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An Interdisciplinary Approach to Doctoral Education Building Toxic Free Europe: Examining Implications of Phthalates

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PHTHALATES AND THEIR METABOLITES IN THE IRISH ENVIRONMENT – A WASTEWATER EPIDEMIOLOGY APPROACH

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ABSTRACT:

Phthalate esters are a group of synthetic organic chemicals commonly used as plasticizers in polyvinyl chloride (PVC) and other materials. Over 213M kg of phthalates are produced globally each year with end uses including cosmetics and personal care products, food packaging, flooring, paints, tubing and medical devices. Due to their high production volume and continuous release, phthalates are emerging contaminants ubiquitous in the environment. Research has shown that the widespread exposure to these chemicals has been associated with numerous adverse health effects including impaired reproductive health in males, decreased neurological development in children, cancer and obesity.
This project aims to identify the levels of phthalate diesters in various matrices in the Irish environment, and constitutes the first application of wastewater epidemiology to determine phthalate exposure in an Irish population. Phthalates and phthalate biomarkers are analysed in influent wastewater at wastewater treatment plants to assess phthalate exposure through mathematical modelling. A meta-analysis on health risk data serves to relate the level of exposure to an associated risk, providing the first step in phthalate risk assessment within the Irish environment. Results will inform on the feasibility of using wastewater biomarkers for future compliance monitoring. Metabolites from the following phthalates are considered for investigation in wastewater: benzylbutylphthalate, dibutylphthalate, diethylhexylphthalate, diisobutylphthalate, di-n-octylphthalate and diisononylphthalate.

KEYWORDS: Phthalate metabolites, LC-MS, sewage, sludge, wastewater epidemiology

INTRODUCTION

Phthalates are synthetic organic compounds, commonly used in plastic and particularly PVC products, with a wide range of end uses including food packaging, cosmetics and personal care products, medical devices, tubing and flooring. Due to the extensive presence and environmental persistence of phthalates, their effects on health have been frequently studied. It has been found that phthalates act as endocrine disruptors leading to a range of adverse effects including hypospadias, reduced anogenital distance, cryptorchidism, impaired neurological development in children and precocious puberty, with children and women at the highest risk of exposure (Kay et al., 2014; Ejaredar et al., 2015; Goodman et al., 2014).

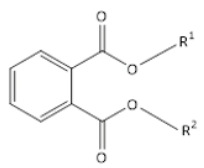
Due to these findings, certain phthalates have been legislated for including; Benzylbutylphthalate (BBP), Dibutylphthalate (DBP), Diethylhexylphthalate (DEHP), Di-n-octylphthalate (DNOP) and Diisodecylphthalate (DIDP). However, research is far from complete and with new replacement phthalates having being introduced it is vital that the health effects of these compounds are also assessed (CHAP Report, 2014).

The ubiquitous nature of phthalates in the environment raises a valid concern for their effects on human health. Phthalates are colourless, odourless compounds that are liquid at room temperature. They are added to give a product flexibility and resilience due to their fluidity, stability, and low volatility. Phthalates are heavily used throughout PVC manufacturing with soft PVC containing up to 40% DEHP (Koch et al., 2006). The most common exposure of phthalates in humans identified in the literature is through food consumption (at least 67% of total exposure), but drinking water, air, dermal contact, and cosmetics all contribute to total exposure (Das et al., 2014). This project aims to investigate a number of matrices including influent from WWTPs, dry recyclables, drinking water sources and municipal solid wastes to define a link between the exposure roots of phthalates and their resulting impact on human health.

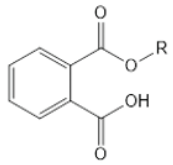
Phthalates including DBP, BBP, and DEHP have been banned or limited in manufacturing (in particular for items such as children's toys) and as a result these have typically been the most widely studied phthalates. Due to this legislation a number of higher molecular weight plasticizers were

introduced as substitutes to reduce leaching from plastics. However, new research may indicate that these substitute plasticizers have an equally negative impact on human health and this warrants further study. Current phthalate research is lacking in a comprehensive sample range of phthalates and there has yet to be conclusive evidence of phthalates' impact on human health. This research is timely as legislation on Endocrine Disrupting Chemicals (EDCs) is increasing (U.S. Environmental Protection Agency, 2014; European Commission, 2006). As there is relatively little information on the human health impacts of phthalates and the extent of phthalate contamination in Ireland, this information is pertinent.

Humans readily metabolise phthalates, generally excreting the phthalate as a number of phthalate metabolites within 1-2 days (Anderson et al., 2011) (see Figure 1). This metabolism occurs in at least two steps; hydrolysis (Phase 1) and conjugation (Phase 2) (see Figure 2). The simpler forms of phthalates such as DEP and DBP are usually excreted as their corresponding phthalate mono-ester, whereas highly branched phthalates undergo more extensive biological transformations (Saravanabhavan et al., 2012). These phthalate metabolites (or biomarkers) can be monitored to infer a subject's phthalate body burden. Knowledge of the metabolism of phthalates will be important for consideration in identifying biomarkers for phthalate body burden as only a fraction of the phthalates are excreted in their unconjugated forms (Frederiksen et al., 2007). Using the metabolites can therefore give us a more accurate representation of the direct effects on humans. A large quantity of the research looks at monoesters due to the ease of analysis and the evidence it yields on human consumption. Although further oxidative metabolites may give more accurate information on the human consumption of phthalates, these more highly conjugated metabolite standards are very difficult to source (Koch et al., 2006).

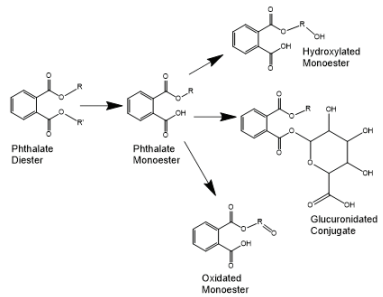


Phthalate



Phthalate Metabolite

Figure 1: Phthalate Metabolic Pathway



It has been determined that phthalates can produce a "cocktail effect" and can have additive effects. When a subject was exposed to a mixture of phthalates, the resulting effect was stronger than if exposure was restricted to the most potent component (CHAP Report, 2014). Most studies involve focusing on the effects of isolated phthalates. This project will monitor a diverse range of phthalates which aims to give a greater understanding of how these can influence human health. The physical properties of the phthalate and its metabolite will influence how it affects human health and how it is monitored.

Previous studies on phthalates in the environment have focused solely on either analytical methods or on human health effects. This project will aim to offer a holistic view of phthalates to monitor the pathways of exposure, the extent of contamination and link this to health impacts from source to sink.

RESEARCH METHOD

Wastewater epidemiology is an emerging method of gaining insight on human health and behaviour at a population level. This method utilizes human excretion products (metabolites/biomarkers) of certain compounds as they enter the wastewater system. Analysing parent compounds (in this case phthalate di-esters) to examine a population's exposure to phthalates is ineffective due to phthalates' extensive metabolism and the large quantity of phthalates that enter the sewage system through industrial disposal. Wastewater based epidemiology was first used in 2005 as a means of assessing cocaine use in Italy (Zuccato et al., 2005). Since then it has grown to monitor a wide range of biomarkers

for multiple xenobiotics, infectious agents, stress and cancer (Bicchi et al., 2009; Ryu et al., 2016; Yang et al., 2015; Bisseux et al., 2018). If the biomarker is considered stable in wastewater then the calculated level can be attributed to human exposure. Phthalates will be examined as a candidate for wastewater based epidemiology in this research. Human exposure to phthalates is unavoidable and as small study sizes are labour intensive and cannot capture the exposure of the general public, wastewater epidemiology is an attractive method of analysis as it provides a cost-effective and unbiased means of determining human phthalate body-burden.

