WCSR Advice 2023-19

SCIENTIFIC COMMITTEE REACH (WCSR)

TOWARDS A MORE EFFECTIVE REACH IN PROTECTING HUMAN HEALTH AND THE ENVIRONMENT

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CONTENTS

Context

This paper is an advice by the Belgian Scientific REACH Committee (WCSR), which is a group of experts that offers advice on the dangers and risks of chemical substances to public health and the environment, in particular within the context of the implementation of REACH. This paper presents the expert opinion of the committee on the current functioning of REACH, as well as why and how REACH could improve in the future.

1. Why should the REACH procedure be improved

1.1. High and often increasing incidence or prevalence of ”diseases of civilization"

In the last hundred years the socioeconomic conditions and the availability of many food items have improved markedly in the western countries, and the progress in medical science and techniques has allowed to limit the impact of diseases on mortality and severe morbidity. However, although life expectancy has risen, the incidence and prevalence of many diseases of civilization has increased after correction for ageing. The incidence of cancer and the prevalence of diabetes, cardiovascular diseases, the metabolic syndrome, obesity, allergies and problems with fertility have increased whereas the percentage of people free of chronic diseases that can be related to chemicals has, at least until recently, not risen. Also, the prevalence of neurodegenerative diseases and neurodevelopmental disorders has increased during the past decades and there are indications that, since more than a decade, cognitive capacities have decreased in some Western countries whereas they had increased over previous decades. See annex 1 for a comprehensive text and references.

1.2. Man-made products contribute substantially to the risk of “diseases of civilization”

Growing evidence from epidemiological and molecular-epidemiological studies indicates that pollutants and man-made products contribute substantially to the risk of diseases of civilization. This has been substantiated for cancer, cardiovascular diseases, diabetes, reproductive disorders such as early puberty, male and female infertility, diabetes, obesity, neurodegenerative diseases, disorders of neurodevelopment and cognition and immune system related diseases. According to Global Burden of Disease study, all forms of pollution combined were responsible in 2015 for 21 % of all deaths from cardiovascular disease, 26 % of deaths due to ischaemic heart disease, 23 % of deaths due to stroke, 51 % of deaths due to chronic obstructive pulmonary disease, and 43 % of deaths due to lung cancer. See annex 2 for a comprehensive text and references. Annex 3 entails much data and references on biological and health effects of real life low dose exposures.

Modern science and technology, and the use of 'next generation' weight-of-evidence assessment approaches, are not embedded in the regulation of chemicals - Over the past decades, many new technologies and methods have become available that allow to collect data on different toxicological endpoints in a faster way by using less or even no animals. However, the current regulatory framework is not compatible with the use of data generated via these methodologies as it is focused on collecting data for specific endpoints, mainly via traditional (in vivo) testing and depending on tonnage level. In order to better integrate these modern science and technologies, new regulatory frameworks are needed. Within this context, as suggested by Cronin et al. (2021) and Fentem et al. (2021), regulatory frameworks that integrate biological

activity and kinetics from different sources and comparing the outputs with estimates of exposure could be of interest.

2. How should the REACH procedure be improved

2.1. Independent toxicity testing

In the current regulatory framework for REACH, the type and number of toxicological tests that needs to be performed is driven by the tonnage level. Although the tonnage level is an indirect indication of the exposure, the limitation with this approach is that it can lead to the generation of data which is not appropriate or insufficient to evaluate the hazards/risks most relevant for the proposed use. There are limitations on the number of toxicological tests performed on chemicals. Previous reviews of submitted registrations dossiers showed insufficient characterization/description of the uncertainties associated with the hazard and risk assessments, and also issues with what data was chosen to report (1). The EU Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) made the industry in charge of commissioning the tests to testing laboratories. However, due to conflicts of interests, this approach is highly susceptible to bias (2). There is a lack of control with third parties who only have limited access to the data and a lack of control by authorities with limited resources to check compliance to the REACH regulation (2).

Each decision a scientist makes throughout the testing process could influence the final result of the test (3). Funding bias happens when scientists make choices to maximise the chance of an output that is wished for by the party funding the test or research (3). The scientist performing the testing can also be biased by institutional demands (4). Several behind-the-scenes practices influence the final result, while they are hard to detect (3). However, precautions against such problems can be easily checked in audits (3). An example of conflicts of interest can be seen in the research of Bisphenol A (BPA). Research studies funded by industry did not report significant effects when assessing low doses of BPA while more than 90% of the research studies funded by governmental agencies did find significant effects for exposure to low doses of BPA (5). Within this context, a positive publication bias, (i.e. the bias that might occur due to the fact that authors are more likely to submit, or editors are more likely to accept, positive results than negative or inconclusive results), should on the other hand also be taken into account.

Currently, the industry only has to deliver a study summary, which must contain sufficient and accurate information on the complete study (2). Ingre-Khans et al. (2) recently investigated study summaries and found several kinds of errors: from typing errors, to unclear and incomplete reporting, to omission of information which was considered relevant for assessment of the chemical. The latter is highly concerning for the accuracy of these summaries, which are used for decision making (2). As a result, the reliability and quality of the data that is delivered by the industry can be questioned.

Many of the dossiers submitted to the European Chemicals Agency (ECHA) are noncompliant, with the majority lacking information needed to assess the risks and hazards (6–9). ECHA considers compliance a priority with a focus on long-term effects on human health and on the environment and aims to improve compliance and quality of dossiers in a gradual and planned way (6–9). To improve transparency of data, a

potential solution can be to make full study reports available or to allow independent assessment of the data (2). Also for the risk assessment, all available data should be accessible (2). However, the majority of the data received from those studies is considered confidential (2).

To minimise bias and to avoid conflict of interests, it is worth to consider to perform the testing and registering of the chemicals by an independent third party (2). In order to ensure that studies are performed in a qualitative way with adherence to GLP (1). The independent laboratories would have to be subject to more audits to confirm conformity to GLP and to check the quality of their data. Finally, there is need for close collaboration between these laboratories performing the testing and REACH regulators to identify needs for testing and to perform checking of data.

2.2. Follow-up of substances after putting on the market

Currently, under REACH, substances are only tested for certain endpoints, depending on tonnage per year used. Once allowed on the market, no mechanism is in place to monitor how the risk associated with these substances evolves. Registrants have requirements to keep their registrations up to date, however, in 55 % of the companies selected for an inspection system to update the registrations were absent and 18% had registration dossiers that had not undergone an obligatory update (8). Further, there is no follow-up on epidemiological, long-term effects of these substances after being put on the market. Scheepers and Godderis (10) described the importance of post-market evaluation mechanisms, that are still lacking in REACH. A molecular epidemiological approach could be more sensitive than classical epidemiology (11) and is essential in the post-market evaluation. Substances are also mainly evaluated for their intended use separately, while new applications for products can arise over time (10). These new applications of the substances go hand in hand with exposure to new unidentified populations, which were not considered at the pre-market assessment (10). Despite, the obligation to update the chemical safety report when a new, non-covered use arises is included in REACH, it is clear that the update is not always done. This causes uncertainties of the long-term effects of substances on human health and on the environment. Like ECHA mentioned in their report on the operation of REACH and CLP (8), REACH only demands restricted information regarding environmental risks. However, between, 2016 and 2020, an acceleration was seen in the restriction of substances with risks to the environment and since 2020, persistent organic pollutants are integrated in the portfolio of ECHA (8,9).

The REACH regulation also includes the regulation of mixtures effects, but no guidance documents for their assessment exist, while various stakeholders have highlighted their importance (12). The absence of information on mixture effects pressures the testing strategy for chemicals (13). It has been shown that certain mixtures show more toxicity than the active ingredient, while REACH requirements only focus on pure chemicals (10,13). Compared to the USA, the European Union does not have a regulatory framework for assessment of mixture effects (14). While international guidelines are limited to effects on human health, the European Commission together with the relevant scientific and industrial public would go for guidelines concerning both human health and the environment (14). The European Commission also stated the need for the industry to develop exposure scenarios for mixtures (7). Martin et al. (15) showed that risk assessments

for mixtures of chemicals in published mixture studies usually can be predicted using the addition concept, however, the potential synergistic or antagonistic action of chemicals should be considered.

2.3. Increase amount and types of testing

By now it is evident that classical toxicological tests do not provide sufficient protection against adverse health effects. A more comprehensive approach is needed. In annex 3 a narrative comprehensive text and references concerning the growing evidence for the importance of mixture effects, epigenetic and transgenerational effects, low dose and non-monotonic effects, and of exposures in early life is to be found. Also the more recent discovery of the existence of ligand specific effects of exogenous substances binding to nuclear receptors is of concern. Chemicals that bind to receptors with transcription factor functions might have effects that differ from the effects of the physiological ligands and hormones. These effects cannot easily be predicted and might lead to adverse health effects.

REACH requires certain tests based on what tonnage level the chemical is used with more extensive testing for chemicals that are used in a higher tonnage levels (7). While this approach has proven reasonable for marketed substances, it might lead to issues with new substances of lower tonnages that can require market access with less comprehensive testing (7). Testing of chemicals currently takes a long time, while many substances are not yet properly tested with data lacking on their hazards or risks. Moreover, there are indications that certain chemicals can exert non-monotonic effects, and thus can lead to more effects when exposed to low doses than in higher doses (17). Required testing to gain market access does not directly address endpoints regarding endocrine disrupting abilities, neurotoxicity or immunotoxicity, which can be important consequences of exposure. Due to long latency periods, most toxicity tests and thus also the required testing under REACH is not predictive for long term effects of exposure to chemicals. Thereby, there is a lack of studies and investigation into and many uncertainties about toxicological endpoints.

Risk assessment and containment are important concepts in preventing diseases (16). A proper risk assessment can only be made when all information is available on hazardous properties of the chemical substance taking into account the current state-of-the-art in toxicology (16). However, due to the limitations in the current testing requirements under REACH and the lack of continuous surveillance and updating, not all available toxicological information on substances is considered. Further, the available information that is required comes mostly from only in vitro, or when obliged, in vivo experiments, while the animals differ physiologically from humans (16). However, despite the fact that human data are key, they are often missing due to the difficulty to collect and obtain certainly in the early phase of an application (16) . In order to obtain more insights in the toxicological profile of chemicals and to integrate modern science and technologies, we would need an adapted regulatory framework.

