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Review Article

Effects of diesel exhaust, heavy metals and pesticides on various organ systems: Possible mechanisms and strategies for prevention and treatment

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Environmental pollutants have a significant impact on the ecosystem and disrupt balance between environment, human and non-human components that result in deleterious effects to all forms of life. Identifying environmental factors for potential imbalance are extremely crucial for devising strategies for combating such toxic dysregulation. Automobile exhaust (in air), heavy metals (in food and water) and pesticides (in air, food, soil and water) are the most common environmental pollutants and their short and long term exposures can cause hazardous effects in humans leading to systemic disorders involving lungs, kidney and immune systems. Mechanisms involved in genesis of such toxic effects have revealed complex, interactive pathways. Strategies for the protection of homeostasis and health, *viz*., general preventive measures, nutritional supplements and herbal agents have been described, to counter these pollutants induced damaging effects on various body systems.

Keywords: Antioxidants, *Azadirachta indica*, Diesel exhaust, Environmental toxicants, Heavy metals toxicity, Oxidative stress, Pesticides

Environmental pollution is the contamination of the ecosystem that causes instability, disorder, harm or discomfort to the physical systems or living organisms. Environmental factors have important links with infectious as well as non-infectious diseases of both acute and chronic nature. Global burden of disease attributable to selected sources of environment like water sanitation and hygiene, urban outdoor and indoor pollution, occupational carcinogens, noise and airborne particulates has been assessed to be 8-9%, measured either in terms of mortality or 'disability adjusted life years'(DALYs). DALYs incorporates number of years lived with a disability due to disease or injury, weighted according to its severity (based on expert assessments of the relative impact of some 500 different conditions and disease sequelae)[1]. Such hazardous events prompted to implement legislation and the Clean Air Act of 1956 was implemented. Pollution began to draw major public attention in the United States between the mid-1950s and early 1970s, when Congress passed some of the regulatory acts; the National Environmental Policy Act, Clean Air Act, and Clean Water Act[2,3] .

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Environmental toxicology

Impact of air pollutants on health—WHO estimates that 2.4 million people die each year from causes directly attributable to air pollution, with 1.5 million of these deaths attributable to indoor air pollution[4]. A study by the University of Birmingham has shown a strong correlation between pneumonia related deaths and air pollution from motor vehicles[5]. Direct causes of air pollution related deaths include aggravated asthma, bronchitis, emphysema, lung and heart diseases, and respiratory allergies. Principal stationary pollution sources include chemical plants, coal-fired power plants, oil refineries, petrochemical plants, nuclear waste disposal activity, incinerators, large livestock farms (dairy cows, pigs, poultry, etc.), PVC factories, metals production factories, plastics factories, and other heavy industry[6]. Though globally man made pollutants from combustion, construction, mining, agriculture and warfare also contribute significantly in the air pollution equation[7]. Agricultural air pollution comes from contemporary practices which include clear felling and burning of natural vegetation as well as spraying of pesticides and herbicides. Air is polluted by the release of chemicals and particulates like carbon monoxide, sulphur dioxide, chlorofluorocarbons (CFC) and nitrogen oxides produced by industries and motor vehicles into the atmosphere[8].

A survey by the Central Pollution Control Board and the All India Institute of Medical Sciences of New Delhi showed that a majority of people living in Delhi suffered from eye irritation, cough, sore throat, shortness of breath and poor lung functioning. One in 10 people have asthma in Delhi[9].

Effect of diesel exhaust on health—Motor vehicle emissions are one of the leading causes of air pollution[10,11]. Vehicle emissions are responsible for 70% of the country's air pollution. Diesel is being increasingly used in motor vehicles and industries because it is a cheaper fuel. Fine particles or microscopic dust from unfiltered diesel engines are rated as one of the most lethal forms of air pollution caused by industry, transport, household chores and oil-fired power stations. Diesel emission is a complex mixture of thousands of gases and fine particles (commonly known as soot) that contains more than 40 toxic air contaminants. These include many known or suspected cancer-causing substances, such as benzene, arsenic and formaldehyde. It also contains other harmful pollutants, including nitrogen oxides[10,11]. Exposure to diesel exhaust (DE) is an environmental and occupational health concern. The microscopic suspended particles in diesel exhaust are less than one-fifth the thickness of a human hair and are small enough to penetrate deep into the lungs, where they contribute to a range of health problems. Exposure to fine particles has been associated with increased frequency of childhood illnesses and can also reduce lung function in children. Since children's lungs and respiratory systems are still developing, they are more susceptible than healthy adults to fine particles[12]. Acute effects of diesel exhaust exposure include irritation of the nose and eyes, lung function changes, respiratory changes, headache, fatigue and nausea which manifest as rhinitis and asthma. Chronic exposures are associated with cough, sputum production and lung function decrements. In addition to symptoms, exposure studies in healthy humans have documented a number of profound inflammatory changes in the airways, notably, before changes in pulmonary function can be detected. It is likely that such effects may be even more detrimental in asthmatics and other subjects with compromised pulmonary function. Diesel emissions can trigger asthma and in the long run even cause lung cancer[13,14]. NO_2 there are both epidemiological and laboratory-based evidences suggesting that increased exposure to liquid petroleum and gas-derived air pollutants [nitrogen dioxide (NO_2), ozone, and respirable particulate matter] may play a role in the clinical manifestation of both allergic and non-allergic airway disease[13-15].

Diesel contains a number of potentially neurotoxic substances[16] and exposure to other mid-distillate fuels has resulted in neurological disorders including drowsiness, neurasthenia and decreased sensorimotor speed[17]. Several case studies have shown acute renal failure (secondary to acute renal tubular necrosis) as a potential complication following acute exposure to diesel[18-21]. Signs of oliguria (progressing to anuria), nausea, abdominal cramps and diarrhea have been reported. Exposure of the eyes to diesel may cause transient pain and/or hyperaemia[22]. Acute dermal exposure may result in local irritation (erythema, pruritis) which is generally more severe than that seen with other middle distillate products[23]. Incorporation of additives (such as biocides) may augment dermal sensitivity to diesel[24]. There are limited evidence to suggest that long-term pulmonary residual effects may occur following chemical pneumonitis (as a result of aspiration-induced pneumonitis)[25,26].

Few studies have reported the toxicity of diesel *per se*. Exposure of animals to diesel exhaust in simulation chambers have shown alterations in biochemical and cellular constituents of airway lavage. Therefore, we have investigated the time-course of the development of changes in protein content and elastase inhibitory capacity (EIC) of the bronchial airway lavage following diesel exhaust (DE) exposure. Morphological and histopathological changes in lungs of these rats have been correlated with suspended particle matter (SPM) deposition and lung/body weight ratio[27].

