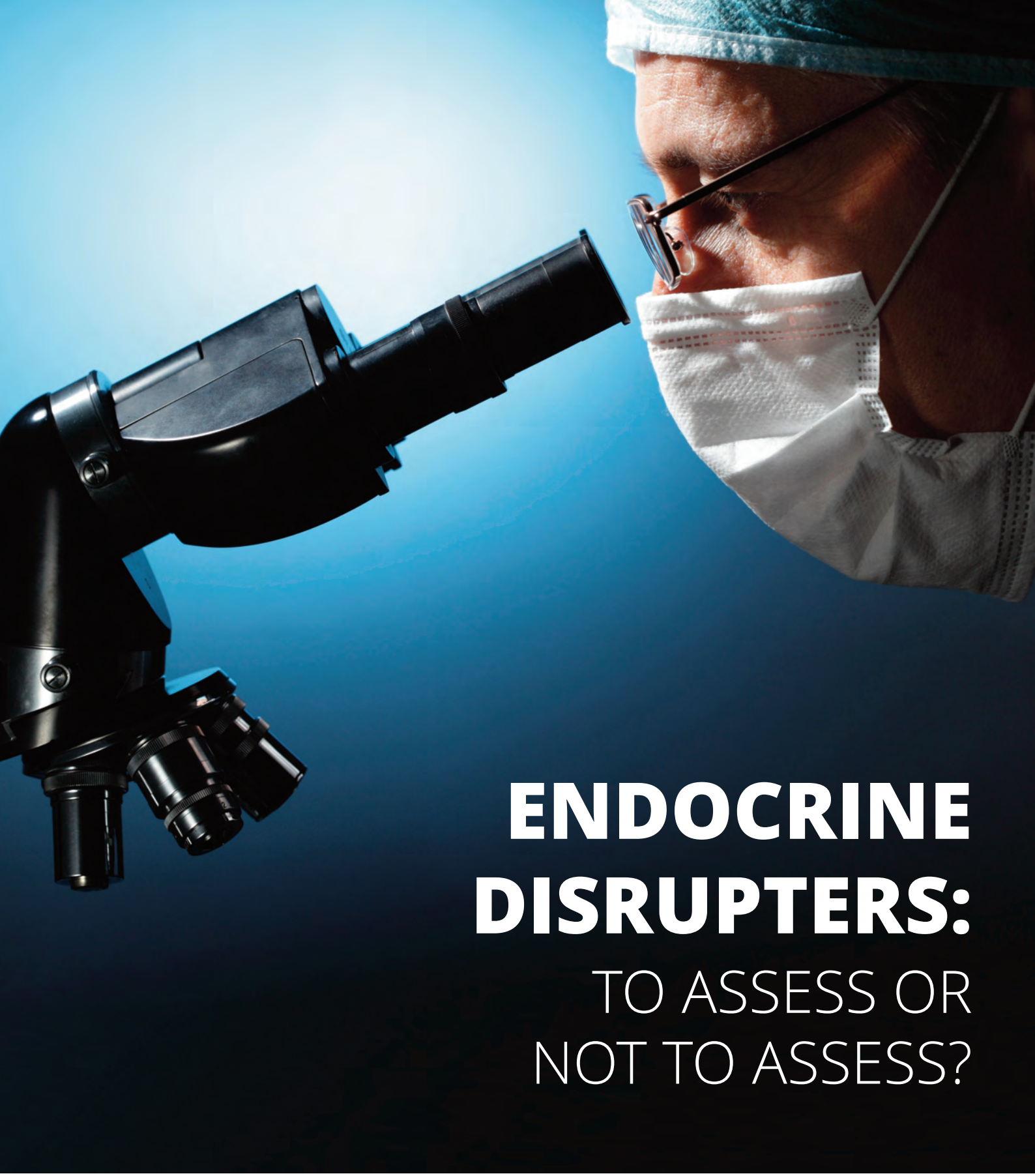

ENDOCRINE DISRUPTERS:
TO ASSESS OR NOT TO ASSESS?

