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Review

Assessment of Endocrine-Disrupting Properties in Cosmetic Ingredients: Focus on UV Filters and Alternative Testing Methods

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Abstract

Endocrine-disrupting chemicals are substances capable of interfering with hormonal systems, potentially leading to adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife. Various experimental models are currently available to assess the endocrine-disrupting potential of substances. However, in the context of cosmetic ingredients, the ban on animal testing for safety and efficacy evaluations in Europe and other regions necessitates the use of in vitro or in silico approaches. Concerns have been raised regarding the possible endocrine-disrupting properties of certain cosmetic compounds, prompting the development of a priority substance list that includes several ultraviolet (UV) filters. This review provides a comprehensive overview of the main methodologies employed to evaluate endocrine-disrupting effects, with a particular focus on different endocrine organs. It also compiles and analyzes literature data related to commonly used UV filters such as benzophenones, avobenzone, homosalate, octocrylene, octinoxate, and 4-methylbenzylidene camphor. A major limitation identified is the lack of validated in vitro methods for assessing disruptions in specific endocrine organs, such as the thyroid and pancreas. This gap hinders accurate interpretation of experimental results and highlights the urgent need for further research to clarify the safety profiles of UV filters and other cosmetic ingredients.

Keywords: endocrine-disrupting chemicals; ultraviolet filter; in vitro methods

Academic Editor: Enzo Berardesca

Received: 15 July 2025
Revised: 10 August 2025
Accepted: 12 August 2025
Published: 16 August 2025

Citation: Maddaleno, A.S.; Guardia-Escote, L.; Vinardell, M.P.; Teixidó, E.; Mitjans, M. Assessment of Endocrine-Disrupting Properties in Cosmetic Ingredients: Focus on UV Filters and Alternative Testing Methods. *Cosmetics* **2025**, *12*, 175. https://doi.org/10.3390/cosmetics12040175

1. Introduction

Endocrine-disrupting chemicals (EDCs) are substances that can interfere with the hormonal systems of organisms, leading to adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife [1].

These compounds can mimic or block hormones, disrupting the delicate balance of the endocrine system. Cosmetics, which are widely used personal care products, can contain a variety of ingredients that have been identified as EDCs, raising concerns about their potential health impacts, particularly to vulnerable populations such as pregnant women, children, and individuals with weakened immune systems [2]. The European Commission launched a call for data on cosmetic ingredients that might have had endocrine-disrupting properties. The aim was to collect scientific evidence to support the evaluation of certain

substances used in cosmetics. This information was intended to help assess potential health risks related to hormonal disruption and to guide future regulatory actions within the European Union [3]. In oncology, endocrine disruptors have been associated with breast, testicular, and prostate cancer [4]. The increasing awareness of the presence of EDCs in cosmetics has prompted consumers and regulatory bodies to scrutinize the safety of these products. Many cosmetic ingredients, such as parabens, phthalates, and certain synthetic fragrances, have been linked to hormonal disruptions, leading to debates about their continued use in consumer products. In response to public concern, some companies have begun reformulating their products to eliminate or reduce the presence of these harmful substances, while regulatory frameworks are being reassessed to better protect consumers from potential risks associated with endocrine disruptors in cosmetics [2].

1.1. Endocrine System and EDCs

The endocrine system, together with the nervous system, is responsible to regulate the physiological functions of the body by secreting hormones to bloodstream [5]. It is composed of various endocrine glands (Figure 1), as the pituitary and pineal glands at the encephalon, the thyroid and parathyroid glands in the neck and the adrenal glands in the abdomen next to the kidneys, besides the gonads (ovaries and testes) and certain parts the pancreas [6]. However, other non-endocrine tissues and organs also include cells that can synthetize and release hormones such as the heart (atrial natriuretic peptide), adipose tissue (leptin, adiponectin), the intestine or kidneys (erythropoietin, calcitriol, renin) [7,8].

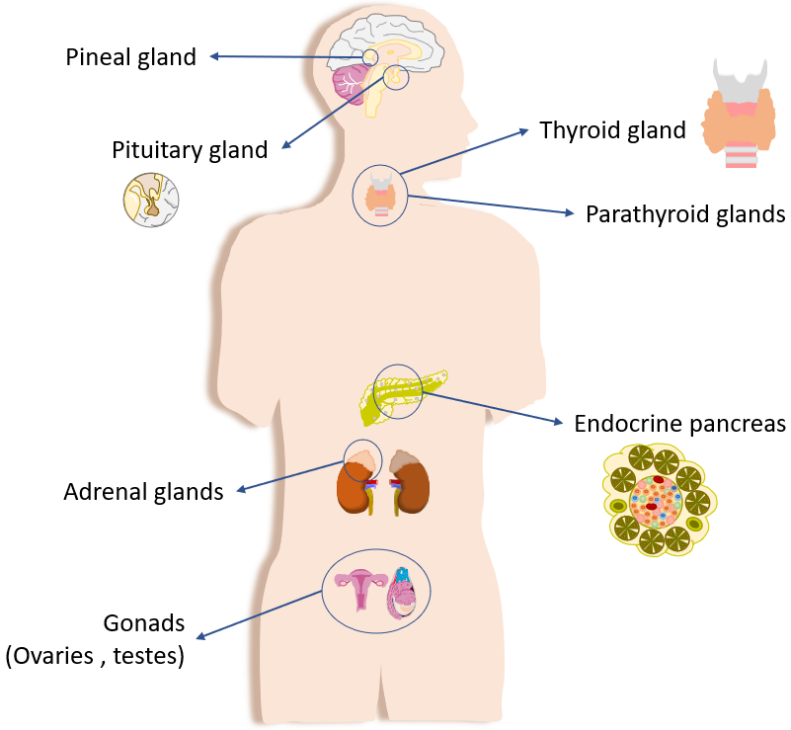

Figure 1. Main glands of endocrine system.

While endocrine disruptors have the potential to interfere with multiple hormone systems, the majority of studies have focused on a limited subset of endocrine glands, with thyroid and reproductive hormone systems representing the most frequently reported targets of disruption [8]. In this section, we address the main elements of the endocrine system involved in chemical-induced disruption.

1.1.1. Hypothalamic–Pituitary–Thyroid (HPT) Axis

The thyroid gland is essential for regulating metabolism, growth, and development, and its function is influenced by endogenous factors like thyroid-stimulating hormone and iodide. However, synthetic chemicals, known as thyroid system disrupting chemicals (TSDC), including industrial pollutants and pesticides, can disrupt thyroid hormone balance, posing risks to human health. These chemicals interfere with hormone synthesis, signaling, metabolism, and excretion, leading to adverse effects like neurological and metabolic disorders [9]. Growing concerns over thyroid-related disorders prompted regulatory guidance in 2018 to improve the identification of endocrine disruptors, emphasizing mechanistic studies [10].

Currently, the collected human data in different epidemiological and observational studies present inconsistent associations between thyroid function parameters and potential thyroid disrupting agents making it difficult to determine whether these associations reflect true endocrine disruption or are the result of confounding factors. For example, in the case of triclosan while some studies report alterations in triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) concentrations, others find no significant effects, likely due to variations in study design, exposure levels, population characteristics, and the timing of hormone measurements [11,12]. Similarly, Coperchini et al. in 2021 found that the clinical relevance of thyroidal function found with exposition to polyfluoroalkyl substances is still unclear [13]. For this reason, mechanistic studies are essential to establish biological plausibility and to delineate dose–response relationships. Such studies can also help identify critical windows of susceptibility, such as during fetal development or adolescence, during which thyroid disruption may have lasting consequences. Ultimately, integrating mechanistic data with epidemiological evidence will be crucial for the accurate identification and regulation of thyroid-disrupting chemicals.

1.1.2. Parathyroid Glands

Parathyroid glands regulate calcium and phosphate plasma levels by the secretion of parathyroid hormone (PTH) together with vitamin D. Consequently, PTH plays an important role in the homeostasis of physiological functions such as bone metabolism or nerve transmission. Although primary hyperparathyroidism is a relatively common endocrine disorder, its increased incidence has risen concerns about the potential role of environmental factors including chemical pollutants [14]. However, methods to identify parathyroid EDCs are not yet addressed and solely epidemiological studies are found in the literature. One explanation could be that the mechanism by how such pollutants altered the secretion of PHT still needs clarification. Some authors indicated that modifications in PHT levels can be attributed to alteration in Ca^{2+} transport and metabolism, while others to a direct (parathyroid) or indirect (renal) cell toxicity and finally to inactivation of vitamin D receptors [14–16].

1.1.3. Hypothalamic–Pituitary–Adrenal (HPA) Axis

The hypothalamic–pituitary–adrenal (HPA) axis represents a core component of the stress response system, contributing to the regulation of physiological homeostasis and the modulation of responses to endogenous and exogenous stressors [17].

The mammalian adrenal gland secretes steroid hormones, particularly glucocorticoids such as cortisol, predominantly regulated by adrenocorticotrophin (ACTH) released from the anterior pituitary. ACTH secretion is controlled by hypothalamic corticotrophin-releasing hormone and arginine vasopressin. The HPA axis is self-regulated through negative feedback exerted by cortisol on both the hypothalamus and pituitary [18].

As summarized by Yilmaz et al. in 2020, studies have reported that EDCs can affect adrenal gland function and hormonal regulation, particularly by interfering with the biosynthesis and metabolism of steroid hormones [19].

The inhibition of the steroidogenic pathways is the major mechanism of action by EDCs regarding to the adrenal cortex [20].

The in vivo assessment of disruptions in the HPA axis presents significant challenges. The process of sampling individuals causes significant stress, which is itself a major activator of the HPA axis [18]. Moreover, some commonly used animal models lack the CYP17 enzyme, rendering them incapable of synthesizing cortisol. This limitation highlights their utility as models for identifying compounds that specifically target this critical enzyme in steroidogenesis [20].

1.1.4. The Hypothalamic–Pituitary–Gonad (HPG) Axis

The hypothalamic–pituitary–gonad (HPG) axis regulates the hormones, driving the growth and maturation of germ cells, and the synthesis of gonadal steroids in female and male gonads [21].

Hypothalamus releases gonadotropin-releasing hormone (GnRH) which stimulates the pituitary glands to secrete the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH). In the female reproductive system, LH and FSH are essential for the production of estrogens and progesterone. LH acts upon thecal and granulosa cells in the ovary, and FSH regulates estradiol and 17b-HSD-1 [22]. In males, LH acts on Leydig cells in the testes, stimulating the production of testosterone and insulin-like peptide 3 (INSL3), while FSH acts on Sertoli cells in testes, which release other hormones and support spermatogenesis. Testosterone acts by binding to the androgen receptor (AR). The primary source of androgens is the testes, whose development and function are controlled by hormonal actions through both endocrine and paracrine pathways [23]. While testosterone and estrogen are conventionally considered the primary sex hormones for males and females, respectively, estradiol—a major estrogen derivative—serves an essential regulatory function in male sexual physiology [24].

EDCs can affect female gonads by altering estrogen (E) signaling pathways and interacting with estrogen receptors (ER). In male gonads, these compounds may disrupt hormonal function through interference with androgens (A) and their binding to AR. Studies revealed that EDCs can act as agonists and can bind and activate various hormonal receptors such as AR, ER, estrogen-related receptor (ERR) or aryl hydrocarbon receptor (AhR), chimeric antigen receptor (CAR) and pregnane X receptor (PXR). Moreover, EDCs can be antagonistic by binding to the receptors [25].

Folliculogenesis is considered the primary biological process affected by EDCs in relation to female reproductive system development. EDCs may lead to infertility by interfering with the development of follicles from the primordial to the antral stage. EDCs can cause miscarriage, endometriosis, cancer, semen quality alteration and testicular dysgenesis [21]. Overall, the disruption of the HPG axis by EDCs highlights their capacity to interfere with key regulatory pathways that govern sexual development and reproductive function in both sexes. Understanding these mechanisms is essential for evaluating the potential risks of EDC exposure on human fertility and for establishing effective strategies to mitigate their impact.

1.1.5. Pancreas

An increasing body of research indicates a connection between EDC exposure and the onset of various pancreatic disorders, including diabetes, pancreatitis, and pancreatic cancer [26–28]. This is the case of a widespread endocrine disruptor as bisphenol-A that

impacts the mouse endocrine pancreas. BPA produces rapid non-genomic effects on insulin-secreting beta-cells and glucagon-secreting alpha-cells in freshly isolated pancreatic islets of Langerhans. Disruption of blood glucose homeostasis resulting from BPA exposure may increase the susceptibility to type II diabetes [29].

2. Overview of the Main In Vitro Current Models for Identifying Endocrine-Disrupting Activity

The main in vitro assays for detecting EDCs are grouped according to the hormonal mechanism of action they assess (e.g., estrogenic, androgenic, or thyroid activity, or interference with steroidogenesis). Many are validated or included in the battery of assays recommended by the OECD (Organisation for Economic Co-operation and Development Conceptual Framework for Testing and Assessment of Endocrine Disruptors).

Several laboratories are developing methods to study EDCs, however a recent study by the EURL-ECVAM concluded that most of the methods require updating, revision and optimization before they can be reliably implemented across different laboratories. To help in this exercise, specific training by the method developer was requested for some of these methods [30].

Such alternative test methods are needed to infer a causal relationship between molecular initiating events and adverse outcomes such as perturbed brain development. There is still a need to develop more extensive alternative test batteries to cover the range of potential key events along the causal pathway between initial chemical disruption and adverse outcomes in humans [31].

Under the new paradigm, toxicity is initiated when a chemical interacts with and alters the structure or function of a molecular target. This interaction sets off a series of events that propagate through cellular, tissue, organ, and organismal levels, ultimately linking the initial molecular disturbance to an adverse effect. The Adverse Outcome Pathways (AOP) framework systematically organizes relevant knowledge, such as that derived from validated methods, by mapping the relationship between the molecular initiating event (the molecular target) and a sequence of key events (KE) that constitute a causal chain leading to an adverse outcome [32,33]. The KE are casually linked to the adverse outcome under consideration, and they are measurable. Adverse outcomes are typically the endpoints reported in studies conducted following standard in vivo OECD Test Guidelines, though they may also arise from findings in other toxicological or epidemiological research. Within an AOP, the linkage between an upstream and a downstream KE is defined as a key event relationship (KER). KERs capture empirical and mechanistic evidence supporting the causal association between the two KEs, including detailed descriptions of the underlying biological processes—referred to as biological plausibility—that mediate this connection [34].

