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REVIEW

Effects of 6-methoxybenzoxazolinone (6-MBOA) on animals: state of knowledge and open questions

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Abstract
6-methoxybenzoxazolinone (6-MBOA) is a secondary plant metabolite predominantly found in monocotyledonous plants, especially Gramineae. In damaged tissue, 2-β-D-glucopyranosyloxy-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA-Glc) is hydrolyzed to DIMBOA, which spontaneously decomposes into 6-MBOA. It is commonly detected in plants consumed by voles and livestock and can also be present in cereal-based products. Discovered in 1955, this compound is renowned for its ability to trigger animal reproduction. However, there is a lack of research on its functional and mechanistic properties, leaving much of their potential unexplored. This review aimed to comprehensively summarize the effects of 6-MBOA on animal reproduction and human health, as well as its defensive role against herbivores. Studies have shown that 6-MBOA effectively inhibits the digestion, development, growth, and reproduction of insects. 6-MBOA may act as a partial agonist of melatonin and exert a regulatory role in mammalian reproduction, resulting in either promoting or inhibiting effects. 6-MBOA has been theorized to possess anti-tumor, anti-AIDS, anti-anxiety, and weight-loss effects in humans. However, insufficient attention has been paid to its defense properties against mammalian herbivores, and the mechanisms underlying its effects on mammalian reproduction remain unclear. In addition, research on its impact on human health is still in its preliminary stages. The review emphasizes the need for further systematic and comprehensive research on 6-MBOA to fully understand its diverse functions. Elucidating the effects of 6-MBOA on animal reproduction, adaptation, and human health would advance our understanding of plant–herbivore coevolution and the influence of environmental factors on animal population dynamics. Furthermore, this knowledge could potentially promote its application in human health and animal husbandry.

Keywords 6-MBOA · Herbivore · Plant secondary metabolite · Reproduction · Chemical defense · Human health

Abbreviations

2-HHPAA	2-Hydroxy-N-(2-hydroxyphenyl)-acetamide
2-HPAA-GlcA	N-(2-glucuronopyranosyloxy-phenyl)-acetamide
6-MBOA	6-Methoxybenzoxazolinone
6-MBOA-Glc	6–β-D-glucopyranosyloxy-methoxybenzoxazolin-2-one
ARC	Arcuate nuclei
BOA	2-Benzoxazolin-2(3H)-one
BPH1	Benign prostate hyperplasia
BXs	Benzoxazinoids
CNS	Central nervous system
CYP11a1	Cytochrome P45011a1
DIBOA	2,4-Dihydroxy-2H-1,4-benzoxazin-3(4)-one
DIBOA-Glc	2-β-D-glucopyranosyloxy-4-hydroxy-1,4-benzoxazin-3-one
DIMBOA	2,4-Dihydroxy-7-methoxy-1,4-benzoxazinone
DIMBOA-Glc	2-β-D-glucopyranosyloxy-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one
ECB	European Corn Borer
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
HBOA	2-Hydroxy-1,4-benzoxazin-3-one
HBOA-Glc	2-β-D-glucopyranosyloxy-1,4-benzoxazin-3-one
HBOA-GlcA	2-Glucuronopyranosyloxy-1,4-benzoxazin-3-one

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HDMBOA	2-Hydroxy-4,7-dimethoxy-2H-1,4-benzoxazin-3(4H)-one
HDMBOA-Glc	4,7-Dimethoxy-2-[3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxy-1,4-benzoxazin-3-one
HMBOA	2-Hydroxy-7-methoxy-1,4-benzoxazin-3-one
HMBOA-Glc	2-β-D-glucopyranosyloxy-7-methoxy-1,4-benzoxazin-3-one
HMPMA	3-Hydroxy-1-methylpropylmercapturic acid
HPG	Hypothalamic-pituitary–gonadal
LNCaP	Prostatic carcinoma cell line
LH	Luteinizing hormone
PSA	Prostate-specific antigen
PSMs	Plant secondary metabolites
STAR	Steroidogenic acute regulatory protein
T	Testosterone
TRIBOA-Glc	(2R)-4,7-dihydroxy-3-oxo-3,4-dihydro-2H-1,4-benzoxazin-2-yl beta-D-glucopyranoside
UGTs	UDP-glycosyltransferases

Introduction

Plant secondary metabolites (PSMs) are diverse molecular compounds not essential for basic plant metabolism and growth, but critical for plant interactions with their environment and coping with biotic and abiotic stressors. PSMs protect plants against herbivores, bacteria, fungi, viruses, and competing plants (Bennett and Wallsgove 1994; Wink 2016). 6-methoxybenzoxazolinone (6-MBOA), a cyclocarbamate compound originally discovered in *Coix lachryma*, is a natural PSM (Koyama and Yamato 1955). It is derived from hydroxamic acid compounds, which mainly include 2,4-dihydroxy-7-methoxy-1,4-benzoxazinone (DIMBOA), 2,4-dihydroxy-2H-1,4-benzoxazin-3(4)-one (DIBOA), and other derivatives. These compounds are present in intact plant cells in the form of both diglycosides and monoglucosides (Baumeler et al. 2000). Wounding of tissue results in hydrolysis and degradation of glycosides of DIMBOA and DIBOA to 6-MBOA and BOA, respectively (Sanders et al. 1981; Epstein et al. 1986) (Fig. 1). These degradation products are present at much lower concentrations compared to the hydroxamic acid compounds (Tanwir et al. 2013). 6-MBOA is widely distributed in monocotyledonous plants, such as wheat, maize, and bamboo (Wahlroos et al. 1959; Argandoña and Corcuera 1985; Bailey and Larson 1991; Talbott and Talbott 2013; Dai et al. 2014) and occurs in a limited number of dicotyledonous plants (Niemeyer et al. 1986; Niemeyer 1988). Additionally, 6-MBOA is present in common cereal-based food items in our daily diet (Fomsgaard et al. 2011; Pihlava and Kurtelius 2016). 6-MBOA demonstrates allelopathic activities towards other plant species and various bacteria, fungi, and soil microbial communities (Wang et al. 2001; Wang and Ng 2002; Martyniuk et al. 2006; Acharya et al. 2021) and has multiple effects on herbivorous animals. 6-MBOA was found to act as a crucial environmental signal to initiate the seasonal reproductive activities of wild voles (Berger et al. 1981), sparking significant interest among researchers in its involvement in animal reproduction.