For diesel toxicity, animals were exposed to 1 part DE diluted with 5 parts of clean air in a simulation chamber for 15 min/day for 1, 7, 14 and 21 days. After completion of various exposures, biochemical parameters including elastase inhibitory capacity (EIC) and protein content of the bronchial airway lavage (BAL) and histopathological changes along with lung/body weight ratio were assessed. EIC (index of the protection against destruction of elastin, lung connecting tissue) in the BAL reached maximum after 1 week, remained elevated up to two weeks of exposure and surprisingly showed a decreasing trend after three weeks. Since EIC is

considered to have a protective role in tissue injury and inflammation, the increase in EIC during first and second week could have been due to the sudden stress during the initial stage, where the body tends to protect against the DE effects by increasing the EIC level. However, with the continued exposure, there was a fall in EIC, indicating initiation of inflammatory changes. Protein contents of BAL fluid were maximum on day 14 which might have been due to increased leakage of proteins and some enzymes due to increase in permeability. However, the EIC values were relatively lower on days 14 than on day 7, suggesting that EIC levels did not follow the pattern of exudation. Changes in protein contents in BAL were represented by a bell shaped curve, while that of EIC formed a linear line. The results suggested that the changes in EIC are not due to increase in pulmonary permeability, whereas increase in protein content might have been due to increase permeability[27].

Histopathological study of control rat lung showed few inflammatory cells (Fig. 1). After 7 days of DE exposure, the rat lungs showed mild inflammation comprising mainly of lymphocytes and plasma cells with few carbon laden particles within alveoli, whereas after 14 days marked lymphocytes aggregation and edematous changes in alveolar septa and bronchioles were observed indicating marked lung inflammation. Lung section of 21 days of DE exposed rats showed comparatively less marked lymphocytes aggregation around bronchioles which could be due to tolerance of the lung tissue to the consistent high levels of SPM allergen in DE. However, there was thickening of alveolar walls and small blood vessels with exudates within the lumen and around the bronchial walls. Changes in lung/body weight ratio and SPM deposited on filters (simulation chamber) correlated well with EIC, protein content in BALF and histopathological changes. Biochemical findings accompanied with chronic structural changes in the lungs of rats following exposure to DE could be relevant to the clinical observation of increased incidence of chronic lung diseases after continued DE exposure[27].

Adverse effects of diesel exhaust are being now a subject of many recent studies and efforts are being made to explain the cellular and molecular mechanisms of pulmonary immune/inflammatory responses to DE exposure. *In vitro* studies have suggested that human fibroblasts, B-lymphocytes, alveolar macrophages, and epithelial cells/cell lines may be involved during such responses. Similarly, studies of B-lymphocytes have demonstrated that exposure to DE enhances the synthesis of immunoglobulin E by these cells[28]. Various studies have demonstrated that exposure of nasal or bronchial epithelial cells to NO_2, ozone, and DE results in significant synthesis and release of pro-inflammatory mediators, including eicosanoids, cytokines, and adhesion molecules. Nam *et al*.[29] have demonstrated that DE exposure increases the expression of antimicrobial peptide and inflammatory cytokine at the transcriptional level in IL-1beta-primed A549 epithelial cells. They have suggested that the increase is mediated at least partially through NF-kappa B activation which can, thus, enhance the airway-responsiveness especially of the patients suffering from chronic respiratory disease. Both the organic and the particulate components of DE exposure cause oxidant lung injury. The particulate component induces alveolar epithelial damage, alter thiol levels in alveolar macrophages and lymphocytes, and activate production of reactive oxygen species (ROS) and pro-inflammatory cytokines and causes a sustained down-regulation of CYP2B1 in the rat lung[30]. The organic component, on the other hand, is shown to generate intracellular ROS, leading to a variety of cellular responses including apoptosis and induction of cytochrome P450 family enzymes that are critical to the polycyclic aromatic hydrocarbons (PAH) and nitro-PAH metabolism in the lung as well as in the liver and the induction of heme oxygenase-1 (HO-1), a cellular genetic response to oxidative stress. They induce IL-4 and IL-10 productions which may skew the immunity toward Th2 response, whereas the particulate component may stimulate both the Th1 and Th2 responses. Long-term exposures to DEP, carbon black (CB), TiO2, and DE (devoid of the organic content), have been shown to produce tumorigenic responses in rodents. However, no correlation has been found between tumor development and DE chemical-derived DNA adducts formation. Both the organic and the particulate components of DE have been shown to enhance the respiratory allergic sensitization, cause DNA damage, and induce the development of lung tumors under long-term exposure[30].

