## Appendix:

## Supplemental Digital Content to Article on Overview of Health Benefits of Thyme
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### Methods Used in Searching the Literature on Thyme

A search of the PubMed literature database was completed in order to identify relevant research publications. Search terms included: *Thymus vulgaris*, thyme, carvacrol, thymol, p-cymene, and α-terpineol. Carvacrol and thymol were selected for study, since they are major components of thyme oil and the most extensively studied biologically-active constituents of thyme. p-Cymene and α-terpineol were chosen because there is a growing scientific literature addressing their biological actions, and, in some thyme essential oils, these can be major constituents. Full reports of publications and English abstracts of foreign-language articles from peer-reviewed journals were primary sources of information. The quality of some studies' methodologies varied, particularly in regard to adequately describing the composition of test samples. Nonetheless, these were included in this discussion so that the variety of information can be evaluated and issues for future reseach can be identified. Commercial and governmental reports also were supplementary sources.

### Health Benefits of Thyme:

### Antioxidant actions:

### In Vivo

*In vivo* studies generally provide support for antioxidant properties of thyme depending on the samples tested. Rabbits were fed diets supplemented with 3% dried thyme for 11 weeks and the meat was evaluated during subsequent retail display (1).

Compared to controls, meat from thyme-fed rabbits exhibited significantly lower lipid peroxidation and higher n-3 fatty acid levels suggesting that thyme preserved the integrity and functionality of muscle cell membranes. In other studies the effect of thyme oil administration was assessed in rat and rabbit models. In these studies several tissues were examined, and doses varied from 5 to 42 mg/kg for periods of time from 1 week to 28 months. For example, in rats treated with aflatoxin to induce oxidative stress, oral administration of thyme oil (5-7.5 mg/kg) for 4 weeks led to decreased liver and kidney malondialdehyde and lipid peroxide levels, as well as increased liver content of superoxide dismutase (SOD), catalase (CAT), and GPx, compared to controls (2). Furthermore, in three rat studies designed to evaluate age-related tissue changes during a 21-month period, dietary supplementation with thyme oil (42.5 mg/kg/day) helped maintain higher polyunsaturated fatty acid levels in liver, kidney, brain and heart tissue, and helped increase SOD, and GPx levels that had declined with age in control rats (3-5). These results are similar to those reported in mice (6). In ageing rats the effect of thyme oil on the retina was determined following oral administration of 3.9 mg of oil daily for 17 months (7). Compared to controls, docosahexaenoic acid (DHA) levels in retinal phospholipids was significantly higher. This increase was associated with a decrease in oleic acid levels. The authors suggested that this oil may be useful in prevention of age-related macular degeneration. In a study with rabbits fed diets supplemented with thyme oil (0.5 g/kg diet, ~25 mg/kg bw) significant increases in total SOD activity in the blood and in GPx activity in the liver were observed, as well as a decrease in malondialdehyde levels in duodenal tissue, compared to controls (8). In contrast, compared to controls, rats fed a diet supplemented with thyme oil (618 mg/kg diet, ~20 mg/kg bw) for 7 days

resulted in no effect on hemoxygenase and GPx activities in colons of animals or those treated with dextran sulfate sodium (DSS) to induce colitis as well as in colons of those without DSS treatment (9). It is worth noting that considerably different oil compositions were reported for the thyme oils samples evaluated in these studies. The major components detected were either p-cymene, carvacrol or thymol. Since many of the oils with varying composition nonetheless provided antioxidant activity, this suggests that any benefits of the oil are likely not due to a single constituent. This is supported by the observation in one study that when administered at the same doses thymol alone did not provide significantly higher antioxidant benefits than whole oil (3).

The antioxidant actions of methanol and aqueous extracts also have been reported and are inconsistent. For example, a study of oxidative stress induced by N-nitrosodiethylamine (NDEA) showed that, compared to controls, rats fed diets supplemented with an undefined methanol extract of thyme (0.5% w/w) for 2 weeks did not exhibit a change in blood glutathione levels but did show enhanced SOD and GPx activities in erythrocytes and heart (10). Lipid peroxidation was decreased in liver, kidney and spleen but not in lungs and heart. Another methanol extract of thyme was given orally (100 mg/kg) to streptozotocin (STZ)-treated rats for 28 days (11). Compared to controls, animals administered the extract had increased glutathione levels in erythrocytes, retinas and lens. In addition lipid peroxidation was decreased in erythrocytes and retinas. An undefined ethanol extract of thyme was given orally (500 mg/kg) to aflatoxin-treated rats for 6 weeks (12). Animals administered the thyme extract showed significantly lowered liver peroxidation and increased activities of GPx and SOD, compared to controls. In contrast, an aqueous extract of thyme that was given

(100 mg/kg, i.v.) to rats failed to exert a significant NO-scavenging ability in blood 30 min after injection (13). Inconsistent antioxidant effects among these studies likely were due to differences in experimental protocols, in tissue-specific responses, in the inducers of oxidative stress used, and in compositions of the undefined extracts.