ISTITVTO SVPERIORE DI SANITA'

ENDOCRINE DISRUPTERS

Doing research on endocrine disrupting chemicals? Still worthwhile?

Endocrine Disrupting Chemicals (EDC) are chemical substances that can damage our health and/or the health of environmental organisms by altering the hormone function. Thus, EDC -like mutagenic or carcinogenic substances- are a group made by highly diverse substances, from the standpoints of chemical structures and usages (pesticides, plasticisers, persistent pollutants...) sharing the same action. Indeed, the endocrine system is the most complex signalling network in the organism; EDC, therefore, may act through a number of mechanisms and targets.

Why such a fuss about EDC among EU and non-EU agencies, scientists, industries and NGOs? From my toxicologist's viewpoint the fuss is justified. EDC are hazardous for next generation's health, since hormones are crucial for development, from embryo through to puberty. Each hormone regulates several, often many, organs and tissues: for instance, besides reproduction estrogen function impinges on bone, fat, brain, etc. Hence, an "estrogen-mimicking" EDC may display patterns of multiple effects, depending on the sex and age of the exposed organism. Last but not least, EDC are widepread in environment, products, foods and some may also bioaccumulate in our bodies: tiny amounts of one EDC might sum up with other substances with similar action and elicit some adverse effect, just like the pyramid of minions achieves to change a light bulb.

Europe and some Member States have devoted substantial resources to research on EDC for about 20 years. So one EU citizen could legitimately say "So much knowledge gained, stop putting money on research, let's regulate the hazards": my answer is "**Yes** and **No**".

"**Yes**" because gaps of knowledge must not prevent taking action whenever it is supported by knowledge. For instance, current EU regulations on pesticides and biocides require that EDC are identified and restricted: this can be done, based on available knowledge, and delays would be unjustified.

"**No**" because the available evidence presents a few "holes" of major relevance for risk assessment.

The first one is an old, yet still ongoing, story which is essential for risk assessment. How can we define a "safe dose" for EDC? EDC that interact with nuclear receptors may elicit a cellular response at very low doses, that may be qualitatively different from ones that are elicited at higher doses (e.g., stimulating at lower and antagonising at higher concentrations): research is still needed to understand whether these low-dose responses are linked to adverse effects, especially in developing organisms which are considered to be more susceptible.

Then, are we able to assess hazards to all main EDC targets?

Most EDC research still concentrates on effects on the reproductive cycle, whose importance cannot be disputed. Yet, as already mentioned above, major hormones do regulate a number of organs and tissues. As an example, estrogen balance regulate bone metabolism, with recognised effects in post-menopausal women. However, skeletal health is not a usual target in toxicological testing, either in vitro and in vivo. Most important, the current testing tools, either regulatory in vivo tests or novel in vitro assays, do not appropriately identify effects related to the major, endocrine disease of today's

world, type II diabetes; the same applies to the endocrine component of obesity, which is connected to diabetes in the so-called "metabolic syndrome". Experimental, and to a lesser extent epidemiological, research shows that some environmental chemicals increase the risk of diabetes and/or obesity; in general such substances belong to the small group of thoroughly investigated ones, like arsenic or bisphenol A. However, the absence of robust endpoints and assays jeopardises the consistent identification of substances, (beyond the "usual suspects") that elicit effects relevant to such top-class public health issues, as the metabolic syndrome. Adverse outcome pathways (AOP) are a novel toxicological approach, building causative chains from molecular changes through to pathological conditions at organism level; Indeed AOP could support understanding of the full spectrum of EDC effects.