Upon its discovery, 6-MBOA was recognized as a phytochemical compound involved in plant defense mechanisms against insects (Wahlroos et al. 1958; Klun and Brindley

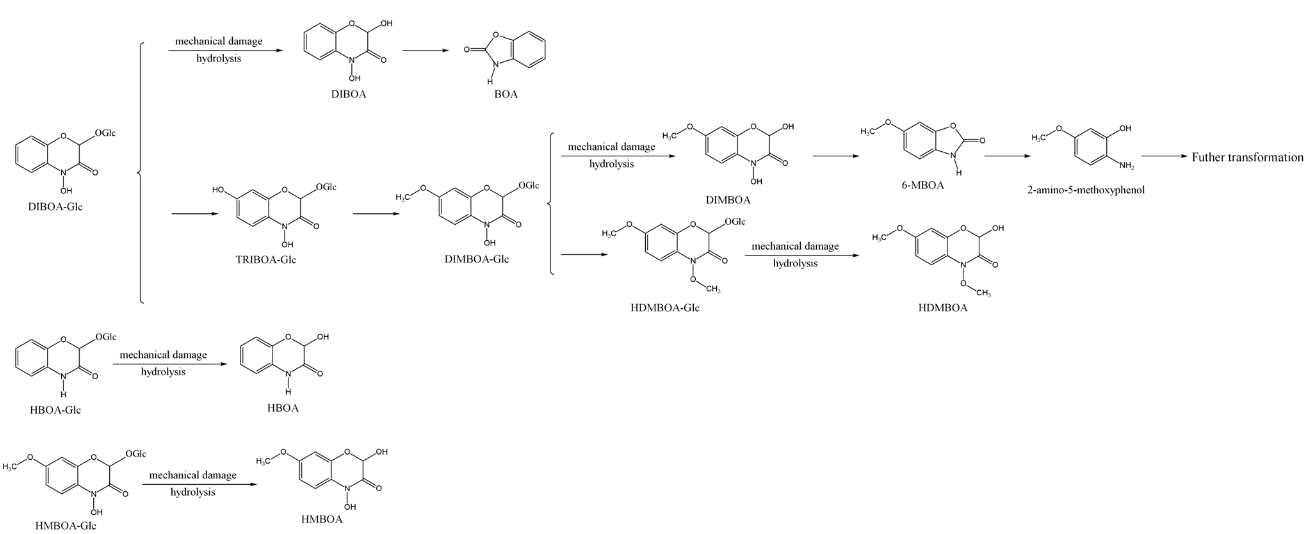


Fig. 1 Transformation between some BXs (referenced from Wouters et al. (2016), Macías et al. (2007), and Villagrasa et al. (2008)). Note: The "Glc" represents "Glycoside."

1966; Jiang et al. 2007). The insecticidal properties of 6-MBOA include its ability to inhibit reproduction, feeding, and digestion (Campos et al. 1988; Houseman et al. 1992; Dowd and Vega 1996). Because of its structural similarity to melatonin, 6-MBOA is believed to act on the reproductive system of animals (Chen et al. 2016). It can affect the reproductive capabilities of herbivorous animals, including invertebrates (Hansen 2006), birds (Brake et al. 1985; Berger et al. 1987), and mammals (Berger et al. 1981; Dai et al. 2016, 2017). The effects of 6-MBOA on animal reproduction vary, encompassing both promotion and inhibition, which are contingent on the species and environmental factors. Moreover, 6-MBOA impedes the digestion and growth of herbivorous mammals by influencing the composition of the cecal microflora (Dai et al. 2022). In addition, 6-MBOA has been theorized to possess antitumor, anti-AIDS, anti-anxiety, and weight-loss effects in humans (Adhikari et al. 2015). Despite approximately 70 years of research reporting the diverse effects of 6-MBOA on animals, researchers have not arrived at a consensus regarding its functions, and the exact mechanisms remain to be comprehensively elucidated. Given the widespread occurrence of 6-MBOA in plants consumed by voles and livestock (Dai et al. 2014) and in cereal-based products, it is crucial to elucidate its functionalities and underlying mechanisms. This review acquaints the reader with the chemical properties and natural distribution of 6-MBOA, extensively summarizes its impact on animal reproduction, defense, and human health, and delves into the limitations of existing research. By undertaking this approach, we aimed to foster in-depth investigations into the functions of 6-MBOA in animals, facilitating the comprehension of the coevolution between plants and herbivores with 6-MBOA as a pivotal mediator, and its improved utilization in animal husbandry and health products.

Chemical properties and natural sources of 6-MBOA

Chemical properties of 6-MBOA

6-MBOA, a natural product in powdered form, has a molecular weight of 165.15 g/mol, a melting point of 151–156 °C, a boiling point of 292.97 °C, and a density of 1.345 g/cm^3. It is soluble in organic solvents, such as acetone, dichloromethane, methanol, and tetrahydrofuran. From a chemical perspective, 6-MBOA was identified as 6-methoxy-2-benzoxazolinone, a cyclocarbamate compound (Sanders et al. 1981) comprising a benzene, ketone, an amino group, and two ether bonds (Fig. 1).

Natural derivation of 6-MBOA

Benzoxazinoids (BXs) are a class of compounds consisting of indoles with a 2-hydroxy-2H-1,4-benzoxazine-3(4H) ketone skeleton and their derivatives (Gao et al. 2017). The structural diversity of BXs has led to their classification into hydroxamic acids, lactams, benzoxazolinones, and methyl derivatives (Niemeyer 2009; Hanhineva et al. 2011) (Table 1, Fig. 1). Among these, the most biologically active compounds are hydroxamic acids (Niemeyer 2009) and their derivatives, which play crucial roles as secondary metabolites in phytochemical defense mechanisms (Fomsgaard et al. 2004). These compounds are primarily found in monocotyledonous plants, specifically Gramineae, although rare occurrences have been observed in dicotyledonous plants, such as *Acanthaceae* and *Scrophulariaceae* (Niemeyer et al. 1986; Niemeyer 1988; Dai et al. 2014). As the most abundant hydroxamic acid derivative in the Gramineae family (Argandoña and Corcuera 1985), DIMBOA exists primarily as a glycoside in plants (DIMBOA-Glc) (Hofman and Hofmanova 1969). Following tissue damage in young plants, DIMBOA-Glc is hydrolyzed by β-glucosidase, leading to the formation of DIMBOA, which spontaneously decomposes into 6-MBOA (Epstein et al. 1986).