Fentem et al. (18) stated that if the obligated testing remains based on tonnage levels, protection from the chemical substances to environment and consumers would fail and registrants would have large volumes of data that has to be generated. Therefore, definition of several surrogates for chemical exposure other than tonnage levels is important in the further improvement of protection from chemicals (18).

In 2010, it was already noted that REACH requirements are strict endpoint-related and that endpoints such as respiratory toxicity, obesity, neurodevelopment, and neurodegeneration are not investigated, with highly relevant and incident diseases such as neuropsychiatric disease, metabolic syndrome, endocrine and reproductive disorders and pulmonary diseases as potential consequences (13). ECHA suggests that this is a consequence of the generous use of read-across data (6). The strictly defined endpoints allow for an easy evaluation of compliance, but bring doubts whether those tests can be used to predict all health hazards those chemicals give rise to (13). ECHA recognizes the relevance of endpoints that are currently not included in the column 1 information such as immunotoxicity (19). Tests on endpoints such as developmental neurotoxicity and immunotoxicity should in REACH be performed when available data has shown concern for these endpoints (8). However, subjectivity hits in on how the concern should be established (8). Even less substances receive a mutagenic or carcinogenic classification, which is reflective of the focus within REACH, but the conditions to include testing for mutagenicity and carcinogenicity could be reviewed (8). The past eight years no endocrine disrupting chemicals (EDC) which were discussed by the EDC Expert Group resulted in restriction or inclusion in the Authorisation list (20).

While Article 25 of the REACH legislation states that testing on vertebrate animals only would be undertaken as a last resort and recital 37 states that it should be approached by using other alternative testing strategies whenever possible, ECHA has rejected read-across or non-animal tests proposed by industry when they were not considered to fulfil the standard information requirements (for instance when the read-across justification by industry was lacking or insufficient). In 2018, the European Court of Justice ruled against ECHA, after it had demanded a developmental toxicity study in animals to Esso Raffinage to eliminate data gaps , while Esso Raffinage argumented to be able to demonstrate safety with information from other sources (18). This leads to the perception that there is a lack of willingness of ECHA to consider alternative testing strategies, however, ECHA is concerned about the robustness of the alternative approaches to replace standard animal tests (22,23). Several studies have also been performed on in silico profiling, in vitro profiling, toxicokinetic modelling, and read-across methods in order to promote their use to their full potential as a transparent and consistent methodology (24–26). ECHA published a document describing the read-across assessment framework (19), and launched a Quantitative structure-activity relationship (QSAR) Toolbox, allowing to extract each other's results (9). According to some researchers, this document does seem to be very demanding to make an acceptable read-across (22). Due to the adaptation of the requirements for safety information for REACH in 2016, an increase in alternative testing strategies is seen, with more adaptations such as read-across and a more widely use of in vitro testing (9). However, in 2018, it was noted that in vitro tests were only used at a low level as complete replacements, while much effort was put into their validation (22). For example, between 2008 and 2016, for only 11% for the skin irritation, and 7% for eye irritation only in vitro results were submitted (22,23). There are also delays regarding updating the Test Method Regulation (TMR), which should be updated when ECHA regards a method "appropriate", suggesting like Taylor (22) mentioned an overly cautious approach of ECHA to consider them as complete replacements.

An important point of criticism in REACH is the need for more chemical control before entry to the market is allowed, especially on long term effects to human health and the environment (8,21,27). A no data, no market principle is proposed and acknowledged by ECHA by a more extensive completeness check since July 2016 (6,8,20).

2.4. Need for faster decision making under the current REACH process

Another point of criticism in REACH is the lengthiness before regulatory action is taken and the low output of substance evaluations (6,21). This results in the piling up of substances that need regulatory action and meanwhile consumers and workers stay exposed. Due to the large amounts of substances to be tested and the slow process of doing so under REACH, very little action is taken up until now. Until 2018, only 94 substances had completed the substance evaluation (6). Only a quarter of the chemicals identified with a very high concern, have been added to the Authorisation list, with half of the uses of such chemicals still allowed awaiting a decision by ECHA (20). Therefore, there is need for more ambitious goals to ensure a safer environment and health. This includes more stringent obligations for industry to provide information and to proof the absence of risks before the substance can be allowed on the market.

When risks emerge for a substance, the authorities have the decision whether the chemical can remain on the market with restrictions for the uses that resulted in risks or if the substance gets banned from the market (10). The report of Late Lessons From Early Warnings of the European Environment Agency concludes there to be a lack of mechanisms to respond to early warning signals, with the recommendation to reduce the delay between early warning and taken actions (16). One of the reasons for the slow evaluation process is also the lack of environmental and human data and proposals have been made to set in place (inter)national expert groups with a centralised expert group at EU level to ensure collaboration to evaluate new and emerging risks (6,16). With chemical substances evaluated based on priority, some substances will not get evaluated, and thus remain on the market without restrictions (Armstrong et al., 2021). In thirteen years, only 27 substances were restricted by REACH (20).

In a non-paper, the European Environmental Bureau wrote that in the best case scenario, it takes three and a half years for identification and classification for a hazardous substance (20). When legal timeframes are respected and depending on the duration of additional testing, a substance evaluation is completed after 7-9 years, which in many cases finds even more postponement throughout the process (6). For example, triphenyl phosphate was flagged with concerns about endocrine effects (6). The evaluation in the Community rolling action plan was postponed four years in a row (6). After 7-9 years to clarify a suspected concern, the implementation of regulatory actions may take 5-7 years, leading to 12-16 years before chemicals of concern are regulated (6). However, listing substances of very high concern in the Candidate list only takes six months (20). Compliance checks of the safety data can take more than five years (20).

Since 2019, authorities have been using a grouping approach for similar substances where possible, to increase efficiency and effectiveness of regulatory action (8). Based on structural similarity, substances can exhibit similar physico-chemical, toxicological and ecotoxicological effects and therefore can be considered as members of a group. Within these groups, data regarding health endpoints can be predicted by the data of another member of the group, also known as read-across data (1). In 2018, the European Commission also suggested to use a grouping approach next to improving the non-compliance present in the registration files (7).

3. Remediation of shortcomings

For the classification of chemicals (no-restriction, restriction, ban), no discrimination is made between hazards to human health or to the environment.

In order to faster obtain more insights in the toxicological profiles of a larger number of chemicals while at the same time relying less on animals, new regulatory frameworks are needed. As highlighted by Cronin et al. (28) , such frameworks should integrate data on biological activity and kinetics from different sources and compare the outputs with estimates of exposure. To increase the speed and scope of testing we propose to include fast battery testing as well as toxicokinetic testing. Further, alternative methods should be considered such as new in silico and in vitro testing methods to expand the knowledge of the chemical substances before they enter the market. We also think it is necessary to include testing with endpoints such as endocrine disrupting features, neurotoxicity, immunotoxicity, and others as obligation under REACH to cover these effects before market access. It would be in everyone's interest to make all testing data publicly available. In the ECHA strategic plan 2019-2023 final, they describe their desire to have robust data about all chemicals in Europe and to have all registrations dossiers updated with appropriate and complete data about hazards of substances (21). In 2022, the European Environmental Bureau published a non-paper in which they again stated the need for this kind of policy, suggesting it has not been implemented yet (20).

To guarantee objectivity and completeness of test results, testing should ideally to be performed by independent laboratories. In order to ensure that studies are performed in a qualitative way with adherence to GLP, these independent laboratories would have to be subject to more audits. Ideally, financing of the tests should run via a test fund that is managed by REACH policy makers and fed by the industry.

Once a chemical has been allowed to be put on the market there should be a follow-up for example by an independent organization. Once substances of concern have been identified, there is a need for epidemiological studies but also a molecular-epidemiological approach should be followed in addition to epidemiology. Indeed, the molecular-epidemiological approach is much more sensitive than classical epidemiology, and can lead to the detection of effects at a point in time where no irreversible health damage has occurred, permitting preventive measures and constant follow-up. Santos et al. (6) showed that for many chemicals for which concerns were demonstrated regulatory action to confine the risks remains absent. According to the European Commission (7), improvement is needed with the notification of substances of very high concern in articles, initiatives like digital products passports could help in this regard. Several articles propose mechanisms as pharmacovigilance and the 'disease first' method as 'early warning systems' as post-market surveillance systems (10,16). Important is the awareness of health professionals to the potential risk chemicals might have even with the regulations in place (16). Due to the probable rareness of these diseases and their long latency times, Palmen et al. (16) suggests the need for international surveillance systems. These forms of secondary prevention have the potential to prevent new cases in an early phase and to fill knowledge gaps (16).

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Annex 1 – The issue: high and often increasing incidence or prevalence of "diseases of civilization"

That chronic non-communicable diseases are an important cause of human suffering is certain (WHO, 2022). In the last hundred years the socioeconomic conditions and the availability of many food items have improved markedly in the western countries. However, although life expectancy has risen, the incidence and prevalence of many diseases of civilization has increased after correction for ageing (Belgian Superior Health Council 2019). In this annex the term "diseases of civilization" is used to designate cardiovascular diseases, cancer, diabetes, obesity, female and male reproductive dysfunction, disorders of neurodevelopment and cognition, and immune system related diseases. The incidence of cancer and the prevalence of diabetes, the metabolic syndrome, obesity, allergies and problems with fertility have risen.

It is clear that the progress in medical science and techniques has allowed to limit the impact of diseases on mortality and severe morbidity, but, at least until recently, the percentage of people free of chronic diseases that can be related to chemicals has not risen. In the Netherlands the life expectancy at birth without chronic illness (see RIVM website in references) has decreased for men from 54.5 years in 1981 to 48.1 years in 2012 and for women from 53.9 in 1981 to 40.5 in 2012 (Van Duuin & Stoeldraijer, 2014)). Correspondingly, in Flanders the prevalence of self-reported chronic diseases has increased with 34% from 2001 to 2018. This was however not the case for Brussels and Wallonia. (https://www.healthybelgium.be/en/health-status/non-communicable-diseases/overview#references and https://www.sciensano.be/en/biblio/gezondheidsenquete-2018-chronische-ziekten-en-aandoeningen).