Different AOPs have been proposed recently for identifying endocrine disruptors at different levels [35–38].

2.1. Reproductive System

EDCs can interfere with the reproductive system by affecting the synthesis, action, or metabolism of sex hormones. To assess these effects, a variety of in vitro assays have been developed to detect interactions with hormone receptors, disruptions in steroidogenesis, or functional responses in target cells.

2.1.1. Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS) Assay

The ISO 19040-1:2018 [39] and ISO 19040-2:2018 [40] describe methods for the determination of estrogenic potential of water and wastewater. These methods use a reporter

gene in genetically modified yeast strains *Saccharomyces cerevisiae* or *Arxula adeninivorans*, respectively. The ISO 19040-3:2018 [41] is a standard that describes an in vitro bioassay using genetically modified human cells that express the human estrogen receptor alpha (hERα) along with a reporter gene (typically luciferase). When estrogenic substances are present, they bind to the receptor and activate the expression of the reporter gene. The resulting signal (e.g., luminescence) is proportional to the estrogenic activity of the tested sample, which allows for sensitive and quantifiable detection [41]. However, the assay only detects activation of the estrogen receptor alpha and does not identify other mechanisms of endocrine disruption (e.g., antagonism or other receptor pathways).

The XenoScreen® YES/YAS assay (Xenometrix AG, Allschwil, Switzerland) is a commercially available in vitro method designed to detect estrogenic and androgenic activity using genetically modified *Saccharomyces cerevisiae* strains. These yeast strains express the hERα or androgen receptor (hAR) along with a reporter gene, typically encoding β-galactosidase. Upon binding of a hormonally active compound to the receptor, the reporter gene is activated, allowing quantification of receptor-mediated transcriptional activity through colorimetric detection.

This assay is commonly applied to assess endocrine activity in various matrices, including environmental samples, chemicals, pharmaceuticals, and cosmetic ingredients. Unlike the ISO 19040 series [39–41]—specifically developed for the testing of aqueous samples—the XenoScreen® YES/YAS assay enables the direct testing of a broad range of substances, including pure compounds and complex formulations, offering greater flexibility in screening applications.

This assay has been widely applied in the evaluation of endocrine-disrupting activity of various chemicals. For instance, it has been used to assess the estrogenic potential of phthalates [42], the effects of triclosan and parabens [43], and to investigate how chitosan-modified TiO_2 nanoparticles reduce estrogen (E2) levels and exhibit anticancer properties by inhibiting MCF-7 breast cancer cell growth [44]. Additionally, the assay has contributed to safety assessments of parabens, supporting their evaluation as non-hazardous at relevant exposure levels [45].

The XenoScreen® YES/YAS assay is a widely used in vitro method for detecting estrogenic and androgenic activity in various matrices, including environmental samples, pharmaceuticals, and cosmetics. Unlike the ISO 19040 series [39–41], which is specifically designed for aqueous samples, this assay allows direct testing of pure compounds and complex formulations, offering greater flexibility. Additionally, it has been successfully applied to assess a broad range of hormonally active substances. However, compared to ISO standards using human cells (e.g., ISO 19040-3) [41], the use of yeast strains may limit physiological relevance.

2.1.2. Cell Proliferation Assay (E-SCREEN Assay)

The E-SCREEN assay was developed by Soto et al. in 1995 [46] to identify estrogenicity of chemicals using the proliferative effect of estrogens on specific cells. Estrogens have the ability to induce the proliferation of cells of the female genital tract. To this end, the number of MCF-7 cells (breast cancer cell line) is compared under estrogen-free conditions, in the presence of 17β-estradiol (used as a positive control), and across a range of concentrations of the test chemicals [46].

The biologically relevant approach of the E-SCREEN assay directly reflects the proliferative effect of estrogens on target cells, making it useful for identifying hormonally active compounds. However, as it relies on cell proliferation, it may be influenced by cytotoxicity or non-specific mitogenic effects of test substances, which could interfere with result interpretation.

2.1.3. Transactivation System with Stably Transfected Cells to Detect Estrogen Receptor Agonists or Antagonists

OECD Test No. 455 is a performance-based guideline that describes in vitro assays using stably transfected transactivation systems to identify compounds that act as agonists or antagonists of the ER. This guideline includes mechanistically and functionally similar test methods, aiming to support the development of new or improved assays based on the same biological principle. It describes two reference test methods using the (h)ERα-HeLa-9903 cell line and the BG1Luc-4E2 cell line, which express ER and have been stably transfected with an ER responsive luciferase reporter gene [47].

These assays offer a mechanistically relevant and standardized approach for identifying estrogenic activity. The guideline promotes harmonization and supports assay development using consistent biological principles. However, as with other in vitro receptor-based assays, it may not capture effects requiring metabolic activation or involving non-receptor-mediated pathways.

2.1.4. Estrogen Binding Affinity Assay

The OECD test guideline 493 describes an in vitro method using human recombinant estrogen receptors (hrER) to detect chemicals with ER binding affinity. The guideline presents two mechanistically and functionally equivalent protocols designed to identify compounds that bind to the ER. These reference methods also support the development of new or adapted assays based on similar principles. The two key protocols forming the basis of this performance-based guideline are: the Freyberger–Wilson (FW) in vitro Estrogen Receptor Binding Assay, which uses the full-length human recombinant ERα, and the Chemical Evaluation and Research Institute (CERI) assay, which utilizes the ligand-binding domain of the human ER.

The main goal of the assay is to evaluate a test chemical's ability to compete with a radiolabeled ligand ([^{3}H]17β-estradiol) for binding to the ER. The test includes two main steps: a saturation binding experiment to determine receptor–ligand binding parameters and confirm ER specificity, followed by a competitive binding experiment to assess how the test compound competes with the radioligand. While primarily intended as a screening tool, the assay also provides mechanistic insights into the interaction of chemicals with the ER [48].

This assay offers valuable mechanistic insight and receptor specificity through competitive binding analysis. However, as a binding-only assay, it does not assess downstream functional responses (e.g., transcriptional activation), and its primary role remains as a screening tool rather than a stand-alone test for endocrine disruption.

2.1.5. Transactivation System with Stably Transfected Cells to Detect Androgen Receptor Agonists or Antagonists

OECD TG 458 describes in vitro assays using Androgen Receptor TransActivation (ARTA) to detect AR agonists and antagonists. This assay is based upon the transcription and translation of a reporter gene, such as luciferase gene, following binding of a chemical to the receptor and subsequent transactivation. This guideline includes assays with AR-EcoScreen™ cell line (Otsuka Pharmaceutical, Tokyo, Japan), the AR-CALUX® cell line (BioDetection Systems B.V., Amsterdam, The Netherlands), and 22Rv1/MMTV_GR-KO cell line [49].

The use of multiple validated cell lines, such as AR-EcoScreen™, AR-CALUX®, and 22Rv1/MMTV_GR-KO, supports robust detection of AR-mediated activity. However, as with other receptor-based assays, this method does not account for metabolic activation or complex endocrine interactions beyond direct receptor binding and transactivation.

2.1.6. Aromatase (Human Recombinant) Assay

United States Environmental Protection Agency (EPA) offers a series of test guidelines established by the Office of Prevention, Pesticides and Toxic Substances (OPPTS). These OPPTS are methodologies and protocols to generate data to inform regulatory decisions. A set of guidelines in the OPPTS 890 series are a full screening battery under the Endocrine Disruptor Screening Program (EDSP) to identify substances that have potential to interact with the estrogen, androgen, or thyroid hormone.

Among these guides, there are some in vitro methodologies to identify endocrine-disrupting capacity such as OPPTS 890.1200: Aromatase (Human recombinant). This as-say is part of the EPA's EDSP and serves as a Tier 1 screening tool to identify substances that may interfere with estrogen biosynthesis. This guideline describes an in vitro methodology to identify if a chemical inhibits the activity of the human aromatase enzyme (CYP19), which converts androgens to estrogens. Human recombinant aromatase, which is expressed in microsomal preparations, and a radiolabeled substrate ([1β-3H]—Androst-4-ene-3,17-dione), are used. Human recombinant microsomes are commercially available. Inhibition is assessed by measuring the release of tritiated water, which reflects enzymatic activity [50].

This assay provides a mechanistically relevant and human-specific approach to detecting interference with hormone synthesis. However, as a biochemical assay, it does not account for cellular uptake, metabolism, or downstream hormonal effects, limiting its scope to enzyme-level interactions.

2.1.7. H295R Steroidogenesis Assay

OECD TG 456 outlines an in vitro assay to assess the impact of chemical substances on the synthesis of 17β-estradiol (E2) and testosterone (T). The assay uses the human H295R adrenocortical carcinoma cell line, which expresses the genes responsible for key enzymes involved in steroidogenesis. Cells are exposed for 48 h to seven different concentrations of the test compound. Additionally, known inhibitors and inducers of hormone production are included as negative and positive controls, respectively. Hormone levels in the culture supernatant are then measured using commercial assay kits and/or analytical techniques [51].

The use of human cells expressing relevant steroidogenic enzymes enhances biological relevance. However, the assay's complexity, longer exposure time, and dependence on accurate hormone quantification can present technical challenges.

2.2. Thyroid Gland

Currently, there are no validated guidelines for assessing EDCs that affect the thyroid gland. However, various methodologies are currently under development and validation. The OECD has published recommendations for the development and use of in vitro and ex vivo thyroid assays, as well as for identifying gaps in the thyroid signaling pathways that are not yet addressed and require further research. Experts from the OECD categorized the assays into three levels depending on their current potential for inclusion in the OECD Test Guidelines work plan: Level A (in vitro/ex vivo assays that are ready for validation in the short term), Level B (in vitro/ex vivo assays that could be developed for potential validation in the long term) and Level C (assay gaps—no in vitro/ex vivo assays identified to cover a specific mode of action or disrupting pathways) [52].

Vergauwen et al. published in 2024 [53] a comprehensive inventory of test methods relevant to the assessment of thyroid hormone system disruption. The publication emphasizes the significant advancements achieved in the development of in silico models (12 identified computational approaches), in vitro assays (67 methods), and in vivo (29 assays) testing strategies. This overview describes the current methodological landscape and contributes to

the identification of existing tools and knowledge gaps that must be addressed to improve the regulatory assessment of thyroid-disrupting chemicals [53].

Moreover, in 2024, the OECD published a report summarizing the validation status of in vitro assays for assessing thyroid disruption, based on the activities of the European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL) [54]. In this section the basis of the thyroid methods from the EU-NETVAL and their validation status will be discussed.

2.2.1. Thyroid Method 1b: Thyrotropin-Stimulating Hormone (TSH) Receptor Activation Based on cAMP Measurement

Intracellular adenosine 3′,5′ cyclic monophosphate (cAMP) is produced by the activation of the G protein linked membrane based TSH receptor. TSH receptor is expressed on the basal membrane of thyroid follicle cells. The assay used TSH receptor transfected into the cell Chinese Hamster Ovary (CHO) cell line and was categorized at Level B [52]. The 2024 OECD report noted that there was currently insufficient information to support the validation of the method. Specifically, it highlighted the need to identify positive reference chemicals for the agonist mode, further develop the antagonist mode, and define appropriate positive reference chemicals for it. Addressing these gaps is essential for progressing toward future validation [54]. This indicates that the assay employs a relevant receptor and established cell system, which is promising for mechanistic studies and potential future regulatory use once validation is complete.

2.2.2. Thyroid Method 2a: Thyroperoxidase (TPO) Inhibition Based on Oxidation of Amplex UltraRed® (TPO-AUR)

The TPO inhibition assay using Amplex UltraRed oxidation is based on the protocols described by Paul et al. (2014) [55] and Paul Friedman et al. (2016) [56]. This method is designed to detect inhibitors of TPO, the enzyme responsible for catalyzing thyroid hormone (TH) synthesis. For this purpose, extracts from FTC-238 human follicular thyroid carcinoma cells transfected with recombinant human TPO are incubated with the test chemicals. TPO activity is then assessed by measuring the oxidation of Amplex UltraRed (AUR; Thermo Fisher Scientific, formerly Life Technologies, Carlsbad, CA, USA), a commercially available peroxidase substrate, which produces a fluorescent signal upon reaction [53].

The assay uses a relevant human-derived cell line expressing recombinant human TPO and a sensitive fluorescent detection method, which provides a mechanistic approach to identify TPO inhibitors. According to OECD experts, results from this assay often exhibit high variability between runs and elevated background signals. To address these issues, the EU-NETVAL laboratory optimized the standard operating procedures (SOPs) using five reference chemicals: methimazole (MMI), ethylene thiourea (ETU), rosmarinic acid, triclosan, and 2,4,6-tribromophenol. Despite these improvements, significant variability remained. Therefore, the OECD experts recommended confirming the SOP and transferring it to additional laboratories to continue the validation process [54].

2.2.3. Thyroid Method 2c: Tyrosine Iodination Using Liquid Chromatography (LC) (TYRO-IOD)

The tyrosine iodination assay using LC is based on previously described methods [57–61]. This assay measures the TPO-catalyzed oxidation of iodide, a critical step in thyroid hormone biosynthesis. The oxidized iodine species produced by TPO enable the iodination of tyrosine residues to form monoiodotyrosine (MIT) or to diiodotyrosine (DIT), on thyroglobulin within the follicular lumen of thyrocytes [52].

Specifically, the assay monitors the conversion of L-tyrosine to iodotyrosine catalyzed by TPO. This enzyme is present in extracts obtained from FTC-238 human follicular thyroid

carcinoma cells transfected with recombinant human TPO. The resulting iodinated products are quantified by LC-MS/MS.

The assay uses a sensitive and specific LC-MS/MS technique, employs biologically relevant human TPO-expressing cells, and targets key physiological steps in thyroid hormone synthesis, enhancing its relevance for detecting TPO inhibitors. According to OECD (2024), the method is considered ready for transfer to additional laboratories to assess inter-laboratory reproducibility as part of the validation process [54].