Carvacrol and thymol tested alone generally exhibited consistent antioxidant actions. In this regard, four rat studies demonstrated an antioxidant benefit of thymol. Rats given thymol orally (42.5 mg/kg) for 21 months evidenced significant increases in the polyunsaturated fatty acids (PUFAs) 20:4(n-6) and 22:6(n-3) in heart, liver and kidney, compared to age-matched controls. In the brain dietary thymol exposure resulted in an increase in GPx activity, and in 20:1(n-6) and DHA levels, compared to controls. SOD levels did not change, however (3,4). In a shorter study oral dosing with thymol (7.5 mg/kg) for 7 days prior to isoproterenol-induced myocardial infarction resulted in lower plasma lipid peroxidation and a restoration of GSH, ascorbic acid and vitamin E, compared to controls (14). However, antioxidant endpoints in the myocardium were not measured. Such measurements as well as histological examination of the heart would have been of particular importance, since isoproterenol-induced serum creatine kinase MB activity decreased upon oil dosing. High fat-diet-fed rats given thymol (14 mg/kg) orally twice per day for 4 weeks also exhibited decreased lipid peroxidation and increased SOD and CAT levels in serum (15). In contrast, chickens provided diets supplemented with thymol (29 ppm) for 21-41 days showed no effect on any of the C16 and C18 fatty acids measured in adipose tissue or plasma (16). This lack of effect on fatty acids may have been due to the dose of thymol examined (~1.5 mg/day) and the animal model. Effects on lipid peroxidation, similar to those in rats, were observed in

mice administered a single oral dose (300 mg/kg) of thymol (17). Antioxidant benefits similar to those for thymol were observed in rats given carvacrol orally (25-50 mg/kg) 14 days prior to induction of hepatotoxicity by thioacetamide (18) and those orally dosed with carvacrol (20,40,80 mg/kg) for 9 days prior to isolation of pancreatic islets (19). However, in this latter study it is noteworthy that only the 20 mg/kg dose significantly lowered $H_2O_2$-induced lipid peroxidation, whereas at the 80 mg/kg dose there was an opposite response in lipid peroxidation and islet viability declined. This underscores the importance of evaluating multiple doses of individual phenolics in a variety of tissues not only for potential benefits but also for any toxic effects. Compared to controls, stomachs of mice given oral carvacrol (25 mg/kg) one hour prior to ethanol-induced gastric ulcers exhibited a 174% increase in CAT activity (20). When chickens were given an equal mix of carvacrol and thymol to provide supplementation of diets with 60, 100 and 200 mg total phenolics/kg diet for 17 days, thigh muscles and serum showed increases in SOD and GPx levels and a decrease in lipid peroxidation, compared to controls. The ratio of polyunsaturated to saturated fatty acids in these tissues also increased. No negative effects of the phenolic-supplemented diets on hematological parameters were detected (21). In an injection study carvacrol had an antioxidant action in nerve tissue of rats (22) administered the neurotoxic oxidant methotrexate (MTX). Compared to controls, animals dosed with carvacrol (73 mg/kg, i.p.) one day prior to MTX showed a significant reduction in lipid peroxidation and total oxidant status (μmol $H_2O_2$ equivalents/L) in sciatic nerve tissue 7 days later.

**Anti-inflammatory Actions:**

**In Vitro**

Individual constituents of thyme have been evaluated *in vitro* for antiinflammatory activity and the effects are mixed. For example, carvacrol (2-10 μg/ml) inhibited NO and $H_2O_2$ production in glucose-stimulated human monocytes (23). Moreover, carvacrol (100-400 μM) suppressed LPS-induced COX-2 mRNA and protein expression in macrophage cells which was associated with activation of PPARγ (24). It also inhibited ($IC_{50}$=0.8 μM) COX-2 catalyzed prostaglandin E2 biosynthesis in an *in vitro* COX-2 assay (25). In contrast, carvacrol also stimulated p38MAPK, activated ERK, and triggered intracellular $Ca^{++}$ mobilization ($EC_{50}$=10-30 μM) in monocytic cells (26). Intracellular $Ca^{++}$ mobilization is a response of immune-responsive cells to activation of cell surface receptors that can have diverse impacts on cell functions depending on intracellular signaling pathways impacted. Another thyme constituent thymol added to cultures of neutrophils (10-20 μg/ml) activated $Ca^{++}$ channels and triggered a corresponding decrease in elastase, a marker of inflammatory disease (27). $CD4^+$ T-helper (Th) lymphocytes play important roles in modulating immune and inflammatory responses particularly the balance of Th-type 1 (Th1) and Th-2 cell responses. Th 1 cells can secrete the cytokines IL-2, IL-3, IL-10, TNF-α/β and interferon (IFN)-γ, whereas Th-2 produces IL-3, IL-4, IL-5, IL-6 and IL-10. Addition of thymol (0.05-5.0 μM) to murine primary splenocytes resulted in changes in Th1/Th2 cytokine secretion profiles usually associated with anti-inflammatory potential (28). Another thyme constituent p-cymene did not affect NO and $H_2O_2$ production when added to cultures of glucose-stimulated monocytes (23). However, p-cymene when added to LPS-stimulated macrophage-like cells (53-214 μg/ml) suppressed inflammatory cytokine production and mRNA

expression (29). When provided to oral buccal cell cultures (3.1 mM) the thyme constituent α-terpineol suppressed IL-6 formation and inhibited IL-6 receptor gene expression (30). These diverse and inconsistent *in vitro* activities of thyme constituents underscore the importance of *in vivo* confirmation of any anti-inflammatory actions.