A world-wide increase in the incidence of cancer is observed (Sasco, 2008). In Flanders, the incidence of cancer has risen for men till 2004 with a cumulative incidence of 35.3% and for women till 2014 with a cumulative incidence of 28.3% ages 0-74 after exclusion of non-melanoma skin cancer. In Flanders a decrease has been observed in recent years for men, and to a lesser extent for women. In 2020 in Flanders the cumulative incidence of cancer for ages 0-74, after exclusion of non-melanoma skin cancer, amounted to 32.20 % in men and 27.10 % in women (data from the Belgian National Cancer Registry).

Although age-adjusted atherosclerotic cardiovascular disease mortality rate trends decreased globally (Barquera et al., 2015), the leading cause of death, globally, remains cardiovascular diseases; their prevalence is incessantly progressing in both developed and developing nations according to the report of the World Health Organization (Balakumar et al., 2016). However, probably due to medical progress, the mortality from cardiovascular disease in Belgium diminished from 36.0 % of total mortality in 1998 to 28.4 % in 2015 (National Institute for Statistics).

The prevalence of obesity, diabetes, the metabolic syndrome have risen in the past decades. Overweight and obesity have increased markedly in the last 20 years in most OECD (Organisation for Economic Co-operation and Development) countries, not only in adults, but also in children, and an increase in children has also been observed in Belgium between 2000 - 2001 and 2013 - 2014 (https://www.oecd.org/els/health-systems/Obesity-Update-2017.pdf, accessed 26/9/2018). From 2003 - 2004 to 2011 - 2012, overall prevalence of the metabolic syndrome increased in the USA from 32.9 % (95 % confidence interval (CI), 31.6 % - 34.2 %) in 2003 - 2004 to 34.7 % (95 % CI, 33.5 % - 36.0 %) in 2011 - 2012 (Aguilar et al., 2015).

In the second half of the 20th century it became obvious that a relentless increase in diabetes type 2 (diabetes mellitus (DM)) affecting the economically affluent countries, is gradually afflicting also the developing world (Ginter & Simko, 2010). The global prevalence of type 2 diabetes is estimated to have doubled over the past 30 years and now includes rapidly rising numbers of children and adolescents (The Lancet editorial, 2018). In the USA age-adjusted prevalence of type 2 diabetes for adults age 65+ increased at an average annual percentage change of 2.31 % between 1992 and 2012

(Akushevich et al., 2018). The incidence of childhood-onset type 1 diabetes has increased worldwide. Throughout Europe the reported annual increment varied between 2 % and 5 % according to the observed population. In Belgium a secular trend of increasing incidence was noted in children, but a decreasing incidence in the age group 15 - 39 years was observed, indicating an earlier onset of diabetes type 1 (Gorus et al., 2004). According to the Belgian "Diabetes Liga" prevalence of diabetes has more than doubled in the past decades and the International Diabetes federation estimates that 8.0 % of the Belgian population suffers from diabetes, predominantly (about 90 %) from diabetes type 2 (https://www.diabetes.be/diabetes-cijfers, accessed 26/9/2018).

In Flanders (Comhaire et al., 2007) and many other regions in the world the incidence and prevalence of problems with male fertility has increased. A review by Sengupta et al. (2017) identified an overall 57 % diminution in mean sperm concentration over the past 35 years ($p = 0.0002$), which, when analyzed for each geographical region, identified a significant decline in North America, Europe, Asia, and Africa.

According to the World Allergy report allergic diseases are increasing in prevalence worldwide (Pawankar et al., 2008). In the UK, the prevalence of allergic disorders has risen importantly over several decades, but rates have stabilized over the past decade (Gupta et al., 2007). In the UK admissions for some systemic allergic diseases have however risen sharply in the last decade which may indicate a rising incidence of these conditions (Gupta et al., 2007).

In most countries, the prevalence of asthma has been reported to increase in the last few decades (Eder et al., 2006).

Chronic obstructive pulmonary disease (COPD) is a leading cause of world-wide mortality and disability. On average about 5 – 15 % of adults in industrialized countries have COPD defined by spirometry (Anto et al., 2001). The World Health Organisation has predicted that COPD will become the third most common cause of death in the world by 2030 (cited by Diaz-Guzman & Mannino, 2014). In recent years the COPD morbidity and mortality have however decreased in some developed countries (Diaz-Guzman & Mannino, 2014).

There are indications that since more than a decade, cognitive capacities have decreased in some Western countries (Teasdale & Owen, 2005; Dutton & Lynn, 2013), whereas these cognitive capacities had increased over the previous decades in the 20th century (Flynn, 1987; Pietschnig and Voracek, 2015). Moreover, the prevalence of neurodevelopmental disorders has increased during the past decades: autistic spectrum disorders and attention deficit hyperactivity disorders (Bellanger et al., 2015).

The prevalence of neurodegenerative diseases has increased. In the Netherlands, the incidence of Persistent Cognitive Decline increased among 65 - 88 year-olds from 2.5 % to 3.4 % between 1992/1993 and 2015/2016, and in Belgium the importance of Alzheimer disease as a cause of death has increased with 35.4 % between 2005 and 2016 (healthdata.org).

Finally, it seems probable that the prevalence of certain types of behavioral problems has increased (Bor et al., 2014).

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Annex 2 – Man-made products and pollutants, in particular carcinogenic agents, mutagenic agents and endocrine disruptors, contribute substantially to the risk of diseases of civilization

It is well known that the causes of diseases of civilization are multifactorial, and that inherited traits, nutrition (WHO technical staff, 2014) and behaviour, including the amount and regularity of physical exercise (American Cancer Society, Kushi et al., 2012), play an important role. This text is meant to present the main lines of evidence indicating that pollutants and man-made products contribute substantially to the risk of diseases of civilization. This item is developed in somewhat greater detail with relation to cancer but is also addressed in relation to other diseases. That pollution poses a serious threat to human health is increasingly recognized. The Global Burden of Disease study, a multinational study (WHO, World Bank, Harvard School of Public Health, 2015), estimates that pollution-related disease was responsible in 2015 for 16 % of total global mortality (Cohen et al., 2017). According to the global burden of disease study, all forms of pollution combined were responsible in 2015 for 21 % of all deaths from cardiovascular disease, 26 % of deaths due to ischaemic heart disease, 23 % of deaths due to stroke, 51 % of deaths due to chronic obstructive pulmonary disease, and 43 % of deaths due to lung cancer.

1. Numerous substances are mutagenic, carcinogenic or endocrine disruptors

For about four decades, the human population has been exposed to an increasingly large array of synthetic chemicals. Only about 1 % of those chemicals have been studied so far since scientific research is time-consuming and costly (Trasande et al., 2016), or because testing was simply not requested or not deemed necessary.

Animal tests with 127 substances, selected because they were produced in huge quantities and/or because of the existence of an important human exposure, showed that 26 (20 %) of these substances were carcinogenic (Huff, 1993). It is probable that a large percentage of reactive chemical substances are genotoxic carcinogens (see Alberts et al., 1994, p 243; Huff & Hoel, 1992).) According to a report by Dhooge et al (1998) two to three percent of the

substances with a high production volume might have an oestrogenic activity. So far, 1,409 chemicals (last updated September 2017) have been listed as potential EDC based on data published in the peer-reviewed literature (TEDX, 2017). Some 82,000 chemicals are registered for commercial use in the USA alone (Duncan, 2006), and in Europe almost more than 140,000 chemicals were preregistered for a later full registration within REACH (Backhaus et al., 2010). The European Union (European Chemicals Agency (ECHA)) has listed 145,297 chemicals as pre-registered before 2008 (last updated 11 August 2017). An estimated 2,000 new chemicals are introduced annually for applications in everyday items such as foods, personal care products, prescription drugs, household cleaners, and lawn care products (Duncan, 2006). In the European Union there are about 100,000 substances on the market and about 2,000 chemical substances are produced or imported in large quantities. The Toxic Substances Control Act Chemical Substances Inventory contained in February 2017 more than 67,000 chemicals (https://www.epa.gov/tsca-inventory/about-tsca-chemical-substance-inventory#howare). In polluted air or in emissions to environmental air more than 2,800 different chemical substances were already identified in 1992 (Lewtas, 1993). The US Environmental Protection Agency considers 10,517 substances for testing related to endocrine disruption. The literature contains data on testing for endocrine disruption of 1,036 substances (http//endocrinedisruption.org/).

2. Cardiovascular disease (CVD)

According to the Lancet commission on pollution and health (Landrigan et al., 2017) all forms of pollution combined were responsible, worldwide, in 2015, for 21 % of all deaths from cardiovascular disease.

There are now studies suggesting a direct link between EDCs and CVD, independently of those EDCs acting as obesogens or diabetogens (Gore et al., 2015.). Dioxin exposure (Humblet et al., 2008), organochlorine pesticides (Min et al., 2011) and dichlorodiphenyltrichloroethane (DDT) (La Merrill et al., 2013) were found to be associated with CVD in epidemiological studies. There is evidence that Bisphenol A (BPA) acts directly as a cardiovascular disruptor in rodents (Gore et al., 2015) and that internal exposure to BPA is associated with CVD in humans (Gore et al., 2015).

Carcinogenesis and atherosclerosis might have several fundamental biological mechanisms in common (Botto et al., 2001). So several of the pollutants contributing to the risk of cancer might also contribute to the risk of CVD. In accordance with this is, for instance, the fact that fine airborne particles increase risk of cardiovascular disease by inducing atherosclerosis (Landrigan et al., 2017). Also, fine particulate air pollution is associated with several risk factors for cardiovascular disease, including: hypertension, increased serum lipid concentrations, increasing oxidative stress, increasing insulin resistance, promoting endothelial dysfunction, and enhancing propensity to coagulation (Landrigan et al., 2017). Ionizing radiation is another example of an exogenous factor inducing, at low level exposures, as well cardiovascular diseases as cancer. A systematic review and meta-analysis has been performed to summarize information on circulatory disease risks associated with whole-body ionizing radiation exposures. This review supports an association between circulatory disease mortality and low and moderate doses of ionizing radiation (Little et al., 2012). Tumor suppressor molecules are activated in the complex environment of atherosclerotic plaque, and regulate growth arrest, cell senescence and the apoptosis of vascular smooth muscle cells, which may protect against the progression of atherosclerosis (Suzuki et al., 2014.).

