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FOCUSED REVIEW

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The effect of sunscreen 4-methylbenzylidene camphor in different and reproductive models, its bioaccumulation and molecular effects on ligand-receptor interaction, and protein expression

Cassandra Lax[1] | **Elin Wicksell**[1] | **Anastasia Grip**[1] | **Jenni Viivi Linnea Niemi**[1] | **Wen Liu**[1] | **Alexandra Rafeletou**[1] | **Błażej Kudłak**[2] | **Helgi B. Schiöth**[1]

[1]Department of Surgical Sciences, Functional pharmacology and neuroscience, Uppsala University, Uppsala, Sweden

[2]Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, Gdańsk, Poland

Correspondence
Helgi B. Schiöth, Uppsala University, Department of Surgical Sciences, Functional pharmacology and neuroscience, Uppsala, Sweden.
Email: helgi.schioth@neuro.uu.se

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Abstract

4-Methylbenzylidene camphor (4-MBC) is a photo-absorbing UV filter prevalently used in cosmetics, which can be absorbed into circulation and cause systemic effects. 4-MBC is continued to be released in the environment despite the growing knowledge about its bioaccumulation and endocrine disrupting effects. Previous reviews have mentioned UV-filter together but this review considers 4-MBC alone, due to its prevalence and concerning health effects. This review considers 4-MBC's potential effects on human health regarding systemic and molecular effects, with the main focus on reproduction. Also, the potential bioaccumulation and interactions with receptor systems such as the oestrogen receptors β and α, and progesterone receptor are covered. Additionally, previous studies about 4-MBC's effects on mRNA and protein expression, especially in the prostate and the brain are analysed. Furthermore, 4-MBC is reported to act with inflammatory pathways by activating p38 MAPK and NF-κB, leading to the production of inflammatory TNF-α and IL-6. 4-MBC was also found to induce apoptosis and inhibit cell proliferation and DNA repair. In conclusion, 4-MBC has wide-ranging effects in many different models interacting with multiple pathways causing long-term effects even at low doses and this knowledge can guide governmental risk assessment, regulation divisions and chemical industries.

KEYWORDS
4-MBC, bioaccumulation, endocrine disruption, reproduction, sunscreen

Elin Wicksell and Anastasia Grip contributed equally to this work.

1 | INTRODUCTION

4-Methylbenzylidene camphor (4-MBC), also called Enzacamene, is used in topically applied personal care products (PCPs) to provide a UV-B filter and may therefore help in the prevention of skin cancer. Concentrations up to 4% are approved for use in most of Europe but are not allowed for use in Japan and USA. 4-MBC has been detected in urine samples even without sunscreen application, suggesting exposure from other sources, such as plastics, food packaging and textiles.[1,2] 4-MBC is frequently named to be an endocrine disruptor that causes reproductive toxicity and anti-androgenicity, for instance by inhibiting spermatogenesis, decreasing plasma 11-ketotestosterone levels, and down-regulating mRNA level of hormone receptor genes such as hERβ and hERα.[3,4] Additionally, 4-MBC is reported to affect placental and perinatal development. Detrimental effects on reproduction are shown in studies using many animal models as well as with human tissues such as trophoblast cells, endometrial cells and human spermatozoa.[4–6] 4-MBC is reported to induce transcriptomic responses in the HPG-axis by significantly increasing the levels of androgen receptors (ARs), oestrogen receptors, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) in the medaka fish brain. Due to its endocrine effects, 4-MBC is also reported to have thyroid-disturbing effects.[4] Furthermore, 4-MBC is reported to have adverse effects on the development of muscles and the heart in zebrafish.[7,8] Overall, 4-MBC affects cell proliferation, induces oxidative stress and cell apoptosis.[3] Potential interactions with oestrogen receptors, progesterone receptors and macrophages have been suggested as it has shown the potential to act as an endocrine disruptor, as well as trigger an inflammatory cascade.[9–11] It seems thus clear that 4-MBC can have adverse effects on multiple organismal levels, from development to reproduction.

4-MBC has been studied using many different experimental models since it was detected in aquatic ecosystems and proven to bioaccumulate in bodies of water in studies dating back to 2005.[12,13] Several of the published studies highlight the negative effects of this UV filter, either alone or in combination with other UV filters.[1,8,14] Meanwhile, its synergistic toxicity with other compounds, such as bisphenols (BPs) is emerging; any BP mixed with 4-MBC showed a strong synergy effect.[15] Overall, the main focus of most studies is the potential endocrine disruptive effects, whereas a few studies evaluate other effects such as induction of inflammatory cascades.[11] To our best knowledge, this article is the first article that solely focuses on how 4-MBC may affect reproduction. This is crucially important as we continue to discharge 4-MBC in our environment without adequate knowledge about how it affects our health. Therefore, there is a growing need for a thorough analysis of 4-MBC's effects and the aim of this review is to comprehensively analyse all the current knowledge about 4-MBC's effects on the body with focus on reproduction. We summarize the studies that involve 4-MBC and go through them considering both systemic and molecular effects. We start by clarifying 4-MBC's function as a UV-filter and discussing its bioaccumulation in nature to illustrate its high prevalence in the environment. After that, we start to discuss the 4-MBC molecular effects in ligand-receptor interactions, and RNA and protein expression levels. Then we discuss shortly 4-MBC's effects on cell apoptosis and proliferation. Finally, 4-MBC's systematic effects are analysed, and this is divided in 4-MBC's effects on inflammatory cascade, hormone levels and other effects. Parallel in the analysis, we pay special attention to which animal models, such as the rat and fish models, are used for studying 4-MBC's toxic effects. Rats and fish models are useful for reproductive studies as it is possible to follow them through multiple generations and see the transgenerational effect of the compound. We analyse these animal models and review their accuracy for studying 4-MBC's effects and how well the results can be applied to humans.

