ENDOCRINE DISRUPTERS: A NETWORK OF KNOWLEDGE

A network of knowledge on endocrine disrupting chemicals: the activity of the European Teratology Society

The **European Teratology Society** (ETS) is the only European scientific society specifically devoted to research on risk factors for human development. Endocrine disrupting chemicals (EDC) are currently the "hottest" topic in toxicological rik assessment, and the intrauterine and post-natal development are specifically vulnerable to EDC modes of action: thus, EDC-related topics have featured prominently in ETS conferences, including the most recent ones (Budapest, 2017; Berlin, 2018). The ETS has identified and tackled EDC issues by making avail of its multi-voice network of knowledge, involving scientists from academy, industry and public institutions. EDC are substances that induce adverse effects through endocrine mechanisms; this seems rather straightforward, if one do not take into account the inherent complexity of the endocrine system:

- How the new strategies for toxicological risk assessment can help in regard of EDC? The use of Adverse Outcome Pathways (AOP) will elucidate the plausible link between molecular events (e.g., receptor binding) or biomarkers (e.g., changes in hormone levels) and adverse effects. Beyond individual AOP, the input of chemically-specific pharmacokinetics and the interlinking of different AOPs in ontologies lead to Integrated Approaches to Testing and Asessment (IATA). In perspective, IATA may exploit the big and increasing amount of data in toxicology, in order to predict potential adverse effects, including endocrine disruption, of chemicals in a more robust and time-effective way;

- In the meamwhile, in the current scenario best practices should be implemented in order to achieve a robust identification of chemicals of concern, such as better exploitation of data bases and pharmacokinetics and mechanistic information in order to assess the human relevance of animal data;

- Cancer and EDC, a still controversial question. Differently from the "classical" genotoxic (i.e., DNA-damaging) carcinogens, such as acrylamide or aflatoxin B1, several EDC can act as tumour promoters through epigenetic and/or proliferative mechanisms, especially in tissues with a strong and direct hormone regulation (breast, prostate, testis, thyroid). The intrauterine phase may be a critically vulnerable window: the evaluation of potential EDC transplacental carcinogenesis calls for an interdisciplinary approach. Improved testing of chemicals, and especially the development of predictive biomarkers to be used in mother-child cohorts would estimate and reduce ongoing and future risks;

- The evaluation of thyroid effects may be the current priority EDC topic, in the fields of both chemical safety and public health: indeed, many chemicals, in particular pesticides, can target several steps of thyroid function, including pituitary regulation, hormone synthesis, hormone catabolism, Thyroid-targeting EDC primarily affect neurodevelopment: ad-hoc toxicological investigations may model dose-response and lifestage-response relationships, in order to identify brain "phenotypes" and functional impairments related to the subtle, yet adverse, effects of low-intensity thyroid disruption. Moreover, hormone level changes should be evaluated as a pattern, which is much more predictive that single biomarkers (e.g., T4 level in serum/plasma). Last but not least, batteries of non-animal tests are needed in order to reliably screen thyroid-targeting EDC; moreover, intact non-mammalian organisms, like the zebrafish embryo, may deserve attention as they can offer a number of molecular, biochemical, morphological and even behavioural endpoints.

Overall the ongoing ETS debate on EDC illustrates the added value provided by an interdisciplinary network of knowledge in tackling ongoing and emerging issues.

The programmes, abstracts and full-lenght papers of ETS conferences are published in a dedicated yearly issue of the journal Reproductive Toxicology.

Endocrine disrupting chemicals: sustainability and/or resilience?

A “sustainable” development meets the needs of the present without compromising the ability of future generations to meet their own needs. For example, “sustanaible food safety” encompasses the actions that, by enforcing the safety and nutritional quality of today’s food, can prevent or reduce the risks and burden of poor health for generations to come. The current concern about Endocrine Disrupting Chemicals (EDC) stems from the programming function of hormones during development: scientific evidence supports that chemicals able to interfere with the programming role of hormones during prenatal and postnatal development can cause adverse effects that do not become evident until later in life.

