

# Analysis and Bioanalysis: an Effective Tool for Data Collection of Environmental Conditions and Processes

Natalia Szczepańska<sup>1</sup>, Katarzyna Owczarek<sup>1</sup>, Błażej Kudlak<sup>1</sup>, Andrzej Pokrywka<sup>3</sup>, Zofia Mazerska<sup>2</sup>, Agnieszka Gałuszka<sup>4</sup>, Jacek Namieśnik<sup>1\*</sup>

<sup>1</sup>Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, 11/12 Narutowicza, 80-233 Gdańsk, Poland

<sup>2</sup>Department of Pharmaceutical Technology and Biochemistry, Faculty of Chemistry, Gdańsk University of Technology, 11/12 Narutowicza Str., 80-233 Gdańsk, Poland

<sup>3</sup>Faculty of Medicine and Health Sciences, University of Zielona Góra, Zyty 26, 65-046, Zielona Góra, Poland

<sup>4</sup>Geochemistry and the Environment Division, Chemistry Institute, Faculty of Mathematics and Science, Jan Kochanowski University in Kielce, 25-406 Kielce, 15G Świętokrzyska Str, Poland

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## Abstract

Reliable information that allows us to estimate the state of the environment and to forecast changes in the ecosystem are constantly required. Increasing environmental consciousness and dynamic development of analytical techniques are the main reasons for determining the wide range of pollutants occurring at very low concentrations in complex matrix samples. The presence and concentration of many of those pollutants in the environment are not yet subjected to legal regulations, so development of new methodologies to ensure good quality of results and their control are essential. This paper presents the target and development directions currently observed in environmental monitoring and biomonitoring. A lot of attention has been paid to a wide range of compounds that are endocrine disruptors. The overview of analytical approaches and procedures used to detect and to determine biologically active compounds can be found in this paper as well.

**Keywords:** biomonitoring, bioluminescent signal system, endocrine disrupting compounds, environmental pollution, organic trace pollutants, xenobiotics

## Introduction

A broad spectrum of various pollutants can be found in the individual elements of the environment, both living (biota) and non-living. Chemists very often pay attention only to chemical compounds, which are treated as substances foreign to the average chemical composition of individual elements of the environment or occur at levels higher than the so-called mean composition.

Environmental research includes a broader and broader spectrum of chemical individuals, where the group of xenobiotics should be included. The term xenobiotic relates to the chemicals that are strange to the living organisms and undergo metabolic transformation in the organism.

Attention should also be paid to legal aspects connected with the presence of specific pollutants in the individual elements of the environment. Fig. 1 presents information about the classification of environmental pollutants according to their legal status [1].

Compounds that need to be detected, identified, and determined can be divided into two groups in respect to their legal regulations [1]:

1. Compounds that are already subject to legal regulations because their physicochemical properties as well as immediate and distant toxic effects (as a result of ecotoxicological tests) and appropriate methodologies are already available and it is possible to obtain reliable information about changes in the content of these analytes in various types of environmental samples. Thus, it was possible to propose appropriate standards defining the highest concentration of a given xenobiotic in a defined environmental element. These normative values are called the maximum admissible concentration (MAC) in European countries and the threshold limit value (TLV) in the United States [2, 3].
2. The group of compounds that is not subjected at this time to legal regulations contains xenobiotics and other compounds detected (but not determined) in the environment because new appropriate analytical methodologies were not yet introduced into the analytical practice. Thus, it was not possible to detect and determine analyses occurring in tested environmental samples at very low levels. It is said that the determined compounds have so far been called non-identified pollutants. The group of pollutants that are not subject to legal regulations

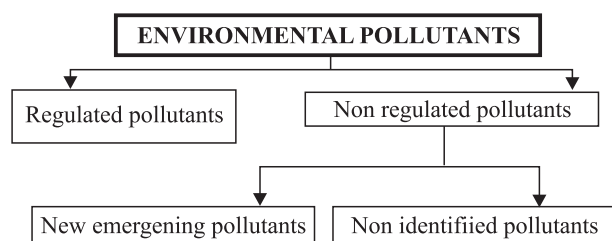


Fig. 1. Classification of environmental pollutants according to their legal status

include the so-called newly emerging pollutants [4]. These compounds have been introduced into the individual elements of the environment as a result of new manifestations of human pressure, e.g., a new technology of manufacturing products or consumer goods. As a result, it has not yet been possible to define the ecotoxicological properties or develop and validate appropriate analytical procedures that could make it possible to obtain reliable information about the levels of these compounds in various environmental samples.