Distribution of 6-MBOA in plants

The distribution of 6-MBOA is primarily influenced by the specificity of the plant organs and tissues, as well as the growth and developmental stages. For instance, 6-MBOA is found in the roots, leaves, and seeds of wheat (*Triticum spelta*), with significantly higher content in the roots than in other parts of the plant (Villagrasa et al. 2006). Similarly, in *Aphelandra squarrosa* and *A. fuscopunctata*, the 6-MBOA content is high in the root tip but low in the aboveground plant parts (Baumeler et al. 2000). Furthermore, germination

Table 1 Classification of the benzoxazinoids (referenced from Gao et al. (2017))

Classification	Representation
Hydroxamic acids	DIBOA(2, 4-dihydroxy-1, 4-benzoxazin-3-one)
	DIMBOA(2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one)
Lactams	HBOA(2-hydroxy-1,4-benzoxazin-3-one)
	HMBOA(2-hydroxy-7-methoxy-1,4-benzoxazin-3-one)
Benzoxazolinones	BOA(2-benzoxazolin-2(3H)-one)
	6-MBOA(6-methoxy-benzoxazolin-2-one)
Methyl derivatives	HDMBOA(2-hydroxy-4,7-dimethoxy-(2H)-1,4-benzoxazin-3(4H)-one)

noticeably increases 6-MBOA content in wheat grains (Zivkovic et al. 2023). During the early stages of wheat growth, the content of 6-MBOA is the highest and mainly concentrated in the meristem region of wheat seedlings. As development progresses, 6-MBOA gradually shifts to the wheat roots (Epstein et al. 1986; Mogensen et al. 2004). Plant samples with the same weight but of shorter height host a higher number of individual plants or branches, as well as a greater number of initial centimeters that contain a higher content of 6-MBOA. Consequently, the overall 6-MBOA content in shorter plants is greater, indicating a potential correlation between 6-MBOA content and plant height (Epstein et al. 1986). In *Leymus chinensis*, a vital forage grass for livestock and a favored plant among wild voles, the 6-MBOA content is highest during the early germination stage and gradually declines thereafter (Dai et al. 2014). Additionally, the 6-MBOA content in plants is influenced by various factors, including light intensity (Ahman and Johansson 1994), temperature (Epstein et al. 1986), and moisture (Richardson and Bacon 1993). Recent studies have reported the presence of 6-MBOA in the human diet. For example, beer produced from germinated or malted wheat or rye contains substantial amounts of BXs (Pedersen et al. 2011; Pihlava and Kurtelius 2016). Grain products such as bread made from rye, wheat, and other grains, including the simplest rye bran, are also abundant sources of 6-MBOA (Fomsgaard et al. 2011). Herbivorous animals, domestic animals, and humans consume 6-MBOA through the intake of plants and grain products, subtly affecting their physiology and overall health. Furthermore, while metabolic pathways and specific regulatory genes involved in the synthesis of 6-MBOA-related substances in plants have been elucidated (Wouters et al. 2016; Gao et al. 2017), how environmental stresses, such as global warming, influence the production of 6-MBOA-related substances in plants via these metabolic pathways and gene expressions remains unresolved due to the current paucity of research on the topic.

Role of 6-MBOA in animal defense

Defensive role of 6-MBOA against insects

Hydroxamic acid was initially investigated as a phytochemical defense compound with antibacterial and anti-insect properties, aiding in the prevention of bacterial and fungal infestations as well as insect ingestion by plants (Wahlroos et al. 1958; Klun and Brindley 1966; Fomsgaard et al. 2004; Jiang et al. 2007). 6-MBOA, a derivative of hydroxamic acid, also exhibits anti-insect effects and contributes significantly to plant resistance against herbivorous animals. Klun and Brindley (1966) discovered that inbred maize lines that demonstrated greater resistance to the first-brood larvae of the European corn borer (*Ostrinia nubilalis*) (ECB) exhibit higher levels of 6-MBOA. Larvae fed 6-MBOA experience a marked reduction in pupation rate and a significant increase in pupation time. Campos et al. (1988) determined that concentrations of 1.5 mg/g or higher prolong the development and adult emergence time of ECB larvae, increasing mortality rates. An assessment using different concentrations of 6-MBOA in feed and employing [^{3}H] MBOA as a tracer established a significant increase in larval development and adult emergence time. Furthermore, 6-MBOA has been found to inhibit trypsin activity in ECB (Houseman et al. 1992) and the food intake of leafhopper (*Dalbulus maidis*) (Dowd and Vega 1996). Even low concentrations of 6-MBOA have been shown to reduce aphid (*Sitobion avenae*) reproduction, thus preventing economic losses (Hansen 2006). When fed with wild type maize, the specialist western corn rootworm (*Diabrotica virgifera virgifera*) still exhibited a more significant response to BXs than the generalist congeneric southern corn rootworm (*D. undecimpunctata howardi*). This was evident in differences in body weight between the two species (Miller et al. 2014). However, further research is needed to verify the definite effect of 6-MBOA, a component of BXs, on these corn rootworms. These observations suggest that 6-MBOA may affect the reproductive, developmental, digestive, and feeding processes of insects. However, the precise mechanisms by which 6-MBOA acts against these insects remain largely unexplored. Some insect species have been reported to exhibit tolerance to DIMBOA, a precursor of 6-MBOA. For example, the fall armyworm (*Spodoptera frugiperda*) can glycosylate DIMBOA through stereoselective reglycosylation via insect UDP-glycosyltransferases (UGTs), forming nontoxic products (Wouters et al. 2014). Alternatively, the insect can utilize UGTs to metabolize DIMBOA to the HMBOA (2-hydroxy-7-methoxy-1,4-benzoxazin-3-one) which is then degraded to 6-MBOA spontaneously in the gut. The final excretion product is a mixture of (2S)-DIMBOA-Glc, (2S)-HMBOA-Glc, and 6-MBOA-Glc (Israni et al. 2020). This is one of few reports of the degradation adaptation mechanism of insects to DIMBOA, which could provide insights into the response mechanism of animals to 6-MBOA. Presently, 6-MBOA has been definitely observed to exhibit effects on a limited range of insects, including ECB, leafhoppers, and aphids. The absence of literature on the defensive properties of 6-MBOA against other insects prompts the question of whether these insects possess adaptations or tolerance mechanisms to 6-MBOA. Moreover, it is also urgent to study whether the feeding of insects can influence the production of 6-MBOA in plants, and how it affects this process.