2.2.4. Thyroid Method 3a: Thyroxine-Binding Prealbumin (TTR)/Thyroxinebinding Prealbumin (TBG) Binding Using Fluorescence Displacement (ANSA) (TTR-ANSA)

In vivo exposure to EDCs, particularly those involved in thyroid disruption, has been shown to reduce serum T4 levels. This effect occurs because these chemicals can bind to thyroid hormone transport proteins, displacing T4 [62]. Cao et al. introduced an innovative fluorescence displacement method to study the binding interactions between chemicals and hormone-binding proteins [63]. This method uses a fluorescent probe, 8-anilino-1-napthalenesulfonic acid (ANSA) that remains non-fluorescent in solution but emits strong fluorescence once attached to serum transport proteins, specifically to thyroxine-binding prealbumin (TTR) and thyroxine-binding globulin (TBG). When a compound interacts with TBG or TTR at the probe's binding site, it competes for that spot, displacing the probe from the protein and leading to a measurable decrease in fluorescence [63,64]. Biological plausibility of this assay is strong due to that represents a plausible biological process that has been documented for a number of chemicals [52]. For the validation of the method, further refinement of fluorescence interference criteria is needed, followed by inter-laboratory reproducibility testing once the SOP is updated [54]. This assay provides a direct and measurable approach to detect chemical interactions with key thyroid hormone transport proteins, supporting its relevance in endocrine disruption assessment.

2.2.5. Thyroid Method 3b: Thyroxine-Binding Prealbumin Binding Using Fluorescence Displacement (TTR FITC T4)

The TTR FITC-T4 assay aims to assess whether test substances interfere with the binding of thyroid hormones to the human serum transport protein transthyretin (TTR). In this method, T4 is labeled with fluorescein (FITC-T4). If a compound competes with FITC-T4 for the TTR binding site, it displaces the labeled hormone, resulting in a decrease in fluorescence [65]. The assay provides a direct, fluorescence-based method to detect displacement of thyroid hormones from a key human transport protein, which is biologically relevant for assessing potential endocrine disruption. For the SOP, further work is needed on the criteria for interference with the fluorescence readout and also transferability to at least one or two laboratories [54].

2.2.6. Thyroid Method 4a: Deiodinase 1 Activity Based on Sandell–Kolthoff Reaction (DIO 1)

Deiodination is the major pathway regulating T3 bioavailability in mammalian tissues. Although type I deiodinase (D1) catalyzes the conversion of T4 to T3 less effectively than types II and III, D1 is considered to be the major source of circulating T3 [52]. Inhibition of Deiodinase 1 activity method measures the activity of D1 in human liver microsomes. The uninhibited enzyme liberates free iodide from the substrate and is measured using the Sandell–Kolthoff method, providing a biologically relevant and quantifiable endpoint [53]. The assay is ready to be transferred to at least one other laboratory, suggesting that further validation and reproducibility studies are still required [54]. Although the assay is biologically relevant and quantifiable, the current protocol relies on human liver microsomes, which may introduce variability due to biological source differences.

2.2.7. Thyroid Method 4b: Inhibition of THs Glucuronidation Using Liquid Chromatography/Mass Spectrometry (GLUC LC/MS)

Glucuronidation is a key metabolic pathway for T4 and T3. It is a phase II biotransformation reaction in which glucuronic acid is conjugated to lipophilic molecules, increasing their solubility and facilitating their excretion. This method evaluates the potential of test chemicals to inhibit the metabolism and excretion of T4 and T3. Thyroid hormones are incubated with human liver microsomes containing UDP-glucuronosyltransferases (UGTs), the enzymes responsible for glucuronidation. After incubation, the samples are purified and analyzed using LC-MS to separate and identify acyl- and phenolic-glucuronide conjugates [53,66]. Additional data on inactive compounds are needed to confirm the assay's specificity and ensure its transferability across laboratories [54].

2.2.8. Thyroid Method 6a: Human TH Receptor Alpha (TRα) and Human TH Receptor Beta (TRβ) Reporter Gene Transactivation Measuring Agonist Activities. (TRα and TRβ Reporter Assays)

The assay measures the activation of thyroid hormone receptor α and β using HEK293 cells transfected which expresses high-level expression of the human TRα and TRβ coupled with a highly responsive luciferase reporter gene (commercially available kit from INDIGO Biosciences). Measurements of agonist TR functional activity is measured by quantification of changes in luciferase expression [53]. OECD experts concluded that the assay is valid for the detection of TR agonists. However, investigating the detection of antagonistic activity is highly recommended [54].

2.2.9. Thyroid Method 6b: TR CALUX Human TH Receptor Beta (TRβ) Reporter Gene Transactivation Measuring Agonist and Antagonist Activities (TR CALUX)

TR CALUX is a method that asses the activation of TRβ using TRβ CALUX® cells which originated from a human osteoblastic osteosarcoma U2OS line. These cells express a functioning human TRβ coupled with a luciferase reporter gene. Chemicals with agonistic or antagonistic activities on TRβ can be detected by changes in the expression of luciferase activity [53]. Recommendations for validations are to add more active agonist and antagonist chemicals and transfer to other laboratories to confirm reproducibility [54].

2.2.10. Thyroid Method 8a: T-Screen Assay Measuring Cell Proliferation of GH3 Cells Using Alamar Blue/Resazurin (T-SCREEN)

The T-screen assay is a proliferation assay based on the growth of a rat pituitary tumor cell line (GH3) in serum free-medium. The proliferative effect of T3 is driven by well-characterized, high-affinity TRs, which, upon binding to thyroid hormones, interact with thyroid hormone response elements in the nucleus to regulate gene expression. The T-Screen assay is therefore suitable for detecting both agonistic and antagonistic activities of test compounds at the level of the thyroid receptor [52,67–69]. In this assay, chemicals are tested both in the absence and presence of T3 to evaluate their potential interference with TH signaling at the cellular level. GH3 cell growth is assessed using AlamarBlue™ (Thermo Fisher Scientific, Invitrogen, Carlsbad, CA, USA); its colorimetric absorbance increases in response to TH agonists and decreases when TH antagonists are present [53]. Experts group from the OECD concluded that the assay is valid for the detection of TR agonists but the response measured by cell proliferation is unspecific of thyroid modalities. For this reason, other hormones and growth stimulators would need to be tested to assess specificity [54].

Various in vitro tests are being developed to evaluate disruption in the reproductive and endocrine systems. Each assay presents its own advantages and limitations, reflecting differences in sensitivity, and biological relevance (Table 1). While several in vitro methods

targeting the reproductive system have already been accepted and integrated into regulatory frameworks, many tests focused on thyroid disruption are currently under validation and are progressing toward regulatory acceptance.

Table 1. Overview of In Vitro Assays for Assessing Endocrine Disruption in the Reproductive and Thyroid Systems: Endpoints, Advantages, Limitations, and Validation Status.

Organ/System	Test	Activity Determined	Advantages	Disadvantages	Validation Status
Reproductive system	YES assay	Estrogenic interaction	Sensible and quantifiable detection	Only detection of hERα	Regulatory accepted
	XenoScreen YES/YAS assay	Estrogenic/ Androgenic interaction	Activity of pure compounds and complex formulations	Poor physiological relevance	-
	E-SCREEN assay	Estrogenic activity	Easy detection of cell proliferation by hormonally active compounds	Activity can be influenced by cytotoxicity or non-specific mitogenic effects	-
	Transactivation system with stably transfected cells to detect estrogen/androgen receptor agonists or antagonists	Estrogenic/ Androgenic activity	Mechanistically relevant and standardized approach	Effects of metabolic activation or involving non-receptor-mediated pathways not detected	Regulatory accepted
	Estrogen binding affinity assay	Estrogen activity binding	Specific, mechanistic, and suitable for screening ER-binding compounds	Lack of functional responses detection	-
	Aromatase assay	Steroidogenic activity	Mechanistically relevant and human-specific approach	Biochemical assay	Regulatory accepted
	H295R Steroidogenesis Assay	Steroidogenic activity	Human-specific approach	Complexity	Regulatory accepted
Thyroid gland	TSH receptor activation based on cAMP measurement	TSH receptor interaction	Relevant receptor and established cell system	Lack of positive reference chemicals for the agonist mode and of antagonist mode development.	Validation finalized *
	TPO-AUR	TPO inhibition	Human-specific approach	High variability	Validation finalized *
	TYRO-IOD	Tyrosine iodination activity	Human-specific approach	Inter-laboratory reproducibility confirmation required	Validation finalized *
	TTR-ANSA	TTR/TBG binding activity	Strong biological plausibility; direct and measurable approach	Further refinement of fluorescence interference is needed	Validation finalized *
	TTR FITC T4	TTR binding activity	Human-specific approach; direct and measurable approach	Fluorescence interference	Validation ongoing
	DIO 1	Inhibition of D1	Biologically relevant and quantifiable endpoint	Variability due to biological source of human liver microsomes	Validation ongoing

Table 1. *Cont.*

Organ/System	Test	Activity Determined	Advantages	Disadvantages	Validation Status
Thyroid gland	GLUC LC/MS	TH glucuronidation inhibition	Sensitive LC-MS analysis	Additional data of inactive compounds required	Validation finalized *
	TRα and TRβ reporter assays	Activation of TRα and TRβ	Agonistic activity detection	Lack of antagonistic activity detection	Validation finalized *
	TR CALUX	Activation of TRβ	Agonist and antagonist detection	More active agonist and antagonist chemicals are required	Validation finalized *
	T-SCREEN	T3 interaction	Agonist and antagonist detection	Unspecific for thyroid modalities	Validation finalized *

* While the validation is complete, the OECD has provided further recommendations to support and confirm the validity of the method.

3. Overview of the In Silico Models for Identifying Endocrine-Disrupting Activity

Many chemicals are identified as endocrine disruptors, but the mechanisms by which most EDCs operate remain poorly understood. Comprehensive in vitro testing of all chemicals—potential EDCs—employed in industrial, agricultural, or food preservation applications for endocrine system effects is both resource-intensive and time-prohibitive. Computational techniques such as virtual screening, quantitative structure–activity relationships, and molecular docking are established tools widely utilized in pharmaceutical development. These methodologies could similarly advance EDC research [70].

Different in silico tools have been developed in recent years to predict different toxicology effects. Among them there are in silico models for identifying different endocrine disruptors. These models can identify androgen active chemicals [71], as well as estrogenic activity [72,73]. Examples of these in silico quantitative structure–activity relationship (QSAR) tools and, even, servers, are OECD, VEGA HUB or CAESAR. Expanding knowledge of functional and structural data has enabled the assessment or prediction of potential interactions between known or putative EDCs and various molecular targets through docking studies or more computationally intensive approaches [74].

VirtualToxLab™ (Biographics Laboratory 3R, University of Basel, Basel, Switzerland) is an in silico technology developed at the Biographics Laboratory 3R that enables prediction of the endocrine-disrupting potential of pharmaceuticals, chemicals, and natural products. This platform employs an automated protocol to calculate binding affinity of target molecules against a panel of 12 proteins known or suspected to mediate adverse effects, subsequently estimating the resulting toxic potential. VirtualToxLab™ is accessible to science-oriented organizations for a nominal fee [75,76]. This in silico model has been used for many researchers [77,78].

Recently, authors have developed an in silico model for predicting the binding of chemicals to human thyroid hormone distributor protein transthyretin (hTTR). The newly developed QSARs provide valuable tools for screening potential endocrine disruptors affecting the thyroids [79].

Given the multifaceted nature of endocrine disruption mechanisms, conventional in silico models targeting individual pathways prove insufficient for identifying EDCs. To overcome these constraints, research has focused in employing both single-label and multilabel modeling approaches across six endocrine disruption targets such as AR, ERα, thyroid receptor, glucocorticoid receptor, peroxisome proliferator-activated receptor gamma, and aromatase. Authors determined that single-label models are suitable for identifying po-

tential EDCs, whereas multilabel models are more appropriate for predicting the probable mechanisms of action of established EDCs [80].

In silico models provide a valuable alternative to in vitro testing for identifying EDCs, offering faster and less resource-intensive screening. Established computational methods such as QSAR, molecular docking, and virtual screening enable high-throughput assessment of large chemical libraries, predicting interactions with key endocrine targets like estrogen or androgen receptors. Tools like VirtualToxLab™ and multilabel modeling approaches further enhance mechanistic understanding by evaluating multiple pathways.

However, these models have limitations. They often focus on single targets, which may not capture the complexity of endocrine disruption. Their performance depends on the availability and quality of input data, and advanced models can be computationally demanding. While suitable for prioritizing chemicals, in silico predictions usually require experimental validation to confirm biological relevance.

4. Overview of the Main Current Models for Identifying Endocrine-Disrupting Activity Using Partial Replacement

According to the 3Rs principal replacement refers to technologies or approaches which replace the use of animals in experiments where they would otherwise have been used. The replacement can be divided in two categories: full replacement and partial replacement. Partial replacement includes the use of some animals that, based on current scientific thinking, are not considered capable of experiencing suffering. This category comprises invertebrates such as *Drosophila melanogaster*, nematode worms, *Daphnia* spp., and social amoebae, as well as early life stages of vertebrates, such as zebrafish (*Danio rerio*) embryos before the onset of independent feeding. It also encompasses the use of primary cells and tissues obtained from animals euthanized solely for this purpose—i.e., not subjected to procedures that cause suffering [81].

Several alternative test methods have been developed to evaluate endocrine activity while aligning with the 3Rs principle, particularly through the use of partially replacing models such as *Daphnia magna*, zebrafish embryos, and amphibian larvae. The following assays exemplify established approaches for detecting endocrine-disrupting effects across various endocrine pathways and developmental stages.

4.1. Short-Term Juvenile Hormone Activity Screening Assay Using Daphnia Magna

This Test Guideline (TG) describes a short-term juvenile hormone (JH) activity screening assay using Daphnia magna to detect the potential of chemicals with JH [82]. Adult female Daphnia carrying developing embryos within their brood chambers are exposed for a period of 7 days to a minimum of three concentrations of the test substance and a water control. The primary endpoint of this assay is the male ratio within the second brood offspring. The male ratio (number of male offspring divided by total offspring number) in the second brood must be calculated for each parental organism (representing individual replicates), with the mean male ratio subsequently determined for each test condition. The development of this method was based on the studies of male offspring production in Daphnia which is regulated by JH signaling pathway [83,84]. The method allows for clear quantification of endocrine-related effects in a whole-organism context. However, the assay is limited to a single endpoint—the male ratio—which may not capture other potential endocrine-related changes and requires careful brood monitoring and individual-level data analysis, which can be labor-intensive.