Numerous research centres and design studios are researching the development of new methodological solutions and design work connected with the construction of new types of control and measuring devices that make it possible to obtain reliable information about the presence and levels of various groups of xenobiotics in individual elements of the environment as well as various processes and transformations they undergo.

Fig. 2 presents a classification of analytical approaches that can be used for this purpose.

Special attention should be paid to measuring systems based on the use of bioluminescent bacteria for detecting toxic substances. One such system is known as the bioluminescent signal system (BSS) [5, 6].

The advantages of such measuring systems are [5-7]:

- Universality: the possibility of developing a biotest solution intended for detecting a broad range of toxic factors.
- High sensitivity: the possibility of detecting toxic substances at concentrations ranging from  $10^{-12}$  to  $10^{-15}$  mol/l.
- High speed of measurements: bioluminescence reactions occur very fast (in milliseconds).
- Relative simplicity and availability of reagents.
- Simplicity and a relatively low cost of appropriate devices (bioluminometers) intended for fast measurements of appropriate signals.
- High numerical values of the S/N parameters (no noise occurs in some cases).
- Easy signal registration – the process can be easily automated and computerized so does not need highly qualified personnel for routine measurements.
- Toxic, explosive, or radioactive materials or reagents need not be applied.

The use of such a system, just like in the case of other methodological and instrumental solutions, which determine numerical values of summary parameters will constitute the first stage of research. It indicates the general level of environmental pollution of xenobiotics. The next step will involve analytical research to detect, identify, and determine individual chemicals/xenobiotics when the numerical value of summary parameters changes in an adverse direction.

## Various Analytical Approaches

The chemical analyst's basic task involves obtaining reliable information about the composition of individual