Defensive role of 6-MBOA against herbivorous mammals

6-MBOA has been identified in the diets of herbivorous mammals. For example, *L. chinensis* is a preferred forage grass in the Inner Mongolian grassland and is consumed by Brandt's voles (*Lasiopodomys brandtii*). Our study revealed that 6-MBOA does not affect the food consumption of Brandt's voles but inhibits the growth of male individuals following intragastric administration for a 15-day period. Furthermore, the alpha and beta diversities of the cecal microflora vary after 6-MBOA treatment. The presence of butyrate, a short-chain fatty acid, in the cecum significantly increases after 6-MBOA treatment, resulting in changes in protein digestion and absorption, as well as the degradation and metabolism of foreign substances in the cecal microflora. Additionally, the abundances of the genera *Quinella*, *Caproiciproducens*, *Anaerofilum*, *Harryflintia*, and unidentified *Spirochaetaceae* in the cecum are enhanced in a dose-dependent manner following the administration of 6-MBOA. Our findings revealed that 6-MBOA has the potential to exert an effect on Brandt's voles by modulating the abundance of cecal bacteria, leading to alterations in the levels of short-chain fatty acids and pathway intermediates, ultimately impeding the growth of voles. We propose that 6-MBOA functions as a digestion-inhibiting PSM in the interactions between mammalian herbivores and plants (Dai et al. 2022). This represents the first identification of changes in the cecal microbiota in response to 6-MBOA in Brandt's voles, marking a significant advancement in our understanding of the mechanism of action of 6-MBOA in Brandt's voles. However, additional experiments are required to elucidate the specific mechanism of action.

Before the discovery of the effect of 6-MBOA on the gut microbiota of Brandt's voles, previous studies indicated its inhibitory effect on bacterial and fungal microorganisms (Wang et al. 2001; Wang and Ng 2002; Martyniuk et al. 2006; Acharya et al. 2021). However, it is worth noting that certain bacteria and fungi, including *Staphylococcus aureus*, *Bacillus subtilis*, and *Fusarium verticillioide*s, *F. subglutinans*, *F. cerealis*, and *F. graminearum*, exhibit tolerance to 6-MBOA. Additionally, some fungi possess the ability to metabolize it into non-toxic 3-hydroxy-1-methylpropylmercapturic acid (HMPMA), possibly explaining their tolerance to 6-MBOA (Glenn et al. 2001). These findings suggest a potential role of the gut microbiota in the adaptation of animals to 6-MBOA. Consequently, it is justifiable to explore the response of animals to 6-MBOA by investigating the changes in the cecal microflora of male Brandt's voles. The defensive role of 6-MBOA in herbivorous mammals has been largely overlooked by researchers, with only the response of Brandt's voles to 6-MBOA being investigated. PSMs are chemical compounds that play a crucial role in the defense against herbivores and affect herbivore physiology and behavior (Freeland and Janzen 1974; Hughes 1988). In response, herbivores have developed various strategies such as gut microbial detoxification and biotransformation by cytochrome 450s in the liver and gut (Jones and Megarrity 1986; Ding and Kaminsky 2003; Dearing et al. 2005; Sundset et al. 2010; Johnson et al. 2018). For example, the koala (*Phascolarctos cinereus*) is a tree-dwelling herbivore with a highly specialized diet, relying solely on eucalyptus leaves for sustenance. While eucalyptus leaves would be toxic or fatal to most other mammals due to toxic secondary metabolites such as tannins and formylated phloroglucinols, the koala has evolved adaptations to detoxify and tolerate these compounds. Metagenomic sequencing of the koala's gut microbiome and genome sequencing of the koala have revealed commensal bacteria that aid in degrading these toxic plant metabolites. Furthermore, expansion of the cytochrome P450 family genes have been observed, aiding the koala in detoxifying toxic phenolic compounds in eucalyptus trees (Shiffman et al. 2017; Johnson et al. 2018). Japanese wood mouse (*Apodemus speciosus*) can adapt to consuming plants with high tannin content through their gut-dwelling tannin-degrading bacteria (Shimada et al. 2006). White-throated woodrat (*Neotoma albigula*) can aid their adaptation to consuming foods with high oxalic acid content through oxalate-degrading bacteria (Miller et al. 2014). Future investigations should comprehensively examine the responses of both insects and herbivorous vertebrates to 6-MBOA, employing isolation of 6-MBOA-degrading bacteria, gut bacteria transplantation, metagenomic sequencing of the gut microbiome, and genome sequencing of herbivores to elucidate gut microbial degradation and biotransformation enzyme detoxification. These approaches will enhance our understanding of animal adaptive mechanisms to PSMs and the co-evolution of plants and herbivores. Additionally, some forage grasses, such as *L. chinensis*, also contain 6-MBOA; therefore, exploring the physiological response of livestock to defensive 6-MBOA would be beneficial for the advancement of animal husbandry.

Role of 6-MBOA in animal reproduction

Effects of 6-MBOA on rodent reproduction

PSMs have been hypothesized to function as signaling molecules in the regulation of reproductive processes in conjunction with environmental cues such as photoperiod, nutrition, rainfall, and temperature (Reiter 1993; Paul et al. 2008; Visser et al. 2010). Notably, 6-MBOA, a PSM, has been shown to exert an influence on animal reproductive function. Studies investigating the effects of 6-MBOA on rodent reproduction were mainly conducted during the 1980s and the 1990s, initiated by Berger et al. (1981) (Table 2). Among these studies, the most extensively examined rodents were the voles of the *Cricetidae* family. Berger et al. (1981) conducted winter field experiments on non-breeding populations of montane voles (*Microtus montanus*) fed oats coated with 6-MBOA and demonstrated that this compound triggers reproductive activity,