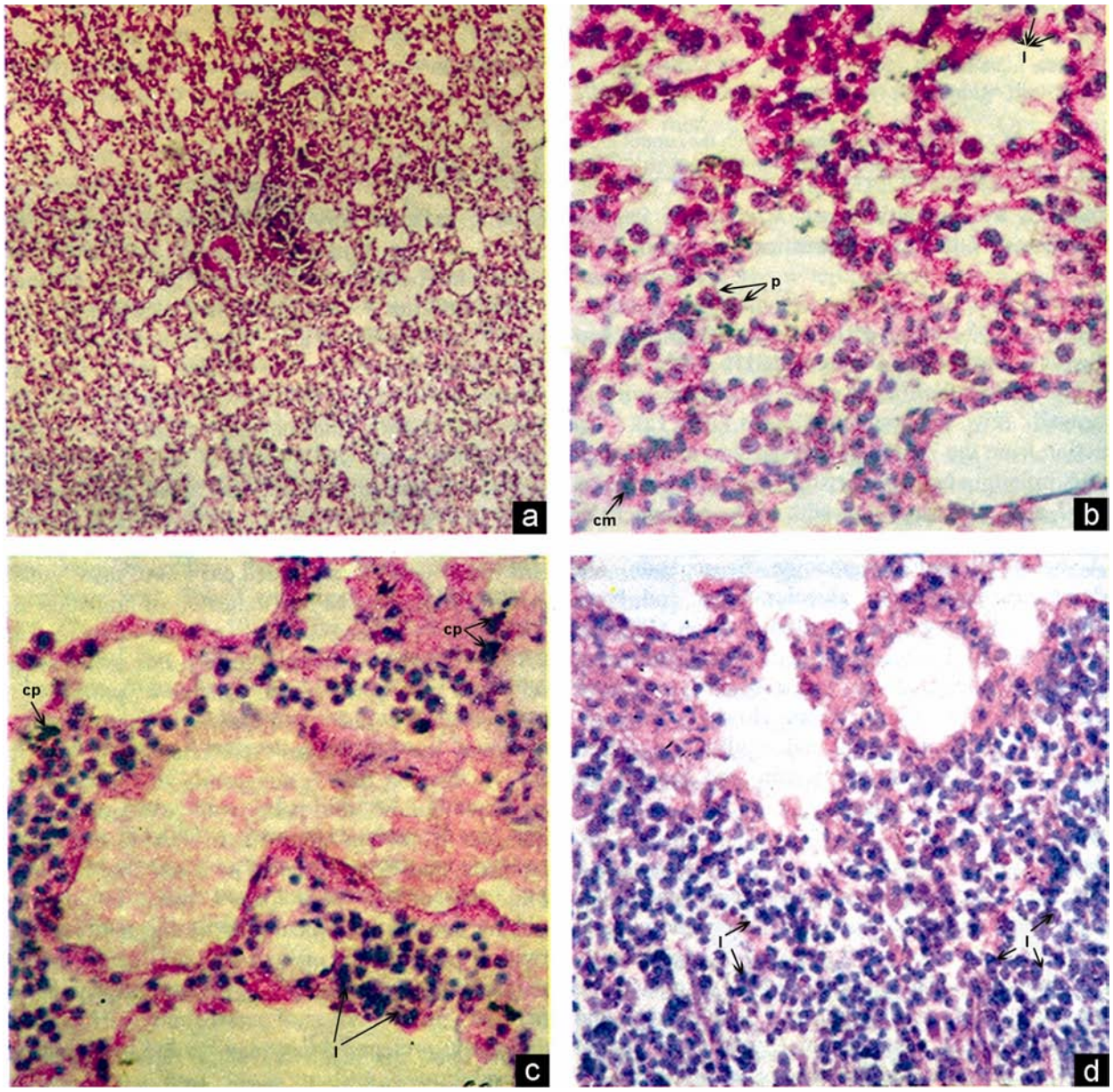

Fig. 1 — Sections of rat lung after exposure to diesel exhaust for various durations (a) non-exposed, showing few inflammatory cells, 115 ×. (b) 7 days, showing mild inflammation comprised of lymphocytes (I) and plasma cells (p), few carbon laden macrophages (cm), 410 ×. (c) 14 days, showing lymphocytes aggregation (I), emematous changes in alveoli and bronchi with carbon laden particles (cp), 410 ×. (d) 21 days, showing lymphocytes aggregation (I), thickening of alveolar and small blood vessel walls, 205 ×.

Industrial pollutants

Effect of heavy metals on health—Heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Heavy metals enter the body through drinking, eating, inhaling, skin and eye contact. Once in the body, they do damage on the cellular level by causing dangerous free radicals production. They can cause developmental retardation, cancer, kidney damage, and even death in some instances of exposure to higher concentration of mercury, gold, and lead. There are also associated with the development of autoimmunity that can lead to the development of diseases of joints (such as rheumatoid arthritis), kidneys, circulatory and central nervous systems[31]. Increased use of coal increases metal exposures because coal ash contains many toxic metals and can be breathed deeply into the lungs. In India, high-ash coal is used as a primary energy source so the health implications are ominous. Mining

itself, not only of heavy metals but also of coal and other minerals, is another major route of exposure. Uncontrolled smelters have produced some of the world's only environmental "dead zones," where little or no vegetation survives[32]. There are many studies on occupational medicine and toxicology with many heavy metals, but due to limitation of this review, the present review focuses on lead, mercury and cadmium toxicity.

Lead toxicity—Lead poisoning in adults can affect the peripheral and central nervous systems, the kidneys, and blood pressure but the most important is the central nervous system (CNS). Lead has a differential effect on neurotransmitter release: Spontaneous neurotransmitter release is enhanced, whereas stimulated release is inhibited[34]. Lead interferes with myelin formation and affects the integrity of the blood-brain barrier[35]. Lead also interferes with the synthesis of collagen and affects vascular permeability. At high enough doses, this results in brain edema and hemorrhage . Lead mimics or competes with calcium and inhibits its entry into cells[36]. Lead exposure has been reported to decrease lifespan. Lustberg and Silbergeld [37] compared the mortality of 4292 subjects with blood lead levels of 20-29 μg/dl to those with levels <10 μg/dl. Subjects with higher lead levels, had a 46% increased all-cause mortality, 39% increased cardiovascular mortality, and 68% increased cancer mortality.

Even at concentrations as low as picomoles, lead competes with calcium for binding sites on cerebellar phosphokinase C and thereby affects neuronal signaling[38]. Lead has also been shown to induce apoptosis in a number of experimental systems, including rat midbrain, rat fibroblasts, rodent lung and rodent retinal rod cell[39,40]. Lead enters in mitochondria and produces swelling and distortion of mitochondrial cristae and thus uncouples energy metabolism, inhibit cellular respiration, and alters calcium kinetics[41]. Lead has been classified as an animal carcinogen, and a recent study has shown an increase in standard mortality rates from cancer, in lead trade workers (e.g., smelters, painters and fender repairmen) but others have not[42]. Recently, ecological investigations have correlated ambient lead levels with crime rates and shown an association between lead exposure and crime. Stretesky & Lynch[43] compared homicide rates in 3311 counties in the United States and reported a four-fold increase in homicide rates in those counties with the highest air lead levels compared to controls.

Mercury toxicity—Mercury is toxic at higher doses. Major factors in mercury toxicity effects are not only the dose of exposure, but also the susceptibility factors like immune reactivity, degree of other toxic exposures and synergism systemic detoxification ability based on blood allele type or metallothionein function, sulfur detoxification deficiencies or other inhibited enzymatic processes related to or methylation[44]. They can play a larger role in effects than dose among a population with significant exposure to mercury and at extremely low levels of exposure. Toxic metals such as aluminum and lead have been documented to have synergistic effects with mercury.

Recent studies found that prenatal mercury exposures from mother's amalgams appear to be major factors in children with chronic neurological conditions like autism and ADHD[45,46]. Mercury depletion of GSH and damage to cellular mitochrondria and the increased lipid peroxidation in protein and DNA oxidation in the brain appear to be a major factor in conditions such as autism, Parkinson's disease, etc[47]. The chronic, low-dose fetal and lactational organic and inorganic mercury intoxication have been found to have significant correlations between seizure susceptibility and cortical mercury level[48].