Disruption of the endocrine homeostasis in adulthood also deserves attention; however, the issue of the EDC-related health risks is largely and mostly relevant to the broader issue of developmental origins of health and disease. Exposure to EDC is, therefore, mainly a long-term concern for the health of the developing organism, hence, it is definitely relevant to the health prevention aspects of sustainability. The European Union approach -as implemented in the regulations on chemical substances (REACH), pesticides and biocides- considers EDC as posing equivalent concern as established categories of “substances of very high concern” (e.g., carcinogens): as a consequence, substances identified to EDC have to be restricted (i.e., banning at least from main usages) and replaced with safer substitutes. The European approach to EDC is, thus, adressing sustainable development: true, the envisaged steps need a robust, consistent and transparent strategy for identifying EDC, which has still to be fully implemented.

The experience of “legacy contaminants”, however, tells that updated, stricter regulations may not be the happy end of the story. Legacy contaminants are chemicals,which remain in the environment long after their producyion and use have been banned. Beyond environmental persistence, legacy contaminants may bioaccumulate along food chains, thus posing a long-standing issue of food safety: PCB and polybrominated flame retardants are examples of bioaccumulating EDC. Widespread pollutants need not to bioaccumulate in order to pose a “legacy” problem. For example, in intensively farmed areas residues of banned pesticides can be found in water comparments, which may represent an environmental reservoir of outdated, but still potentially active, chemicals.

Disposal of chemical waste

Another situation is the disposal of chemical waste from factories. Different from the EU polict, where hazards related to EDC have primarily to be identified, these scenarios call for risk assessment (i.e., integrating hazard and exposure) in order to adress risk management. Here we enter, at least partly, in the domain of resilience: the system has been stressed and it needs adaptation in order to go on functioning. Resilience measures need not be complex, they should be adress the specific risk situation and achieve the intended protection goals: banning the use of certain sources of household water while providing suitable alternative sources can be viewed as a basic resilience strategy. In farming scenarios food-producing animals may bioaccumulate EDC from long-term toxic spills in soils and pastures: a resilience strategy might identify actions in order to preserve an adjusted farming activity in the polluted areas. For example, alternative sources of feed and fodder for animals might be identified; alternatively, the farming system might be converted to agricultural productions that are less liable to bioaccumulation. Research on decontamination systems for environmental media or food-producing organisms is worthwhile, e,g., bioremoval using microbes or plants. Decontamination appears to aim at “restoring” the system, but it needs adaptative measures and changes: risk assessment-based protection goals of the decontamination procesd, workable tools for monitoring and adjusting the ongoing process as well as (important for policy makers) resources devoted to achieve the determined goals.

It may be realistic to assume that as we are improving the sustainability of chemicals we use (and hopefully we will do it), we will also face new environmental “legacies”. Them toxicologists could have to challenge their skills in order to support the resilience of our living environment.

The European Commission roadmap: Towards a More Comprehensive EU Framework on Endocrine Disruptors

Alberto Mantovani of Italy's Istituto Superiore di Sanità assesses the European Commission's efforts to develop a comprehensive and concise regulatory framework for endocrine-disrupting chemical substances

In June, an important public consultation took place: the European Commission (EC) sought feedback on its roadmap, Towards a More Comprehensive EU Framework on Endocrine Disruptors.

Endocrine-disrupting (ED) chemical substances alter the functions of the hormonal system and consequently cause adverse effects. As we already discussed in previous articles on Open Access Government, ED are a hot issue in the global scenario of chemical safety, due to multiple hazards, widespread exposure and the numerous uncertainties that hinder risk assessment and management.

The EC has acknowledged that ED are a main issue to protect human health and environmental quality since at least 2004 (the launch of the first EU Environment & Health Strategy). Nevertheless, the EC has been much criticised by stakeholders, such as environmentalist organisations and some Member States (for example, France and Sweden) because of its perceived slowness to take action.

