



The pilot study on Endocrine Disruptors

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ABSTRACT

Endocrine disruptors have been described as “exogenous chemical substances or mixtures that alter the structure or function(s) of the endocrine system and cause adverse effects at the level of the organism, its progeny, populations, or subpopulations” (EPA, 1998).

Several experimental studies reported that also very low doses of endocrine disruptors can affect the endocrine system causing diseases and altering the development of mammalian (humans included) and non-mammalian species. Among the diseases associated with the exposure to endocrine disruptors cancer, cardiovascular risk, modulation of adrenal, gonad and thyroid functions, and endometriosis are those that mainly catch the public concern considering their social cost.

This paper describes the research activity planned in the pilot study on Endocrine Disruptors granted by CNR in the general context of the Environment and Health Inter-departmental Project (PIAS-CNR).

1 INTRODUCTION

Over the past 50 years, some chemical pollutants, such as pesticides, flame retardants, alkylphenols, polychlorinated biphenyls, phthalates and metals have been released into the environment in an increasing way. Some of these substances, owing to their ability of interfering with hormonal activity, are called “Endocrine Disruptors” (EDs). According to the definition proposed by the Agency for Environmental Protection (EPA) of the United States, the Endocrine Disruptors are “Exogenous agents that interfere with synthesis, release, transport, binding, action or elimination of natural hormones responsible for the maintenance of homeostasis and the regulation of developmental processes and/or behavioural problems”. The effects of these compounds on endocrine functions

in animals, and hence in humans, result in an increase of the incidence of endocrine-related cancers, increased risk of cardiovascular diseases, reduced fertility and in the alteration of development processes. Endocrine disruptors reach living organisms through air, soil and water. The major route of transmission, however, remains the aquatic environment, where these substances bioaccumulate through the food chain. Even at very small doses EDs perform their harmful activity (1).

The concerns regarding the exposure to EDs are mainly due to: 1) the adverse effects observed in some wild animals, fish and ecosystems; 2) the increase of some human diseases related to the endocrine system; and 3) the alterations of the endocrine functions observed in laboratory animals after exposure to some environmental chemical pollutants. Already in 1996, the USA-EPA identified

the endocrine disruptors as one of six priority areas of research.

Human health effects associated with the presence of environmental endocrine disruptors have been recognized throughout foetal development, loss of reproductive capacity, changes in sexual behaviour, and onset of cardiovascular diseases (through obesity) and endometriosis. In addition, it has been observed an excessive cell proliferation and carcinogenesis as well as effects on the neurological and immune system.

2. STATE OF THE ART: INTERNATIONAL AND NATIONAL INITIATIVES

The Endocrine Disruptors are one of the major topic of the International and European research on risk assessment in food and environmental safety. The major international agencies have proposed to study the problems associated with the exposure to Endocrine Disruptors from different points of view. Just to give an example, the International Program for Chemical Safety (IPCS) of the World Health Organization in 2002 published the Global Report Assessment on the endocrine disruptors knowledge (http://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/index.html), whose main objective was the critical review of the scientific evidence of the association between exposure to Endocrine Disruptors and the damage to human health or ecosystems.

Moreover, the Organization for Economic Co-operation and Development (OECD) has dedicated its attention mainly to the development and harmonization of strategies to identify Endocrine Disruptors and characterize their effects on humans and ecosystems with the establishment of the Working Group of Endocrine

Disruptors Testing and Assessment (http://www.oecd.org/document/62/0,2340,en_2649_34377_2348606_1_1_1_1,00.html).

Europe has not underestimated the problem of EDS. The first definition of the problem took place during the European Workshop on “The Impact of Endocrine Disruptors on Human Health and Wildlife (Weybridge 2-4/12/1996). Ten years later there was a new European Workshop on the “Impacts of Endocrine Disruptors” (Helsinki, 8-10/11/2006).

Resources devoted to the research on Endocrine Disruptors in the last three European Research Framework Programs have been more significant. Among the major projects, we must remember: INUENDO, ANEMONE, the cluster of CREDO projects and the CASCADE network of excellence.