2 | APPROACH

The articles were identified on PubMed using search words such as just "4-methylbenzylidene camphor" and "4-MBC," or together with "female," "male," "embryo," "rat," "endocrine," "androgen," "oestrogen," "progesterone," "inflammation," "receptor," "mechanism of action," "photophysical properties," "photochemical properties" or "reproduction."

3 | CHARACTERISTICS OF 4-MBC

As briefly touched upon in the introduction, 4-MBC is a chemical compound that acts as a UV filter and shows signs of bioaccumulation both in the environment and in humans. In this section of the article, these aspects of 4-MBC are elaborated.

3.1 | Basics of the chemical structure

4-MBC's generic name is Enzacamene, and the IUPAC name is (3E)-1,7,7-trimethyl-3-[(4-methylphenyl)methylidene]bicyclo[2.2.1]heptan-2-one (see Figure 1). It is a small molecule, with an average weight of 254.373 and a

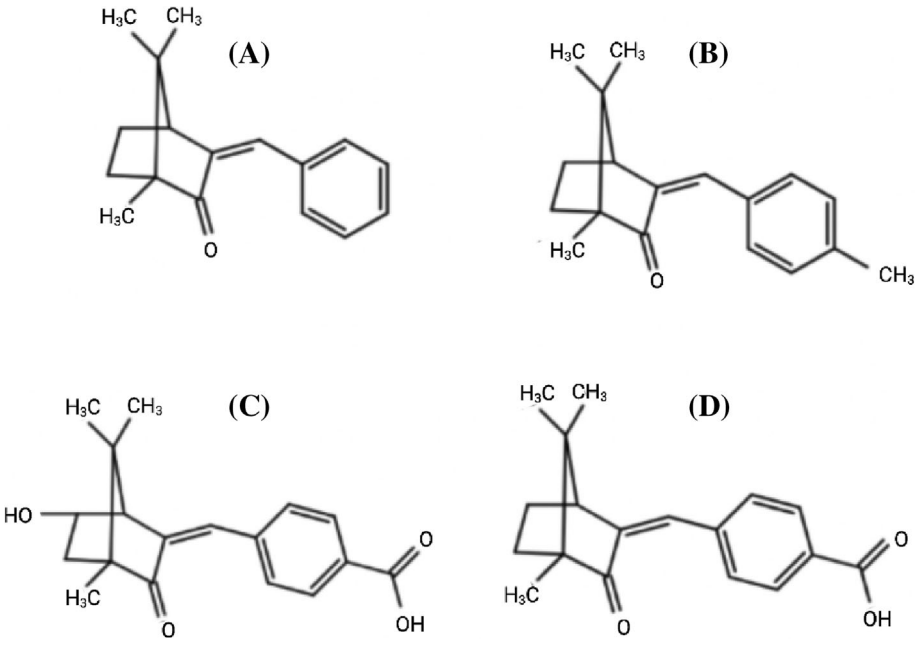

FIGURE 1 (A)4-Methylbenzylidene camphor, (B) 3-benzylidene camphor, (C) cx-MBC-OH and (D) cx-MBC.

chemical formula of $C_{18}H_{22}O$. It has a melting point between 66–70°C and an XLogP3-AA of 4.5. Other common names for 4-MBC are (+/−)-3-(p-methylbenzylidene)camphor, 3-(4-methylbenzylidene)camphor and 3-(p-Methylbenzylidene)-D-camphor.

3.2 | 4-MBC's function as a UVB filter

4-MBC's ability to absorb light at the UVB's wavelength (285–315 nm) is high as the molar attenuation coefficient for 4-MBC is 24 500 M − 1 cm − 1 at 300 nm. The light absorption occurs through a reversible photo-induced trans–cis isomerization. This scatters the photon energy, thus leading to protection from harmful rays. However, as 4-MBC is topically applied, it can be absorbed through the skin and thus may induce multiple possible mechanisms besides protection against UVB, such as endocrine disruption, or inducing inflammatory responses.[11,16]

3.3 | Bioaccumulation of 4-MBC in the environment and in humans

4-MBC accumulates extensively in living organisms and in the environment due to its chemical stability and lipophilicity (log Kow 5.1).[12] In 2005, 4-MBC was measured to be prevalent in water bodies and fish in Switzerland. In untreated wastewater, the 4-MBC concentration was 2.3–6.5 μg/L, and in treated wastewater, it was 0.2–2.3 μg/L.[12] Also, sediment samples collected from New Zealand, China, Brazil and Greece, because these are locations where different sunscreens are frequently used, contained 4-MBC in various concentrations.[13,15,17,18] Surface water collected from a subset of these locations, as well as Antarctica, was also positive for 4-MBC.[19] Interestingly, in Antarctica, 4-MBC was detected in 100% of the samples collected from effluent wastewater in concentrations ranging from 321 to 11 700 ng/L.[20] Additionally, 4-MBC has been found in low concentrations (up to 166 ng/g on a lipid basis) in white fish (*Coregonus* species), roach (*Rutilus rutilus*), perch (*Perca fluviatilis*), edible clams (Manila clam, *Ruditapes philippinarum*) and mussels (Mediterranean mussel, *Mytilus galloprovincialis*).[12,21–23]

Apart from environmental samples, 4-MBC has also been detected in samples collected from humans and some studies have focused on pregnant women. It has been found in 20.4% of human breast milk samples, and consequently, human babies are exposed to UV-filters at a concentration of approximately 48.37 ng/g lipid in human milk.[24,25] It is still unknown whether the 4-MBC found in the milk is directly from applying the PCP or if it can be stored in adipose tissue to be released later. Additionally, 4-MBC has been found in 20.7% of serum samples from pregnant women that participated in a study in Denmark.[26] Thus, the presence of 4-MBC in the human body is evident.