3. Diabetes

Many EDCs produce insulin resistance and alter insulin production and secretion by directly acting on adipocytes, liver, and Beta-cells in the absence of overweight or obesity (Gore et al., 2015). There is substantial evidence, including prospective studies, linking some persistent organic pollutant (POP) exposure to type 2 diabetes in humans, including organochlorine pesticides such as trans-nonachlor, hexachlorobenzene, dichlorodiphenyldichloroethylene (DDE), polychlorinated biphenyls (PCBs), and dioxin-like chemicals. Notably, nonmonotonic relationships and low-dose effects appear in humans (Lee et al., 2014). Also internal exposure to bisphenol A, and exposure to arsenic and phthalates were found to be associated with the risk of type 2 diabetes (Gore et al., 2015).

Experiments in vitro and on animals have produced evidence for diabetogenic activity of several chemicals, including perfluorooctane sulfonate (PFOS) (Gore et al., 2015).

In a systematic review, Dimakakou et al. (2018) found a consistent positive association between ambient air pollution and type 2 diabetes.

4. Obesity

The origin of obesity is multifactorial and is influenced by both genetic and environmental factors. The "obesogen hypothesis" suggests that prenatal or early-life exposure to certain EDCs predisposes some individuals to gain fat mass and become obese. Bisphenol A, phthalates and persistent organic pollutants have been found to be associated with obesity in some epidemiological studies, but the evidence for this associations is limited (Gore et al., 2015).

In vitro experiments have shown that low concentrations of tributyl tin (TBT), some phthalates, parabens, 4-nonylphenol, the fungicide triflumizole, the pesticide tolylfluanid, the brominated flame retardant 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) and bisphenol A promote adipogenesis (Gore et al., 2015). Activation of peroxisome proliferator-activated receptor gamma (PPARgamma) and the retinoid X receptor (RXR) are an important mechanism that can lead to adipogenesis (Gore et al., 2015). But adipogenesis can also be stimulated through other mechanisms, involving estrogen receptors, a glucocorticoid receptor or the aryl hydrocarbon receptor (AhR) (Irigaray et al., 2006; Gore et al., 2015).

Animal studies show obesogenic effects of environmental estrogens, tributyltin, some phthalates, the flame retardant tetrabromobisphenol, the anti-stick chemical perfluorooctanoic acid, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the polychlorobiphenyls 126 and 77, DDT and the organophosphate insecticides chlorpyrifos, diazinon and parathion (Gore et al., 2015).

5. Female reproductive dysfunction

Several studies indicate that EDCs can adversely affect the ovary, uterus, vagina, anterior pituitary, and/or steroid production, which can lead to reproductive disorders such as early puberty, infertility, abnormal cyclicity, premature ovarian failure/menopause, endometriosis, fibroids, and adverse pregnancy outcomes (Gore et al., 2015).

Bisphenol A, some phthalates, the pesticide methoxychlor (MXC) and the dioxin TCDD were reported to disturb ovarian development in animals (Gore et al., 2015).

Disturbance of ovarian function in animals was reported (Gore et al., 2015) for postnatal exposure to: bisphenol A, some phthalates, the pesticides MXC, endosulfan, malathion, chlorpyrifos, cypermethrin, imidacloprid, fenvalerate,

trifluralin, bifenthrin, diuron, and 2,4-dichlorophenoxyacetic acid; diethylstilbestrol; the dioxin TCDD, several PCB congeners (Gore et al., 2015). Multiple studies consistently show that a variety of pesticides alter ovarian steroidogenesis in laboratory animals (Gore et al., 2015). Bisphenol A, some phthalates, and the pesticide heptachlor were reported to disturb ovarian steroidogenesis in women (Gore et al., 2015).

Interestingly, the effects of EDCs on the ovary may be transgenerational in nature because studies indicate that both fetal and neonatal exposure to MXC caused epigenetic alterations in ovarian genes in adults (Zama & Uzumcu, 2009; Uzumcu et al., 2012).

Bisphenol A, some phthalates, several pesticides including a mixture of organophosphorus pesticides (dichlorvos, dimethoate, and malathion), endosulfan, fenvalerate, DDT, hexachlorocyclohexane, benomyl, carbendazim, the herbicide pendimethalin, the antibacterial agent triclosan, and the antistich chemical perfluorooctanoic acid (PFOA) all were reported to disturb uterine structure/function (Gore et al., 2015).

Diethylstilboestrol induces adenosis lesions in the cervix and vagina in women, and in utero exposure causes clear cell carcinoma of the vagina (Smith et al., 2012).

Bisphenol A, the phthalate DEHP (bis(2-ethylhexyl) benzene-1,2-dicarboxylate), the pesticide atrazine (ATR) and diethylstilbestrol adversely affected the function of the anterior pituitary gland in animals (Gore et al., 2015). Endocrine disrupting substances were reported to be associated with diverse effects on puberty, but the results of these studies are mixed (Gore et al., 2015).

Bisphenol A was observed as well in women as in animals to adversely affect fertility (Gore et al., 2015) and prenatal BPA exposure may have transgenerational effects on female fertility in mice (Ziv-Gal et al., 2015). Experimental studies show an association between phthalate exposure and reduced fertility in animals, but only limited information exists on phthalate exposure and fertility outcomes in women (Gore et al., 2015). Several studies indicated that pesticide exposures reduce fertility or cause infertility in animal models (Diamanti-Kandarakis et al., 2009; Gore et al., 2015) but the data on pesticide exposure and infertility in humans are equivocal (Gore et al., 2015).

Studies in women, including a prospective cohort study, showed that BPA exposure is associated with premature ovarian failure and early menopause in women (Yang et al., 2009; Souter et al., 2013). In a cross-sectional survey using the US National Health and Nutrition Examination Survey (NHANES), women with high levels of phthalate metabolites or with high levels of the pesticides β-hexachlorocyclohexane (β-HCH) and mirex had an earlier mean age at menopause compared to women with low levels (Grindler et al., 2015). Animal studies are consistent with the effect of the pesticides in the study of Grindler et al. because they indicated that exposure to pesticides may cause premature ovarian failure (Gore et al., 2015). In utero exposure to diethylstilbestrol was associated with an increased lifetime risk of early menopause in women (Gore et al.,2015).

DDE (a DDT metabolite), the dioxin TCDD and PCBs were shown to induce premature reproductive senescence in female animals, and for DDE this was also observed in women (Gore et al., 2015). Urinary levels of propylparaben (a preservative in personal care products) were associated with a trend toward lower antral follicle counts as well as higher day-3 follicle-stimulating hormone (FSH) levels (indicators of ovarian aging) (Smith et al., 2013).

The potential effects of EDCs on premature ovarian failure may be transgenerational in nature because developmental exposure to a pesticide mixture (permethrin and N,N-diethyl-m-toluamide) increased ovarian insufficiency in the F3 generation of rats (Manikkam, 2012a). Similarly, TCDD increased the incidence of ovarian insufficiency in the F3 generation of rats (Manikkam, 2012b).

Earlier studies showed that the dioxin TCDD was associated with an increased risk of endometriosis in nonhuman primates and women. A positive association between dioxin-like PCBs and an increased risk of endometriosis was also observed in women. Recent studies indicated that TCDD exposure disrupted cannabinoid signaling in the human endometrium, leading to increased inflammation in the endometrium and that it inhibited progesterone responsiveness in humans and animal models. Exposing mice to TCDD caused a progesterone-resistant phenotype in adults that persisted over multiple generations, suggesting that TCDD exposure had transgenerational effects on endometriosis (Gore et al., 2015).

6. Male reproductive dysfunction

Genetic mutations affecting androgen production or action cause testicular dysgenesis syndrome (TDS), including cryptorchidism, hypospadias, impaired semen quality, and markedly increased risk of testicular cancer (Skakkebaek et al., 2001).

Chemical compounds that disrupt androgen production or action can cause testicular dysgenesis symptoms such as hypospadias, cryptorchidism, and impaired spermatogenesis in experimental animals and cause structural alterations in the testis resembling the abnormalities seen in human testicular cancer (Fisher et al., 2003). Animal models show that antiandrogens can act in a dose-additive or even synergistic manner, which has challenged the current no adverse effect levels because the adverse outcomes have appeared when the animals have been exposed to a combination of chemicals far below their individual no-observed-adverse-effect levels (NOAELs) (Christiansen et al., 2009; 2012). In addition to antiandrogens, estrogens and dioxins cause similar effects, via their cognate estrogen receptors (ERs) and AhRs, respectively (Gore et al., 2015). Perfluorinated chemicals such as PFOS and PFOA have been associated with disruption of male fertility in as well animal experiments (Song et al., 2018) as in observations on humans (Di Nisio et al., 2018; 2019). In the Flemish biomonitoring program however no adverse effects on fertility were observed at the levels of internal exposure to perfluorinated chemicals measured in Flanders.

One meta-analysis suggested an increased risk of hypospadias in sons of parents exposed to pesticides, but in general results concerning the link between pesticides and hypospadias in men are rather inconsistent (Gore et al., 2015). In animal studies, hypospadias is a common outcome in male pups that have been exposed to antiandrogens in utero. Some of the chemicals inhibit testosterone production (e.g., phthalate esters [benzyl butyl phthalate (BBP), dibutyl phthalate (DBP), DEHP, diisononyl phthalate (DINP)]), whereas others block the androgen receptor (AR) (e.g. the pesticide DDE, and fungicides vinclozolin and procymidone). Despite their dissimilar mechanism of action, these chemicals act in a dose-additive manner, with increased likelihood of adverse effects of low intensity exposures to individual chemicals in the mixture (Gore et al.,2015).

7. Disorders of neurodevelopment and cognition

Many studies report an association between exposure to air pollution and disturbance of neurodevelopmental processes, neurodegeneration and impairment of cognitive development. A search in Pubmed on the seventh of September 2018 for articles published in the period 2015 - 2018 mentioning "air pollution" and one of the terms "cognition", "cognitive", "neurdegen*" or "neurodevelopm* in the title or abstract resulted in a list of 175 articles.