A study of children of mothers consuming a marine diet which contains mercury, found that there are significant cardiovascular effects as birth mercury blood level increases from 1 ug/L to 10 ug/L[49]. At seven years of age, clear dose-response relationships were observed for deficits in attention, language, and memory and mercury levels. Thus a level of mercury exposure below current Government health safety limits, is documented to have significant cardiovascular effects and the recommended limit for mercury has been decreased from the former limit of 10 ug/L in blood. Toxic effects include damage to the brain, kidney, and lungs. Chronic exposure to mercury can facilitate overgrowths of pathogenic bacteria, viruses, and yeast leading to chronic conditions. Thyroid imbalances related to genetic susceptibility or toxic exposures can strain the adrenal glands and result in imbalances. Toxic exposures can facilitate digestive problems related to leaky gut, chronic maldigestion, exposure to gut pathogens, and/or suppression of protective microorganisms by toxic exposures[50].

Cadmium toxicity—Cadmium is a highly toxic environmental pollutant and produces as an inevitable by-product of zinc (or occasionally lead) refining,

since these metals occur naturally in the raw ore. In non-smoking population the major exposure pathway is through food, via the addition of cadmium to agricultural soil from various sources (atmospheric deposition and fertilizer application) and uptake by food and fodder crops. Additional exposure to humans arises through cadmium in ambient air and drinking water. Cadmium is bio-persistent and, once absorbed by an organism, remains resident for many years (over decades for humans) although it is eventually excreted. Cadmium is a dangerous ingredient in cigarettes, so smokers and second hand smokers are at risk. Average daily intake for humans is estimated as 0.15 µg from air and 1µg from water[51].

Consuming foods and liquids contaminated with cadmium, over time, can lead to severe GIT problems. The first organ reached after uptake into the GI-blood is the liver. Here cadmium induces the production of Metallothionein. After consecutive hepatocyte necrosis and apoptosis, Cd-Metallothionein complexes are washed into sinusoidal blood. From here, parts of the absorbed cadmium enter the entero-hepatical cycle via secretion into the biliary tract in form of Cadmium-Glutathione conjugates. Enzymatically degraded to cadmium-cysteine complexes in the biliary tree, cadmium re-enters the small intestines[52].

Cadmium is efficiently retained in the kidney (half-time 10-30 years) and the concentration is proportional to that in urine. Cadmium is nephrotoxic, initially causing kidney tubular damage. Cadmium can also cause bone damage, either via a direct effect on bone tissue or indirectly as a result of renal dysfunction. After prolonged and/or high exposure the tubular injury may progress to glomerular damage with decreased glomerular filtration rate, and eventually to renal failure. Furthermore, recent data also suggest increased cancer risks and increased mortality in environmentally exposed populations[53]. Recent studies have demonstrated that chronic cadmium administration induces oxidative stress. High exposure can also lead to obstructive lung disease and has been linked to lung cancer, although data concerning the latter are difficult to interpret due to compounding factors. In addition, the metal has been linked to increased blood pressure and effects on the myocardium[53,54]. Hypertension has also been reported following chronic administration of cadmium. However the mechanisms involved are not clear and controversial.

Several possible mechanisms have been reported for the cadmium induced hypertension, such as sodium retention, direct vasoconstrictor action and involvement of sympathetic system. There has been a considerable debate and controversy regarding the role of renin in the hypertensive effect of cadmium. A study was conducted to investigate the onset of hypertension and nephropathy by measuring changes in blood pressure and renal functions after different durations of exposure to cadmium to determine if hypertension to cadmium is cause or consequence of renal damage or unrelated to the renin system. Onset of hypertension and nephropathy after 1, 2, and 4 weeks of exposure to cadmium chloride (1 mg/kg, ip) was studied in rats by measuring changes in blood pressure and renal function (urinary output, electrolytes, serum creatinine, inulin clearance and Na^+ K^+ ATP ase). Na^+ K^+ ATPase is responsible for active sodium extrusion from the cell and is decreased in hypertension. Significant decrease in body weight and rise in blood pressure were observed as early as one week of exposure. There was no significant change in fluid intake, urine output, urinary sodium and potassium levels, thus excluding it to be the cause for hypertension. However, microalbuminuria was detected in 50% of the animals after 2 weeks. Na^+ K^+ ATP ase was depressed after 1 week with maximum lowering occurring after 4 weeks. There were no detectable changes in urine output, electrolytes, inulin clearance and serum creatinine even after 4 weeks. It was concluded that hypertension and tubular lesion set in earlier than glomerulopathy as indicated by microalbuminuria and the latter could be the consequence of rise in blood pressure[55].

Studies further suggested that the renin-angiotensin system (RAS) may be involved in the hypertensive effect of cadmium[55] and experiments were conducted to delineate the central vs peripheral component of the RAS involved in this effect. Intravenous administration of CdCl (1 mg/kg) produced a biphasic response, i.e. a transient fall followed by a marked and consistent rise in blood pressure. The peak hypertensive effect was accompanied by raised PRA levels. Pretreatment with captopril (1 mg/kg, iv), losartan (1 mg/kg, i.v.) or captopril + losartan attenuated the pressor response to Cd by 62%, 42% and 100% respectively in separate groups. Central administration of Cd (10 micrograms/rat, i.c.v.) showed a biphasic response similar to that observed after i.v. route. However, it was not accompanied by

raised PRA levels. Prior treatment with losartan (10 micrograms/rat, i.c.v.) completely abolished the pressor response to Cd (i.c.v.) whereas it was not affected significantly by captopril (10 microgram/rat, i.c.v.). Blockade by Losartan suggested that cadmium probably modulates the central angiotensin receptor. Since it has been suggested that calcium sites are in close proximity to AII receptors, cadmium might be having an agonistic effect on these sites. There have been reports of blockade of effects of cadmium by Ca^{2+} Channel blockers. Further lack of responsiveness to captopril could have been due to relatively low expression of key enzymes involved in the synthesis of angiotensin-II in the brain RAS system. Campbell *et al.*[56] also reported that local RAS's viz. brain, heart, kidney and adrenal gland do not contribute significantly to the circulating levels of active renin or angiotensin. On the other hand, centrally administered losartan only partially reduced the pressor response to i.v. Cd. The results provided evidence for a differential involvement of central vs peripheral renin-angiotensin system in the hypertensive effect of Cd[57].

Organo-chemical pollution

Pesticides—There is growing public concern about the impact of pesticides on human health[58]. Over 98% of sprayed insecticides and 95% of herbicides reach in air, water, soil[58] and cause water pollution or contribute to soil contamination.

