Forest Organic Volatile Compounds: Do Monoterpenes Affect Physiological Processes That Promote Health?

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Review

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Abstract: This paper considers whether forest volatile organic compounds (VOCs) and/or their metabolites have health-promoting activities, and whether this is through 'mindfulness' practices and/or activation of health-promoting physiological processes. The VOCs considered are the monoterpenes D-limonene, a-pinene, D^3-carene and 1,8-cineole. Clearly, some of the health benefits of forest bathing come from 'mindfulness' practice that promote parasympathetic activities. There is also clear evidence that the 4 monoterpenes at high enough concentrations can activate physiological processes that lead to better health outcomes, particularly with decreases in anxiety and decreases in inflammation. These processes appear to involve activation of the A_{2A} receptor and down-regulating activation of Nuclear Factor kappa B. The question is whether the monoterpenes can do so at the concentrations encountered in a forest setting. There is some research that indicates that forest atmospheric concentrations of monoterpenes can activate physiological processes that promote health but more research is required to definitively establish this. This review ends with suggestions on how to unequivocally answer the question whether the 4 monoterpenes considered at concentrations found in a forest, or their metabolites, can activate physiological processes that promote health, in particular physiological processes that decrease anxiety and inflammatory conditions and promote better endothelial function.

Keywords: A_{2A} receptor; anxiety; asthma; D^3-carene; 1,8-cineole: forest volatile organic compounds; health; D-limonene; monoterpenes; NF kappa B; Nrf2; a-pinene

1. Introduction

Forest bathing for health has become popular in recent years. This paper considers the question of whether forest volatile organic compounds (VOCs), particularly monoterpenes, can activate physiological processes that promote health.

'Forest Bathing' for health has a long history. For example, in year 77 of the Common Era Pliny the Elder published the first ten volumes of his Natural History series and wrote in Chapter 19 of Volume V [1]: "It is a well-known fact that forests planted solely with trees from which pitch and resin are extracted, are remarkably beneficial for patients suffering from phthisis[1], or who are unable to recover their strength after a long illness: indeed it is said, that in such cases to breathe the air of localities thus planted, is more beneficial even than to take a voyage to Egypt, or to go on a summer's journey to the mountains to drink the milk there, impregnated with the perfumes of plants."

The practice of Shinrin-Yoku (forest bathing) was made popular in Japan during the 1980s [2] and now many physicians and other healthcare providers throughout the world prescribe encounters with nature. Forest bathing does not necessarily involve exercise but is simply being in the presence of a forest for some time, although some studies on the effect of forests on health are dynamic involving activities such as walking. Today there are formal nature prescription programs in North America where healthcare providers prescribe visits to green spaces [3]. One example is the PaRx

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[1] tuberculosis

prescription program developed by Dr Melissa Lem in combination with the British Columbia Parks Foundation that allows registered healthcare providers to prescribe free access to Provincial and National parks. This program was approved by the Canadian Medical Association and has now spread across Canada.

Potential Mechanisms Involved in Forest Bathing for Health

There are many primary research articles that demonstrate there are health benefits to static forest bathing such as increasing the sense of well-being, e.g., [4] and decreasing depression, anxiety as well as increasing the anti-oxidant status of blood in a chronic stroke population e.g. [5]. There are a number of reports that indicate forest bathing by urban children with asthma improves symptoms, e.g., [6,7]. Here children are taken out of an urban environment to a forest environment. We do not know whether such improvements in asthma symptoms are due to leaving a more polluted air environment to going to a less polluted air environment, 'mindfulness' aspects of the forest environment, or due, in part, to the presence of monoterpenes. One study showed a positive correlation between lung function and total monoterpene exposure [7], although daily concentrations of monoterpenes measured were low and ranged from 38 to 62 ng/m^3. Both static forest bathing as well as dynamic forest bathing show that a forest environment improves cardiovascular function (decreased systolic blood pressure and increased arterial compliance) while an urban environment does not, e.g., [8–10]. There are many reviews on this topic, e.g., [11,12].

Does forest bathing promote health through practices associated with mindfulness or do the volatile molecules emitted by the forest activate physiological processes that promote health? Forest bathing exposes one to 2 situations that can impact health through promotion of the activation of the parasympathetic nervous system: i) a calming environment that promotes activation of the parasympathetic nervous system, and ii) an atmosphere containing volatile forest molecules. The pleasant scent of many of volatile forest molecules in turn can promote activation of the parasympathetic nervous system just by delightful odours, for example, Christmas wreathes, or bring back pleasant memories. These effects may be mediated through 'mindfulness' practices. There is clear evidence that practicing mindfulness promotes activation of the parasympathetic nervous system and decreases blood pressure in hypertensive patients [13], increases antioxidants in blood [14] and decreases depression and anxiety [15]. There is also evidence that simply viewing forest imagery has some positive physiological and psychological effects when compared to viewing urban imagery [16].

Can one simply substitute images of forests for bathing in an actual forest? Or do VOCs activate physiological processes that promote health beyond 'mindfulness' practices? Can forest VOCs appear in the blood of forest bathers in sufficient concentrations that may have physiological effects? Inhalation of VOCs is an efficient way of delivering these compounds to blood. As an example, Chen and colleagues demonstrated that inhaling D-limonene by mice was 65 times more efficient in delivering the compound to blood than ingesting the same quantity [17]. In a natural setting do VOCs enter the blood stream in concentrations that may have physiological effects? For some of these molecules the answer is yes. After a 60 min walk in the Tsubetsu forest of Eastern Hokkaido, comprised mainly of spruce and fir trees, blood levels of participants' a-pinene, the dominant monoterpene in this forest's atmosphere, increased from 2.6±1.7 to 19.4±2.1 nM [18]. This is not that surprising since the lungs have an enormous surface area available for molecular uptake and a-pinene, like other hydrocarbon monoterpenes, is lipophilic [19] and should readily enter the blood whose lipophilic molecules should act as monoterpene sinks. Could a concentration of 19.4 nM be sufficient to activate any physiological process? Perhaps. In my own laboratory we have seen cell physiological responses to sulforaphane at 10 nM with near maximal responses seen at 50 nM [20].

Not only do monoterpenes readily enter the blood but they will readily move from blood into fatty tissues. A study in mice exposed to monoterpene vapours have shown that within 30-90 min of exposure brain levels reached over 90 µg/g for a-pinene, over 50 µg/g for D-limonene and over 70 µg/g for 1,8-cineole [21]. In these experiments 50 µL of the VOC was applied to a filter paper placed

into a 5 L sealed cage where each mouse was placed. For example, with a-pinene, which has a density of 0.858 g/mL and had a 95% purity, 40.75 mg of a-pinene would have been placed on the filter. Unfortunately, the concentration of monoterpenes in the cage was not measured; however, if all, for example, the a-pinene volatilized, then the concentration would have been 8.15 mg/L, many orders of magnitude higher than would be found in a forest, but these experiments do show the lipophilicity of the monoterpenes and that fats can act as stores for monoterpenes.