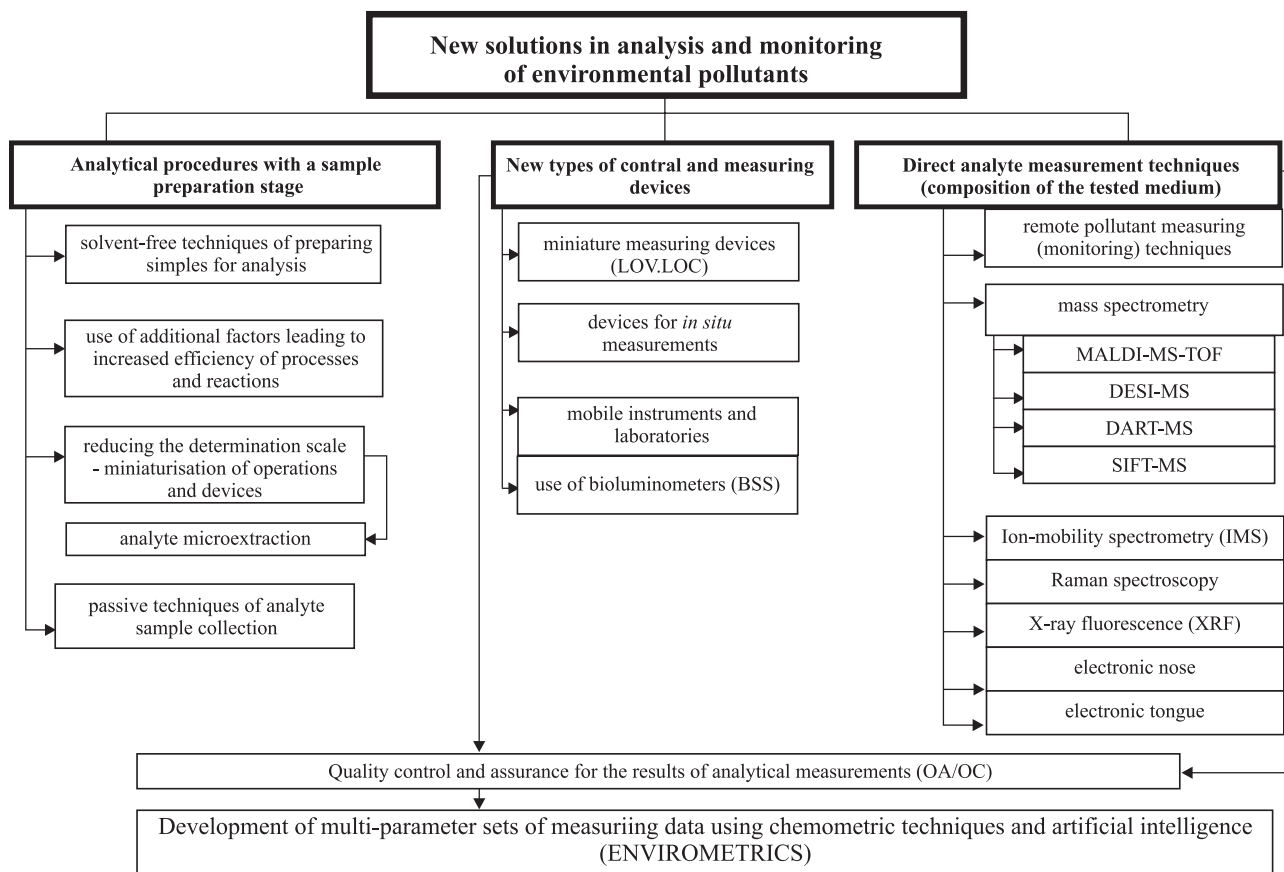


Fig. 2. Classification of analytical approaches that can be used to obtain information about the condition of individual elements of the environment, and processes that occur in them.