Table 2 Reproductive response of rodents to 6-MBOA

Family	Species	Method of 6-MBOA application	Response	Source
Cricetidae	*Microtus montanus*	Feeding, injection, or implantation	Promotion	(Berger et al. 1981, 1987, 1992; Sanders et al. 1981; Frandsen et al. 1993)
		Injection	Inhibition	(Gower and Berger 1990)
	M. townsendii	Feeding	Promotion	(Korn and Taitt 1987)
	M. pinetorum	Implantation	Promotion	(Schadler et al. 1988)
	M. ochrogaster	Feeding	Promotion	(Nelson 1991; Nelson and Blom 1993)
		Feeding	Inhibition under LP	(Nelson 1991)
	M. arvalis	Injection	No significant effect	(Krol et al. 2012)
	Lasiopodomys brandtii	Injection	Promotion under SP	(Dai et al. 2016)
		Injection	Inhibition under LP	(Dai et al. 2017)
	Lemmus sibiricus	Injection	Promotion	(Negus and Berger 1987)
	Peromyscus leucopus	Feeding	Inhibition	(Martin et al. 2008)
	Mesocricetus auratus	Implantation	No significant effect	(Anderson et al. 1988)
	Phodopus sungorus	Feeding	No significant effect	(Diedrich et al. 2014)
Heteromyidae	*Dipodomys ordii*	Injection and feeding	Promotion	(Rowsemitt and O'Connor 1989)
Muridae	*Mus musculus*	Implantation	Promotion under SP and inhibition under LP in females; no significant effect on males	(Nelson and Shiber 1990)
	Rattus norvegicus	Injection and implantation	Promotion	(Butterstein et al. 1985; Butterstein and Schadler 1988)
	Gerbillus harwoodi	Feeding	Promotion	(Alibhai 1986)

Feeding means “feeding 6-MBOA in the diet;” Injection means “intraperitoneal injection of 6-MBOA;” Implantation means “implantation of 6-MBOA-filled silastic capsules;” *LP* means “long photoperiod;” *SP* means “short photoperiod.”

as evidenced by increased uterine and testicular weight. Subsequent laboratory experiments by Sanders et al. (1981) and Berger et al. (1987) revealed that intraperitoneal injection of 6-MBOA and implantation of 6-MBOA-filled silastic capsules stimulate reproductive activity in montane voles. Specifically, Sanders et al. (1981) found that the intraperitoneal injection of 6-MBOA in juvenile and mature female voles results in increased uterine weight, whereas implantation of capsules in females leads to increased litter size, number of litters, and female offspring (Berger et al. 1987). In contrast, Butterstein et al. (1985) observed that the effect of 6-MBOA on the reproductive system of rats (*Rattus norvegicus*) is age dependent. Their laboratory experiments showed that injection of 6-MBOA in prepubertal females results in increased ovarian and uterine weights, whereas implantation of silastic capsules in mature females leads to increased ovarian weight and corpora lutea number. By feeding 6-MBOA to Townsend’s voles (*M. townsendii*) in winter, Korn and Taitt (1987) observed an acceleration in the recruitment of young voles and sexual maturation, thereby advancing the breeding season of females by 4 weeks. After the implantation of a capsule containing 6-MBOA into the abdominal cavity of prepubertal and mature female pine voles (*M. pinetorum*), there was an increase in the weight of the ovaries, uterus, and body mass of both prepubertal and adult females. Additionally, the level of follicle-stimulating hormone (FSH) in prepubertal females exhibited a significant increase, whereas the vaginal opening remained closed (Schadler et al. 1988). Consequently, it was initially postulated that 6-MBOA acts as a cofactor to stimulate the mammalian reproductive system (Schadler et al. 1988). In female Ord’s kangaroo rats (*Dipodomys ordii*), injection of 6-MBOA results in increased uterine and ovarian weights, whereas field feeding experiments have shown an increase in population (Rowsemitt and O'Connor 1989). In female *Lemmus sibiricus*, the injection of 6-MBOA leads to an increase in uterine weight (Negus and Berger 1987). In Harwood’s gerbils (*Gerbillus harwoodi*), feeding in the field leads to an increase in testicular and ovarian weights (Alibhai 1986). Dai et al. (2016) observed reproduction-stimulating effects in male Brandt’s voles by injecting 6-MBOA during a short photoperiod. Moreover, 6-MBOA has the potential to decrease the testicle size in white-footed mice (*Peromyscus leucopus*) under both long and short photoperiods, thus influencing their reproductive systems (Martin et al. 2008). However, it is important to note that the promotion of reproduction by 6-MBOA has not been observed in any rodent species. According to Anderson et al. (1988), the implantation of a silastic capsule containing 6-MBOA in male Syrian hamsters (*Mesocricetus auratus*) under both long and short photoperiods does not improve their reproductive capacity. Similarly, Diedrich et al. (2014) reported that the administration of 6-MBOA to Djungarian hamsters (*Phodopus sungorus*) under both long and short photoperiods does not enhance reproduction. Additionally, Krol et al. (2012) found that the injection of 6-MBOA into female common voles (*M. arvalis*) under both long and short photoperiods has no effect on their reproductive organs.

Effects of 6-MBOA on the reproduction of non-rodent animals

In addition, researchers have discovered the effects of 6-MBOA on the reproductive processes of non-rodent species. For example, the reproduction of grain aphids (*Sitobion avenae*) is affected by 6-MBOA, with the greatest decrease in reproduction rate observed at a concentration of 0.1 mM (Hansen 2006). Interestingly, 6-MBOA has been found to enhance reproduction in birds, specifically quail (*Coturnix coturnix*) (Berger et al. 1987), although a delayed effect has been observed in single-comb white-horn pullets (Brake et al. 1985). Furthermore, the reproduction of male and female New Zealand White doe rabbits (*Oryctolagus cuniculus*) is significantly promoted by 6-MBOA (Rodriguez-De Lara et al. 2007; Fallas-Lopez et al. 2011). In contrast, 6-MBOA does not promote the reproduction of St. Croix White ewes, female minks (*Mustela lutreola*), or mares (*Equus caballus*) (Ginther et al. 1985; Vaughan et al. 1988; Willard et al. 2006). Studies on prepubertal gilts have indicated that 6-MBOA does not affect gonadal development or gonadotropins (Guthrie et al. 1984). The potential influence of 6-MBOA on the reproductive capabilities of cattle and sheep is yet to be documented and thus remains unknown. This lack of information impedes the comprehensive assessment of the impact of 6-MBOA on animal husbandry practices, given that certain forage grasses contain this phytochemical compound.