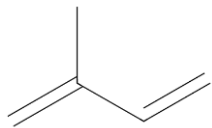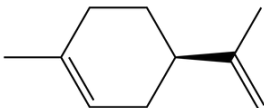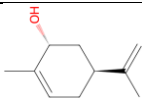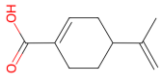Evidence That Exposure to Forest Monoterpenes Can Impact Physiological Processes

Is there any evidence that VOCs alone may positively affect health? That forest VOCs may directly affect physiological processes directly is demonstrated by experiments by Ahn and colleagues [22,23]. Mice were administered lipopolysaccharide (LPS) intraperitoneally or into the lungs. LPS is present in the membranes of gram-negative bacteria and initiates a strong systemic inflammatory response by binding to and activating the Toll-like Receptor-4 in cell membranes [24]. Following administration of LPS, mice were divided into the four groups: i) controls with no further treatment, ii) a group treated with dexamethasone, a potent anti-inflammatory steroid, iii) a group exposed to a panel of Korean pine in their cages, and iv) a group exposed to a panel of Japanese larch (or Hinoki cyprus) in their cages. The wood panels were shown to emit a number of VOCs with hexanal and the monoterpenes b-phellandrene and a-pinene being dominant (96-280 μg/m^3 in the cage atmosphere). As expected, administration of dexamethasone greatly reduced a variety of inflammatory markers. Exposure to volatiles emitted by the pine, larch and cyprus panels also significantly decreased many of the inflammatory markers examined such as interleukin-1b (IL1b), IL13, tumour necrosis factor-a (TNFa) and nuclear factor kappa B (NFkB) mRNA. Although the concentrations of VOCs were high (an order or two of magnitude greater than would be encountered in a forest) in these experiments, it strongly suggests that some of the forest VOCs act directly on physiological processes that ameliorate systemic inflammation.
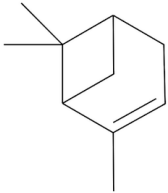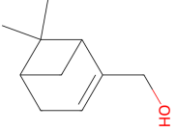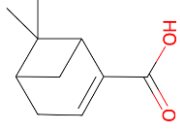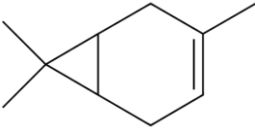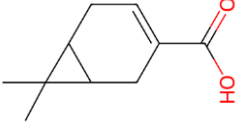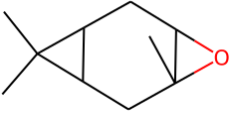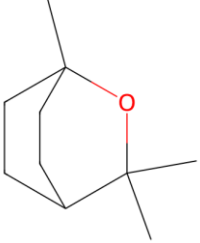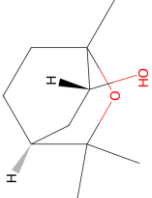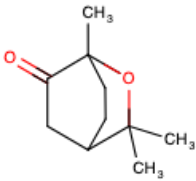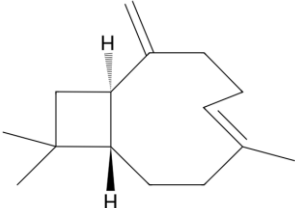
In conclusion, forest bathing has three potential mechanisms of action: one mediated by processes activated by 'mindfulness', a second by going from a more polluted environment to a less polluted environment and a third mediated by direct actions of VOCs on physiological processes.

2. What Are the Volatile Organic Compound Molecules in Northern Temperate Forests?

It is estimated that the earth's vegetation releases about a billion tonnes of VOC carbon annually into the atmosphere [25,26]. More than half of the emissions is isoprene (~634 megatonnes), with monoterpenes at ~89 megatonnes and sesquiterpenes at ~36 megatonnes forming most of the rest. The basic building unit of the terpenes is isoprene. Isoprene has the formula of C_5H_8, while that of monoterpene is $(C_5H_8)_2$ and sesquiterpene is $(C_5H_8)_3$ – see Table 1.

Table 1. Terpenoids, including the 4 monoterpenes and some of their metabolites considered in this paper. Chemical structures obtained either from Chemspider or Phytohub.

Terpene	Parent Compound	Metabolite	Metabolite
Isoprene 63.12 daltons			
Monoterpene D-Limonene 136.238 daltons		 Carveol 152.237 daltons	 Perillic acid 166.22 daltons

Monoterpene a-Pinene 136.238 daltons		Myrtenol 152.237 daltons	Myrtenic acid 166.220 daltons
Monoterpene D³-Carene 136.238 daltons		D³-caren-10-carboxylic acid 180.247 daltons	D³-Carene epoxide 152.237 daltons
Monoterpene 1,8-Cineol 154.252 daltons		2-hydroxy1,8-cineole	2-oxo-1,8-cineole
Sesquiterpene b-Caryophyllene 204.357 daltons			

The majority of the terpenes are emitted by living plants but a fraction also comes from decaying plant matter [27]. There is great variability amongst species in individual terpene chemical species emissions [28]. Even within a genus, terpene emissions can vary immensely, for example the emission rate (ng/hr/gram branch) of D³-carene for *P. albicaulis* is reported to be 651 and for *P. longaeva* it is 9.5 while for limonene it is 321 vs 12.2, respectively [29]. Coniferous forests also emit more monoterpenes and less isoprene compared to deciduous forests. This is demonstrated in a study examining the VOCs in the August air of the Tharandt forest in Germany where the dominant tree species were European spruce (72%), Scots pine (15%) European larch (10%) and 3% deciduous trees and in the Hohe Holz forest in Germany where the dominant tree species were European beech, sessile oak, Silver birch, common hornbeam, and a few newly planted European spruce and European larch [30]. The coniferous forest had about 1.8 $\mu g/m^3$ of maximum isoprene concentrations forming 12% of total terpenes while the deciduous forest had a maximum isoprene level of 12 $\mu g/m^3$ forming 93% of total terpenes The dominant monoterpenes in the coniferous forest was a-pinene at 6 $\mu g/m^3$ while the dominant monoterpene in the deciduous forest was sabinene at 0.65 $\mu g/m^3$.

Most of the studies on VOCs in forests examine terpene emissions. Because of the presence of unsaturated bonds in many terpenes, they are susceptible to oxidation and their half-lives can vary [31]; hence, it is important to analyze the composition of the atmosphere and not just pay attention to terpene synthesis and emission.

2.1. Isoprene

Although there is a large emission of isoprene by vegetation, isoprene is a very reactive chemical and its lifespan in the atmosphere is only about one hr [32]; hence, isoprene levels tend to be relatively low in the atmosphere of a forest. For example in a mixed temperate forest in Britain isoprene levels at a temperature of 25 °C was 2 ppb [33] – that is 5.57 $\mu g/m^3$ at 25° C using the formula: $\mu g/m^3$ =

molecular weight x concentration (ppb) ÷ 24.45 (i.e., the volume of one mole in litres at standard temperature and pressure), or 0.08 nmoles/L of air. In a mixed temperate forest in Estonia, the maximum level of isoprene was 5.3 μg/m³ of air reached in summer but could be as low as 0.2 μg/m³ in winter [34].

Isoprene is also a by-product of cholesterol catabolism [35]; hence, even in the absence of isoprene in the atmosphere, isoprene is normally present in human blood and exhaled via the lungs: such human-derived blood plasma levels are in a similar concentration range as found in the atmosphere of forests, ranging from 15 to 70 nmoles per litre of blood [36]; hence, atmospheric isoprene likely will have little influence on blood levels.

2.2. Monoterpenes

There are a number of studies that have examined monoterpene concentrations in the atmosphere of forests. There are a range of concentrations of specific monoterpenes reported. Some of the differences in concentrations of monoterpenes may be due to differing heights that the samples were taken since there is increased concentrations of monoterpenes the higher into the canopy that one samples [37] while others may be due to the time of year or time of day when collected since there are seasonal [38] and diurnal changes in concentrations [39]. Some differences could be due to the amount of light or temperature [40], or possibly calculation errors.

One should keep in mind that depending upon analytical technique used, only a subset of the monoterpenes will be identified and quantitated; hence, there are differences amongst the studies on the species of monoterpenes analyzed and reported. There are also differences in reporting concentrations. A range in concentrations of monoterpenes have been reported, for example in a hemiboreal forest in Estonia, a-pinene atmospheric concentrations were reported to be 23 μg/m³ in August at ground level [34], 6 μg/m³ in August in a pine forest in Germany at 13 m above the ground [30], up to 6.5 μg/m³ above the canopy in a boreal coniferous forest in Finland [41] and up to 54 μg/m³ a few centimetres above the ground in a Scandinavian pine forest [42].