4-MBC as a chemical can be degraded into metabolites. It is metabolized in the body mainly through the two metabolites, 3-(4-carboxybenzylidene)-6-hydroxycamphor (cx-MBC-OH) and 3-(4-carboxybenzylidene)camphor (cx-MBC).[20] In rats, this UV filter undergoes extensive biotransformation by cytochromes P450 to

3-(4-carboxybenzylidene)-6-hydroxycamphor and 3-(4-carboxybenzylidene)camphor. These metabolites have been detected in low levels in rat urine, whereas the glucuronidated forms are believed to be excreted via faeces.[20] In rats, the blood concentration of 4-MBC and its metabolites reach the peak 10 h after exposure.[27] In humans, these metabolites have been found to reach the highest plasma levels after 12 and 24 h, respectively. Dermal application of 4% 4-MBC (w/w) covering 90% of the body surface was tested in order to mimic maximum dermal exposure.[20] It resulted in a mean dermal 4-MBC dose of 22 mg/kg bw, and 4-MBC has a half-life of 20–31 h in humans.[20] The initial chemical, 4-MBC, peaked in the plasma after 6 h with a half-life of 9 h, reaching the level of quantification at 48 h.[28] There is thus evidence that 4-MBC and its metabolites can be present in the human body for at least 2–3 days after exposure, but how it is accumulated and how long it may have detrimental effects remains to be studied further.

4 | 4-MBC AS AN ENDOCRINE DISRUPTOR

The role of 4-MBC as an endocrine disruptor is highlighted by many studies that have been conducted both on cell lines and on animal models. The endocrine-disrupting effects can be divided into different categories depending on the cellular-organismal level that 4-MBC interferes with and the effect that it has.

4.1 | Potential ligand-receptor interactions

There are not a lot of studies that demonstrate 4-MBC's direct interaction with a receptor. Three studies were found that attempted to illustrate competition binding in radioligand binding assays.[9,29] One study suggested that 4-MBC displaced 16alpha125I-estradiol from human oestrogen receptors β (hERβ) but not α (hERα).[14] The experiments in these papers can be considered very preliminary. The second paper is more extensive, suggesting that through the usage of human Ishikawa cells in vivo, 4-MBC could displace a labelled ligand at both hERβ and hERα at very high doses.[29] These results show that 4-MBC can bind to both hERβ and hERα in vivo *but* bind preferentially to hERβ.[29] The binding to both receptors was weak as an agonist but strong as an antagonist. However, 4-MBC has not been illustrated to be able to bind to hERα in vivo and the binding to hERα in vitro is likely due to active ligands such as 3-(4-carboxybenzylidene)-6-hydroxycamphor or 3-(4-carboxybenzylidene) camphor.[20] It has also been shown theoretically that 4-MBC can fit into the pocket of the TAK1, also called MAP3K7.[11] MAP3K7 is a serine/threonine protein kinase that when activated by IL-1 can form a complex required for activation of NF-κB. This was shown in a molecular docking study where the binding energy was determined to be −7.5 kcal/mol for 4-MBC. Lower binding energies indicate a higher possibility for stable binding. Via simulations, 4-MBC could be seen to potentially form a hydrophobic packing as its benzene ring is surrounded by hydrophobic amino acid residues.[11] In conclusion, present data suggest a direct interaction between 4-MBC and multiple receptors. However, antagonist activity seems to be low, and the biological relevance is debatable.

4.2 | Messenger RNA (mRNA) expression dysregulation

4-MBC has been found to alter mRNA expression in various pathways. In more detail, 4-MBC down-regulates the mRNA expression of hERβ, hERα, AR and insulin-like growth factor 1 (IGF-1) in adult rats.[30] In the prostate, AR mRNA and ER-α mRNA showed lower expression in the ventral lobe than in the dorsolateral lobe, whereas ER-β mRNA and IGF-1 mRNA were decreased in both lobes.[31] Similar results were observed in male Japanese medaka. Furthermore, 4-MBC was shown to decrease mRNA expression of progesterone receptors in the ventromedial hypothalamic nucleus (VMH) in the brain of female rats. Interestingly, the same effect was not seen in male rats.[32]

4.3 | Protein expression alterations

4-MBC has been suggested to affect different protein levels. Western blot analysis suggested that 4-MBC causes ER-α protein to be undetectable in the ventral prostate lobe and decreased in the dorsolateral prostate lobe in Long Evans rats. HER-β protein levels were unaltered in the ventral lobe and raised in the dorsolateral lobe. AR protein levels were decreased after 4-MBC exposure.[31] The expression of PR on the other hand, increases in the VMH and medial preoptic region (MPO).[33]

Additionally, 4-MBC was found to increase the production of the oestrogen-responsive gene products vitellogenin (VTG) and choriogenin (CHG). These gene products are produced in the liver as oestrogen-responsive precursor proteins and are commonly used as biomarkers for assessing disrupting effects on endocrine pathways.[27] In copepod *Tigriopus japonicus*, 4-MBC also induced transcription of an invertebrate ecdysone

receptor (EcR), which is important for development and reproduction in invertebrates. Furthermore, gene transcription of P53, an important gene for apoptosis and DNA repair, was significantly induced.[34]