Mechanism of 6-MBOA effect on animal reproduction

The mechanism underlying the effect of 6-MBOA on animal reproduction has consistently remained a focal point in the realm of research investigating the interactions between 6-MBOA and animals (Fig. 2). Butterstein and Schadler (1988) surgically implanted a capsule filled with 6-MBOA into female Sprague–Dawley rats and removed their pituitary glands. They observed that the effects of 6-MBOA were evident only when FSH was administered in a dose-dependent manner. This implies that 6-MBOA might function as a regulatory factor in the hypothalamic-pituitary–gonadal (HPG) axis, affecting rodent reproductive processes. 6-MBOA does not seem to exhibit direct estrogenic activity, as indicated by its lack of effect in ovariectomized animals (Sanders et al. 1981) (Fig. 2). Its β-adrenergic agonist property is demonstrated by its stimulation of adenylate cyclases, suggesting that 6-MBOA may have diverse effects, including direct actions on gonadotropin synthesis and release (Sweat and Berger 1988) (Fig. 2). This is supported by the uterotrophic effects observed in montane voles with the use of the mixed adrenergic agonist ephedrine and antidepressant imipramine.

Given the structural resemblance between 6-MBOA and melatonin (Fig. 3), which is synthesized by the pineal gland and regulated by the light/dark cycle (Reiter 1980), researchers have examined the relationship between 6-MBOA and melatonin, as well as the influence of photoperiod on the reproductive consequences of 6-MBOA. Studies have shown that 6-MBOA enhances melatonin production by activating serotonin N-acetyltransferase activity in the pineal glands of rats (Yuwiler and Winters 1985) (Fig. 2). From these findings, it has been theorized that the observed association between progonadal effects and the consumption of plants containing 6-MBOA in montane voles could be attributed to the excessive stimulation of melatonin receptor sites. Additional potential explanations include non-pineal effects, such as the inhibition of melatonin receptors in the central nervous system or gonads, as well as a direct impact of this compound on gonadal function (Yuwiler and Winters 1985) (Fig. 2). Alternatively, the pro-gonadal effects of 6-MBOA could also arise from adrenergic stimulation of gonadotropin release, whereas it may exert anti-gonadal effects by stimulating melatonin synthesis (Sweat and Berger 1988). This phenomenon can explain the steep dose–response curve observed in MBOA-induced uterotropic effects as well as the inhibitory effects observed at higher doses of MBOA (Sanders et al. 1981; Sweat and Berger 1988). The predominant hypothesis concerning this mechanism suggests that 6-MBOA competes with melatonin for receptor sites in tissues (Ellis 1972; Sweat and Berger 1988), potentially diminishing the inhibitory effects of melatonin on growth and sexual maturation (Gower and Berger 1990) (Fig. 2). Short-term implantation of 6-MBOA under a short photoperiod has been found to enhance reproduction in female house mice (*Mus musculus*), whereas long-term implantation inhibits reproduction (Nelson and Shiber 1990). In male montane voles, 6-MBOA has no discernible effect on the reproductive system during a long photoperiod, possibly because the maximum development of gonads is facilitated by extended daylight exposure at this time (Gower and Berger 1990). However, during short photoperiods, high doses of 6-MBOA inhibit reproduction (Gower and Berger 1990). The intraperitoneal administration of 6-MBOA results in an

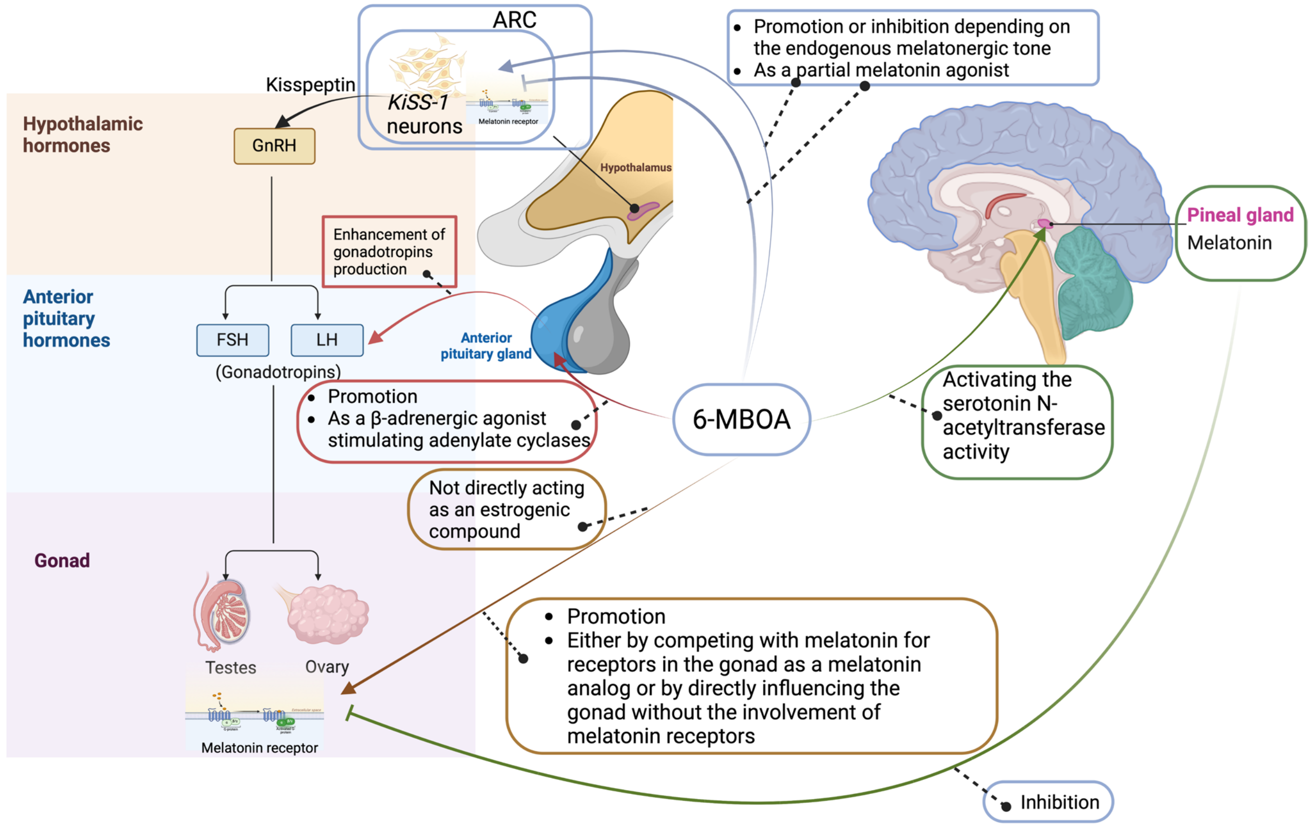
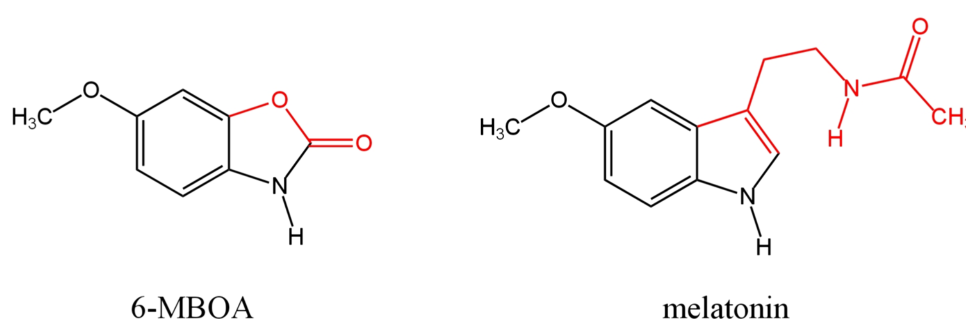


