicals in a mixture belonging to same or different category can produce effects greater than the effects of each component when tested singly. This inference has been investigated

experimentally by combining chemicals at level below or equal to no observable adverse effect level (Wade *et al.,* 2002; Walter *et al.,* 2002; Kortenkamp, 2008). The papers dealing in the toxicology of mixtures are gradually increasing but several of these studies, tested two or more compounds at relatively higher doses. Initial studies were conducted with plankton where they work on the planktonic micro fauna and showed a decline in the plankton diversity in the polluted regions (Thomas *et al.,* 1980 Nanda, 2002). Similarly, studies on fish toxicity were carried out to assess the effects of mixtures of more than two chemicals (Konemann, 1981; Khangarot and Ray, 1990). Recent studies have been documented on effect of chemical mixture on higher organisms and on different cell lines exhibiting the additive and interactive effects of the mixture toxicant (Hotchkiss *et al.,* 2004; McDermott *et al.,* 2008; Pomati *et al.,* 2008).

Several environmental laws including the Safe Drinking Water Act amendments (SDWAs), the Clean Air Act, the Food Quality Protection Act have acknowledged the significance of potential exposure of chemical mixtures. The scientific literature on mixture toxicity has few publications describing method for conducting additive or interaction studies. The most noTable examples are the testing of various petroleum products together and chemicals mixture from drinking water (Chapin *et al.,* 1989; Simmons *et al.,* 1994; Patrick-Iwuanyanwu *et al.,* 2011). Another example of the testing mixtures is the studies of commercial formulations of polychlorinated biphenyls congeners (Kostyniak *et al.,* 2005). Few studies have been conducted on the assessment of combined toxicity of defined chemical mixtures consisting of Metals/ pesticides/ fertilizers (Benjamin *et al.,* 2006; Jadhav *et al.,* 2007; Wielgomas and Krechniak, 2006; Shalaby and El-Mageed, 2010). Gruener and Lockwood (1980) examined the mutagenicity to hamster embryo cells of chemical mixtures of drinking water. Similarly, genotoxicity of chemical mixtures have also been studied in different cell lines (Lah *et al.,* 2005). Combinations of several nephrotoxicants were also studied in the form of mixture on animals (Jonker *et al.,* 1993). Systemic toxicity studies with a couple of chemicals demonstrated development of cellular and

sub cellular lesions (Brzoska *et al.,* 2003; Jadhav *et al.,* 2007). Very low levels of 25 different pollutants in groundwater were reported to behave differently and induce toxicity in experimental animals (Chapin *et al.,* 1989; Heindel *et al.,* 1995). However there is a lack of information on prolonged, repeated toxicity studies on mixtures at low doses. The study on heterogeneous mixture may alter basic toxicological findings and understanding acquired by single compound exposure. We believe that exposure to heterogeneous toxicant mixture may provide better understanding of the toxic potentials of industrial complexes. With increase in the number of chemicals in a mixture formulation there may be greater chance of chemical interaction, therefore for a model mixture toxicological study three to four component mixture is generally considered suitable.

3. REVIEW OF INTERNATIONAL LITERATURE

3.1. Single Compound, Specific Group and Heterogeneous Mixture Toxicity

Majority of research regarding the potential impacts of contaminants on life is derived from laboratory toxicity studies on single chemicals. With the improvement of technology and methodology, the toxicology gradually evolved from a science of high dose to a science of environmentally relevant doses. It also progressed from insensible conclusions to insightful end point which includes measurement of biochemical and functional changes in different systems. These changes toward low doses and more sensitive end points are encouraging as detailed information on mechanism of action can be obtained. The existing literature indicated abundance of toxicity information on single compounds on several model species. For each study, test animals were exposed to different concentrations of test chemical to obtain the valuable information of both behavioral and physical responses. Toxicological studies conducted earlier during mid 20^{th} century were designated to identify lethal dose (LD_{50}). The most common test was the acute toxicity test, in which

animals were given a single dose of chemical. As part of many toxicology studies, lethality studies are still conducted, but as a first step towards providing some insight into the toxic potentials of the new chemical.

Now a day's investigations on structure- activity relationships and molecular mechanisms of action are fundamental of single compound toxicity studies. A great deal of effort has already been devoted to elucidate the toxic responses and mechanism of toxicity and there is a growing body of literature which reflects the same (Table 1). For example, the evidences suggest that carcinogenic action of certain dioxin and related compounds is due to interactions of specific protein receptor (Mandal, 2005; Schwarz and Appel, 2005). In contrast, the study of toxicity mechanism of metals has suggested the generation of reactive electrophilic species which undergoes covalent binding to biological macromolecule ultimately leading to cancer (Stohs and Bagchi, 1995; Flora *et al.,* 2008).

Of all the toxicity studies that address the assessment of mixtures, the majority focus on binary mixtures or two chemicals at a time. The assessment was carried out at relatively high chemical concentrations and mostly chemicals belonging to same class were selected (Chukwuand and Okhumale, 2009). The studies were also carried out on combined toxicity (more than two) of defined chemical mixtures consisting of nephrotoxicants, pesticides, endocrine disrupting chemicals or carcinogens (Jonker *et al.,* 1996; Crofton *et al.,* 2005). In these studies the mixtures were formulated with those compounds whose mode of action and pharmacological properties were similar or comparable. Few mixture studies were concentrated on very complex mixtures such as industrial effluent, diesel fuel mixture or contaminated water samples (Shelby *et al.,* 1990; Huang *et al.,* 2008). The studies on environmentally relevant concentration of mixture (i.e. at low doses) are very less to rule out the potential for interactions amongst low doses of many different chemicals in living organisms.

Our environment (either at waste site, in the aquatic bodies, soil or the air environment) consist of chemical mixtures of several different compounds that are not produced together, but may occur together. For example a waste site might contain several heavy metals together with organophosphate, pesticides and some solvents. Therefore, studies on toxicity of mixture derived from different compounds (in terms of their nature and structure) are essentially required. But unfortunately this type of toxicological database is inadequate for risk assessment of chemical mixture.

4. REVIEW OF INDIAN LITERATURE

4.1. Single Compound, Specific Group and Heterogeneous Mixture Toxicity

Voluminous literature is available to disclose the occupational health hazards due to exposure to chemicals in industries; to determine the mode of action of toxic chemicals; to conduct safety evaluation of chemicals used in industries and everyday life. Literature is also available on remedial or preventive measures to safeguard health and environment from contaminants.

Several of Indian researchers have suggested that a variety of accompanying changes in antioxidant defense enzyme is associated with production of reactive oxygen species due to chemical exposures. A review written by Chowdhury (2009) revealed that exposure to different heavy metals caused an irreversible toxic insult to male reproductive system and could interfere with the spermatozoa directly in semen.