The most abundant monoterpenes in Korean cyprus and pine forests were a-pinene, b-pinene, D-limonene, myrcene and sabinene [43], with similar monoterpenes found in a beech forest, albeit at lower concentrations. In a study of a German pine forest the most abundant monoterpenes were a-pinene, b-pinene, D-limonene, sabinene, D³-carene and camphene whereas in a deciduous forest they were the same but a lower concentrations [30]. In a study of a Finnish boreal forest the major monoterpenes were a-pinene, camphene, b-pinene, myrcene, D³-carene, p-cymene, D-limonene, 1,8-cineol and terpinolene [44]. There are differences between summer and winter atmospheric concentrations with a-pinene, D³-carene and b-pinene dominating in summer while camphene, p-cymene and a-pinene dominating in winter. A Scandinavian pine forest air sampled at night had the following monoterpenes: a-pinene, b-phellandrene, camphene, b-pinene, D-limonene, D³-carene and myrcene [42].

Reported levels of sesquiterpenes were much lower than the common monoterpenes measured; hence, this manuscript will not deal with sesquiterpenes.

There is an enormous literature on the effect of monoterpenes on a variety of disease conditions. For example, there are studies showing that D-limonene ameliorates disease in animal models of stroke [45], Parkinson's disease [46], cardiac infarction [47], Type 2 diabetes [48], peritoneal adhesions [49], inflammatory bowel syndrome [50], to name a few. In this manuscript only 4 monoterpenes, D-limonene, a-pinene, D³-carene and 1,8-cineole (Table 1), and two health conditions (respiratory inflammatory problems and anxiety) will be considered.

3. Pharmacokinetics and Metabolism

We should keep in mind that monoterpenes are metabolized by the various tissues and organs in the body and any therapeutic effect may be mediated by a metabolite rather than the parent monoterpene. This makes understanding the mechanisms underlying any therapeutic effect complex.

The majority of studies examining monoterpene metabolism are following administration of the monoterpene either intraperitoneally or perorally; hence, the intestine, liver and kidneys are primarily involved in the monoterpene metabolism. Lung tissue also contains xenobiotic metabolizing enzymes [51]; thus, it is very possible that the metabolites that are relevant for the therapeutic effects may be missed in the metabolic studies where the monoterpene is administered intraperitoneally, perorally or dermally. Effects via inhalation may be quite different from effects where the monoterpene is administered via other routes. For example, Heuberger and colleagues found different physiological and psychological responses whether the monoterpenes 1,8-cineole and linalool were applied dermally or via inhalation [52].

We should also keep in mind that the pharmacokinetics of inhaled volatile lipophilic compounds such as monoterpenes is complex since blood concentrations are affected by uptake into the blood, movement into lipophilic blood molecules such as low density lipoprotein, movement into fatty tissue such as brain or adipose tissue, metabolism by the liver, kidneys and lungs and exhalation of the parent compound by the lungs [53]. Following the end of exposure to a VOC such as a-pinene [53], there is a rapid (5-10 min) drop in the blood concentration followed by a slower (hours) decline. The rapid drop involves the metabolism of the compound in the aqueous phase of the blood as well as exhalation of the compound while the slower decline is due to movement of the compound from the fat molecules and fat tissue into the blood. All inhalation studies examining pharmacokinetics in humans used very high concentrations of monoterpenes, many of orders higher than found in forests.

Finally, many of the monoterpenes emitted by forests are also present in many of our food items [54] and this must be kept in mind when examining plasma levels.

D-Limonene: D-Limonene is by far the dominant monoterpene at 1.5 μg/L (i.e., 11 nM) found in several studies of blood levels of monoterpenes [55,56] – in these studies it is likely that the plasma D-limonene had a dietary origin. D-Limonene generally enters the blood either through inhalation or by ingestion. By far the majority of studies involving monoterpenes have been on D-limonene.

Inhalation of D-limonene at 10, 225 and 450 mg/m^3 in human volunteers resulted in 65% of the inhaled D-limonene transferred from air to blood [57]. It should be noted that Kohlert and colleagues question whether the difference between the concentration in inhaled air versus exhaled air can be considered the amount of monoterpene transferred to blood [58]. Elimination from the blood following inhalation of the higher doses was in 3 phases with half-lives of 3, 33 and 750 min, with the slow elimination phase due to movement from adipose tissue into the blood. Following ingestion of 9.3 mg (i.e., 68 μmoles) by human volunteers there was no detectable D-limonene in the plasma at 1 hr [59], with the major metabolites detected being limonene-8,9-diol, limonene-1,2-diol followed by perillic acid. Other metabolites that have been reported in various studies include: dihydroperillic acid; dihydroperillic acid, methyl ester; perillic acid, methyl ester; perrilyl alcohol; and carveol [60–62]. It seems very possible that many of the therapeutic effects attributed to D-limonene may be due to one or more of its metabolites, and possibly explains why such high concentrations of D-limonene are required to observe an effect in cultured cells. Which of the metabolites may be responsible for the therapeutic effects of inhaled D-limonene is not clear, and especially so since we do not know which are the dominant metabolites formed from inhaled D-limonene. Perillic acid seems to be a compound of interest since it appears to be a Michael electron acceptor. Michael electron acceptors activate the Nrf2 signalling pathway [63,64] – see section IV: Discussion.

a-Pinene: Pharmacokinetics of inhalation of a-pinene was similar to that of D-limonene [65]. Capillary blood concentrations of a-pinene increased rapidly for the first half hour and then more slowly to reach 9 μM and 19 μM at two hr for the 225 μg/L and 450 μg/L air concentrations, respectively. At the 10 μg/L air concentration, the blood concentration stabilized at about 30 min at 0.5 μM, likely because metabolism equilibrated with transfer of a-pinene from air to blood. It was estimated that 60% of a-pinene inhaled transferred to blood. After the 2-hr exposure to the lipophilic a-pinene, elimination from the blood occurred in 3 phases, a rapid phase with a half-life of 4.8 minutes that involved, exhalation of the non-lipid-bound parent compound and metabolism by the liver,

kidneys and lungs and then slower phase clearances (38 min and 695 min half-lives) as the parent compound moved from lipid molecules and adipose tissues into the blood.

Inhalation studies in rodents also looked at the accumulation of a-pinene in mammary glands, i.e., a fat rich tissue and observed an accumulation within mammary glands that accounted for the third slow phase of a-pinene elimination from the blood [66]. Mice were exposed to either 50 ppm (279 μg/m³) or double that concentration to a-pinene for 6 hr/day for seven consecutive days. Inhaled doses were calculated to be 70 and 140 mg/kg/day at the two atmospheric concentrations respectively. Assuming the transfer from air to blood is the same as in humans [65], with 60% absorption, 42 and 84 mg/kg transferred to the blood each day. a-Pinene accumulated in female mammary gland fat at 1.18 and 2.5 mg/g lipid at the two atmospheric concentrations. Even 24 hr following the 7-day exposure to a-pinene there were still 10 and 20 ng a-pinene/mL, respectively, in the blood due to the slow release from the fat stores., i.e., 73 and 147 pM.

Blood samples were obtained from human subjects before and at 1 to 5 hr after consumption of 9 mg of a-pinene [67]. Within 1 hr of consumption there was no detectable a-pinene in the blood. Four major metabolites were detected in the blood: myrtenol, cis- and trans-verbenol, myrtenic acid and possibly a hydroxylated form of myrtenic acid. Myrtenic acid has the characteristics of a Michael electron acceptor.

A study in mice observed the presence of cis- and trans-verbenol as well as myrtenol in urine following inhalation of air containing 1 ppm (5.6 mg/m³) a-pinene for a period of six hr [68].