Studies suggest that pesticides may be related to various diseases, including cancers, as well as having neurological, mental and reproductive effects. Associations between non-Hodgkin lymphoma, leukemia, prostate cancer, multiple myeloma, and soft tissues sarcoma have been reported[59]. Children may be more susceptible to the effects of pesticides due to increased exposure via food and breast milk, underdeveloped detoxification pathways, and longer life expectancy in which to develop diseases with long latency periods. Organophosphate pesticide use has increased as they are less damaging to the environment and less persistent than their organochlorine counterparts[60]. But these are also associated with acute health problems such as abdominal pain, dizziness, headaches, nausea, vomiting, as well as skin and eye problems[61]. Additionally, many studies have indicated that pesticide exposure is associated with long-term health problems such as respiratory problems, memory disorders, dermatologic conditions, cancer, depression, neurological deficits miscarriages, and birth defects[62-66].

According to researchers from the National Institute of Health (NIH), USA, licensed pesticide applicators that used chlorinated pesticides on more than 100 days in their lifetime were at greater risk of diabetes[67]. There are concerns that pesticides used to control pests on food crops are dangerous to people who consume those foods. Chemicals that are no longer used but that are resistant to breakdown for long periods may remain in soil and water and thus in food. Studies now suggest neurotoxic effects on developing animals from organophosphate pesticides at legally tolerable levels, including fewer nerve cells, lower birth weights, and lower cognitive scores[68].

Dichloro diphenyl trichloro ethane (DDT)—DDT and its metabolites, DDD and DDE, have been shown to be recalcitrant to degradation. The daughter compound, DDE, may result from aerobic degradation, abiotic dehydrochlorination, or photochemical decomposition. DDE has also occurred as a contaminant in commercial-grade DDT. However, when DDT was repeatedly applied to the soil, the DDE concentration may remain unchanged for more than 20 yr. and continues to contribute to toxicity. Effects of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) and its metabolites, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethene (DDE), 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (DDD) and 2,2-bis(p-chlorophenyl) acetic acid (DDA) were comparatively evaluated on humoral and cell mediated immune (CMI) responses in rats. Rats were given a diet containing 200 ppm of the various test compounds for 6 weeks and were subsequently immunized with ovalbumin. DDT, DDE and DDD, all induced differential degrees of humoral and cellular immune suppression. There were (a) increases in albumin/globulin ratios, (b) suppression of IgM and IgG levels, and (c) attenuations in ovalbumin induced antibody responses. In CMI studies, there were marked inhibitions of (a) leucocyte and macrophage migration factors, and (b) delayed type hypersensitivity (DTH) reaction. Whereas, these effects were most marked with DDE and DDD, DDA did not elicit such immunomodulatory effects. The results showed that suppression of immune responses by immediate DDT metabolites, DDE (and DDD and not DDA) is an important determinant of the toxicity of DDT (DDE > DDD > DDT) and the influence of this environmental pollutant in health and disease[69].

As there are reports of involvement of free radicals in the xenobiotic toxicity, the role of free radicals and oxidative stress during immunotoxicity of DDT and endosulfan and the protective effects of antioxidants like ascorbic acid and tocopheral showed beneficial effects. The effects of subchronic DDT, lindane and endosulfan exposure was determined on oxidative stress markers and immune responses in rats. Oral administration of DDT, (100 and 200 ppm) and lindane (40 and 80 ppm) dose dependently increased thiobarbituric acid reactive substance (TBARS) levels in serum after 8 wk of treatment. SOD activity in red blood cells (RBC) was also dose dependently increased by these compounds. In addition, such DDT or lindane exposure markedly suppressed the humoral immune response as assessed by anti-sheep RBC antibody titres. Simultaneous treatment with ascorbic acid (100 mg/kg) markedly attenuated the effects of DDT and lindane on (a) lipid peroxidation, (b) SOD activity and (c) humoral immune suppression. These results indicate the possible involvement of free radicals in organochlorine-induced immunotoxicity[70].

Organophosphates—Organophosphate pesticides are also widely used for agriculture and public health programmes in India. Inadvertent exposure of the population to these xenobiotics may result in both immediate and delayed effects. Hence risk assessment to such pesticide exposure is of paramount importance. In a study, effects of subchronic exposure to malathion was evaluated on adaptive immune responses in experimental animals. Using varying antigens, it was shown that both humoral immune (antibody titres, plaque forming cell count and immunoglobulin levels) and cell mediated immune (leucocyte migration inhibition, macrophage migration inhibition) responses were suppressed after malathion exposure, and these changes were time dependent. It was also shown that the threshold doses of malathion exposure needed to induce immonutoxicty was dependent on the animal species, type of the antigen, and method of immunological assay. Immunotoxicological studies could therefore be effectively used as a marker for their safety evaluation[71].

Possible prevention and treatment of environmental toxicity

Antioxidants—As there are reports of involvement of free radicals in the xenobiotic toxicity, the role of free radicals and oxidative stress during immunotoxicity of DDT and protective effect of antioxidant, vitamin C was evaluated. The effects of subchronic DDT and lindane exposure was determined on lipid peroxidation, Superoxide dismutase (SOD) and humoral immune response in rats. Oral administration of DDT, (100 and 200 ppm) and lindane (40 and 80 ppm) dose dependently increased thiobarbituric acid reactive substance (TBARS) levels in serum after 8 wk of treatment. SOD activity in red blood cells (RBC) was also dose dependently increased by these compounds. In addition, such DDT or lindane exposure markedly suppressed the humoral immune response as assessed by anti-sheep RBC antibody titres. Simultaneous treatment with ascorbic acid (100 mg/kg) markedly attenuated the effects of DDT and lindane on (a) lipid peroxidation, (b) SOD activity and (c) humoral immune suppression. These results indicate the possible involvement of free radicals in organochlorine-induced immunotoxicity[70].

Recently, we studied the effects of endosulfan exposure on immunotoxicity and protective effect of combined therapy of L-ascorbic acid plus alpha-tocopherol and with N-acetylcysteine. Endosulfan exposure (8 and 16 mg/kg) to rats significantly decreased the activities of superoxide dismutase and catalase, level of reduced glutathione and increased lipid peroxidation. The primary and secondary anti-SRBC antibody titers, plaque forming cells counts and delayed hypersensivity reaction, and the TH1 or TH2 cytokines levels were significantly suppressed in a dose dependent manner. L-ascorbic acid and alpha-tocopherol produced a synergistic reversal of oxidative stress parameters following endosulfan exposure. N-acetylcysteine produced significant reversal of altered oxidative stress parameters and immune response after endosulfan exposure. The results clearly demonstrated a significant attenuation of the oxidative stress markers and immunotoxicity with a combined therapy of L-ascorbic acid plus alpha-tocopherol and with N-acetylcysteine[72].