4.4 | Induction of apoptosis and inhibition of cell proliferation

In the HTR8/SVneo human trophoblast cell line, 4-MBC was found to induce apoptosis and production of reactive oxygen species while inhibiting cell proliferation and invasion[4] (Figure 3A). Trophoblast cells are present in the early developing embryo and will go on to develop the placenta. If the trophoblasts die or are adversely affected, the placenta may not form correctly.[4] In zebrafish, apoptosis was induced via inhibited Bcl-2 proteins expression, Bax and cleaved Caspase-3. Potential binding interactions were also found with the zebrafish proteins Abcb4 and CYP450 8A. The function of the human ortholog to Abcb4 is undetermined; however, there is a theory that it is involved in the production of bile in hepatocytes.[35] 4-MBC also activated pathways for down-regulating PCNA by 55.0% (at 20 μM) and 75.6% (at 50 -μM), which plays an important role in DNA replication and repair.[35]

4.5 | Triggering of inflammatory cascade

Aside from endocrine effects, 4-MBC has been shown to have modulatory effects on macrophages by activating NF-κB and p38 MAPK pathways.[11] These pathways lead to an increase of inflammation-associated cytokines IL-6 and TNF-α. (Figure 3B). The authors presented a log Kow of 5.92 for 4-MBC, which means that 4-MBC mostly interacts with receptor proteins coupled with the release of IL-6. 4-MBC caused the macrophages to produce significantly more IL-6 and TNF-α compared to the control. The mRNA expression also increased 1.1-fold ($p < 0.05$) for TNF-α and 1.5-fold for IL-6 ($p < 0.001$).[11]

4.6 | Hormonal dysregulation

4-MBC have been suggested to affect testosterone synthesis in vitro using a Human Embryonic Kidney 293 (HEK293) cell line. The cell line was transfected with plasmids expressing human 17β-hydroxysteroid dehydrogenases (17β-HSDs) type 1, 2, 3 and 5, which are involved in several processes of hormone synthesis.[16] Subtype 17β-HSD3 catalyses the final step of testosterone synthesis in testicular Leydig cells, converting 4-androstene-3,17-dione (AD) to testosterone. This step was inhibited by 4-MBC at an IC50 of 10.7 μM.[16] Another subtype, 17β-HSD2, that converts testosterone to AD, and estradiol to estrone were shown to be inhibited by 4-MBC with an IC50 of 5.9 μM.[16] Additionally, subtype 17β-HSD1 was also weakly inhibited by 4-MBC with an IC50 of 70 μM.[16]

Furthermore, 4-MBC has been suggested to affect thyroid hormone levels by increasing TSH and T3 and decreasing T4 in rats.[31,36] However, 4-MBC did not change deiodinase or thyroid peroxidase activity.[31] One possible explanation for this pattern has been suggested to be a decrease in the activity of Dio-1, which is an enzyme that promotes the inactivation or activation of thyroid hormones.[37]

4.7 | Other off-target effects

4-MBC has been suggested to cause a premature induction of the acrosome reaction by causing a cationic channel of sperm (CatSper)-mediated rise in Ca2+. The premature acrosome reaction disturbs the spermatozoa's ability to fertilize the ovum.[5] Furthermore, 4-MBC competitively inhibits progesterone-mediated calcium influx, which desensitizes sperm to progesterone's effects.[5,38] Sperm in the mussel *M. galloprovincialis* has also suggested that 4-MBC induces oxidative stress in spermatozoa as well as physiological and functional impairments in vitro.[22] In females, 4-MBC does not seem to be able to terminate early pregnancy and it is not observed in trophoblasts that normally provide nutrients in early pregnancy.[6,39]

Additionally, 4-MBC exposure resulted in the delayed separation of the prepuce from the glans penis in male Long Evans rats after doses of 0.7, 7, 24 and 47 mg/kg/day.[30] 4-MBC administration also caused decreased ventral and dorsolateral prostate weight and increased thyroidal weight.[30] 4-MBC has even been shown to affect brain development in female rats leading to severely impaired sexual interaction and decreased perceptive behaviour with an unexposed male.[32,33,40]

In smaller aquatic organisms, such as the marine copepod *Tigriopus japonicus*, exposure to 4-MBC caused mortality and decreased reproduction in F0 generations at low concentrations (0.5, 1, 5 and 10 μg/L).[41] Mortality increased with higher concentrations, and a concentration of 1000 μg/L caused 100% mortality in copepod *Tigriopus japonicus*. The number of eggs produced by the F0 generation was found to be significantly decreased to 65 ± 16 ($p < 0.01$, 10 μg/L). However, in generation F1–3 mortality and reproduction were not as significantly affected.

