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Steroid Hormones in the Aquatic Environment – Review of the State of Knowledge and Comparison of Surface Water Pollution between Europe and Asia

Hormony steroidowe w środowisku wodnym – przegląd stanu wiedzy i porównanie zanieczyszczenia wód powierzchniowych w Europie i Azji

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Abstract: Steroid hormones are fundamental regulators of physiological processes in both animals and humans. The aim of this mini review is to present key findings in recent years regarding the presence and impact of steroid hormones on the natural environment. The high biological activity of individual steroid hormones combined with the absence of antagonistic effects when they occur simultaneously (e.g. in mixtures present in sewage) poses a significant threat to aquatic ecosystems in many water bodies. These compounds enter the aquatic environment through effluents of large livestock farms, industrial (pharmaceutical) plants and households. Over the past decade, concentrations of steroid hormones in European surface waters have increased significantly. Despite this, European countries have some of the cleanest surface waters in the world in terms of hormonal pollution. Nearly one-third of global emissions of animal-derived hormone pollutants are concentrated in India and Brazil, with India exhibiting the highest levels of anthropogenic steroid pollutant emissions. Even with removal efficiencies of 70-80%, wastewater treatment plants processing large water volumes discharge significant amounts of steroid hormones, which destabilise populations of many aquatic vertebrate and invertebrate species. Both Europe and Asia face serious threats to aquatic organisms from steroid hormones, but the nature and sources of this problem differ between regions.

Keywords: steroid hormones, water waste, surface water

Streszczenie: Hormony steroidowe są podstawowymi regulatorami procesów fizjologicznych zachodzących w organizmie zwierząt i człowieka. Celem niniejszego skróconego przeglądu literatury jest przedstawienie najistotniejszych informacji uzyskanych w trakcie badań przeprowadzonych w ostatnich latach na temat obecności oraz wpływu hormonów steroidowych na środowisko naturalne. Zarówno wysoka aktywność biologiczna poszczególnych hormonów steroidowych, jak i brak efektu antagonizmu przy ich jednoczesnym występowaniu (np. w mieszaninach obecnych w ściekach), stanowią istotne zagrożenie dla ekosystemów wodnych w wielu zbiornikach wodnych. Do środowiska wodnego trafiają z odpływów wielkich ferm hodowlanych, zakładów przemysłowych (farmaceutycznych) i gospodarstw domowych. Na przestrzeni ostatnich 10 lat stężenie hormonów steroidowych w wodach powierzchniowych Europy znacznie wzrosło. Pomimo to państwa Europy posiadają jedne z najczystszych wód powierzchniowych na świecie pod względem zanieczyszczeń hormonalnych. Blisko jedna trzecia globalnej emisji hormonalnych zanieczyszczeń pochodzenia zwierzęcego koncentruje się w Indiach i Brazylii, przy czym to Indie wykazują najwyższy poziom emisji steroidowych zanieczyszczeń pochodzenia antropogenicznego. Nawet przy efektywności usuwania sięgającej 70-80%, oczyszczalnie ścieków przetwarzające duże objętości wody pozostawiają znaczne ilości hormonów steroidowych, które doprowadzają do zachwiania stabilności populacji wielu gatunków kręgowców i bezkręgowców wodnych. Zarówno Europa, jak i Azja stoją w obliczu poważnego zagrożenia, jakie hormony steroidowe stanowią dla organizmów wodnych, jednak charakter i źródła tego problemu różnią się w zależności od regionu.