**In Vivo**

Thyme oil, carvacrol and thymol have been evaluated *in vivo* for their inflammation suppressing capacities. For example, mice given diets containing combinations of thyme oil (0.1-0.4%) and oregano oil (0.05-0.2%) for 7 days exhibited an attenuation of trinitrobenzene sulfonic acid (TNBS)-induced colitis and mortality, compared to controls (31). In colon tissue from these oil-treated mice, protein levels of pro-inflammatory cytokines IL-1β and IL-6 decreased, and macroscopic inflammatory injury of the colonic tissue was reduced. It was noted that there was no dose dependency of the responses with only the intermediate levels (0.2% thyme oil, 0.1% oregano oil) being efficacious. The reason for the narrow range of active concentrations was not explained and the individual contribution of thyme oil can not be determined. In another study rats were provided a diet containing thyme oil (618 mg/kg diet) for 7 days prior to induction of colitis by DSS treatment. The oil contained thymol as the major constituent, and the diet was designed to provide 2 mmol thymol/kg diet (9). The oil counteracted the DSS-induced elevation of COX-2 and vascular cell adhesion molecule (VCAM) expression in liver and decreased intestinal inflammation, compared to controls. Of interest, thyme oil substantially increased the expression of the tight junction protein Cldn3, suggesting that it improves intestinal barrier function. A third study in rats (32) evaluated oral administration (250,500,750 mg/kg) of a thyme oil that contained

carvacrol (46%) and α-terpineol (23%) as major components. The oil was given one hour prior to interpleural injection of carrageenan, and animals were evaluated 4 hr later. All doses decreased inflammatory exudate, but only the highest dose decreased leukocyte and polymorphonuclear cell migration.

Carvacrol when evaluated individually *in vivo* also exhibited anti-inflammatory actions when tested against diverse inflammatory stimuli. For example, in mice fed a high-fat diet supplemented with carvacrol (0.1% w/w) for 10 weeks, the production of pro-inflammatory cytokines in visceral adipose tissues was suppressed, compared to high-fat controls, in part due to inhibition of toll like receptor 2 (TLR2)- and TLR4-mediated signaling (33). Dietary supplementation of carvacrol also was able to significantly reverse the high fat diet-induced up-regulation of adipose tissue genes and proteins associated with inflammation. Several studies examined the anti-inflammatory actions of carvacrol in paw and ear edema models using a variety of inducers of inflammation and routes of administration (32,34-36). Although an anti-inflammatory effect of carvacrol was usually detected, the effective doses varied. For example, oral carvacrol (100,200,400 mg/kg) was given to rats 1 hr prior to carrageenan-induced peritonitis (32). When examined 4 hr later pleural exudate and migration of leukocytes to the injury site decreased, particularly at the 400 mg/kg dose. Oral dosing with carvacrol (25,50,100 mg/kg) prior to induction of paw edema by histamine or substance P (34) resulted in a response that was not dose-dependent. The 50 mg/kg dose was effective in inhibiting edema induced by histamine and the 100 mg/kg dose was effective in suppressing edema induced by substance P, compared to controls. Topical application of carvacrol (10-40 mg/ear) to rats 1 hr prior to croton oil, decreased ear edema only at the

lowest dose, compared to controls (32). Topical treatment of mice with carvacrol (0.1 mg/ear) 1 hr prior to administration of TPA or arachidonic acid (34) suppressed ear edema for 6 hr. When mice were given carvacrol (25-100 mg/kg, i.p.) 30 min prior to carrageenan-induced pleurisy all doses suppressed recruitment of leukocytes to the pleural cavity and decreased TNF-α levels in the exudate (35). However, the magnitude of carvacrol's anti-inflammatory effects were not dose-dependent, the reason for which was not determined. Injection of mice with carvacrol (25,50,100 mg/kg, i.p.) 30 min prior to carrageenan treatment led to decreased paw edema for 4 hr at the 2 highest doses, compared to controls (35). Similarly, mice given the same doses of carvacrol (i.p.) 40 min prior to treatment with Freund's adjuvant (CFA) resulted in a significant decrease in paw edema at the 2 highest doses, an effect lasting up to 24 h-post-CFA (36). In this study expression of numerous mediators of inflammation (COX-2,IL-1β, $PGE_2$) in paw tissues were lowered and antiinflammatory IL-10 levels were increased for mice given carvacrol at the 50 and100 mg/kg doses. When this experiment was subsequently conducted using IL-10 knockout mice that were treated with 100 mg/kg carvacrol, no suppression of paw edema was observed, compared to controls. Collectively, these findings from the edema models suggest that carvacrol exerts its anti-inflammatory properties due to its capacity to suppress production of inflammatory mediators and to modulate vascular events involved in inflammation (34,36). The lack of dose dependency in some studies may be due to variability in responses of different inflammatory mediators and to different administration protocols chosen. These data also point to the fact that carvacrol likely affects multiple signaling pathways, such as those

associated with protein kinase C (PKC), arachidonic acid and COX-2, as well as events triggering IL-10 release.