The biomarkers used for phthalate exposure are their mono-ester metabolites (Frederiksen et al., 2007). Attribution of phthalate body-burden in a population is calculated through multiplying the measured phthalate mono-ester metabolite concentrations in wastewater by the daily flow rates of that Wastewater Treatment Plant (WWTP) to find daily sewer loads. From this value, the total consumption of the phthalate is estimated by applying a specific correction factor, which considers the average excretion rate of a given phthalate and the molecular mass ratio of the parent phthalate di-ester to its mono-ester metabolite. Finally, the daily consumption can be found by dividing these daily values by the number of people served by the WWTP, (Equation 4). This value is expressed per thousand population (Zuccato et al., 2008).

$$Correction\ Factor = \left(\frac{MW\ diester}{\%\ Excreted\ as\ Monoester} \times \frac{1}{MW\ Monoester}\right)$$

$$Estimated\ Daily\ Intake = \frac{Concentration \times Flow\ Rate \times Correction\ Factor}{Population} \qquad Equation\ 4$$

This current model for the analysis of down-the-drain chemicals is very basic and does not account for the in-transit and in-sewer transformations that can occur with unstable metabolite compounds. Down-the-drain metabolites can be lost through degradation or formed through enzymatic formation from their parent compounds in transit to the WWTP. At the treatment plant, there is an even greater likelihood of these transitions occurring due to the wastewater treatment process. (Figure 3). Although many studies examine the removal and stability of phthalate di-esters in the wastewater treatment plant, very little is known about their mono-

ester metabolites in this environment with only one study to date that investigates phthalate monoesters from a wastewater epidemiology perspective (Gonzalez-Marino et al., 2017). There are significant gaps of knowledge in the rate of enzymatic formation or removal of phthalate mono-esters within the wastewater treatment plant, these variables have also not been accounted for with metabolites of other compounds. Any risk-based predictions determined through this method show an unknown degree of variation as a consequence.

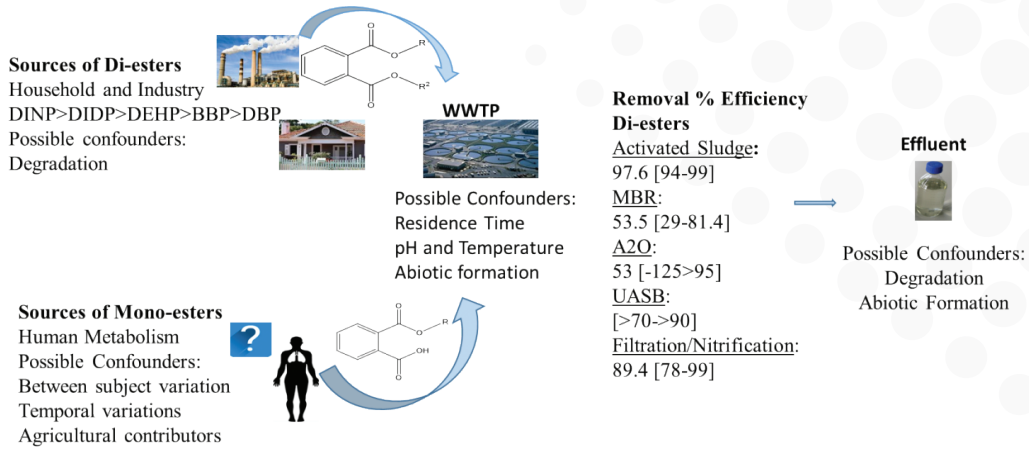


Figure 2: Confounders in Wastewater Epidemiology

To circumvent these degrees of uncertainty two approaches are being taken; analysing influent alone for risk-based decisions and the addition of further control factors to the model. As phthalate mono-ester metabolites will theoretically be transformed at a greater rate in-sewer, biomonitoring data from WWTP effluent may be misleading when compared to wastewater influent (human effluent). This indicates that perhaps the best predictor of a community's exposure to phthalates is the analysis of influent alone. As the current model does not account for confounding factors, the data from influent should be assessed using a new model that accounts for confounding factors like residence time.

The available literature has illustrated that in-sewer transformation is compound-specific and influenced by environmental factors. Some compounds seen in the literature (e.g. MDMA, KET and MDPV) remain stable at neutral pH and temperatures up to 20°C. However, drugs like

THCCOOH, fentanyl, mephedrone and cathinones have higher levels of variability (McCall et al., 2016). In order to compare results between different studies and environments, a standardised method with quality controls/correction factors for stability of compounds in addition to in-transit and in-sewer transformation should be developed. This will allow for a higher degree of accuracy when informing policy.

MAIN RESULTS AND DISCUSSION

Most phthalates were found in all environmental matrices, confirming the ubiquitous nature of phthalate contamination.

Further presentation of results is not possible at this point, as the work is unpublished at this stage. Authors may be contacted by email for discussion.

CONCLUSION

The literature on phthalate health effects in humans remains limited. Validation of LC-MS methods for the determination of phthalate diester and mono-esters for a variety of environmental matrices are completed as part of this research. Data on odds ratio have been collected for the assessment of risk to specific adverse effects and will be applied to the wastewater data collected in Ireland for the final risk assessment. This research is the first application of wastewater epidemiology to determine phthalate exposure in an Irish population; and has made clear the need for improved methodologies in mathematical modelling of transformation of phthalates and their metabolites during transport from household to WWTP, i.e. in-sewer transformation, both biotic and abiotic. Further research urgently needs to address this deficit.

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SYSTEM-INTEGRATION OF LOW-DIMENSIONAL ELECTRICAL DEVICES FOR INTELLIGENT SENSOR PLATFORMS

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ABSTRACT

Electrical devices based on one-dimensional (e.g. nanowires, nanotubes) and two-dimensional (e.g. Graphene and Transition Metal Dichalcogenides) materials have risen to prominence for their unique advantages as efficient electrical transducers. Other than remarkable electrical properties, feasibility of system-integration, and surface adaptations play essential roles towards developing real applications as sensor platforms. In this contribution, we present top-down nanofabrication of nanoscale transducers based on metal, semiconducting nanowires and graphene based platform aiming at overall system-integration of state-of-the-art nanoscale transducers for point-of-care chemo/bioanalytical systems. Going beyond realizing a holistic framework for system-integration, we focus further on realization of advanced functional layers and readout strategies for high throughput multiplexed readout of molecular analytes and monitoring of complex biological processes in natural sample environments.

INTRODUCTION

Efficient detection of substances and monitoring of events is central to the development of intelligent sensor designs that form the basis of data-driven industrial revolution in the coming years[1]. One of the key thrust areas involve creation of environment friendly technologies leaving a minimal impact on the climate and nature. Sensor networks dealing with climate monitoring can accelerate global monitoring of different types of biological and a biological pollutants in water sources and in air – providing real-time information for detail study and decision making

by responsible agencies[2]. Micro and nanoscale technologies are at the forefront dealing with sensor challenges which often require ultra-fast molecular-level detection of a range of substances in a complex media such as fresh water bodies, sea-water and biological media[3]. Developing system-integrated approaches for each these application fields require synergies among active transducer material, development of a functional layer, sample treatment and readout strategies etc. For example: an intelligent sensor platform for monitoring of water quality against plastic pollution in the seas and other water bodies would ideally distinguish a family of phthalate and other plastic pollutants in a highly complex water matrix with thousands of biotic and abiotic substances and require advanced surfaces and interfaces withstanding fouling in natural environments for longer deployment. In addition, such intelligent platforms require standalone capabilities of multiplexed Analog-to-Digital conversion of sensor signals, preliminary data processing and transmission over wireless communication to the network.

Realization of nanoscale field-effect transistors (nanoFETs) out of silicon and other one dimensional materials in the previous decade opened possibilities towards development of system-integrated sensor platforms with multiplexed detection capabilities[4, 5]. Few of such nanoscale sensor platforms were optimized towards multianalyte sensing using various techniques[6, 7]. Examples include integration of multiple sensor spots having different specificities or by analysing dynamic response patterns of a blind sensor towards different analytes. Design of nanomaterials based sensor platforms has become mainstream ever since with their advantages of high surface-to-volume translating into effective receptor-analyte interactions and unique physicochemical properties. Silicon, nanoscale metal electrodes, carbon nanotubes (CNTs) and other semiconductor nanowires are prime examples of one-dimensional nanomaterial for the development of sensor platforms that have been deployed for electrical sensing in chemical and biological media[8]. Use of conventional materials such as metals and silicon for the development of one dimensional sensor-arrays are advantageous for their high-level integration in a standard cleanroom environment.

Availability of sophisticated nanofabrication processes over large areas yielding devices with reproducible sensor characteristics is an essential requirement that benefits integration of intelligent sensor designs.

In this contribution, we present low-dimensional devices based on one dimensional (silicon nanowires, metal nanoelectrode arrays) and two dimensional (graphene based) nanomaterials, that have been system-integrated, in order to realize sensor platforms with highly reproducible sensor characteristics. Top-down nanofabrication of silicon-based nanoscale ion-sensitive field-effect transistors (nanoISFETs) by using 4 inch top-down fabrication approach combining nanoimprint lithography and photolithography processes is presented at first[9]. In the following, we present a similar top-down fabrication approach for the realization of metal-based nanoscale interdigitated electrode arrays as an active electrochemical transducer interface[10]. Finally, a unique nanofabrication strategy for the realization of graphene-based two-dimensional (2D) electronic devices at wafer-scale is presented which combines advantages of top-down lithography approaches with cost-effective chemically sourced graphene oxide (2D) as active transducer material[11].