Dietary supplements—Further, we conducted studies to evaluate the influence of dietary protein on immune responsiveness after subchronic DDT exposure in albino rats. Rats were given 20%, 12% and 3% protein diets and exposed to DDT (20, 50 or 100 ppm) for 4 weeks. DDT (50 and 100 ppm) induced humoral and cellular immune suppression only in rats fed on 3% protein diet. There was (a) an increase in the albumin/globulin ratio, (b) suppression in IgM and IgG levels, and (c) attenuation in the tetanus toxoid-induced antibody responses. Further, in

rats immunized with tetanus toxoid, the leucocyte and macrophage migration inhibition were also attenuated. Moreover, these animals maintained on 3% protein diet showed depression in humoral and cellular immune responses to antigen in a dose-dependent pattern after exposure to DDT at dose levels which were not immunosuppressive for rats on 12 or 20% protein diet. These results suggested that dietary protein content may predispose to the immunotoxic effects of DDT exposure, and also be a crucial determinant in DDT detoxification[73].

Herbals drug therapy—In recent years, there has been an upsurge in the use of herbal agents from plant sources for a variety of disease states. As the respiratory, hepatic and immune systems are crucial targets for xenobiotic toxicity the therapies are aimed at protecting these vital organs and their functions from such environmental challenges. Several studies have suggested that plants like *Ocimum sanctum* (Tulasi), *Withania somnifera* (Ashwagandha), *Emblica officinalis* (Amla), *Azadirachta indica* (Neem), *Allium sativum* (Lahsuna), *Curcuma longa* (Haldi), *Tinospora cordifolia* (Guduchi), etc. to name a few, have important role in preventing and alleviating human disease[74,75]. Imuunomodulation appears to be a key factor which is common to all these agents and xenobiotic toxicity. In different experimental situations, all the above mentioned plants have shown differing degrees of immunopotentiation in normal and emotionally/environmentally stressed situations[74].

Recent studies with *Azadirachta indica* have shown that it has immunomodulatory potential. The effects of *Azadirachta indica* (AI, Neem) were evaluated on tests of humoral and cell-mediated immune responses after 3 weeks of oral AI (leaf extract) treatment in ovalbumin immunized mice. At the dose levels tested, AI (10, 30 or 100 mg/kg), had no appreciable influence on different organ (liver, spleen, thymus)/body weight indices, when compared to controls. In tests for humoral immune responses, AI (100 mg/kg) treated mice had higher (1) IgM and IgG levels, and (b) anti-ovalbumin antibody titres, when compared to the vehicle treated group. In tests for cell-mediated immune responses, there was an enhancement (%) of (a) macrophage migration inhibition, and (b) footpad thickness after AI (100 mg/kg) treatment. These results suggest the possible immunopotentiating effects of AI[75].

Further, these immunomodulatory effects were also seen during stressed situations, where stress-induced immunesuppression was reversed with AI leaf extract pretreatment. Another study showed that, by using gamma glutamyl transpeptidase (GGT) as an immune marker, AI pretreatment attenuated the stress-induced suppression of GGT activity in different lymphoid tissue, and these effects were comparable with diazepam and ascorbic acid. Interestingly, pesticides like DDT and lindane are known to cause immune suppression and also lower GGT levels[76]. It is therefore possible that *Azadirachta indica* (Neem) could protect the biological system from the damaging effects of pesticide exposure. Similar complex immunomodulatory effects have also been reported with *Ocimum sanctum*, *Withania somnifera*, *Tinospora cordifolia*, and many other herbs[74].

The other area that plays a crucial role in environmental toxicant induced pathophysiology is hepatic function. Hepatoprotective agents not only protect the liver from such toxicants, but also help to eliminate these agents by detoxifying them. Some herbal hepatoprotectives which have been used against xenobiotic induced toxicity include *Picrrorhiza kurroa* (Kutki), *Andrographis paniculata* (Kalmegh), *Phyllanthus niruri* (Bhumyamlaki), *Nycanthis arbor*-stristis (Harshingar), etc. Experimental and clinical studies have shown that most of these agents protect the liver from a variety of hazardous situations resulting from xenobiotic exposure[76].

Recently, the importance of nutrition in protecting the living organism against the potentially lethal effects of reactive oxygen species and toxic environmental chemicals has been realized. Reports on the role of bioflavonoids as antioxidants and their potential use to reduce the risks of coronary heart disease and cancer in human beings have opened a new arena for future research. The biological antioxidant defense system is an integrated array of enzymes, antioxidants and free radical scavengers which could be used as a prophylactic measure against such xenobiotic toxicity. Dietary supplements containing glutathione reductase, glutathione-s-transferase, glutathione peroxidase, phospholipid hydroperoxide glutathione peroxidase, superoxide dismutase (SOD) and catalase, together with the antioxidant vitamins C, E and A, could also be helpful. These individual components get utilized in various physiological processes and for chemoprotection and therefore require replenishment from the diet. Other components of the diet like

carbohydrates, proteins and lipids are important for maintaining the levels of various enzymes required in body's defense system providing protection against carcinogens[77,78].

Herbal drugs are of particular significance and studies have indicated that they may become viable alternatives in the prophylaxis and treatment of xenobiotic toxicity. Environmental toxicologists are constantly conducting specialized laboratory and field studies to answer relevant questions. A multidisciplinary global initiative is mandatory to prevent or minimize risks to biological and ecological system as a result of such toxicant exposure.

References

1 Ezzati M, Lopez A D, Rodgers A, Vander Hoorn S & Murray C J L, The Comparative Risk Assessment Collaborating Group. Selected major risk factors and global and regional burden of disease, *Lancet,* 360 (2002) 1347

2 Kallman M, World Resources Institute: August 2008 Monthly Update: *Air Pollution's Causes, Consequences and Solutions* (World Resources Institute: August 2008 Monthly Update) Submitted by on Wed, 2008-08-20 18:22. Retrieved on April 17, 2009.

3 Briggs D J & Wills J, Presenting decision-makers with their choices: environmental health indicators for NEHAPs. in *Environmental health for all. risk assessment and risk communication for national environmental health action plans,* edited by D J Briggs, R M Stern, T L Tinker (Dordrecht: Kluwer Academic Publishers), 1999, 187

4 *WHO Member State. Estimated deaths & DALYs attributable to selected environmental risk factors*, (WHO) 2002.

5 Guardian Media Group, Study links traffic pollution to thousands of deaths, in : *The Guardian* (London, UK) 15 April, 2008.

6 Beychok M R, in *Aqueous wastes from petroleum and petrochemical plants* (John Wiley, London) LCCN 67019834

7 *Declaration of the United Nations Conference on the Human Environment*, (UNEP, Stockholm)1972.

