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Endocrine disruptors (ED) and human exposure

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ABSTRACT

Increasing cases of malfunctions in the reproductive system, such as spermatic decrease in count and functionality, early puberty in boys and girls, increase of breast, prostate and testicle cancer and malformations associated with hormonal problems raise a sort of generalized concern. Simultaneously, cases of alterations in the reproductive function of an increasing amount of animal species can be observed. These are due to the exposure to persistent chemical substances such as pesticides, detergents, dioxins and furans. As a response, in the last decades an intense research activity has been promoted in order to describe these compounds according to their capacity to alter the endocrine- reproductive system homeostasis, naming them endocrine disruptors. Among the recognized disruptor compounds, the following can be listed: phytoestrogens, organochlorinated pesticides, alkyl-phenol polyethoxylates, polychlorinated biphenyls, chlorophenols, phthalates, artificial estrogens, dioxins, furans and some aromatic polycyclic hydrocarbons; that is to say, compounds that can be found in everyday life. The concern lies in that they can be found in the environment at very low levels and have been proved to have harmful effects on fauna species as well as on laboratory animals at these low levels. The greatest difficulty to determine the effects on human beings is that they are exposed to several ED simultaneously. The main exposure risk lies on the early prenatal and postnatal development, when the organs and the nervous system are transforming and the increasing incidence of neurodegenerative diseases of unknown etiology could be explained by environmental factors of substances which can be promoters, such as some pesticides. This work tries developing better global data, especially in countries outside North America and Europe, on status and trends of environmental contamination, exposure, and health outcomes.

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INTRODUCTION

In the last decade, the evidence that numerous chemical, natural and artificial products can interfere in the endocrine system and cause harmful effects in human beings and fauna has become stronger. The scientific community calls these chemical products endocrine disruptors (ED). They can be found in many of the products we use daily, including plastic bottles, food cans, detergents, toys, cosmetics, foods and pesticides. There is enough information on ED potential effects on human health, but the main concern lies in that they can be found in the environment at very low levels and have

been proved to have harmful effects on fauna species as well as on laboratory animals at these low levels. The greatest difficulty to determine the effects on human beings is that they are exposed to several ED simultaneously. ED's are natural or artificial products that can interfere in the production or the functions of the endocrine system hormones and thus, they can bring about negative effects on health. Many of them are connected to problems of development, reproduction, the nervous system, the immune system in fauna and laboratory animals. Therefore, it is surmised that these chemical products are affecting human health in a similar way, resulting in a decrease in fertility, and progres-

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sive increases of some diseases including endometriosis and cancers. Several studies on animals have elucidated the mechanisms by which ED's affect the endocrine system and alter hormone functions. People can be exposed to ED's through foods and drinks that they consume, the medicines they take and the cosmetics they use. Therefore, exposures can occur early, at low levels, through diet, air, water, the skin and can pose a greater risk during early postnatal and prenatal development, when the organs and the nervous system are transforming, early reproductive senescence and cancer, the effects becoming evident after a long period of time. This research shows the author's experience, who has worked for almost 20 years, with human exposure to diverse chemical products, today considered ED's, as well as assays with these pollutants in several systems (plant, human cells and bacteria).

MATERIALS AND METHODS

Sample

In occupational exposure studies, the exposed and control individuals' blood, urine or sperm samples were used. In the research on some environmental pollutants, samples of water, plastic materials, cereal grains, were used. For ecotoxicity research, several systems such as plant, human cells and bacteria were used. Moreover, the levels of several pollutants were studied as well as other biochemical parameters related to exposure.

Genotoxicity and mutagenesis

This one was determined by different tests: Sister Chromatid Exchange (SCE)^[1], chromosome aberrations (CA)^[2] and micronuclei (MN)^[3] in human lymphocytes. Chromosome aberrations in *Allium cepa*^[4] and the Ames test^[5].

Reproductive function

For this, human sperm collected after 4 days of sexual abstinence was used, according to Wyrobek's method^[6].