Basic transducer principles, surface modification, biofunctionalization techniques for the sensor applications of the above mentioned sensor platforms are discussed in brief, while considering the imminent challenges associated with their deployment in real scenario[12]. Typical examples of chemical and biosensing using the 1D and 2D electrical sensors are given demonstrating their analytical advantages. In addition, selection of appropriate readout mechanisms and tools, optimization of sensor surfaces against non-specific interactions with the environment, robust receptor-analyte interactions in terms of specificity, sensor surface regeneration, and chemical/biofouling issues will be discussed. As the nanofabrication and integration technologies mature in the coming years, application oriented development of intelligent point-of-care solutions are expected to become a reality in near future.

RESULTS AND DISCUSSION:

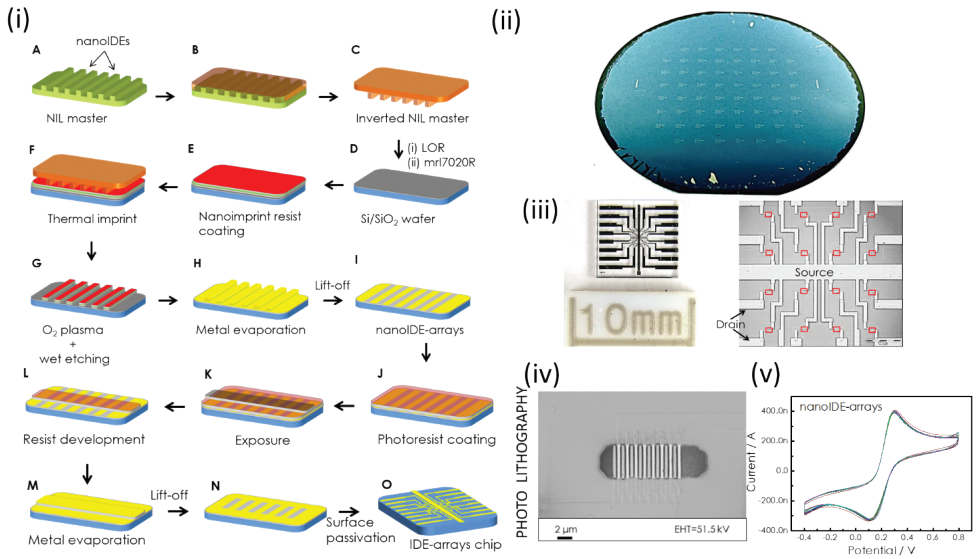

Figure 1: Scaling down electrochemical transducers of metal as nanoscale interdigitated electrode arrays (NEAs), using top-down nanofabrication approach. (i) Nanofabrication process flow using nanoimprint lithography (NIL) starting with realization of a NIL master with desired nanoscale features, transfer of the nanoscale features to an intermediate stamp, and following process steps towards transferring the features onto the desired substrate (4 inch Si/SiO2 wafer). (ii) The NIL process ends with the fabrication of nanoscale features on the silicon wafer as shown in image, (iii) NIL follows with photolithography steps for electrical contacts, where a typical sensor chip is shown having 16 individually addressable sensor-spots on a chip, (iv) scanning electron micrograph showing such NEAs which are also passivated with an oxide layer, reducing the interaction with outer environment only through this localized sensor spot on the chip, (v) electrochemical behaviour of 16 NEAs sensor spots on a typical chip using cyclic voltammetry measurements exhibiting identical sensor characteristics proving a high-quality nanofabrication process.

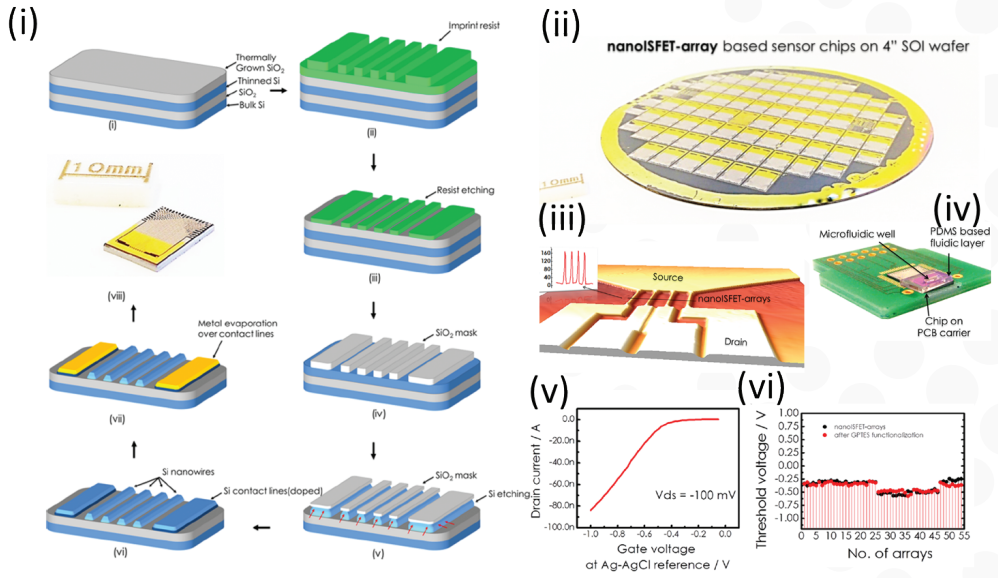

Figure 2: Wafer scale fabrication of ion sensitive field-effect transistors with silicon nanowires down to 100 nm scale. (i) Nanofabrication process flow is illustrated which started with nanoimprint lithography on silicon-on-insulator substrates as 4 inch wafer in order to structure nanowires of silicon, followed with photolithography steps for metallic source-drain contact lines, a typical sensor chip with nanoscale ISFETs is shown in the end of the process-flow, (ii) Photograph of a 4 inch wafer after the complete fabrication process, (iii) atomic force microscopy image of a set of fur silicon nanowires from the sensor chip showing the topography and vertical height, (iv) a fully encapsulated sensor chip after wire-bonded onto a chip career and with a fluidic layer on top for easy handling of liquid sample, (v) typical field-effect curve of a nanowire channel while deployed as an ion-sensitive field-effect transistor in buffer solution, and (vi) demonstration of near-identical sensor behaviour by device-to-device threshold voltage comparison of different nanowires channels over entire wafer.

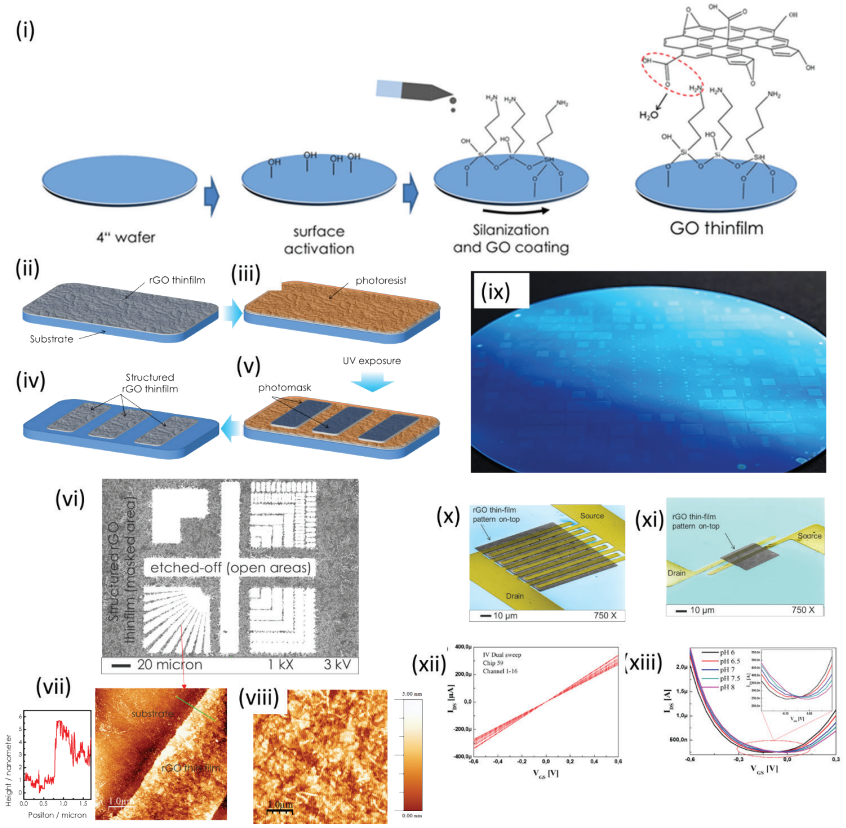

Figure 3: Top-down nanofabrication of two-dimensional devices based on Graphene. (i) Fabrication process starts with the realization of graphene oxide thin-films on glass or silicon wafers in the sequence shown as above where silanization is carried out to create suitable functionalities on the substrate enabling covalent binding of graphene oxide that was chemically produced in newly worked out exfoliation method. Graphene oxide flakes are spin-coated over the surface modified wafers yielding continuous and uniform thin-layers, (ii) graphene oxide films are thermally reduced to form semiconducting reduced graphene oxide or rGO thin-films, (iii-v) rGO films can be readily used in photolithography process and structured into a variety of configurations, (vi-vii) SEM and AFM images show detail surface characterization of test structures produced after the lithography process, (ix) photograph of a wafer with patterned rGO thin-film, (x, xi) SEM images showing electrical devices with interdigitated and single pair source-drain electrodes, (xii-xiii) electrical characteristics of the rGO devices, graphs showing current-voltage measurements taken from 16 devices from a single chip showing near identical resistance, and pH sensing behaviour of a typical rGO in ISFET configuration.