Besides testing EDC in the lab, a lot of identified or possible EDC are present in our living environment. Is there a health risk ongoing? Should urgent measures be taken to reduce such risk? Then we come to epidemiological studies, which currently show a good ground for improvement. The main issue is how to assess the "early exposure-late effect" scenario which is the foremost problem with EDC: in practice, the exposure in the womb or as a kid does matter definitely more than the current EDC levels in body fluids of fully-grown adults. But how do we cope with this? An answer could be creating and exploiting biobanks, and finding biomarkers of effect that can link developmental exposures to adult health risks. Not to say that adult exposure does not matter: here too, substantial advances are needed, including models and tools for exposure characterisation and relevant biomarkers. Biological plausibility of endpoints and findings is a main requirement for epidemiological studies: here "cross-fertilisation" between epidemiology and toxicology will greatly help. Finally, and again, also epidemiological research should take into account substances other than the "usual suspects".

All that said, many EDC are useful substances for consumers, not just for industry: pesticides to protect crops, plasticisers, preservatives, sunscreens for our everyday life, flame retardants, etc. Yet, restrictions are required to protect our health. Sunstitution of high-concern substances is invoked by the EU Regulation REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals). There is the chance that chemicals candidates for replacing hazardous substances just appear to be less hazardous because their toxicity was insufficiently investigated. The challenge, therefore, is to identify EDC of priority concern, in particular because of widespread use and exposure, and search for substitutes through a robust testing strategy that considers EDC-related as well as other high-concern activities (genotoxicity, bioaccumulation, etc.). As a consequence, the substitutes would be confidently identified as less hazardous. Since the need to screen among numerous potential substitutes requests the development of cost-effective screening strategies, making the best possible use of non-animal (in silico, in vitro) tools (see the project LIFE EDESIA).

So, we do not need "more research" on EDC; rather, we need "fit-for-purpose" research to support risk managers and policy makers in Europe and worldwide.

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To assess, or not to assess endocrine disrupters, that is the question

Endocrine disrupters, (ED from here) are a current challenge in the international arena of hazardous chemicals evaluation. The debate on ED often reaches high-pitched tunes especially in Europe, due to the great move for chemical safety represented by the REACH (Registration, Evaluation, Authorisation and restriction of CHemical Substances) programme. Somebody coming into this arena, who is unaware of ED, might ask whether a (Shakespearean) tragedy is going on.

Indeed:

- The fatal flaw: all heroes in Shakespeare's tragedies have a weakness in personality that eventually leads to their downfall (ED themselves, slowly drawn toward banning due to their hazards);

- Fall of the nobleman: many characters in Shakespeare's tragedies have extreme wealth and power, making their downfall more tragic (let's look to industry and the European Commission and their investments in economy, reputation, etc.);

- External pressure: Shakespeare's tragic heroes often fall victim to external pressure from others (let's look to public opinion, media and ONGs);

