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IS EXPOSURE TO ENVIRONMENTAL OR INDUSTRIAL ENDOCRINE DISRUPTING ESTROGEN-LIKE CHEMICALS ABLE TO CAUSE GENOMIC INSTABILITY?

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1. ABSTRACT

Human and wild life populations are continually exposed to a wide variety of environmental estrogen-like chemicals. Most research to date on environmental endocrine disrupting estrogen-like chemicals has focussed on screening of estrogenic activity of environmental or industrial chemicals, their bioaccumulative properties and toxicokinetics, and developing the structure-activity relationship between environmental or industrial chemicals and estrogen-receptor. Whether estrogen-like chemicals also possess the ability to alter the stability of the genome is not clear. It is very important to understand the effects of estrogen-like chemicals at the genome level. This article evaluates the current status of knowledge of the potential of producing genomic instability in response to the exposure of estrogen-like chemicals, which might help in understanding the mechanisms of some of the adverse effects. We and others have shown several structural, numerical, and functional changes at the cellular levels in response to DES exposure. Some other phenolic estrogen-like chemicals, such as, bisphenol A, phenylphenol and nonylphenol, also follow some of the pattern of effects similar to DES. These compounds also alter cell cycle kinetics, induce DNA damages, and produce telomeric associations and chromosomal aberrations. Whether weak or strong, the estrogenic response of a chemical, if not overcome, will add extra estrogenic burden to the system, and particularly those endocrine disrupting environmental and industrial estrogen-like chemicals capable of producing genomic instability will induce additional burden of genomic instability. Though, estrogenically some of these compounds may be weak, however, they may have different activities in generation of genomic instability. For example, nonylphenol is weak in estrogen-like action compared to DES, however, it is equal or more potent in producing telomeric associations in MCF-7 cells compared to DES. Additive or synergistic extra-burden of estrogenicity and genomic instability could produce detrimental effects compare to estrogenic action alone. Screening of endocrine disrupting environmental estrogen-like chemicals for their ability to produce genomic instability and analysis of molecular basis of some of the adverse human health outcomes as a result of exposure of these types of chemicals should lead to a better understanding of how these environmental estrogen-like chemicals may influence the development of some adverse effects in humans and wildlife.

2. INTRODUCTION

The number of chemicals in the environment that have been recognized to have potential to disturb development of the endocrine system and of the organs that respond to endocrine signals in organisms, which are classified as endocrine-disruptors, has been growing fast (1). Some of the recent findings have raised the level of concern that some of these environmental and industrial chemicals may interfere with the endocrine system of both humans and wildlife (1-4). In this study, we will focus on those environmental and industrial endocrine disrupting chemicals that have estrogen-like activity, i.e., estrogen-like chemicals. It is becoming evident that many chemicals, both natural and synthetic, exhibit estrogen-like activity (2-13, table 1). A large number of pesticides and industrial chemicals possessing estrogen-like activity are ubiquitous in the environment and make their way into the food chain (1-21).

Table 1. Examples of some known or suspected environmental estrogen-like endocrine-disrupting chemicals

