Exposure of population to genotoxic factors from the environment

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INTRODUCTION

Genotoxic factor is any factor that can show alternation of the DNA structure. Genotoxic factors are common and prevalent factors in our modern life, and carry an important and significant risk in our society. Some of those factors react with the environment exclusively and induced genotoxic related diseases and other agents react with other substances or compounds that already present in the environment. Genotoxic factors can cause genotoxic related diseases that among them are: tumors, infections, abortions and many other diseases. Genotoxic factors can be react with the human body in several ways, among them are: inhalation, drinking, eating, contamination by blood or saliva.
We have several tools to detect the exposure of human population to mutagenic and carcinogenic factors, among them ar We use the comet assay- that is an assay the measure the DNA damage, particularly DNA strand breaks. The comet assay is a single cell gel electrophoresis assay; it is simple, rapid and sensitive technique for analyzing and quantifying DNA damage. In this method the cell is embedded in agar and exposed to DNA-damaging agent such as UV radiation or chemical mutagen. The cell is then permeabilized by adding detergent and an electric field applied. If the cell's genomic DNA has been broken into small fragments then these fragments move out of the cell by electrophoresis and form a streak or "tail" leading away of the cell. This looks a bit like a comet, hence the name of the assay.
Other important method is Ames test. Ames test is a biological assay that assesses the mutagenic potential of chemical compounds. The test serves as a quick assay to estimate the carcinogenic potential of compounds. The procedure of Ames test is connecting to several strains of Salmonella typhimurium that carry mutations in genes involved in histidine synthesis. This bacteria require histidine for growth.
The variable being tested is the mutagen's ability to cause a reversion to growth on a histidine-free medium. The tester strains are especially constructed to have both frameshift and point mutations in genes require to synthesize histidine, which allows for detection of mutagens acting via different mechanisms.
Some compounds are quite specific, causing reversions in just one or two strains. The tester strains also carry mutations in the genes responsible for lipopolysaccharide synthesis, making the cell wall of the bacteria more permeable, and in the excision repair system to make the test more sensitive. Rat liver extract is added to stimulate the effect of metabolism, as some compounds, like benzopyrene , are not mutagenic themselves but their metabolic products are. The bacteria spread on a histidine-free agar plate in the middle of which the mutagen to be tested is added. The plates are then incubated for 48 hours. The mutagenicity of the substance is proportional to the number of colonies observed. We can mention other methods that employed in the analysis of genotoxic related diseases –like-Allium cepa root test, Anaphase aberration test , micronucleus test,Trad/MCN test and many others.

One of the main elements to control the genotoxicity related diseases is IARC. IARC is part of world health organization (WHO) and is kind of UN specialized agency. IARC has 16 participating state It coordinates and conducts research on causes of human cancer and the mechanisms of carcinogenesis, and to develop scientific strategies for cancer control. It involved in epidemiological and laboratory research, and disseminates scientific information through publications, meeting, courses and fellowships. IARC published 5 categories of agents that human populations are exposed.

Category 1 is carcinogenic substances like arsenic, tobacco, aflatoxin, asbestos, benzene, HBV, nickel, silica.

Category 2a- is probably carcinogenic substances-like –
Adriamicin, lead, diesel engine exhausts, mixtures from coal tar, cobalt.

Category 2b-possibly carcinogenic to humans- like – cobalt, welding fumes, coffee (urinary bladder),dry cleaning, printing.

Category 3-not classified as carcinogenic-in this category are agents that there is inadequate evidence or the limited scientific data.
Among agents are-coal dust, diazepam, polyprophylene, toluene,ink,crude oil.
Category 5-probably not carcinogenic-when there is scientific evidence for the lack of mutagenecity.