Such criticisms were largely justified, even though ED are relevant to a broad range of regulatory fields (pesticides, biocides, environmental contaminants, food packaging, cosmetics, occupational health, etc.) Thus, taking effective and consistent action toward ED may be rather complex.

Nevertheless, in the last three years, the ED issue has started to move at an increasing speed, spearheaded by the new regulatory framework of pesticides and biocides.

The new Communication from the EC sets out the ambition to provide a comprehensive, yet concise, EU framework on ED, describing key issues, taking stock of present achievements and outlining concrete steps for the future.

The Communication received 44 comments from institutions, associations and individual scientists. Some comments were quite strong, as – in my opinion – they failed to recognise that this Communication represents a step forward.

Unfortunately, most comments came from a narrow group of countries of Central and Northern Europe, as a significant part of the EU seems to be weakly involved in the debate.

We (the Italian National Health Institute, Dept of Food Safety, nutrition, veterinary public health) considered that overall, the roadmap can be a good (yet slow-coming) compromise among different driving forces. Our comments came from a careful reading of the draft text because a devil might hide in the details.

In the section 'Problem the Initiative Aims to Tackle' (page 2 of the Communication), one point states: "Science on endocrine disruptors progresses quickly, but there are a number of scientific aspects that are not entirely understood."

The main research issue of concern for health services and citizens is the potential (and plausible) role of ED in some major public health issues such as cancer (breast, thyroid, testis) or diabetes and metabolic syndrome.

In our opinion, pointing out the potential involvement of ED in major public health problems, and the need for a science-based answer to the related concerns will

strengthen the relevance of the actions foreseen in the roadmap for the benefit of all EU citizens, beyond the domain of chemical regulation.

Even more important, in the same section we strongly recommend avoiding the wording "extraordinary complexity" in regard to the concerns raised by ED: it seems to recall a hopeless situation which might only be dealt with either through straight precaution or by overlooking the problem. The consistent message of the roadmap should be that more science, science providing effective inputs into the regulations and policies targeted to protection goals are expected to minimise the ED-related risks for health and environment.

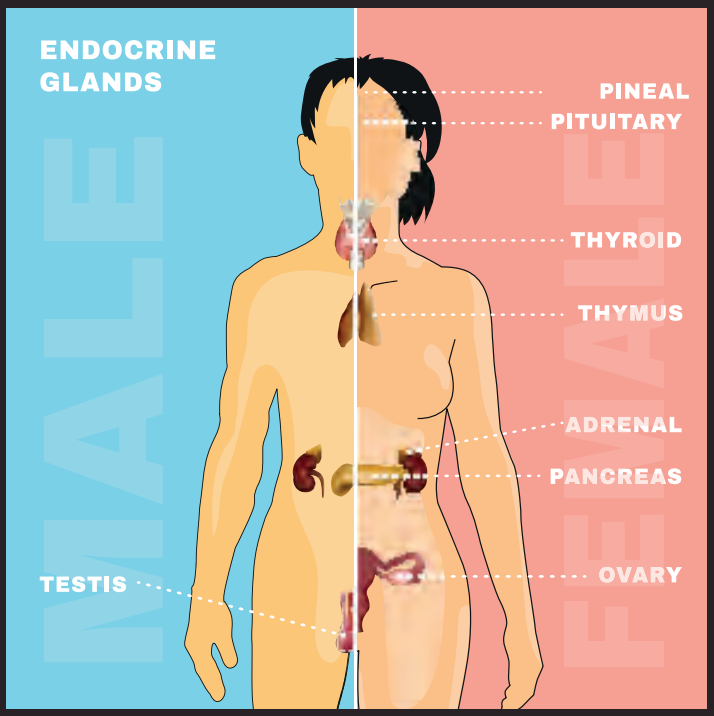
Coming to 'What Does the Initiative Aim to Achieve and How' (page 3), we consider that there is a missing element between the two (well-defined) goals namely, addressing knowledge gaps and linking science and regulation. This additional goal can be identified as a robust and consistent testing and assessment strategy for ED, ensuring the adequate power to predict hazards for health and environment and reducing uncertainties. By making full avail of knowledge and technologies, testing can also be more efficient, ie, more cost- and time-effective, hence, delivering earlier responses to the requests by regulators, policymakers and the public.