In insect *Chironomus riparius* larvae, 4-MBC exposure (0–10 mg/L) did not result in a significant increase in mortality rate ($p > 0.05$).[33] In zebrafish embryos, 4-MBC (0.083–0.77 mg/L) exposure from 0 to 96 h post-fertilization (hpf) increased mortality and malformations. At the highest concentration tested (0.77 mg/L, 96 hpf), survival rate of the zebrafish embryos was 65%. Morphological abnormalities, such as notochord curvature, delayed absorption of the yolk sac and pericardial oedema were the most prevalent malformations. Of surviving embryos exposed to the highest concentration (0.77 mg/L), 100% presented malformations.[8]

5 | DISCUSSION

Articles about 4-MBC's environmental accumulation and systemic and molecular effects on human health were collected and summarized. The aim was to create a comprehensive and extensive summary of any potential reproductive effects 4-MBC might have. 4-MBC is a UV filter that bioaccumulates in the environment, acts as an endocrine disruptor and causes highly diverse adverse effects. Several studies suggest that 4-MBC has endocrine effects via functional antagonistic events related to hERβ, hERα and PR. The studies of 4-MBC's direct interaction with any specific hormonal receptor often use high doses to receive effects, which do not realistically mimic the environmental exposure. However, there are a number of studies suggesting that 4-MBC interacts with steroid signalling even in low doses, mainly through effects on the expression of RNA or a protein. Some of 4-MBC's possible interactions are summarized in Figure 2. Moreover, 4-MBC is also shown to have several other effects, such as thyroid hormone regulation, premature acrosome reaction in the sperm and promotion of inflammatory reactions.[11] There are also interesting cell studies showing 4-MBC effects on cell proliferation, invasion, induction of apoptosis and production of reactive oxygen species (Figures 2 and 3). The studies on 4-MBC may suggest that its effects are mainly related to transcriptional regulation; however, the data do not convincingly point out a single protein of interaction at this stage.

Ecological studies show 4-MBC's ability to interfere with multiple trophic levels even at low concentrations.[42] Furthermore, sunscreens often contain several different UV-filters, which makes it more challenging to interpret the additive toxic effects. This can occur even if there are no obvious adverse effect levels (NOAELs) individually.[43,44] In a recent study where mixtures of BPs and UV-filters were tested as a mixture, it was found that mixing any three BPs with 4-MBC can lead to strong synergy effects.[45] The particular mixtures contained any pair of three BPs (e.g., BPA + BPS, BPA + BPF and BPS + BPF), together with one sunscreen component, that is, oxybenzone, OXYB, 4-MBC or cinnamates (2-ethylhexyl 4-methoxycinnamate, EMC). These mixtures showed strong synergy or overadditive effects. However, mixtures containing two UV filters (any pair of OXYB, 4MBC and EMC) and one BPs (BPA, BPS or BPF) had a strong propensity towards concentration-dependent underestimation. This clearly demonstrates how difficult it may be to estimate the toxicity of such compounds in mixtures and that some of the complex effects of 4-MBC can be related to the complex interaction with other pollutants.

This article highlights the increasing concerns that 4-MBC may have detrimental effects on reproduction at different levels. 4-MBC can disturb the embryo implantation in the uterus wall in females and the production of testosterone and acrosome reaction in males.[4,5,16] 4-MBC has been suggested to affect testosterone's interaction with its receptor on Sertoli cells and disrupt the regulation of tight-junction protein claudin-3 expression.[5] 17β-HSD3 has also been suggested to be inhibited by 4-MBC, which could impact the blood testicle barrier negatively and thus endanger the spermatozoa.[5,16] However, the literature presents a high number of different experimental models and more standardized studies are needed to better understand the interactions in a more complex system than cell cultures. The systematic use of whole genome transcriptomics and epigenomics could be useful to identify the main pathways. These whole genome analyses could also result in the identification of the key nodes that are affected in the systems. Such key nodes are often targeted by pharmaceuticals such as GPCRs, kinases, solute carriers, ligands and number of enzymes.

An interesting question is the relevance of the studies of 4-MBC toxicity and if the toxicity could indeed be harmful in real life outside the laboratory. The realistic risk of the findings can be deduced from the measured prevalence of 4-MBC in the humans. 4-MBC concentrations measured in the human serum vary around 0.18–6.04 ng/ml in a study in Denmark whereas lower concentrations of approximately 48.37 ng/g have been measured in human breast milk.[24,25] These concentrations can be compared to concentrations used in the 4-MBC studies. Some studies showed an effect even at the low concentrations of 0.1 mg/L, which are close to the measured concentrations in humans. According to these concentrations, the results could be relevant for the public exposure of 4-MBC. Importantly, 4-MBC's toxic effects have been shown directly in humans for instance in testosterone concentration, which strengthens the argument that the numerous cited results in this article are relevant in practice.[14] However, although the 4-MBC concentrations relevant to public exposure concentrations are able