4.2. EASZY Assay: Detection of Endocrine Active Substances, Acting Through Estrogen Receptors, Using Transgenic Tg(cyp19a1b:GFP) Zebrafish Embryos

The EASZY assay is a mechanism-based in vivo screening methodology designed to detect endocrine-active compounds functioning as ER agonists through induction of green fluorescent protein (GFP) expression under control of the cyp19a1b promoter. This assay enables detection of estrogenic activity in test chemicals using transgenic (cyp19a1b:GFP) zebrafish embryos subjected to 96 h exposure during embryonic developmental stages. Newly fertilized Zebrafish eggs (up to 4 h post-fertilization) are exposed to the test chemical under semi-static conditions with a total renewal of the test medium every 24 h [85,86]. The use of whole organisms allows for biologically integrated responses in a vertebrate model. However, the assay requires maintenance of transgenic zebrafish lines, semi-static exposure conditions with daily medium renewal, and careful timing of embryo exposure, which may increase technical complexity and resource demands.

4.3. Larval Amphibian Growth and Development Assay

The test design entails exposing X. laevis embryos at Nieuwkoop and Faber (NF) stage 8–10 via the water route to four different concentrations of test chemical as well as control(s) until 10 weeks after the median time to NF stage 62 in the control with one interim sub-sample at NF stage 62. The endpoints evaluated are endocrine modes of action targeting estrogen-, androgen-, or thyroid-mediated pathways [87]. This assay allows for the detection of multiple endocrine target pathways in a vertebrate model, offering comprehensive in vivo assessment. However, the extended exposure duration and use of amphibian embryos require significant time and resources, and careful developmental staging, which may limit throughput and increase assay complexity.

4.4. Xenopus Eleutheroembryonic Thyroid Assay (XETA)

The XETA was designed as a screening assay to provide a medium throughput and short-term assay to measure the response of eleutheroembryos to potential thyroid active chemicals. It is a relevant amphibian model because its early development and thyroid hormone-dependent metamorphosis are very well-characterized. The measured endpoint is the induction of fluorescence in eleutheroembryos. When transcription of the genetic construct is activated or inhibited following chemical exposure, eleutheroembryos express increased or decreased levels of GFP, resulting in higher or lower fluorescence compared to unexposed individuals, in which fluorescence remains at the basal levels [88]. This setup allows for efficient detection of thyroid-related endocrine activity in a biologically relevant organism. However, the reliance on a single endpoint—GFP fluorescence—may limit the detection of other thyroid-mediated effects, and the performance of the assay depends on the stability and responsiveness of the genetic construct, which may introduce variability.

4.5. Daphnia Multigeneration Test for Assessment of Endocrine-Active Chemicals (DMGT)

This long-term in vivo assay with Daphnia magna is responsive to JH agonists which lead to the production of male offspring. It is a method used to assess the potential endocrine-disrupting effects of chemicals by observing their impact on multiple generations of the crustacean, Daphnia magna. The method consists of three linked exposure experiments. It begins with <24 h-old neonates, exposes them continuously to dilutions of the test chemical, allows them to grow to adulthood, then produce at least three successive broods for 21 days. The second test takes neonates from the third or subsequent brood in each concentration for a further 21 days. The third solely takes control neonates from the third or subsequent brood in the first test and again exposes them for 21 days to the same range of concentrations as in the other tests. At the end of each test, all individual neonates

are sexed by observation of their longer first antenna. This test provides valuable insights into the potential risks associated with long-term exposure [89].

The endpoints determined are related to reproduction, growth, and survival. By observing changes in these endpoints across generations, the test can help identifying chemicals that interfere with hormonal or endocrine systems, which can have significant consequences for aquatic organisms and potentially for human health. This assay provides valuable insights into the long-term and generational effects of chemical exposure, making it a powerful tool for identifying substances that interfere with hormonal systems. However, its extended duration, multigenerational design, and labor-intensive nature may limit its throughput and require substantial resources, which can be a drawback for routine screening purposes.

All these methods are used with purposes of ecotoxicological effects but support the potential endocrine disruptor effects of the chemical studied.

5. Limitations

Numerous methods have been developed and validated to assess endocrine disruption related to reproductive system endpoints; however, there is still a lack of official guidelines for evaluating thyroid disruption. For example, although some methods to assess thyroid-related effects are currently under development and validation, they have not yet been formally approved. Moreover, there is no available data or in vitro studies available concerning endocrine disruptors that affect other components of the endocrine system, such as the pituitary gland or hypothalamus.

The use of human cells in research offers significant advantages over laboratory animals, primarily because it eliminates the need for interspecies extrapolation. This is due to the fact that human cells possess more relevant morphological, physiological, and biochemical characteristics [90]. Human cell lines, as animal cell lines, are highly accessible, easy to handle, and useful for advancing knowledge in basic cell and molecular biology, pathology, and the development of new therapies [91].

One of the main challenges in the study of EDCs is the limited understanding of their metabolic pathways. To address this, Lopardo et al. in 2018 investigated metabolic markers associated with chemicals suspected of endocrine-disrupting potential [92]. However, data from various in vitro studies have often produced inconsistent or ambiguous results, highlighting a lack of correlation between different cell models [93].

High-throughput mechanistic assays are abundantly available in the suite of ToxCast and Tox21 assays, which screen thousands of chemicals for a variety of toxicity pathways, including endocrine disruption. These types of assays have not undergone international validation, and therefore regulatory authorities use the data only in certain contexts [94]. Contrary to the results observed in different studies related to the endocrine disruptor effects of different UV filters, as part of a weight-of-evidence analysis, a recent study showed that the UV filters octisalate, homosalate, octocrylene, octinoxate, and avobenzone have low endocrine disruption potential risk based on an examination of ToxCast/Tox21 data with weak activity occurring at concentrations >100-fold what would be achieved in human plasma. The exception is oxybenzone where the bioactivity and plasma concentrations are separated by less than 10-fold. The ToxCast/Tox21 database is a program organized and maintained by several agencies including the U.S. National Toxicological Program (NTP) and the U.S. EPA. EDSP21 is an in vitro HTS program publicly available within Tox21 that is focused on measures of the endocrine system. The EDSP21 screening program is designed to identify and prioritize estrogen, androgen, and thyroid disruptors as well as steroidogenesis, the so-called EATS [95].

6. EDCs in Cosmetics

Absorption of chemicals from cosmetics is possible, with the skin being the main route of exposure. The health of the skin represents a critical determinant in the efficiency of the skin's protective barrier. Exposure is also possible through inhalation (fragrances) and through ingestion as, for example, in the case of lipstick [2]. Furthermore, indirect routes should be considered, as cosmetics ingredients can enter water systems [96], and bioaccumulate in fatty tissue of fish or seafood, posing environmental concerns and impacting human health, as some EDCs can have toxic effects at low doses [97].

The potential routes of entrance of EDCs through the use of cosmetics can be by skin, ingestion and inhalation depending on the cosmetic formulation (Figure 2).

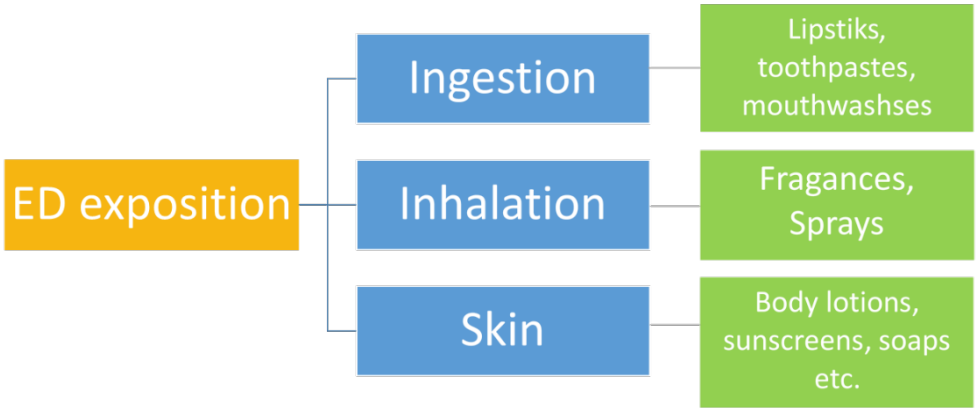

Figure 2. Routes of exposition to EDCs via cosmetics.

In the Cosmetic Regulation, there are no specific provisions for EDCs. To address potential risks to human health, the Commission may introduce measures that prohibit or restrict the use of substances [2]. For identified or potential endocrine disruptors that are not classified as carcinogenic, mutagenic, or toxic for reproduction (CMR) substances, their use in cosmetics is governed by the general provisions of Article 31 of the Cosmetic Regulation, which requires a scientific opinion [98]. Among the substances evaluated by the Scientific Committee on Consumer Safety (SCCS) for endocrine-disrupting properties (Table 2) are UV filters like benzophenone and homosalate [99], which are particularly concerning due to their potential to disrupt thyroid hormone function. These opinions indicate that substances with endocrine-disrupting properties should be investigated based on the safety assessment by the SCCS, considering the limitations associated with the bans on animal testing, and considering data from in vitro and in vivo studies.

6.1. Ultraviolet Filters

UV filters are key ingredients in sunscreens, providing protection against skin cancer and photoaging. Use of sunscreen products is increasing due to the awareness of skin cancer which causes individual and economic damage [100]. These compounds absorb or block UV radiation by reflection and/or scattering. Depending on their chemical structure these filters can be organic, absorbing UV light, or inorganic, with metal oxides such as titanium dioxide and zinc oxide [101].

Beyond their use in sun protection products, UV filters are also incorporated into plastics, adhesives, paints, textiles, furniture, and various other materials to prevent UV-induced degradation [102].

Suncare products are predominantly administered topically, with dermal absorption representing the principal route of exposure. Nevertheless, secondary exposure pathways, such as inhalation or accidental ingestion, may also contribute to overall systemic uptake [101].

Table 2. List of priority cosmetic ingredients to be assessed by the SCCS as potential endocrine disruptors with their function in cosmetic industry.

Cosmetic Ingredient	Function
Benzophenone-3	UV filter
Kojic Acid	Antioxidant, whitening
4 methylbenzylidene camphor	UV filter
Propylparaben	Preservative
Triclosan	Preservative
Homosalate	UV filter
Resorcinol	Dye
Octrocrylene	UV filter
Triclocarban	Preservative
Butylated hydroxytoluene (BHT)	Antioxidant, fragrance
Benzophenone-4	UV filter
Benzyl salicylate	Fragrance, UV absorber
Genistein	Skin conditioning
Daidzein	Skin conditioning
Buthylparaben	Preservative
Tert-butylhydroxyanisole /Butylatedhydroxyanisole/BHA	Antioxidant
Benzophenone-1	UV filter
Benzophenone-2	UV filter
Benzophenone-5	UV filter
Methylparaben	Preservative
Cyclopentasiloxane/ decamethylcyclopentasiloxane/D5	Emollient, hair conditioning
Cyclomethicone	Emollient, hair conditioning
Salicylic acid	Antiseborrheic, hair conditioning, fragrance
Butylphenyl methyl propional/BMHCA	Fragrance
Triphenyl phosphate	Plasticiser
2-tert-butyl-4-methoxyphenol	Antioxidant, fragrance
2-Ethylhexyl trans-4methoxycinnamate or octinoxate	UV filter

Multiple studies have shown that UV filters can be systemically absorbed, with these compounds being detected in various human fluids and tissues, including amniotic fluid, urine, fetal and cord blood cells in pregnant women, as well as in breast milk, placental tissue, blood plasma and serum, urine, and seminal fluid [100].

Although the protective capacity of UV filters, an increasing number of studies, both in vivo and in vitro, indicates that several UV filters might have endocrine disruptive effects [103,104]. According to Krause et al. (2012) Benzophenone-3, 4-methylbenzylidene camphor, and 2-ethylhexyl 4-methoxycinnamate are classified as substances of significant concern due to their potential adverse effects on human health [103].

6.1.1. Benzophenones and Derivatives

Residues and metabolites of benzophenones have been detected in human urine and breast milk. Among them, benzophenone-3 (BP-3) and its derivatives have shown high toxicity and estrogenic activity in various studies [102]. Benzophenone compounds have been associated with carcinogenicity, as well as reproductive and developmental toxicity.

Importantly, the substitution of functional groups significantly influences the interaction of benzophenones and their derivatives with biomolecules. BP-3 is of particular concern due to its adverse effects on cancer cell proliferation and migration, reproductive capacity, sex differentiation, and neurodevelopment [105]. Similarly, benzophenone-8 and its metabolites BP-3 and benzophenone-1 (BP-1) have been shown to disrupt progesterone-dependent decidualization in human primary endometrial stromal cells [106].

Multiple studies have proposed different mechanisms of action for BP-3 and other benzophenones [107,108]. Notably, benzophenone filters have been shown to interfere with steroidogenesis. BP-1 and benzophenone-2 (BP-2) strongly inhibit 3β-hydroxysteroid dehydrogenases (3β-HSDs) in human, rat, and mouse gonadal tissues—enzymes responsible for converting pregnenolone to progesterone. BP-2, in particular, is the most potent inhibitor of human 17β-hydroxysteroid dehydrogenase 1 (17β-HSD1), which catalyzes the conversion of estrone to estradiol, a key step in estrogen activation [109].

Docking analyses have revealed that the 4-hydroxyl group of these benzophenones forms hydrogen bonds with critical residues such as Ser123 in human 3β-HSD2, Asp127 in rat 3β-HSD1 [110], and Ser142 in human 17β-HSD1 [109], supporting their inhibitory potential at the molecular level.

Furthermore, BP-2 has been linked to thyroid axis disruption. In larval tadpoles, exposure to BP-2 resulted in concentration-dependent increases in the severity of thyroid follicular cell hypertrophy and hyperplasia [111].