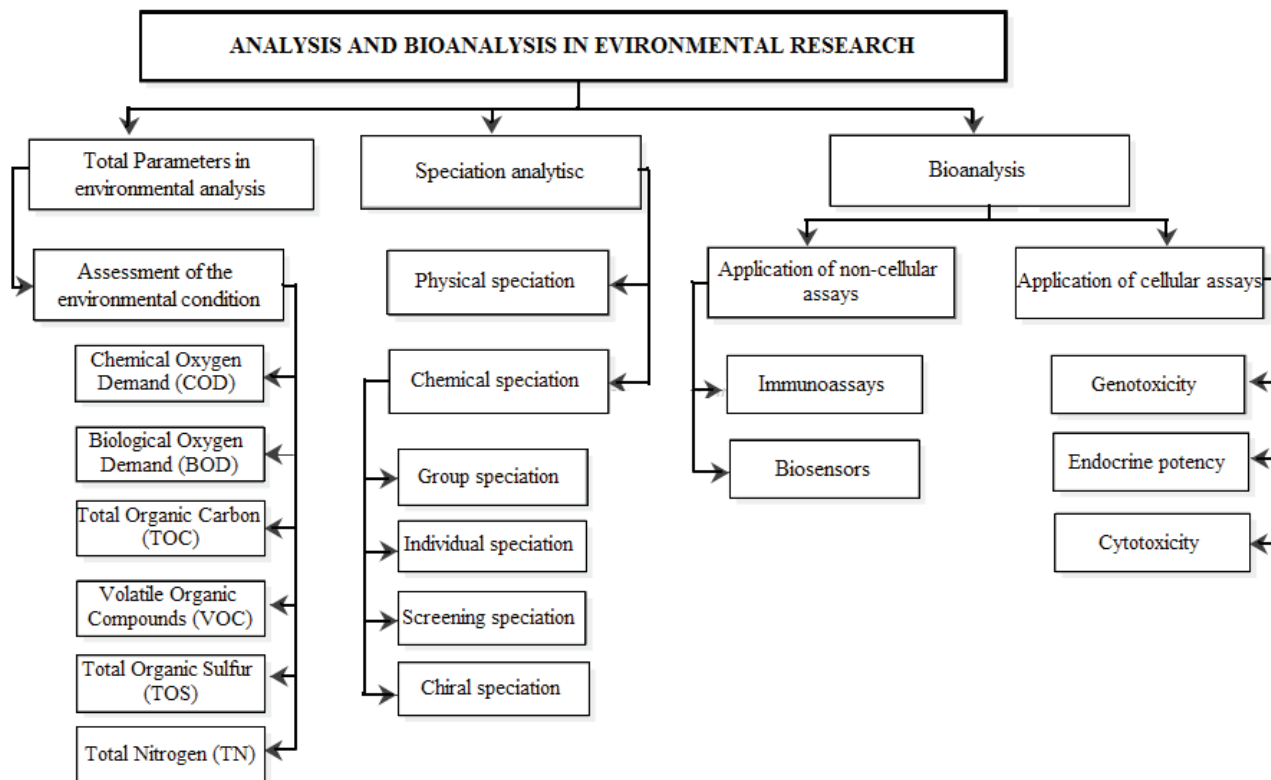


Fig. 3. A schematic classification of analytical and bioanalytical methodologies used in environmental research.

Table 1. Information about selected endocrine disruptors [29].

COMPOUND	ABBREVIATION / ACRONYM	NOTES
17 $\beta$ -estradiol and 17 $\alpha$ -estradiol	$\beta$ -E2 and $\alpha$ -E2	Natural oestrogens
Estrone	E1	Oestrogen, metabolite E2
Estriol	E3	Natural oestrogen
17 $\alpha$ -ethinylestradiol	EE2	Synthetic oestrogen generally used in combined contraception
Testosterone	Test	Natural androgen
Progesterone	Pg	Natural androgen
Dehydroepiandrosterone	DHEA	Androgen, sex hormone precursor
Levonorgestrel	LNor	Synthetic progestogen commonly used in contraception using only progestogens
Norethindrone	Nore	Synthetic progestogen commonly used in contraception using only progestogens
Cortisone	Cor	Glucocorticoid – synthetic cortisone homologue
Prednisone	PD	Glucocorticoid – synthetic cortisone homologue

elements of the non-living environment and biota (see Fig. 3.). Three types of analytical tools are required in this respect:

1. Control and measuring devices that can be used for analytical research both directly at a specific material object or on the representative samples, usually after preparing them for analysis (both offline and online), *in situ*, or after transporting them to an appropriately equipped laboratory [8].
2. Reference materials with a different metrological value as well as standard substances and solutions (mixtures) that are components of appropriate quality control and quality assurance systems for measuring data (QA/QC). The use of such systems is necessary when the results of measurements are to constitute a basis for reliable information about the condition of tested objects and the processes occurring. These materials are used for:
  - Validation of individual stages and whole analytical procedures.
  - Calibration of measuring devices [9] (one point that constitutes a part of this operation is zeroing by an application of an appropriate solvent blank).
3. Analytical procedures ensuring the appropriate preparation of representative samples so that it is possible to detect, identify, and quantitatively determine a defined group of ingredients, i.e., analytes. As a result of using appropriate operations included in the procedure, it is possible to [10]:
  - Simplify or replace the matrix with one that is compatible with the measuring instrument.
  - Removing some ingredients from the sample matrix (interferents).
  - Chemical transformation of analytes (derivatisation) to change their physicochemical properties, which should facilitate their extraction (retaining), separation, and quantitative determination.

Information presented in this drawing unambiguously indicates that information about the condition of the environment can be obtained not only by using analytical methodologies, in which samples collected from the non-living part of the environment and chemical operations are used before analysis. More and more often, it is living matter that is the object of interest and various types of biological material are used in the analytical process.

Two approaches can be distinguished in bioanalysis: Observation of the condition of living matter to obtain information about the condition of the non-living part of the environment. This approach is often defined as biomonitoring. It should be added here that the close term “biomonitors” is used in the literature together with the term “bioindication” to show the character (qualitative/quantitative) of information obtained about the condition of the environment using biomonitors/bioindicators [11].