5. SYSTEMIC AND MALE REPRODUCTIVE TOXICITY OF SELECTED COMPOUNDS

The selected heavy metals (i.e. Cd and Cr) were linked with malformation associated with heat stress protein, androgen binding protein,

cadherin and many other stressor proteins (Murthy *et al.,* 1991; Aruldhas *et al.,* 2005; Patra *et al.,* 2011). However the toxicity of these metals were involved in producing cellular impairments at structural and function level in different systems. The metal Cd accumulates in renal cortex and results in malfunctioning of the kidneys. The cortex region was more affected whereas glomeruli as well as proximal tubules showed wall thickening (Mohammad, 2010). Ramesh and Satakopan, in 2010 showed tissue damage in liver due to cadmium poisoning and suggested that elevation of reactive oxygen species and lipid peroxidation was the mechanism of action. The impact of chromium VI exposure was studied by Dey et al in 2003 on liver, kidney, testis, spleen, cerebrum and cerebellum of male Wistar rats. Due to transformation of chromium VI to chromium III inside the cell, increase in lipid peroxidation and decrease in acid and alkaline phosphatase activities were observed as a consequence of reactive oxygen species produced in most of the organs.

As far as toxicity of selected organic contaminant is concerned, damage to various organs has been directly linked to the compound itself and also to their metabolites (Reddy *et al.,* 2006). The metabolites formed during the process of detoxification were linked to depletion of natural antioxidant enzymes and/or direct damages to cells. Treatments of PE in rats were linked to reduce the activity of succinic dehydrogenase and adenosine triphosphatase where histopathological studies revealed focal degeneration of seminiferous tubules and edema of interstitium in testis (Seth *et al.,* 1976).

The field of mixture toxicity is relatively unexplored in India. Jadhav *et al.* (2006) has concluded that the mixture of eight heavy metals induced genotoxicity in rat bone marrow and spleen cells. He also suggested that additive or synergistic effects of exposure to metal contaminants at MPL levels derived by WHO are unlikely to induce oxidative stress and result in adverse effects on the cytogenetics of male rats.

6. PRESENT STATUS OF ENVIRONMENT IN GUJARAT

Gujarat is one of the top industrialized states in India and consistently, it has maintained a higher pace of industrialization. The industrial belt of Gujarat is known as "Golden Corridor", runs along the main north – south highway. This industrial belt is considered as the robust backbone of Gujarat's economical development. This area includes the large industrial estates in Ahmedabad, Nandesari, Ankleshwar and Vapi (Fig. 4). These areas contain thousands of individual industrial units, including dye factories, pesticide, textile, pulp and paper producer, pharmaceutical, engineering and chemical companies *etc.* The chemicals used or produced by these industries are listed in Table 3. This rapid industrial development throughout the state has resulted in generating abundant industrial wastes having both organic and metal contents (Table 4) (Labunska *et al.,* 1999). These industrial wastes are discharged through Asia's first and longest effluent channel into estuarine region of Mahi River after proper effluent treatment for detoxification (Fig. 5). It starts from Dhanora (take off point) near Nandesari Industrial area, where it receives treated effluent from industries located in industrial complex in between Vadodara and Nandesari, and other industries at Nandesari through common effluent treatment plant. It receives effluent from oil refinery, fertilizers and chemicals factories at Koyali and chemical industries mostly of pharmaceuticals and dyes from Padra taluka of Vadodara district and Jambusar taluka of Bharuch district. A small river, called Mini River passes in between Nandesari industrial area and nearby industrial complex All these industries release various contaminants into this river. The Mini River meets River Mahisagar and ultimately meets the Gulf of Khambhat leading to Arabian Sea. Therefore, in this way the released industrial effluent is diluted into the environment at various points.

6.1. Chemical Pollution: Present and Future Scenario

Gujarat's rivers are bearing the burden of industrial pollution. All the major rivers and streams of Gujarat are in bad state due to effluent

discharged by industries (Fig. 6 and 7), as the quality of effluent at final discharge point is always with high organic content and also not meeting with standard discharge norms. Several times, because of the high costs involved in effluent treatment, the chemical factories dump their waste materials in open or in effluent channel without any treatment processes illegally. Not only this has polluted the ground water but it has also spoiled the fertile lands. Moreover, despite the fact that effluent is rich in toxic hazardous wastes, it is regularly used by farmers for irrigating the farms on the sides of channel (Fig. 8), leading to toxic and hazardous organic and metal compounds being absorbed in the food chain. Studies carried out in our department earlier, on environmental impacts of the effluent along the effluent channel, have demonstrated the high level of heavy metals in vegetables, fruits, food grains, fish muscle and even in human hairs (Sharma, 1995). The study clearly showed that over the years the soil had accumulated several toxic compounds and as a result of this biomagnification was observed in all edibles items (Table 5).

The nonstop dumping of effluent wastes of diverse kinds into the Mahi and Mini River made their water unreceptive for aquatic life and inappropriate for human consumption. The inhabitants of nearby areas, reported about the contamination of water tanks and wells. These kinds of complaints became a regular feature in this area and local newspapers or television news is reporting same types of evidences on daily basis. In fact, families staying in the region face severe health problems. The local population in all these industrialized areas has complained of skin irritation, perforation of nasal septum, high blood pressure (among youngsters), renal stone, asthma, cancer and infertility. These industrial units also release noxious and poisonous gases other than solid and liquid hazardous wastes (Fig. 9). The chemical zone has the strong odour of chemicals and pesticides. Landfills also contribute towards the emission of toxic gases into the air.

The government of Gujarat is going ahead with its various infrastructure development plans, to make the second most urbanized Indian state into a dream destination for business, but ignored the impact of chemical

pollution caused by the industrial discharge on public health and the environment. In Nandesari village around 200 hectares of agricultural land has been turned into a chemical industrial estate. Moreover, according to the future planning for economic development, government has targeted Gujarat's 1600 kilometer Arabian Sea coastline for port based industries. Mega cement plants are coming up on the coast in Kachchh and Saurashtra region, while huge refineries of the Reliance and Essar group of industries are under implementation alongside the protected marine national park in the Gulf of Kachchh.

With massive investment in the petrochemical, chemical and pharmaceutical industries, the state is facing problem of pollution in the major industrialized areas. Moreover, its future economic development planning can lead to severe horrified changes in environment and health, if the steps of environment protection measures were neglected or not followed strictly. Therefore, the studies like "impact assessment of industrial chemicals or industrial effluent on systems" and "toxicity report of assessment of industrial complex mixture" will draw the attention of higher authorities to take the necessary action to reduce the level of pollution.

7. TOXICOLOGICAL PROFILE OF SELECTED COMPOUNDS

It is very clear that all living organisms are exposed to various types of environmental contaminants; majority of these are harmful. Cadmium (Cd), chromium (Cr), 1, 2 dichlorobenzene (DCB) and phthalic acid di butyl ester (PE), selected for mixture toxicity study are considered to be among the most toxic compounds. Exposure to these toxicants, as a result of industrial and environmental pollution, represent serious health hazard to human and other organisms. The compounds have been found to produce wide range of biochemical and physiological dysfunctions in laboratory animals and humans. Depending on dose, route and duration of exposure the compounds can produce both acute and chronic tissue injury and can damage various organs. The mechanisms of cellular toxicity because of these four compounds

are well known at molecular level (Fig. 10). A brief description of research findings is given below and basic toxicological information is tabulated (Materials and Methods-Table 1) with their available LD_{50} values.

Chronic Cd exposure leads mainly to the nephrotoxicity (Mohammad, 2010). At chronic doses it can also produce skeletal damage, severe damage in nervous, endocrine and immune systems, as well as linked to cancer (Jarup *et al.,* 1998; Goyer *et al.,* 2004). Acute Cd exposure primarily affects the liver, inducing hepatocyte swelling and fatty changes. Cd caused testicular edema and exerted deleterious effect on the vascular structure of testis (Siu *et al.,* 2009; Marettova *et al.,* 2010). Histopathological observation revealed injury of germinal epithelium and damages to the interstitial tissue (Mason *et al.,* 2005) after Cd exposure.

