Endocrine disrupting chemicals. Harmful substances and how to test them

Produtos químicos como desreguladores endócrinos: substâncias danosas e como devem ser testadas

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Abstract *This paper presents an analysis of the opinions of different groups from: scientists, international regulatory bodies, non-governmental organizations and industry; with an interest in the problem of identifying chemical substances with endocrine disrupting activity. There is also discussion of the consequences that exposure to endocrine disruptors may have for human health, considering concrete issues related to: the estimation of risk; the tests that must be used to detect endocrine disruption; the difficulties to establish an association between dose, time of exposure, individual susceptibility, and effect; and the attempts to create a census of endocrine disruptors. Finally, it is proposed that not all hormonal mimics should be included under the single generic denomination of endocrine disruptors.*
Key words *Toxic Substances; Chemical Compound Exposure; Endocrine Disruptors*

Resumo *Este artigo apresenta uma análise das opiniões de diferentes grupos, inclusive de cientistas, agências regulatórias internacionais, organizações não-governamentais e indústrias, interessados na questão da identificação de substâncias químicas com atividade desreguladora endócrina. Os autores discutem também o impacto da exposição aos desreguladores endócrinos sobre a saúde humana, considerando as seguintes questões: estimativa de risco; testes utilizados para detectar distúrbios endócrinos; dificuldades na identificação de uma associação entre dose, tempo de exposição, suscetibilidade individual e efeito e tentativas no sentido de mapear os desreguladores endócrinos. Finalmente, os autores argumentam que nem todos os agonistas hormonais devem ser incluídos sob a denominação genérica de desreguladores endócrinos.*
Palavras-chave *Substâncias Tóxicas; Exposição a Produtos Químicos; Desreguladores Endócrinos*

Introduction

Endocrine disruptors are chemical substances, exogenous to the human or animal organism, which have hormonal activity and can thus alter the homeostasis of the endocrine system. Many of these compounds interfere with the development of the endocrine system and affect the functioning of organs that respond to hormonal signals. The endocrinal and reproductive effects of endocrine disruptors may be a consequence of their ability to: (a) mimic natural hormones, (b) antagonize their action, (c) alter their pattern of synthesis and metabolism, or (d) modify the expressions of specific receptors (Colborn & Clement, 1992).

The homeostasis of the endocrine system does not, despite its name, mean that hormonal levels are maintained constant or static, but rather that the system adapts physiologic functions within the range necessary for an optimal performance. This is why it is difficult to define what normal endocrine activity is. For instance, at the simplest level, the concentrations of circulating hormones vary according to the age and gender of the individual and the precise timing of the measurement. Thus, any realistic attempt to estimate the consequences of exposure to endocrine disruptors must take into account the hormonal pattern of each individual and how small variations from normality can affect the functioning of the system as a whole. In fact, a clear distinction has been drawn between endocrine disruptors and potential endocrine disruptors. The latter denomination is reserved for chemical compounds whose effects on live animals are not documented, despite evidence of their hormonal activity in *in vitro* assays (European Workshop on the Impact of Endocrine Disruptors on Human Health and Wildlife, 1996).

This paper presents an analysis of the opinions of different groups such as: scientists, international regulatory bodies, non-governmental organizations and industry, who have proposed analytical systems and screening tests to identify chemical substances with possible endocrine disrupting activity. There is also discussion of the consequences that exposure to endocrine disruptors may have for human health, with a point-by-point consideration of the issues related to the estimation of risk: the tests that must be used to detect endocrine disruption; the difficulties to establish an association between dose, time of exposure, individual susceptibility, and effect; and the attempts to create a census of endocrine disruptors. Finally, it is proposed that not all hormonal mimics should be included under the single generic denomination of endocrine disruptors.