It is obvious that if cell-based assays are used, two types of information can be obtained [12, 13]:

1. The toxic effect of individual compounds on the body when samples containing just this one component are tested (reference samples).
2. The endocrine genotoxicity or cytotoxicity effects of environmental or biological samples with an unknown composition are estimated; in this case further types of summary parameters can be referred to for preliminary assessment of the presence of compounds inducing specific toxic effects in samples.

### Endocrine Disruptors – Example of Nonregulated Environmental Organic Pollutants

A specific group of compounds that interest environmental researchers is endocrine disruptors, also known as endocrine-disrupting compounds. Compounds responsible

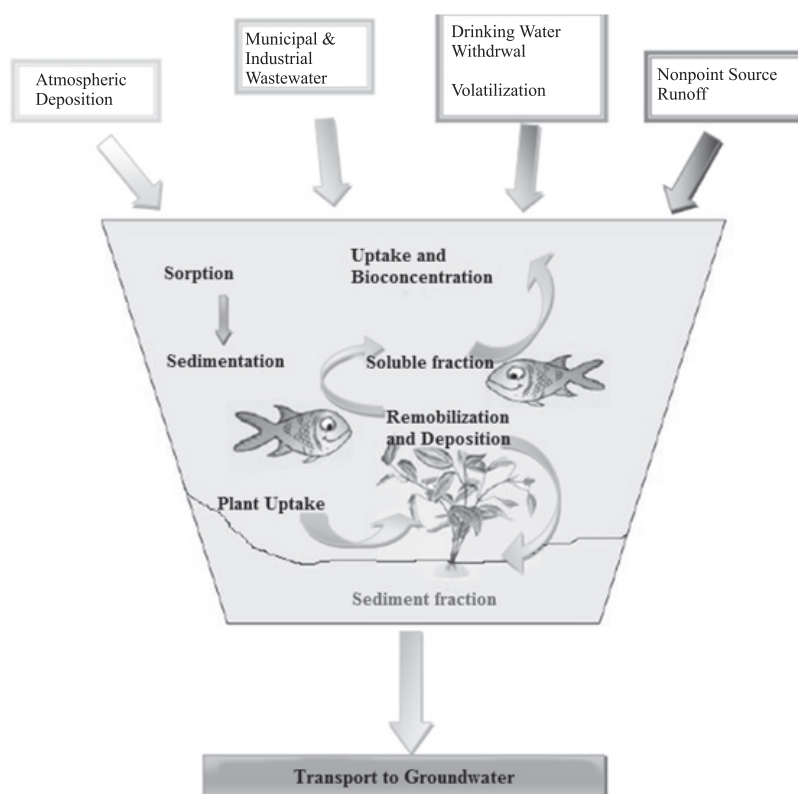


Fig. 4. Schematic presentation of the environmental fate of endocrine disruptors.

for interfering with the body's endocrine system are "exogenous substances or their mixtures influencing the function(s) of the endocrine system, thus inducing adverse effects in a given organism or its offspring or subpopulation" [14].

Chemical compounds responsible for interfering with the endocrine system (EDC) manifest their influence by [15] imitating endogenous hormones, antagonizing the synthesis of normal hormones or their metabolism, and modifying the level of hormonal receptors.

Compounds from the EDC group have the potential ability to influence reproduction and development as they are under the control of hormonal signals. Table 1 presents information about selected endocrine disruptors.

Endocrine action is assigned to many other compounds, which quite commonly occur in various elements of the environment. The following compounds can be listed as examples [16-18]:

- Polychlorinated biphenyls (PCB),
- Bisphenol A,
- Steroid oestrogens,
- Alkylphenols,
- DDT and its metabolites,
- Halogenated pesticides (e.g. methoxychlor),
- Polycyclic aromatic hydrocarbons (POM) (some of them),
- Tributyltin (TBT),
- Phthalate,
- Some pharmaceutical products.