Chromium cause hepatic and renal toxicity in animal and workers and lipid peroxidation has been considered to cause membrane damage and tissues injuries by chromium (Stohs and Bagchi, 1995). Studies of workers in chrome pigment industry revealed a correlation between exposure to Cr VI and lung cancer (Levy *et al.,* 1986; Langard, 1993). Moreover Administration of chromium VI through intraperitoneal injection during organogenesis in rat revealed embryo and fetotoxic effects (Marouani *et al.,* 2011). Experimental observations also indicated that different doses (20, 40, 60 mg/kg) of chromium in rat caused diminution of tubular diameter, nuclear size of testicular cells and reduction in cell population of spermatogenic cells (Chowdhury, 2009).

The toxicity of DCB was found to be associated with pathological findings of hepatic centrilobular necrosis, hepatocellular degeneration and depletion of lymphocytes in thymus and spleen (Aiso *et al.,* 2005; Younis et al 2003). High dose to male rats showed renal tubular degeneration while mice exhibited multifocal mineralization of myocardial fibers and skeletal muscle (NTP, 1985). Hematological changes were also observed at 500 mg/kg body weight in rats (NTP, 1985).

The PE is used extensively in the manufacture of plastic and has been characterized as a developmental and reproductive toxicant in the rat (Foster *et al.,* 2001; Bao *et al.,* 2011). Oral doses of PE when given to juvenile and adult male rats produces a testicular lesion characterized by sloughing of germ cells and vacuolization of Sertoli cell cytoplasm (Boekelheide *et al.,* 2000). Pubertal rats are more sensitive than adult rates to the toxicity of PE. It has also been shown that PE readily crosses the placenta and dietary administration throughout gestation period to adulthood resulted in malformation of male reproductive tract (Saillenfait *et al.,* 1998).

8. UNDERSTANDING OF MECHANISM OF TOXICITY

8.1. **Metals** (Fig. 10)

During distribution phase, metals exit the blood and enter into the extracellular space where it can be reduced, resulting in the formation of reactive oxygen species and cause lipid peroxidation of cellular membrane. The metals move readily into the cells simply by diffusion or through intercellular spaces/pores of capillary endothelium cellular membrane which is generally facilitated by transport protein. At first NADPH oxidase increased the production of H_2O_2 because of action of metal induced alterations. The increased H_2O_2 level disrupts the phospholipid membrane layer due to lipid peroxidation leading to production of reactive oxygen species (ROS). Enhance generation of ROS can overwhelm cell's endogenous antioxidants status and result into severe oxidative stress condition (Ercal *et al.,* 2001). This oxidative stress condition leads to various dysfunctions of protein and DNA structures and of cellular membrane. Oxidative damage can affect the efficiency of other organelles like mitochondria and further increases the rate of ROS production. However, enhance mitochondria ROS level can produce DNA strand breaks that leads to the activation of the DNA repair enzyme poly (ADP-ribose) synthetase (PARS). Excessive activation results in the depletion of its substrate, adenosine triphosphate (ATP) and nicotinamide adenine

dinucleotide (NAD), leading to cellular dysfunction or even death. In addition, receptor metal complex in membrane cause elevation of concentration of calcium ions and activation of various kinases via initiating cal-modulin Ca^{2+} (Cal-M Ca) system. The process starts transcription factor in the nucleus and mitochondria leading to the production of stress proteins that can act as mediator of cellular injury. Depleted thiol level and cellular lipid peroxidation can also alter intracellular calcium homeostasis and induce damages in protein and membrane through protease activation, endonuclease induction.

8.2. **Organic Compounds** (Fig. 10)

Organic compounds exert its toxicity via binding to the intracellular aryl hydrocarbon receptor (Ah receptor) with the help of Ah receptor nuclear translocator (ARNT) protein. Ah receptor dissociates from its chaperon protein (HSP 90) and forms a heterodimer with ARNT in nucleus, upon its binding with organic compound. The heterodimer binds with DNA at xenobiotics responsive element (XRE) and activate genes of many drug metabolizing enzymes to activate their transcription and translation (Zhang, 2011). Biologically inert compound is activated through induction of cytochrome P450 to electrophilic derivatives and thus leads to adverse changes in normal function of cell. The aryl hydrocarbon receptor also functions as a modulator of cellular signalling pathways via protein kinase C activation (Long *et al.*, 1998). However little is known about the ability of protein kinases to regulate the activity of the Ah receptor or ARNT.

In addition to metals, organic compounds can also produce ROS that in turn can cause damages to protein, lipid or DNA. These toxic responses can initiate necrosis, apoptosis, carcinogenesis and hypersensitivity and hamper the activity of other organelles like mitochondria (Henkler and Luch, 2011). Moreover, ROS escape form leaky channels of mitochondria and elevate the level intracellular free radical. To stabilize itself, free radical can steal an electron and break down other biomolecules like fatty acid and can initiate oxidative stress condition.

9. QUALITY OF LIFE AND REPRODUCTION

Chemical contaminants in the environment are big problem because they have reduced the quality of lifestyle and threatened the human health. Dermatitis, premature ageing of the skin, digestive disorders, bronchitis, fatigue *etc* are the few effects of environmental pollutants on health. Few studies have revealed that exposure to solvents, pesticides and metals are associated with the occurrence of abortion, low birth weight, birth defects, childhood leukemia, cancer and other end points related to growth and development. Pollution also cause diseases associated with the progress of civilization, such as increase in rate of allergic incidences, cardiovascular diseases and diabetes (Bertazzi *et al.,* 1998; Brook *et al.,* 2004). Also the pathological conditions of the male reproductive organs are becoming more common. In past 50 years incidences of infertility have increased and the most frequent causes, for 90% of the total, are associated with spermatogenesis impairment and other are related to disorders of accessory glands, erection, ejaculation and coitus (Queiroz and Waissmann, 2006).

9.1. Current Global Trends of Male Reproduction and Toxicology

Concerns that chemical exposures in the environment have been detrimental to male sexual development and fertility has been heightened by reports of declining sperm count over the past few decades (Carlsen *et al.,* 1992; Skakkebaek *et al.,* 2001). The reports of testicular cancer and other male reproductive tracts such as hypospadias and cryptochidism have progressively increased in many countries over past century (Toppari *et al.,* 1996; Skakkebaek *et al.,* 2001; Saradha and Mathur, 2006). Environmental chemical exposures especially to estrogenic compounds and various occupational factors have been correlated with these observed changes in male reproductive health and fertility. For this reason, the significance of possible trends of semen quality and other reproductive disorders due to

influence of occupational and environmental hazards, are now considered as most active areas of toxicological research. The male reproductive system is complex; its full reproductive capacity is dependent on disparate physiological processes. Therefore, an accurate picture of male reproductive capacity cannot be obtained through the measurement of sperm count. So, clinicians and researchers are using sophisticated methodologies to assess the male reproductive health and fertility. The method for assessing impact of hazardous substances on reproductive functions includes questionnaires to determine reproductive history and reproductive hormone profiles, semen analysis, assays of sperm function and biomarkers of DNA damage.