Tests of endocrine disruption

Several existing tests and bioassays of very different types have been proposed by distinct international bodies to identify hormonal mimics/antagonists, for the purpose of assessing the risk of exposure to chemical compounds and eventually using the assays as regulatory instruments for international application (Comisión de las Comunidades Europeas, 1999). However, despite the major efforts that have been made in a very short period of time, the charge has been laid that the hormonal effects attributable to some chemicals compounds suspected of being endocrine disruptors cannot be measured with the toxicological tests currently in use (Miller & Sharpe, 1998). The following have been noted:

a) A chemical compound may have endocrine disruption-related effects at a lower dose than the currently accepted No Observable Adverse Effect Level (NOAEL).

b) Many of the toxicity tests are not designed to detect effects that occur after exposure during early development.

c) Very few tests evaluate the combined effect of several chemical compounds.

d) Alterations of endocrine function can have repercussions in any organ and at any moment in the life of an individual, because of the special actions of hormones during development and in the maintenance of homeostasis during critical periods.

The development of tests to detect endocrine disruption is a simple problem to solve, but at the same time is not without complexity. It is simple to determine whether a given chemical compound is a hormonal mimic-agonist or antagonist. It must not be forgotten that many of the bioassays that served over seventy years ago to define estrogenic hormones can also be used to identify chemical compounds, natural or not, with estrogenic activity.

In 1936, Dodds & Lawson published in Nature the first demonstration that chemical compounds without the perhydrophenanthrenic ring, a characteristic of the sex hormones, acted as potent estrogens in the ovariectomised rat (Dodds & Laws, 1936). The increase in uterine weight and transformation of the vaginal epithelium after bisphenol treatment were as great as those induced by natural estrogen. Shortly afterwards, Dodds was awarded the

Nobel Prize for his discovery of the highly potent synthetic estrogen diethylstilbestrol (DES). The purpose of Dodds' research was to identify new compounds that could be used as a therapeutic tool in clinical practice, and DES was prescribed to women for over 30 years for the prevention of miscarriage. Unfortunately, its dramatic impact on the developing fetus was not observed until well into the 1970s, when the daughters of these women were shown to have an increased risk of vaginal cancer. The administration of DES to pregnant women was eventually prohibited, due to the proven risk of disease in their children. This illustrates how hormonal activity tests can fail to show endocrine disruption; in other words, they do not predict damage that has further-reaching consequences than the hormonal effect observed over the short term.

The complexity of the task of screening chemical substances for endocrine disruption derives from our need to predict effects, beyond the simply-observed hormonal action, which are implicated in the pathogeny of endocrine-related diseases (Ashford & Miller, 1998).

At any rate, it is clear that we can select some well-established and adequately standardized bioassays for the demonstration of hormonal action. They can be used to classify chemical compounds according to their ability to mimic or antagonize natural hormones. From this starting-point, the definition of endocrine disruption would require the development of tests with longer observation periods than that considered by transgenerational effects. To summarize, a long enough time has surely elapsed since Dodds' 1936 experiments for us to be able to identify a modest series of *in vivo* and *in vitro* bioassays that are useful to classify chemical compounds under suspicion after their analysis with an adequate battery of tests for each hormonal function. The scientific literature of the last 70 years can also yield essential information on chemical compounds attributed with hormonal activity in any system.

The European Parliament and Council (1997) instructed the European Commission to develop screening tests for endocrine disruptors. The Commission delegated this responsibility (European Parliament, 1998) to the International Committee of Experts in Endocrine Disruption (EDTA) of the Organization for Economic Cooperation and Development (OECD). The EDTA members discussed with groups of experts from the United States, Japan and other nations the types of tests and bioassays that should be implemented and how the screening program should be set up. To date, three assays have been approved by the different groups of experts: the uterotrophic test in immature rats; the Herberger prostate assay; and Protocol 407, extended to assess transgenerational effects.

However, the EDTA took over four years to decide whether Dodds' 1930's uterine test or Hershberger's rat prostate androgenicity test were appropriate for citizens of the new millenium to assign estrogenicity or androgenicity to a handful of chemical compounds; far too long, given the need to produce solutions for immediate action. Furthermore, excessive effort has been devoted to producing data already published by scientists over the last 70 years, for the sole reason that they were not generated for the purposes of regulatory policy or international trade.