DNA DAMAGE IN LEUKOCYTES OF WORKERS OCCUPATIONALLY EXPOSED TO ARSENIC IN COPPER SMELTERS:

Inorganic arsenic compounds are classified as known human carcinogens, although the risk is known, millions of people worldwide are still exposed to those compounds in highly contaminated drinking water. This problem is especially prevalent in the following countries: Bangladesh, China, India, Thailand, Argentina, Chile and US. In the most contaminated regions the concentration of inorganic arsenic compounds exceeds the limit of 50 microg/l but can be as high as 4400 microg/l. The exposure can be prevalent in several industries: mining, pesticide production, glassware manufacturing. Inhalation is the principal route of exposure in occupational setting. The epidemiological data indicate the chronic exposure induces cancer in number of internal organs as: lung, kidney, liver, urinary bladder. Inhalation is associated with lung cancer while ingestion of contaminated water is associated with skin, liver, kidney. The mechanism of action is still a matter of controversy. The models in vivo and in vitro indicate that genotoxic mechanism may be responsible to carcinogenicity of those compounds. Arsenic compounds induce micronuclei and increases the frequency of chromosomal aberrations and sister-chromatid exchanges both in animals and humans. But others models shows other non-genotoxic mechanisms, although some studies indicate DNA damage, it is claimed that the compounds do not directly interact with the DNA and it may potentiate other toxic action of other physical and chemical agents. Therefore it has been regarded as co-mutagen. Insufficient data exist regarding the genotoxic effects of arsenic in populations who are chronically exposed via inhalation in work environments where the level of arsenic is usually low and where other agents coexist. In our study we bring data from polish worker from who work at copper smelters, in our study we use Comet assay, to asses the DNA damage in leukocytes of the workers, in addition we measure the level of arsenic derivatives in urine.

The mean urinary concentration of the total arsenic metabolites and DMA (dimethylarsenate) which was the main metabolites was higher in the exposed group versus the control group BUT not significantly higher than the controls. The level of DNA damage in leukocytes was expressed as the median tail moment determined from measurements in 50 cells per individual, the study revealed a significantly higher tail moment in the leukocytes of workers ($13.2x10^{-3}$) then leukocytes from the control group ($2.1x10^{-3}$) A 3 hour incubation of the cells in culture medium resulted in reductions in the median tail moment, reductions noted among cells from the workers were especially significant. The average median tail moment for the

workers was reduced from $13.2x10^{-3}$ to $3.2x10^{-3}$ after the incubation. For the control group was apparent "repair" of damaged DNA because of the incubation, but the overall reduction (smokers + nonsmokers) was not statistically significant. However, after the incubation, the median tail moments for cells from the workers (3.2x10-3) was several times higher than the tail moment of the cells from the controls (0.9x10-3) Preincubation with FPG significantly increases the DNA damage in leukocytes from both workers and control group, the median tail moments for leukocytes from workers and controls without FPG digestion, 13.2×10^{-3} and 2.1×10^{-3}, respectively , were increased to 70.1 x10-3 in cells from the workers and to 8.6 x 10-3 in cells from the control. Multiple regression analysis revealed no effect on confounding factors such as age, smoking status, and time of exposure (only in workers) on the level of DNA damage in leukocytes both in workers and controls. Univariate regression analysis revealed no correlation between the level of exposure to arsenic compounds and the level of DNA damage in leukocytes.

Urinary excretion of total arsenic species by workers 57 microg/l and by controls 12.3 microg/l indicates that workers at the copper smelters had a relatively low-level of exposure to arsenic compounds There were periodic measurements of air born arsenic compounds in the breathing zones of the workers; the measurements confirmed that most of the arsenic levels in the plants did not exceed the permissible level 50 microg/l We can see a significant increase in the level of DNA damage and oxidative DNA damage in the leukocytes of the workers. Although most of the fragmented DNA in the exposed group was repaired after a 3-hour incubation in culture medium, the level of DNA damage after this period of repair was still significantly higher in the cells from the workers than from controls. The results indicate that occupational exposure in the smelters mainly induced oxidative DNA damage in the workers leukocytes. This conclusion is based on the five fold increase in the level of DNA damage in workers leukocytes after FPG treatment. Although it has been difficult to associate arsenic exposure to the induction of DNA damage in occupational settings, cytogenetic assays conducted on populations exposed to arsenic in drinking water CLEARLY indicate positive genotoxic effects. Consumption of arsenic compound in drinking water is associated with increased frequencies of micronuclei in lymphocytes and in epithelial cells from the oral cavity and bladder, and increased frequencies of chromosomal aberrations in lymphocytes. The majority of the populations with long time exposure showed higher incidences of chromosomal aberrations, sister chromatid exchanges , and / or micronucleus formation. In several human biomonitoring studies, the cytogenetic effects did not correlate with the level of arsenic exposure, although correlations could sometimes be found at high levels of arsenic exposure. The level of exposure to arsenic compounds was low and did not correlate with the level of DNA damage in leukocytes.