CHEMICAL	PRIMARY USE/SOURCES	REFERENCE
1. PESTICIDES		
1.1. Herbicides	Herbicide and fungicide	23
2,4-dichlorophenol	Traizine herbicides	144
Atrazine		
1.2. Insecticides		
Dicofol, Dieldrin, o,p'-DDT, Methoxychlor, Chlordecone, Toxaphene, Heptachlor	Organochlorine insecticide	18, 145,146
2. OTHER INDUSTRIAL CHEMICALS		
2.1. Polychlorinated biphenyls pcbs		
Trichloro-4-biphenylols	Adhesives, fire retardants, waxes	45, 147
2.2. Pthalates	Plasticizer	23
2.3. Alkylphenols		
4-nonylphenol	Lubricants, surface active agents	17,20
4-octylphenol	Detergents, paints, herbicides	20
Nonylphenoxycarboxylic acid	Detergents, paints, herbicides	24
Bisphenol A	Manufacture of polycarbonate plastics	65
Naphthol	Rubber industry, dyes, perfumes, etc	79
α-Phenylcresol		79
Tert-Amyl phenol		79
2.4.polycyclic aromatic hydrocarbons		
Benzo[a]pyrene	Combustion of fossil fuels	4
3,9-dihydroxy-dmba		148
3. NATURAL ESTROGEN-LIKE CHEMICALS		
3.1. Phytoestrogens		
Coumestrol	Alfalfa	1,52,149,150
Genistein	Soya beans	55, 151
Diadzin	Soya beans	55, 151
Equol	A metabolite of coumestrol	55, 55,151
Formononetin	Red clover, alfalfa	152
Biochanin A	Red clover, alfalfa	152
Pelargonin	Flowers of pelargonium zonale	153
Mirestrol	Natural products	155
D^9-tetrahydrocannabinol	Cannabis sativa marijuana	156
β-Sitosterol	Plant oils, legumes, wood	157
3.2. Mycotoestrogens		
Zearalenone	Moldy fusarium sp. Corn, wheat	155
3.3. Microbial estrogens		
Enterolactone	Intestinal microbial metabolite	158
4. SYNTHETIC ESTROGENS USED AS DRUGS		
Diethylstilbestrol DES	Oral contraceptive	159
17α-ethinylestradiol	Oral contraceptive	159
5. DRUGS NOT INTENDED FOR USE AS ESTROGENS BUT HAVE ESTROGENIC ACTIVITY		
Cimetidine	Histamine h2-receptor antagonist drugs	160
Digitalis	Cardiac glycosides	160
Sulfonamide	Sulfonamide antimicrobials	155

Adverse effects of estrogen-like chemicals have been extensively reviewed (1,4,5, 13, 21) and will not be discussed here. Recent epidemiological and laboratory findings have aroused the growing concern that exposure to an estrogen-like chemical present in the environment might cause deleterious effects to wildlife as well as to human beings (1-21). Because of their hydrophobicity, environmental or industrial estrogen-like chemicals enter the body easily by diffusion through biological membranes, are difficult to excrete in unchanged form in the urine and bile, and accumulate in hydrophobic compartments of the cell, where they can disturb normal cellular functions. A major concern is that the profound and permanent effects that exposure to environmental and industrial estrogen-like chemicals may produce during critical periods in development can alter the future well-being of wildlife and humans, although chronic exposure after maturity can also present a health risk. The role of environmental estrogen-like chemicals in the etiology of some of the human cancers and reproductive health hazards has been implicated, although the linkage between these two processes is highly controversial (21). However,there is a general agreement

that human populations are continually exposed to a wide variety of environmental estrogen-like chemicals. Most research to date on environmental endocrine disrupting estrogen-like chemicals has focussed on (a) screening of estrogenic activity of environmental or industrial chemicals, (b) analyzing their bioaccumulative properties and toxicokinetics, (c) developing *in vitro* and short term *in vivo* assay to evaluate estrogenic activity, and (d) assessing the structure-activity relationship between environmental or industrial chemicals and estrogen-receptor. However, recently we have begun to recognize the toxicology of estrogen-like chemical action from a different perspective (1). It is important to understand the effects of estrogen-like chemicals at the genome level. Therefore, this review article will critically evaluate the current status of knowledge of the potential of producing genomic instability in response to the exposure of estrogen-like chemicals, which might help in understanding the mechanisms of some of the adverse effects. Only a limited number of estrogen-like compounds, such as DES, bisphenol A, nonylphenol, PCBs and DDT have been used to assess the biochemical and molecular changes at the cellular level (1). Whether these compounds also possess the ability to alter the stability of the genome is not clear. A large number of xenobiotics is known to cause cellular instability through structural, numerical, and functional alterations in the genome. Such alterations may include extra or missing copies of microsatellite DNA, telomere length reduction due to loss of telomeric DNA sequences, transcriptional silencing, chromosomal deletions, frameshifts, amplifications, rearrangements, translocations and other changes that interfere with the integrity of the genome. More recent findings have shown that in addition to estrogen receptor-mediated action some of the same compounds are also capable of producing instability in the genome (1). Estrogenically some of these compounds may be weak, however, they may have different activities in generation of genomic instability. An understanding of genomic alterations may help to explain some of the permanent adverse effects which may result if multiple or chronic exposure of environmental concentration of estrogen-like chemical(s) occurred at a critical stage of life. Therefore, in this paper we have critically evaluated the ability of environmental endocrine disrupting estrogen-like chemicals to produce genomic instability.