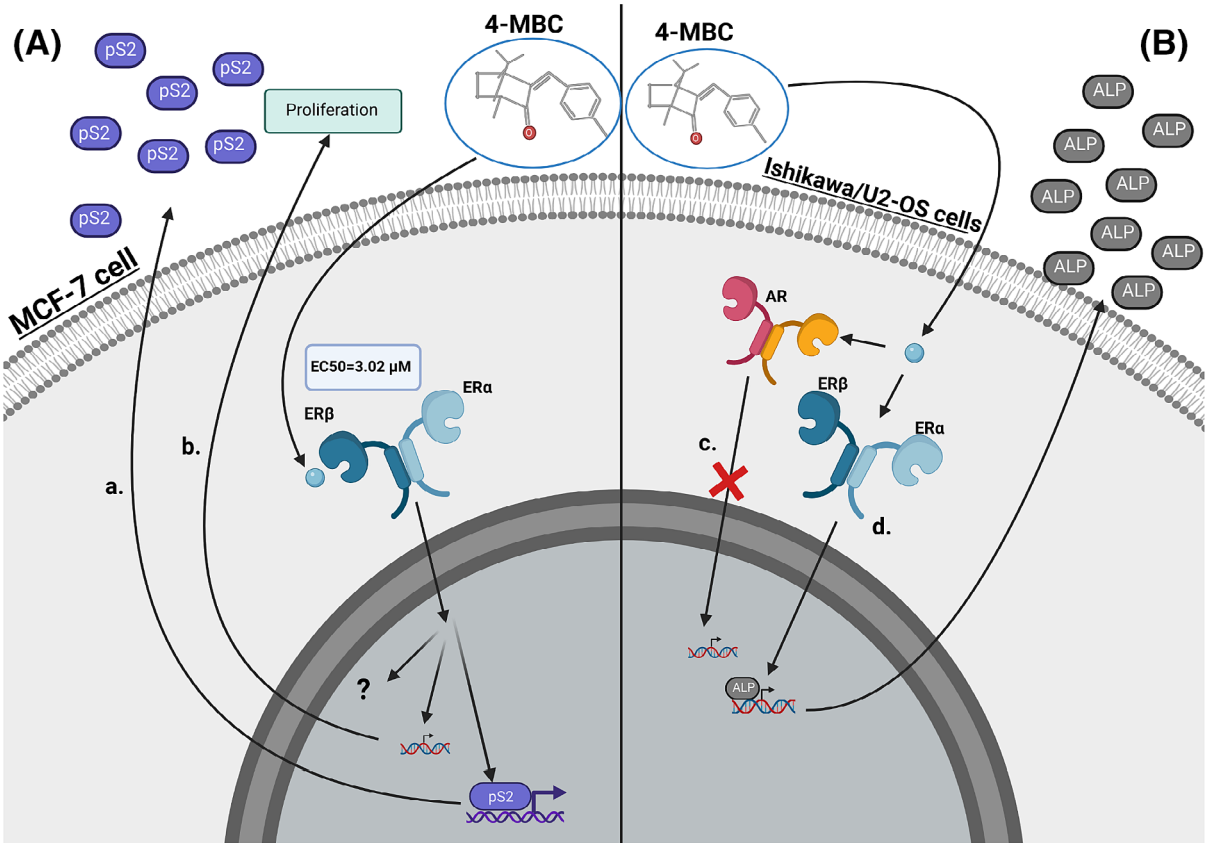

FIGURE 2 A. The effects of 4-MBC on MCF-7 (A), Ishikawa and U2-OS cells (B); results compiled from several studies.[20,29] (A) The study evaluated MCF-7 cells exposed to both 4-MBC and a mixture of 4-MBC with other common UV-filters (BP-1, BP-3, OMC). pS2 was used as a marker for estrogenicity in MCF-7 cells. Another study with the same cells and same estrogenicity marker showed similar results.[28] (B) With proliferation as a marker for estrogenicity, 4-MBC showed binding to ER, and they were also able to determine that 4-MBC binds to ERβ via competition experiments with 16α125I-estradiol.[28] B. Testing 4-MBC in Ishikawa and U2-OS cells. (C) Using U2-OS cells and dihydrotestosterone (DHT) as a positive control for androgen receptor (AR) agonist, the study found that 4-MBC had anti-androgenic potential.[20] This contradicted another study conducted that found no AR activity. However, this was explained as them using cells with a lower endogenous expression of AR (MDA-kb2 cells) meaning the specificity may not be accurate. (D) Alkaline phosphatase is a marker for estrogenic activity in Ishikawa cells (with endogenously expressed oestrogen receptors (ER)) and was used in a study to binding to ERα and β. The results confirmed a preferential binding to ERβ but further investigation showed that it was much weaker compared to other xenoestrogens tested.[29]

to harm model animals, it is unclear which concentrations could cause harm to humans. Therefore, the question remains how well these model animals can be used to reflect the toxic effects in humans. The studies on 4-MBC have taken advantage of several different animal models whereas the rat model has been one of the most valuable ones for functional studies and to study sexual dimorphism. 4-MBC was seen to delay puberty in male rats, as well as decrease prostate weight, increase testis weight, and alter androgen and oestrogen receptor expression in the prostate.[30] However, in female rats, 4-MBC was found to severely impair sexual behaviour.[33] Prenatally 4-MBC exposed female rats also exhibited lower progesterone mRNA expression in the hypothalamus, with similar progesterone expression levels as males. Rats are in general a good model organism to study 4-MBC effects on reproduction as rats and humans have many biological similarities. However, one important difference is the organization of the placenta. Rats, however, have a placental barrier made up of three layers forming a hemotrichorial labyrinth instead of single layer of hemomonochorical villous as human have. As the mechanism of the exchange of 4-MBC across the placenta is unknown, it is not clear if the results obtained from rats in the uterus can be translated to human physiology. It is speculated that the exact mechanism behind how 4-MBC penetrates the placenta could be critical to determine whether the rat is an adequate model animal for 4-MBC's effects or not.