The lack of QUANTITATIVE CORRELATION between the level of arsenic metabolites in urine and DNA damage in the leukocytes of copper[smelters workers suggests that DNA damage was caused not only by exposure to arsenic compound but also to other agents that present in the environment of copper smelters.

The increase in DNA damage in leukocytes of the workers, however, indicates that employees of copper smelters are at increased risk of developing genotoxic related diseases.

GENOTOXICITY OF DRINKING WATER DISINFECTANTS IN PLANT BIOASSAYS-

Municipal drinking water is usually supplied by wells or by surface sources like rivers or lakes, which contain varying amounts of natural and anthropogenic organic compounds. Toxic compounds can be formed in finished drinking water by the constituents of the untreated water reacting with the disinfectants used for water treatment , especially chlorine. Many of these disinfected by-products (DBPs) have been identified and are known to be mutagenic or carcinogenic in experimental models. Epidemiological studies of populations using chlorinated drinking water obtained from surface sources have demonstrated some evidence for a cancer hazard, spontaneous abortion and other reproductive and developmental effects. Several monitoring studies have detected the presence of mutagenic activity in chlorinated drinking water, using bacterial mutagenicity tests.

We saw other epidemiological studies in Finland USA and Taiwan that that show an association between the mutagenicity of chlorinated drinking water and tumors of the urinary and gastrointestinal tract. Drinking water is usually disinfected with the object of not only killing pathogenic microorganisms present in the raw water , but also maintaining small amounts of free disinfectant to prevent acquiring infectious waterborne disease organisms during water distribution Therefore health hazards could result from chronic exposure to these free disinfectant residues. Chloride is the most widely used disinfectant; it was found to be carcinogenic. Hydrogen peroxide is other disinfectant that shows mutagenic activity in both vitro and vivo short-term tests. Water samples have been successfully studied with short-term plant tests for chromosomal damage, such as: allium cepa root anaphase aberration test, the vicia faba root micronucleus test, and the tradescantia micronucleus test. Anaphase aberrations are the first manifestation of the clastogenic /aneugenic effects of genotoxic compound: like : anaphase disturbances , such as chromatin bridges , chromosome fragments , and lagging chromosomes , represent the generating events of micronuclei , which appear at the next interphase and arise from the exclusion of genetic material from daughter nuclei. Therefore, both those cytogenetic endpoints give a good measure of genotoxic effects, measuring both clastogenicity amd aneugenecity.n

Aqueous solutions if single compounds have been evaluated for genotoxicity in the laboratory, using the Trad/MCN test, The Allium cepa test and the vicia faba/MN test. Anaphase aberration test conducted in Allium cepa root tips has been used to study urban and industrial wastewater, drinking water , fresh water. Disinfectant –treated surface water samples have been analyzed with these tests to study the genotoxicity associated with DBP formation. We saw in situ studies in the river water who discouosly disinfected in a pilot plant with a new disinfectant, peracetic acid using the Trad/MCN test. Also the genotoxicity of lake drinking-water disinfected in a pilot plant with two widely used biocides , chloride dioxide (ClO_2) and NaClO and with PAA using a battery of in vivo and vitro tests.