Basagana et al. (2016) performed a longitudinal observational study on 2,618 schoolchildren (average age 8.5 years). Children completed computerized tests assessing working memory, superior working memory, and inattentiveness during four visits. An interquartile range increase in indoor traffic-related PM2.5 (particulate matter - fine particles

with a diameter of 2.5 µm or less) was associated with reductions in cognitive growth equivalent to 22 % (95 % CI: 2 %, 42 %) of the annual change in working memory, 30 % (95 % CI: 6 %, 54 %) of the annual change in superior working memory, and 11 % (95 % CI: 0 %, 22 %) of the annual change in the inattentiveness scale. Traffic was the only source of fine particles associated with a reduction in cognitive development.

In a systematic review, Dimakakou et al. (2018) found a consistent positive association between ambient air pollution and both type 2 diabetes and neurodegeneration risk, such as dementia and a general decline in cognition. Neuroimaging studies found cerebral white matter, cortical gray matter, and basal ganglia might be the targets of traffic-related air pollution (de Prado et al., 2018). Seelen et al. (2017) report, based on a case control study including 917 amyotrophic lateral sclerosis patients and 2,662 controls, that long-term exposure to traffic-related air pollution is associated with increased susceptibility to amyotrophic lateral sclerosis (ALS). Risk of ALS was significantly increased for individuals in the upper exposure quartile of PM2.5 absorbance [odds ratio (OR)=1.67; 95 % confidence interval (CI): 1.27, 2.18], NO2 (OR=1.74; 95 % CI: 1.32, 2.30), and NOx concentrations (OR=1.38; 95 % CI: 1.07, 1.77).

The Lancet commission on pollution and health considered that air pollution is causally associated with decreased cognitive function, attention-deficit or hyperactivity disorder and autism in children and neurodegenerative disease, including dementia, in adults (Landrigan et al., 2017). Pollutants known to be toxic to the developing brain (in addition to lead) include mercury, combustion by-products such as polycyclic aromatic hydrocarbons and fine particulate matter, organophosphate pesticides, brominated flame retardants, phthalates, and polychlorinated biphenyls (Landrigan et al., 2017).

Decreased school performance and scoring on intelligence tests and even mental retardation were observed in individuals exposed in utero to the radioactive fallout of the atomic bombs in Hiroshima and Nagasaki, particularly when exposure occurred between weeks 8 and 15 of pregnancy (Otake et al., 1998; Schull et al., 1999). In the medical field, low doses of ionizing radiation to the brain in infancy have been shown to influence cognitive abilities in adulthood (Hall. et al., 2004). In order to increase the statistical power and to have more dosimetric and biological data allowing to understand the mechanisms of the cognitive and cerebrovascular effects after an exposure to low ionizing radiation doses, the project CEREBRAD was developed and supported by the EU Euratom 7th framework programme (FP7) with a multidisciplinary approach (human epidemiology, animal studies and mechanistic studies). This project unveiled effects at doses previously assumed to be harmless. Persistent effects (DNA (deoxyribonucleic acid) damages, inflammation) were observed in animal studies at low doses (20 and 100 mGy) several months after exposure (corresponding to years in humans) (Benotmane A., in European Commission 2018). Combined exposures to radiation and other environmental agents decreased significantly the dose at which brain effects are observed (Eriksson et al., 2010). Interestingly, compared to the offspring exposed to maternal alcohol intake or to infectious agents (zika virus (ZIKV)), the neuropsychological development and the transcriptomic modifications of those prenatally exposed to ionising radiation are highly similar, including induction of genes involved in premature neuron differentiation (Benotmane A., in European Commission 2018).

EDCs also contribute to impairment of intellectual development, increased risk of autistic spectrum disorders and attention deficit hyperactivity disorders (Trasande et al., 2015; Attina et al., 2016). According to Bellanger et al. (2015), polybrominated diphenyl ether and organophosphate exposures contribute to IQ loss in the European population. The mechanism is likely involving interaction with the developmental effects of thyroid hormones in the brain, particularly during prenatal and early postnatal life. The most harmful chemicals appear to be organophosphate pesticides in the EU and polybrominated flame retardants in the U.S.A. Importantly, the cost of EDC effects in the

EU has been estimated by Trasande et al. (2015) to be 157 billion euros per year of which the vast majority (84 %) is related to neurodevelopmental disorders.

8. Immune system related diseases

8.1. Endocrine disruption, risk of asthma, allergies and some autoimmune diseases

There are some indications that endocrine disrupting substances can increase the risk of asthma, allergies and some autoimmune diseases. Developmental exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) may increase the risk of autoimmune responses (Rooney et al., 2008). Prenatal exposure to the persistent environmental pollutant and model Ah receptor agonist, TCDD, has been shown to permanently suppress postnatal cell-mediated immunity (Rooney et al., 2008). More recently, skewing of select adult T and B cell responses toward enhanced inflammation has also been described in C57BL/6 mice after prenatal TCDD (Mustafa et al., 2011). Prenatal exposure to polychlorinated biphenyls showed a positive association with asthma, eczema/hay fever, and frequent ear infections (Parker-Lalomio et al., 2017).

8.2. Reactive substances directly interfering with immunological reactions

Many drugs but also environmental pollutants may cause adverse reactions in susceptible individuals that are reminiscent of autoimmune syndromes (Pieters, 2000; Pieters et al., 2001; 2003). Reactive chemicals or metabolites may provoke formation or release of immunosensitizing neo-antigens (a.o. hapten-carrier complexes or cryptic epitopes). Indeed, reactive chemicals, such as tetrachlorobenzoquinone, the reactive metabolite of hexachlorobenzene, can alter endogenous macromolecules through covalent or non-covalent binding, resulting in the formation of a novel antigen in which the chemical functions as hapten, leading to autoimmune reactions. Reactive chemicals can also alter the structure of an endogenous macromolecule, such that epitopes, that were previously hidden, become exposed (Ezendam et al., 2003). In addition reactive chemicals but also certain inert chemicals may trigger macrophages and other inflammatory cells to release proinflammatory products that, via elicitation of costimulatory help, support hapten- or neo-antigen-specific T cell activation. In addition, chemicals may influence immunoregulatory processes and modulate for instance the balance between type 1 and type 2 responses (Pieters, 2003).

Examples of pollutants that can induce autoimmune diseases are trichloroethylene (Cooper et al., 2009), hexachlorobenzene (Ezendam et al., 2003) and heavy metals (Chen et al., 2002).

9. Cancer

Cancer is a disease due to the clonal proliferation starting in a single cell due to a disturbance of the control of cell division. Cancer is a disease of the social organization of cells in tissues, and cancer cells divide when they should not, and move when they should not. During this clonal proliferation a process called tumor progression occurs and cancer cells acquire the capacity to invade surrounding tissues and to metastasize to other organs. Clonal proliferation and tumor progression can be stimulated by tumor promotors (see also paragraph 3 in annex 3). Mutations, and genetic instability leading to further mutations form the basis of carcinogenesis, tumor progression and resistance to therapy (Alberts et al.,1994).

Under age 75 cancer (all cancers together) is the most frequent cause of mortality in the western world, before cardiovascular diseases (Belpomme et al., 2007; Clapp et al., 2006).

9.1. A small increase in mutation rate leads to important increase in cancer risk

It is known since the beginning of the 1970's that cancer rests fundamentally on the accumulation of several mutations in the same cell, most often in a stem cell. The number of mutations necessary to the malignant tumoral transformation varies in function of the type of tumor (Renan, 1993; Alberts et al.,1994), but is generally between 3 (leukemia's) and 7 (carcinoma's). The fact that several mutations (in the same cell) are needed is in agreement with the increase of the risk with age (often with the 3rd, 4th or 5th power of age) and is the main line of defense against carcinogenesis, as the probability of accumulating several mutations in the same cell is very low. An important implication of the fact that multiple mutations in the same cell are a necessary condition for the malignant tumoral transformation is that the chance of this transformation occurring increases exponentially with the mutation rate. In a model where this transformation would depend on 6 mutations, a doubling of the mutation rate would lead to an increase of the likelihood of malignant tumoral transformation with a factor 64. So the important message is: a small increase in mutation rate already leads to an important increase in risk of cancer.

9.2. Carcinogenesis also rests on changes in gene expression

Not only mutations, but also changes in gene expression can contribute to carcinogenesis. The important impact of tumor promotion and of receptor binding and otherwise endocrine disrupting agents rests mainly on changes in gene expression.

As well genotoxic carcinogens (carcinogens acting primarily through causation of mutations) (Godderis et al., 2012) as non-genotoxic carcinogens (acting primarily through effects on gene expression) (Van Delft et al., 2004) affect expression of genes in human cells, but in different ways (Van Delft et al., 2004).

Prenatal low intensity exposures to bisphenol A, induced in the mammary gland tubes of mice, changes the expression of genes that can contribute to carcinogenesis (Vandenberg, 2008; Wadia et al., 2013; Wang, 2014). Diethylstilbestrol induces the precancerous condition vaginal adenosis by disrupting SMAD/RUNX1-mediated cell fate decision in the Müllerian duct epithelium through a downregulation of the RUNX1 gene (Laronda et al., 2013). Bisphenol A affected the gene expression in human prostate stem cells and stimulated their proliferation (Ho et al., 2015; Calderon-Gierszal & Prins, 2015). This is particularly relevant because lifetime cancer risk is strongly correlated with the total number of divisions of the stem cells (Tomasetti & Vogelstein, 2015).

9.3. Endocrine disrupting agents and cancer

Endocrine disrupting agents are substances that disrupt hormonal and homeostatic systems. They act through nuclear receptors, non-nuclear steroid receptors, non-steroid receptors (for instance receptors for neurotransmitters, "orphan" receptors such as the AhR), and through interference with enzymatic reactions related to the biosynthesis or metabolism of endogenous hormones. The most .important endocrine disrupting substances are xenoestrogens, antiestrogens, antiandrogens and substances disrupting thyroid function and metabolism (De Coster & van Larebeke, 2012). Endocrine disruptors can have widely different chemical structures and comprise substances used as industrial liquids, plastic components, pesticides, medical drugs, pollutants arising from combustion processes and heavy metals

such as cadmium and lead (De Coster & van Larebeke, 2012). There is substantial evidence indicating the importance of endocrine disruption in the causation of breast cancer, uterine cancer, ovarian cancer, cancer of the vagina, prostate cancer and testicular cancer (Gore et al., 2015).