D³-Carene: Pharmacokinetics of inhaled D³-carene was performed where human subjects were exposed to D³-carene at 10, 225 and 450 μg/L for 2 hr [69]. Maximum blood concentrations reached were ~1, 12 and 26 μmol/L. It was estimated that 70% of the D³-carene inhaled transferred to blood. The typical 3 phase elimination from blood was observed, an initial rapid phase with a half-life of 4.5 min, an intermediate phase of 35 min and a final slow phase of 30 hr. Similar results were seen by Kohlert and colleagues [58].

Following oral administration of 10 mg D³-carene the main metabolites found in urine were D³-caren-10-carboxylic acid (chaminic acid), D³-caren-9,10-dicarboxylic acid, likely a saturated version of D³-caren-10-carboxylic acid (dihydrochaminic acid), D³-caren-3,4-diol-9-al, and D³-caren-3,4,9-triol [70]. It was reported that at least one of the metabolites has a half-life of 3 hr. Of interest is that D³-caren-10-carboxylic acid, D³-caren-9,10-dicarboxylic acid and D³-caren-3,4-diol-9-al appear to be reactive molecules. Studies using human liver microsomes showed that D³-caren-10-ol and carene and D³-carene epoxide can also be formed from D³-carene [71]. D³-caren-10-carboxylic acid has the characteristic of a Michael electron acceptor.

1,8-Cineole: 1,8-Cineole is an oxidized monoterpene. In a study where human subjects inhaled air passing over 4 mL of 1,8-cineole for 20 min 1,8-cineole was rapidly transported from air to blood with plasma levels peaking at 14-19 min [72]. Peak plasma levels ranged from 3 to 7.4 nM, likely due to differing rates of metabolism amongst the individuals. The elimination of 1,8-cineole from plasma was biphasic with the first phase with a half-life of 6.68 min and the second with a half-life of 104.6 min. There were differences between the male and female subjects likely due to varying levels of fat tissue that would have absorbed 1,8-cineole. In another inhalation study where the blood analytical approach was designed to minimize volatilization of 1,8-cineole demonstrated that plasma levels of 1,8-cineole after 45 min reached anywhere from 1 to 3 μM [73].   The concentration of 1,8-cineole in the air breathed in was not determined in these studies.

Following consumption of 1 mg 1.8-cineole it was shown that the parent compound was rapidly metabolized resulting in the formation of the following metabolites found in human plasma: 2-hydroxy-1,8-cineole, 3-hydroxy-1,8-cineole, 7-hydroxy-1,8-cineole and 9-hydroxy-1,8-cineole [74]. All compounds peaked at 45 min with 2-hydroxy-1,8-cineole being the dominant metabolite at 86 nM followed by 9-hydroxy-1,8-cineole at 33 nM, with the parent compound peaking at 19 nM, indicating rapid metabolism. In a study of human milk, where participants consumed 100 mg 1,8-cineole [75], ten metabolites of 1,8-cineole were identified; a2-hydroxy-1,8-cineole, b2-hydroxy-1,8-cineole, a3-hydroxy-1,8-cineole, 7-hydroxy-1,8-cineole, 9-hydroxy-1,8-cineole, 2-oxo-1,8-cineole, 3-oxo-1,8-

cineole, 2,3-dehydro-1,8- cineole, a2,3-epoxy-1,8-cineole and 4-hydroxy-1,8-cineole. There was considerable variability in the relative concentrations of metabolites found from sample to sample; however, 2-hydroxy-1,8-cineole was the dominant metabolite with at least a ten-fold higher concentration than the other metabolites [76]. Of interest is the oxidation product of 2-hydroxy-1,8-cineole, 2-oxo-1,8-cineole as well as the epoxide, may be Nrf2 activators.

Remarks: All four monoterpenes are lipophilic compounds whose inhalation pharmacokinetics are similar. Because of their lipophilicity they will absorb onto fat molecules and accumulate in fat tissue.

4. Therapeutic Effects

With forest bathing the exposure to VOCs is via the lungs. The highest concentration of VOCs would be experienced by the bronchiolar and alveolar epithelial cells followed by the endothelium of pulmonary capillaries, the pulmonary veins and then the endothelium of the systemic arterial circulation. It seems likely that the epithelium of the pulmonary system and the endothelium of the vascular systems play important roles in the potential therapeutic effects of VOCs. The importance of the endothelium to health was recognized over 70 years ago by Dr Rudi Altschul who wrote: '…one is as old as one's endothelium" [77]. Modern research findings are in support of the importance of the endothelium to one's health [78–80]. As noted above, the lung tissues have xenobiotic metabolizing enzymes but little attention has been paid to metabolites formed that may be unique to lung xenobiotic metabolism. Similarly, little attention has been paid to potential effects of VOCs and their metabolites on vascular function.

This will not be an exhaustive review of the literature and will focus on two health issues: inflammatory respiratory disorders and anxiety.

4.1. Anti-Inflammatory Effects: Asthma and Other Respiratory Problems

There are a number of reports that indicate forest bathing by urban children with asthma improves symptoms, e.g., [6,7]. Here, children are taken out of an urban environment to a forest environment. We do not know whether such improvements in asthma symptoms are due to leaving a more polluted air environment to going to a less polluted air environment, 'mindfulness' aspects of the forest environment, or due, in part the presence of monoterpenes. One study showed a positive correlation between lung function and total monoterpene exposure [7], although daily concentrations of monoterpenes measured were low and ranged from 38 to 62 ng/m^3.

D-Limonene: Several studies have examined the effect of inhalation of D-limonene to ameliorate lung damage. In one, mice were sensitized to antigens from the mite *Dermatophagoides farinae* as well as diesel exhaust particles [81]. Here, mice were divided into 3 groups: the first group were sensitized to the mite antigens and diesel exhaust particles. In a second group, mice inhaled 0.01% (w/w) D-limonene that was nebulized using a sonicator for a 20 min period daily. Following this, the mice were administered mite antigen as well as diesel exhaust particles while a third group of mice were administered only the saline. After 48 hr the mice were administered acetylcholine to initiate airway hyperresponsiveness. The D-limonene group had greatly reduced airway hyperresponsiveness compared to the mice who received only the mite antigens and diesel exhaust particles. The D-limonene inhalation group showed greatly decreased eosinophil infiltration, IgE levels, decreased inflammatory cytokines such as IL5 and decreased tissue pathological changes.

Unfortunately, blood D-limonene was not measured in these experiments; however, the authors do report that mice were exposed over a 20 min period each day to 1 mg/kg D-limonene. If, as in humans, 65% of the D-limonene is transferred to the blood in the mice, then the mice absorbed 650 µg D-limonene/kg daily, i.e., 32.5 µg/kg/min. How does this compare to an exposure to D-limonene in a forest walk? Dudek and colleagues have reported that D-limonene may have an atmospheric concentration of 18 µg/m^3 (i.e., 18 ng/L) in a mixed pine and hornbeam forest [82]. A 75 kg human walking on a flat surface breathes in 0.47 L/min/kg [83] and thus would breathe in ~8.5 ng/min/kg

and absorb ~5.5 ng/min/kg, i.e., , almost 4 orders of magnitude less than the mice in the experiments by Hirota and colleagues.