In Italy, some public research institutions, such as ISPESL and ISS, supported the research on endocrine disruptors with funds from the Ministry of Health. A recent survey, organized by the Interuniversity Consortium INBB and the ISS, evidenced the existence of more than one hundred research groups actively working in this field. The CNR addressed this issue and several initiatives were promoted in joint action between the Department of Earth and Environment and the Department of Medicine. The most important is the Environment and Health Inter-departmental Project, PIAS-CNR, under the responsibility of Dr. Fabrizio Bianchi. The final goal of PIAS is to understand the links between pollution sources and their effects on human health, since, as previously reported, the environment directly or indirectly affects human health. The prevention of environmental origin diseases requires a number of actions either on attitudes and lifestyles or on laws and other institutional measures

designed to guarantee the safety of the population exposed to environmental hazards. This is the goal of the pilot project on “Endocrine Disruptors”, activated by CNR within the scope of action of PIAS, and concisely described in the following pages. This study represents the logical conclusion of a series of PIAS initiatives taken in this scientific area. The project sees the participation of three research units belonging to three CNR Institutes, namely the Institute of Clinical Physiology (CNR-IFC, Pisa), the Institute of Genetics and Biophysics (CNR-IGB, Naples), and the Water Research Institute (CNR-IRSA, Brugherio).

Many research activities, even very good ones, have been excluded from this project solely for the scarcity of available funds. We hope that the results that will be produced from this project may serve as a basis and stimulus for future initiatives on this research field.

3. GROUND AND CONTENT OF THE PILOT STUDY

The project is divided into three experimental lines, that at first glance might seem unrelated, but that in reality are converging into a single objective: the study of the epidemiological and experimental links between some social diseases and the exposure to endocrine disruptors. These social diseases are the cardiovascular risk, which is among the principal causes of mortality in Italy, and endometriosis, which affects about 15% of women worldwide.

To better analyze the epidemiological link between exposure to endocrine disruptors and the above mentioned diseases, the population of a territorial district recognized as highly polluted: Gela (Sicily, Italy) has been chosen as to be studied from an epidemiological point of view.

Gela is sadly known for its pollution since air, soil and water are polluted by high concentrations of Endocrine Disruptors (with estrogenic or androgenic or arylc activity) and heavy metals. Perhaps this is why Gela is characterized by a higher level of malformations and cancer, kidney and cardiovascular diseases, as well as diseases of the reproductive tract and thyroid with respect to the national average. So Gela is the ideal place to verify the existence of a direct link between “Environmental Pollution and Health”. Since food is the principal mean by which EDs reach man, and since the population under study lives in a coastal-marine area, attention will be paid to determine the concentration of some EDs in some fish types of larger consumption by the indigenous population. Finally, *in vivo* experiments of EDS prenatal exposure in mice will be carried out in order to verify the possible occurrence of endometriosis.

We will now describe the experimental approach planned for each of the three research lines.

3.1 Line 1: Endocrine Disruptors and cardiovascular risk, occurrence of endometriosis, modulation of adrenal, gonad and thyroid functions in Gela population.

One hundred adult voluntaries of both sexes and resident in the Gela area will be recruited with the aim of studying the possible relationship between the levels of toxic pollutants in their biological fluids and the risk or occurrence of cardiovascular diseases, as well as alterations of thyroid, gonad and adrenal functions, using exposure biomarkers and responses to a specific questionnaire. In particular, personal, medical history, lifestyle, environmental and professional exposure, will be collected together with the weight,

height, waist circumference and blood pressure of each recruited person. Blood analysis will include: blood count, α PTT, PT, fibrinogen, PCRD, total cholesterol, HDL, LDL, triglycerides, blood glucose, uric acid, creatinine, BUN. Hormones dosage will be carried out testing for LH, FSH, PRL, P, E2, DHEA-S, total and free testosterone, 4α androstenediol, Cortisol ACTH, $\Delta 4$ -androstenedione, 17α -OH Progesterone, TSH, FT3, FT4, aldosterone, PRA. A 24 hours urine collection is planned for the determination of creatinine clearance, electrolytes, and cortisol.