The Committee of Experts (EDSTAC) of the American Environmental Agency (EPA) attempted with mixed fortunes to establish their own battery of tests for the pre-screening of chemical substances, based on *in vitro* assays. Only the androgenicity and estrogenicity tests proved to be of utility. However, the position taken by the American Government markedly contrasts with the repeated claims by industry representatives in the OECD that only *in vivo* bioassays are useful to detect endocrine disruption, an attitude that has defeated any attempt to standardize any *in vitro* tests.

Attention has been called more than once to the passivity of the European Commission on this issue, especially given the emphatic moves by the Joint Research Center (JRC) to develop alternatives to animal experimentation (SCTEE, 1999). Despite the great interest shown by some groups in the defense of *in vitro* tests (cell cultures and biochemical analyses), the various proposals have always been countered by the industry representatives, who seem to regard bioassays that use intact animals as being more to their interests.

To summarize, some members of the OECD have been pressured by their parliaments to start endocrine disruptor screening programs on a unilateral basis. The American scientists and experts that are following this process consider it to be more complex than expected, because:

a) there have been great difficulties in deciding which test to implement;

b) bioassays have only been developed for a few hormonal activities (estrogenicity, androgenicity) within the totality of hormonal functions;

c) there is a very long list of more than 83,000 chemical substances to be tested.

Dose, time and susceptibility

The European administration, through the Directorate-General Environment (DGENV) (Comisión de las Comunidades Europeas, 1999), expect the experts eventually to produce a series of tests that can be applied to the list of chemical compounds with hormonal activity and identify the true endocrine disruptors (Comisión de las Comunidades Europeas, 1999). However, things appear to be not so simple. It has been questioned more than once whether these tests, designed to detect hormonal mimics, are appropriate to identify the long-term effects of hormonal dysregulation or to assess the risk of continued exposure to these substances.

Many national experts share the view that a lot of time is spent on screening and characterization procedures while many critical questions remain unanswered. Some of the most important of these have been raised by scientists in studies published over the last few years and they must be addressed as a matter of priority:

a) Are there different levels of sensitivity to hormonal effects among the different subjects of observation?

b) Can dose–response curves adopt paradoxical forms (U and inverted-U) or be non-monotonic?

c) What is the baseline effect that can be attributed to the endogenous hormones of *in vivo* models?

d) Can the phenomena of additivity, synergy and antagonism be considered in any of the proposed models?

e) Is there an account taken of the differences between sub-populations or interspecies variability?

Census of endocrine disruptors

Professional interest in the emerging field of endocrine disruptors may have multiple motivations. Sometimes, a group has contributed scientific information on one of the chemical substances under suspicion and wishes to learn more about the assessment of the risk. Some professionals may hold a position of responsibility in the area of environmental health and must formulate general recommendations of a preventive nature. For other individuals, the sole interest may be to participate in one of the research programs funded by international agencies. Commercial interests can also focus people's attention on substances under investigation by toxicologists. Finally, there are those who simply wish to know what is happening and which substances are covered by the term endocrine disruptor.

Many scientists and experts agree that a census of the chemical substances under suspicion would be of great value to the investigation of this issue. It would serve to orient research programs, facilitate the accumulation of information, and allow the panels of experts to publish their conclusions sooner. The data already available allows this process to be started, despite the fact that the current information on endocrine disruptors is often anecdotal and based on data published for very different purposes over the last 50 years. There are at least 13 lists of endocrine disruptors drawn up by different administrations and organizations. German, Swedish, British, American, Norwegian and Japanese lists compete with others produced by various non-governmental organizations.