3. NUMERICAL CHANGES IN THE GENOME

An increasing number of observations suggests that exposure of natural estrogen or environmental estrogen-like chemicals produces numerical aberrations or biochemical or molecular events which may be involved in numerical impairments of genome (22-29). The proposed cellular targets of attack by environmental estrogen-like chemicals include spindle microtubules (MT), MT-associated proteins, kinetochores and centromeres, centrioles and centrosomes, as well as DNA. DES and natural estrogen have been shown to be capable of producing chromosomal abnormalities both *in vitro* and *in vivo* (24-34). In addition, it has been shown that both DES and estradiol are potent inhibitors of mitosis *in vitro*, and are capable of inducing genomic mutations in cultured cells (reviewed in 24). The results of similar types of studies suggested that estrogen induces two types of genetic changes; numerical chromosome changes (aneuploidy) with no apparent DNA damage and structural chromosomal aberrations induced by estrogen catechol metabolites presumably through DNA damage (24). DES-induced aneuploidy has also been reported in epithelial cells from the uterine cervix of neonatal mice (25). How DES or other estrogen-like chemicals produce aneuploidy *in vivo* is not clear. Metzler and his associates have shown using an *in vitro* system that DES and metabolites of DES alter polymerization/depolymerization of purified microtubular proteins (23) and binding of DES and its metabolites to tubular proteins in an *in vitro* system (97). Therefore, one may possibly think that DES and/or DES metabolites may interfere with spindle apparatus during mitosis resulting in abnormal segregation of chromosomes. Other environmental endocrine disrupting chemicals with estrogen-like activity have also been shown to inhibit MT assembly. For example, bisphenol A disrupted cytoplasmic MT complex and induced micronuclei (MN) in cultured Chinese hamster V79 cells (29). *p*-Nonylphenol**,** another environmental endocrine disrupting chemicals with estrogen-like activity, is also a MT inhibitor (22). Pentachlorophenol, extensively used as a fungicide and bactericide, causes a concentration-dependent inhibition of MT assembly (22,23). Some hydroxylated polychlorinated biphenyls, such as, 4-hydroxy-2',4',6'-trichlorobiphenyl, 4-hydroxy-2,2',5'-trichlorobiphenyl**,** and 3-hydroxy-2',5'-dichlorobiphenyl, have been shown to inhibit MT assembly under cell-free conditions to cause aneuploidy and MN induction (22,23). A comparison of DES, E_2 and coumestrol on their ability to induce MN indicate that coumestrol seems to have clastogenic properties and is the most potent MN inductor in human chorionic villi cells (22,23). However, whether such events occur *in vivo* or are responsible for the development of aneuploid cells *in vivo* remains to be shown. Microtubule disruptive activities of some natural estrogens do not correlate with their hormonal carcinogenesis. Estrone is known to stimulate growth and produce tumorigenesis, but it has no effect on the microtubule network (28). The 17 α-estradiol is noncarcinogenic and hormonally very less active (35,36), however its microtubule disruption potential is equal to that of DES or 17 β-estradiol (28). Whether aneuploidy and binding of DES and its metabolites to spindle proteins play a role in the development of cancer remains to be ascertained.

4. STRUCTURAL CHANGES IN THE GENOME

4.1. Covalent interaction with the genome

Previous studies using sister chromatid exchange and Salmonella mutation assays suggested that phenolic compounds, such as bisphenol A (BPA), phenylphenols (both are estrogen-like chemicals), are nongenotoxic (37, 38). Using the ^{32}P-postlabeling technique, we investigated covalent modifications in DNA caused by *in vitro* or *in vivo* exposure to BPA or o-phenylphenol (39-43). These compounds form DNA adducts both *in vitro* and *in vivo*. In addition to DES, bisphenol A and phenylphenol, from their structure it appears that there are other industrial and

environmental estrogen-like chemicals, such as some alkylphenols (octylphenol, nonylphenol) and biphenyls, which may be converted to genotoxic metabolites. Recent demonstration of the ability of the DES adducts to stop replication of cytochrome oxidase III (CO III) gene suggests that DES (44)and other phenolic estrogen-like chemicals capable of being converted to genotoxic metabolites (such as bisphenol A and other alkylphenols, and some chlorinated biphenyls) may produce instability by producing mutational changes in mitochondrial or nuclear genome through obstruction of replication.