Słowa kluczowe: hormony steroidowe, ścieki, wody powierzchniowe

Introduction

Steroid hormones are primary regulators of physiological processes in animals and humans. They can be divided into five main groups, distinguished on the basis of their physiological behaviour in the human body (Skałba 2008):

- mineralocorticoids (responsible for regulating renal tubular function)
- glycocorticoids (responsible for carbohydrate metabolism in the human body)
- oestrogens (inducing the appearance of secondary female characteristics)
- progestogens (hormones responsible for the stimulation and proper functioning of the reproductive process)
- androgens (inducers of secondary male characteristics)

Although steroid hormones were initially classified according to their mode of action in the human body, subsequent studies have shown that they affect animals (including fish, lizards, birds and snails) in a similar way (DeQuattro et al. 2012, Gupta and Kanungo 1996, Callard et al. 1972). An important feature of individual steroid hormones is their lipophilic nature, which enables them to bind to transport proteins in the bloodstream and allows even relatively large molecules to enter the target cell (Miller 1988). All steroid hormones have very similar biosynthetic pathways but differ in the target receptor they interact with in a given organism. Complexes (hormone + receptor) regulate the transcription process of specific gene domains, thereby introducing a wide range of physiological changes in the body. Most steroid hormones are metabolised in liver cells (hepatocytes), and their prolonged use can lead to liver damage and impaired body functioning (Wincewicz et al. 2007). Corticosteroid hormones act in a similar manner; when taken in high doses over a long period of time, they can cause damage to the circulatory system and increase blood pressure in both humans and animals (Grennan and Wang, 2019; Dodic et al. 1999). In addition, high concentrations of steroid hormones have a negative impact on the developing human brain during puberty and induce permanent structural changes in the nervous system, altering the overall organisation of neural networks (Neufang et al. 2009). In both humans and animals, steroid hormones affect the process of cell proliferation in many tissues, thereby contributing to the formation of cancerous cells (arising from random mutations during numerous cell divisions) or increasing the likelihood of such cells developing. The most common target tissues in the process of carcinogenesis are those with high oestrogen activity. Despite decades of cancer research, the statement from Segaloff's 1975 work remains valid: it is not possible to unequivocally assign specific types of cancer to a particular steroid hormone; the observed cancerous changes appear to be species-specific.

Progestogens are divided into four main groups depending on when they were introduced into contraceptive treatment (Chlewicka et al. 1996):

- Generation I (discovered between the 1950s and 1960s these include norethynodrel)
- Generation II (discovered in the 1970s, these include norgestrel, levonorgestrel and norethysteron)
- Generation III (discovered in the 1980s and 1990s, these include

- desogestrel, gestodene and norgestimate)
- Generation IV (introduced at the beginning of the 21st century it includes drospirenone)

The complete history of the discovery of steroid hormones and their gradual application in various fields of science is extensive and complex, but it can be summarised by a few key research achievements in medicine (Fig. 1). At the same time, there was a gradual development of new methods of regulating the growth and development of farm animals in veterinary medicine using synthetic steroid hormones, which led to numerous changes in the global steroid hormone market and in the natural environment itself (Fig. 2).

In recent years, there has been an increase in demand for synthetic steroid hormones, contributing to the continued growth of this market segment. The global market for hormone therapy preparations grew from USD 12.01 billion in 2022 to USD 12.66 billion in 2023, reflecting a compound annual growth rate (CAGR) of 5.4%. Analysts of the synthetic hormone market in the Asia-Pacific region forecast growth from USD 2.41 billion in 2023 to USD 4.89 billion in 2033, with an estimated compound annual growth rate (CAGR) of 7.3% (GlobeNewswire 2023). In 2022, North America held the largest share of the steroid hormone market, while the Middle East and Africa currently stand out as some of the fastest growing regions, actively implementing innovative therapies involving steroid hormones (GlobeNewswire 2023).

1. Sources of Steroid Hormone Pollution in the Aquatic Environment

Steroid hormones enter the environment through multiple routes, but most commonly through sewage and drainage systems from large factories, industrial plants and households (Zhang et al. 6.2021). Wastewater from animal husbandry is subject to much less stringent treatment than municipal wastewater, and natural methods of spreading animal manure on fields often lead to even greater amounts of steroid hormones being released into the natural environment (Combalbert et al. 2011). While hormones may undergo partial degradation in water, many of them are highly persistent and capable of bioaccumulating, which facilitates their circulation in aquatic ecosystems and soils (Schoenborn et al. 2015).