Beyond the mammalian models, fish and aquatic animals are good model organisms to study how 4-MBC affects embryogenesis, as the fish embryos lack the

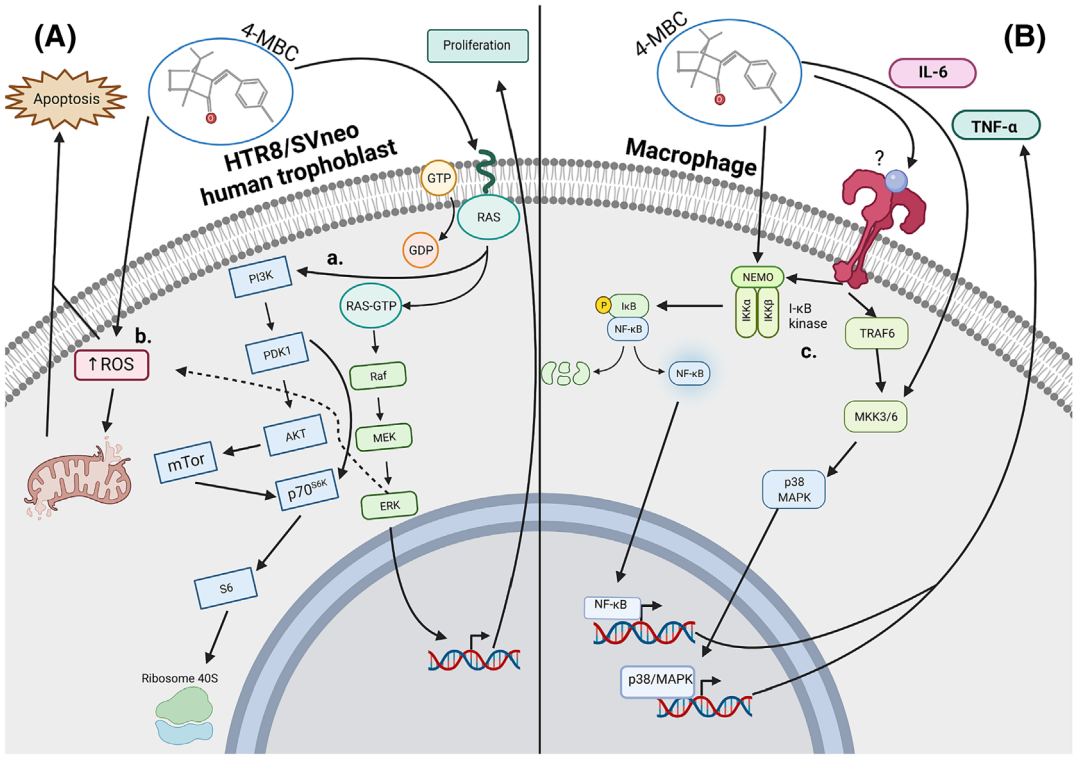

FIGURE 3 A. The effects of 4-MBC on HTR8/SV neo human trophoblast cells.[4] The picture shows how the PI3K/AKT and ERK pathways are activated, as well as the induction of reactive oxygen species, leading to apoptosis. (A) The results showed that every kinase measured was activated by 50 μM 4-MBC. (B) in the same study, oxidative stress was measured. This is induced by ROS accumulation and is thus used as a marker to study oxidative stress. It was increased by 4-MBC. B. the effects of 4-MBC on macrophages and how they activate inflammatory pathways.[11] (c) cytokines were studied TNF-α and IL-6 using a limulus amebocyte lysate test (LALT) and Western. Both IL-6 and TNF-α were significantly increased after exposure to 4-MBC compared to the control, TNF-α with a P-value < 0.05 and IL-6 with a P-value < 0.001. When adding SB203580 (p38 MAPK inhibitor) and BAY 11–7082 (NF-κB inhibitor) to 4-MBC exposed cells the expression of IL-6 and TNF-α significantly decreased.[11]

protective placenta and are easy to study in terms of progression. Some very useful model organisms within the 4-MBC research are molluscs (*Mytilidae*), American bullfrog (*Lithobates catesbeianus*), copepod (*Tigriopus japonicus*) and the freshwater caddisfly (*Sericostoma vittatum*).[46–48] Some other organisms involved in studying 4-MBC's effects are Japanese medaka (*Oryzias latipes*), Harlequin fly (*C. riparius*), zebrafish (*Danio rerio*), Senegalese sole (*Solea Senegalensis*) and Iberian green frog (*Pelophylax perezi*). The downside of many of these organisms, such as Japanese medaka and Harlequin fly, is that they are sensitive fish useful for ecotoxicity tests but less useful for studying 4-MBC's effects directly on human health. Zebrafish, Iberian green frog and senegalese sole are examples of model organisms more commonly used for studying 4-MBC's effects on human health mainly because of their short life cycle and rapid pollutant absorption. In multiple studies, an important measurement was mortality, which unfortunately is a relatively unspecific measure as it does not provide specific data. The use of multiple species to determine the overall toxicity is important, as seen by the different results in EC50 compiled from multiple studies (Figure 4).[41,49,50] Other commonly used cells for 4-MBC experiments are HEK293 cells, HTR8/SV neo human trophoblast cells and mouse neuro-2A cells. HTR8/SV neo cells are especially useful for studying 4-MBC's effect on the function of placental tissue as the cells are from human first-trimester placenta transfected with the gene encoding for simian virus 40 large T antigen. Neuro 2A cells, on the other hand, are a fast-growing mouse neuroblastoma cell line that has the ability to differentiate into neurons and are used for studying axonal growth, neuronal differentiation and signalling pathways. HEK293 cells are a more versatile cell line that is used for studying 4-MBC's effects in many fields.