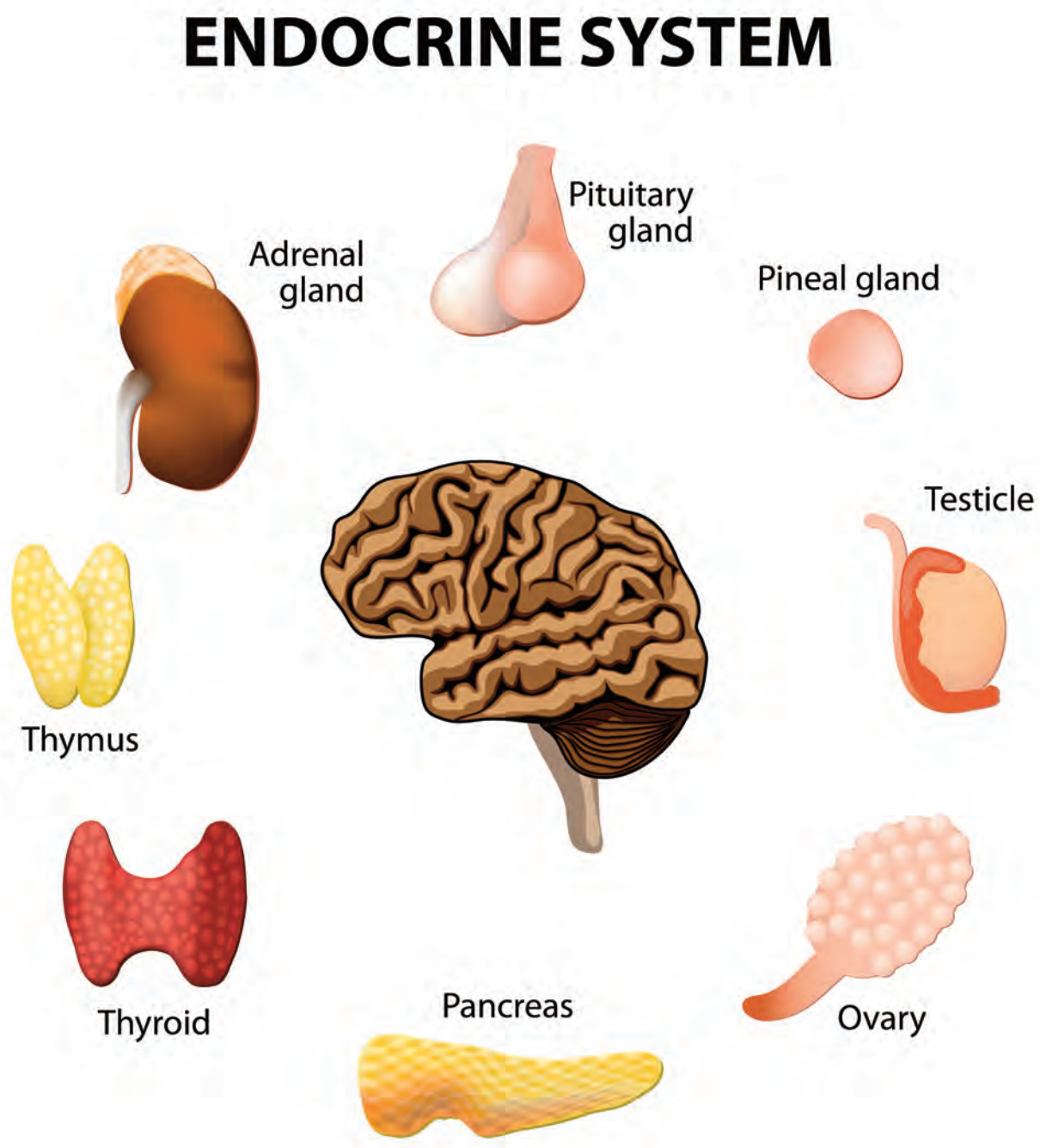
- And finally, the hero, who has the opportunity for redemption and victory, but never takes advantage of these in time, which leads to ruin (unfortunately, scientists seem to fit this role).

ED are substances that can cause adverse effects on health by altering the endocrine system function, according to the definition by the World Health Organization (WHO) in 2012. The safety of chemicals is evaluated by the risk assessment process, where i) critical hazards are identified (e.g. liver toxicity), and ii) safe levels of exposure are set based on the identified critical hazard(s), taking into account all the uncertainties; the safe levels are then compared with certain exposure conditions (e.g. intended usage levels of a pesticide in fruits) to determine if an appreciable risk does exist.

Together with the great mainstream of toxicology, we are convinced that the risk assessment framework should be adopted whenever possible, with updates from new scientific developents. Indeed, several toxicologists keep maintaining that ED assessment is "business as usual." ED can cause reproductive disorders and/or tumours in toxicological tests with laboratory animals, if that's the case, then let's set safe levels for such effects.

Unfortunately, what complicates ED risk assessment is the burden of uncertainties.