In fact, the development of more predictive, more efficient and uncertainty-lowering strategies is the main link between research and regulation, as well as a requirement for implementing the EU regulation on chemicals REACH.

A further goal states that "the EU legislative framework is adequately implemented and remains fit for purpose" (page 3). Under this respect, the roadmap has to make avail of the essential concept of "One Substance – One toxicology": the same criteria for hazard identification/characterisation must be used throughout the different regulatory schemes.

In practice, the same substance may fall under different legislative domains, for instance: pesticide, biocide, industrial intermediate and water contaminant.

Irrespective of specific decisions related to legislation and exposure, this substance should be consistently identified as ED or non-ED across the different regulatory domains.



A more general point is the recognition of ED as a global health topic, as endorsed also by the United Nations Environmental Programme (UNEP). The EU currently has a leading role in the regulatory action on EDC and a tight collaboration with OECD on the development of testing strategies.

Further to OECD, the EU roadmap should look toward broader international cooperation with countries that are major players in the global market, as well as in most research fields (including toxicology); yet these countries still have a limited presence in the international debate on ED. Examples include Russia, China, Brazil and India or world areas such as the Middle East or sub-Saharan Africa. Making use of its agencies (ECHA, EFSA), the EU could play a significant role in more inclusive international action on EDC.

Last but not least, a critical aspect is to define the level of evidence that is sufficient to trigger action. This is an important issue for all stakeholders and should have due visibility in the roadmap.

Research is slowly but steadily tackling the many uncertainties that lead to postponing regulatory decisions: the time comes when regulators have adequate information and tools in order to make science-based decisions to protect the public and the environment. So, when is enough, enough?

How to screen for endocrine disrupting chemicals (EDC)

Alberto Mantovani from Istituto Superiore di Sanità, Rome, Italy explains how to screen for endocrine disrupting chemicals (EDC)

Endocrine disrupting chemicals (EDC) are internationally recognised as a major issue for the regulation of chemical safety: for instance, the European regulatory framework requires that pesticides and biocides identified as EDC should be excluded from the EU market (but for exceptional derogations such as negligible exposure/risk). Thus, in principle, all new and existing substances should undergo a robust and consistent testing for their potential to act as EDC; a cost-effective testing strategy should concentrate on a first-step of screening.

The development of screening tests and/or batteries meet the current interest toward the increased use of non-animal assays in toxicity testing. However, the legitimate enthusiasm should not hide some of the critical considerations: EDC are substances whose endocrine activity is plausibly linked to an adverse effect; the endocrine system is a complex signalling network regulating development and all body functions.

Finally, screening for EDC should be part of a decision tree and decisions (for example, to proceed with additional testing) should be taken based on screening results. The complexity of molecular/cellular events that may be relevant to endocrine disruption can be managed with the help of pathophysiology, according to the current approach of Adverse Outcome Pathways (AOP).

For instance, the thyroid function is, in fact, a "thyroid axis," which includes multiple targets and tissues. The pituitary gland regulates the thyroid function through the thyroid stimulating hormone-TSH. Within the thyroid gland, critical targets are enzymes deputed to hormone biosynthesis and to iodine uptake, as this trace element is all-essential for thyroid function. In the target tissues, like the developing brain, gene expression cascades are triggered through specific nuclear receptors (the TRs). The liver also acts as an "endocrine" organ, as hepatic metabolism regulates thyroid hormone levels. A less simplified scheme would include also the upstream hypothalamic signalling, the proteins transporting thyroid hormones in the blood and the cross-talk with other endocrine axes.

This rather long list of potential targets shows that efficient and standardised screening for thyroid-targeting EDC should include several assays that cover a representative sequence of events, such as TSH release, thyroid hormone production, iodine uptake, TR interaction and liver metabolism. Whereas it might be unfeasible to develop assays for each component of the thyroid axis, the components most likely to targeted by chemicals and/or to lead to adverse effects (with the support of AOP) should be included in the battery.