8 Badami M G, Transport and uban air pollution in India. *Environ Menage*, 36 (2005) 195.

9 Central Pollution Control Board Report. Epidemiological study on effect of air pollution on human health (adults) in Delhi, *Central Pollution Control Board Report* (Central Pollution Control Board, Delhi, India) 2006, 1.

10 Sydbom A, Blomberg A, Parnia S, Stenfors N, Sandstrom T & Dahlen S E, Health effects of diesel exhaust emissions, *Eur Respir J*, 17 (2001) 733.

11 David J M & Zali O, Motor vehicle air pollution: Public health impact and control measures. in : *WHO Publication Series* (WHO, Geneva), PEP/92.4, (1992), 189

12 Mehiri Ben R N, Louzir B, Cherif J, Beji M, & Daghfous J, Respiratory effects of diesel exhaust emission, *Tunis Med,* 83 (2005)127.

13 Pope C A, Dockery D W & Schwartz J, Review of epidemiologic evidence of health effects of particulate air pollution, *Inhal Toxicol*, 7 (1995) 1

14 Pope C A , Barnett R T, Thun M J, Calle E E, Krewski D, Ito K & Thurston G D, Lung cancer, cardiopulmonary mortality, and long term exposure to fine particulate air pollution, *JAMA*, 287 (2002) 1132

15 Pope C A & Dockery D W, Epidemiology of particle effects. Iin: *Air pollution and health*. Holgate, edited by S T, Samet, J M, Koren, S H, Maynard, Air Pollution and Health (Academic Press, San Diego), 1999, 673.

16 Ritchie G D, Still K R Alexander W K, Nordholm, A F, Wilson C L, Rossi J & Mattie D R, A review of the neurotoxicity risk of selected hydrocarbon fuels, *J Toxicol Environ Health B Crit Rev,* 4 (2001) 223

17 Risher J F & Rhodes S W, Toxicological profiles for fuel oils. (U S Department of Health and Human Services, USA) 1995

18 Barrientos A, Ortuno M T, Morales J M, Tello F M & Rodicio J L, Acute renal failure after use of diesel fuel as shampoo, *Arch Intern Med,* 137 (1977) 1217.

19 Environmental Health Criteria 171, Diesel fuel and exhaust, in : *Proceedings of international programme on chemical safety (IPCS)*, (WHO, Geneva), 1996

20 International Agency for Research on Cancer (IARC), Occupational exposures in petroleum refining: Crude oil and major petroleum fuels, in : *IARC Monographs on the evaluation of carcinogenic risks to humans* (Lyon, France) 1989.

21 Crisp A J, Bhalla A K & Hoffbrand B I, Acute tubular necrosis after exposure to diesel oil, *Br Med J*, 2 (1979) 177.

22 CONCAWE, Gas oils (diesel fuels / heating oils), Report Number 95/107, 1996

23 Koschier F J, Toxicity of middle distillates from dermal exposure, *Drug Chem Toxicol,* 22 (1999) 155.

24 Bruynzeel D P & Verburgh C A, Occupational dermatitis from isothiazolinones in diesel oil, *Contact Dermatitis,* 34 (1996) 64.

25 Eade N R, Taussig L M & Marks M I, Hydrocarbon pneumonitis, *Pediatrics*, 54 (1974) 351.

26 Litovitz T & Greene, A E, Health implications of petroleum distillate ingestion, *Occup Med*, 3 (1988) 555.

27 Lall S B, Das N, Das B P & Gulati K, Biochemical and histopathological changes in respiratory system of rats following exposure to diesel exhaust, *Indian J Exp Biol*, 36 (1998) 55.

28 Devalia J L, Bayram H, Rusznak C, Calderón M, Sapsford R J, Abdelaziz M A, Wang J & Davies R J, Mechanisms of pollution-induced airway disease: *in vitro* studies in the upper and lower airways, *Allergy* 52 (1997) 45

29 Nam H Y, Ahn E K, Kim H J, Lim Y, Lee C B, Lee K Y & Vallyathan V, Diesel exhaust particles increase IL-1beta-induced human beta-defensin expression via NF-kappaB-mediated pathway in human lung epithelial cells, *Part Fibre Toxicol*, 3 (2006) 9

30 Ma J Y & Ma J K, The dual effect of the particulate and organic components of diesel exhaust particles on the alteration of pulmonary immune/inflammatory responses and metabolic enzymes, *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev*, 20 (2002) 117

31 Glover-Kerkvliet J, Environmental Assault on Immunity *Environ Health Persp*, 103 (1995) 236

32 Young J E, "Mining the Earth, in" *Worldwatch*, Paper No. 109 (Worldwatch Institute, Washington, D.C.) 1992, 21

33 Eaton D L & Robertson W O, Toxicology. in *Textbook of clinical occupational and environmental medicine*, edited by L Rosenstick and M R Cullen, (WB Saunders, Philadelphia) 1994, 116

34 Bressler J & Goldstein G, Mechanisms of lead neurotoxicity, *Biochem Pharmacol,* 41 (1991) 479

35 Krigman M R, Neuropathology of heavy metal intoxication, *Environ Health Persp,* 26 (1978) 117

36 Simons T, Lead-calcium interactions in cellular lead toxicity, *Neurotoxicology,* 14 (1993) 77

37 Lustberg M & Silbergeld E, Blood lead levels and mortality, *Arch Intern Med,* 25 (2002) 2443

38 Markovac J & Goldstein G W, Picomolar concentrations of lead stimulate brain protein kinase C, *Nature,* 334 (1988) 71

39 Tavakili-Nezhad M, Barron A J & Pitts D K, Postnatal inorganic lead exposure decreasesthe number of spontaniously acive midbrain dopamine neurons in the rat, *Neurotoxicology,* 22 (2001) 259

40 Iavocoli I, Carelli G, Sgambato A, Masci O, Ardito R, Cittadini A & Castellino N, *et al*., Lead inhibits growth and induces apoptosis in normal rat fibroblasts, *Altern Lab Anim,* 29 (2001) 461

41 Needleman H, Lead poisoning, *Annu Rev Med*, 55 (2004) 209

42 Landrigan P J, Boffetta P & Apostoli P, The reproductive toxicity and carcinogenicity of lead: a critical review, *Am J Ind Med,* 38 (2000) 231

43 Stretesky P B & Lynch M J, The relationship between lead exposure and homicide. *Pediatr Adolesc Med,* 155 (2001) 579.