Fig. 2 The potential molecular pathways underlying the impact of 6-MBOA on animal reproduction. Note: The blunthead represents inhibitory effects, while the arrowhead represents promoting effects

Fig. 3 The structure of 6-MBOA and its structural similarity to melatonin (referenced from Gower and Berger (1990)). Note: The black section is the same structure between 6-MBOA and melatonin



increase in the relative testis weight of Brandt's voles, independent of dosage, under short photoperiod conditions (Dai et al. 2016). Furthermore, the serum levels of luteinizing hormone (LH) and testosterone (T) as well as the mRNA levels of steroidogenic acute regulatory protein (*StAR*) and cytochrome P45011a1 (*CYP11a1*), which are key enzymes for testosterone synthesis, are elevated in the testes (Dai et al. 2016). Nevertheless, 6-MBOA does not significantly affect the mRNA levels of *KiSS-1* in the arcuate (ARC) or anteroventral periventricular nuclei. Conversely, under long photoperiod conditions, 6-MBOA leads to a decrease in the mRNA level of *KiSS-1* in the ARC as well as a reduction in the circulating levels of LH, FSH, and T (Dai et al. 2017) (Fig. 2). The levels of *StAR* and *CYP11a1* in the testes as well as the relative testis weight decrease upon 6-MBOA administration, suggesting an inhibitory effect on the reproductive system of adult male Brandt's voles under a long photoperiod. It has been proposed that 6-MBOA may act as a partial melatonin agonist, exerting its effects via the *KiSS-1/GPR54* system, and the testes of the HPG axis to suppress reproductive activity in male Brandt's voles maintained under long-day photoperiod conditions (Dai et al. 2017) (Fig. 2). Similarly, a promoting effect of 6-MBOA has been observed under a short photoperiod, indicating that the photoperiod remains the main regulatory factor for the reproduction of Brandt's voles and that 6-MBOA serves as a modulatory signal for the photoperiod. In conclusion, the effects of 6-MBOA on the melatonin pathway are contingent on the endogenous melatonin tone and can exhibit either agonistic or antagonistic effects (Dai et al. 2017) (Fig. 2). In accordance with the hypothesis proposed by Gower and Berger (1990), 6-MBOA may function as a partial agonist of melatonin signaling. Furthermore, there is insubstantial evidence that 6-MBOA directly targets the melatonin receptor. Further investigation of its precise mechanism is needed.

Transmission of 6-MBOA information between animal generations

Researchers have also examined the process and timing of 6-MBOA transmission in parental communication with offspring in rodents. Female montane voles implanted with a silastic capsule containing 6-MBOA produce significantly larger seminal vesicles in male offspring and significantly increased uterine weight in female offspring (Berger et al. 1992). In montane voles from various regions, the reproductive rate of female offspring from 6-MBOA-treated females is also significantly enhanced, and the time of the first embryo birth is significantly advanced with an increase in litter size (Berger et al. 1992). Frandsen et al. (1993) investigated the effects of 6-MBOA on female montane voles during pregnancy and lactation. They observed no notable discrepancies in reproductive development among the progenies during the two treatment periods. Nonetheless, a significant difference was observed between the control group progeny and those of mothers who received 6-MBOA during pregnancy and lactation. Offspring from the latter group exhibited increased body length, uterine weight, and testicular weight. These findings suggest that 6-MBOA enhances procreation during pregnancy and lactation. Additionally, females receiving 6-MBOA only during pregnancy had larger uteri, indicating that the uterus is more sensitive to 6-MBOA during pregnancy than during lactation (Frandsen et al. 1993). The addition of 6-MBOA to the diet of female prairie voles (*M. ochrogaster*) during pregnancy and lactation eliminates the delay in reproductive maturation in male offspring caused by short photoperiod exposure. However, this effect is reversed under a long photoperiod. However, no difference is observed in female offspring, possibly indicating varying responses to photoperiod (Nelson 1991; Nelson and Blom 1993). These studies suggested that 6-MBOA can be transmitted between parents and offspring as an information molecule during pregnancy and lactation, thus influencing offspring reproduction (Nelson and Blom 1993). Unfortunately, there have been no further investigations into the transmission of 6-MBOA information between parents and offspring since the 2000s, with such observations being limited to female montane and prairie voles with their offspring. Consequently, a significant research gap exists regarding the processes and mechanisms of 6-MBOA information transmission. It is imperative to study this phenomenon in various animal species to elucidate the underlying mechanisms.

Despite decades of effort by scientists to elucidate the mechanism underlying the impact of 6-MBOA on reproduction, progress in this area has been slow, and numerous aspects of this phenomenon still lack clarity. Additionally, the hypothesis on this matter awaits definitive verification. When evaluating the effects of 6-MBOA on animal reproductive function, it is crucial to consider the internal mechanisms through which animals adapt and metabolize this compound. Given that 6-MBOA is a plant-defensive PSM, it is possible that its reproductive-related function is a side effect of the coevolution between plants and herbivores. Because of its potential impact on animal reproduction, it is believed that 6-MBOA ought to serve as a crucial factor in the regulation of wild animal populations by functioning as an environmental signal. Considering the present situation, it is imperative to embark on a novel avenue of investigation to delve into the mechanism of action of 6-MBOA in animal reproduction, apart from its structural similarity to melatonin. Network pharmacology may assist in elucidating the underlying mechanisms. Tannin, as a plant defensive PSM, can mediate autophagy in the testis and possibly affect the reproductive function of male Brandt's voles by regulating antioxidant levels (Dai et al. 2020). Therefore, to determine whether 6-MBOA functions on animal reproduction via its influence on oxidative levels, autophagy, and apoptosis, is a new direction to explore the mechanism of 6-MBOA on animal reproduction.