Evidence of carvacrol's anti-inflammatory action also was reported in liver, stomach, and lung models. Rats given carvacrol orally (25,50,100 mg/kg) for 14 days after administration of acetic acid to the stomach, exhibited accelerated return of chronic gastric ulcers to normal mucosal patterns at all doses, compared to controls. The authors noted that this ulcer model closely resembled the pathology and healing events for human ulcers, especially because of the difficulty and lengthy time period encountered in their treatment (34). In another stomach model, carvacrol was provided orally (12.5,25,50 mg/kg) to mice 1 hr prior to induction of gastric lesions by ischemia/reperfusion, ibuprofen or ethanol (20). Only the 25 and 50 mg/kg doses suppressed gastric damage by all three induction protocols, compared to controls. Pretreatment of mice with L-NOARG, an inhibitor of NO synthase activity, partially reversed carvacrol's gastroprotective benefits. The authors suggested that carvacrol's effects may involve prostaglandins and $K_{ATP}$ channel opening. With regard to the liver, giving carvacrol to rats (20 mg/kg, p.o.) for 1 week after D-galactosamine-induced hepatotoxicity, resulted in suppression of liver expression of TNF-α, IL-6, iNOS, COX-2 and NF-κB levels that had been upregulated by galactosamine (37). In another rat study oral administration of carvacrol (25,50,100 mg/kg) for 2 weeks prior to dosing with thioacetamide resulted in a significant decrease in liver damage, liver sinusoidal congestion and inflammatory cell migration, compared to controls (18). Carvacrol treatment also markedly inhibited NF-κB expression in a dose-dependent manner and increased the expression ratio of antiapoptotic Bcl2 to proapoptotic BAX.

In an injection study mice were given carvacrol (20,40,80 mg/kg, i.p.) 1 hr prior to a LPS challenge to induce acute lung injury (38). After 7 days those administered carvacrol evidenced a significant increase in survival, compared to controls. Specifically, groups of mice receiving 20, 40, and 80 mg/kg showed 28%, 62% and 76% increases in survival, respectively, compared to controls. For the 40 mg/kg and 80 mg/kg groups inflammatory infiltration, focal areas of fibrosis and pulmonary congestion were reduced as well. In an accompanying experiment, when mice were treated with the carvacrol doses after i.n. administration of LPS, a significant, dose-dependent decrease in the inflammatory cytokines TNF-$\alpha$, IL-1$\beta$, and IL-6 was observed in bronchoalveolar lavage fluid, compared to controls. The authors suggested that carvacrol may be effective as an agent in strategies to prevent and treat endotoxin-induced acute lung injury and acute respiratory distress syndrome. In an investigation involving sciatic nerve tissue, carvacrol dosing (73 mg/kg, i.p.) one day prior to methotrexate (MTX) administration suppressed IL-1$\beta$ and TNF-$\alpha$ levels in this tissue, compared to controls (22). In a novel study, cultures of bone marrow derived dendritic cells (BMDCs) were exposed to carvacrol and a physiological (thermal) stress *in vitro* to induce a tolerogenic phenotype in DCs (39). Tolerogenic DCs are able to dampen immune responses associated with autoimmune diseases. Subsequent prophylactic injection (i.p.) of these combination-treated DCs into arthritic mice suppressed disease progression, compared to controls. In contrast, DCs treated only with thermal stress were unable to inhibit arthritis progression. Of interest, this treatment of DCs led to alteration of mRNA expression for both pro- and anti-inflammatory mediators (chemokines and cytokines), although this mixed gene expression profile was associated overall with anti-inflammatory activity. Combined

treatment also led to very high levels of the immuno-regulatory molecules MT1 and Hsp70, compared to cells only treated with thermal stress. Based on this experiment and previous evidence that oral carvacrol is protective in this arthritis model (40), the authors suggested that carvacrol may offer unique opportunities to develop dietary-based strategies against chronic inflammatory diseases. In humans subgingival irrigation with 0.02% carvacrol of patients with advanced periodontitis reduced clinical and immunological inflammatory indices (41).

Compared to carvacrol there is less consistent *in vivo* evidence for thymol's anti-inflammatory activity. In studies using rodent models of chemically-induced paw edema and peritonitis, acute treament with thymol (10-100 mg/kg, i.p.) 1 hr prior to administration of carrageenan led to reduced inflammation, a lower influx of leukocytes into injured areas and improved wound healing (42). In an ear edema model in rodents thymol (10 mg/ear) administered topically 1 hr before dosing with a chemical irritant (croton oil) did not decrease edema but rather increased ear edema, compared to controls (32), an effect partly mediated by histamine and prostanoids. This irritation due to thymol was similar to that of the croton oil. In a third study both acute and chronic models of inflammation were used in evaluating thymol. When thymol (2 mg/ear) was applied once prior to a chemical irritant, the subsequent ear edema was significantly reduced, compared to controls. However, when thymol was applied to the ears for multiple days along with the chemical agent croton oil, the result was a significant increase in ear edema and cutaneous damage, indicative of proinflammatory responses (43). The authors cautioned against chronic use of thymol to treat skin inflammation. In contrast, other authors (44) consider topical thymol as a potential therapeutic for mast