A fast, robust and cost-effective ("high-throughput") screening battery can support and speed the evaluation of data-poor, high exposure substances (for example, some industrial chemicals or food additives), which are major concerns for regulators. Many in vitro assays are currently developed for EDC, thus regulators should ask toxicologists about scientific aspects, such as the prediction of potential adversity or the profiling of chemicals through the integrated evaluation of the screening battery results.

Other questions should pivot on screening optimisation: comparing candidate assays for concordance and/or redundancy; liability to automatisation; standard operating procedures and quality assurance criteria and so on. The use of case studies is of major value for such purposes. A screening battery for EDC should aim at the optimal balance between reducing complexity, as far as possible, and the capacity to cover an appropriate range of mechanisms.

Assessing endocrine disrupting chemicals (EDC)

Alberto Mantovani presents the contribution of the Istituto Superiore di Sanità (ISS) to the international assessment of endocrine disrupting chemicals (EDC)

The Istituto Superiore di Sanità (ISS), as the reference scientific public body of the Italian Health Ministry and National Health Service, has a major involvement in the regulatory and risk assessment activities on chemicals at international level.

The experts at ISS contribute to the development of new toxicity testing guidelines, as well as to the updating of existing guidelines, which is the remit of the programme on guidelines for the testing of chemicals of the Organisation for Economic Co-operation and Development (OECD). The OECD guidelines are accepted internationally as standard methods for safety testing and are regularly updated with the assistance of national experts from OECD member countries.

The OECD provides specific attention to endocrine disrupting chemicals (EDC) as an emerging topic where many uncertainties still exist. For over 10 years, the ad hoc OECD advisory group on the testing and assessment of EDC has developed and updated a conceptual framework, as well as engaging in the validation work of new tests, with special emphasis on in vitro assays on mechanisms and in vitro/in vivo assays to assess EDC effects on environmental biota. In this vein, the ISS experts, on behalf of Italy as an OECD member country, have identified a potentially significant gap, that is the lack of guidelines for testing the effects on post-natal, pre- and peripubertal development, as a life stage specifically vulnerable to EDC. As consequence, OECD is considering how to investigate hazards for post-natal development.

ISS also contributes to the EU expert group that supports the Advisory Group on Environmental Exposure and the Impact of EDC within the United Nations Environment Programme (UNEP). The current UNEP priority is awareness-raising among policy-makers, including developing countries or countries that currently have only a minor involvement in international programmes on EDC.

In the European Union (EU), the main fields where ISS toxicologists are involved are the European Chemical Agency (ECHA) and the European Food Safety Authority (EFSA).

Implement the EU legislation

The ECHA's task is to implement EU legislation on chemicals for the benefit of human health and the environment, as well as for innovation and competitiveness, by fostering the replacement of high-concern chemicals. The current priority of ECHA is the identification of high-concern substances: carcinogens, genotoxicants, reproductive/developmental toxicants, persistent bioaccumulative and toxic or very persistent and very bioaccumulative; in addition, EDC are considered as substances giving rise to an equivalent level of (high) concern, where scientific evidence indicates probable serious effects to human health or the environment. The ISS experts have been active on EDC at ECHA, for example, by requesting additional studies to clarify whether the UV-screener octabenzone is an EDC.

EFSA is the first EU authority entirely devoted to risk assessment; due to its solid activity, established since 2003, EFSA is taking a spearhead role in the development of risk assessment methods and concepts. Accordingly, ISS scientists contribute their qualified and independent expertise to ECHA and EFSA activities. The contributions concern the assessment of substances (pesticides, plasticizers, nano-sized materials and so on), as well as novel approaches for assessing toxicological emerging hazards, such as EDC; for example, the use of adverse outcome pathways (AOP) in order the strengthen the potential of in vitro, mechanistic studies for predicting adverse effects on human health.

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