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THE STATE OF KNOWLEDGE ABOUT PHTHALATES AS ENDOCRINE DISRUPTORS

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ABSTRACT

Phthalates are recognized as endocrine disrupting chemicals and could interfere with normal hormonal function. Due to their ubiquitous presence in everyday products, the exposure to phthalates even at low concentration could be related to different health problems. Therefore, phthalates are under special attention among scientists. Up to now more than 3000 scientific papers could be found in PubMed base using keyword “phthalates”. It is suspected that detectable concentrations of phthalate metabolites are related to the disruption of glucose and lipid homeostasis, increased body mass index, atherosclerosis, fertility disorders, hypothyroidism, liver damage, carcinogenicity, etc. Mostly, people are exposed to phthalates via food and drinks that have absorbed the chemicals. However, the absorption through the skin by using cosmetics, personal care products and medical devices is not negligible. In vitro studies demonstrated that besides nuclear receptor, phthalates act as agonist for PPAR alpha and gamma. Also, phthalates behave as full ER alpha and partial ER beta agonist. Still it is difficult to draw a direct line between phthalates and health disorders. People are rarely exposed to one phthalate, and therefore the investigation of exposures to mixtures of phthalates and other environmental pollutants is important to gain a better insight into the associations between environmental exposures and biomarkers. More epidemiological studies conducted on numerous women and men of different ages worldwide are necessary in order to convince authorities to completely restrict their production and usage.

KEYWORDS: Phthalates, Endocrine disruptors; Health disorders.

INTRODUCTION

It is estimated that more than 200 different chemicals could be found in human during randomized biomonitoring after common daily activities. Although for some chemicals, the health risk associated with their levels in blood/urine is clarified, there is still a gap in the knowledge for a wide range of compounds, and the possible adverse effects related to the detected low concentration levels (ppb or even ppt) (Milić et al. 2015).

The Network of Reference Laboratories for Monitoring of Emerging Environmental Pollutants (NORMAN) provides the open access list (available online http://www.norman-network.net) of the most frequently detected substances in the environment. On the list, the chemicals are divided into groups based on the structure and activity. It is believed that of 85 000 known chemicals approximately 1000 are recognized as potential endocrine disrupting chemicals (EDCs). The US Environmental Protection Agency (EPA), defines EDCs as “exogenous agents that interfere with synthesis, secretion, transport, metabolism, binding action or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction and developmental process” (Medic Stojanoska et al. 2017). Until the second millennium the disease risk due to the continuous exposure to EDCs was significantly underestimated. In past, the detection of EDCs in biological and environmental samples presented a real analytical challenge because of their low concentrations and diversity in physico-chemical properties. Thanks to the development of sophisticated instrumental analytical tools, it became possible to detect various types of EDCs in ng/L concentrations. Consequently, since 2005, NORMAN plays a significant role as an independent organization between science and policy in the development of knowledge about EDCs (Milić et al. 2013).

EDCs could be found in most man made products such as pesticides, solvents, ingredients in household products and materials, additives or contaminants in food, pharmaceuticals and industrial ingredients. The continual exponential growth of scientific data about the health problems caused by exposure to EDCs become a subject of growing concerns among stakeholders and even policymakers in developed countries. However, in the developing countries the knowledge about the EDCs impact on public health is limited. EDCs are associated with various reproductive health problems, sex ratio in humans, thyroid related disorders, neurodevelopment in children, hormone related cancers, adrenal disorders in humans and wildlife, disruption of immune function, immune diseases, metabolic disorders and wildlife population sustainability loss (De Toni et al. 2017; Kabir et al. 2015).

Among different EDCs, humans are commonly exposed to phthalates. Phthalates are chemicals that are added to plastics to improve their properties (flexibility, transparency, durability and longevity) (Figure 1). Phthalate esters could be easily released from the food contact materials and therefore the main route of human exposure to phthalates is through the consumption of food and drinks. In human diester phthalates are metabolized to monoester phthalates that are even more biologically active than parent compound. The 'obesogenic' property of phthalate esters is related to the several facts: packaging size and material properties can lead to higher migration; higher temperature and long storage time increases leaching; food chemistry governs migration of chemicals from packaging materials (Benjamin et al. 2017). Some developed countries regulated the tolerable daily intake and maximum level of migration from packaging (dibutylphthalate (DBP,) benzylbutylphthalate (BBP), bis-(2-ethylhexyl) phthalate (DEHP)). However, fragranced cosmetics and personal care products, as well as medical equipment and devices present a significant source of phthalate exposure and thus the intake of phthalates via inhalation and skin present the risk for consumers. No state or global authority regulates the safety of fragrance chemicals such as phthalates. Moreover, no state or global authority regulates which fragrance chemicals should appear in which products.

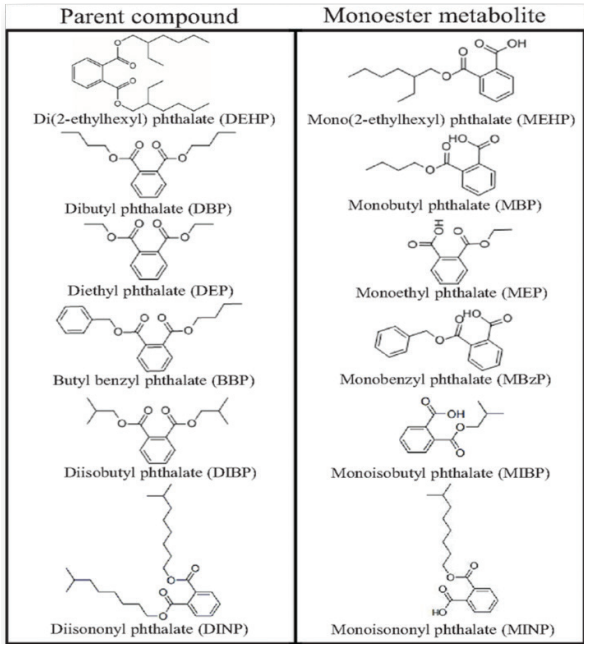

Figure 1. Chemical structures of common phthalates and their monoester metabolites

Based on all, avoiding phthalates altogether is tricky due to the variety of daily products that contain them. The newest evidences about the possible impact of phthalates on human health will be highlighted.

RESEARCH METHOD

The literature was searched using PubMed, ISI Web of Science, Google Scholar and Medline database. Only scientific peer-reviewed papers published on English were used. Besides systematic review papers, the inclusion criteria were original research papers, cross-sectional and case-control studies. The obtained results both on animals and humans were included. After literature search, titles and abstracts were scanned and only full texts of recently published papers were selected for full read. The findings of only recently published papers in eminent journals were included.

MAIN RESULTS AND DISCUSSION

The epidemiological studies conducted on both humans and animals showed that phthalates act as obesogenic and promote Types 2 Diabetes Mellitus (T2DM) and other metabolic disorders. Two big cross-section studies conducted by the National Health and Nutrition Examination Survey found the significant correlation between urinary MBP, MEP and MBzP (Figure 1) levels and insulin resistance index. Additionally, MnBP, MiBP, MCPP and ΣDEHP were significantly associated with glycemia, insulinemia and insulin resistance. Therefore, DEHP, DEP and DBP are labeled as phthalates with the greatest impact on the development of metabolic disorders due to the fact that MEP, MBP, MBzP and MEHP belong to the most frequently detected monoester metabolites in the urine with the frequency rate above 75% (James-Todd et al. 2016; Medic Stojanoska et al. 2017).