cell-associated skin inflammation, such as that encountered in eczema. In their studies, ears of mice were topically treated with 20 μl thymol (200 μM, 2 mM, 20 mM) 24 hr before antigen challenge in the passive cutaneous anaphylaxis (PCA) protocol. Compared to controls, thymol dose-dependently suppressed PCA-associated ear swelling, indicating its capacity to decrease mast cell responses. Based on additional cell culture studies, it was determined that thymol impaired mast cell survival via activation-induced cell death-associated apoptosis. Of note, thymol administration to the ears did actually lead to local mast cell degranulation, but did not result in later swelling. The differences in response to topical thymol between the current and previous studies (43) deserve further characterization. Taken together, the animal model of inflammation as well as the timing and method of thymol administration may contribute to either pro- or anti-inflammatory outcomes. It is of interest that thymol is one of several natural compounds used in the design of anti-inflammatory prodrugs to reduce the effects from traditional anti-inflammatory drugs (45-47).

Two other thyme constituents p-cymene and α-terpineol also display *in vivo* anti-inflammatory properties. p-Cymene administered (10-100 mg/kg, i.p.) for 4 hr to carrageenan-treated mice resulted in a significant decrease in leukocyte migration into the peritoneal cavity, compared to controls (48). In a mouse model of acute lung injury, mice were treated with p-cymene (25-100 mg/kg, i.p.) 1 hr prior to LPS dosing (49). Compared to controls, bronchoalveolar lavage fluid collected from p-cymene-treated mice showed a significant decrease in proinflammatory cytokines. Pulmonary congestion and edema were lower in treated animals, and inflammatory cell migration into the lungs was suppressed. p-Cymene appeared to impede cytokine production in tissues by

inhibiting NF-κB, ERK1/2 and MAPK pathways (49,50). Similarly, dosing of mice with p-cymene (54-214 mg/kg, i.p.) for 1 hr prior to LPS challenge markedly suppressed the production of TNF-α and IL-1β and increased IL-10 secretion, compared to controls (29). In a study using the carrageenan-induced pleurisy model, treatment with α-terpineol (25-100 mg/kg, i.p.) for 30 min prior to carrageenan significantly inhibited neutrophil influx into the pleural cavity, compared to controls (51).

**Respiratory Problems and Cough:**

**In Vitro**

The early history of research from the 1930s to the 1980s into potential antitussive effects of thyme has been reported (38,52). In these early studies thymol and thyme oil were often evaluated for secretomotoric, secrolytic and spasmolytic activities in tracheal and ileal muscle tissue samples. For example, the effects of volatile oils of 22 plants were studied on guinea pig tracheal and smooth muscle preparations and compared to the relaxant effects of catecholamines and phosphodiesterase inhibitors (53). Use of isolated guinea pig tracheal strips is a model considered to resemble human airway smooth muscle in its sensitivity and reaction to drugs. Based on these comparisons, thyme oil belonged to a group of plant oils which had predominantly relaxing effects. Its potency with tracheal smooth muscle was more modest ($EC_{50}$=56 μg/ml) compared to others (e.g. clove oil=3.8 μg/ml), whereas it was a potent inhibitor of the phasic contractions of the ileal myenteric plexus-longitudinal muscle ($EC_{50}$=6.9 μg/ml). In addition, an undefined chloroform extract of thyme as well as thymol and carvacrol were examined for relaxant effects on isolated guinea pig tracheal strips. Although thymol and carvacrol exhibited tracheal relaxant actions, the total antispasmodic activity of the

extract could not be accounted for by the combined activities of these two compounds. More recently the contributions of specific fractions and individual thyme constituents have been examined for their antispasmodic activity *in vitro* along with possible mechanisms. Variable potency has been reported among studies. In one report hexane, dichloromethane, methanol and aqueous extracts were evaluated in guinea pig tracheal chains in organ bath (0.4-1.6g %) for dose-dependent relaxant effects (54). A bronchodilatory action was noted for the hexane, dichloromethane and methanol fractions. The active constituents in the hexane fraction were not determined, although they were speculated to be the relatively non-polar thymol and carvacrol. The effectiveness of the dichloromethane and methanol fractions suggest that other compounds such as polar terfenadines, glycosides and saponins may contribute to a relaxant effect as well. Multiple mechanisms for the n-hexane relaxant activity were suggested including actions involving muscarinic receptors, $Ca^{++}$-channel antagonism, and $K^{+}$-channel opening. It is of interest that the aqueous extract was a relaxant when tracheal chains were treated with the spasmogen methacholine and a contractant when KCl was used as spasmogen. The reasons for these different responses were not determined. Various alcoholic extracts were examined in six reports using *in vitro* assays. For example, a methanol extract of undefined composition was found to exhibit an antispasmodic action toward guinea pig ileum ($EC_{50}$=1.7 mg/ml) possibly due to effects on anticholinergic and serotonergic pathways (55). An undefined ethanol extract was evaluated in isolated guinea-pig trachea cultures (0.2-2.0 % extract) in another experiment. This extract reversibly antagonized the contraction of the trachea muscle, but the degree of relaxant activity was dependent on the individual spasmogen tested,