44 Tingting L & Woods J S, Cloning, expression, and biochemical properties of CPOX4: A genetic variant of coproporphyrinogen oxidase that affects susceptibilitly to mercury toxicity in humans, *Toxicol Sci,* 109 (2009) 228

45 Grether J, Croen L, Theis C, Blaxill M, Haley B & Holmes A, Baby hair, mercury toxicity and autism, *Int J Toxicol*, 23 (2004) 275

46 Mutter J, Naumann J, Sadaghiani C, Schneider R & Walach H Alzheimer disease: Mercury as pathogenetic factor and apolipoprotein E as a moderator, *Neuroendocrinol Lett*, 25 (2004) 331

47 Echeverria D, Woods J S, Heyer N J, Rohlman D S, Farin F M, Bittner A C Jr, Li T & Garabedian C, Chronic low-level mercury exposure, BDNF polymorphism, and associations with cognitive and motor function, *Neurotoxicol Teratol*, 27 (2005) 781

48 Szasz A & Barna B, Effects of continuous low-dose exposure to organic and inorganic mercury during development on epileptogenicity in rats, *Neurotoxicology*, 23 (2002) 197

49 Weihe P, Debes F, White R F, Sorensen N, Budtz-Jorgensen E, Keiding N & Grandjean P, Environmental epidemiology research leads to a decrease of the exposure limit for mercury, *Ugeskr Laeger,* 165 (2003) 107

50 Clifton J C, Mercury exposure and public health, *Pediatr Clin North Am,* 54 (2007) 237

51 WHO, Sources of human and environmental exposure : cadmium, in *Environmental Health Criteria*, (WHO, Geneva) 1992, 134

52 Godt J, Scheidig F, Grosse-Siestrup C, Esche V, Brandenburg P, Reich A & Groneberg D A, The toxicity of cadmium and resulting hazards for human health, *J Occup Med Toxicol*, 1 (2006) 22

53 Jarup L & Akesson A, Current status of cadmium as an environmental health problem, *Toxicol Appl Pharmacol,* 238 (2009) 201.

54 Peters J L, Perlstein T S, Perry M J, McNeely E & Weuve J, Cadmium exposure in association with history of stroke and heart failure, *Environ Res,* 110 (2010) 199

55 Lall S B, Das N, Rama R, Peshin S S, Khattar S, Gulati K & Seth S D, Cadmium induced nephrotoxicity in rats, *Indian J Exp Biol,* 35 (1997) 151

56 Campbell D J, Kladis A, Skinner S L. & Whitworth J A, Characterization of angiotensin peptides in plasma of anephric man, *J Hypertens,* 9 (1991) 265

57 Lall S B, Peshin S S, Gulati K, Khattar S, Das N & Seth S D, Involvement of renin-angiotensin system in hypertensive effect of cadmium in rats, *Indian J Exp Biol,* 35 (1997) 338

58 Cohen M, Environmental toxins and health–the health impact of pesticides, *Aust Farm Physician*, 36(2007)1002.

59 McCauley L A, Anger W K, Keifer M, Langley R, Robson M G & Rohlman D, Studying health outcomes in farm worker populations exposed to pesticides, *Environ Health Persp,* 114 (2006) 953

60 Jaga K & Dharmani C, Sources of exposure to and public health implications of organophosphate pesticides, *Rev. Panam Salud Publica,* 14 (2003) 171.

61 Ecobichon D J, Toxic effects of pesticides, in *Casarett and Doull's toxicology: The basic science of poisons,* edited by C D Klaassen & J Doull (MacMillan, New York) 1996, 643.

62 O'Malley M A, Skin reactions to pesticides, *Occup Med,* 12 (1997) 327.

63 Daniels J L, Olshan A F & Savitz D A, Pesticides and childhood cancers, *Environ Health Perspect*, 105 (1997) 1068.

64 Beseler C L, Stallones L, Hoppin J A, Alavanja M C, Blair A, Keefe T & Kamel F, *et al*., Depression and pesticide exposures among private pesticide applicators enrolled in the Agricultural Health Study, *Environ Health Perspect,* 116 (2008) 1713.

65 Eskenazi B, Bradman A & Castorina R, Exposures of children to organophosphate pesticides and their potential adverse health effects, *Environ Health Perspect*,107 (1999) 409

66 García A M, Pesticide exposure and women's health, *Am J Ind Med*, 44 (2003) 584.

67 Montgomery M P, Kamel F, Saldana T M, Alavanja M C & Sandler D P, Incident diabetes and pesticide exposure among licensed pesticide applicators: Agricultural Health Study, 1993-2003, *Am J Epidemiol*, 167 (2008) 1235

68 Kamel F & Hoppin J A, Association of pesticide exposure with neurologic dysfunction and disease, *Environ Health Perspect*, 112 (2004) 950

69 Banerjee B D, Ray A & Pasha S T, A comparative evaluation of immunotoxicity of DDT and its metabolites in rats, *Indian J Exp Biol,* 34 (1996) 517

70 Koner B C, Banerjee B D & Ray A, Organochlorine pesticide-induced oxidative stress and immune suppression in rats, *Indian J Exp Biol,* 36 (1998) 395

71 Banerjee B D, Pasha S T, Hussain Q Z, Koner B C & Ray A, A comparative evaluation of immunotoxicity of malathion after subchronic exposure in experimental animals, *Indian J Exp Biol,* 36 (1998) 273

72 Pal R, Ahmed T, Kumar V, Suke S G, Ray A & Banerjee B D, Protective effects of different antioxidants against endosulfan-induced oxidative stress and immunotoxicity in albino rats, *Indian J Exp Biol,* 47 (2009) 723

73 Banerjee B D, Saha S, Mohapatra T K & Ray A, Influence of dietary protein on DDT-induced immune responsiveness in rats, *Indian J Exp Biol,* 33 (1995) 739

74 Gulati K, Ray A, Debnath P K & Bhattacharya S K, Immunomodulatory Indian medicinal plants, *J Nat Remed,* 2 (2002) 121

75 Ray A, Banerjee B D & Sen P, Modulation of humoral and cell-mediated immune responses by *Azadirachta indica* (Neem) in mice, *Indian J Exp Biol,* 34 (1996) 698.

76 Koner B C, Banerjee B D & Ray A, Effects of stress on gamma glutamyl transpeptidase (GGT) activity in lymphoid system of rats: Modulation by drugs, *Indian J Exp Biol*, 35 (1997) 222

77 Lall S B, Singh B, Gulati K & Seth S D, Role of nutrition in toxic injury, *Indian J Exp Biol,* 37 (1999) 109

78 Devasagayam T P, Tilak J C, Boloor K K, Sane K S, Ghaskadbi S S & Lele R D, Free radicals and antioxidants in human health: Current status and future prospects, *J Assoc Physic India*, 52 (2004) 794