In vitro and in vivo studies tried to unravel the mechanism of phthalate interference with hormonal function. Probably, they impair normal endocrine function through mediation of estrogen receptor alpha, beta and gamma (ERα, ERβ and ERγ). However, it is suspected that peroxisome proliferator-activated receptors (PPAR) are crucial in the phthalate governed development of diabetes and increased adipogenesis (Benjamin et al. 2017). In the Table 1 the possible mechanisms of phthalates action are summarized.

Table 1. The possible mechanisms of phthalates action

	PHTHALATE	EFFECT
PPARα	Agonist	Fatty acid uptake and oxidation, gluconeogenesis
PPARγ	Agonist	Adipocyte differentiation and adipogenesis, increased insulin sensitivity
ERα	Agonist	Lipid accumulation and induced differentiation of preadipocytes into adipocytes
ERβ	Partial agonist	ERβ inhibits ERα when coexpressed
ERRγ	No sufficient data	Disturbed energy homeostasis
PXR	Activator of PXR mediated transcription	Obesity and insulin resistance
TR	Antagonist	Insulin resistance and glycemic control disorders
NPY	Up-regulation	Impaired apetite control

Based on the literature data, women and children are more vulnerable groups. The increased exposure to phthalates at young age led to the more pronounced negative effects. Beside the increase in body weight (BW) in newborns and children, phthalates impaired the fat distribution and even presents the risk for the development of the obesity later due to the in utero exposure. Gestational phthalate exposures are associated with the decrease in anogenital distance among male infants, maternal serum concentrations of CRH, testosterone, and thyroid hormones through pregnancy. Pubertal and adult exposure to phthalates could also be related to the testicular toxicity, the prolonged cycles of reproductive hormones, suppressed or delayed ovulation and eventually lead to the formation of smaller pre-ovulatory follicles and decreased oestradiol levels in serum. Recently published investigation on animals suggested that chronic exposure of female mice to the mixture of phthalates and alkylphenols had impact on follicle development, puberty onset, and fertility (Cathey et al. 2019; Repouskou et al. 2019).

Regarding the lipid metabolism in the two recent cross-sectional studies conducted in Serbia on more than hundred adults MEHP was positively correlated with waist circumference while negative correlation was found with HDL. Also based on the statistically significant linear correlation between MEP urinary levels and AST, TG and derived parameters (such as VAI and ration of TG and HDL), the presence of phthalates in the urine increased the risk of cardiometabolic adverse effects and insulin resistance (Medic Stojanoska et al. 2015; Milošević et al. 2017a, 2017b). Although there is a significant body of evidence about the possible health disorders that are associated with phthalates levels, many of them are still in use. Since the early 2000s the European Union has restricted the use of phthalates in consumer products and five phthalates were banned. Also the eight types of phthalates are banned or restricted in certain children's products in the USA. Canada has also banned and restricted the use of some phthalate ester in cosmetics. More epidemiological studies conducted on numerous women and men of different ages worldwide are necessary in order to convince authorities to completely restrict their production and usage.

CONSLUSIONS

It is difficult to assert direct linkage between phthalate exposures and health disorders that may occur years or even decades after continuous intake of low concentration. People are rarely exposed to only one phthalate but until now the safety and regulation of chemicals are on the individual basis. Still it is unclear if EDCs effects are additive and/or do EDCs might multiply the effects by acting on the same hormone systems. Hence, future studies should be focused on adverse effects of mixtures of phthalates and other EDCs in order to broaden knowledge about associations between environmental exposures and biomarker levels. Improved testing for EDCs and successful methods for evaluation of obtained evidences are necessary in order to improve legislation and restrict the production and usage both of phthalates and EDCs in general. The reduction of exposures and thereby vulnerability to disease is not possible without creation of environment for scientific advances, innovation and disease prevention.

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THE ASSESSMENT OF NEUROTOXIC EFFECT OF LOW MOLECULAR WEIGHT PHTHALATES

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Phthalates (dialkyl ester of ortho-phthalic acid) are extensively used as a plasticizer in many products, especially medical devices, furniture materials, cosmetics, and personal care products. They are not chemically bound to plastic polymers and over time, easily migrate out of these products and into the environment

Approximately 2–50% of phthalates easily migrate out of products into food, saliva, blood, etc. contaminating those who are in close contact. Experimental investigations evaluating the biological impact of phthalate exposure on developing organisms are critical given that estimates of phthalate exposure are considerably higher in infants and children compared to adults.

Phthalates are well known as endocrine disruptors for last two decades. Besides this, phthalates' chronic exposure is associated with hepatotoxicity, mutagenicity and cancer. Recent limited, but growing, evidence link exposure to certain phthalates with neurobehavioral outcomes. In animals, a number of studies have reported that phthalate exposure was associated with altered neurobehaviors, including impaired self-righting ability [13], [14]. Regarding high lipophylicity of phthalates and their potential to penetrate BBB, abovementioned suggests neurotoxicity of these compounds. The precise mechanism of phthalates' action on CNS is still unknown and needs intensive biochemical study in contest of link to increased frequency of hyperactivity, autism spectrum disorders and other behaviour disturbances in children.

KEYWORDS:
low-molecular phthalates, CNS, neurotoxicity, behaviour disorders.

Most of people, particularly in Europe and North America have daily contact with organic and man-made polymers, including coatings, elastomers, adhesives, blends, plastics, fibers, caulks, ceramics, and composites. Use of plastics provides cheaper, lighter, stronger, safer, more durable and versatile products and consumer goods that serve to improve our quality of life. PVC containing the softener phthalate DEHP was first manufactured in 1931. Phthalates are produced in high volume, over 500 million pounds per year (EPA 2006). Phthalates can be divided into three categories based on the length of the ester side chains: low molecular weight, high molecular weight and transitional or mid-molecular weight. Low molecular weight phthalates have short, straight side chains of three or less carbon atoms, while transitional phthalates can have side chains that are either straight or branched and contain four to six carbon atoms.

Nowadays neurological disorders prevalence increase with high speed. According to the statistics of National Research University Higher School of Economics in USA, between 1990 and 2015, the number of deaths from neurological disorders has increased by 36.7 percent (V. L. Feigin et al, 2013). Plastic usage is also growing every year. Phthalates are released to the environment from multiple sources including industrial releases, the disposal of manufacturing, processing and industrial wastes, municipal solid waste, land application of sewage sludge, and release from products containing phthalates. With annual production of about 6.0 million tons, phthalates have been detected in water, air, sediments, soil, food and so on. The large production volume and wide application has made the presence of phthalates almost ubiquitous. Phthalates will permeate substances that are lipid soluble more readily than substances that are water soluble (Schettler, 2006). The concentration of DEHP in a food product is directly associated with the date of packaging with higher concentrations of DEHP found in food packaged at an earlier date (Kueseng et al., 2007). Concentrations of DEHP in plastic toys have been reported to be as high as 40% with higher DEHP concentrations resulting in increased DEHP migration into saliva (Lyche et al., 2009).

Phthalates have been detected in amniotic fluid, breast milk, saliva, blood and urine of humans at all ages. Data from the NHANES (CDC, 2009)

indicates widespread exposure of the general population to phthalates. Biomonitoring data from amniotic fluid and urine have demonstrated that humans are exposed to phthalates in utero, as infants, during puberty, and in adult life, and that people are exposed to several phthalates at once. For example, NHANES (National Health and Nutrition Examination Survey) detected a DEHP urinary metabolite in 78% of the 2541 samples tested with women having a higher exposure than men (CDC, 2009). Children have been reported as having the highest exposures; specifically, to DEHP, DBP, BBP and DnOP. Humans are exposed to phthalates in one of four ways: ingestion, inhalation, absorption and/or intravenous (Latini et al., 2003; Schettler, 2006).

Human organism and other mammals could slowly metabolize esters of phthalic acid. The primary metabolite of DEHP (mono-2-ethylhexyl phthalate (MEHP)) has also been found in breast milk (mean 11 mg/L; range 1.5–1410 mg/L) (Lottrup et al., 2006; Main et al., 2006). Estimates of human exposure from urinary metabolites suggest that adults are exposed to approximately 3 to 30 mg/kg body weight per day (Koch et al., 2006; Latini, 2005). At least one phthalate metabolite was detected in the urine of all infants, with seven or more detected in the urine of 81% of infants (Sathyanarayana et al., 2008). There is limited, but growing, evidence linking exposure to certain phthalates with neurobehavioral outcomes.