Level of pollutant concentration in the environment

In some researches in which occupational exposure studies were carried out, the pollutant concentration was analyzed in the air to which

individuals were exposed. The method consisted in making the environmental air flow through tubes containing activated carbon (SKC inc. PA) connected to personal air-sampling pumps (Zambelli, Milano, Italia, 2S model 0.21), working at a constant flux of 100 ml/min. The ethylene oxide sample (EO) was immediately dissolved in carbon disulfide and determined by gas chromatography^[7]

Level of concentration in exposed individuals

Blood and urine samples were used in order to determine the level to which they were exposed. For that reason, gas chromatography^[8], atomic absorption spectrophotometry^[9] and spectrophotometric methods were used.

Biochemical analyses

In some studies, blood analyses were carried out to determine the hepatic function (GOT, GPT, FA), kidney function (creatinine, urea), prostate function (FAT and FAP) with colorimetric methods^[10] and hormone analyses (Estradiol, progesterone, 17-cetosteroids) by radio-immune assay^[11].

Statistical analyses

In this study, several statistical methods were used such as Student's "t" test, Pearson's correlation coefficient, Dunnett's test, Fischer-Irwin exact probability test (Z), X² test, Kolmogorov-Smirnov test^[12] ANOVA test, Tukey's multiple comparison test^[13], variance analysis^[14].

RESULTS

Sperm quality

In male workers, exposed to lead in a battery factory, spermatoc characteristics were studied. In comparing the exposed groups with the controls, there were notable differences in asthenospermia and teratospermia^[25]

The sperm of exposed individuals at pesticides fenitrothion, monocrotophos, parathion, aldrin, endosulfan and glyphosate were studied. Mobility alterations were found in the sperm of exposed group with respect to the control group^[26].

The reproductive function in farmers who were exposed to 2,4-dichlorophenoxyacetic (2,4-D) was studied. Significant levels of asthenospermia,

necrospermia and teratospermia were found in exposed farmers compared with unexposed controls. Over time, asthenospermia and necrospermia diminished but the abnormal spermatozoa (teratospermia) continued^[27].

The prostate

Prostatic function was studied in men exposed to lead in a battery factory. Prostatic and acid phosphatases were the variables studied. The total acid phosphatase activity was significantly high in exposed workers when they were compared with the controls. The same did not occur with the prostatic acid phosphatase^[28]

Neurobehavior

SCE, hemogram, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), cholinesterase, organochlorides and organophosphates in peripheral blood were studied in individuals exposed to pesticides fenitrothion, monocrotophos, parathion, aldrin, endosulfan and glyphosate and non-exposed control ones. A significant increase in eosinophils and organophosphates and a decrease of white blood cells was found in the exposed group with respect to the control group^[26].

Cancer

Lead (lead oxide) effect was determined in individuals exposed to it in a battery factory. According to our results, people exposed to lead must be considered "in risk" so that can behave like as a clastogenic agent, determined by the sister chromatid exchange (SCE).

Women occupationally exposed to ethylene oxide (EO), used in the sterilization of medical instruments, were studied at a hospital. Chromosome aberration frequency in exposed individuals were significantly increased compared with controls. A significant relationship between the frequencies of SCE and CA and EO exposure was demonstrated^[29].

Exposed individuals, of different ages and backgrounds who had been drinking water from well, containing more than 0.13 mg/l (0.13 ppm) arsenic, for a period of at least 20 years, were studied. SCE data showed that arsenic at concentrations used by the population (0.13 mg/l) induced a significantly elevated response. Other health effects of arsenic at these con-

centrations were found, e.g., hyperkeratosis, melanosis, keratosis and basal cell carcinoma^[30].

For 14 months the quality of the Tercero river, Córdoba, Argentina water was studied. Water was genotoxic and clastogenic over several biological systems^[31].

The trihalomethanes (water by-products upon chlorination) from two treatment plant of the eastern region of Cordoba, Argentina, that use hypochlorite as disinfectant were studied. The amount and speciation of trihalomethanes (THMs) in tap water was analyzed. According to the results of the tap waters, the THM which predominates is chloroform being the average for the city of Bell Ville 102.6 ug/l and 100.3 ug/l for San Marcos city^[32].