9.4. Risk of cancer increases strongly with duration of exposure

That cancer is a disease affecting mainly older people is evident. The fundamental reason for this is that cancer rests on the accumulation of different mutations in the same cell, and this accumulation increases with time (Alberts et al., 1994). But through an experiment on a very large number of rats (4,080) Peto et al. (1991a, 1991b) could demonstrate that duration of exposure in itself, independent of age, is more important than intensity of exposure. Lung cancer risks depend far more strongly on the duration than on the daily dose-rate of cigarette smoking (Peto, 1986; Flanders et al., 2003). For example, a three-fold increase in the daily dose-rate may produce only about a three-fold increase in effect, while a three-fold increase in duration might produce about a 100-fold increase in effect (Peto, 1986) This implies that chronic exposures to environmental or life style factors have a more important impact on the risk of cancer than short term accidental exposures to the same dose.

9.5. Epidemiology points to life style and occupational or environmental agents

Epidemiological data indicate that in the vast majority (probably about 80 %) of cases of cancer exogenous factors (life style, environment) play an essential role (Higginson & Muir, 1977; van Larebeke, 1997). Indeed, there are huge differences (generally a factor of 10 or more) in the age standardized incidence of each type of cancer between different geographical area's having good cancer registers. Not only between industrial countries and developing nations, but also between industrial nations. It is highly likely that these important differences cannot be explained by differences in diagnostic capabilities, and for some cancer types the highest incidences are recorded in third world countries.

Studies on migrants indicate that differences between populations inhabiting different geographical area's are not primarily due to genetic factors, as migrants and their descendants adopt, with time, the cancer incidence pattern of the area in which they immigrated (Thomas & Karagas, 1987). Also important changes in cancer incidence in function of time, in the same population, have been described (Devesa et al., 1995; Tominaga, 1995).

Parents of children suffering of cancer do not, themselves, show an increased risk of cancer (Olsen et al.,1995).

The Finish twin study (Verkasalo et al., 1999) and the Swedish family cancer data base (Hemminki & Vaittinen, 1997) both point to a limited impact of inherited genetic factors on the incidence of cancers, also concerning breast cancer. Even for BRCA mutant carriers external factors are important in determining the eventual occurrence of breast cancer. The cumulative incidence at age 50 amounts to 24 % for such women if born before 1940, and to 67 % if born after 1940 (King et al., 2003). In Iceland the cumulative incidence at age 70 in women carrying the BRCA mutation was 18.6 % (95 % CI = 11.0 % to 29.5 %) in 1920 and 71.9 % (95 % CI = 45.9 % to 100 %) in 2002 (Tryggvadottir et al. 2006).

There is substantial epidemiological evidence for the link between air pollution and cancer, mainly lung cancer. The risk of lung cancer is clearly increased by exposure to polluted air (Cislaghi & Nimis, 1997; Raaschou-Nielsen et al.,

2010; Katanoda et al., 2011; Allen et al., 2013; Heinrich et al., 2013). Air pollution, assessed in terms of biological activity by the limitation of lichen diversity, was, for men, clearly associated with mortality due to lung cancer (Cislaghi & Nimis, 1997). According to the Global Burden of Disease study in 2015, all forms of pollution combined were, in 2015, responsible for 43 % of deaths due to lung cancer.

Possibly, as one would expect on mechanistic basis, also the risk of other forms of cancer might be increased by exposure to polluted air, including breast cancer (Crouse et al., 2010), bladder cancer (Liu et al., 2009) and kidney cancer (Soll-Johanning et al., 1998).

A recent prospective cohort study found an increase in the risk of breast cancer in women in association with a more frequent use of beauty or skincare products (Taylor et al., 2018).

A recent prospective cohort study on 94,668 French adults found that a higher frequency of organic food consumption was associated with a reduced overall risk of cancer (hazard ratio for quartile 4 vs quartile 1, 0.75; 95 % CI, 0.63 – 0.88, P for trend = 0.001) (Baudry et al., 2018).

The International Agency for Research on Cancer has published lists of risk factors it considers to be proven, probable or possible human carcinogens. These lists are available on the website of the IARC (http://www.cancer-environnement.fr/478-Classification-des-substances-cancerogenes.ce.aspx). Also there are thousands of publications in the international scientific literature describing associations between risk factors and an increase in the incidence or mortality from cancer.

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Annex 3 – effects that are not sufficiently covered by classical toxicological tests

1. Effects of real life exposures, low dose and non-monotonic effects

1.1. What is meant by "Low dose".

There is by now extensive evidence for the existence of low dose effects based on experimental, and molecular epidemiological studies. Low-dose effects have been defined by the National Toxicology Program as any biological changes 1) occurring in the range of typical human exposures or 2) occurring at doses lower than those typically used in standard testing protocols, i.e. doses below those tested in traditional toxicology assessments (2). Other definitions of low dose include 3) a dose below the lowest dose at which a biological change (or damage) for a specific chemical has been measured in the past, i.e. any dose below the lowest observed effect level or lowest observed adverse effect level (LOAEL), or 4) a dose administered to an animal that produces blood concentrations of that chemical in the range of what has been measured in the general human population (i.e. not exposed occupationally, and often referred to as an environmentally relevant dose because it creates an internal dose relevant to concentrations of the chemical measured in humans) (Vandenberg et al., 2012). Similarly, with regard to genotoxic agents the term low

dose is used to indicate low intensity exposures such as those occurring through diet or through environmental exposures.

1.2. Evidence for low dose effects of endocrine disrupting agents

Physiological hormones have low dose effects typically in the picomolar to nanomolar range (Vandenberg et al., 2012). The free concentrations that actually bring about effects in cells are even lower, for example 0.1–9 pg/ml for estradiol (Vandenberg et al., 2012). The mechanisms responsible for low dose effects of substances binding to receptors are known. At low hormone levels, a 10-fold increase in hormone concentration can have a 9-fold increase in receptor occupancy, whereas at high doses of hormone, a 10-fold increase in hormone concentration produces a less than 1.1-fold increase in receptor occupancy (Welshons et al., 2003). Also, low-dose effects have been observed in man-made chemicals from a number of classes with a wide range of uses including natural and synthetic hormones, insecticides, fungicides, herbicides, plastics, UV protection, and other industrial processes. Furthermore, low-dose effects have been observed in chemicals that target a number of endocrine endpoints including many that act as estrogens and antiandrogens as well as others that affect the metabolism, secretion, or synthesis of a number of hormones (Vandenberg et al., 2012; Vandenberg & Pelch, 2021). For instance, in animals Bisphenol A was shown to have low dose effects on the mammary gland (Markey et al., 2001; 2003; Moral et al., 2008; Murray et al., 2007) and on spermatogenesis (Hass et al., 2016). It should be noted that the study of low dose effects is apparently affected by conflicts of interest: As of December 2004, there were 115 published in vivo studies concerning low-dose effects of BPA. Among government-funded published studies, 94 of 104 (90%) report significant effects at doses of BPA < 50 mg/kg/day. If we consider industry-funded studies, not a single one (0 of 11, or 0%) reports significant effects at the same levels (Vom Saal &Hughes, 2005). By now (March 2022) a very large number of papers in the peer reviewed literature report low dose effect of a series of man-made products (bisphenol derivatives, phthalates, tributyl tin, nonylphenol, perfluorinate substances, insecticides, fungicides, PCBs, dioxin-like substances, organophosphorus flame retardants, organophosphorus pesticides), affecting molecular biological and physiological functions. These include DNA methylation and epigenetic effects, neurodevelopment, behavioral, cognitive and other neurological aspects including transgenerational effects on behavior, processes involved in carcinogenesis, thyroid function, metabolism and regulation of body fat, regulation of the immune system and immune system related diseases, male and female fertility including transgenerational effects on Leydig cells of the testis, fluid and electrolyte homeostasis, the blood-testis barrier). Even antagonistic interactions between EDCs were observed (Shi et al., 2021).

Regarding endocrine disrupting agents, even infinitesimally low levels of exposure - indeed, any level of exposure at all - may cause endocrine or reproductive abnormalities, particularly if exposure occurs during a critical developmental window (Sheehan et al., 1999). Balaguer et al. (2017) describe three mechanisms explaining high-affinity interactions (and so possible low dose effects) between EDCs and nuclear receptors. The mycoestrogen α-zearanol, although structurally different from 17β-estradiol, displays a similar interaction with the ligand binding pocket of the estrogen receptor α. In contrast, organotins such as TBT do not recapitulate any of the specific interactions made by the classical ligands, but use a Sn–S covalent interaction to bind to and modulate the transcriptional activity of the Retinoid X Receptor - peroxisome proliferator-activated receptor (RXR-PPAR) heterodimer at nanomolar concentrations. In the third reported mechanism, a pesticide and a pharmaceutical compound were found to interact with each other in the Pregnane X Receptor Ligand binding pocket, forming a 'supramolecular ligand' that is a more potent activator than either of the two chemicals alone.