Some phthalates were reported to have the potential to cause decreased testicular weight and seminiferous tubular atrophy, increased DNA damage in men's sperm, premature breast development in girls, shortened pregnancy and decreased anogenital distance in newborn male babies (summarized by Schecter et al.). These reproductive defects most likely result from the estrogen disrupting activity of certain phthalates. High maternal levels of phthalate metabolites (particularly MEHP) are correlated with shortened gestational periods and an increase in preterm birth (Meeker et al., 2009; Whyatt et al., 2009). Several phthalates are listed as reprotoxic category 1B substances under EU Regulation (EC) 1272/2008 – Classification, Labeling and Packaging of Substances and Mixtures (CLP Regulation).

In animals, a number of studies have reported that phthalate exposure

was associated with altered neurobehaviors, including impaired self-righting ability, impaired spatial learning and reference memory, increased hyperactivity, and decreased grooming behavior. Moreover, several studies have reported that phthalate exposure is associated with deficits in social functions, reduced intelligence, attention deficit hyperactivity disorder (ADHD) at school age, and autism spectrum disorders (ASDs). Recent studies have shown that prenatal phthalate exposure is associated with alterations in childhood behavior and executive functioning. Phthalates might induce neurotoxicity, but little is known about the mechanisms by which this occurs.

Because human phthalate exposure occurs via personal care products taken by a pregnant female, one concern is whether prenatal exposure can affect developmental plasticity. Perinatal exposure, that is, the time right before and the time right after birth (during the infant nursing period) to phthalates has also been reported to have detrimental consequences on both functional and structural plasticity aspects of the hippocampus. Perinatal exposure to phthalates (DEHP) may also be associated with increased vulnerability to anxiety- and depressive-like behaviors. Prenatal and perinatal exposure is of particularly attention, since at early stage of development metabolism rate of xenobiotics is very low. Therefore, lipophilic compounds stay in organism longer, easily cross BBB that enhances their toxicity. Some papers suggest the link of phthalates' exposure to ADHD (Kim et. al., 2009), ASD (Testa et. al., 2012) and other cognitive or behavior impairments (Zhang et. al., 2009). Unfortunately, studies of neurobehavioral outcomes for humans, following phthalate exposure, are limited and need following biochemical investigation.

RESEARCH METHOD

For our research two low-weight phthalates were selected: 2-ethylhexyl phthalate (DEHP) and dibutyl phthalate (DBP). DEHP can be found in both industrial and consumer plastic products including automotive upholstery, perfumes, carpets, insulation, mattress pads, shower curtains, clothing, toys, umbrellas, straws, pesticides, medical tubing and medical bags, among other products. DEHP is considered to be a known animal and possible human reproductive and developmental toxicant (CPSC, 2010). Dibutyl phthalate (di-n-butyl phthalate, DBP) is one of themost commonly used phthalate esters and is an endocrine-disrupting chemical (EDC).

DBP is widely used as plasticizer in a variety of household industries and consumer products, such as toys, food containers, furniture, cosmetics and personal care products, latex adhesives, cellulose plastics, varnishers, and dye solvents (Heudorf et al.2007). Both of them are considered as compounds, exposure to which is increased particularly in early childhood. Researchers suggest that target structure for DEHP and DBP neurtoxicity is hippocampus [Rowdhwal et. al, 2018], but studies on other brain structures were not conducted.

Therefore, the main goal of our research is study of neurotoxic effect and its biochemical mechanism of these compounds in rats during prenatal and perinatal exposure. The following tasks are scheduled for this purpose:

- Assessment of DEHP and DBP prenatal and perinatal subchronic exposure's effect on the behavior and learning ability;
- The target brain structure/s of neurotoxic action of DEHP and DBP will be determined;
- Time- and dose-dependency of neurotoxic effect will be estimated.
- The precise molecular mechanism, underlying toxic effect on neurons, including apoptosis and signal transduction disorders, will be determined:
- The changes of key molecules in plasma membranes and inside neural cell, involving in toxic effect, will be detected.

All these together, would give us the possibility to determine the precise mechanism of phthalates' toxicity in the immature brain. This stage is especially vulnerable to any toxic action and changes made in this age usually are linked to further behaviour, cognitive and psychiatric disorders. Additionally, this knowledge would be useful for increase the awareness of society about potential risk of phthalates' exposure in early childhood particularly.

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BIOREMEDIATION USING MICROALGAE

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ABSTRACT

Adverse effects of human activity are leading to water contamination by an increasingly complex range of emerging pollutants, which are not readily treated by conventional means, and, through the urban water cycle, can enter ground and surface waters, can persist in the environment, bioaccumulate through the food chain and reach drinking water. These include pharmaceuticals, pesticides, plasticisers and several other groups of contaminants.

In this paper, we discuss the potential use of the microalgae Nannochloropsis sp. for the removal of phthalates from water, based on the previous studies with pharmaceuticals and pesticides. This species remained alive in the presence of the pollutants studied, and removed them from water with varying efficiencies.

INTRODUCTION

Due to anthropogenic activities, the lakes, the rivers and streams, the seas and oceans of the earth are heavily polluted. Water pollution is a serious environmental threat, mostly caused by pollutants released from industrial and sewage discharges and runoffs from agriculture. Not only it affects habitats and entire ecosystems, but also, specifically, human health. Many of the pollutants found in water bodies are considered disruptors to the endocrine system of humans and wildlife; they are called endocrine disrupting chemicals (EDCs)1,2. Through the urban cycle of water, pollutants such as phthalates, bisphenols, organochlorine pesticides, heavy metals and so many others, are found in ground, surface and drinking waters2. The conventional drinking and wastewater treatment, which include aeration, sedimentation, flocculation, coagulation, flotation, activated sludge, ozonation, chloronation and oxidation processes, do

not completely remove most of these pollutants2. Also, the excessive concentration of nitrates and phosphates in the water bodies lead to the eutrophication with the inevitable consequences of having microalgal blooms, sometimes with toxic and lethal species, that reduce the oxygen present in the water causing the death of the native flora and fauna.

One potential contribution to solve the water pollution problem consists on the use of biological agents, together with existing methods. The biological treatment processes include bacteria, fungi, protozoa, enzymes and microalgae. The bioremediation using microalgae has several advantages over the conventional methods. The potential solution to the concerns regarding all aspects of the environment and the economic valorization of the resulted biomass are among the most important advantages of this treatment.

In our research, we assessed the performance and efficiency of free and immobilised cells of microalgae Nannochloropsis sp. for the removal of five pharmaceuticals and one neonicotinoid insecticide, chosen for their occurrence or persistence in the environment.

Pharmaceuticals and their metabolites are released to the environment by domestic, hospital, and pharmaceutical industry wastewaters. The concentrations of these compounds that are innocuous to humans could be deadly to non-target organisms. Conventional wastewater treatment technology does not guarantee effluents of high quality; the apparently clean water may be loaded with pollutants.

Other pollutants found in effluents include pesticides. The large-scale use of pesticides has been one of the major causes of the dramatic disruptions of our environment. Pesticides, such as imidacloprid, are imposing increasing pressure on hydric resources and ecosystems. This neonicotinoid insecticide has been implicated in bee health decline and in the toxicity to other beneficial insects, and, due to its persistence in the environment and its accumulation in the food chain, poses a threat to human health.

Therefore, it is important to test possible environmentally-friendly solutions for the elimination of pollutants from water, such as bioremediation.

EXPERIMENTAL

Several methods were employed to carry out relevant tasks in our research. These include extraction techniques and chromatographic, spectroscopic, and hyphenated techniques. A rapid reverse phase high-performance liquid chromatography (RP-HPLC) methods were developed and validated for the simultaneous quantification of paracetamol, ibuprofen, olanzapine, simvastatin and simvastatin acid, and of imidacloprid, in the context of microalgae bioremediation. The methods were validated according to the guidelines of the US Food and Drug Administration (FDA), the International Conference on Harmonization (ICH), Eurachem and SANTE/ 11813 20173,4,5.

RESULTS AND DISCUSSION

In studies of the bioremediation potential of Nannochloropsis sp., the drug alprazolam was used. Analysis of UV–Vis spectroscopy revealed a high percentage of alprazolam removal from water. Epifluorescence microscopy images of Nannochloropsis sp. cells (Figure 1) showed the fluorescence of alprazolam in whole cell indicating the diffusion of the drug.