Overall ED affects the most complex regulatory network of the body, distributed in several, highly different tissues (pituitary, gonads, thyroid, adrenals, pancreas...). Thus, ED may hit a number of targets with a number of mechanisms. True, the current methods can reliably identify ED effects on reproduction and thyroid. However, a number of other targets, such as adrenals, growth hormone or parathyroids, are evaluated less reliably or might even escape evaluation. The first and main example is the endocrine component of the so-called "metabolic syndrome" (diabetes, obesity and hypertension), which is a main cause of disease worldwide.



We have no validated tools to screen chemicals for their potential to cause, or increase the risk of, metabolic syndrome. This holds true also for several other endocrine-related diseases, associated to ED exposures by some epidemiological findings. Yet, no robust experimental framework can currently screen chemicals for mechanisms related to endometriosis, polycystic ovary syndrome or osteoporosis. Thus, we can identify part, but not all, of the spectrum of potential ED effects.

Moreover, ED effects depend on lifestage. One great issue of ED assessments are “low dose” effects. The same term “low dose effect” is unclear: it may indicate an adverse effect observed at doses lower than the “mg/kg body weight” magnitude order usually investigated in standard toxicological assays, it might even hint to the uncertainty about a “lower threshold of effect” for ED ithat interact with hormone nuclear receptors. Whatever the interpretation, there is sound evidence that organ-

isms during prenatal and, to a lesser extent, postnatal development are more susceptible and, therefore, ED effects may be elicited at significantly lower exoosures than in adults. But, again, no validated tools are available to screen chemicals for some highly relevant ED effects on developmental programming. For instance, independent research showed that the developmental exposures to certain ED alter the differentiation of target tissues, making them more prome to develop cancer later in life. Bisphenol A (a diffused, and much debated, plasticizer with estrogen-like action) increases the proliferation of mammary tissue in developing rodents; this effect has been taken into account by the European Food Safety Authority (EFSA) when evaluating bisphenol A (2015), as well as reducing the previous tolerable daily intake by one magnitude order. Yet, toxicological testing must deal with the "universe of chemicals", beyond the small bunch of highly investigated "usual suspects": no standardised test is currently available to screen chemicals for their endocrine-related effects on the programming of cancer predisposition.

The uncertainty burden (which includes also other issues besides those discussed above) may imply either i) that ED risk assessment requires a particularly great amount of data (like the EFSA assessment of bisphenol A) or that ii) a precautionary approach is needed, as called by NGOs and part of the scientific world, such as the Endocrine Society. An international workshop organised this year by the Federal Institute for Risk Assessment (Germany) under the auspices of the European Commission pointed out that considerable uncertainties and debate exist on ED assessment; conversely, most ED can be identified here and now, based on WHO definition. The workshop delivered a set of general criteria to identify ED. In addition, to keep criteria as straight as possible, I deem that sub-categories such as

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"possible ED" should be avoided, unless temporary, i.e., indicating substances for which more studies are needed in order to identify whether they are ED or not. The process should pinpoint in a specific way chemicals of concern: if the system indicates that "almost every substance might be an ED", the legitimate reaction by risk managers could be "then, if everything is an ED, nothing is an ED". The approach pivoting on ED identification is currently undertaken by the European Commission, albeit with much debate, exitation and painful delay. ED should be identified in a consistent way across different regulatory contexts (REACH, biocides, pesticides) alike other "high-concern" hazardous substances (e.g. carcinogens). Then, the work of risk managers will start: regulations require that, whilst considering socio-economic impacts, restriction measures are launched for ED, with substitution featuring prominently.

So, the answer is that identification, rather than assessment, is the priority action to date.

But, some ED, even after drastic restrictions, may persist in the environment and enter the food chains, like brominated flame retardants. Here risk assessment is required: you cannot ban foods.

Boosting research in Italy

Stefania Giannini, Italian Minister of Education, Universities and Research, outlines to Adjacent Government how the Ministry are investing in research talent to boost science in Italy…

What makes a European Country competitive and attractive in the 21st century are not natural resources, or merely the cost of employment protection legislation. Conversely, its ability to innovate, to create and to disseminate knowledge will ensure a sustainable future to our citizens and encourage investors to bet on Italy.