Role of 6-MBOA in human health

In addition to its roles in animal reproduction and defense, 6-MBOA, a component of BXs, is believed to have various effects on human health. A trial in healthy individuals demonstrated that BXs levels in plasma reached a peak 3 h after food intake and remained detectable for up to 36 h, suggesting that BXs have potential to impact human health (Jensen et al. 2017). BXs have demonstrated their anti-cancer properties, as DIBOA, a specific type of BX and active ingredient in Cernilton (a medication used for prostate disease), can inhibit the growth of prostate cancer cells, thereby improving benign prostate hyperplasia (BPH1) and chronic prostatitis (Zhang et al. 1995). In a study involving subcutaneous transplantation of an androgen-sensitive prostatic carcinoma cell line (LNCaP) into nude mice and feeding them diets containing rye bran for 9 weeks, it was observed that mice in the experimental group, compared to those in the control group fed diets containing corn starch and sucrose, had smaller palpable tumors and increased tumor cell apoptosis. However, the specific mechanism underlying this effect requires further investigation (Bylund et al. 2000). A human trial consisting of 17 patients with prostate cancer who were given a daily intake of whole grain rye and bran products or refined wheat products demonstrated significant reductions in the plasma concentration of prostate-specific antigen (PSA) with the consumption of whole grain rye and bran products (Bylund et al. 2000). Additionally, it was observed that in these patients, BXs levels significantly increased in plasma and prostate-specific antigen (PSA) concentration decreased, inversely correlating with BXs metabolites such as HBOA-Glc, 2-HHPAA, HBOA-GlcA, and 2-HPAA-GlcA (Bylund et al. 2000; Nordin et al. 2022). 6-MBOA inhibits HIV replication by suppressing HIV-1 reverse transcriptase activity. This inhibitory effect is dependent on the concentration of 6-MBOA (Wang and Ng 2002). A patent revealed that cereals containing BX compounds, with 6-MBOA as a major component, offer significant benefits to the central nervous system (CNS) (Fomsgaard et al. 2011; Adhikari et al. 2015). These benefits include appetite reduction, mood enhancement, improved sexual function, and relief from symptoms associated with fibromyalgia and sleep apnea disorders. Breads made from rye grains or a combination of wheat and rye along with medicinal plants and young cereals containing BXs are particularly advantageous. Moreover, 6-MBOA possesses anti-cancer, anti-inflammatory, analgesic, and antibacterial properties (Fomsgaard et al. 2011; Adhikari et al. 2015). Human experiments have shown that extracts from monocotyledonous plants (such as maize, wheat, and bamboo) containing 6-MBOA have anti-stress and relaxation effects, effectively improving sleep quality and alleviating stress by influencing serotonin levels (Talbott and Talbott 2013; Kalman et al. 2015; Talbott et al. 2023).

Although BXs and 6-MBOA are considered beneficial to human health, there is limited systematic research on their effects. In particular, the specific health benefits of 6-MBOA in appetite suppression and weight loss as well as its mechanism of action are not well understood and lack experimental evidence. Furthermore, the role of 6-MBOA in HIV treatment and the enhancement of mood and sleep quality require more direct evidence and thorough research. Therefore, it is not currently justifiable for application in the human healthcare industry. Moving forward, it is essential to thoroughly investigate the physiological effects of 6-MBOA in mice and rats using in vivo and in vitro methods to facilitate its potential applications in human health.

Perspective

The PSM 6-MBOA is present in forage grass consumed by livestock and is favored by wild voles, and in crops consumed by insects. It is also found in cereal-based products. Therefore, the study of 6-MBOA holds significant value across various domains, including ecology, zoology, animal husbandry, and human health. The diverse functions of 6-MBOA in animals can also potentially result from the coevolution of plants and herbivorous animals. In the future, we can elucidate the responses of 6-MBOA-related substances in plants to insects, herbivorous mammals, and environmental changes through transcriptomics and metabolomics. This will enhance our understanding of the interactions between animals and plants producing 6-MBOA-related substances.

However, its defensive function in herbivorous mammals such as voles and livestock remains unknown. Recent studies have indicated that 6-MBOA may affect the growth and digestion of voles by modulating the gut microbiota. This finding opens new avenues for studying the interactions between PSMs and mammals. To deepen our understanding of the evolutionary dynamics between plants and herbivores, further comprehensive investigations should be conducted to determine how herbivorous mammals adapt to 6-MBOA through the modulation of their gut microbiota. The utilization of metagenome sequencing and the identification of bacteria capable of degrading 6-MBOA would greatly facilitate the elucidation of the mechanisms underlying the role of the gut microbiota in facilitating the adaptation of herbivorous mammals to 6-MBOA. Previous studies have concluded that 6-MBOA may function as a partial melatonin agonist and play a regulatory role in mammalian reproduction. However, its effects are largely dependent on species differences and photoperiod, ultimately resulting in either the promotion or inhibition of reproductive functions. Therefore, the mechanism by which 6-MBOA influences animal reproduction is complex and not yet fully understood. Future animal studies should aim to expand the scope and embark on novel avenues of investigation with the assistance of network pharmacology to better elucidate the mechanisms by which 6-MBOA acts. Understanding the role and mechanism of 6-MBOA in animal reproduction would not only deepen our understanding of how environmental cues influence seasonal reproduction and population dynamics of animals, but could also pave the way for the application of 6-MBOA in animal husbandry. New technologies, such as transcriptomics and metabolomics, can help elucidate the effects of 6-MBOA on animal physiology and its mechanisms, thereby gaining a comprehensive understanding of 6-MBOA's effects on animals. This understanding can further elucidate the ecological function of 6-MBOA, facilitating its use in ecosystems. Despite some observed potential health benefits of 6-MBOA in humans, current research is primarily at the preliminary stage and lacks robust evidence to support its claims. Therefore, rigorous and comprehensive studies are necessary prior to the safe and effective application of this natural PSM to human health.

Author contribution Xin Dai conceived the idea for this review. Jia-Yi Shi conducted the literature search and wrote the initial manuscript. Ke-Han Gu assisted with the literature search. Sheng-Mei Yang and Wan-Hong Wei assisted with the drafting and revision of the work, and Xin Dai drafted and revised the manuscript.

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Declarations

Conflict of interest The authors declare no competing interests.

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