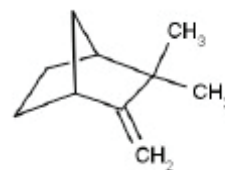
These compounds undergo various processes in

the environment, both in the non-living part of the environment and in organisms that were exposed to them in their habitat. The environmental fate of endocrine disruptors is shown schematically in Fig. 4 [19].

Compounds included in this group, just like other types of xenobiotics, may undergo the bioaccumulation process in tissues and organs of organisms at higher trophic levels. This thesis is confirmed by data on toxaphene presented in Table 3.

Toxaphene is an insecticide found in more than 670 products. Toxaphene is characterised by toxicity, stability, and the ability to bioaccumulate in animals and to travel long distances. Toxaphene is poorly soluble in water, so it can be found in the air, soil, or sediments on the bottom of lakes and streams (see Table 2. for details) [20].

In the 1970s, toxaphene was one of the most commonly used pesticides in the world [21, 22].



Toxaphene was used for fighting pests feeding on cotton, grain, fruits, nuts, and vegetables. In the 1970s, fishing and hunting agencies also used toxaphene for killing fish species that were considered undesirable. It was also used for fighting ticks and other acari in domestic

Table 2. Toxaphene concentrations in samples from various parts of non-living environment and biota accumulated in the Arctic areas of Canada [21].

ELEMENT OF THE ENVIRONMENT	CONCENTRATION (ppb)
Air	0.0007
Snow	0.0009 – 0.002
Seawater	0.0003
Zooplankton	3.6
Arctic cod	14 – 46
Arctic char	44 – 157
Ringed seal oil	130 – 480
European sturgeon oil	1380 - 5780
Narwhal oil	2240 - 9160

animals and poultry. Toxaphene is currently banned in the USA and in 57 other countries worldwide, while in other 12 countries its use is strictly restricted. At the beginning of the 1990s, toxaphene was produced in Africa and Latin America; it is estimated that its use is greatest in Africa [20, 23].

Thus, there is no doubt that the following aspects of the presence of endocrine disruptors in the environment are of significant importance:

- Detection and determination of the content of a broad

spectrum of endocrine compounds in environmental samples of various types and origins.

- Determination of the endocrine properties of individual compounds (using specific laboratory tests).
- Estimating the total endocrine potential of representative environmental samples.

In the last case, the determination of the total endocrine effect of all compounds present in the tested sample is considered. Such a parameter is to be treated as one of numerous parameters used to estimate the presence of various pollutants in the samples. Fig. 5 presents information about the analytical tools that can be used to obtain the aforementioned types of analytical information [15].

Even a brief analysis of literature data leads to the conclusion that so far, operations of sample preparation for analysis constitute a significant element of analytical methodologies that have been typically used in analytical practice. Therefore, it is possible to [10]:

- Simplify the matrix composition of the sample as a result of analyte transfer, e.g., to an appropriate portion of a solvent.
- Increase analyte concentration in the obtained extracts at the level above the limit of quantification of the control and measuring device used.
- Remove interferences.

Operations that make it possible to obtain such effects are highly labour- and time-consuming. The diagram presented in Fig. 6 may be used as a source of information about various methodological approaches in this area.