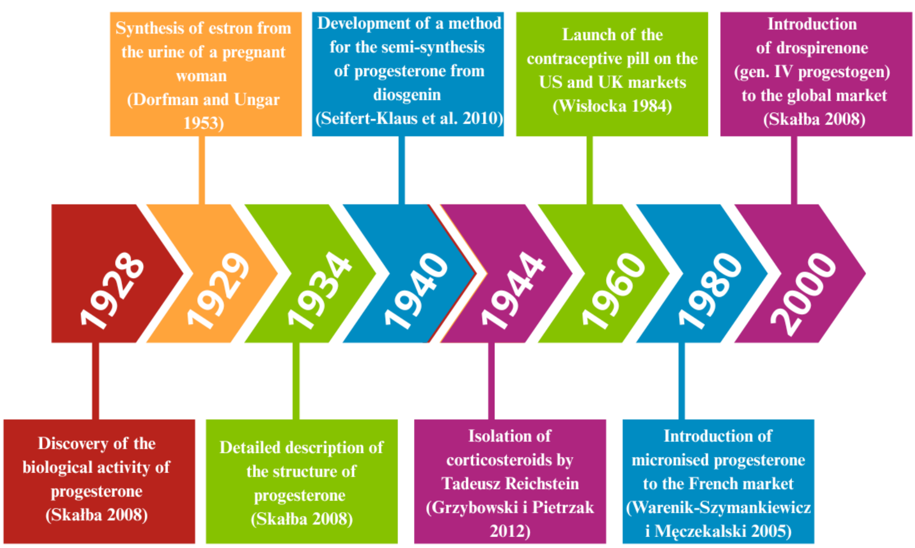

Figure 1. A timeline showing the most important events in the history of steroid hormones in medicine

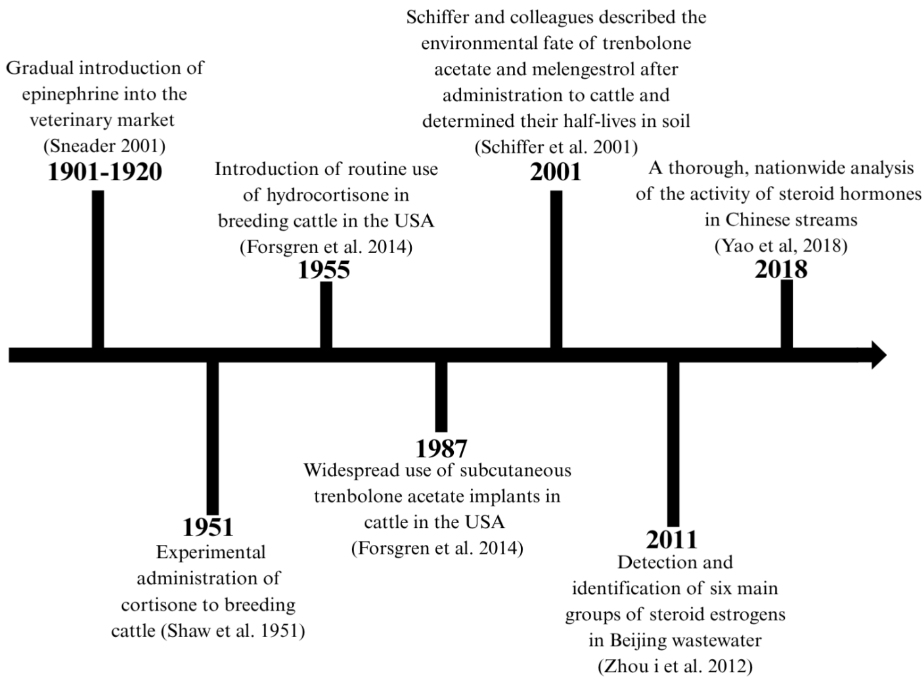

Figure 2. Graphical visualization in the form of a timeline of the most important events related to the history of steroid hormones in veterinary medicine and research into the effects of steroid hormones on the environment in Asian countries