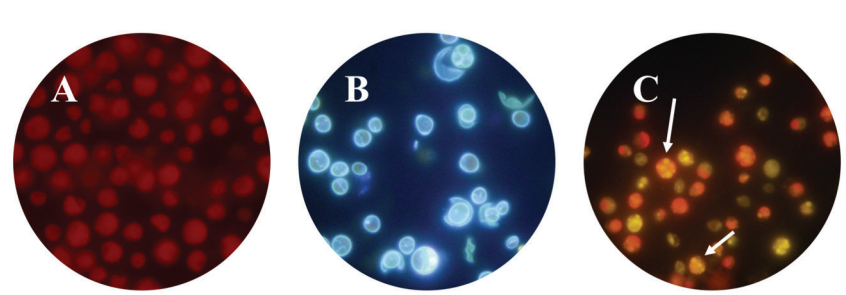

Figure 1 (A) Epifluorescence microscopy images of microalgae cell showing the red autofluorescence of chlorophyll from Nannochloropsis sp. chloroplast, (B) Nannochloropsis sp. cells cultivated in the presence of alprazolam, and (C) Nannochloropsis sp. cells cultivated in the presence of olanzapine.

Cultures of Nannochloropsis sp. immobilized in polymer beads (Figure 2) grew in population after the first hours and remained stable, with a slight decrease after 60 hours of culture.

Figure 2 Nannochloropsis sp. cells immobilised in PVA beads.

Upon analysis by RP-HPLC (Figure 3), the chromatograms revealed that the removal of paracetamol, and ibuprofen by Nannochloropsis sp. after 24 hours of culture was significantly higher in immobilised cells. In the group of free cells, the concentration of olanzapine decreased 50% of the initial concentration (50 µg mL-1) after 60 hours of culture, suggesting more affinity to the molecule of olanzapine than to paracetamol and ibuprofen, presumably reflecting the electrostatic interactions and other forces between them and the components of the membrane.

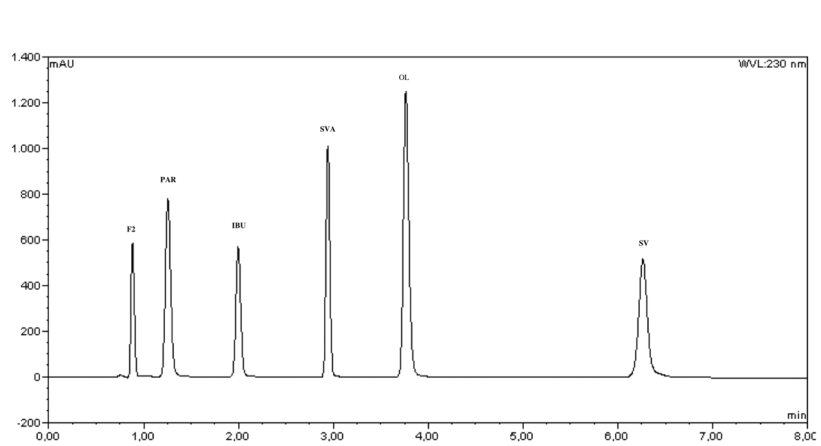

Figure 3 Chromatograms of the standards PAR, IBU, SIMA and SIM considered for the development and validation method.

In the studies using imidacloprid (Figure 4), a cell density of 5.5 x 107 removed 4.39 µg mL-1 from an original content of 9.59 µg mL-1 of the insecticide in the first 20 hours, grown autotrophically. The study showed that the removal of the imidacloprid by the marine microalgae Nannochloropsis sp. is both effective and light dependent.

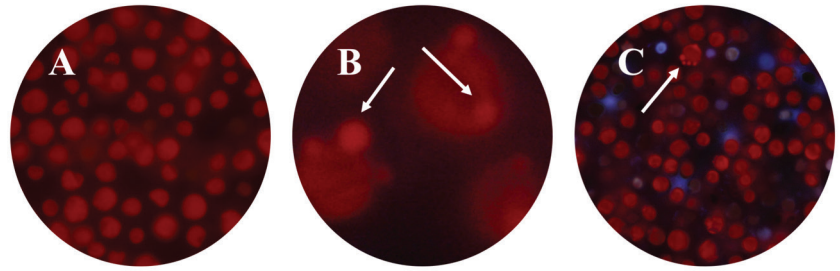

Figure 4 (A) Epifluorescence microscopy images of microalgae cell showing the red autofluorescence of chlorophyll from Nannochloropsis sp. chloroplast, (B) Nannochloropsis sp. cells grown in medium with 10 µg mL-1of imidacloprid, after 7 days of cultivation, and (C) detail of Nannochloropsis sp. cells showing specific staining (red) associated to the cellular internalisation of the imidacloprid.

CONCLUSION

The microalga Nannochloropsis sp. was found to remain alive in the presence of the four pharmaceuticals and imidacloprid, and to remove them from water, although with different efficiencies. Comparing with the free cells, the immobilised ones showed a higher resilience in the presence of the pharmaceuticals. The imidacloprid removal is more efficiently when a source of light is present, while aeration is another factor that might contribute to the removal.

From the results obtained in our research, it became apparent that the microalga Nannochloropsis sp. could be considered as a promising specie in the removal of pollutants from effluents.

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PHTHALATES IN THE NICU

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THE IMPORTANCE OF PREMATURITY

Each year, worldwide, 10 % of children or 15 million babies are born preterm and are at risk of death and life-long disability for many reasons1. Studies show that children born before 32/40 weeks of gestation, very preterm by definition, have a 40% risk of cognitive impairments (20% in term born peers), 10-20% risk of motor impairment (maximum 5% in term born peers), and 2-10 times more adverse social/behavioral or psychiatric problems. Physiological immaturity, enhanced susceptibility to infections and social inequalities are among the known factors of adverse outcome in this population. One factor less mentioned in the literature is the early and intense exposure to environmental pollutants in the neonatal units where these babies are cared for(1). The last 20-30 years have seen the emergence of the field of developmental care in the NICU, which aims to lessen the adverse impact of the NICU environment. Among targeted endpoints, there is noise reduction, respect of sleep/wake cycles, pain control, kangaroo mother care, and more recently, the limitation of chemical exposure when possible.

MEDICAL DEVICES IN THE NICU

The NICU is a highly technicized environment for infants needing critical care, in which infants are exposed to chemicals through cleaning solutions, hygiene products, diapers, hand disinfection, nutrition, enteral and parenteral drugs, and medical devices.

Phthalates have been used to enhance the flexibility of plastics since the 1930s. Concerns regarding their toxicity have arisen since the 1990s, especially for neonates and children, and their use in childcare products was banned in 2006 in Europe. In the NICU, medical devices may contain phthalates and notably DEHP, and Swiss regulations stipulate, “Devices which could possibly release phthalates to the body

of the patient should be labeled accordingly". In 2014, and again in 2017, Fischer Fumeaux et al undertook a systematic review of all the medical devices used in their tertiary care NICU, with the labels first, and when the information was lacking, by contacting the industry. Among 354 devices, the labelling disclosed DEHP in 10%, and in 13 % the label did not show the composition (2). Devices related to ventilation procedures were the most at risk of containing DEHP. In pediatric intensive care units, Malarvannan G et al have also shown the presence of phthalates and alternate plasticizers, the health effects of which are unknown(3), and recommend monitoring this exposure. Leaching from the device to the environment and the infant depends on physico-chemical properties such as temperature or lipid content. In 2014, Mallow et all showed that the daily doses received by a critically ill 2 kg infant in the NICU might exceed 160'000 times the hepatotoxic limit, 4000 times the reproductive toxicity limit, with certain procedures, such as ECMO, putting the infant especially at risk(4). Recently, Jenkins et al showed an association between phthalate metabolites and hypertension in premature infants after NICU(5).

PHTHALATES AND HEALTH EFFECTS IN NEONATES

Epidemiological studies suggest that prenatal exposure to phthalates through the amniotic fluid is associated with reduced gestational length, reduced birthweight, birth-size and birth head circumference, as well as with endocrine disruption and reproductive effects. These effects seem to be different in males and females. Postnatal exposure happens through breastmilk and even more through formula, often exceeding the reference dose of antiandrogenicity, with possible associations to obesity, asthma.

PHTHALATES AND NEURODEVELOPMENT

Prenatal exposure to phthalates has been linked in epidemiological studies to behavioral issues such as hyperactivity and attention deficit (6,7), oppositional behavior (8,9), depressive symptoms, as well as with measures of general intelligence (10).

In children hospitalized in intensive care, the exposure linked to medical devices is associated with attention deficit(11).

Finally, there are conflicting results regarding the association of prenatal phthalate exposure with autistic spectrum disorder(12).