4.2. Telomere Loss

The ends of chromosomes consist of a specialized structure, the telomere, composed of repeats of TTAGGG making up a total of 5-15 kilobase pairs, depending on age and proliferative activity of the tissue. There is a loss of telomeric sequences following every cell division estimated to be between 50 and 65 base pairs/cell division in human fibroblasts and embryonic kidney cells *in vitro* (45). We have recently determined the length of the telomere in the mammary gland of Noble rats exposed to estrone or DES. A significant reduction in telomere length was observed in response to exposure of DES and estrone (46). The major function of the telomere is to provide stability to chromosomes and protect underlying unique coding sequences from degeneration. Telomeric loss can lead to chromosome instability and genetic changes of possible significance for tumor development (47-49). Based on these findings, it is logical to think that some of the environmental estrogen-like chemicals may contribute to genomic instability through loss of telomere repeat sequences.

4.3. Telomeric Associations

Telomere-deficient chromosomes show telomere-telomere associations. Most cancer cells exhibit telomeric associations. Recently, we determined the effects of exposure of MCF-7 breast cancer cells to environmental estrogen-like chemicals (diethylstilbestrol, bisphenol A, nonylphenol) on telomeric associations. Exposure of MCF-7 cells to DES, bisphenol A or nonylphenol induced a dose-dependent increase in telomeric associations (50). Whether these effects are the result of a direct interaction of these chemicals at chromosome level or an indirect effect through interaction with nuclear proteins, remains to be examined. The telomeric associations have been implicated to contribute instability in the genome presumably by facilitating homozygosity, translocation, amplification, and other rearrangements. The significance of telomeric associations remain obscure, although they clearly represent a form of chromosome instability present in pathologic tissue (51), and play a role in the formation of ring chromosomes, conceivably because of the stickiness of the telomere region (52-53). In normal cells, telomere repeats appear to be necessary for chromosome stability, so telomere loss or shortening results in fusion events and chromosome instability. Thus, these findings suggest that exposure of cells to some environmental estrogen-like chemicals may potentially be involved in the induction of instability in the genome through telomeric associations and/or reduction in telomeric length. Studies of perturbation in cell cycle coupled with genomic instability would provide insights as to how environmental or ovarian estrogen may produce adverse effects to human health and wild life.

4.4. Chromatid/chromosomal breaks

In addition to aneuploidy and telomeric loss/associations, DES, estradiol, and some other environmental estrogen-like chemicals have been shown to be produced chromatid/chromosomal breaks (32-34). For example, recently, we have demonstrated that exposure of MCF-7 cells to environmental estrogen-like chemicals, bisphenol A, nonylphenol, and DES increases breaks at chromatid and chromosomal levels and gap formation (50). Perinatal exposure to estrogen induces chromosomal aberrations in the same target tissues in which tumors develop after *in vivo* administration of estrogen (24,25) . We and others have shown oxidative damage to DNA by DES exposure (reviewed in 95). Formation of free radicals from estrogen or estrogen-like chemicals may explain some of the cytogenetic changes observed in response to estrogen-like chemical exposure (32-34,50).

5. IMPAIRED EXPRESSION OF TRANSCRIPTION-REGULATING AND DNA REPAIR PROTEINS AND PROTOONCOPROTEINS INDICATING GENOMIC INSTABILITY

While it is widely believed that unrepaired DNA or chromosomal damage by chemical mutagens is likely the major cause of genomic instability, alterations in the nuclear proteins associated with the regulation of gene expression or transcription and DNA repair can also play an important role in this process (56). We have shown that DES quinone, one of the metabolites of DES, binds to pure nonhistone proteins, RNA polymerase and DNA polymerase, and inhibits transcriptional activity both *in vitro* and *in vivo* (58-59). Tyrosine phosphorylation of p53 and several other phosphoproteins is increased in response to exposure to DES and increased tyrosine phosphorylation of p53 and of other transcription related proteins including RNA pol II in response to DES exposure coincides with that of perturbation of cell cycle and DNA damage (61-67). Based on these data it appears that transcriptional regulation of some of the growth regulating genes through enhanced tyrosine phosphorylation may be involved in the maintenance of cellular integrity by controlling DNA damage and cell cycle perturbation.