Another inhalation study examined the ability of D-limonene to protect lungs from smoke damage [84]. Rats were divided into three groups: Control, Vehicle and Experimental. Vehicle and Experimental rats were exposed to smoke for three 9-min periods in a closed acrylic box interrupted by 30 sec exposures to ambient air. Following this the Vehicle group was exposed to the nebulized saline Vehicle while the Experimental group was exposed to nebulized D-limonene dissolved in saline for a period of 30 minutes. The D-limonene concentration in the chamber air was 4.5 μg/L, i.e., 33 nmoles/L, which is many orders of magnitude higher than what is found in forest environments, e.g., [30]. Tracheal TNFa was significantly increased in the Vehicle group compared to the Control Group and D-limonene greatly attenuated the TNFa response to smoke. The Ferric Reducing Ability of Plasma (FRAP) was significantly reduced in the Vehicle group compared to the Control group and FRAP was significantly increased in the Experimental group compared to the Vehicle group. The Vehicle group also had significantly decreased reduced-glutathione (GSH), catalase and superoxide dismutase activities in the lung while D-limonene inhalation countered these decreases. The lung histopathology was improved in the Experimental group compared to the Vehicle group showing less inflammatory cell infiltration, less edema and fewer vessels showing hyperemia. Thus, the D-limonene reduced the oxidative stress and inflammation caused by smoke inhalation. This study used atmospheric D-limonene almost three orders of magnitude higher than what can be encountered in a forest.

In an ovalbumin model of asthma in the mouse, a 20 min exposure to aerosolized 0.01% solution of D-limonene reduced airway hyper-responsiveness in sensitized mice to methacholine [85]. D-Limonene also reduced the number of inflammatory cells in bronchoalveolar lavage fluid and reduced the concentration of ovalbumin-specific IgG. The concentration of D-limonene was not measured, but would be higher than that found in a forest environment.

Computational docking modelling showed that D-limonene can bind to adenosine receptors A_{2A} and A_{2B}. The researchers determined that D-limonene had no protective effects on an ovalbumin model of asthma in A_{2A} receptor knockout mice, concluding the protective effects of D-limonene in this asthma model were mediated by activating the adenosine A_{2A} receptor.

a-Pinene: In a mouse model of rhinitis, a-pinene ameliorated symptoms in response to the allergen, ovalbumin [86]. Experimental mice received, daily, a-pinene perorally at doses ranging from 0.1 to 10 mg/kg. All doses ameliorated the allergic responses to ovalbumin including nasal IgE, TNFa, Macrophage Inflammatory Protein-2, and decreased numbers of inflammatory and mast cells infiltrating the nasal mucosa. The increased activation of Nuclear Factor Kappa B (NFkB) in response to ovalbumin was decreased following a-pinene administration. NFkB is a transcription factor that plays a central role in inflammation and when activated promotes the expression of dozens of pro-inflammatory genes [87,88]

Are the doses used relevant to forest bathing. Even at the lowest dose each mouse would have received 100 μg a-pinene/kg daily. Is this relevant to forest bathing? It is hard to say but if the difference in efficiency of transfer is 65 times greater for inhalation than ingestion of a-pinene is similar to that found with D-limonene [17], then one can make the reasonable assumption that 100 μg/kg ingested is equivalent to breathing in 1.5 μg/kg a-pinene. Let us consider the German forest where a-pinene has been reported to be ~6 μg/m³ or 6 ng/L [30]. A mouse has a lung tidal volume of ~0.18 mL and a respiratory rate of about 255 breaths per min [89]. In 1.0 min the mouse would have breathed in and out 0.0459 L and breathed in 0.294 ng a-pinene. Assuming 60% is transferred to the blood, then each minute the mouse would absorb 0.1764 ng a-pinene. Assuming a mouse has an average weight of 25 g, this is equivalent to 7.056 ng/kg/min. A mouse would receive a total dose of 1.5 μg/kg in 3.5 hr. If the forest atmosphere contained 23 μg/m³ as reported by Noe and colleagues [34], then the mouse would have breathed in 1.5 μg a-pinene in a little under an hr. One can question whether the experiments described are relevant to forest bathing.

Inhaling the metabolite, myrtenol, was also tested in an ovalbumin model of asthma in rats and shown to improve inflammatory markers and increase anti-oxidant status in the rat hippocampus [90]. The rats were sensitized to ovalbumin and exposed to a nebulized ovalbumin for 30 min a day for a period of 28 days and then exposed to different concentrations of myrtenol in the air they breathed in. Optimal results were obtained when rats were exposed to a total of 8 mg/kg myrtenol in the 30 min period. Assuming that 60% of the myrtenol entered the blood, then rats received a dose of 4.8 mg myrtenol/kg over the 30 min exposure period, i.e., 151 μg/kg/min or 1.05 μmole/kg/min. This dosage exposure does not seem relevant to human forest bathing.

D³-Carene: In an ovalbumin model of asthma, mice were administered intranasally one of the following: saline, the 0.9 μg steroid budesonide, 120 ng D³-carene or 1200 ng D³-carene [91]. Assuming the mice were 25 g in weight and transference 1,8-cineole to blood was the same as in humans (70%) [69], then the mice received 3.36 or 33.6 μg D³-carene/kg, i.e., 24.7 or 247 nmoles/kg D³-carene. Both budesonide and D³-carene, particularly the lower dose, decreased the inflammatory markers such as bronchiolar lavage polymorphonuclear leukocytes, bronchiolar epithelial thickness, plasma IgE and supressed the increase of the cytokines IL4, IL5 and IL13. Whether such effects are mediated by D³-carene or one of its metabolites is not known; however, we do know the parent compound is rapidly metabolized [69] while some of the metabolites such as D³-caren-10-carboxylic acid have a half-life of three hr [70]. Of interest is that D³-caren-10-carboxylic acid is a likely Michael electron acceptor and activator of Nrf2.

These results show that D³-carene has promises as an inhaler to treat asthma attacks but can one get sufficient D³-carene to have therapeutic effects during a forest walk. A northern boreal forest in Norway comprised mainly of spruce and some pine has a typical D³-carene atmospheric concentration 2 m above the ground of around 4 ppb, i.e., 22 μg/m³ (22 ng/L) [92]. Petersson [42] reported that D³-carene could be as high a concentration of 39 μg/m³ (39 ng/L) on a summer night in a pine forest in southern Sweden when measured a few cm above the forest floor. A 75 kg human walking on a flat surface breathes in 0.47 L/min/kg [83] and would take 7.7 hr to breathe in 4.8 μg D³-carene/kg in the Norwegian forest to deliver 3.36 μg D³-carene/kg to the blood. Lying on the forest floor in Sweden would still take over 4 hr to breathe in 4.8 μg D³-carene/kg. Thus, D³-carene has promises as an inhaler to treat asthma but it is not likely that sufficient D³-carene can be obtained through forest bathing to affect the symptoms of asthma, **if** the lower dose administered to the mice is near the optimal dose.

1,8-Cineole: In an ovalbumin-sensitized form of asthma in Guinea pigs, exposure to an atmosphere containing 79 mg 1,8-cineole/m³ for 15 min ameliorated the response to an ovalbumin challenge [93]. It decreased the inflammatory cytokines TNFa and IL1b in broncho-alveolar lavages and decreased airway hyperresponsiveness to carbachol. Again, these are very high concentrations of 1,8-cineole compared to what one may encounter in a forest. Although the oxidized monoterpene 1,8-cineole is considered to be one of the major monoterpenes emitted from North American forests [94], there are few studies examining the emission rate or forest atmosphere levels of 1,8-cineole. One such study is by Hellén and colleagues who report maximal levels of 19 ppt (0.12 μg/m³) in a boreal forest from southern Finland [44] while Hakola and colleagues reported that 1,8-cineole can form up to 8% of all monoterpenes in the forest atmosphere of a northern boreal forest [95].