Allium cepa test – 0.1 mg and 0.2 mg/l NaClO increased anaphase aberrations , but only at unadjusted (acid) pH . ClO_2 was positive only at 0.2 mg/l at neutral pH ,whereas all the test concentrations of PAA were negative at both pHs. The mitotic indices were consistently above 1% , indicating that treatments produced little toxicity and allowed adequate rates of cellular division. Their where different kinds of cellular aberrations, with one exception , no significant induction in the specific DNA , and mitotic spindle damage was detected at either pH for any of the disinfectants . Only 0.2 mg/l NaClO treatment at unbuffered (acid) pH produced a statistically significant increase in anaphase aberration induction. Vicia faba-

Mitotic indices ranged from 7.2 + 0.9% to 9.3 + 0.3% (mean+ standard error) for the treated samples and controls. A statistical analysis of the data indicated no alternations in the proliferating activity if the roots of the controls and treated groups , at the same pH Micronucleus frequency was increased significantly by all the disinfectants at both fixation times. Under unbuffered (acid pH) conditions , positive responses were found for the entire concentration range tested(0.1-0.5 mg/l) compared with the controls at the closest pH values.

At the buffered pH , significant increases in micronucleus frequency were detected only at the two highest test concentrations of NaClO and ClO2 and at 0.5-2 mg/l of PAA. The micronucleus frequencies observed after the 6 hour exposure and 42 hour recovery time were almost always higher than those detected after 6 hour exposure and 66 hour recovery time. This time dependent decrease may be due to the progressive dilution of micronuclei during cell proliferation. Analysis of micronucleus induction in control roots indicated that pH had no significant effect on vicia faba micronucleus frequency. We can see the analysis of three disinfectants: NaClO , ClO2,PAA in Trad/MCN test. NaClO and PAA were consistently negative at the test concentrations.
The longest NaClO exposure induced toxic effects at highest doses,preventing a complete analysis of the test concentrations. For this reason ClO2 and PAA doses of 1,2 and 10 mg/l were not evaluated with 24 hour exposure, ClO2 produced positive responses in the Trad/MCN test , but mainly after the 6 hour exposure under acid (unbuffered) conditions. Under natural conditions , only the 24 hour exposure to ClO2-0.2 mg/l increased the frequency of micronuclei. The highest concentrations 10 mg/l of NaClO at acid pH and ClO2 at natural pH produced toxicity in the early tetrads.

The results of this study indicate that two widely used drinking water biocides, NaClO and ClO2 , and new PAA have clastogenic/aneugenic activity. In particular ClO2 showed at least some positive responses in all the plant tests, and some of the positive responses were concentrations similar to those typically present in tap water. NaClO was genotoxic in two plant tests, mainly at higher concentrations, and under unbuffered (acid) pH conditions. PAA showed positive responses in only one plant test , and the activity was higher at acid pH. The three plant tests used to evaluate the disinfectants detect somewhat different genetic endpoints. Allium cepa test measured the formation of anaphase chromosome aberration,the tests conducted in vicia faba conducted in vicia faba and transcantia detected DNA damage as micronuclei.
These plant tests differed in terms of sensitivity. NaClO and ClO2 treatments gave positive results with the allium cepa test only under acid and neutral conditions, respectively , whereas PAA produced no increase in aberrations at either pH . The vicia faba test was the most sensitive,as it gave positive results with all the disinfectants at most of the test concentrations. Doses as high as 10 mg/l were used in the Trad/MCN test only with the shorter (6 hours) exposure , because initial experiments indicated that 24 hour exposures resulted in toxic effects at higher doses. Only Cl02 treated water gave positive results in this assay, and mainly under acid conditions. At neutral pH , ClO2 showed positive results both in the allium cepa test and Trad/MCN test at a concentration of 0.2 mg/l , a concentration that is often present in tap water to maintain disinfectant activity during water distribution. All the disinfectants acidified the treatment solutions to varying degrees. the pH of the exposure solutions affected the activity of the disinfectants , especially in the vicia faba test, in which genotoxic activity was found at lower concentrations if all the three disinfectants at unadjusted (acid) pH than at neutral pH. This did not appear to be due to a direct effect of the acid pH. This did not appear to be due to a direct effect of the acid pH ,since pH had no effect on the responses in the untreated controls. The greater genotoxicity of NaClO at acid