The European Parliament asked the European Commission (European Parliament, 1998) to produce a census of endocrine disrupting chemical compounds. This responsibility was delegated by the DGENV to the international consultants BKH and TNO, who in May 1999 finally presented a list of around 560 compounds that had been demonstrated in scientific publications to interfere with a hormonal system. The list was approved by the Committee of Experts of the DGENV, and was then modified after the receipt of information provided by industry on two specific areas: production volume and environmental persistence. This caused the original list to be shortened, from 553 to 29 chemical substances plus some of their congeners, so that the census now contains 70 substances.

This priority list is at the very least incomplete, inaccurate and confusing. It basically includes organochlorine compounds – pesticides, PCBs and dioxins – which are adequately characterized, widely-studied for persistence and toxicity, and in most cases already controlled by strict regulations. Derivatives of tributylstannyl, some phthalates, and bisphenol-A, make up the rest of the census.

The aim of this list was to provide the scientific community with a set of families of chemical compounds to which special attention should be devoted. The resulting research would yield the information required to definitively include or exclude them from a census of compounds whose use should be regulated. The list that has been proposed is unlikely to meet these objectives and the scientific re-

search it stimulates will prove fruitless if the production and use of the compound under study is already prohibited. Industry representatives frequently declare their commitment to this particular aspect of chemical toxicology, and have it in their power to lend considerable assistance. They could simply release information on the uses and applications of compounds that are not on the priority list, but could present a risk of inadvertent exposure for the population and are possible candidates to be included in the census.

Why all types of hormone mimics should not be included in the census

It is remarkable to see how some censuses of endocrine disruptors, and more specifically of estrogenic xenobiotics, list pesticides and chemical products used in agriculture together with phyto- and mycoestrogens, natural products contained in some vegetable organisms, and drugs used in the hormonal treatment of very different diseases, both in human and veterinary medicine. Some lists even include natural hormones and degradation products that are naturally found in the urine of healthy individuals.

Why include such different chemical compounds in a single list of xenoestrogens? Differences in chemical structure are to be expected, given the diversity of chemical compounds with similar activities. More critical is the variability in our current knowledge of these substances and of their uses and exposure sources. There is good, sometimes excellent information on the compounds used in pharmacology. There is also an acceptable degree of information on natural hormones, whether of animal, plant or fungal origin. Animal species have been in contact with many of these substances during long evolutionary periods and have developed adaptive mechanisms and forms for the elimination and neutralization of their adverse effects. In the pharmacological setting, society has set in place rigorous systems to control the administration of hormones used in pharmaceutical products and exposure to them, and their availability is strictly regulated by physicians, pharmacists and other health care professionals. However, the case of artificial estrogenic xenobiotics is distinct in two important ways. First, the toxologic information on them is virtually non-existent. Second, we do not have enough data on their production or use to allow effective action to be taken to reduce or eliminate exposure to them.

To summarize, while there is an acceptable level of information on natural and pharmacological estrogenic xenobiotics, our knowledge of artificial xenoestrogens is largely anecdotal. There appear to be no reasons to include such different chemical compounds under the single generic denomination of endocrine disruptor. It has been suggested that some of the parties involved in the risk evaluation process may support the single-list option as a way of increasing the complexity of an already complex issue and thus delaying any restrictive decisions and measures.

Conclusions

Can we use available screening tests and assays to demonstrate an association between exposure to endocrine disruptors and disease? If we seek irrefutable evidence of a cause-effect relationship between exposure to individual chemical compounds with endocrine disrupting activity and disease, then the answer to this question must be no. This level of evidence cannot be attained, at least not with the instruments currently available to toxicologists and epidemiologists, because of the universality of exposure, the complexity of the pathogenic mechanisms of diseases, and the time delay between exposure to a substance and the onset of disease. As the European Parliament (1998) itself proposed, the precaution principle must be adopted in the evaluation of scientific data, in the decision-taking, and in the preventive activity launched to preserve the health of populations that are most at risk and in greatest need of protection, such as pregnant women and children.

Acknowledgements

This work was supported by a 96/99 Research Project from the Health Department of the Andalusian Regional Government.

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Submitted on 30 March 2001
Final version resubmitted on 25 April 2001
Approved on 15 May 2001