In addition, many farmers use manure from these farms because of its significantly lower market price, which means that many oestrogens, progestogens and androgens end up in arable soils, from where they are then washed out with precipitation (Zhang et al. 6.2021). Although many modern animal farms, such as pig, cattle and poultry farms in South Asian countries, are equipped with integrated wastewater treatment systems, a significant proportion of steroid hormones still find their way into nearby water bodies. This is largely due to the limited effectiveness of current technologies in removing these compounds from livestock wastewater, leading to their gradual accumulation in the natural environment (Zhang et al. 3.2021). It should also be noted that only 17% of all steroid hormone emissions into the natural environment originated from humans in 2015 (Zhang et al. 6.2021). Due to the process of biomagnification – the accumulation of exogenous substances in the food chain via the consumption of aquatic organisms by terrestrial vertebrates or humans – the threat posed by steroid hormones is not limited to aquatic organisms alone (Schoenborn et al. 2015).

2. The Effects of Steroid Hormones on Aquatic Organisms and Aquatic Ecosystems

In recent years, the concentrations of steroid hormones in the aquatic environment have increased significantly, and their negative impact on reproduction has been observed in many aquatic organisms, as evidenced by declining fish and amphibian populations in European countries (Ojoghoro et al. 2021). Due to its chemical properties, water is one of the best solvents, enabling hormones to easily penetrate the tissues of living organisms and cause hormonal imbalances. High concentrations of exogenous hormones in an animals disturb normal metabolism. The endocrine glands, under the strong influence of steroid hormones, may become impaired, resulting in

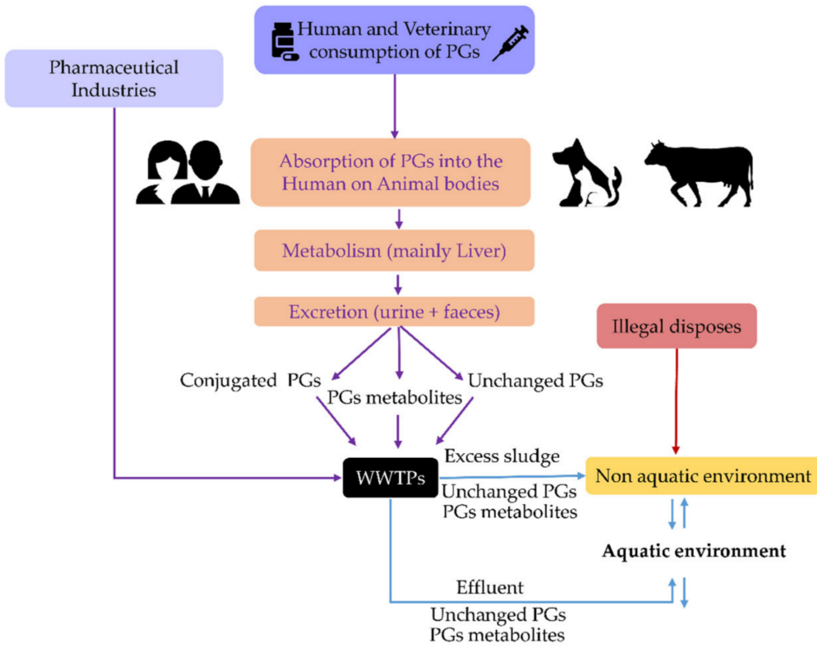

Figure 3. Examples of progestin pathways into the natural environment, taking into account the breakdown of progestogens (Source of the illustration: Rocha and Rocha (2022), Figure 3, based on literature analysis, including Besse and Garric (2009)