The SCCS on the basis of safety assessment, and considering the concerns related to potential endocrine-disrupting properties of BP-3, concluded that the use of BP-3 as a UV filter up to a maximum concentration of 6% in sunscreen products, either in the form of body cream, sunscreen propellant spray or pump spray, is not safe for the consumer. The use of BP-3 as a UV filter up to a maximum concentration of 6% in face cream, hand cream, and lipsticks is safe for the consumer. The use of BP-3 up to 0.5% in cosmetic products to protect the cosmetic formulation is safe for the consumer. The SCCS has concluded that the currently available evidence regarding the endocrine-disrupting properties of BP-3 is inconclusive and, at best, equivocal. This assessment encompasses all available data sources, including in silico modeling, in vitro assays, and in vivo studies, whether evaluated individually or collectively. While the SCCS acknowledges that certain studies provide indications of potential endocrine effects associated with BP-3 exposure, the committee determined that the overall body of evidence remains insufficient to definitively establish whether BP-3 functions as an endocrine-disrupting substance. Consequently, the SCCS has identified the need for additional investigations to resolve this uncertainty [112].

Recently the SCCS has studied the potential endocrine disruption effect of BP-2 and Benzophenone 5 (BP-5) as substances with potential endocrine-disrupting properties in cosmetic products. BP-5 is considered safe when used as UV filter up to a maximum concentration of 5% in sunscreen, face and hand cream, lipstick, sunscreen propellant spray and pump spray [113]. Another recently study from the SCCS has considered Benzophenone-1 not safe when used as a light stabilizer in cosmetic products because of its evidence of endocrine-active substance due to its estrogenic activity and weak anti-androgenic activity and potential activity against thyroid modality [114].

In 2021, the state of Hawaii banned the sale of sunscreens containing BP-3 and octinoxate due to evidence showing these chemicals are harmful to coral reef ecosystems [115].

6.1.2. 4-Methylbenzylidene Camphor

4-Methylbenzylidene camphor (4-MBC) is accumulated in sediments, organisms and watershed systems in general [116]. Studies have shown that 4-MBC can bind to human estrogen receptors hERα and hERβ in vivo, with a preference for hERβ, acting as an antagonist. In vitro experiments using the HEK, MCF-7 and PALM cell lines have demonstrated its ability to interfere with testosterone synthesis, estrogenic potential and a strong capacity as hAR antagonist [117,118]. In animal models, 4-MBC has also been observed to alter thyroid hormone levels by increasing TSH and T4, while reducing T3 concentrations in rats [118]. In addition, in adult zebrafish, 4-MBC have presented decreasing in fertilization [119], and developmental toxicity in zebrafish embryos [120]. Based on the safety assessment

of 4-MBC performed by the SCCS, this UV filter has been forbidden in Europe since 2023. The opinion concluded that there is adequate evidence that 4-MBC may function as an endocrine disruptor with effects on both thyroid and estrogen systems. Effects on the androgen system are less clear, as only in vitro evidence exists [121].

6.1.3. Homosalate

Homosalate has shown estrogenic activity and antagonism to hAR [117]. The SCCS opinion on homosalate stated that, based on the safety assessment, it is safe as a UV filter at concentrations up to 7.34% in face creams and pump sprays. Nevertheless, concerns remain regarding its potential endocrine-disrupting properties [122]. More recent studies showed alteration in hormonal thyroid function and in growth of zebrafish embryo/larvae [123] and toxicity in FRTL-5 cells and upregulation of mRNA expression levels of TPO and Tg genes [124]. Fish exposed to homosalate exhibited significant reductions in gonadosomatic index, testosterone concentrations, and expression of multiple genes (such as hsd3b2, cyp17a1, and hsd17b1), alongside significant elevations in hepatosomatic index, liver steatosis, 17β-estradiol concentrations, and vtg gene expression. These outcomes demonstrate that homosalate's estrogenic and anti-androgenic actions are mediated through steroidogenic pathways. Lee et al. revealed that homosalate, alone or in combination with avobenzone, decreased testosterone levels in H295R cells, according to their studies in male zebrafish [125].

6.1.4. Octocrylene

Octocrylene has been shown to activate the ER pathway, impacting processes such as growth, development, and reproduction in both in vivo (zebrafish larvae) and in vitro (ZFL cell line) models [126]. Additionally, similar to ethylhexyl 4-methoxycinnamate (EHMC), BP-3, and avobenzone, octocrylene has been reported to lower thyroid hormone levels in fish larvae, suggesting a potential for thyroid disruption, as well as to induce hypoactivity and/or anxiety-like behaviors [127]. Octocrylene is considered safe as UV filter, according to the opinion of the SCCS at concentrations up to 10% [98].

6.1.5. Ethylhexyl Methoxycinnamate or Octinoxate

Recent in vivo studies have shown that octinoxate can disrupt thyroid hormone regulation. In zebrafish larvae, octinoxate exposure led to decreased levels of T3 and T4, along with upregulation of genes related to neurotoxicity. In adult zebrafish, transcriptional changes associated with nephrotoxicity were observed [128]. Similarly, in rats, thyroid hormone disruption was reported following exposure to octinoxate during the lactational period, along with immunological alterations in the offspring [129]. Octinoxate, like BP-1, BP-2, 4-MBC, and homosalate, has been shown to increase motility and invasiveness in breast cancer cell lines (MCF-7 and MDA-MB-231), suggesting a potential role of these UV filters in promoting breast cancer progression. Furthermore, octinoxate demonstrated estrogenic activity in MCF-7 cells, although it did not stimulate cell proliferation [130].

Recently, in the last plenary meeting of the SCCS in June 2025 the opinion about EHMC or octinoxate has been adopted. The SCCS has determined that available evidence demonstrates octinoxate exhibits endocrine-active properties, specifically estrogenic activity and weak anti-androgenic activity in both laboratory and animal studies. After reviewing the provided data and considering concerns about octinoxate's potential endocrine-disrupting characteristics, the SCCS concludes that octinoxate can be safely used as a UV filter at concentrations up to 10% in sunscreen lotions, face and hand creams, lipsticks, sunscreen propellant sprays, and pump sprays, whether used individually or in combination with other products [131]. The studies supporting this opinion about the endocrine disruptor

activity of octinoxate are in more cases old studies performed in vitro and in vivo between 2001 and 2018.

The established OECD test guideline 456 [51], which utilizes human adrenal H295R cells and, facilitates the measurement of testosterone and estradiol production to identify potential EDCs has been used to study octinoxate. The authors showed that steroid profile alterations caused by 10 μM of octinoxate, characterized by increased corticosteroids, resulted from elevated CYP11B2 and 3bHSD2 mRNA expression levels. This indicates that octinoxate does not directly influence the activity of these enzymes but instead modifies their expression levels [132].

Lorigo et al. [133] performed a first review in 2018 of the studies concerning the animal and human effects of octinoxate as endocrine disruptor. In this review authors indicated that the main concern is the lack of information in humans because there are more studies performed in rodents. From these studies in rodents it can be concluded octinoxate may disrupt thyroid hormone synthesis, metabolism, distribution, and function through multiple targets and various mechanisms [133]. Only two epidemiological studies were found, suggesting the possibility of neonatal exposure resulting from maternal exposure to this UV filter, but they were not validated and it is difficult to obtain clear conclusions and more conclusive studies are necessary.

More recently Lorigo et al. in 2024 [134] performed a new review concluding that octinoxate exposure poses considerable risks to human health. The endocrine-disrupting effects manifest through cardiovascular, thyroid, reproductive, and immunological alterations. Documented associations with hypothyroidism development, hypertensive pregnancy disorders, pubertal changes, respiratory diseases, and breast cancer warrant serious attention. Future epidemiological and experimental studies are critically needed to enhance toxicological assessment of this endocrine-disrupting chemical and improve human health outcomes [134].

6.1.6. Avobenzone

Avobenzone (butyl methoxydibenzoylmethane) is an endocrine disruptor that directly binds to estrogen receptor β and acts as an estrogen agonist. In vitro studies demonstrated that avobenzone inhibited the proliferation of HTR8/SVneo cells, the immortalized human trophoblast cell line. Human trophoblast cells form the placenta through a precisely controlled differentiation process during the early pregnancy of women, then avobenzone inhibits the proliferation of human trophoblast cells and induces apoptosis [135].

The co-exposure to avobenzone and homosalate enhanced sex hormone disruption induced by homosalate [125].

A study performed in zebrafish larva receiving avobenzone and octinoxate showed a significantly lower larvae survival rate together with an increased ratio of T3 to T4. Significant decrease in T4 level indicates feedback in the hypothalamus to maintain hormonal homeostasis. The results suggested that both avobenzone and octinoxate affects the thyroid hormone receptor and the feedback mechanisms of the hypothalamus–pituitary–thyroid axis [136]. The SCCS concluded that avobenzone is safe as UV filter at concentrations up to 5% [98].

Based on the available data concerning the potential endocrine-disrupting properties of various UV filters, Table 3 provides a comprehensive summary of the most significant biological effects identified for each UV filter, along with safety considerations at the maximum permissible concentrations established within the European regulatory framework following the recommendations of the SCCS after their evaluation and the corresponding reference. Furthermore, 4-Methylbenzylidene camphor has been prohibited from use due to documented adverse effects as an endocrine disruptor on the thyroid and estrogen systems.

Table 3. UV filters described as endocrine disruptors, their activities and regulatory status.

UV Filters	Activities	Regulatory Status	References
Benzophenone and derivatives	Estrogenicity, carcinogenic, reproductive, and developmental toxicity Thyroid hormone levels decrease, hypoactivity	BP-1 considered not safe as light stabilizer BP-2 not permitted BP-3 at concentrations up to 0.5% in cosmetic products and up to 6% as UV filter; not safe in body cream, sunscreen propellant spray or pump spray BP-5 safe at concentrations up to 5% in various cosmetic products and as UV filter	[102,105,112–114,127]
4-Methylbenzylidene camphor	Estrogenicity, testosterone synthesis modulation, regulation of thyroid hormones, developmental toxicity and fertilization decrease	Banned in Europe	[117–121]
Homosalate	Estrogenicity, growth alteration, hormonal thyroid function alteration	Safe as UV filter at concentrations up to 7.34% in face creams and pump sprays	[117,122–125]
Octocrylene	Estrogenicity, growth, development and reproduction toxicity, thyroid hormone levels decrease, hypoactivity	Safe as UV filter at concentrations up to 10%	[98,126,127]
2-Ethylhexyl 4-methoxycinnamate/ Octinoxate	Thyroid hormone levels decrease, hypoactivity, neurotoxicity, estrogenicity	Safe as UV filter at concentrations up to 10%	[127–134]
1-(4-tert-Butylphenyl)-3-(4-methoxyphenyl) propane-1,3-dione/Avobenzone	Thyroid hormone levels decrease, hypoactivity Inhibition of trophoblast cell proliferation	Safe as UV filter at concentrations up to 5%	[98,125,127,135,136]

Beyond their potential endocrine-disrupting effects in humans, several organic UV filters have raised increasing environmental concern due to their persistence, bioaccumulation, and toxicity in aquatic ecosystems. Numerous studies have reported their presence in marine waters, sediments, and surface microlayers as a result of widespread sunscreen use and coastal tourism [137]. Certain filters, such as BP-3, 4-MBC, EHMC, and octocrylene, have been shown to induce acute and chronic toxicity, genotoxicity, oxidative stress, reproductive and developmental toxicity, coral bleaching, and endocrine disruption in marine organisms, particularly at the base of the food web, with possible cascading effects on higher trophic levels [137]. These findings have led to regulatory actions in multiple regions, including Hawaii, the U.S. Virgin Islands, and parts of Mexico and the Netherlands, which have banned sunscreens containing the most harmful UV filters. Similarly, octocrylene and EHMC have been identified as among the most toxic cosmetic ingredients for aquatic life [138]. However, despite increasing awareness, a robust environmental risk assessment remains hindered by substantial data gaps, particularly for freshwater ecosystems, where information on chronic toxicity, bioaccumulation, and persistence is still lacking for many of the 32 organic UV filters approved for use in the EU [139].

7. Conclusions

The widespread use of chemicals in cosmetics and other consumer products has raised significant concern due to their potential to interfere with the endocrine system by mimicking, blocking, or altering the physiological function of endogenous hormones. Numerous

cosmetic ingredients have been identified as EDCs, particularly those affecting key hormonal pathways. These substances can enter the human body through multiple routes of exposure, including ingestion, inhalation, and transdermal absorption, contributing to systemic effects that may not be immediately evident.

In recent years, public awareness and scientific scrutiny regarding the presence of EDCs in cosmetic products have increased considerably. In response, regulatory authorities are progressively working toward the implementation of specific frameworks to evaluate and manage the risks associated with these substances. Significant advancements have been achieved in the assessment of EDCs acting on the reproductive axis, especially those interacting with estrogenic and androgenic pathways, for which several validated guidelines and standardized testing protocols are already available.

Conversely, the evaluation of endocrine disruption involving the thyroid axis remains incomplete. While various test methods are currently under development and validation, no official regulatory guidelines have yet been adopted. An even greater gap exists regarding the study of EDCs that may affect other endocrine components, such as the pituitary gland, pineal gland, hypothalamus, or pancreas. The absence of comprehensive data and testing strategies for these targets highlights a critical need for the development of novel, robust, and mechanism-based in vitro and in silico approaches to address these less explored pathways of endocrine disruption.

Notably, among the 27 priority cosmetic ingredients identified by the SCCS for evaluation as potential EDCs, 9 are UV filters. Compounds such as benzophenone and its derivatives, homosalate, octocrylene, octinoxate, and the recently banned 4-methylbenzylidene camphor have all shown evidence of endocrine-disrupting activity. Given the restriction on animal testing for cosmetics in the European Union, there is an urgent need to further develop alternative non-animal methods—particularly in vitro assays and predictive in silico models—to effectively assess the endocrine-disrupting potential of cosmetic ingredients, with a strong focus on UV filters due to their widespread use and biological relevance.