In a study examining the effects of protective effects of 1,8-cineole against the harmful effects of smoking, mice were exposed for 72 minutes intermittently to the smoke of 12 cigarettes each day for 5 days in the presence or absence of 1,8-cineole in the atmosphere [96]. The 1,8-cineole was administered as an aerosol into 4 L chambers in which the mice were placed for 15 min daily. The aerosolized 1,8-cineole used was 1, 3 and 10 mg, i.e., the chambers contained 250 mg/m³, 750 mg/m³ or 2,500 mg/m³, concentrations of at least 5 orders of magnitude greater than can be found in a forest. Protective effects were seen at 3 and 10 mg aerosolized concentrations as shown by reduced inflammatory cell infiltration of lungs, reduced NFkB activation, decreased levels of inflammatory cytokines such as IL1b, IL6 and less oxidative stress.

There are other animal studies showing that 1,8-cineole has positive effects on insults to the respiratory system [97]; however, these studies typically used high doses of 1,8-cineole and usually administered intraperitoneally.

A randomized, placebo-controlled, double-blinded clinical trial with asthma patients is described where 200 mg 1,8-cineole was ingested 3 times daily for a period of 6 months [98]. The average weight of the patients was ~77 kg; hence, patients consumed a dose of 16.8 μmoles/kg thrice daily. Patients who consumed 1,8-cineole showed marked improvements compared to the placebo group. But, again, such studies, although showing promising therapeutic effects of 1,8-cineole, are not relevant to forest bathing.

In vitro studies examining the response of primary cultures of human monocytes to LPS show that low micromolar concentrations of 1,8-cineole inhibit release of prom-inflammatory cytokines [99,100]. These studies also showed that 1,8-cineole at these low concentrations synergized with the effect of nebulizer steroid budesonide.

4.2. Anxiolytic Effects

Donelli and colleagues reported on a propensity score matching study of over 500 patients with anxiety that were introduced to a 3 hr period of low intensity exercise in a forest environment at several locations in Italy [101]. Extent of reductions in anxiety were related to total atmospheric monoterpene levels as well as the dominant monoterpene, a-pinene, levels. Total monoterpene concentrations ranged from 0.10 to 0.99 μg/m³.

In a controlled laboratory setting Kim and colleagues examined how a mixture of a-pinene, b-pinene and D-limonene affected stress levels in human subjects [102]. They demonstrated that 20% of the subjects could detect each monoterpene at a concentration of 3 ppb (16.7 μg/m³) and all could detect the each monoterpene at a concentration of 15 ppb (83.5 μg/m³). In subsequent experiments they chose a mixture of a-pinene:b-pinene:D-limonene at ratios of 1.0:0.5:0.5. Subjects inhaled 7 ppb (39 μg/m³), 15 (83.6 μg/m³) or 20 ppb (111.4 μg/m³) of total monoterpenes. These levels can be found in Norway spruce and Scots pine forests as measured by Hov and colleagues [92] at a level 2 m above the ground forests, where they report that the concentration sum of the a-pinene, b-pinene and D-limonene can be as high as 21 ppb (i.e., 117 μg/m³). Breathing an atmosphere of 39 μg/m³ of total monoterpenes for 2.5 min decreased subject stress as measured by brain alpha waves and heart rate variability with decreasing stress with higher levels of the monoterpene mixture. Thus, we have evidence that inhaling monoterpene mixtures at a concentration that can be found in a forest has health benefits. Whether this is due to 'mindfulness' mediated effects due to the pleasantness of the odours or whether these effects were mediated by direct action of the monoterpenes on physiological processes is unclear.

A study with human subjects breathing air that passed over 2 mL of 1,8-cineole for a period of 20 min, showed an with increased global cerebral blood flow [103], even in an anosmic subject. This indicated that the increased global cerebral blood flow was not mediated via olfactory perception. Thus, it is very possible that the decrease in anxiety in the studies described above was mediated by monoterpenes activating physiological processes independent of olfaction.

D-Limonene: There are a number of animal studies examining the anxiolytic effects of D-limonene but they all used high doses of D-limonene that would not be encountered in forest bathing; hence, only two studies will be considered. An elevated plus maze experiment in mice showed that inhaled D-limonene had anxiolytic effects similar to that of the GABA$_A$ diazepine receptor agonist diazepam [104]; however, the anxiolytic effect of D-limonene was not blocked by flumazenil, the GABA$_A$ diazepine receptor antagonist. This is interesting since the GABA$_A$ receptor is the target of anxiolytic drugs [105]. The concentration of D-limonene used in these experiments was enormous, since the animals were exposed to air concentrations greater than 1 μg/cm³ and this high concentration might have been able to outcompete flumazenil for the receptor binding site.

Another elevated plus maze study used 1 to 10 mg D-limonene/kg in mice showed that prior intraperitoneal injection of D-limonene significantly decreased the anxiety of mouse placed in an

elevated maze at the 5 and 10 mg/kg doses administered intraperitoneally [106]. These doses are also quite high, but lower than in the inhalation experiments, being 36.7 to 73 μmoles/kg. The authors also showed that the 10 mg D-limonene/kg decreased GABA content and increased dopamine content in the striatum of the mice. In vitro experiments with PC12 cells suggested that the decrease in GABA content was due to increased release of GABA from the neurons. The administration of an A_{2A} receptor antagonist eliminated the anxiolytic effect of D-limonene.

a-Pine: There are few studies examining the anxiolytic effects of a-pinene or its metabolites. There is one paper that examines the ability of a-pinene to ameliorate anxiety in rats and this is in Persian but has an English abstract [107]. The author states that injection of 2 mg (14.7 μmoles) and 4 mg (29.4 μmoles) a-pinene/kg significantly reduced anxiety as determined by the elevated pus maze test, comparable to that of diazepam. The administration of a-pinene also decreased malondialdehyde and increased GSH levels in the hippocampus.

Myrtenol, a metabolite of a-pinene, administered intraperitoneally at 25, 50 and 75 mg/kg significantly increased the time rats were present in the open in the elevated plus maze and Light-Dark Transition test with the lowest dose having the greatest anxiolytic effect [108]. The $GABA_A$ receptor antagonist flumazenil was able to reverse the anxiolytic effect of myrtenol. Since 25mg/kg had the greatest effect, it is unfortunate that lower doses were not tested.

D^3-carene: There are no studies examining the effect of D^3-carene on anxiety amelioration; however, one study examined the ability of D^3-carene to enhance sleep [109]. D^3-Carene was injected intraperitoneally at doses ranging from 12.5 mg (92 μmoles)/kg to 100 mg (734 μmoles)/kg, which are quite high doses. The mice were also injected with pentobarbital and time to sleep as well as sleep duration were measured. Doses of 25 mg/kg or greater significantly deceased the time to sleep while doses of 50 mg/kg or greater significantly increased the duration of sleep. This sleep-enhancing effect was mediated through the $GABA_A$ benzodiazepine receptor since it was inhibited by flumazenil.

1,8-Cineole: There are a number of papers that describe the anxiolytic effects of 1,8-cineole in rodents. I will consider only two. In the first rats were injected intraperitoneally with 1 mg 1,8-cineole (7.34 μmoles)/kg and subjected to the elevated plus maze [110]. This dose of 1,8-cineole decreased anxiety and was blocked by flumazenil, an antagonist of the $GABA_A$ benzodiazepine receptor.

A more relevant study is described by Dougnon and Ito [111]. Here, 1,8-cineole was dissolved in triethyl citrate and then placed on filter papers in closed cages with total volumes of 61.2 L [111]. Total amounts of 1,8-cineole placed in each cage ranged from 4 ng to 100 μg. Thus, if all of the cineole volatilized, mice would encounter an atmosphere containing anywhere from 65 ng/m^3 to 1.63 mg/m^3. After volatilization for 60 min mice were introduced into the cages and monitored every 5 min for a variety of anxiety tests including the Light-Dark box. Decreases in anxiety was seen in the Light-Dark box test at 6.5 μg 1,8-cineole/m^3 with lower and higher concentrations having no effect on anxiety. Flumazenil, a $GABA_A$ benzodiazepine antagonist blocked the anxiolytic effect of 1,8-cineole. A concentration of 6.5 μg 1,8-cineole/m^3 can possibly be encountered in a forest setting.