pH could be due to the higher percentage of undissociated HClO , wheras for PAA it could be due to dissociation products , such as H2O2. The genotoxicity of surface water after treatment with the same disinfectants was recently evaluated in situ by means if allium cepa, vicia faba / MN and Trad /MCN tests. Concentrations of free disinfectants were very low (<0.1 mg/l) and pH values were close to neutral. Thus , the results of the present study indicate that the genotoxic activity detected in the treated surface water were most likely due to DBPs generated by interactions between organic compounds present in the surface water and these disinfectants. NaClO and ClO2 treatments resulted in the formation of several halogen-containing DBPs , whereas none were observed for PAA treated water. DBPs were presumably not generated by the disinfectants in redistilled water , therefore the genotoxic activities detected in this present study may be due to oxidative stress induced by free disinfectants and their dissociation products (HClO and H2O2) although these responses should be confirmed in other systems , the results of this study indicate that plant tests , especially the vicia faba test , are capable of detecting the genotoxicity of disinfectants , at concentrations typically present in tap water(0.1-0.2 mg/l)

The decrease of genotoxic effects observed under neutral vs. acid pH conditions should be taken into account when surface disinfected waters are under study, the pH of which may vary considerably, since trace amounts of free disinfectants are necessary for preventing waterborne infectious diseases, and are ingested daily by a large number of people , these findings could be very relevant to managing human cancer risk.

DNA DAMAGE IN LYMPHOCYTES OF RURAL INDIAN WOMEN EXPOSED TO BIOMASS FUEL SMOKE AS ASSESSED BY THE COMET ASSAY-

Biomass is the principal fuel used by rural agricultural communities in developing countries and its combustion is suspected to be responsible for the significant part of the global disease burden. Data suggest that around 90% of rural households and 30% if urban households rely on biomass fuels (BMF) for cooking, and because the number of households involved, the resulting global indoor pollution has been estimated to outweigh the total global outdoor exposure. BMF used for domestic cooking include wood, crop residues m agricultural wastes, animal dung, but wood is the most prevalent. BMF emits combustion emits a complex mixture of organic compounds and gases , which include carbon monoxide (CO) , nitrogen and sulfur oxides, aldehydes , polycyclic aromatic hydrocarbons , volatile organic compounds, chlorinated dioxins, respirable particular radicals and free radicals. In humans prolonged inhalation of BMF smoke causes : chronic bronchitis , acute respiratory infection, chronic obstructive pulmonary disease, lung cancer , chronic interstitial pneumonitis, lung fibrosis , cor pulmonale, pulmonary lung hypertension, tuberculosis , asthma , and alternation of immune system. Although the combustion products produced by burning liquefied petroleum gas (LPG) are generally regarded as less toxic than BMF smoke , a study in Chinese population showed that individuals exposed for more than 5 years to LPG combustion products exhibited adverse symptoms such as irritation in the respiratory tract , nose and eyes, leading to pharyngitis, rhinitis and conjunctivitis. LPG combustion products such as sulfur dioxide, nitrogen dioxide , total suspended particulate (TSP) and total hydrocarbons have also been shown to be mutagenic and genotoxic in both human and animals. The single-cell gel electrophoresis or Comet assay is a rapid, simple and sensitive technique for quantitative and qualitative analysis of DNA damage.