Limitations of the current research on 4-MBC's effects relate to relatively dated technologies and the lack of larger-scale studies. A large part of the research was performed in the middle of the 2000s but has since tapered off a bit, even though the results were inconclusive. We lack studies that show how a low dose of 4-MBC over

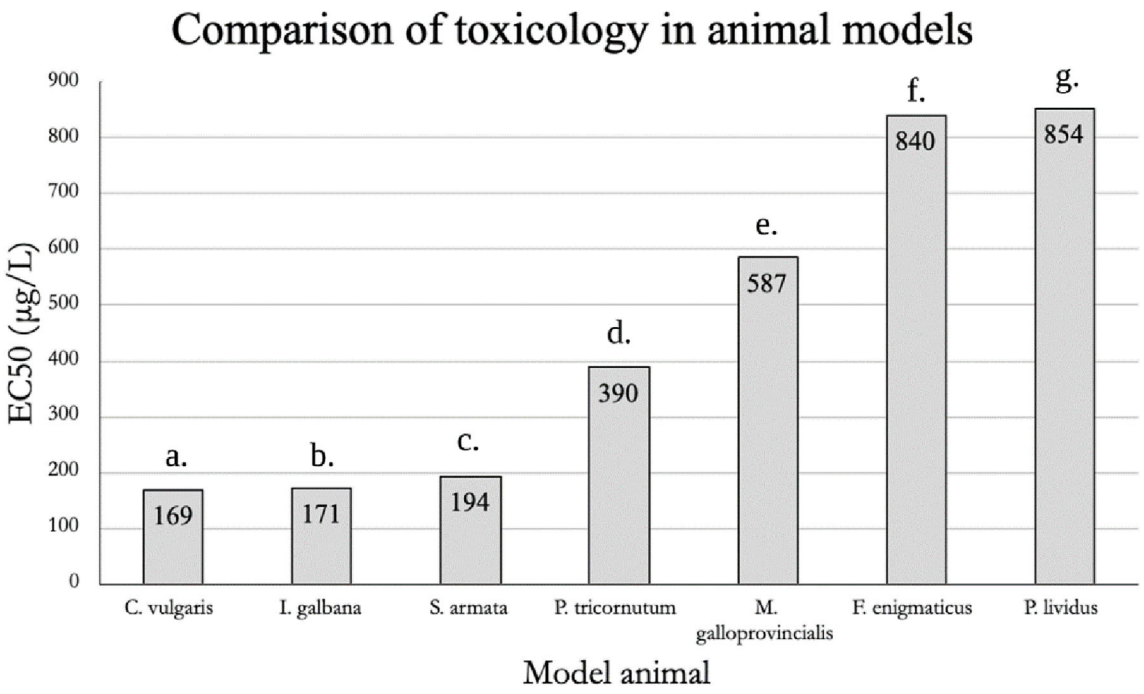

FIGURE 4 The graph shows the different EC50s for ecotoxicology measured in different model animals.[46–48] This illustration demonstrates a comparison from different experimental models, showing that different model organisms differ in sensitivity to 4-MBC. Thus, 4-MBC can be deemed toxic for different animals in different concentrations. (A) *Chlorella vulgaris* is a green microalga and toxicity was assessed using a growth inhibition test. This study was determining acute toxicity, and when interpreting their data according to the acute toxicity test classification criteria, 4-MBC was classified as a very high toxicity level compound. (B) *Isochrysis galbana* is an autotroph and it was the most sensitive to 4-MBC.[47] (C) *Siriella armata* is a type of crustacea and reacted similarly to *P. lividus* to 4-MBC.[47] (D) *Phaeodactylum tricornutum* is a diatom and was used in a study to compare growth inhibition to exposure to 4-MBC. 4-MBC showed a growth inhibiting effect, however not as strong as the other UV filters tested.[46] (E) *Mytilus galloprovincialis* is a mussel, and among the UV filters tested in the study 4-MBC was the most toxic to this animal.[47] (F) *Ficopomatus enigmaticus* is a tube worm and was exposed to 4-MBC for a larval development assay. In this animal model, 4-MBC showed the highest toxicology compared to the other compounds.[46] (G) *Paracentrotus lividus* is a sea urchin and in the study, their larva was used to test the toxicity of multiple UV filters, among them 4-MBC. The result was that the two most toxic UV filters for *P. lividus* were EHMC and 4-MBC.[47]

time could potentially affect human health, which would provide a more accurate model of real-life exposure. Additionally, more research on 4-MBC levels in the environment would be needed, as well as the bioaccumulation and how long a relevant amount of 4-MCB stays in the human body.

In conclusion, several studies have established 4-MBC's damaging effects on hormonal balance, inflammation, prenatal development, fertilization, brain development and sexual development. However, so far there have been no reviews that compile information on the reproductive effects of specifically 4-MBC. There are many potential mechanisms for 4-MBC's action, and the broadness of the action does not suggest a single type of interaction with regulatory proteins but rather an effect that is mediated through complex transcriptional regulation. Exposure to 4-MBC is likely to have endocrine disruptive effects but it can also activate some more distinct pathways, for example, the p38 MAPK and NF-κB.[12] These pathways can then lead to the up-regulation of two inflammatory cytokines TNF-α and IL-6 and cause broader systemic effects. Overall, it is still unclear to what extent 4-MBC is absorbed and accumulated in the body or how its damaging effects can be avoided. More studies are needed to assess 4-MBC environmental concentrations, specific transcriptional regulations, interactions with other compounds and toxic effects in mixtures. More research regarding 4-MBC's inflammatory properties could be of interest from a reproductive health point of view. Furthermore, the next steps for research on 4-MBC's effects could be considering its effect on epigenetics, either alone or combined with other compounds. No studies so far have focused on, for example, the epigenetic profiles of embryos or germline cells after exposure of 4-MBC.

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CONFLICT OF INTEREST STATEMENT

The authors of this review have no competing interests.

ORCID

Helgi B. Schiöth https://orcid.org/0000-0001-7112-0921

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