A small clinical trial demonstrated that inhaling 1,8-cineole for 5 min before selective nerve root block decreased anxiety in human patients [112]; however, a high concentration of 1,8-cineole in air was breathed in.

5. Discussion

5.1. Pharmacokinetics

The pharmacokinetics of all 4 monoterpenes is similar. The elimination of the parent compound from the blood generally occurs in 3 phases with the first phase being rapid (~5-10 min half-life) where the compound in the aqueous phase is metabolized and volatilized from the lungs, a second slower phase (~30-60 min half-life) likely involving movement of blood from lipophilic blood compounds such as low and high density lipoproteins into the aqueous phase of blood as well as some transfer from adipose tissue into blood, and a final slow phase that may have a half-life of 10 or more hr where the monoterpene is transferred from adipose tissue to the blood. The exception is

the oxidized monoterpene 1,8-cineole where the slow phase has a half-life under 3 hr. This is likely due to 1,80-cineole being less soluble in lipids - it has an octanol-water partition coefficient of only 2.8 compared to 4.4 to 4.5 for D³-carene, D-limonene and a-pinene [113].

There is rapid metabolism of the parent compound with many metabolites present for a longer time in the blood. There have been few studies examining the half-lives of the metabolites. These are important studies to do since many of the therapeutic effects may be mediated by the metabolites rather than the parent compound. It should be noted that the majority of monoterpene metabolism studies used ingested monoterpene rather than inhaled monoterpene.

5.2. Mode of Administration

It is clear that the 4 monoterpenes considered have anxiolytic and anti-inflammatory effects; however, the doses examined experimentally are generally well above those that would be encountered in forest bathing. As mentioned, since the parent compounds are rapidly metabolized, it is likely that many of the therapeutic effects recorded are mediated by metabolites. The metabolites formed are derived by the Phase 1 xenobiotic metabolizing enzymes that are present in different tissues [114]. Ingested monoterpenes are primarily metabolized by the intestine, liver and kidneys [54]. However, the lung epithelium from the bronchioles to the alveoli also have xenobiotic metabolizing enzymes, including both cytochrome P450 (CYP_{450}) and non-CYP_{450} oxidoreductases [51]. Although the activities of these xenobiotic metabolizing enzymes generally are lower in the lungs than other organs such as the liver, the enormous surface area of the lungs makes this a less important issue. That route of entry may make a difference in the therapeutic effects of monoterpenes is demonstrated by the experiments of Heuberger and colleagues [52]. Their experiments exposed human subjects to 1,8-cineole either by inhalation or by transdermal applications. The dermal absorption of the 1,8-cineole improved speed of attentional performance while inhalation had the opposite effect but increased the sense of well-being. Large doses were used in these experiments: 20 min inhaling an atmosphere containing 6.8 mg/L and 86.4 mg applied transdermally. If we assume that 70% of the inhaled 1,8-cineole was transferred to blood then each subject would have inhaled 45 mg of the monoterpene, quite comparable to what would have been absorbed transdermally. These experiments do point out that inhalation of a monoterpene compared to other means of administration may make a difference in physiological responses.

Most monoterpene inhalation studies are at concentrations far greater than could possibly be found in a forest environment. The exceptions are the study by Kim and colleagues who demonstrated that a mixture of a-pinene, b-pinene and D-limonene with a combined concentration of 39 $\mu g/m^3$ breathed in for 2.5 min decreased anxiety in human subjects as demonstrated by heart rate variability and brain alpha waves [102] and the experiments by Dougnon and Ito [111] who showed that 6.5 μg 1,8-cineole/m^3 can reduce anxiety in mice. More such inhalation studies using concentrations of monoterpenes found in forest environments need to be performed to understand how VOCs may affect health.

5.3. Possible Therapeutic Mechanisms of Action

The focus of this manuscript are the mechanisms involved in decreasing anxiety and inflammation in the respiratory system.

5.3.1. Anti-Inflammatory Mechanisms

In the studies referenced in this paper there are two possible mechanisms that should be investigated further: activation of the adenosine receptor A_{2A} and activation of NFkB. As mentioned previously, NFkB plays a central role in promoting the expression of pro-inflammatory genes [87]. NFkB normally exists as a dimer that is bound to a member of the Inhibitor of kappa B proteins (IkBs) [115]. NFkB is activated by signalling pathways that activate IkB kinase (IKK) to phosphorylate specific serine residues on IkB that result in the degradation of IkB via the ubiquitin-proteasomal

pathway, thus exposing the nuclear localization signal allowing the NFkB to translocate to the nucleus to bind to kB elements, thereby promoting the expression of dozens of pro-inflammatory genes. Factors that regulate and inhibit the activation of NFkB are complex [115].

There are also connections between the adenosine receptor, A_{2A} and NFkB. There are a number of papers that show activating the A_{2A} receptor decreases activation of NFkB [116,117]. Furthermore, Sands and colleagues showed that overexpressing the A_{2A} receptor gene in C6 glioma cells decreased the inflammatory response to stimuli such as LPS by inhibiting the phosphorylation of IkB [118], perhaps through inducing the Dual Specificity Phosphatase 1 (DUSP1) [119].

Suppressing activation of NFkB seems to be the main anti-inflammatory mechanism for many monoterpenes [120,121]. The exact mechanisms involved are still not clear.

5.3.2. Anxiolytic Mechanisms

The anxiolytic mechanisms appear to involve the promotion of the activation of the $GABA_A$ receptor since it is blocked by flumazenil. This may not involve monoterpenes or their metabolites directly acting on the $GABA_A$ receptor. They may act on mechanisms that ultimately result in the production of an endogenous ligand such as inosine [122] or a neurosteroid [123] for the benzodiazepine binding site of the $GABA_A$ receptor or 2-arachidonoly glycerol for the a2 $GABA_A$ receptor subunit [124].

Most studies examining the anxiolytic effects of monoterpenes use high concentrations of the monoterpene; however the studies by Kim and colleagues [102] and the experiments by Dougnon and Ito [111] show that concentrations of monoterpenes that can be found in forest environments can decrease anxiety. There are interesting observations by Hajagos-Tóth and colleagues that examined mechanisms whereby D-limonene promotes contraction of the myometrium of the pregnant rat [125]. These experiments were performed ex vivo and showed that D-limonene at 1 pM concentration promoted myometrial smooth muscle contraction and this was blocked either by a calcium L channel blocker or by a non-specific adenosine receptor blocker. Concentrations of D-limonene even as low as 0.1 pM caused an increase in cAMP in the myometrium. The researchers propose that D-limonene activated A_{2A} receptors resulting in an increase in intracellular cAMP that in turn enabled Protein kinase A to phosphorylate L channels allowing movement of calcium ions into the cytoplasm thereby causing contraction. These observations should be investigated further since they may lead to an understanding of the mode of action of low concentrations of D-limonene or its metabolites. D-Limonene is known to bind to the adenosine A_{2A} receptor but with half-maximal binding being around 5 μM [126] and the concentration to cause half maximal cAMP production is ~5 μM, clearly much higher than seen in the experiments by Hajagos-Toth and colleagues. It may well be possible that D-limonene binds to a yet to be discovered macromolecule that interacts with the A_{2A} receptor such as how 2-arachidonoyl glycerol promotes activation of the $GABA_A$ receptor [124].