Exposure of neonatal mice to estrogen have been shown to alter c-fos and HER2/neu expression (24,68). The c-fos protein is a crucial part of the primary genome which responds to stimuli by extracellular signals. It transduces these signals through regulation of some secondary genes yet to be identified. Induction of TGF-a and bFGF-like proteins in hamster kidney slices, of TGF-a, PDGF and its receptor in the reproductive tract of CD-1 mice, and of TGF-beta in rat granulosa cells in response to DES exposure

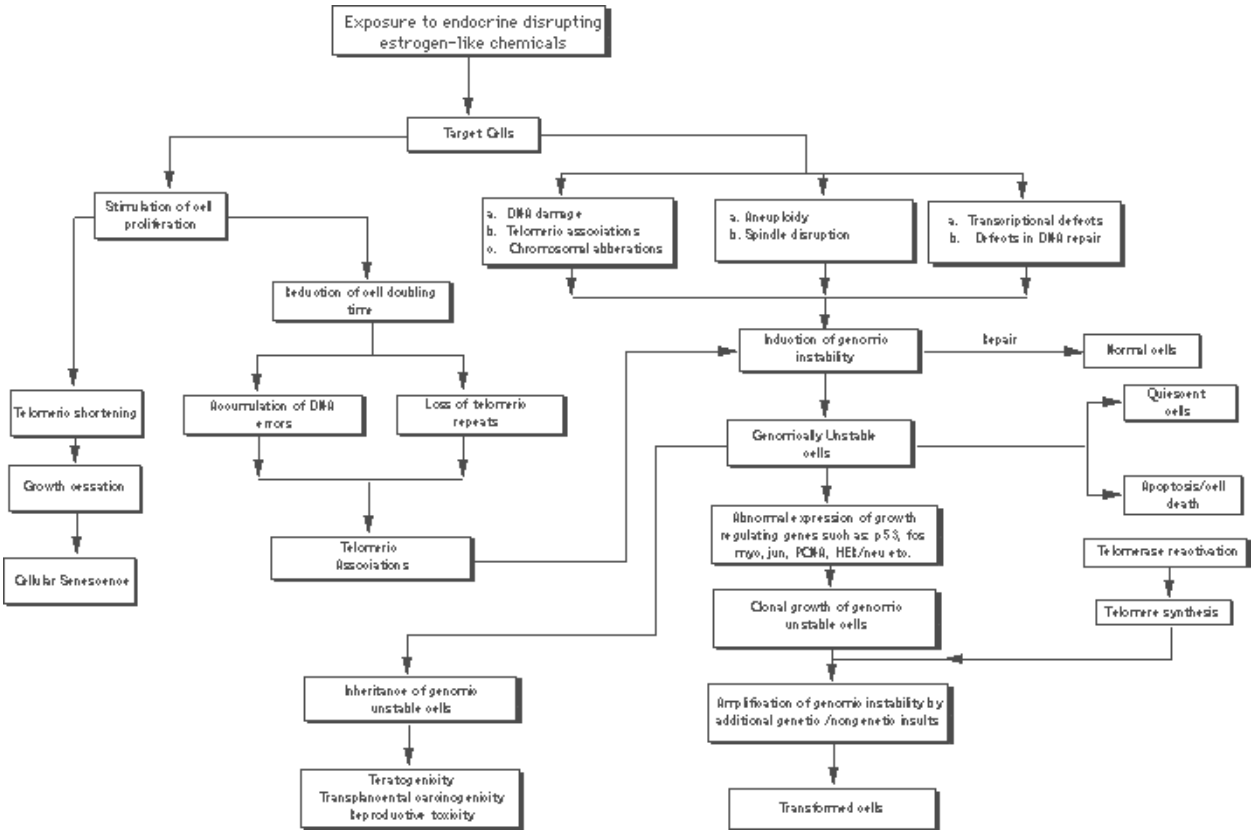

Figure 1. A scheme of possible mechanisms of induction of genomic instability associated with exposure of environmental estrogen-like chemicals and the role of genomic instability in some of the adverse effects.