10. THE SYSTEMS STUDIED

10.1. Blood, Liver and Kidney: Toxic Manifestations

A toxicant is distributed to tissues throughout the body via blood. The simplest approach to testing chemical toxicity in animals is to evaluate the effects on the circulating blood cells. If the balance between cell production and removable is disturbed, health will be impaired. A reduction in red cell concentration in the blood affects the oxygen and nutrient supply to various vital organs. Wintrobe 1981 listed many drugs and chemicals that have been reported to cause blood dyscrasias. Also, it is expected that the hematopoietic organs are readily affected by wide varieties of chemicals. It is therefore, understandable that examination of the whole blood profile is one of the important actions performed in the health surveillance.

Liver and kidney play important role in transforming and clearing chemicals and is therefore susceptible to the toxicity from chemicals. Liver is the largest internal organ in the body and histologically divided into lobules. The lobules are filled with hepatocytes which are responsible for bile secretion and perform varieties of metabolic functions. Nearly 75% of blood coming to the liver arrives directly from the gastrointestinal organs and thus brings xenobiotics in undiluted form. Between each row of hepatocytes are small

cavities called sinusoids. The main functions of sinusoids are to destroy old red blood cells, to remove foreign particles from blood and to detoxify toxins and other harmful substances. These chemicals or harmful substances are subjected to various processes of metabolism to make them suitable for elimination. The process is generally divided into two phases. Phase 1 reaction involves oxidation, reduction, hydrolysis and many other chemical reactions and also prepares the foreign substance for phase 2 reaction. However many foreign substances can be metabolized directly by phase 2 reactions. Moreover, these processes can generate metabolites which are more active and potentially toxic.

Kidney serves as a natural filter of the blood and removes wastes. The kidney excretes waste such as urea and creatinine and also responsible for reabsorption of glucose and amino acids. The outer portion of the kidney consists of the cortex and the medulla containing millions of nephrons. The nephrons are composed of two main parts the renal corpuscle and the renal tubule. Inside the renal corpuscle is the glomerulus and the renal tubule consists of the proximal tubule, the loop of Henle and the distal tubules.

The metabolites that are excreted from the kidneys may also cause cellular damage. Thus several processes are responsible inducing hepatic and renal injury. Chemicals can produce wide varieties of clinical and pathological hepatic and renal injuries. Several biochemical markers are generally used to indicate extent of liver and kidney damage. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) activities are the most common used enzymes to assess hepatocellular damage. Significant elevation in serum SGOT and SGPT indicate the toxic responses of chemical. Besides these two markers of liver function test other markers like alkaline phosphatase, lactate dehydrogenase activities are also used to evaluate the toxicity. Urea and creatinine are nitrogenous end products of metabolism removed from the kidneys and both are used as clinical serum biomarker of renal damage. Elevation in urea and creatinine level can serve as clinical indicator of poor kidney function.

10.2. Male Reproductive System

The male reproductive system consists of the testes and their associated excurrent ducts to conduct sperms to the outside. It also includes various accessory organs that stores sperms and produces non sperm components of the ejaculate. The spermatozoa pass from the testis to the ductuli efference, ductus epididymis and vasa deferentia to prostatic ampulla of vas deference. At this site the secretions of seminal vesicle and prostate glands mix to form semen, which upon ejaculation passes through the prostatic urethra. It also includes the intromittent organ, the penis.

The emphasis has been given to investigate the toxicological effects on testis, epididymis and on indicative functional markers of other reproductive accessory organ.

The outer border of the testis is demarcated by a thick band of connective tissue, called as tunica albuginea. The testis has seminiferous tubular and interstitial compartment. The bulk of the volume of the testis is filled with the seminiferous tubules. The lining cells of the tubules comprise the seminiferous epithelium. Some (spermatogonia) of them are source of sperm and some (Sertoli cells) are there to maintain the production. 14 distinct stages are distributed in the form of continuous wave in the seminiferous tubule (Fig. 11).

Sertoli cells, the somatic constituent interact directly with developing germ cell throughout spermatogenesis. Several studies have shown that these cells have an indispensible role in the development and movement of germ cells and exhibit cyclic variations in their morphology and physiology with respect to spermatogenic cell association. Cyclic variations in Sertoli cell's lysosomes population, activities of acid phosphatase, ATPase, SDH, LDH, and other enzymes and microtubule organization at different seminiferous epithelial stages have been reported. The functions of Sertoli cells includes: 1) providing structural support, 2) creating an impermeable

barrier, 3) participating in germ cells movement and spermiation, 4) nourishing germ cell via their secretory products. Tight junctions are found between adjacent Sertoli sells at the level of blood testis barrier where they coexist with basal ectoplasmic specialization and desmosomes gap junction. The BTB physically divides the seminiferous epithelial into two distinct compartments: a basal compartment where spermatogonia and early spermatocytes are found, and an adluminal compartment where more developed germ cells are sequestered from the systemic circulation (Knobil and Neill, 1994).

The principle interstitial cells that are found adjacent to the seminiferous tubules are Leydig cells. They produce testosterone in the presence of luteinizing hormone. The testosterone regulates the process of spermatogenesis, epididymal sperm maturation, accessory sex organ function and sexual behavior.

Chemicals, either in isolation or in mixtures, may have adverse effects on all organs including liver, kidney, heart, brain, and reproductive organs. However, the majority of recognized chemicals that cause toxicity will alter structure and function of vital organs like liver, kidney and reproductive organs like testis and epididymis either as a consequence of their own metabolism or by direct action. Therefore the emphasis will be given to evaluate the toxicity effect of chemicals mixture on systemic and reproductive organs.

11. LACUNAE OF KNOWLEDGE

Mixtures in the environment are usually composed of multiple components with dissimilar chemical structures and modes of actions. This type of mixture (with different chemical structure and actions) has been least frequently studied. The majorities of studies have analyzed the effect of combinations of chemicals belongs to same class of compounds. There is very less evidence on the combined action of environmentally realistic mixtures, composed of chemicals from different chemical and functional classes. To explore the limitation in order to answer of question that does the

chemical mixture disobey dose response rule or to further demonstrate the conjecture that concentration and dose addition might be applicable as a general rule for describing the joint action of chemical mixtures.

12. INITIATION OF RESEARCH IDEA

Published mixture studies were mainly conducted with aim to explain the combined action of selected pure compounds in terms of their individual effects. Existing studies of chemical mixtures largely focused on investigation of effects and interaction of binary mixture, or on complex mixtures such as pesticides, environmentally relevant mixture of pharmaceuticals, organohalogen compounds etc. Few toxicity studies are focused on complex chemical mixture exposure in the form of effluent (released from various industries), diesel exhaust, and tobacco smoke; where the actual identity of the chemical was partially known. There is lack of toxicity studies of properly defined mixture using environmentally doses. In this context, it would be especially valuable to obtain further insight into question as to whether low, individually non toxic concentrations of dissimilar compounds might lead to a significant mixture effect. Therefore, the decision was reached to study a chemically defined mixture given for relatively longer exposure periods at low doses (can be consider as realistic doses). It was decided to investigate the adverse effect of a known chemicals mixture of few of the industrial effluent contaminants on systemic and reproductive toxicity.