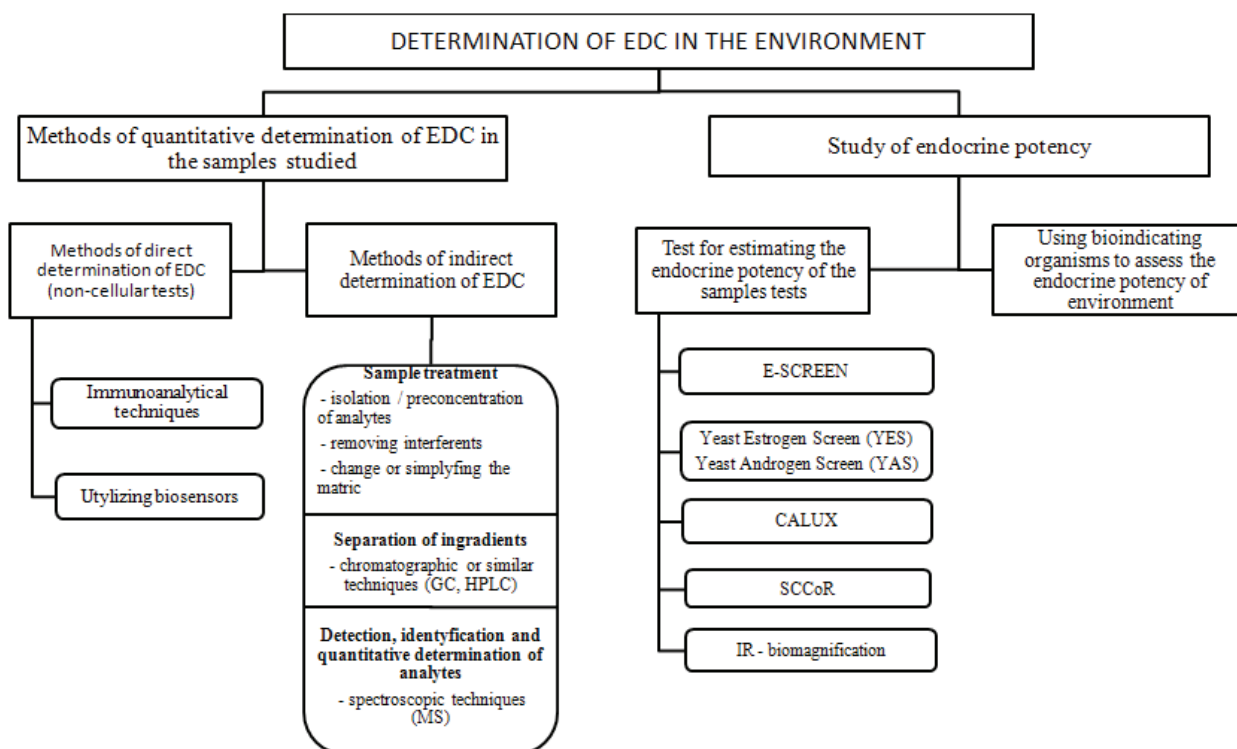


Fig. 5. Classification of analytical approaches used for the detection and determination of endocrine disruptors in environmental samples and for the assessment of endocrine disruption levels for various types of samples.