Author Contributions: Conceptualization, A.S.M., E.T., L.G.-E., M.M. and M.P.V.; writing—original draft preparation, A.S.M. and L.G.-E.; writing—review and editing, A.S.M., E.T., L.G.-E., M.M. and M.P.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Fouyet, S.; Ferger, M.-C.; Leproux, P.; Rat, P.; Dutot, M. Advancing Endocrine Disruptors via In Vitro Evaluation: Recognizing the Significance of the Organization for Economic Co-Operation and Development and United States Environmental Protection Agency Guidelines, Embracing New Assessment Methods, and the Urgent Need for a Comprehensive Battery of Tests. *Toxics* **2024**, *12*, 183. [CrossRef]
2. Kalofiri, P.; Biskanaki, F.; Kefala, V.; Tertipi, N.; Sfyri, E.; Rallis, E. Endocrine Disruptors in Cosmetic Products and the Regulatory Framework: Public Health Implications. *Cosmetics* **2023**, *10*, 160. [CrossRef]
3. European Commission. 16 May 2019. Call for Data on Ingredients with Potential Endocrine-Disrupting Properties Used in Cosmetic Products. European Commission Newsroom. Available online: https://ec.europa.eu/newsroom/growth/items/651201/en (accessed on 4 August 2025).
4. The Lancet Oncology. Endocrine disruptors-the lessons (not) learned. *Lancet Oncol.* **2021**, *22*, 1483. [CrossRef]
5. Wagner, H. Essentials of human physiology. In *The Psychobiology of Human Motivation*; Routledge: Abingdon, UK, 2021; Volume 1, pp. 11–24. [CrossRef]

6. Chou, K. Endocrine System and Endocrine Disruptors. In *Reference Module in Biomedical Sciences*; Elsevier: Amsterdam, The Netherlands, 2019; ISBN 9780128012383. [CrossRef]
7. Bany Bakar, R.; Reimann, F.; Gribble, F.M. The intestine as an endocrine organ and the role of gut hormones in metabolic regulation. *Nat. Rev. Gastroenterol. Hepatol.* **2023**, *20*, 784–796. [CrossRef]
8. Environment, Climate Change and Health (ECH). *State of the Science of Endocrine Disputing Chemicals—IPCP-2012*; Bergman, Å., Heindel, J.J., Jobling, S., Kidd, K.A., Zoeller, R.T., Eds.; United Nations Environment Programme and the World Health Organization: Geneva, Switzerland, 2013; Available online: https://www.who.int/publications/i/item/state-of-the-science-of-endocrine-disrupting-chemicals (accessed on 7 May 2025).
9. Clavel Rolland, N.; Graslin, F.; Schorsch, F.; Pourcher, T.; Blanck, O. Investigating the mechanisms of action of thyroid disruptors: A multimodal approach that integrates in vitro and metabolomic analysis. *Toxicol. Vitr.* **2024**, *100*, 105911. [CrossRef]
10. European Chemical Agency (ECHA) and European Food Safety Authority (EFSA) with the Technical Support of the Joint Research Centre (JRC); Andersson, N.; Arena, M.; Auteri, D.; Barmaz, S.; Grignard, E.; Kienzler, A.; Lepper, P.; Lostia, A.M.; Munn, S.; et al. Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. *EFSA J.* **2018**, *16*, e05311. [CrossRef]
11. Paul, K.B.; Hedge, J.M.; Bansal, R.; Zoeller, R.T.; Peter, R.; DeVito, M.J.; Crofton, K.M. Developmental triclosan exposure decreases maternal, fetal, and early neonatal thyroxine: A dynamic and kinetic evaluation of a putative mode-of-action. *Toxicology* **2012**, *300*, 31–45. [CrossRef]
12. Homburg, M.; Rasmussen, Å.K.; Ramhøj, L.; Feldt-Rasmussen, U. The Influence of Triclosan on the Thyroid Hormone System in Humans—A Systematic Review. *Front. Endocrinol.* **2022**, *13*, 883827. [CrossRef]
13. Coperchini, F.; Croce, L.; Ricci, G.; Magri, F.; Rotondi, M.; Imbriani, M.; Chiovato, L. Thyroid Disrupting Effects of Old and New Generation PFAS. *Front. Endocrinol.* **2021**, *11*, 612320. [CrossRef]
14. Hu, X.; Saunders, N.; Safley, S.; Smith, M.R.; Liang, Y.; Tran, V.; Sharma, J.; Jones, D.P.; Weber, C.J. Environmental chemicals and metabolic disruption in primary and secondary human parathyroid tumors. *Surgery* **2021**, *169*, 102–108. [CrossRef]
15. Tyc, H.J.; Kłodnicka, K.; Teresińska, B.; Karpiński, R.; Flieger, J.; Baj, J. Micro- and Nanoplastics as Disruptors of the Endocrine System—A Review of the Threats and Consequences Associated with Plastic Exposure. *Int. J. Mol. Sci.* **2025**, *26*, 6156. [CrossRef]
16. Babić Leko, M.; Pleić, N.; Gunjača, I.; Zemunik, T. Environmental Factors that Affect Parathyroid Hormone and Calcitonin Levels. *Int. J. Mol. Sci.* **2022**, *23*, 44. [CrossRef]
17. Karaca, Z.; Grossman, A.; Kelestimur, F. Investigation of the Hypothalamo-pituitary-adrenal (HPA) axis: A con-temporary synthesis. *Rev. Endocr. Metab. Disord.* **2021**, *22*, 179–204. [CrossRef]
18. Hinson, J.P.; Raven, P.W. Effects of endocrine-disrupting chemicals on adrenal function. *Best Pract. Res. Clin. Endocrinol. Metab.* **2006**, *20*, 111–120. [CrossRef]
19. Yilmaz, B.; Terekeci, H.; Sandal, S.; Kelestimur, F. Endocrine disrupting chemicals: Exposure, effects on human health, mechanism of action, models for testing and strategies for prevention. *Rev. Endocr. Metab. Disord.* **2020**, *21*, 127–147. [CrossRef]
20. Harvey, P.W. Adrenocortical endocrine disruption. *J. Steroid Biochem. Mol. Biol.* **2016**, *155*, 199–206. [CrossRef]
21. Sifakis, S.; Androutsopoulos, V.P.; Tsatsakis, A.M.; Spandidos, D.A. Human exposure to endocrine disrupting chemicals: Effects on the male and female reproductive systems. *Environ. Toxicol. Pharmacol.* **2017**, *51*, 56–70. [CrossRef]
22. Oyola, M.G.; Handa, R.J. Hypothalamic–pituitary–adrenal and hypothalamic–pituitary–gonadal axes: Sex differences in regulation of stress responsivity. *Stress* **2017**, *20*, 476–494. [CrossRef]
23. Li, L.; Lin, W.; Wang, Z.; Huang, R.; Xia, H.; Li, Z.; Deng, J.; Ye, T.; Huang, Y.; Yang, Y. Hormone Regulation in Testicular Development and Function. *Int. J. Mol. Sci.* **2023**, *25*, 5805. [CrossRef]
24. Schulster, M.; Bernie, A.M.; Ramasamy, R. The role of estradiol in male reproductive function. *Asian J. Androl.* **2016**, *18*, 435–440. [CrossRef]
25. Czarnywojtek, A.; Borowska, M.; Dyrka, K.; Moskal, J.; Kościński, J.; Krela-Kaźmierczak, I.; Lewandowska, A.M.; Hjeily, B.A.; Gut, P.; Hoffmann, K.; et al. The influence of various endocrine disruptors on the reproductive system. *Endokrynol. Pol.* **2023**, *74*, 221–233. [CrossRef]
26. Fenichel, P.; Chevalier, N.; Brucker-Davis, F. Bisphenol A: An endocrine and metabolic disruptor. *Ann. Endocrinol.* **2013**, *74*, 211–220. [CrossRef]
27. Kim, K. The Role of Endocrine Disruption Chemical-Regulated Aryl Hydrocarbon Receptor Activity in the Pathogenesis of Pancreatic Diseases and Cancer. *Int. J. Mol. Sci.* **2024**, *25*, 3818. [CrossRef]
28. Linţa, A.V.; Lolescu, B.M.; Ilie, C.A.; Vlad, M.; Blidișel, A.; Sturza, A.; Borza, C.; Muntean, D.M.; Crețu, O.M. Liver and Pancreatic Toxicity of Endocrine-Disruptive Chemicals: Focus on Mitochondrial Dysfunction and Oxidative Stress. *Int. J. Mol. Sci.* **2024**, *25*, 7420. [CrossRef]
29. Ropero, A.B.; Alonso-Magdalena, P.; García-García, E.; Ripoll, C.; Fuentes, E.; Nadal, A. Bisphenol-A disruption of the endocrine pancreas and blood glucose homeostasis. *Int. J. Androl.* **2008**, *31*, 194–200. [CrossRef]

30. Bernasconi, C.; Langezaal, I.; Bartnicka, J.; Asturiol, D.; Bowe, G.; Coecke, S.; Kienzler, A.; Liska, R.; Milcamps, A.; Munoz Pineiro, A.; et al. *Validation of a Battery of Mechanistic Methods Relevant for the Detection of Chemicals that Can Disrupt the Thyroid Hormone System*; Publications Office of the European Union: Luxembourg, 2023. [CrossRef]
31. Ramhøj, L.; Axelstad, M.; Baert, Y.; Cañas-Portilla, A.I.; Chalmel, F.; Dahmen, L.; De La Vieja, A.; Evrard, B.; Haigis, A.-C.; Hamers, T.; et al. New approach methods to improve human health risk assessment of thyroid hormone system disruption–a PARC project. *Front. Toxicol.* **2023**, *5*, 1189303. [CrossRef]
32. Barton-Maclaren, T.S.; Wade, M.; Basu, N.; Bayen, S.; Grundy, J.; Marlatt, V.; Moore, R.; Parent, L.; Parrott, J.; Grigorova, P.; et al. Innovation in regulatory approaches for endocrine disrupting chemicals: The journey to risk assessment modernization in Canada. *Environ. Res.* **2022**, *204*, 112225. [CrossRef]
33. Ankley, G.T.; Bennett, R.S.; Erickson, R.J.; Hoff, D.J.; Hornung, M.W.; Johnson, R.D.; Mount, D.R.; Nichols, J.W.; Russom, C.L.; Schmieder, P.K.; et al. Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. *Environ. Toxicol. Chem.* **2009**, *29*, 730–741. [CrossRef]
34. OECD. *Guidance Document for the Use of Adverse Outcome Pathways in Developing Integrated Approaches to Testing and Assessment (IATA)*; OECD Series on Testing and Assessment, No. 260; OECD Publishing: Paris, France, 2017. [CrossRef]
35. Zilliacus, J.; Draskau, M.K.; Johansson, H.K.L.; Svingen, T.; Beronius, A. Building an adverse outcome pathway network for estrogen-, androgen- and steroidogenesis-mediated reproductive toxicity. *Front. Toxicol.* **2024**, *6*, 1357717. [CrossRef]
36. Li, K.; Cui, K.; Wang, Q. Adverse outcome pathway network approach to identify endocrine disruptor-induced reproductive toxicity. *Curr. Opin. Toxicol.* **2023**, *34*, 100391. [CrossRef]
37. Audouze, K.; Zgheib, E.; Abass, K.; Baig, A.H.; Forner-Piquer, I.; Holbech, H.; Knapen, D.; Leonards, P.E.G.; Lupu, D.I.; Palaniswamy, S.; et al. Evidenced-Based Approaches to Support the Development of Endocrine-Mediated Adverse Outcome Pathways: Challenges and Opportunities. *Front. Toxicol.* **2021**, *3*, 787017. [CrossRef]
38. Wiklund, L.; Pípal, M.; Weiss, J.; Beronius, A. Exploring a mechanism-based approach for the identification of endocrine disruptors using Adverse Outcome Pathways and New Approach Methodologies: A perfluorooctane sulfonic acid case study. *Toxicology* **2024**, *504*, 153794. [CrossRef]
39. *ISO 19040-1:2018*; Water Quality—Determination of the Estrogenic Potential of Water and Waste Water—Part 1: Yeast Estrogen Screen (YES) Assay. International Organization for Standardization: Geneva, Switzerland, 2018.
40. *ISO 19040-2:2018*; Water Quality—Determination of the Estrogenic Potential of Water and Waste Water—Part 2: Human Cell-Based Reporter Gene Assay. International Organization for Standardization: Geneva, Switzerland, 2018.
41. *ISO 19040-3:2018*; Water Quality—Determination of the Estrogenic Potential of Water and Waste Water—Part 3: In Vivo Assay with the Fish Danio Rerio (Estrogenic Gene Expression). International Organization for Standardization: Geneva, Switzerland, 2018.
42. Czernych, R.; Chraniuk, M.; Zagożdżon, P.; Wolska, L. Characterization of estrogenic and androgenic activity of phthalates by the XenoScreen YES/YAS in vitro assay. *Environ. Toxicol. Pharmacol.* **2017**, *53*, 95–104. [CrossRef]
43. Oliver, M.; Kudłak, B.; Wieczerzak, M.; Reis, S.; Lima, S.A.C.; Segundo, M.A.; Miró, M. Ecotoxicological equilibria of triclosan in Microtox, XenoScreen YES/YAS, Caco2, HEPG2 and liposomal systems are affected by the occurrence of other pharmaceutical and personal care emerging contaminants. *Sci. Total Environ.* **2020**, *719*, 137358. [CrossRef]
44. Chauhan, A.; Subbarayan, R.; Ravi, K.; Verma, R.; Rao, S.K.; Girija, D.M.; Gopalakrishnan, C.; Rana, G. Phytomediated Chitosan-Modified TiO_2 NPs for Enhanced Photocatalytic and Potential Application for Breast Cancer Therapy. *Lumin. J. Biol. Chem. Lumin.* **2025**, *40*, e70162. [CrossRef]
45. Svobodova, L.; Kejlova, K.; Rucki, M.; Chrz, J.; Kubincova, P.; Dvorakova, M.; Kolarova, H.; Jirova, D. Health safety of parabens evaluated by selected in vitro methods. *Regul. Toxicol. Pharmacol.* **2023**, *137*, 105307. [CrossRef]
46. Soto, A.M.; Sonnenschein, C.; Chung, K.L.; Fernandez, M.F.; Olea, N.; Serrano, F.O. The E-SCREEN assay as a tool to identify estrogens: An update on estrogenic environmental pollutants. *Environ. Health Perspect.* **1995**, *103* (Suppl. S7), 113–122. [CrossRef]
47. OECD. *Test No. 455: Performance-Based Test Guideline for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists and Antagonists*; OECD Guidelines for the Testing of Chemicals, Section 4; OECD Publishing: Paris, France, 2021. [CrossRef]
48. OECD. *Test No. 493: Performance-Based Test Guideline for Human Recombinant Estrogen Receptor (hrER) In Vitro Assays to Detect Chemicals with ER Binding Affinity*; OECD Guidelines for the Testing of Chemicals, Section 4; OECD Publishing: Paris, France, 2024. [CrossRef]
49. OECD. *Test No. 458: Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals*; OECD Guidelines for the Testing of Chemicals, Section 4; OECD Publishing: Paris, France, 2023. [CrossRef]
50. U.S. Environmental Protection Agency. Endocrine Disruptor Screening Program Test Guidelines OPPTS 890.1200: Aromatase (Human Recombinant) Assay (EPA-HQ-OPPT-2009-0576-0004). Office of Chemical Safety and Pollution Prevention. 2009. Available online: https://www.regulations.gov/document/EPA-HQ-OPPT-2009-0576-0004 (accessed on 7 July 2025).