The assay is increasingly used in biomonitoring and epidemiological studies, where it is most often used to detect DNA damage in peripheral lymphocytes. Using the comet assay, higher levels of DNA damage have been reported among persons exposed to a variety of chemicals and ionizing radiation. In our research 142 female volunteers were recruited for the study from villages around the city of Lucknow, India and stratified by the type of fuel for cooking : BMF or LPG. All parameters indicated that there was significantly greater DNA damage in BMF users group than the reference group. All the comet assay parameters indicated that there was significantly greater DNA damage for the BMF users than the LPG users in each of the age groups. When the volunteers were matched for duration of exposure, most comet parameters were significantly higher for BMF than LPG users. Duration of exposure was not found to affect the DNA damage significantly within the groups. We can see that the exposure to BMF has been associated further with other diseases like nasopharyngeal carcinoma, laryngeal cancer and oral cancer, and high potential for lung cancer. Our study reveals a significant increase in the DNA damage in women who use BMF for cooking relative to those who use LPG. The damage may be due to the presence of several known and suspected carcinogens, such as benzene, PAH, formaldehyde, in the smoke generated by burning BMFs. We can see an increase level of DNA damage in BMF users. And increase micronucleus and chromosomal aberration frequencies for Indian women using BMF relative to LPG users. We can see an exposure-dependent increase in the frequency of micronuclei and chromosomal aberration that indicate a cumulative effect of exposure. Although DNA damage was observed in the reference group , the extent of DNA damage in BMF users was significantly higher. The comet assay is a sensitive method for monitoring human genotoxicity and cancer risk. The ability to produce DNA damage in human lymphocytes as measured with the comet assay has been shown to correlate with carcinogenicity of environmental agents, hence, it is likely that the increased cancer incidence reported in BMF exposed women in other countries may partly be due to the DNA damage and genetic instability caused by the carcinogens in the smoke of BMF.

DNA damage in lymphocytes is at least partly related to exposure to BMF smoke, this is evident by significant increase in all the comet parameters for DNA damage, that is, olive tail movement, percentage tail DNA m and tail length. Analyzing the percent distribution of the cells with different olive tail values indicated that there was a significant increase in the percentage of cells with higher levels of DNA damage in the group of volunteers cooking exclusively with BMFs.

The DNA damage may be due to the presence of several suspected and known mutagens in the BMFs. It has been shown using the Ames test that organic residues of smoke particulates from burning wood and cattle dung contain mutagens. Other study claimed that the polycyclic aromatic hydrocarbons (PAH) that was released into the environment and absorbed onto emitted respirable particular matter (PM) are immunosuppressive in laboratory animals , as well as carcinogenic in animals and possibly in humans. Other study from Brazil showed a significant increase in the urinary mutagenecity of rural Brazilians exposed to wood smoke as compared to non-exposed controls. The result of our study along with other results from other studies conducted on rural population exposed to BMF smoke , may help to explain the higher incidence of cancer among people who using BMFs.

MODULATION OF THE GENOTOXICITY OF PESTICIDES REACTED WITH REDOX-MODIFIED SMECTITE CLAY-

As a group pesticides are unique in that they are regulated toxic chemicals intentionally released into the environment. The use of pesticides presents agricultural, economic and health risks and benefits. Another serious concern is their toxicological impact on ecosystems and the environment. The genotoxic potency in mammalian cells is unknown for most pesticides that used nowadays. Further, the impact of these compounds on public health and the environment is also influenced by a variety of factors including environmental degradation and activation for which we have a limited understanding. During the past decade, studies demonstrated that the surface of reduced-Fe-bearing minerals in the environment enhanced the degradation of environmental pollutants like: 2,4,6-trinitrotoluene, nitroaromatics , carbon tetrachloride and organic biocides. Degradation of environment contaminants like: heavy metals and chlorinated and nitro-aliphatic organic chemicals were influenced by the oxidation state of iron in clay minerals, These findings are intriguing and such processes may modulate the mediation of environmental contamination by pesticides, however, little is known about the impact of redox effects of clay in modulating the genotoxicity of pesticides. The primary mechanism of degradation for most pesticides in soil is considered biological. Abiotic processes of degradation, such as volatilization, reduction and oxidation, isomeriztion and hydrolysis , are considerated minor contributory factors. Therefore more attention has been focused on microorganisms' pathways of pesticides degradation. The mineral impact of soils, especially clay minerals, is generally regarded as a catalyst for enhancing or inhibiting the availability of pesticides to the microorganism for degradation. Microorganismal degradation pathways are considered the primary natural degradation mechanism of 2,4-dichlorophenoxy acetic acid(2,4-D) 2,4-D is rapidly degraded by a variety microorganisms found abundantly in most soils and water.