OBJECTIVES

Assessment of the effect of administration of HCM at/ below "no observable adverse effect level" (NOAEL) of individual component chemical.

i. **Assessment of toxicant body burden and distribution profile.**
ii. **Assessment of systemic and male reproductive toxicity.**
   - **Study spermatogenesis kinetics and seminiferous epithelial stage-wise variability of responses.**
   - **Study spermatological parameters.**

Table 1: Experimental studies on chemicals Toxicity

Environmental contaminants and their effects	References
Cadmium: Renal tubular dysfunction, Osteomalacia, anemia, Neurodegenerative changes with reduction in learning and memory retention capacity, and necrosis of periportal liver cells.	Ohta *et al.,* 2000; Kuester *et al.,* 2002; Ognjanovic *et al.,* 2003; Kaoud *et al.,* 2010; Tarasub *et al.,* 2011
Chromium: Causes allergic dermatitis, pulmonary carcinogenicity, nephrotoxicity. Induces testicular damages with degeneration of seminiferous epithelium. Induces neoplasm of epithelium of oral cavity and small intestine.	O'Flaherty, 1993; Pereira *et al.,* 2005; Stout *et al.,* 2009
Mercury: Repeated exposures resulted in severe disturbances in the central nervous system, Dementia, loss of memory, excitability, and insomnia. Induce oxidative injury in renal proximal tubule and liver.	Philbert *et al.,* 2000; Stacchiotti *et al.,* 2004; Al-Madani *et al.,* 2009; Patnaik *et al.,*2010
Lead: Causes damages in prefrontal cerebral cortex, hippocampus, and cerebellum and leads to neurologic disorders like information processing difficulties, memory problems, reduction in sensory and motor reaction times.	Odigie *et al.,* 2004; Reza *et al.,* 2008; Sanders *et al.,* 2009;
Arsenic: Skin lesions, peripheral neuropathy, encephalopathy, hepatomegaly, anaemia, bone marrow depression, and renal necrosis.	Ayala-Fierro *et al.,* 2000; Hughes, 2002; Ebele, 2009;
Nickel: Atrophy of the nasal olfactory epithelium, cause lung inflammation, fibrosis, and alveolitis.	Zhang *et al.,* 1998; Das *et al.,* 2008; Jia *et al.,* 2010
Organohalogen compounds: Toxic to neural and glial cell, suppress immune responses, impair reproductive functions, produce hepatotoxicity, hypothyroidism, cardiovascular toxicity, bone, skin, and tooth toxicity and cancer.	Birnbaum, 1995; Hanberg, 1996; Kakeyama and Tohyama, 2003; Boas *et al.,* 2006; White and Birnbaum, 2009
polycyclic aromatic hydrocarbon: induce cardiovascular disease and pulmonaly dysfunction	Kumagai and Taguchi, 2007;
Phenols: Neurotoxic, hematotoxic, hepatotoxic, nephrotoxic, provoke mutagenesis and carcinogenesis.	Michalowicz and Duda, 2007;
Phthalates: Reduce testicular hormone production; also affect reproductive functions characterized by reduction in fertility, sperm density and motility, and ovarian and testicular weights.	Gray *et al.,* 2000; Lovekamp-Swan and Davis, 2003; Rider *et al.,* 2009; Nguyen *et al.,* 2010;
Pesticides: Affects the tracts in the nervous system and Inhibit the cholinesterases activity. Also affect the immune responses.	Kamanyire and Karalliedde, 2004; Li and Kawada, 2006; Li, 2007

Table 2: Epidemiological studies on toxicity of chemicals

Environmental contaminants and associated epidemiological observations	References
Cadmium: Itai-Itai disease, renal damage	Jarup, 2002
Chromium: highly carcinogenic	Hayes 1997; Linos *et al.*, 2011
Mercury: Minimata disease with characteristic of endoneurial fibrosis and degenerated myelin sheath.	Eto *et al.*,2002
Lead: Anemia, pronounced effect on developing central nervous system of young children	Silbergeld, 1990; Schwartz *et al.*, 1990
Arsenic: Cause skin cancer, hyperkeratosis and hyperpigmentation.	Germolec *et al.*, 1998; Schuhmacher-wolz *et al.*, 2009
Nickel: Lung fibrosis with risk of respiratory cancer, cardiovascular and kidney disease	Ilic *et al.*, 2007
Organohalogen compounds: Erythema followed by acneform eruption, Low birthweight and increase breast cancer incidences, cardiovascular diseases and immunologic disorder	Bertazzi *et al.*, 1998; Schecter *et al.*, 2005
Polycyclic aromatic hydrocarbon: Classified as human carcinogens and cause lung and bladder cancer.	Mastrangelo *et al.*, 1996
Phenols: Hematotoxic and hepatotoxic, provoked mutagenesis and carcinogenesis.	Michalowicz and Duda, 2007
Phthalates: Induce developmental and reproductive toxicity and alter thyroid, respiratory function.	Jurewicz and Hanke, 2011
Pesticides: Organochlorines adversely affect semen quality and caused testicular cancer in males, induce menstrual cycle abnormalities and spontaneous abortions. Organophosphate pesticides caused thyroid toxicity and inhibited cholinesterases activity.	Toft *et al.*, 2004; Jintana *et al.*, 2009; Lacasana *et al.*, 2010;

Table 3: List of hazardous chemicals identified in waste water and solid waste from the industries of Nandesari area near Vadodara

Industry	Hazardous Chemical(s) Used/ Products /By Products
Reliance Industries Limited	Caustic soda, Ethylene, Propylene butadiene, Toluene, Xylenes, Glycol, Ethylene-oxide
Gujarat Alkalies and Chemical Limited	Caustic Sauda, Sodium Hydrochloride, Liquid chlorine, Compressed hydrogen gas, Aluminium chloride
Gujarat State Fertilizers Company	Caprolactam, Methyl ethyl ketoxime, Melamine, Anhydrous ammonia, Nylon 6, Ammonium sulphate
Indian Oil Corporation Limited Gujarat Refinery	Petrol, Diesel, Naphtha, Kerosene, Linear alkyl benene, Hydrocarbons, Hydrogen sulfide, Phenols, Benzopyrene, Ammonia
Deepak Nitrite Limited	Nitric acid
Ester India	Dimethyl hydantoin, Halogenated hydantoins, Succinimide, Acetic acid, Acetone cyanohydrine, Ammonia, Bromine, Isopropanol, Methanol,
Spa Vet Min Private limited	Hydrochloric acid, Residue of rock phosphate
Diamines & Chemicals Limited	Ethylene Amine, N-Methylpyrrolidone, ammonia, sodium hydroxide, Methyl methacrylate, Glyceryl monolaurate
Nahar Pharma Chem	Monochloroacetic acid, Isopropyl alchol, Methyl alcohol, Ammonium hydroxide, Cholo acetetes
Nandesari Rasayanee Limited	Carbon Sludge
Chloritech Industries	Chloral hydrate, Hydrochloric acid, Chlorine
Sodium Metal Private Limited	Acetyl, Trityl Chloride, Diisopropyl propionitrile, Polyporous prilled ammonium nitrate, Soda amine, Isopropyl bromide, Benzyl chloride, Methanol
Ushma Industries	Borax anhydrous, Boric acid, Zinc carbonate, Saccharin, Halazone Usp, Etp Sludge, Hdpe Bags,
Reckon Petrochem	Monochloroacetic acid, Ascorbic acid, Hydrochloric acid
Sodium metals private Limited	Metalic sodium, Benzophenone, Chloro acetophenone, Naphthoic acid, Ethyl amines
Gujarat Dyestuff Industries	Vinyl sulphone esters, Hydrochloric acid
Ntp Tar Products private Limited	Bitumen emulsions
Gandhar petrochemical Private Limited	Vinyl sulphone ester, Acetic acid
Nikunj Chemicals Limited	Chlorinated paraffin, Dicalcium phosphate, Glacial acetic acid, Sodium sulphate, Meat tenderizer, Zinc oxid, Methyl 12 hydroxy stearate, Sulphuric acid, Sodium metabisulphite