Dimethyl terephthalate (DMT), terephthalic acid (TPA), the main components of polyethylene glycol terephthalate (PET), were evaluated for genotoxicity and mutagenicity in the Ames test, UDS by liquid scintillation counting and micronuclei induced binucleate human lymphocytes. Data showed that DMT and TPA at different concentrations (0.5, 5, 50 and 500 ug ml⁻¹) had neither genotoxic nor mutagenic effects^[33]. Fumonisin B₁, B₂ and B₃ (FB₁, FB₂ and FB₃) are a group of toxins produced by different mold species, *Fusarium moliniforme* and *Fusarium proliferatum*. Its compounds were tested in chromosome aberrations (CA), sister chromatid exchange (SCE) and micronucleus (MN) in human lymphocytes, and, in *Allium cepa* (onion), the chromosomal aberrations (CA) assay was used. Cytogenetic studies using different levels of FB₁, FB₂ and FB₃ yielded positive results at high concentrations (5 and 10 ug/g) with FB₁. Regarding the FB₁ cytogenetic aspects, we found an increase in the incidence of genetic damage measured by CA, SCE, MN and CA in *Allium cepa*^[34].

DISCUSSION

Sperm quality

According to our results, people exposed to lead must be considered "in risk". In the reproductive function, a significant level of asthenospermia and teratospermia was observed. Higher proportions of sperm with shape abnormalities, but no differences in sperm count compared with control have been found in men occupationally exposed to lead^[15]

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In an analysis of the semen of men attending an infertility clinic in Calgary, Canada, a lower sperm concentration and motility and an increased rate of abnormal sperm (tapered head) were found among 55 agricultural workers^[16]. Various cases of reversible sterility due to lindane poisoning have been reported^[17]. A decrease in infertility has been shown in men exposed to kepone^[18]. According to the study on the exposure of farm sprayers to 2,4-dichlorophenoxyacetic acid (2,4-D) and the reproductive function, significant levels of asthenospermia, necrospermia and teratospermia were found in exposed farmers when compared to unexposed control individuals. Over time, asthenospermia and necrospermia diminished but the abnormal spermatozoa (teratospermia) continued.

The prostate

The total acid phosphatase activity was significantly higher in exposed subjects when compared to control subjects. This was not the case with the acid prostatic phosphatase. The adverse effects of exposure to inorganic lead on the male reproductive system have been reported^[15].

Neurobehavior

Among the people exposed to different pesticides (fenitrothion, monocrotophos, parathion, aldrin, endosulfan and glyphosate) a significant eosinophil increase was found. Moreover, was found that organophosphate compounds increased in blood sample. Regarding organophosphates, can mention the phenomenon known as "neuropathy promotion". This is an effect by which subclinical injuries of different types can eventually develop a neurodegenerative syndrome, if there is later exposure to promoting substances called "neuropathy promoters". This phenomenon, although it was detected for the first time in studies about delayed neuropathy in organophosphates, seems to be a general mechanism which can occur in injuries caused by other toxic mechanisms (acrylamide, hexanodione) or in physical injuries. Thus the phenomenon of promotion is independent of the causing mechanism, but the delayed neuropathy in organophosphates is a good model of the injury caused by the phenomenon of promotion, and most studies on this striking phenomenon have been carried out on this syndrome.

Cancer

Human exposure to ethylene oxide showed a high frequency of chromosome aberrations (CA) in the exposed individuals as compared to control ones. The broad spectrum of alkylating properties of EO in biological systems including its hydroxyethylation of the 7 position of guanine in DNA. This ability to disrupt DNA probably accounts for the efficacy of EO as a sterilizing agent. EO is mutagenic in microorganisms, plants and animals.

Clinical evidence has been obtained showing that the incidence of cancer, especially leukemia is higher among workers occupationally exposed to a mixture of agents including EO^[19].

Individuals chronically exposed to arsenic in drinking water, at the pollutant agent concentration used by the population (0.13 mg/l) induced a higher SCE response. Exists sufficient evidence that As at relatively higher doses favors both mutagenesis and carcinogenesis. Moreover, Davey^[20] recently reported that As has a powerful endocrine-disrupting effect at low environmental levels.

In the study on river and treated water, the Ames test for genotoxicity showed that the treatment plant, which uses chlorine gas, produces a direct mutagen in the exiting water, as observed in other cases^[21]. The same sample also showed clastogenic activity on human lymphocytes and this could be considered as a warning on the potential effects of the water on somatic and germinal cells.