Soil moisture, temperature, pH and the formulation of 2,4-D , however , are found to alter the degradation rate. Degradation of dicamba in soil is thought to be primarily by microorganisms. While degradation occurs more rapidly under aerobic conditions, dicamba is also degraded under anaerobic conditions. Oxamyl is readily decomposed in both aerobic and anaerobic conditions by microorgasims , and thus the primary degradation pathways is considered biological. Oxamyl is readily hydrolyzed in neutral and alkaline soils and in solution. Recent studies have examined the ability of cations to degrade oxamyl. One study suggested that an increased amount of Fe(2) was responsible for the rapid degradation of oxamyl. Oxamyl was abiotically degraded by Fe(2) and Cu(1) and the presence of redox-modified smectites. The reduced state of Fe in the clay minerals is considered responsible for the degradation of the pesticide. We want to show the effect of Fe oxidation state on the genotoxicity of three of these pesticides. The objectives were to evaluate the capacity of agricultural chemicals to induce genomic DNA damage in mammalian cells, and to assess the biological impact of the Fe oxidation state in clay minerals on their genotoxicity. The direct genotoxicity of 2,4-D ,dicamba, oxamyl without reaction with clay was evaluated. 2-4-D was not genotoxic or acutely toxic in concentration range from 10 microM to 10 microM . Oxamyl directly induced a concentration-dependent increase in genomic DNA damage to CHO cells at concentrations greater than 400 microM. Cell viability was above 80% for all oxamyl concentrations tested In all cases the assays were conducted within the aqueous solubility limits of 2-4-D 900 mg/l dicamba 6500 mg/l oxamyl 280000 mg/l at 25 celsius The direct assays established the concentration range that would be used in evaluating the effect of redox-modified clays on the genotoxic responses of the pesticides in mammalians cells. in all

of our measurements the pesticides were reacted with H20 or reduced smectite or oxidized smectite and analyzed for genotoxiciy.