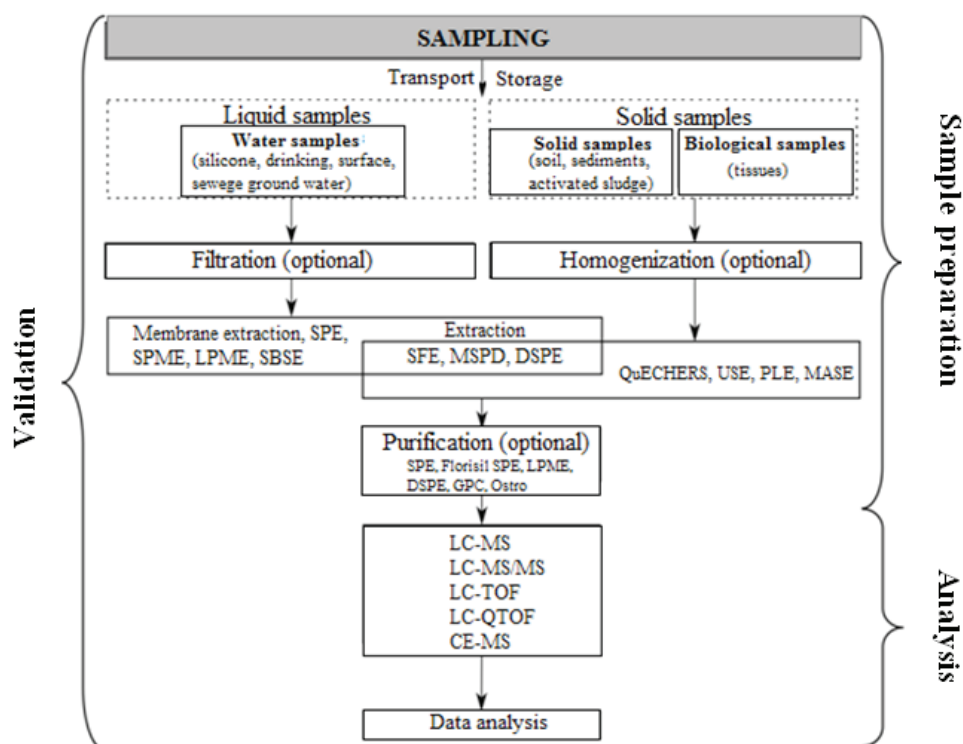


Fig. 6. General diagram of analytical procedures for determining compounds exhibiting biological activity in environmental samples characterised by a complex matrix composition [28].

- Numerous research centres perform research aimed at:
- Developing new analytical methodologies and their validation.
  - Using various procedures to obtain information about the content of various groups of xenobiotics in samples collected from various elements of non-living environment and biota samples.

Table 3 presents selected literature about the occurrence and confirmed levels of selected endocrine disruptors in samples from various elements of non-living environments.

## Conclusions

It is obvious and commonly known that various analytical approaches used in environmental research are assessed and compared in terms of their metrological parameters, as they determine the ability to use them in analytical practice.

The following parameters are most often considered in this respect [24]:

- *Method determination limit* (MDL) and *method quantification limit* (MQL);
- Repeatability and reproducibility;
- Precision and accuracy.

For several years, yet another issue of environmental analysis has been gaining more and more importance, namely the influence of a given type of analytical activity on the condition of the environment itself and on the health of employees of analytical laboratories (environmental

impact assessment, or EIA). It is connected with attempts being made to introduce commonly known principles of green chemistry and more and more popular principles of green analytical chemistry (GAC) into everyday laboratory practice [25, 26].

They are closely related to the sustainable development concept. Therefore, new methodological and device-related solutions are being sought to minimise the use of reagents (including toxic reagents), consumed energy, and produced waste (calculated for one analytical cycle or one analyte) [25]. Elimination or at least reduction of the influence of various operations and activities performed at analytical laboratories on the personnel health by [26]:

- Reducing the determination scale;
- Automating individual operations;
- Integrating analytical procedures.

Therefore, appropriate tools are needed for the assessment of environmental impact and personal exposure of analytical chemists, both for individual analytical procedures and whole laboratories. Two types of such research can be distinguished:

- The use of the life cycle assessment (LCA) for a comprehensive assessment of all aspects of environmental impact in accordance with the principle of “from the cradle to the grave” [25]
- The use of specific tools, such as the analytical ecoscale, for a comparative assessment of various analytical methodologies that can be used for the same analytical research on representative samples from a given material object [27].

Table 3. Occurrence of endocrine disruptors in the aquatic environment.

COMPOUND	MATRIX	CONCENTRATION [ng/L]	LIT.
Estrone E1	Urban wastewater	10-170	27
	Surface waters	0.7-143	28
	Drinking water	0.2-0.6	19
17 $\beta$ -estradiol (E2)	Urban wastewater	2-50	27
	Surface waters	<5-93	29
	Groundwater	6-66	30
	Drinking water	0.35-0.7	30
17-alpha-ethinyl estradiol	Urban wastewater	0.1-8.9	19
	Surface waters	<5-831	29
	Drinking water	0.15-0.6	19
Bisphenol A (BPA)	Urban wastewater	10-2,500	31
	Surface water (river)	8.9-776	31
	Groundwater	51.6-207	31
	Drinking water	3.5-59.8	32
Nonylphenol (NP)	Urban wastewater	0.030-1,016	27
	Surface water (river)	28-1,220	33
	Groundwater	200-760	19
	Drinking water	0.1-1	32
Atrazine (ATR)	Urban wastewater	20-280	27
	Surface waters	0.1-0.3	35

More and more studies are being published that discuss various approaches to the assessment of greenness of analytical methodologies.

### Acknowledgements

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