51. OECD. *Test No. 456: H295R Steroidogenesis Assay*; OECD Guidelines for the Testing of Chemicals, Section 4; OECD Publishing: Paris, France, 2025. [CrossRef]
52. OECD. *New Scoping Document on In Vitro and Ex Vivo Assays for the Identification of Modulators of Thyroid Hormone Signalling*; OECD Series on Testing and Assessment, No. 207; OECD Publishing: Paris, France, 2014. [CrossRef]
53. Vergauwen, L.; Bajard, L.; Tait, S.; Langezaal, I.; Sosnowska, A.; Roncaglioni, A.; Hessel, E.; van den Brand, A.D.; Haigis, A.-C.; Novák, J.; et al. A 2024 inventory of test methods relevant to thyroid hormone system disruption for human health and environmental regulatory hazard assessment. *Open Res. Eur.* **2024**, *4*, 242. [CrossRef]
54. OECD. *Thyroid In Vitro Methods: Assessment Reports by the Thyroid Disruption Methods Expert Group: Reports Assessing the Validation Status of Assays from the EU-NETVAL Activities*; OECD Series on Testing and Assessment, No. 403; OECD Publishing: Paris, France, 2024. [CrossRef]
55. Paul, K.B.; Hedge, J.M.; Rotroff, D.M.; Hornung, M.W.; Crofton, K.M.; Simmons, S.O. Development of a thyroperoxidase inhibition assay for high-throughput screening. *Chem. Res. Toxicol.* **2014**, *27*, 387–399. [CrossRef]
56. Paul Friedman, K.; Watt, E.D.; Hornung, M.W.; Hedge, J.M.; Judson, R.S.; Crofton, K.M.; Houck, K.A.; Simmons, S.O. Tiered High-Throughput Screening Approach to Identify Thyroperoxidase Inhibitors Within the ToxCast Phase I and II Chemical Libraries. *Toxicol. Sci.* **2016**, *151*, 160–180. [CrossRef]
57. Doerge, D.R.; Takazawa, R.S. Mechanism of thyroid peroxidase inhibition by ethylenethiourea. *Chem. Res. Toxicol.* **1990**, *3*, 98–101. [CrossRef]
58. Divi, R.L.; Doerge, D.R. Inhibition of thyroid peroxidase by dietary flavonoids. *Chem. Res. Toxicol.* **1996**, *9*, 16–23. [CrossRef]
59. Freyberger, A.; Ahr, H.J. Studies on the goitrogenic mechanism of action of N,N,N′,N′-tetramethylthiourea. *Toxicology* **2006**, *217*, 169–175. [CrossRef]
60. Tater, A.; Gupta, A.; Upadhyay, G.; Deshpande, A.; Date, R.; Tamboli, I.Y. In vitro assays for characterization of distinct multiple catalytic activities of thyroid peroxidase using LC-MS/MS. *Curr. Res. Toxicol.* **2021**, *2*, 19–29. [CrossRef] [PubMed]
61. Reinen, J.; Rijk, J.; de Laat, E.; Toersche, J.; Freyberger, A.; Wenker, M. Development and standardization of an assay to evaluate the in vitro inhibition of thyroid peroxidase catalyzed iodination using FTC 238 hrTPO cell homogenates. *Appl. Vitr. Toxicol.* **2024**, *10*, 51–64. [CrossRef]
62. Meerts, I.A.; van Zanden, J.J.; Luijks, E.A.; van Leeuwen-Bol, I.; Marsh, G.; Jakobsson, E.; Bergman, A.; Brouwer, A. Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. *Toxicol. Sci.* **2000**, *56*, 95–104. [CrossRef]
63. Cao, J.; Lin, Y.; Guo, L.H.; Zhang, A.Q.; Wei, Y.; Yang, Y. Structure-based investigation on the binding interaction of hydroxylated polybrominated diphenyl ethers with thyroxine transport proteins. *Toxicology* **2010**, *277*, 20–28. [CrossRef]
64. Montaño, M.; Cocco, E.; Guignard, C.; Marsh, G.; Hoffmann, L.; Bergman, A.; Gutleb, A.C.; Murk, A.J. New approaches to assess the transthyretin binding capacity of bioactivated thyroid hormone disruptors. *Toxicol. Sci.* **2012**, *130*, 94–105. [CrossRef]
65. Hamers, T.; Kortenkamp, A.; Scholze, M.; Molenaar, D.; Cenijn, P.H.; Weiss, J.M. Transthyretin-Binding Activity of Complex Mixtures Representing the Composition of Thyroid-Hormone Disrupting Contaminants in House Dust and Human Serum. *Environ. Health Perspect.* **2020**, *128*, 17015. [CrossRef]
66. Tong, Z.; Li, H.; Goljer, I.; McConnell, O.; Chandrasekaran, A. In vitro glucuronidation of thyroxine and triiodothyronine by liver microsomes and recombinant human UDP-glucuronosyltransferases. *Drug Metab. Dispos. Biol. Fate Chem.* **2007**, *35*, 2203–2210. [CrossRef]
67. Ghisari, M.; Bonefeld-Jorgensen, E.C. Effects of plasticizers and their mixtures on estrogen receptor and thyroid hormone functions. *Toxicol. Lett.* **2009**, *189*, 67–77. [CrossRef]
68. Kusk, K.O.; Krüger, T.; Long, M.; Taxvig, C.; Lykkesfeldt, A.E.; Frederiksen, H.; Andersson, A.M.; Andersen, H.R.; Hansen, K.M.; Nellemann, C.; et al. Endocrine potency of wastewater: Contents of endocrine disrupting chemicals and effects measured by in vivo and in vitro assays. *Environ. Toxicol. Chem.* **2011**, *30*, 413–426. [CrossRef] [PubMed]
69. Waring, R.H.; Ramsden, D.B.; Jarratt, P.D.; Harris, R.M. Biomarkers of endocrine disruption: Cluster analysis of effects of plasticisers on Phase 1 and Phase 2 metabolism of steroids. *Int. J. Androl.* **2012**, *35*, 415–423. [CrossRef]
70. Vuorinen, A.; Odermatt, A.; Schuster, D. Reprint of "In Silico methods in the discovery of endocrine disrupting chemicals". *J. Steroid Biochem. Mol. Biol.* **2015**, *153*, 93–101. [CrossRef] [PubMed]
71. Manganelli, S.; Roncaglioni, A.; Mansouri, K.; Judson, R.S.; Benfenati, E.; Manganaro, A.; Ruiz, P. Development, validation and integration of in silico models to identify androgen active chemicals. *Chemosphere* **2019**, *220*, 204–215. [CrossRef] [PubMed]
72. Bhhatarai, B.; Wilson, D.M.; Price, P.S.; Marty, S.; Parks, A.K.; Carney, E. Evaluation of OASIS QSAR Models Using ToxCast™ in Vitro Estrogen and Androgen Receptor Binding Data and Application in an Integrated Endocrine Screening Approach. *Environ. Health Perspect* **2016**, *124*, 1453–1461. [CrossRef]
73. Chen, Q.; Tan, H.; Yu, H.; Shi, W. Activation of steroid hormone receptors: Shed light on the in silico evaluation of endocrine disrupting chemicals. *Sci. Total Environ.* **2018**, *631–632*, 27–39. [CrossRef]

74. Schneider, M.; Pons, J.L.; Labesse, G.; Bourguet, W. In Silico Predictions of Endocrine Disruptors Properties. *Endocrinology* **2019**, *160*, 2709–2716. [CrossRef]
75. Vedani, A.; Smiesko, M.; Spreafico, M.; Peristera, O.; Dobler, M. VirtualToxLab—In Silico prediction of the toxic (endo-crine-disrupting) potential of drugs, chemicals and natural products. Two years and 2000 compounds of experi-ence: A progress report. *ALTEX* **2009**, *26*, 167–176. [CrossRef]
76. Vedani, A.; Dobler, M.; Smieško, M. VirtualToxLab—A platform for estimating the toxic potential of drugs, chemicals and natural products. *Toxicol. Appl. Pharmacol.* **2012**, *261*, 142–153. [CrossRef]
77. Shao, Y.; Zhu, L.; Chen, Z.; Thalmann, B.; Zhou, S.; Hollert, H.; Seiler, T.B. Evidence of increased estrogenicity upon metabolism of Bisphenol F—Elucidation of the key metabolites. *Sci. Total Environ.* **2021**, *787*, 147669. [CrossRef]
78. Nowak, K.; Jakopin, Ž. In silico profiling of endocrine-disrupting potential of bisphenol analogues and their halogenated transformation products. *Food Chem. Toxicol.* **2023**, *173*, 113623. [CrossRef]
79. Evangelista, M.; Chirico, N.; Papa, E. In Silico models for the screening of human transthyretin disruptors. *J. Hazard. Mater.* **2024**, *480*, 136188. [CrossRef] [PubMed]
80. Sun, L.; Yang, H.; Cai, Y.; Li, W.; Liu, G.; Tang, Y. In Silico Prediction of Endocrine Disrupting Chemicals Using Single-Label and Multilabel Models. *J. Chem. Inf. Model.* **2019**, *59*, 973–982. [CrossRef] [PubMed]
81. Eskes, C. The usefulness of integrated strategy approaches in replacing animal experimentation. *Ann. Ist. Super. Sanita.* **2019**, *55*, 400–404. [CrossRef] [PubMed]
82. OECD. *Test No. 253: Short-Term Juvenile Hormone Activity Screening Assay Using Daphnia Magna (JHASA)*; OECD Guidelines for the Testing of Chemicals, Section 2; OECD Publishing: Paris, France, 2024. [CrossRef]
83. LeBlanc, G.A.; Medlock, E.K. Males on demand: The environmental-neuro-endocrine control of male sex determination in daphnids. *FEBS J.* **2015**, *282*, 4080–4093. [CrossRef]
84. Toyota, K.; Watanabe, H.; Hirano, M.; Abe, R.; Miyakawa, H.; Song, Y.; Sato, T.; Miyagawa, S.; Tollefsen, K.E.; Yamamoto, H.; et al. Juvenile hormone synthesis and signaling disruption triggering male offspring induction and population decline in cladocerans (water flea): Review and adverse outcome pathway development. *Aquat. Toxicol.* **2022**, *243*, 106058. [CrossRef]
85. Tong, S.K.; Mouriec, K.; Kuo, M.W.; Pellegrini, E.; Gueguen, M.M.; Brion, F.; Kah, O.; Chung, B.C. A cyp19a1b-GFP (Aromatase B) transgenic Zebrafish line that expresses GFP in radial glial cells. *Genesis* **2009**, *47*, 67–73. [CrossRef]
86. OECD. *Test No. 250: EASZY Assay—Detection of Endocrine Active Substances, Acting Through Estrogen Receptors, Using Transgenic tg(cyp19a1b:GFP) Zebrafish embrYos*; OECD Guidelines for the Testing of Chemicals, Section 2; OECD Publishing: Paris, France, 2021. [CrossRef]
87. OECD. *Test No. 241: The Larval Amphibian Growth and Development Assay (LAGDA)*; OECD Guidelines for the Testing of Chemicals, Section 2; OECD Publishing: Paris, France, 2015. [CrossRef]
88. OECD. *Test No. 248: Xenopus Eleutheroembryonic Thyroid Assay (XETA)*; OECD Guidelines for the Testing of Chemicals, Section 2; OECD Publishing: Paris, France, 2019. [CrossRef]
89. Padilla Suarez, E.G.; Pugliese, S.; Galdiero, E.; Guida, M.; Libralato, G.; Saviano, L.; Spampinato, M.; Pappalardo, C.; Siciliano, A. Multigenerational tests on *Daphnia* spp.: A vision and new perspectives. *Environ. Pollut.* **2023**, *337*, 122629. [CrossRef]
90. Combes, R.D. The use of human cells in biomedical research and testing. *Altern. Lab. Anim.* **2004**, *32* (Suppl. S1), 43–49. [CrossRef]
91. Poletto, E.; Baldo, G. Creating cell lines for mimicking diseases. *Prog. Mol. Biol. Transl. Sci.* **2021**, *181*, 59–87. [CrossRef]
92. Lopardo, L.; Adams, D.; Cummins, A.; Kasprzyk-Hordern, B. Verifying community-wide exposure to endocrine disruptors in personal care products—In quest for metabolic biomarkers of exposure via in vitro studies and wastewater-based epidemiology. *Water Res.* **2018**, *143*, 117–126. [CrossRef]
93. Natsch, A.; Hostettler, L.; Haupt, T.; Laue, H. A critical assessment of the estrogenic potency of benzyl salicylate. *Toxicol. Rep.* **2021**, *8*, 1002–1007. [CrossRef] [PubMed]
94. La Merrill, M.A.; Vandenberg, L.N.; Smith, M.T.; Goodson, W.; Browne, P.; Patisaul, H.B.; Guyton, K.Z.; Kortenkamp, A.; Cogliano, V.J.; Woodruff, T.J.; et al. Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification. *Nat. Rev. Endocrinol.* **2020**, *16*, 45–57. [CrossRef] [PubMed]
95. Onyango, D.O.; Selman, B.G.; Rose, J.L.; Ellison, C.A.; Nash, J.F. Comparison between endocrine activity assessed using ToxCast/Tox21 database and human plasma concentration of sunscreen active ingredients/UV filters. *Toxicol. Sci.* **2023**, *196*, 25–37. [CrossRef] [PubMed]
96. Feng, M.; He, Q.; Meng, L.; Zhang, X.; Sun, P.; Wang, Z. Evaluation of single and joint toxicity of perfluorooctane sulfonate, perfluorooctanoic acid, and copper to Carassius auratus using oxidative stress biomarkers. *Aquat. Toxicol.* **2015**, *161*, 108–116. [CrossRef]
97. Picot Groz, M.; Martinez Bueno, M.J.; Rosain, D.; Fenet, H.; Casellas, C.; Pereira, C.; Maria, V.; Bebianno, M.J.; Gomez, E. Detection of emerging contaminants (UV filters, UV stabilizers and musks) in marine mussels from Portuguese coast by QuEChERS extraction and GC–MS/MS. *Sci. Total Environ.* **2014**, *493*, 162–169. [CrossRef]

98. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products. Available online: https://eur-lex.europa.eu/eli/reg/2009/1223/oj/eng (accessed on 10 July 2025).
99. *SCCS/1544/14*; Memorandum on Endocrine Disruptors. SCCS (Scientific Committee on Consumer Safety): Luxembourg, 2014.
100. Breakell, T.; Kowalski, I.; Foerster, Y.; Kramer, R.; Erdmann, M.; Berking, C.; Heppt, M.V. Ultraviolet Filters: Dissecting Current Facts and Myths. *J. Clin. Med.* **2024**, *13*, 2986. [CrossRef]
101. Maipas, S.; Nicolopoulou-Stamati, P. Sun lotion chemicals as endocrine disruptors. *Hormones* **2015**, *14*, 32–46. [CrossRef]
102. Liu, Y.-Y.; Lin, Y.-S.; Yen, C.-H.; Miaw, C.-L.; Chen, T.-C.; Wu, M.-C.; Hsieh, C.-Y. Identification, contribution, and estrogenic activity of potential EDCs in a river receiving concentrated livestock effluent in Southern Taiwan. *Sci. Total Environ.* **2018**, *636*, 464–476. [CrossRef]
103. Krause, M.; Klit, A.; Blomberg Jensen, M.; Søeborg, T.; Frederiksen, H.; Schlumpf, M.; Lichtensteiger, W.; Skakkebaek, N.E.; Drzewiecki, K.T. Sunscreens: Are they beneficial for health? An overview of endocrine disrupting properties of UV-filters. *Int. J. Androl.* **2012**, *35*, 424–436. [CrossRef]
104. Just-Sarobé, M. Sunscreens and Their Impact on Human Health and the Environment: A Review. *Int. J. Dermatol.* **2025**. [CrossRef]
105. Ma, J.; Yang, B.; Hu, X.; Gao, Y.; Qin, C. The binding mechanism of benzophenone-type UV filters and human serum albumin: The role of site, number, and type of functional group substitutions. *Environ. Pollut.* **2023**, *324*, 121342. [CrossRef]
106. Zhan, T.; Shen, L.; Zhang, Y.; Wan, F.; Qiu, Y.; Jin, Q.; Wu, Y.; Huang, Y.; Zhuang, S. Decidual Disrupting Effects of Low-Dose Benzophenone-Type UV Filters in Human Endometrial Stromal Cells via ER/PR/FOXO1 Signaling. *Environ. Sci. Technol.* **2025**, *59*, 13. [CrossRef] [PubMed]
107. Zhan, T.; Zhang, L.; Cui, S.; Liu, W.; Zhou, R.; Zhuang, S. Dioxybenzone triggers enhanced estrogenic effect via metabolic activation: In Silico, in vitro and in vivo investigation. *Environ. Pollut.* **2021**, *268*, 115766. [CrossRef] [PubMed]
108. Zheng, X.; Ren, X.M.; Zhao, L.; Guo, L.H. Binding and activation of estrogen related receptor γ as possible molecular initiating events of hydroxylated benzophenones endocrine disruption toxicity. *Environ. Pollut.* **2020**, *263*, 114656. [CrossRef]
109. Chen, Z.; Gong, C.; Wang, S.; Lu, H.; Zhu, Y.; Ge, R.S.; Tang, Y.; Ying, Y. Benzophenone UV-filters: Inhibition on human and rat 17β-hydroxysteroid dehydrogenase 1—Insights from 3D-QSAR and docking studies. *J. Steroid Biochem. Mol. Biol.* **2025**, *250*, 106739. [CrossRef] [PubMed]
110. Wang, M.; Yu, Y.; Tang, Y.; Pan, C.; Fei, Q.; Hu, Z.; Li, H.; Zhu, Y.; Wang, Y.; Ge, R.S. Benzophenone-1 and -2 UV-filters potently inhibit human, rat, and mouse gonadal 3β-hydroxysteroid dehydrogenases: Structure-activity relationship and in silico docking analysis. *J. Steroid Biochem. Mol. Biol.* **2023**, *230*, 106279. [CrossRef]
111. Haselman, J.T.; Sakurai, M.; Watanabe, N.; Goto, Y.; Onishi, Y.; Ito, Y.; Onoda, Y.; Kosian, P.A.; Korte, J.J.; Johnson, R.D.; et al. Development of the Larval Amphibian Growth and Development Assay: Effects of benzophenone-2 exposure in Xenopus laevis from embryo to juvenile. *J. Appl. Toxicol.* **2016**, *36*, 1651–1661. [CrossRef]
112. *SCCS/1625/20*; Opinion on Benzophenone-3 (CAS No 131-57-7, EC No 205-031-5), Preliminary Version of 15 December 2020, Final Version of 30–31 March. SCCS (Scientific Committee on Consumer Safety): Luxembourg, 2021.
113. *SCCS/1679/25*; Scientific Advice on the Safety of Benzophenone-2 (BP-2) (CAS No. 131-55-5, EC No. 205-028-9) and Benzophenone-5 (BP-5) (CAS No. 6628-37-1, EC No. 613-918-7) as Substances with Potential Endocrine Disrupting Properties in Cosmetic Products, Preliminary Version of 27 March 2025, Final Version of 26 June 2025. SCCS (Scientific Committee on Consumer Safety): Luxembourg, 2025.
114. *SCCS/1672/24*; Opinion on Benzophenone-1 (CAS No. 131-56-6, EC No. 205-029-4), Preliminary Version of 25 October 2024, Final Version of 27 March 2025. SCCS (Scientific Committee on Consumer Safety): Luxembourg, 2021.
115. Levine, A. Sunscreen use and awareness of chemical toxicity among beachgoers in Hawaii prior to a ban on the sale of sunscreens containing ingredients found to be toxic to coral reef ecosystems. *Mar. Policy* **2020**, *117*, 103875. [CrossRef]
116. Wang, W.; Kannan, K. Mass loading and emission of benzophenone-3 (BP-3) and its derivatives in wastewater treatment plants in New York State, USA. *Sci. Total Environ.* **2017**, *579*, 1316–1322. [CrossRef]
117. Jiménez-Díaz, I.; Molina-Molina, J.M.; Zafra-Gómez, A.; Ballesteros, O.; Navalón, A.; Real, M.; Sáenz, J.M.; Fernández, M.F.; Olea, N. Simultaneous determination of the UV-filters benzyl salicylate, phenyl salicylate, octyl salicylate, homosalate, 3-(4-methylbenzylidene) camphor and 3-benzylidene camphor in human placental tissue by LC-MS/MS. Assessment of their in vitro endocrine activity. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2013**, *936*, 80–87. [CrossRef]
118. Lax, C.; Wicksell, E.; Grip, A.; Niemi, J.V.L.; Liu, W.; Rafeletou, A.; Kudłak, B.; Schiöth, H.B. The effect of sunscreen 4-methylbenzylidene camphor in different and reproductive models, its bioaccumulation and molecular effects on ligand-receptor interaction, and protein expression. *Basic Clin. Pharmacol. Toxicol.* **2023**, *133*, 130–141. [CrossRef] [PubMed]
119. Prakash, V.; Ansari, M.I.; Chauhan, S.S.; Parthasarathi, R.; Anbumani, S. Embryonal exposure to 4-methylbenzylidene camphor induces reproduction impairment in adult zebrafish (*Danio rerio*). *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2025**, *287*, 110051. [CrossRef] [PubMed]
120. Quintaneiro, C.; Teixeira, B.; Benedé, J.L.; Chisvert, A.; Soares, A.M.V.M.; Monteiro, M.S. Toxicity effects of the organic UV-filter 4-Methylbenzylidene camphor in zebrafish embryos. *Chemosphere* **2019**, *218*, 273–281. [CrossRef] [PubMed]

121. *SCCS/1640/21*; Scientific Opinion on 4-Methylbenzylidene Camphor (4-MBC), Preliminary Version of 22 December, Final Version of 29 April. SCCS (Scientific Committee on Consumer Safety): Luxembourg, 2022.
122. *SCCS/1638/21*; Scientific Advice on the Safety of Homosalate (CAS No 118-56-9, EC No 204-260-8) as a UV-Filter in Cosmetic Products in Cosmetic Products, Final Version of 2 December. SCCS (Scientific Committee on Consumer Safety): Luxembourg, 2021.
123. Ka, Y.; Lee, I.; Ji, K. Thyroid and growth hormone endocrine disruption and mechanisms of homosalate and octisalate using wild-type, thrα$a^{-/-}$, and dre-miR-$499^{-/-}$ zebrafish embryo/larvae. *Ecotoxicol. Environ. Saf.* **2024**, *286*, 117170. [CrossRef]
124. Coperchini, F.; Greco, A.; Teliti, M.; Denegri, M.; Croce, L.; Calì, B.; Gallo, M.; Arpa, G.; Chytiris, S.; Magri, F.; et al. In Vitro study of the UV-filter homosalate effects on rat and human thyroid cells. *Environ. Pollut.* **2024**, *363*, 125063. [CrossRef]
125. Lee, S.; Ka, Y.; Lee, B.; Lee, I.; Seo, Y.E.; Shin, H.; Kho, Y.; Ji, K. Single and mixture toxicity evaluation of avobenzone and homosalate to male zebrafish and H295R cells. *Chemosphere* **2023**, *343*, 140271. [CrossRef]
126. Meng, Q.; Yeung, K.; Chan, K.M. Toxic effects of octocrylene on zebrafish larvae and liver cell line (ZFL). *Aquat. Toxicol.* **2021**, *236*, 105843. [CrossRef]
127. Reum Kwon, B.; Jo, A.R.; Lee, I.; Lee, G.; Joo Park, Y.; Pyo Lee, J.; Park, N.Y.; Kho, Y.; Kim, S.; Ji, K.; et al. Thyroid, neurodevelopmental, and kidney toxicities of common organic UV filters in embryo-larval zebrafish (*Danio rerio*), and their potential links. *Environ. Int.* **2024**, *192*, 109030. [CrossRef]
128. Chu, S.; Kwon, B.R.; Lee, Y.-M.; Zoh, K.-D.; Choi, K. Effects of 2-ethylhexyl-4-methoxycinnamate (EHMC) on thyroid hormones and genes associated with thyroid, neurotoxic, and nephrotoxic responses in adult and larval zebrafish (*Danio rerio*). *Chemosphere* **2021**, *263*, 128176. [CrossRef]
129. Ferraris, F.K.; Garcia, E.B.; Chaves, A.d.S.; Brito, T.M.d.; Doro, L.H.; Félix da Silva, N.M.; Alves, A.S.; Pádua, T.A.; Henriques, M.d.G.M.O.; Cardoso Machado, T.S.; et al. Exposure to the UV Filter Octyl Methoxy Cinnamate in the Postnatal Period Induces Thyroid Dysregulation and Perturbs the Immune System of Mice. *Front. Endocrinol.* **2020**, *10*, 943. [CrossRef] [PubMed]
130. Alamer, M.; Darbre, P.D. Effects of exposure to six chemical ultraviolet filters commonly used in personal care products on motility of MCF-7 and MDA-MB-231 human breast cancer cells in vitro. *J. Appl. Toxicol.* **2018**, *38*, 148–159. [CrossRef] [PubMed]
131. *SCCS/1671/24*; Opinion on Ethylhexyl Methoxycinnamate (EHMC) (CAS No. 5466-77-3/83834-59-7, EC No. 226-7757/629-661-9), Preliminary Version of 25 October 2024, Final Version of 26 June 2025. SCCS (Scientific Committee on Consumer Safety): Luxembourg, 2025.
132. Strajhar, P.; Tonoli, D.; Jeanneret, F.; Imhof, R.M.; Malagnino, V.; Patt, M.; Kratschmar, D.V.; Boccard, J.; Rudaz, S.; Odermatt, A. Steroid profiling in H295R cells to identify chemicals potentially disrupting the production of adrenal steroids. *Toxicology* **2017**, *381*, 51–63. [CrossRef] [PubMed]
133. Lorigo, M.; Mariana, M.; Cairrao, E. Photoprotection of ultraviolet-B filters: Updated review of endocrine disrupting properties. *Steroids* **2018**, *131*, 46–58. [CrossRef]
134. Lorigo, M.; Quintaneiro, C.; Breitenfeld, L.; Cairrao, E. Exposure to UV-B filter octylmethoxycinnamate and human health effects: Focus on endocrine disruptor actions. *Chemosphere* **2024**, *358*, 142218. [CrossRef]
135. Yang, C.; Lim, W.; Bazer, F.W.; Song, G. Avobenzone suppresses proliferative activity of human trophoblast cells and induces apoptosis mediated by mitochondrial disruption. *Reprod. Toxicol.* **2018**, *81*, 50–57. [CrossRef]
136. Ka, Y.; Ji, K. Waterborne exposure to avobenzone and octinoxate induces thyroid endocrine disruption in wild-type and thrα-/-zebrafish larvae. *Ecotoxicology* **2022**, *31*, 948–955. [CrossRef]
137. Lebaron, P. UV filters and their impact on marine life: State of the science, data gaps, and next steps. *J. Eur. Acad. Dermatol. Venereol.* **2022**, *36*, 22–28. [CrossRef]
138. Mota, S.; Sousa, E.; Cruz, M.T.; Martins de Almeida, I. Occurrence and ecotoxicity of cosmetic ingredients in aquatic ecosystems: A narrative review. *Emerg. Contam.* **2025**, *11*, 100512. [CrossRef]
139. Kroll, A.; Kienle, C.; Junghans, M. Organic UV-filters and freshwater organisms: Data gaps impede a robust retrospective environmental risk assessment. *Environ. Sci. Eur.* **2025**, *37*, 6. [CrossRef]