organism's inability to produce its own sex hormones, thus disrupting the entire hormonal balance of fish (Ciślak et al. 2023; Kugathas and Sumpter 2011; Forsgren et al. 2014). In amphibians, natural progestogens play an essential role in the development and differentiation of gonads, the maturation of oocytes through the breakdown of the primordial follicle, and the maintenance of normal homeostasis of the hypothalamic-pituitary-gonadal axis (Ziková et al. 2017). Exogenous hormones (both progesterone and oestrogens) disrupt these processes, leading to reduced fertility and lower offspring numbers in amphibians. The effects of steroid hormones on aquatic invertebrates were already demonstrated in 2010, when an archaic progesterone receptor was discovered in representatives of the micro-invertebrate species *Brachionus manjavacas* (Rotifera). This study proved that progesterone and its receptor retain their function in many animals in many phylogenetic lineages, confirming the ancient origin of steroid hormone regulation in both aquatic and terrestrial animals (Stout et al. 2010). In the flatworm *Girardia tigrina*, the effects of steroid hormones are most often studied in relation to the regenerative abilities of these planarians (Kudikina et al. 2019). High concentrations of oestradiol stimulated the regrowth of severed body parts under laboratory conditions, whereas elevated testosterone concentrations significantly impaired the regenerative capacity of flatworms (Kudikina et al. 2019).

The half-life of many sex hormones and glucocorticoids in aquatic animals can last up to 60 hours after initial exposure to elevated concentrations of exogenous hormones (Moschet and Hollender 2009). It should also be noted that the half-life of steroid hormones, both in water and in living organisms, varies depending on the season. This variation is related to the reduced metabolic rate of many aquatic organisms during winter, which enables survival in low temperatures. Consequently, all mechanisms responsible for removing exogenous chemicals from the body are also slowed down by low temperatures (Ciślaket al. 2023). In addition, the degradation rate of steroid hormones in anaerobic sediments is significantly lower than under aerobic conditions, leading to the accumulation of higher concentrations of these hormones in the muddy sediments of rivers and lakes (Moschet and Hollender 2009). Although similar in chemical structure, steroid hormones differ in potency. Oestradiol (E2), the principal ovarian oestrogen, is approximately 12 times more potent than oestrone (E1) and 80 times more potent than oestriol (E3) (Guyton and Hall 2006). Progesterone and testosterone display potencies similar to oestradiol, although their ultimate physiological effects depend on the species and sex of the organism (Guyton and Hall 2006). Both the high biological activity of individual steroid hormones and the absence of antagonistic interactions when they occur simultaneously (e.g. in sewage mixtures) pose a significant threat to aquatic ecosystems in many reservoirs (Runnallis et al. 2013).

Research shows that hormone concentrations in bottom and surface water do not differ significantly in freshwater environments (Moschet and Hollender 2009). In contrast, in saltwater environments, steroid hormones sink more readily to the seabed and accumulate in sediments. As a result, the effects of steroid hormones are usually much more severe for benthic or burrowing animals (Moschet and Hollender 2009). Steroid hormones disrupt the normal functioning of many organisms in both freshwater and marine systems. Studies have shown that steroid hormones play an important role in regulating interactions between the endocrine and immune systems in the marine invertebrate *Mytilus galloprovincialis* (Porte et al. 2006). The presence and role of steroid hormones in the regulation of reproductive system development has been confirmed in the *Octopus vulgaris*: exposure to steroid hormones promotes the preparation of adults for egg-laying, ensuring adequate reserves for embryo development (Di Cosmo et al. 2011). However, rising concentrations of oestrogens and progestogens may cause population declines in octopuses by disrupting vitellogenesis in adult females. An interesting case involves the teleost fish *Fundulus heteroclitus*, which appears to be extremely sensitive to even relatively low concentrations of steroid hormones (1ng/l). Administration of androgens during experiments significantly reduced the amount of naturally produced steroid hormones in fish, which in turn led to the disruption of the entire endocrine system of the fish (Sharpe et al. 2004).