In the study of treated tap water, in order to determine the disinfection sub-products (SPD), chloroform was the one that prevailed. Several epidemiologic studies suggested that many SPDs were harmful to the health and their presence in drinking water could be a risk factor for the population drinking treated water to contract cancer^[22]. The estimation of the quantification of the risk to contract cancer due to water chlorination subproducts remains unknown, but the most reliable epidemiological studies found a statistical significance between exposure to chlorinated water for a long time and the incidence of bladder, kidney and colorectal cancer^[23].

In this study, DMT and TPA at the different concentrations studied (0.5, 5, 50 and 500 ug ml⁻¹) did not show significant toxic effects in the different genetic assays. The absence of DMT and TPA mutagenic ac-

tivity observed in the assays carried out in this study imply that they are not mutagenic. These negative results, however, can not be determinant or conclusive on whether they are carcinogenic.

As regards the cytogenetic aspects of FB₁, we found an increase in the incidence of genetic damage measured by CA, SCE, MN and CA in *Allium cepa*. These results indicate that human lymphocytes cells and plant cells (*Allium cepa*) have a very sensitive cellular response to the mycotoxin fumonisin B₁ as observed at the highest concentrations. Evidence in vitro and in vivo indicates that fumonisin B₁ can damage DNA indirectly by increasing oxidative stress^[24]. Oxidative damage was closely associated with fumonisin B₁-induced hepatotoxicity and induction of putative preneoplastic lesions in vivo, while the plasma and microsomal membranes, and to some extent the mitochondria and nuclei, appeared to be significantly affected by lipid peroxidation^[24].

CONCLUSIONS

Among the compounds studied and the effects on the health of exposed people it can be concluded that pesticides, considered endocrine disruptors, show hematologic alterations such as eosinophilia, leucopenia, persistence in the organism of organochlorinates and alterations in the reproductive function measured as asthenospermia, necrospermia and teratospermia, the latter persistent over time. Among industrial compounds considered endocrine disruptors such as lead, a long exposure can cause genetic damage, as well as a direct prostatic toxicity, which can probably be reflected in the alteration of spermatoc characteristics. Arsenic was shown to be mutagenic and carcinogenic at high doses in drinking water. The phthalates studied, DMT and TPA in vitro, did not appear to be genotoxic, but they can induce, in the kidney, the proliferation of peroxisome which is tumorigenic involving the overproduction of oxidant radicals in cells or simply stimulating cell proliferation. As regards food, important for human health, it can be an inlet for diverse compounds such as micotoxins, which have been proved to have a genotoxic effect in different studied systems. Moreover, the food production process uses the treated water, which

was proved to be mutagenic with clastogenic activity in human lymphocytes. This can be considered a danger for the potential effects of water on somatic and germinal cells. It can be due to disinfection subproducts (SPD). The ethylene oxide used for sterilizing medical instrumental showed a significant increase of chromosome aberrations in occupationally exposed workers. This chemicals is the most likely candidates for high-impact effects in populations at environmentally relevant concentrations. The main concerns that ED show are due to the fact that they are in the environment at very low levels, people are exposed to multiple ED simultaneously and this poses a difficulty to determine the effects on public health. The only evidence showing that humans are susceptible to ED is currently provided by studies of high exposure levels. The understanding of the effects of chronic, low levels of ED are (much more) little. The potential risks to human exposed by ED in many other areas of the world (particularly in developing countries) have not been addressed adequately to date. Extend monitoring of trends in relevant human health outcomes to provide information that is comparable across regions and over time. This type of studies bears no previous research in our country, in connection with the exposition of people to different environmental polluting agents called endocrine disruptors (ED) and their effects on health. This would undoubtedly be the starting point of a stage in which a contribution to the diagnosis would result into benefit for the community since this work aims at contributing with basic information on the effects of health of continuously exposed people to different ED in order to establish a future management of risk thus obtaining better quality of life for the population. This work tries develop better global data, especially in countries outside North America and Europe, on status and trends of environmental contamination, exposure, and health outcomes.

SUPPLEMENTARY INFORMATION

The author in this work tries to contribute data of exposed humans to different polluting agents in a country outside the United States and Europe with a view to develop better global data.

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