with prostaglandin F2α being most efficiently antagonized and carbachol the least (56). Four other studies examined the action of ethanol extracts of thyme on isolated trachea and other tissues in rats and mice. In one experiment, an ethanol extract was reported to inhibit endothelin-induced rat tracheal contractions in a non-competitive manner (3-10 mg/ml). The two doses of extract that were effective contained thymol at concentrations of 10.8 and 21.6 μM. Yet, thymol when assayed alone did not affect endothelin-induced tracheal contractions (57). The authors suggested that this thyme extract, but not thymol alone, may provide relief in diseases related to endothelin hyperreactivity of the bronchus system such as asthma and COPD. This indicates that thymol may not be the principal anti-spasmodic agent. In fact, in rat ileum and trachea smooth muscle preparations exposed to both low
(< 0.005%) and normal (0.038%) thymol-containing ethanol extracts (58), antispasmodic actions were associated with both extracts.

The specific *in vitro* effect of carvacrol individually was determined in isolated guinea pig tracheal chains. In one study, carvacrol (0.4 μg/ml) had bronchodilatory actions and stimulated β-adrenoreceptors in guinea pig trachea (59). Similarly (60) carvacrol exposure (0.2-0.4 μg/ml) of tracheal tissue resulted in stimulation of β-adrenoreceptors and competitive antagonism of muscarinic receptors. Moreover, carvacrol (4 μg/ml) had an inhibitory effect on histamine $H_1$ receptors (61). In a study using trachea isolated from mice, thymol ($EC_{50}$=70μg/ml) was more potent than carvacrol ($EC_{50}$=200μg/ml) in inhibiting $BaCl_2$-induced contractions (58). Differences in effective doses among experiments may be due to experimental protocols used.

Regarding other constituents from thyme, polymethoxy flavones were reported to be spasmolytic for guinea pig tracheal chains (62).

Although thymol and carvacrol possessed antispasmodic activity, other unidentified components of thyme extract were likely contributors as well. Some of these reports (57,58) show that the content of thymol and possibly carvacrol in the extracts may not fully account for the spasmolytic actions of thyme. In this regard the antispasmodic importance of other nonphenolic compounds was evaluated in a bioassay-guided fractionation of a thymol-deprived ethanol extract of thyme (63). Fractions studied, although containing low levels of carvacrol and thymol, nonetheless possessed good antispasmodic activity. Further characterizations determined that luteolin, contributed significantly to the relaxant activity. Possible synergies of luteolin with luteolin-7-glycoside and other unidentified phenolic compounds were likely occurring. In contrast, rosmarinic acid and apigenin were inactive. The authors suggested that specifications of thyme-containing preparations used in medicinal product standardization should include the flavone luteolin in addition to those for thymol and carvacrol. This finding is consistent with an earlier report that luteolin was one of several *Thymus* flavones that produced a concentration-dependent suppression of tone in guinea pig tracheal preparations contracted with carbachol (62).

**In Vivo**

There is also *in vivo* support for antispasmodic actions of thyme extract and constituents. In one study an ethanol extract was administered orally to mice (0.4 and 4.0 ml/kg). These doses were determined to deliver 0.13 and1.3 mg/kg thymol and 0.015 and 0.152 mg/kg carvacrol (64). Both doses of the extract significantly stimulated

mucociliary clearance in mice trachea. The mechanisms for this effect were not determined, although the participation of β2-receptors was likely, since propranolol attenuated this action of the extract. A separate study (59) using similar dosing of rats with thymol and carvacrol also found that both thymol and carvacrol (0.13 mg/kg) increased mucociliary transport *in situ*. Of interest, in another study carvacrol was added to the drinking water of ovalbumin-sensitized guinea pigs (40-160 μg/ml) for 30 days after which tracheal chains were removed and exposed to either ovalbumin or methacoline (65). Compared to controls, the contractility response to both agents was significantly decreased in a dose-dependent manner for tissue removed from the animals consuming the 80 and 160 μg/ml levels of carvacrol in the drinking water. Based on the daily fluid consumption of the guinea pigs, the higher carvacrol dose corresponded to an intake of about 20 mg/day or about 40 mg/kg/day. This altered tracheal responsiveness also was associated with decreased serum concentrations of NO and nitrite. In a similar experiment by the same group, for animals fed the 80 and 160 μg/ml amounts of carvacrol, serum levels of IL-4 and endothelin were significantly decreased while IFN-γ levels were significantly increased, compared to controls (66). The authors suggested that carvacrol could have a therapeutic effect on asthma by stimulating bronchodilation and reducing lung inflammation.