3. Detected Ranges of Steroid Hormone Concentrations in the Aquatic Environment in the EU and Asian Countries

Over the past decade, concentrations of steroid hormones in European surface waters have increased significantly (Grzegorzek et al. 2024). Despite this, European countries still maintain some of the cleanest surface waters worldwide in terms of hormonal pollution. Oestrogen concentrations in the European aquatic environment show significant spatial variability. For example, in Portugal (2018), in the waters of the Aveiro Lagoon, a concentration of 17β-oestradiol (E2) of 120 ng/L was recorded, which is a relatively high concentration (one of the highest) among all EU member states. In Switzerland (2019), in the tributaries and ponds of the Baldegg Lake catchment area, the concentrations ranged from: oestrone (E1) 0.76-5.2 ng/L, 17β-oestradiol (E2) 0.59-10.2 ng/L and oestriol (E3) 0.78-1.8 ng/L. In Belgium (2020), in the Sambra, Ourthe and Lomme rivers, E1 concentrations of 0.275-0.754 ng/L and E2 concentrations of 0.053 to 0.095 ng/L were measured. In Italy (2018), in the rivers of the Apennines and groundwater in the Romagna region, the concentration of E1 reached 6.9 ng/L. In Poland, on the other hand, in groundwater samples from the Krakow area (2019), E1 was detected at a concentration of 5.4 ng/L (according to Ciślak et al. 2023; Jurado et al. 2022; Zhang et al. 2021). The causes of these pollutants vary from region to region. In Romania and other former Eastern Bloc countries, the problem stems mainly from the inadequate adaptation of wastewater treatment infrastructure to current, demanding EU standards; there is a lack of modern, technologically advanced and costly solutions (Lalik et al. 2025). In Portugal, by contrast, pollution is largely the result of intensive livestock farming and seasonal increases in pressure on the aquatic environment caused by the influx of tourists (Tang et al. 2021). Recent studies also show that inland seas, such as the Black Sea and the Sea of Marmara, are heavily polluted with progestogens and oestrogens. The highest concentrations of hormones in these seas, recorded in marine sediments, range from 40 ng/g to as much as 1600 ng/g (Aysel et al. 2023). Such elevated values indicate a long-term process of chemical accumulation resulting from improper sewage management.

On the other side are South Asian countries, where steroid hormone concentrations are significantly higher than in Europe (Ojoghoro, et al. 2021). Record progestogen concentrations recorded in China reached 677 ng/l (Table 1). Particularly concerning are the substantial quantities of substances used in animal production, including synthetic anabolic steroids and growth stimulants, which are added to feed and supplements to accelerate muscle growth and sexual maturation in farm animals. These substances enter both domestic and industrial wastewater and, ultimately, due to insufficiently effective treatment processes, in the aquatic environment, despite the existence of many large and technologically advanced wastewater treatment plants (Liu et al. 2012; Yang et al. 2020). An analysis of pollution sources based on the profiles of detected steroid compounds showed that 54.5% of steroid hormones in water bodies in China came from freshly discharged, untreated sewage. Oestrogenic bioactivity indices ranged from 0.01 to 40.27 ng/L, and among the three oestrogens studied (E1, E2, E3), the largest contribution to total oestrogenicity was attributed to 17β-oestradiol (E2), whose share was estimated at 82.8% (Yao et al. 2018).

The situation is even more serious in India, where the wastewater treatment system is poorly developed and severely outdated. Nearly one-third of global emissions of animal-derived hormone pollutants are concentrated in India and Brazil. India, however, stands out as the country with the highest emissions of anthropogenic steroids – more than twice as high as the emissions of all EU member states. The scale of their global surface water pollution was estimated based on the volume of emissions to the aquatic environment and hydrological data on annual river flow (Zhang et al. 6.2021).