Table 4: Chemicals found in sediment, solid waste, industrial waste water and river water sample collected in the vicinity of the industries estate around Vadodara (Labunska *et al.*, 1999)

Organic compound	Heavy metal
Dichlorobenzenes	Cadmium
Trichlorobenzenes	Chromium
Tetrachlorobenzenes	Cobalt
Pentachlorobenzenes	Copper
Chlorinated benzenamines	Lead
Chlorinated toulenes	Manganese
Chlorinated naphthalenes	Mercury
Polychlorinated butadienes	Nickel
Naphthalene and its derivatives	Zinc
9 H-Fluorene	
Phenanthrene and its derivatives	
Alkyl phenol derivatives	
Biphenyl and its derivatives	
Alkylated benzene derivatives	
Organosulphur compounds	
Linear alkanes and alkanes	

Table 5: Accumulation profile of several toxicants in various eaTables and other items grown in the farms irrigated with industrial effluent directly from effluent channel (Sharma, 1995)

Metal (mg/kg)	Wheat	Bajra	Drumstick	Brinjal	Potato	Tomato	Grass
Copper	89.8	204	39	29	92	11.8	3.4
Chromium	3.4	63.2	3.1	2	1.6	7.6	2.1
Znic	367.4	427.4	86.8	48.1	22.6	10.8	108.4
Nickel	2.1	3.2	2.2	18.8	2.3	2.3	3.9
Lead	2.4	2.9	9.2	26.3	9.8	91.3	40.8
Cadmium	21.3	10.7	7.9	8.8	8.8	6.2	3.0
Iron	41.3	25.7	12	32.1	10.1	31.2	21.2

Fig. 1: Factors that influence the toxicity of substances

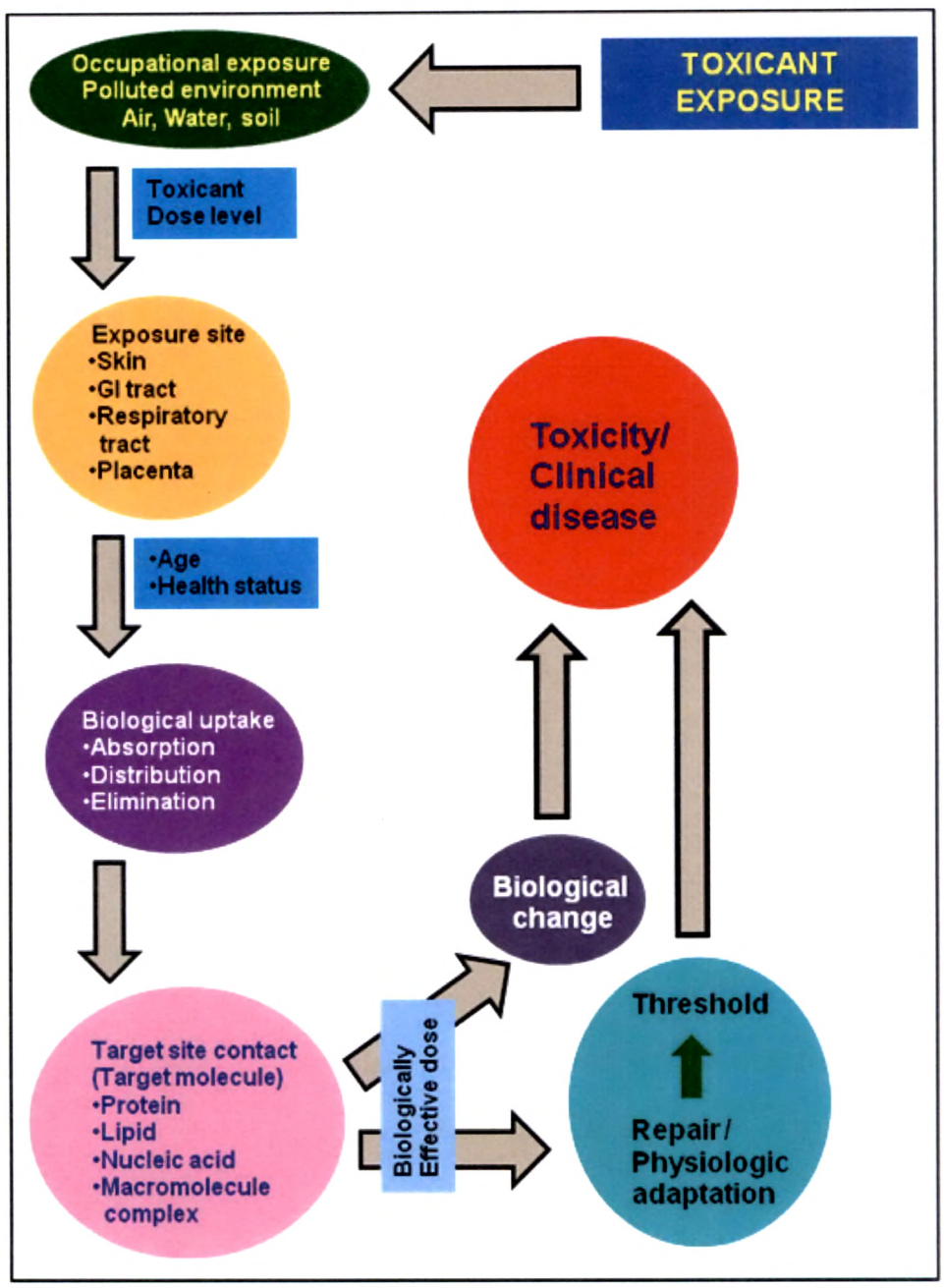


Fig. 2 Diagrammatic representation of possible interaction based study protocol for simple mixtures

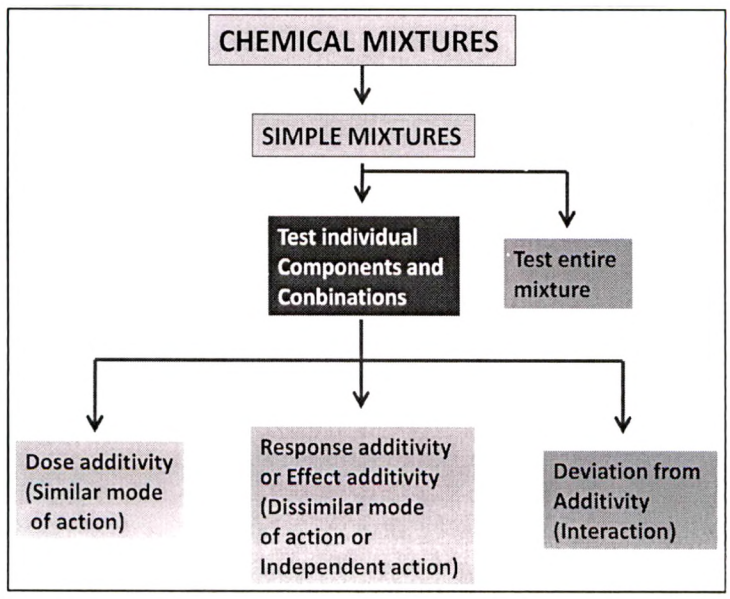


Fig. 3 Digramatic representation of possible interaction based study protocol for complex mixtures