**Neurological actions:**

**Antinociceptive Activity:**

**In Vitro**

Thymol is known to interact with synaptic neural functions on $Na^+$ and GABA-associated $Cl^-$ channels. For example, in two reports cultures of cortical neurons were

treated with thymol (5-200 μM) which led to identification of this phenolic compound as a positive allosteric modulator of the $GABA_A$ receptor (67,68). Not only was thymol able to interact with specific receptor proteins, it also was able to interact with and modulate their surrounding lipid molecules (69). This is relevant in light of the observations that the $GABA_A$ receptor is a focal point for the actions of numerous GABA agonists and positive allosteric modulators, including various drugs and steroids. Such actions can result in diverse anxiolytic, sedative and anticonvulsant or anesthetic responses (67).

Carvacrol added to cultures of colon, corneal epithelial and neural cells was demonstrated to be an agonist of the the transient receptor potential (TRP) V3 channel, which is associated with recognition of warmth and pain sensations. Depending on the cells cultured in these studies, doses varied from 10 μM to 1 mM (70-72). An additional mechanism of action for thymol and carvacrol involves their suppression of neuronal excitability by inhibiting (at concentrations of 0.01-1.0 mM) compound action potentials (73,74). In particular, carvacrol's analgesic action was observed to be a result of direct inhibition of a voltage-gated sodium current (73). These examples of thymol's and carvacrol's *in vitro* actions underscore the variety and complexity of the biological actions of these monoterpenoids on neuronal processes and cell functions in diverse tissues.

**In Vivo**

In *in vivo* studies an alcoholic extract of thyme of unknown composition was injected (100-1000 mg/kg, i.p.) in mice 30 min prior to the performance of three stress tests (75). The antinociceptive effects were measured by the hot plate, tail flick and

formalin tests. The hot plate and tail flick tests use mild exposure to heat as a noxious stimulus and measure nociceptive reflex responses. In the formalin test, a chemical noxious stimulus is used to evaluate responses to acute and long-lasting pain. This latter model detects nociceptive behavior during 3 stages of pain, an initial phase of neurogenic pain, a quiescent phase, and a third, later phase due to inflammatory pain. In these pain models this extract inhibited the pain sensation, compared to controls, but dose-dependent analgesic effects were not consistently demonstrated. Carvacrol also has demonstrated *in vivo* efficacy in suppressing pain in multiple models. For example when tested at 25-100 mg/kg ( i.p.) 30 min prior to stress testing, it inhibited both early neurogenic pain and late inflammatory pain in the formalin test in mice, suppressed nociception due to acetic acid- glutamate- and capsaicin-induced chemical pain, and significantly increased the latency response in the hot plate model of pain in these animals (76). For all these tests carvacrol elicited dose-dependent responses. For other mouse studies, carvacrol (25-100 mg/kg, i.p.) given 30 min prior to testing suppressed mechanical hypernociception and orofacial pain induced by multiple chemicals (35,76,77). Dose-dependent effectiveness of carvacrol was demonstrated.

Taken together these injection studies evaluating multiple models of pain suggest that carvacrol may be acting by several mechanisms. It may be inhibiting peripheral mediators of pain possibly involving prostaglandins. Its effects on central processes include modulation of non-opoid central receptors that involve antioxidant and anti-inflammatory signaling pathways (35,76). Another thyme constituent, the monoterpene p-cymene, also produced suppressive effects on nociceptive responses in chemical and thermal models of pain when given to mice at doses of 25-100 mg/kg, i.p. (78,79).

Moreover, p-cymene reduced nociceptive behavior in both the neurogenic and inflammatory phases of the formalin test. Its actions were mediated by both central and peripheral anticociceptive processes. Of interest, this monoterpene can be metabolized by rodents to carvacrol. Therefore, p-cymene and its metabolite carvacrol may be acting in concert to affect such processes as the arachidonic acid pathway or generation of pro-inflammatory mediators. Another component of thyme, the monoterpene alcohol α-terpineol, reduced nociceptive behavior in mice when measured in four experimental models of pain (51,80). In a thermal model only the highest dose (100 mg/kg, i.p.) suppressed the pain response. However, in several models of mechanical hypernociception, significant pain-alleviating actions were observed, even at the lowest dose (25 mg/kg, i.p.). Similar to data obtained for carvacrol, α-terpineol's mechanisms of action include inhibition of cytokine cascades and the production of inflammatory mediators (51). It is of interest that when evaluated individually in the injection studies, individual thyme constituents, in contrast to extracts, more often than not caused dose-dependent antinociceptive responses. It may be worthwhile to evaluate how the ratio of carvacrol to other extract constituents may be affecting extract potency.

As far as oral dosing is concerned administration of carvacrol (12.5 to 200 mg/kg) was reported to inhibit nociception in three models of pain, acetic acid-induced abdominal constriction, and formalin and hot plate tests. Specifically, carvacrol at doses of 50, 100 and 200 mg/kg suppressed acetic acid-induced contractions. This effect of carvacrol was not reversed when mice were treated with naloxone or L-arginine. This suggests that neither activation of opioid receptors nor the NO pathway are likely involved in carvacrol's antinociceptive effects. It was noted that carvacrol did not impair

motor performance, a potential confounding variable in these model systems (81). In a separate study, oral treatment of mice with a complex of p-cymene and β-cyclodextrin (20-40 mg/kg) improved the analgesic effects of p-cymene, compared to equal doses of p-cymene alone (82). Whether this improvement is due to effects on bioavailability or pharmacological actions is not known. When these studies are considered together, it appears that any antinociceptive properties of thyme extracts are likely mediated by several monoterpene constituents.