Table 1. Couple of the highest concentrations of steroid hormones recorded in surface waters in South Asian countries (Adapted from, Table 3. Ojoghoro et al. 2021)

Class	Steroid	Conc. (ng/L)
Oestrogen	E2	38.1
Glucocorticoids	20α-Dihydrocortisone	54.0-138.0
Glucocorticoids	3β,5β-Tetrahydrocortisol	1.3-69.0
Glucocorticoids	3α,5β-Tetrahydrocortisol	3.9-103.0
Glucocorticoids	Tetrahydrocortisol	2.3-115.0
Glucocorticoids	3α,20β-Control	0.33-93.0
Glucocorticoids	α-Cortolone	1.4-127.0
Glucocorticoids	β-Cortolone	4.1-161.0
Progestogens	Norgestrel	253-677
Progestogens	Progesterone	375

4. Current Methods and Techniques for Monitoring Pollution and Treating Wastewater, Environmental Risk Assessment

Research is currently underway to identify the most effective methods for treating municipal, industrial and domestic wastewater containing high concentrations of steroid hormones. Conventional wastewater treatment systems are primarily designed to eliminate nitrogen, carbon and phosphorus compounds, although in some cases partial removal of other substances – including microplastics, pharmaceuticals and steroid hormones – has also been reported. However, studies show significant variation in the effectiveness of steroid hormone removal, with removal efficiency ranging from 0% to 99%, depending on factors such as the location of the treatment plant, the type of technology employed and the initial concentrations of these compounds (Yazdan et al. 2022). Typical wastewater treatment plants involve three basic stages: preliminary, primary and secondary treatment. Where treated wastewater is discharged into surface water or groundwater an additional tertiary stage of treatment is required (Fig. 4).

The two most common methods used to treat wastewater containing steroid compounds are the microbial mat method and the activated sludge method. In the case of microbial mats, selectively chosen bacteria, arranged in a structured biofilm, absorb pollutants from the flowing water into their cells, after which the used mats are disposed of (Chibata and Tosa 1977). The activated sludge method, on the other hand, involves the decomposition of organic pollutants in the presence of oxygen. This process takes place in an aeration chamber, and the sludge is then separated from the treated water in a secondary settling tank. This technology is highly effective in removing organic pollutants but requires continuous aeration and precise control of biological parameters. For this reason, its implementation may be