The effect of redox-modified clay mineral on the genotoxicity of pesticide to mammalian cells was assessed. Redox-modified ferruginous smectite SWa-1 was reacted with 2,4-D (2mM) dicamba (9mM) oxamyl (550mM) For 2,4-D the supernatant fraction from no-clay control and the supernatants from oxidized or reduced SWa-1 clay had no significant effect on SCGE% tail DNA values. In all cases the redox-modified clays and 2,4-D treatment groups were not acutely cytotoxic. In contrast to 2,4-D , redox-modified clays had a profound effect on the genotoxicity of dicamba. The dicamba and no-clay controls did not induce genomic DNA damage. We saw a significant increase in SCGE % tail DNA values after dicamba was transformed with reduced WSa-1 clay. There was no significant difference in genotoxiciy between the no-clay control (dicamba alone) and dicamba reacted with oxidized SWa-1 clay except at the highest concentration. The acute cell viability for all of the treatment groups was above 75%. The interaction of oxamyl with redox-modified clay minerals generated a response different from that of 2,4-D or dicamba. Oxamyl without clay directly induced genomic DNA damage. Oxamyl with no clay or oxamyl reacted with oxidized clay produced the same level of genomic DNA damage. However, reduced SWa-1 clay significantly reduced the genotoxicity of oxamyl, The acute cell viability did not decline within the concentration range. The reduced-iron bearing clays commonly found in soil can react with pesticides and modified their genotoxicity. 2,4-D alone and following reaction with redox-modified clays was not genotoxic in mammalian cells. We see lack of effect of Fe oxidation state on the transformation of 2,4-D. Abiotic degradation of 2,4-D in water and soil sediments had little effect in environmental toxicology. Dicamba was not directly genotoxic , using the SCGE assay with CHO cells. So the results show that dicamba is not mutagenic. But other studies showed that dicamba induced DNA damage, while no clay control and the oxidized-clay treatment were not genotoxic. The Fe oxidation state drastically altered the genotoxic potential for dicamba. These data parallel results demonstrating that dicamba reacted with reduced SWa-1 clay was more cytotoxic over 72-hr period to mammalian cells than dicamba reacted with oxidized clay.
While examples of the formation of more toxic secondary products from the transformation of the parent pesticide have been reported, the occurrence , rate and mechanism of these processes are unknown. These findings illustrate the formation of more toxic pesticide transformation products as a result of novel, abiotic ,degradation pathways. The analysis of dicamba took place with high performance liquid chromatography and found the parent compound after no-clay and oxidized-clay treatments. The degradation product may be highly toxic to CHO cells because its presence coincides with enhanced cytotoxicity as well as Oxamyl induced direct genomic DNA damage. The direct genotoxicity in genotoxicity. mammalian cells is contrary to most of the published information. Oxamyl is considered nonmutagenic on the basis of negative responses That measured Salmonrlla typhimurium reversion , in vitro cytogenetic damage , DNA repair , unscheduled DNA synthesis , the Rec-assay and host-mediated mutagenesis. The data that showed here warrant further investigation of the genotoxic potential of oxamyl. Reaction with reduced SWa-1 clay significantly decreased the genotoxicity of oxamyl. This results agrees with the degradation of oxamyl by reduced-clay minerals and the concurrent decrease in chronic cytotoxicity and increase in metabolic fitness in mammalian cells. The reduced-clay minerals diminished, but did not eliminate, the direct genotoxic activity of oxamyl alone. This may be due to –a. one or more of the degradation products may be genotoxins. b- the concentrations reacted with clay minerals in our study was higher than that of the earlier studies and the reaction time may have been insufficient for complete degradation of the pesticide. c- the clay-pesticide

transformation system could be saturated leaving small amounts of the parent pesticide in the supernatant. So we can say that environmental genotoxicity of pesticides applied to smectite-bearing soils may be modulated by the oxidation state of clay minerals. Redox cycling in smectite clays may occur in soil after rainfall or irrigation.

The clays undergo swelling and generate reducing conditions because of a change in the oxygen content of the soil. Microbes can also chemically reduce the structural iron in clays. It is this dynamic effect of soil that plays an important role in the molecular transformation of pesticides and may contribute to their bioavailability and impact upon the environment and the public health.

CONCLUSION-

In our work we saw the diversity and the extent of factors or agents that participate in genotoxic related diseases. Our modern life, the industry, the synthetic materials, the medicine, agriculture and etc. etc. become more and more compose with gentoxic factors. Hand by hand with the development of science and medicine it is highly necessary to detect the presence of those factors and to prevent human exposure to those factors. The WHO use IARC to classify those agents and to warn the harmful results of there exposure. It is necessary to perform periodic checking of the presence of those factors. In some cases it is necessary to replace the material/compounds whom are suspected to be compose of genotoxic factors by more appropriate materials(like the Indian women) In other cases it is totally necessary to prohibit the use of those materials (asbestos) It is possible to use protective measures as special clothes, special masks, and special gloves to defend against those genotoxic materials (pesticides, gamma radiation for x-ray and CT, soots) In the medical field it is necessary It is possible to give special programs with instructions how to avoid consumption of toxic fungi (aflatoxin) It is possible to give vaccinations to special groups on high risk for contaminations with infection diseases (HBV) It is possible to avoid special inhalation in special art performances (silica) It is possible to avoid the consumption of certain food and beverages (alcohol, salted fish, areca nut)

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