has been observed (69-71). Alteration of EGF-R expression in hamster kidney (Narayan and Roy, unpublished) and in mouse reproductive tract tissue in response to DES exposure has also been reported (72,73). Recently, we have shown the enhanced expression of both plasma membrane and nuclear IGF-I receptors and an increase in the IGF-1 mediated phosphorylation in DES-treated hamster kidney membranes as compared to that of age-matched controls (75-78). The higher expression of c-fos, c-jun and c-myc in hamster kidney tumor tissues and of c-myc and mdm-2 in murine uterine adenocarcinoma cells compared to that of the control has been observed (79-81). Also neonatal treatment of DES to mice has been shown to increase the expression of c-myc in the prostate (82). Impaired expression of some of these growth regulating genes and protooncoproteins in response to exposure of estrogen-like chemicals suggest some of the adverse effects are probably mediated through the manipulation of a key function in the regulation of cell behavior, although involvement of any growth regulating gene(s) or oncogene(s) in the onset of adverse effects by estrogen-like chemicals remains to be ascertained.

Inhibition of DNA polymerase I activity by DES exposure has previously been reported (83). Our more recent studies revealed that DNA repair enzyme DNA pol b mRNA obtained from DES-induced kidney tumors has several mutations in the catalytic domain compared to that of age-matched control kidney (84-86). Defects in DNA pol β-catalyzed DNA repair system might lead to genetic instability through an increase in replication errors or may allow the accumulation of mutations due to impaired catalytic activity incapable of repairing the lesion. Impaired DNA repair system may specifically allow the accumulation of mutations in protooncogenes, tumor suppressor genes or other cancer associated genes, or may cause genetic instability. We have recently observed mutational change in another gene in DES treated kidneys compared to controls (Roy and Singh, unpublished). A high frequency of genomic rearrangements have been observed in transformed 10T1/2 mouse cell subclones treated with 17 β-estradiol which indicates that 17 β-estradiol and other natural hormones may accelerate the accumulation of mutations (30). Genetic instability manifested by somatic mutation of microsatellite repeats has widespread occurrence in clear cell adenocarcinomas of the vagina and cervix, with evidence of microsatellite instability in all DES-associated tumors examined (31).

Taken together, these findings suggest that in addition to their estrogenic effect, some environmental estrogen-like chemicals produce multiple and multi-types of genetic and/or nongenetic hits which may contribute in the induction of genomic instability (a schematic representation is shown in figure 1). We have shown several structural, numerical, and functional changes at the

cellular levels in response to DES exposure. Some other phenolic estrogen-like compounds, such as, bisphenol A, phenylphenol and nonylphenol, also follow some of the pattern of effects similar to DES(40-44,50,87-90). These compounds also alter cell cycle kinetics, induce DNA damages and produce telomeric associations and chromosomal aberrations. Whether weak or strong, the estrogenic response of a chemical, if not overcome, will add extra estrogenic burden to the system, and particularly those endocrine disrupting environmental and industrial estrogen-like chemicals capable of producing genomic instability will induce additional burden of genomic instability. Though, estrogenically some of these compounds may be weak, however, they may have different activities in generation of genomic instability. For example, nonylphenol is weak in estrogen-like action compared to DES, however, it is equal or more potent in producing telomeric associations in MCF-7 cells compared to DES. Additive or synergistic extra-burden of estrogenicity and genomic instability could produce detrimental effects compare to estrogenic action alone. Genomic instability producing endocrine disrupting environmental and industrial estrogen-like chemicals compared to that of not capable of producing genomic instability should be carefully monitored and thoroughly researched, because genomically unstable cells may produce permanent effects during critical period of development or maturity after chronic exposure leading to cancer or adverse developmental and reproductive outcomes. Further screening of endocrine disrupting environmental estrogen-like chemicals for their ability to produce genomic instability and analysis of molecular basis of some of the adverse human health outcomes as a result of exposure of these types of chemicals should lead to a better understanding of how these environmental estrogen-like chemicals may influence the development of some adverse effects in humans and wildlife.

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