1.3. Non-monotonic dose -effect relationships

Also, the existence of non-monotonic dose -effect relationships has by now been proven. EDCs may exert non-traditional-dose-response curves, such as inverted-U or U-shaped curves (vom Saal et al., 2007). Both of these concepts have been known for hormone and neurotransmitter actions, but only in the past decades have they begun to be appreciated for EDCs. Several mechanisms have been identified and studied that demonstrate how hormones and EDCs produce nonmonotonic responses in cells, tissues, and animals. These mechanisms include cytotoxicity, cell and tissue-specific receptors and cofactors, receptor selectivity, receptor down-regulation and desensitization, receptor competition, and endocrine negative feedback loops (Vandenberg et al., 2012; Vandenberg, 2014). Non-monotonic dose-response curves have been observed for many physiological hormones (in cell culture, in animal studies) and also for man-made substances and metals as well (in cell culture, in animal studies. (See tables 6 and 7 in Vandenberg et al., 2012). Interestingly, in animal studies the endpoints affected by non-monotonic dose-response curves range from higher-order events such as the number of viable offspring (which could be due to alterations in the reproductive tissues themselves or the reproductive axis), to behavioral effects, to altered organ weights, and to lower order events such as gene expression. Also, there are several epidemiological studies reporting non-monotonic dose-response curves for natural hormones in humans (see table 8 in Vandenberg et al., 2012). For instance, in older women age-adjusted incidence of coronary events is highest at the extreme quintiles of bioavailable testosterone blood levels (RR 1.79 and 1.96, compared to the third quintile) (Laughlin et al., 2010). Also, among underweight and obese male manual workers in Vienna (Austria), both higher (hypergonadal) and lower (hypogonadal) bioavailable testosterone concentrations were associated with an increased mental depression score (Kratzik et al., 2007). Also, for man-made chemicals, dioxin-like chemicals and metals reporting non-monotonic dose-response curves were reported in human epidemiological studies. Among non-diabetic Americans, adjusted Odds ratio's for quartiles of non-dioxin-like PCBs were 1.1, 1.3, 1.8 and 1.0 (Lee et al., 2007). By now (March 2022) a very large number of papers in the peer reviewed literature report non-monotonic dose response curves for a series of man-made products (including no-dioxin-like PCBs, perfluorinated substances; chlordecone, an organochlorine insecticide; Bisphenols A, F and S; the phthalate DEHP; the anticonceptive ethinyl estradiol, propylparaben, the herbicide glyphosate, the anti-androgen cyproterone acetate; tranilast, genistein-phthalate mixtures, triclosan, inorganic arsene, dioxins, glyphosate, resveratrol, permethrin, chlorothalonil, nitrate, butylparabenbutylated hydroxyanisole, butylated hydroxytoluene, ,propyl gallate), affecting molecular biological and (patho)-physiological functions. These include the metabolic syndrome, female fertility, male fertility, thyroid and sex-steroid hormones, protein synthesis and energy metabolism, the blood-testis barrier, DNA methylation, mammary gland alterations, bone metabolism and structure, locomotor behavior, androgen receptor agonism, body mass index trajectories over the first 12 years of human life, mitochondrial DNA copy number and telomere shortening, adverse effects in multiple organs, innate immune cells in the rat testes, anxiety-like and exploratory behavior in rats and gene expression changes in the cortical brain, changes in anogenital distance, changes in gene expression involved in oxidative stress and detoxification, interactions with chemotherapy, neurotoxicity, insulin resistance, human breast cancer risk, spindle abnormalities and chromosome misalignment in oocytes.

When nonmonotonic dose-response curves occur, the effects of low doses cannot be predicted by the effects observed at high doses. Although it is still uncertain too which extent these non-monotonic dose-effect curves occur with regard to pollutants or man-made substances, the possibility of their occurrence should be taken into account. Furthermore, endocrine disruption can have opposite effects in function of the developmental stage considered (Parent et al., 2016).

1.4. Low dose effects of genotoxic agents

It is evidently very difficult to collect data on the link between exposures and mutations in human beings. Experiments are ethically unacceptable. The structure, replication and repair of DNA and the interactions of DNA with exogenous agents that can disturb the structure of DNA have, however, been conserved to a high degree throughout the phylogenetic evolution. So, for instance, a given mutagen induces the same primary class of base pair changes in the Ames test strain Salmonella TA100 and in the bacterium E. coli as in mammalian cells in vitro, in rodents in vivo and in the p53 tumor suppressor gene in human cancers associated with exposure to the same mutagen (DeMarini, 2000).

For genotoxic agents there is no critical threshold under which there is no mutagenic effect at all. Generally an approximately linear relation is observed between the dose of a genotoxic substance and the amount of DNA adducts (Phillips et al, 1988; Lutz, 1990). For the induction of double strand breaks by X-rays a strictly linear dose effect relation has been observed between 100 gray (Gy) and 1 mGy (Rothkam & Lobrich, 2003). According to the International Commission for Protection against Environmental Mutagens and Carcinogens (Ehrenberg et al., 1983) and according to Lutz (1990) a linear relation is also generally observed between the dose of a mutagenic chemical and the number of induced mutations (for low doses and for most, but not for all, chemicals, see also Ehrenberg et al.,1996).

In some cases higher doses can have a proportionally larger effect than low doses, especially when the agent in question has, on itself, two different but synergistically acting effects, such as a mutagenic effect and a cell division stimulating effect, or when the dose is very high so that the DNA repair mechanism cannot cope any more (Lutz, 1990).

However, for ionizing radiation very low exposures could be, also in human beings, relatively more mutagenic (per unit of dose) than more intensive exposures (Simonsson et al.,2008; Vandevoorde et al., 2015). This relative increase in mutagenicity at very low doses is however not generally accepted as there are few studies, and a potential bias is explored in a recent publication (Harbron et al., 2017). A relative increase in mutagenicity at very low doses could occur when an exposure occurs in the absence of full expression of the corresponding DNA repair mechanisms. Induction, by environmental carcinogens such as benzo(a)pyrene and by anti-cancer drugs, of the nucleotide excision repair (NER) system, repairing the DNA damage of most environmental and man-made carcinogens, has been observed (Christmann & Kaina, 2013). So it seems likely that low dose hypersensitivity could also occur in relation to exposure to genotoxic chemicals. Also non-targeted effects, effects shown by cells who did not receive a direct hit, including bystander effects (probably mediated through intercellular communication) and induced genetic instability (through the activation of endogenic mutagenic mechanisms) could contribute to larger mutagenic effects per unit of dose at very low doses (Kadhim et al., 2013).

There is a convincing amount of evidence indicating that internal exposures to a series of substances, as occurring in the general population, are associated with mutagenic or genotoxic effects (Bolognesi, 2003; Farmer et al., 2003; Perera & Vineis, 2011; DeMarini, 2013). In the Flemish human biomonitoring on the general population, internal exposure to metabolites of benzene, toluene and phthalates, internal exposure to cadmium, lead, chromium, arsenic, thallium, dichlorophenol, dioxin-like substances and perfluorooctanoic acid were associated with genotoxic effects (van Larebeke et al., 2004; Koppen et al., 2007; De Coster et al., 2008; Franken et al., 2017; De Craemer et al., 2016). In the New Generis study on neonates, transplacental exposure to oxidative fat metabolites, dioxins and PCBs

was associated with mutagenic effects (Kleinjans et al., 2015). In the Environage study on 463 Flemish mother-neonate pairs, prenatal exposure to particulate air pollution with median PM2.5 and black carbon levels of respectively 13.61 µg/m3 (far below the European Air Quality standard of 25 µg/m3) and 0.90 µg/m3 was associated with significant increases in the placenta in mutation rate, methylation of DNA repair genes and methylation of the p53 tumor suppressor gene. Alu mutation rate was associated with greater exposure to PM 2.5 ($r=0.26$, $p<0.0001$) and black carbon ($r=0.33$, $p<0.0001$). (Neven et al., 2018). The Flemish biomonitoring studies suggested that persons with more unfavorable genetic traits concerning genotoxic agents have less chance of surviving until age 50 - 65, probably because they are at a higher risk of morbidity and mortality from chronic diseases (Ketelslegers et al., 2011).

1.5. Effects on gene expression of real life exposures

The Flemish biomonitoring produced a substantial amount of evidence indicating that internal exposures occurring in the general population can be associated with changes in gene expression that could be relevant in terms of risk of cancer. Among a random sample of Flemish adults an association was observed between the expression of a number of genes related to carcinogenesis and internal exposure to pollutants (Van Leeuwen et al., 2008). Internal exposure to pollutants showed an association with tumor-associated protein levels in adults: positive exposure-effect relationships were found for carcinoembryonic antigen (urinary cadmium, t,t'- muconic acid, 1-hydroxypyrene, blood lead, serum levels of p,p'-DDE above the p90), prostate specific antigen above p90 (urinary cadmium), values of p53 above the p90 (higher serum levels of p,p'-DDE, hexachlorobenzene and marker (De Coster et al., 2008). Among Flemish adults De Coster et al. (2013) found significant changes in the expression of a series of genes in association with cadmium, lead, PCBs, dioxin, hexachlorobenzene, p,p'-DDE, benzene, and polycyclic aromatic hydrocarbons. Among Flemish adolescents Croes et al. (2014) observed associations between internal exposure to mercury and a series of genes some of which are linked to the functioning of the nervous system and/or cancer. Among 134 Flemish adults aged 50 - 65, substantial associations, in persons carrying certain genetic polymorphisms, between combined internal exposure to carcinogenic substances (cadmium, lead, polychlorinated biphenyls, p,p′-dichlorodiphenyldichloroethylene, hexachlorobenzene and 1-OH-pyrene) and changes in expression of genes which are known to have a direct link with carcinogenesis were found (Espin-Perez et al., 2015). In Flemish middle-aged men and women sex-specific associations were observed between particulate matter exposure and the expression of genes, some of which featured in pathways related to carcinogenesis such as cell-cell communication, signaling by Type 1 Insulin-like Growth Factor, Insulin receptor signaling cascade, packaging of telomere ends and telomere maintenance (Vrijens et al., 2017).

In the context of the Norwegian BraMat cohort, internal exposure in utero to as well genotoxic as non-genotoxic carcinogens affected expression of genes relevant for carcinogenesis (Hochstenbach et al., 2012).

Ember et al. (2002) proposed that measuring the expression of oncogenes and of oncosuppressor genes is a proper and early molecular epidemiological biomarker of carcinogen exposure and a tool for risk assessment. Measurement of the expression of such genes could also contribute to the development of a more personalised treatment of cancer (Duffy et al., 2016; Kamel & Al-Almoudi, 2017; Yang & West, 2018).

2. Exposures in early life are of critical importance

Past findings and derived concepts indicate that several adult diseases represent late onset consequences of early exposures (Herbst et al., 1971; Skakkebaek et al.,2001; Kleinjans et al., 2015; Martens et al., 2016). Early exposures to EDCs can have huge impact on development and on the risk of diseases such as adult reproductive failure, cancer, obesity, diabetes and metabolic syndrome, and neurodevelopmental disorders among others (Gore et al., 2015). Fetal exposure to dietary carcinogens seems to induce molecular events that indicate increased cancer risks together with other adverse health effects such as reduced birth weight and head circumference (Kleinjans et al., 2015). Childhood cancer, in particular leukemia among boys, can be causally related to the maternal dietary intake of carcinogenic substances during pregnancy (Kleinjans et al., 2015). Fetal exposure to mutagens such as polycyclic aromatic hydrocarbons also increases the risk of cancer and neurodevelopmental disorders (Perera et al., 2011). Telomeres, markers of biological ageing are highly variable at birth and it has been identified recently that maternal exposures to air pollution is associated with telomere length of the next generation (Martens et al., 2017).