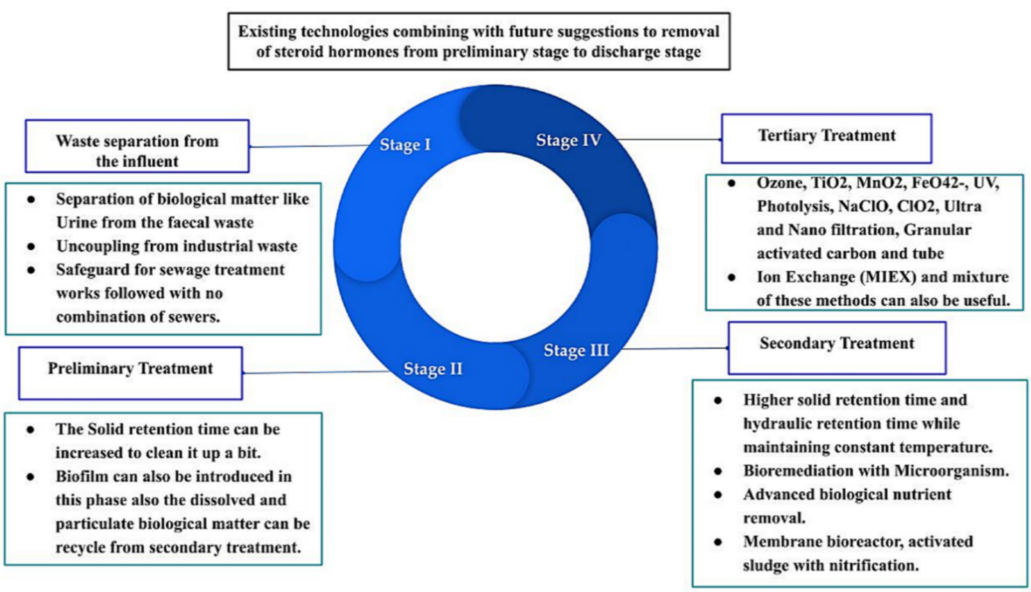

Figure 4. Graphical representation of existing technologies in combination with future proposals for the removal of steroid hormones from the preliminary stage to the stage of wastewater discharge from wastewater treatment plants
Source of the illustration: Yazdan M. et al. 2022, Figure 3.)

Table 2. Efficiency of steroid hormone wastewater treatment using biological methods

Biological Treatment Method	Removal Efficiency (%)
Conventional activated sludge process (ASP)	>65
Moving bed biofilm reactor (MBBR)	84.8
Sponge-based MBBR	89.6-96.2

Adapted from Table 2, Almazrouei et al. 2023.

difficult in less developed countries, where access to advanced infrastructure and energy resources is limited (Chibata and Tosa1977). In response to the growing need for effective and accessible methods of removing steroid hormones from wastewater, new surface water treatment technologies are being developed. Advances in analytical chemistry now allow for the determination of the quantity and complexity of molecules in wastewater, as well as their interactions, thereby enabling more targeted solutions for removing specific groups of steroid hormones (Sosa-Ferrera et al. 2012). One promising and innovative technology is the use of perovskite oxides as materials capable of effectively capturing and eliminating chemical pollutants from the aquatic environment (Bayode et al. 2024). Although this technology is still in the development stage and has not been fully optimised for the removal of specific steroid hormones, preliminary studies indicate its great potential. The high reactivity of perovskite oxides in the degradation of organic compounds structurally and functionally similar to steroid hormones makes this method an attractive alternative to conventional wastewater treatment techniques (Bayode et al. 2024). The European Union's 2022 Water Directive introduces stricter regulations on the presence of micropollutants in wastewater, including steroid hormones (Zembrzuska et al. 2025). Under the new requirements, companies must monitor and report in detail on the levels of pollutants such as pharmaceuticals, hormones and microplastics entering the sewage system. A key element of the new regulations is the introduction of a mandatory fourth stage of treatment (Fig. 4), designed to ensure to ensure more effective removal of persistent micropollutants, supplementing conventional treatment methods (Table 2). This measure represents an important step towards the enhanced protection of aquatic ecosystems and public health (Zembrzuska et al. 2025).

Conclusions

Steroid hormones pose a threat to the natural environment worldwide. Despite considerable efforts, estimating their actual concentrations in surface waters remains extremely challenging due to the many variables that influence the final result of the tests (e.g. the specific section of a river or watercourse from which samples are collected). New methods for detecting steroid compounds in water offer broader possibilities in the fight against surface water pollution. Both Europe and Asia face serious problems with the impact of steroid hormones on aquatic organisms, although the underlying causes differ. In European countries, despite the widespread use of modern wastewater treatment technologies, it remains impossible to completely eliminate pollutants, including endocrine-disrupting substances, before they are released into the aquatic environment. In many Asian countries, on the other hand, sanitary infrastructure is insufficiently developed: the number of modern wastewater treatment plants is limited, and in some countries, such as India, a significant proportion of municipal and industrial wastewater is discharged into the environment without any treatment due to the lack of adequate treatment systems. Extensive research into the effects of steroid hormone pollution on entire aquatic biocoenoses also seems necessary. There is still insufficient research on this topic, and steroid hormone concentrations in both the EU and Asia are constantly increasing, causing further changes in the behaviour and functioning of aquatic organisms. Another challenge is to develop wastewater treatment methods for steroid hormones that is not only highly effective but also inexpensive to implement.

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