PHTHALATES IN THE NICU: WHAT DO NEONATOLOGISTS KNOW

A recent survey shows that nearly half of senior neonatologist are not aware of the issue of phthalates in neonatal care(13). Most units have no system to identify phthalate containing medical devices, nor to monitor potential adverse health outcomes related to this exposure. Marie et al, who showed that 17% professionals felt able to counsel women regarding phthalates in pregnancy, have already showed this lack of awareness(14). It is therefore of major importance to raise the knowledge and awareness of caregivers in neonatal units, while researching safe alternative materials.

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HOW TO BOOST CAREER PERSPECTIVE OF EARLY STAGE RESEARCHERS

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ABSTRACT:

In the last two decades, the number of doctoral graduates has expanded in Europe and this lead to diversifying of their career paths. Doctorate should contribute to knowledge through original research. However, there is a growing range of careers in other sectors and across international settings out of academia including business, industry, non - profit organizations and government. These careers require particular skills and competences that can be transferred from academic to other professional settings and that enhance doctoral graduates employability, ability to manage their own careers, and the sense of their responsibility for making contributions to society.

Doctoral graduates are expected to make effective contributions on the global stage and they need to be sufficiently prepared. Skills acquisition and development are essential for the performance and modernization of labour markets in order to provide new forms of flexibility. Formal education and training should equip doctoral candidates with a broad range of skills which opens doors to personal fulfilment and professional development, social engagement, active citizenship and employment [1]. This requires a new innovative approach that offer to develop a range of various skills that help doctoral candidates to be more effective in their research, but also to work on a broader range of skills there will be useful in their future lives and careers. These skills are often known as transferable skills [2].

KEYWORDS: Early Stage Researchers, Open Science, Career Development, Mental Health

ABOUT EURODOC

Eurodoc, the European Council of Doctoral Candidates and Junior Researchers, is a grassroots federation of national associations of early career researchers in Europe. Eurodoc was established in 2002 and is based in Brussels. As representatives of doctoral candidates and junior researchers at European level, we engage with all major stakeholders in research and innovation in Europe. http://eurodoc.net

MAIN AIMS

According to its Statute, the main goals of Eurodoc are:

- to represent doctoral candidates and junior researchers at the European level in matters of education, research, and professional development of their careers;
- to advance the quality of doctoral programmes and the standards of research activity in Europe;
- to promote the circulation of information on issues regarding young researchers, organise events, take part in debates, and assist in the elaboration of policies about higher education and research in Europe;
- to establish and promote cooperation between national associations representing doctoral candidates and junior researchers within Europe.

MEMBER ASSOCIATIONS

Eurodoc currently has 28 members from 26 countries (Fig. 1) that are associations representing early-career researchers at national level. In addition, the network has 4 observers. Both Eurodoc members and observers belong to the European Union and/or the Council of Europe. Potential members have to fulfil this requirement too.

Fig. 1 Eurodoc on Map.

Eurodoc identify three most important issues for early-career researchers (Fig. 2):

- Career development, especially for non-academic labor market.
- Open Science, what is it and how can be done.
- Rising mental-health issues in academia, especially in the case of doctoral candidates.

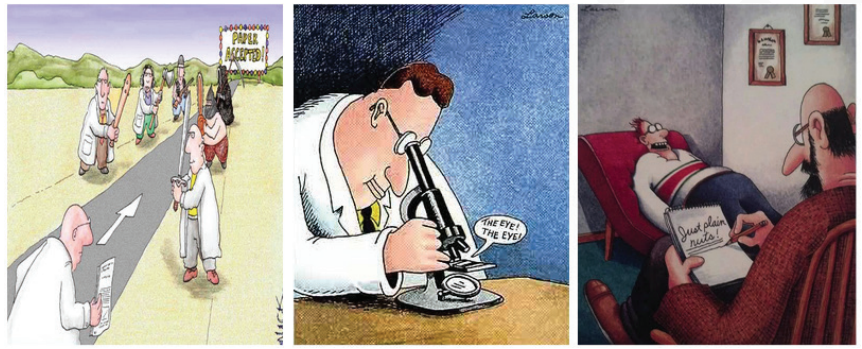

Fig. 2 Career development (a), Open Science (b) and Mental-Health (c).

CAREER PATHS IN OTHER SECTORS

Due to rapid changes in society (Fig. 3), there is a growing range of positions out of academia and across international setting (Fig. 4) [3].

Fig. 3 Technological development and information communication technology (ICT).

According to EUA survey (2009) in Europe > 50 % of doctoral holders found job outside academic sector [4], while according one study in Nature in 2018 already 70 % [5].

Fig. 4 Career paths of doctoral holders in other sectors.

To be more effective in both careers, because positions in academia are more competitive and complex as well: early-career researchers need to develop new skills and competences outside their own area of expertise (especially transferable skills).

Eurodoc WG on Doctoral Training prepared the report on transferable skills and competences (Fig. 5) which are relevant for early career researchers to gather during their doctoral training program and beyond, in order to increase their employability in multiple work sectors.

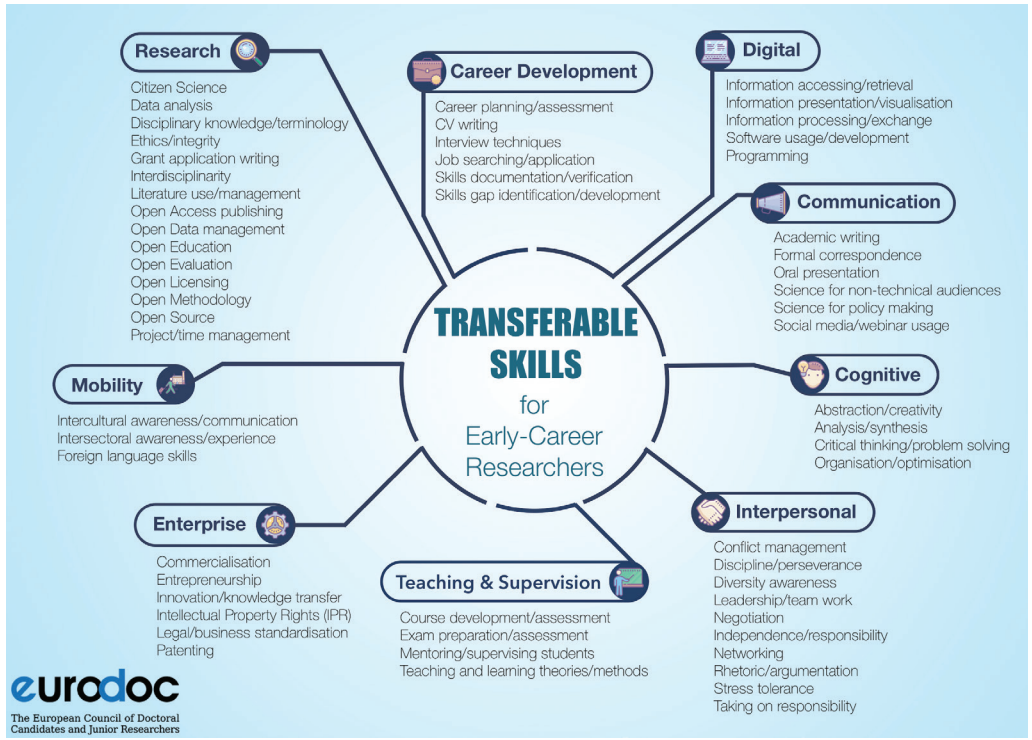

Fig. 5 Eurodoc Report - Identifying Transferable Skills and Competences to Enhance Early-Career Researchers Employability and Competitiveness.

In a recent joint statement on sustainable research career (Fig. 6) with the Marie Curie Alumni Association (MCAA) we call on research institutions,

funding bodies and governments to ensure sustainable researcher careers.

The most critical points to be considered, especially by funding organizations and universities:

1. Provide sustainable career prospects for researchers
2. Deploy career management services at organisations employing researchers
3. Put more emphasis on transferable skills training and recognition
4. Provide a wide variety of networking options and services in and outside of academia

Fig. 6 A Joint Declaration on Sustainable Research Careers

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EURODOC CONTRIBUTIONS

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A balancing act - Mobility and career development (2018, Eurodoc)
DOI: https://doi.org/10.21820/23987073.2018.4.28

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Early career-researchers and mental health
DOI: https://doi.org/10.21820/23987073.2018.2.91

KEY DOCUMENTS ABOUT DOCTORAL TRAINING

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- The Salzburg II Principles (2010, EUA)
- Innovative Principles for Doctoral Training (2011, EC)
- Taking Salzburg Forward (2016, EUA)

HORIZON 2020
BROKERAGE EVENT

Horizon 2020
European Union funding
for Research & Innovation