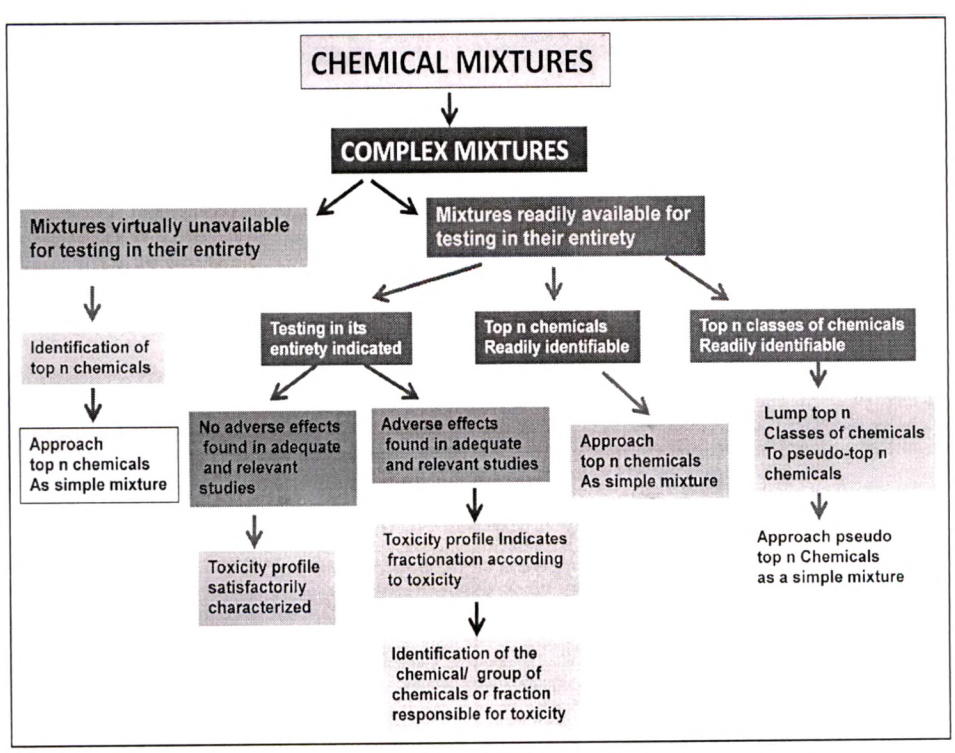


Fig. 4: Major industrial unit in Gujarat along Delhi-Mumbai Industrial Corridor (Source: Government of Gujarat, CLSA Asia-Pacific Markets)

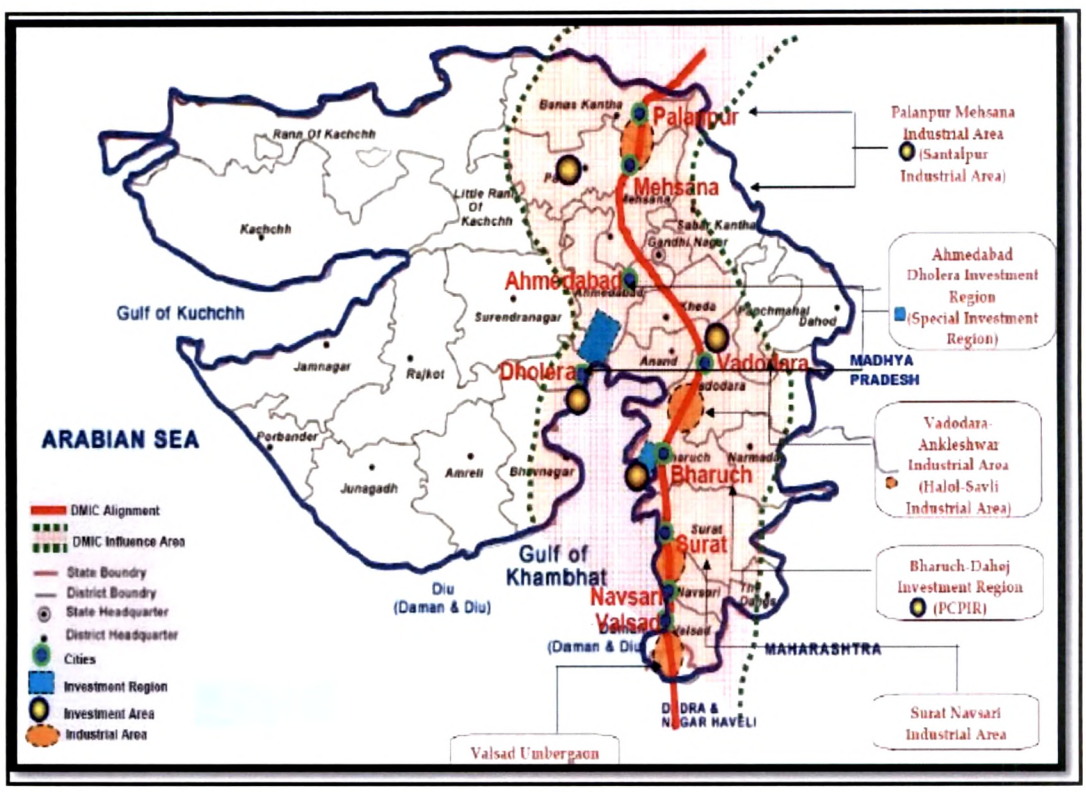


Fig. 5: Showing status of pollution in the chemical industrial zone of Gujarat.

[A] Mini river passing through industrial area near Vadodara and pouring into Mahi River (Source: Google Earth). [B] The effluent released in Mini River. [C] The effluent being released in Mahi River (location shown in Fig. 6)