Women recruited into the study for indirect signs of endometriosis will fill in an additional questionnaire on their pregnancy, offspring and ovarian cycle, and will also undergo blood sample to determine peripheral blood markers of endometriosis such as leukocytes, macrophages, TNF1alfa, CD3, CD 25, IL1, CA125.

On the basis of the number of adults recruited and the analysed biological samples, the research can improve knowledge about the possible correlation between the exposure to some environmental pollutants, typical of industrial and urban areas, and health outcomes, with particular reference to cardiovascular diseases, different forms of cancer and endometriosis.

3.2 Line 2: Endometriosis and Endocrine Disruptors: an in vivo experimental study

Endometriosis is among the diseases supposed to be associated with exposure to EDs. Endometriosis is a recurrent and benign gynaecological disorder characterized by the presence of endometrial tissue outside the uterine cavity. Endometriosis tissues are found on the peritoneal surface in the female pelvis, on the ovaries, on the recto-vaginal

septum, rarely in the pericardium, pleura, and even in the brain (2). Recent statistics report a prevalence of 6-10% among Italian women, but in patients with pain and/or infertility the prevalence rises to 35-60% (3). Several epidemiological data link the occurrence of endometriosis with exposure to various types of endocrine disruptors (4). Reproductive effects were found in monkeys, mice or rats, exposed during foetal life to polychlorinated biphenyls and dioxins (5-7). The possible occurrence of endometriosis in animals exposed during the foetal period to Bisphenol A (BPA), one of the most abundant endocrine disruptors in the environment, is still unknown.

The aim of this research line is to experimentally verify the onset of endometriosis in the offspring of mothers exposed to endocrine disruptors during the prenatal and perinatal life. As reported above, this link has been sufficiently studied in higher animals and in mice or rats, but only in connection to dioxin or dioxin-like compounds exposure (5-7), never in connection to BPA. BPA, on the contrary, has been used to verify the adverse effects on male fertility. There are many scientific papers on BPA estrogenic action (8-12). It has been reported that BPA exposure causes diseases in the developing foetus. It was also found that low levels of BPA exposure during foetal development, for instance, induces earlier puberty (9) and affects the prostate size (13). For this research we will use BALB-C mice exposed to BPA from the beginning of gestation, during lactation and in their early stage of life. The dose-response dependence will be determined together with: a) the morphological and functional changes in the uterus; b) the presence of endometrial tissue outside the uterus in exposed offspring; c) BPA concentrations in some target tissues: muscle, brain, liver, etc.

The involvement in this research of the Italian Endometriosis Foundation will ensure the transfer of the results to the medical community. The Foundation will also permit the access to its national registers in order to allow the comparison between the epidemiological data of the national population and those of the Gela area.

3.3 Line 3: Determination of Endocrine Disruptors concentration in fish of wide consumption coming from an area of high environmental risk

It is well documented that in more heavily populated areas or industrial places, contamination by endocrine disruptors affects not only the system of surface and profound waters (14-17) and the surrounding lands, but also the health of the animals and plants there living (18,19). It must be remembered that in some animal species living in these environments serious diseases and malformations, attributable to contamination by substances that affect the hormone system, have been found (20,21). Many of these substances are characterized by high lipophilicity and resistance to degradation. This means that many endocrine disruptors accumulate in living organisms and increase their concentration along the food chain in the ecosystem (21,22). Fish consumption is one of the major routes by which endocrine disruptors reach the humans (23,24).

In order to answer to this concern, the aim of this research activity is to determine the concentrations of some pollutants, known for their ability to interfere with the endocrine system, in fish of wide consumption and catch in the Gela Sea. The content of Bisphenol A, Octylphenol and Nonylphenol and two ethoxylates of Nonylphenol (mono and diethoxylated) will be determined. The concentration

levels of polychlorinated biphenyls (PCBs), organochlorine pesticides, arsenic, cadmium and mercury will be also determined.

4. EXPECTED RESULTS

From the results that will be reached during this research activity we hope to obtain indications on: 1)- the link between exposure to endocrine disruptors and some social diseases; and 2)- how the environment and the diet operate synergetically in promoting some severe pathologies in wildlife and humans.

We hope also to goad our legislator into achieving a greater consciousness on the danger of these invisible killers so that they can take useful prevention initiatives.

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