6. Future Directions

6.1. Determining Relevant Metabolites

The highest concentration of any monoterpene reported in a forest atmosphere is about 50 μg/m^3 with most reports being under 10 μg/m^3 and often below 1 μg/m^3. If we are to determine whether breathing in monoterpenes at levels found in forests can affect our health by activating physiological processes, then all experiments must be conducted inhaling relevant concentrations of the monoterpenes found in a forest atmosphere. Since lung tissue can also catabolize xenobiotics and some of such metabolites may differ from that formed by the intestine, liver or kidneys, one should determine the metabolites that are formed through inhaling specific monoterpenes and determine the metabolite half-lives since the therapeutic effects may be mediated by monoterpene metabolites, particularly those with long half-lives.

6.2. Inflammation

The majority of therapeutic effects of monoterpenes reported decrease anxiety and/or inflammation. There are many reports that what underlies anxiety and depressive disorders is inflammation [127–129] and inflammation underlies many chronic diseases and diseases related to ageing [130].

A$_{2A}$ Receptor: As pointed out above, activation of the A$_{2A}$ adenosine receptor decreases both anxiety and inflammation. Since the A$_{2A}$ receptor seems to be involved in both anxiety and inflammation, actions of monoterpenes and their metabolites on the A$_{2A}$ receptor should investigated using several different preparations, including the ex vivo myometrium of pregnant rats and in vitro cultures of vascular endothelium and respiratory epithelium.

NFkB: NFkB is a central player in inflammation [87,88]. Activation of NFkB is a consequence of kinase cascades that result in the phosphorylation of IkB that leads to its degradation and exposure of the nuclear localization signal on NFkB allowing translocation to the nucleus and binding to kB elements, thereby promoting the expression of multiple pro-inflammatory genes [115].

Kinase activity is countered by protein phosphatase activity [131]. The catalytic centre of phosphatases are redox-sensitive and are either cysteine centred or centred on a dimetallic cluster containing iron and either zinc or manganese [132]. Protein phosphatases can be inactivated by oxidation of their catalytic centre; hence phosphorylation state of IkB can be due to either activation of protein kinase cascades or to inhibition of protein phosphatases [133]; hence, oxidative stress increases overall kinase phosphorylation status by inhibiting protein phosphatase activity. Thus, cellular redox regulates the likelihood of NFkB activation [87] and decreasing cellular oxidative stress will decrease the probability of NFkB activation [134] and, thus, inflammatory conditions [135].

Nrf2: The Nuclear factor (erythroid-derived) like-2 (Nrf2) signalling system plays a central role in decreasing oxidative stress in a cell [134,136,137]. Nrf2 is normally located in the cytosol bound to a homodimer of Kelch-like ECH-Associated Protein (Keap1). Nrf2 is continually synthesized but Nrf2 bound to Keap1 allows it to be polyubiquinated, tagging it for proteasomal degradation. Electrophiles of a particular geometric and electronic configuration can oxidize critical cysteines on Keap1, dissociating Keap1 from Nrf2, allowing Nrf2 to translocate to the nucleus where it heterodimerizes with small Maf proteins and bind to anti-oxidant (or Electrophile) Response Elements (AREs or EpREs) in the promoter regions of multiple genes, many of whose protein products decrease the probability of forming strong oxidants or scavenge strong oxidants.

Readers should be beware that I view the potential therapeutic effects of bioactive molecules through a lens that that may be skewed to Nrf2 activators. That Nrf2 activators, previously known as phase 2 enzyme inducers, could play a role in health by preventing cancer formation was first proposed by Paul Talalay of John Hopkins University [138]. He is considered to be the founder of the pharmacology of chemoprotection [139]. A review of his many accomplishments can be seen in the paper by Johnson [140]. I had been working on understanding the relationship between oxidative stress and inflammation and became intrigued with Talalay's research and realized that the rise in cellular GSH following an oxidative insult was due to the induction of g-glutamyl-cysteine ligase, the rate limiting enzyme for GSH synthesis. Glutamyl-cysteine ligase has AREs in the promoter regions of both the regulatory and catalytic subunits [141]. I realized that phase 2 enzyme inducers could protect not only against cancer but protect against diseases that have underlying oxidative stress & inflammation components [142]. This led to research on this topic in my laboratory and publication of papers demonstrating that dietary Nrf2 activators (phase 2 enzyme inducers) could ameliorate many chronic diseases, e.g., [143–149]. Many laboratories are now investigating the therapeutic effects of Nrf2 activators [150].

Many of the monoterpenes and their metabolites appear to involve activation of the Nrf2 signalling pathway: D-limonene [151], perillyl alcohol [152], carveol [153,154], a-pine [155], myrtenol [156], and 1,8-cineole [155,157], The D^3-carene metabolites, D^3-caren-10-carboxylic acid and D^3-carene epoxide may also be Nrf2 activators.

I suggest monoterpenes and their metabolites to be screened for their ability to cause Nrf2 activation. A simple approach would be to use cell lines and screen for the ability of the compounds

of interest to increase cellular GSH. The simplest way to measure GSH is to add monochorobimane to living cell cultures allowing the endogenous glutathione S-transferases to form a glutathione-monochlorobimane adduct – this adduct can be excited at 380 nm to emit at 470 nm [158]. This approach allows rapid screening of potential Nrf2 activators. I would suggest a number of different cell lines be examined, including bronchial epithelial, vascular endothelial, hepatic, etc., since there may be differences in the cells abilities to respond to monoterpenes and their metabolites.

6.3. Possibilities of Synergies

To further complicate issues there may well be synergistic interactions amongst the monoterpenes or their metabolites where each compound affects separate signalling components that activate a particular therapeutic pathway. One such example is where an Nrf2 activator phosphorylates serine residues on Keap1 but the likelihood Nrf2 translocating to the nucleus is also determined by the phosphorylation status of particular amino acid residues on Nrf2. Thus, protocatechuic acid results in Nrf2 phosphorylation [159] making it easier for Nrf2 to separate from Keap1 while sulforaphane oxidizes cysteine residues in Keap1 allowing release of Nrf2 [160]. Thus, protocatecuic acid in this example might provide the 'tipping point' in whether low concentrations of an Nrf2 activator may initiate the Nrf2 signalling pathway.

One example of such possible synergies with terpenes is the experiments conducted by Bai and colleagues first on an in vitro model of cardiac hypertrophy followed by an animal model [161]. Again the in vitro and in vivo doses were large (100 μM) but the results clearly showed that combining 1,8-cineole with b-caryophyllene had synergistic effects.

One can conclude that not only must one use monoterpene atmospheric concentrations that may be present in forests but one should use combinations of monoterpenes in concentrations that might be found in forests since therapeutic effects of forest bathing may be due to synergies in the actions of the various monoterpenes. An example of such a study is that by Kim and colleagues who looked at the physiological effects of a combination of b-pinene, a-pinene and D-limonene [102] using blood pressure, heart rate and heart rate variability as the outcomes.

7. Conclusions

There is clear evidence that one of the therapeutic mechanisms of forest bathing is mediated by 'mindfulness' promoting parasympathetic activities. Moving from a more polluted urban environment to a less polluted forest environment perhaps also accounts for some of the therapeutic effects. Clearly, monoterpenes encountered at higher concentrations than are present in forest atmospheres have effects on physiological processes that promote better health outcomes. For example, a Phase III clinical trial has been completed that shows the efficacy of 1,8-cineole in reducing cough frequency and other symptoms of bronchitis [162] and this monoterpene shows great promise as an adjunct therapy for asthma [100]. But there is enough intriguing evidence that some of the forest bathing's therapeutic effects are mediated by the monoterpenes present in the forest atmosphere activating physiological processes that promote health, but this needs further investigation.

For therapeutic mechanisms, two avenues that seem worthwhile to explore: the activation of the adenosine A_{2A} receptor and the activation of the Nrf2 signalling pathway. Because the vascular system is exposed to high levels of monoterpenes and their metabolites, another avenue should be investigated is the effect of these compounds on endothelial funmction.

Conflicts of Interest: There are no conflicts of interest

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