Besides these examples of early disorganization of health for the rest of life, fetal life is also a critical period due to occurrence of unique processes such as brain development. As an example, disruption of thyroid hormone promotion of brain development during fetal and early postnatal life has detrimental consequences on lifelong intellectual abilities (Bellanger et al., 2015).

3. Complex mixtures and synergistic effects

Exposure to combinations of chemicals is the dominant way of exposure in everyday life. More than 300 chemicals have been measured in cord blood samples at birth (Woodruff et al., 2011; Rosofsky et al., 2017; Koppen et al., 2009). It is not known how these chemicals interact and at what exposure levels these combinations may cause biological effects that pose health risks. A major problem in relation to the effect of combined exposures, is the possibility of synergistic interactions between substances with different modes of actions.

That man-made chemicals and pollutants can have synergistic effects through the activation of nuclear receptors has by now been proven. Ligands of the RXR receptor and ligands of the partner receptors (which form active heterodimers with the RXR receptor) can act synergistically to activate heterodimers (Germain et al., 2002). This regulatory control of nuclear signaling pathways by multiple RXR heterodimers allows environmental RXR ligands to potentially trigger a multitude of adverse effects on human health (Balaguer et al., 2017). Delfosse et al. (2015) recently demonstrated that a pharmaceutical estrogen (the contraceptive 17α-ethinylestradiol EE2) and a persistent organochlorine pesticide (trans-nonachlor (TNC)), both exhibiting low efficacy when studied separately, cooperatively bind to the Pregnane X Receptor (PXR), leading to synergistic activation. Both biophysical and cell-based analyses showed that each ligand enhances the binding affinity of the other one, so the binary mixture binds 100-fold more avidly to PXR than TNC and EE2 alone, and induces a substantial biological response at doses at which each chemical individually is inactive (Balaguer et al., 2017). This study provided the first detailed mechanistic explanation and a proof of concept for the synergistic action of a mixture (cocktail) of compounds via their simultaneous interaction with a nuclear receptor (Balaguer et al., 2017).

A major problem in relation to the effect of combined exposures is the possibility of synergistic interactions between substances with different modes of actions. An example of this, important in carcinogenesis, is tumor promotion, an important topic in the early research on carcinogenesis, abundantly studied through in vivo experiments (Slaga,

1983). Tumor promotors such as the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) have a very strong synergetic effect on carcinogenesis when given after an initiating (genotoxic) carcinogen. Exposure to tumor promotors leads to the fact that even a low dose of a carcinogen can induce cancers (Burns et al., 1983; Ehrenberg et al., 1996). Dioxins and some other substances binding on the AhR receptor probably have a tumor promoting activity. Tumor promotion might well be responsible for the human cancer risk in association with exposure to dioxins and dioxin-like substances, as these might act on cells already initiated for carcinogenesis by endogenous or environmental mutagens (McGregor et al. 1998; Schwarz and Appel, 2005; Van Larebeke et al., 2015).

It is not known whether synergistic interactions occur indeed frequently outside of the tumor promotion phenomenon. For instance, as to PFAS mixtures, additivity seems to describe their joint effects (Mumtaz et al., 2021; Dale et al., 2022). However, the fact that synergistic interactions can occur implies that this possibility should be taken into account.

4. Epigenetic and transgenerational effects

Gene expression is not only regulated by transcription factors, but is also influenced, in a longer term, by epigenetic changes including methylation of cytosine residues on DNA, post-translational modification of histones, nucleosome remodeling by "nucleosome remodeling" ATPases (adenosine triphosphatases) and altered microRNA (micro ribonucleic acid) expression. Epigenetic changes can lead to transgenerational effects (Gore et al., 2015).

Much direct experimental evidence now shows that disruption of epigenetic processes by chemicals is a carcinogenic mode of action that leads to altered gene functions playing causal roles in cancer initiation and progression (Parfett & Desaulniers, 2017). Four causal mechanisms participating in pathways to persistent epigenetic gene silencing (of tumor suppressor genes) were considered: covalent histone modification, nucleosome remodeling, non-coding RNA interaction and DNA methylation. Within these four interacting mechanisms, 25 epigenetic toxicity pathway components (SET1, MLL1, KDM5, G9A, SUV39H1, SETDB1, EZH2, JMJD3, CBX7, CBX8, BMI, SUZ12, HP1, MPP8, DNMT1, DNMT3A, DNMT3B, TET1, MeCP2, SETDB2, BAZ2A, UHRF1, CTCF, HOTAIR and ANRIL) were found to have experimental evidence showing that functional perturbations played "driver" roles in human cellular transformation (Parfett & Desaulniers, 2017). A systematic review by Dik et al. (2012) found changes in histone modifications and hence gene expression in association with exposure to xenobiotic stressors, mainly heavy metals. For several environmental exposures including metals (cadmium, arsenic, nickel, chromium, and methylmercury), peroxisome proliferators (trichloroethylene, dichloroacetic acid, and trichloroacetic acid (TCA)), air pollutants (particulate matter, black carbon, and benzene), and endocrine-disrupting/reproductive toxicants (diethylstilbestrol, bisphenol A, persistent organic pollutants, and dioxin), it has been proved that chemicals can alter epigenetic marks, and that the same or similar epigenetic alterations can be found in patients with the disease of concern or in diseased tissues (Baccarelli & Bollati, 2009). Baccarelli et al (2009) found decreased repeated-element methylation after exposure to traffic particles. In a study on 78 gas station attendants, 77 traffic police officers, and 58 unexposed referents in Milan, hypermethylation in tumor suppressor p15 and hypomethylation in Melanoma-associated Antigen 1 MAGE-1 genes were associated with increasing airborne benzene levels (Bollati et al., 2007). In this study altered DNA methylation, reproducing the aberrant epigenetic patterns found in malignant cells, was linked to low-level carcinogen exposure (Bollati et al., 2007).

In a Swedish study children had an increased risk of asthma in the first 6 years of life if their grandmothers smoked during early pregnancy, independent of maternal smoking. Importantly, this exhibited a exposure-response

relationship and was associated with a persistent childhood asthma phenotype. These findings support possible epigenetic transmission of risk from environmental exposures in previous generations (Lodge et al., 2018).

5. Ligand-specific effects

The superfamily of nuclear receptors (NR) is a group of 48 ligand-activated transcription factors that play important roles in metabolism, homeostasis, reproduction and normal development. They are additionally often linked to pathologies such as neurodegenerative and metabolic diseases, inflammation and cancer (Lee et al., 2008; Skerrett et al., 2014; Schulman et al., 2010; Balaguer et al., 2017; Dhiman et al., 2018; Sala & Ampe, 2018). Recently, there is growing evidence supporting the involvement of multiple nuclear receptors other than the estrogen and progesterone receptors, in the regulation of various processes important to the initiation and progression of breast cancer (Doan et al., 2017). Nuclear receptors have evolved throughout the fylogenetic evolution as proteins specially selected for binding to DNA. By binding a ligand they acquire, after additional association with co-activators or corepressors, the capacity to bind to specific DNA sequences (Alberts et al., 1994). But the binding of the ligand is not the only interaction that determines the genomic action of nuclear receptors, coregulators, which are either coactivators or corepressors, also play a role (Dasgupta et al., 2014). The ligand can also intervene in determining which coregulators are bound to the receptor (Li et al., 2018).

Quite recently it has been shown that the type of the ligand (thus the detailed chemical structure of the ligand) is determining to which DNA sequences the ligand-bound receptor binds. For instance, the participation of alternative xenobiotic responsive elements (XREs) (specific DNA sequences) in the AhR transcriptional response suggests that the binding of a particular ligand might adapt the structure of the AhR to permit binding to a particular XRE sequence (Guyot et al., 2013). The model hypothesizes that the AhR-mediated transcriptional response is modulated by selective ligands of the receptor (Guyot et al., 2013), in accord with the selective AhR modulator (SAhRM) concept that was initially described by Safe and McDougal (2002). Selective modulation of sex hormone receptors has been studied for some time (Cappelletti et al., 2003; Shanle & Xu, 2011).

Ligand specific effects could be exploited for therapeutic aims, for instance in the development of hormone replacement therapy without carcinogenic side effects (breast cancer) (Diamanti-Kandarakis et al., 2003). Carbidopa, a drug used for treating Parkinson's disease, is also a SAhRM and inhibits pancreatic cancer cell and tumor growth (Safe, 2017).

However, ligand specific effects were also described for xenoestrogens (Routledge et al., 2000; Watanabe et al., 2003; Shanle & Xu, 2011). Bisphenol AF and bisphenol S, used as replacements for bisphenol A, have also agonistic activity for estrogen receptors. However, bisphenol A, bisphenol AF and bisphenol S differentially recruit coregulators and so have different biological effects (Li et al., 2018).

So it seems likely that the many chemicals that bind to receptors with transcription factor functions might have effects that differ from the effects of the physiological ligands and hormones (for example see Zhang et al.,2022). These effects cannot easily be predicted and might lead to adverse health effects.

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CONFLICT OF INTEREST

No member has declared any conflict of interest.

RAPPORTEUR(S)

The Scientific Committee REACH thanks the rapporteurs Lode Godderis, Nicolas Van Larebeke and Willy Baeyens.

ADOPTION OF THE ADVICE

The Scientific Committee REACH advice was adopted by consensus on 16/05/2023.

LEGAL FRAMEWORK OF THE ADVICE

Cooperation agreement of 17 October 2011 between the Federal State, the Flemish Region, the Walloon Region and the Brussels Capital Region concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

Ministerial decree of 8 July 2014 appointing the members of the Scientific Committee REACH established under Article 3, § 3 of the Cooperation Agreement of 17 October 2011 between the Federal State, the Flemish Region, the Walloon Region and the Brussels Capital Region concerning the Registration, Evaluation, Authorisation and restriction of Chemicals (REACH)

Ministerial decree of 2 June 2016 on dismissal and appointment of members of the Scientific Committee REACH

Ministerial decree of 5 October 2016 on appointment of members of the Scientific Committee REACH

DISCLAIMER

The Scientific Committee REACH reserves, at any time, the right to change this advice when new information and data become available after the publication of this version.

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