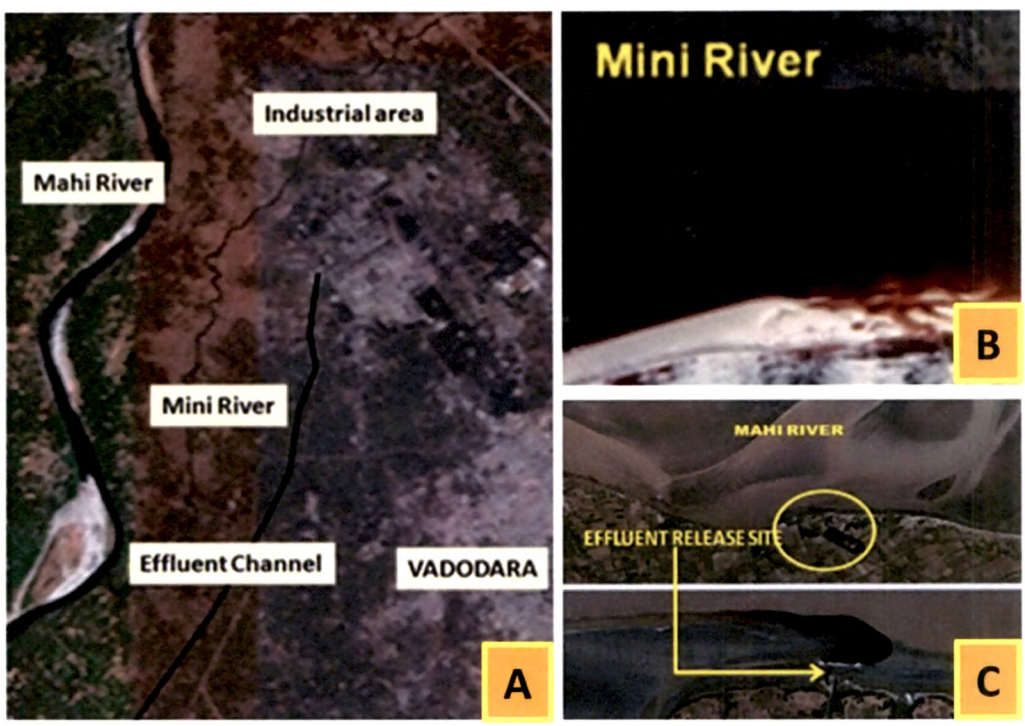


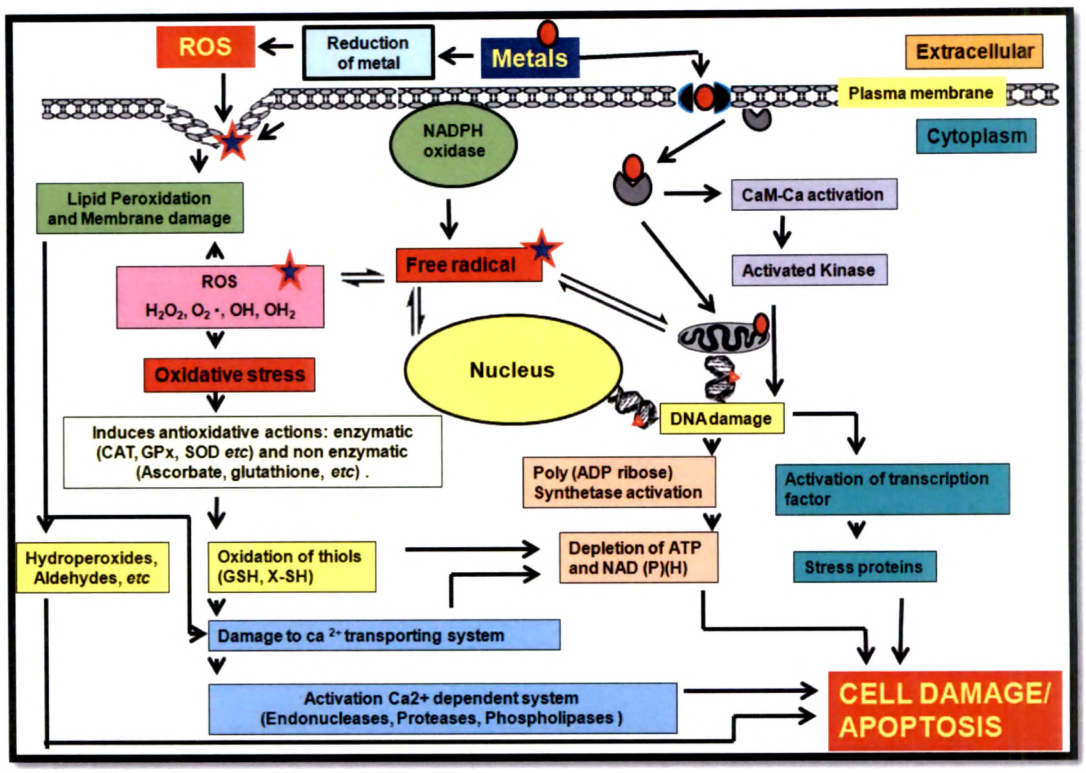
Fig. 6: Showing the industrial effluent releasing site in the lower estuarine area of Mahi River (Source: Google Earth).

Fig. 7: A recent fire in Mini River that passes through the industrial area near Vadodara; the organic sludge load released by industries is described as the reason.

Fig. 8: showing the illegal use of industrial effluent for irrigation

Fig. 9: The pictures of air pollution being generated by the industries in the industrial area around Vadodara

Fig. 10: Mechanism of cellular toxicity of selected compounds.



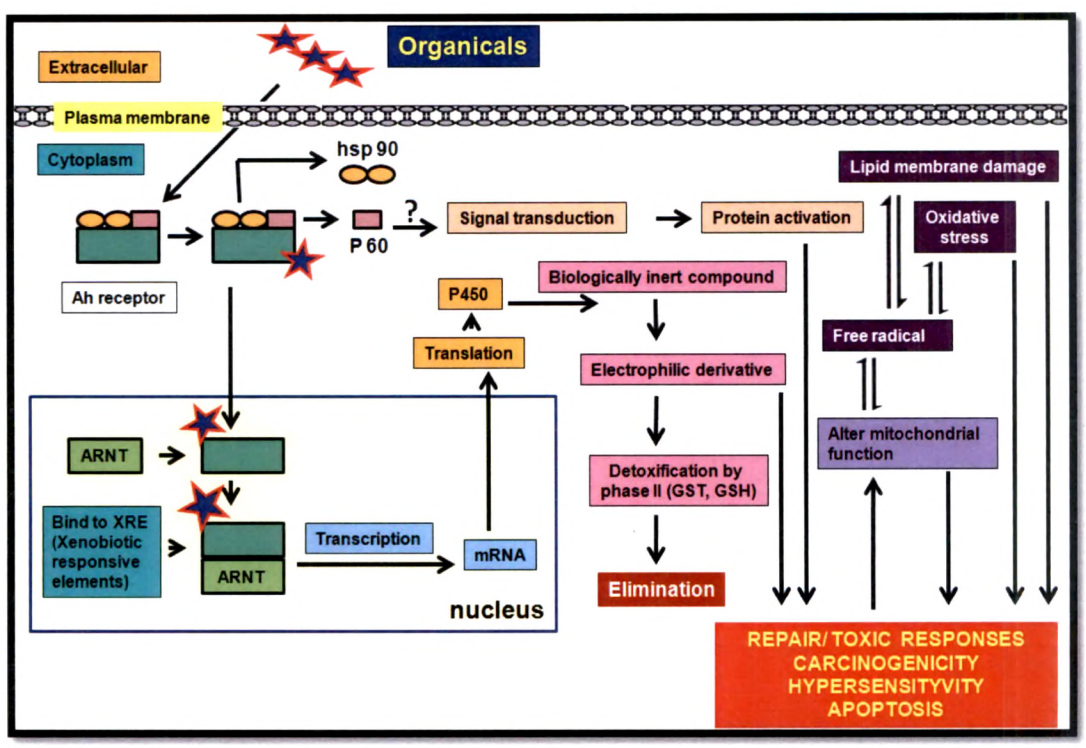


Fig. 11: Distribution of stages in the wave of spermatogenesis in the rat seminiferous epithelium