Since the beginning of our term, the Italian government has invested on the quality of the human capital, considering it as a key factor for the necessary socioeconomic transformation and development. Knowledge and education really make the difference for the future of our globalised societies and economies.

"The NRP will invest €2,5bn in the next three years, an unprecedented budget that allows us to attract additional national resources. More than 40% of the budget will be devoted to the Human Capital Program: we expect to have more than 6000 researchers and PhD students at the end of the plan."

With this belief, in last 3 years, Italy has completely overturned the paradigm for education and research policies.

Starting with universities, our policies are oriented firstly to renew the human capital of professors and researchers. We want to attract the best global and European talents, facilitating brain circulation towards Italy.

This twofold goal will be achieved through the recruitment of 500 new full and associate professors supported by the "Natta fund". Moreover, an extraordinary plan has been launched in order to hire new full professors and more than 1000 researchers in the universities and in the public research agencies.

Stefania Giannini, Italian Minister of Education, Universities and Research

Our wider, but feasible ambition is aimed at stimulating the creation of an ecosystem open to investments and partnerships from the private sector and from foreign countries.

Only such an ecosystem will allow us to fully emerge our strengths that we assume here as a point of departure for the next challenges. The quality of Italian publications, for example, is certified by the high number of citations, on average comparable to Germany and France performance, with some peaks in medicine and engineering.

Having said that, the National Research Program (hereinafter NRP) constitutes the master-plan of

the research for the next 3 years, ensuring that Italian research policies are contextualised in a consistent, predictable and selective framework.

We conceive this plan as the innovative industrial policy for the scientific, economic and social growth of our country.

The NRP will invest €2,5bn in the next 3 years, an unprecedented budget that allows us to attract additional national resources. More than 40% of the budget will be devoted to the Human Capital Program: we expect to have more than 6000 researchers and PhD students at the end of the plan.

Other measures will be carried out in order to reinforce the Public-Private Partnership and Industrial Research Program.

"Starting from the universities, our policies are oriented firstly to renew the human capital of professors and researchers. We want to attract the best global and European talents, facilitating brain circulation towards Italy."

The NRP paves the way for a better innovation ecosystem and selective funding of joint public-private initiatives. It provides the national research system with an intermediate infrastructure of soft-governance, the National Technological Clusters, which are in charge of proposing technology roadmaps on a national level in different fields.

In addition to this comprehensive master-plan, the Italian government has set a challenge towards 2040: to become a world lead in personalised medicine, oncology and neurodegenerative diseases through the development of an intensive, cross-disciplinary project.

Actually, a comprehensive approach to health and ageing (human technologies) does not yet exist, in part because of the necessity to integrate cutting-edge technologies with high-profile basic and translational science in critical areas of medicine, data science, nanotechnologies and nutrition.

Italy wants to fill this gap through a large-scale, cross-disciplinary research infrastructure, named "Human Technopole", which will encompass the synergistic development of fundamental and clinical genomics, nutrition, innovative algorithms for data analysis, multiscale methods in computational life sciences and advanced technologies for food and diagnostics.

The "Human Technopole" will be created in Milan, in the Expo area, by 2018 together with a strong international recruiting action to secure top talents from all over the world. It will host at steady state more than 1,500 researchers (1000 staff units + 500 PhD students), with a strong reverse brain drain effect.

The government will finance the project with €1.5 bn in 10 years.

We conceive it as an asset of a broad strategy that firmly believes in Italian potential to anticipate and create the future through the ideas and the research shared and tested with other scientific communities all over the world.

Italy's present and future competitiveness will depend largely on its ability to transform talent into development, by increasing the knowledge component of our economy and finding new answers to the challenges of society, markets and the environment.

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