**Neurobehavioral and Neuroprotective Studies:**

**In Vivo**

*In vivo* investigations have evaluated the stimulative and sedative effects of thyme oil, α-terpineol and thymol. In one study 0.5 ml thyme oil was inhaled by mice for 10 min before and during a 6 min forced swimming motility test. Compared to controls exposure to thyme oil resulted in a significant decrease in immobility (stimulative effect) both when caffeine-treated overagitated mice and non-treated mice were evaluated (83). In a separate report the opposing actions of two thyme constituents were reported. Following a one hour inhalation period in which mice were exposed to 0.5 ml evaporated thymol, the motility of mice increased by 33%, compared to controls, a result that also was achieved even when mice were artificially induced into overagitation with caffeine prior to the inhalation period. In contrast, also in motility test systems, a 1 hr period of inhalation of α-terpineol (0.5 ml) led to a 45% decrease in motility, compared to controls (84). The basis for these response differences was not explained, but could have been due in part to differences in relative bioavailability and mechanisms of action. These results suggest that different thyme essential oil chemotypes might have different

outcomes in aromatherapy depending on the relative amounts of α-terpineol, thymol or other constituents present.

Several rodent studies provide evidence that carvacrol can modify mood behavior. Anxiolytic-like effects were reported in two mouse studies. In one experiment mice were orally administered 12.5-50 mg carvacrol/kg one hour before the forced swimming and tail suspension tests, two behavioral assays capable of assessing anti-depressant-like activity. In both assays carvacrol lessened depressant-associated behavior in a dose-dependent manner without altering locomotive activity, compared to controls. This effect of carvacrol appeared to be dependent on its interaction with the dopaminergic system, through increases in dopamine levels, while not affecting the serotonergic or noradrenergic systems (85). This suggests that acute carvacrol intake is likely acting in a manner similar to the antidepressant drug buproprion by affecting synaptic levels of dopamine. In contrast, in this situation carvacrol's mechanism of action appears not to involve modulation of serotonin levels, which is the basis by which the selective serotonin reuptake inhibitor antidepressants act. In a similar study (86), carvacrol was administered orally (12.5 to 50 mg/kg) to mice 1 hr prior to the plus maze test, a behavioral test for anxiolytic drugs. It elicited anti-anxiety effects at all doses, without affecting spontaneous locomotor activity or sleep latency, potentially confounding factors. The authors suggested that this activity of carvacrol involved modulation of GABAergic transmission, which is consistent with observations that carvacrol is an allosteric modulator of the $GABA_A$ receptor (87). Some actions of carvacrol on mood may be gender- and dose-related. For example, in one experiment, carvacrol was given orally (0.15 and 0.45 g/kg) to regularly-cycling female rats 2 hours prior to behavioral

testing (88). Rats were evaluated in the diestrus and proestrus phases of the estrus cycle, characterized, respectively, by low and high levels of circulating estrogen. Similar to other reports carvacrol did not alter spontaneous motor activity in either diestrus or proestrus phases. However, only during the proestrus phase carvacrol (only 0.45 g/kg tested) promoted a depressive-like behavioral pattern, which was associated with a reduction in serotonin levels in the prefrontal cortex and nucleus accumbens areas of the brain. Carvacrol also decreased plasma estradiol levels, but only during proestrus and it did not interact with estrogen receptors. In contrast, in another report by the same group, male rats given carvacrol orally (0.45g/kg) 2 hr prior to administering climbing, swimming and immobility tests showed no changes in measures of antidepressant-like activity, compared to controls (89). Furthermore, substantial differences in brain neurochemical content were detected. This dose of 0.45 g/kg led to a significant increase in dopamine levels in the prefrontal cortex and a significant decrease in the hippocampus. On the other hand there were significant decreases in serotonin levels in both brain regions, compared to controls. The authors suggested that regular consumption of carvacrol in low concentrations could influence feelings of well-being in humans. However, it should be noted that the 0.45 g/kg dose corresponds to approximately 50% of the reported $LD_{50}$ value in rats (90), and thus has considerably less relevance to dietary intake of thyme and carvacrol in humans. Moreover, this amount is 9- to 36-fold higher than doses reported to have anxiolytic actions (86). In this regard, cautions were communicated by the authors that carvacrol is lipophilic, likely may traverse the blood-brain barrier, and possibly might accumulate in adipose tissue, making a more thorough evaluation of its chronic intake at low doses warranted (88). These gender- and dose-

related effects of carvacrol are noteworthy and need to be further explored. Additionally, in light of changes in serotonin and dopamine, it may be worthwhile to explore carvacrol's effect on appetite and other behaviors associated with food consumption and energy homeostasis.

Two rat investigations demonstrate that carvacrol possesses cognition-enhancing activity. In one article (91) two rat models of dementia were used. In one of these studies, rats were given an intrahippocampal injection of amyloid β(25-35). Administration of amyloidβ (Aβ) peptides into the brain can produce a number of Alzheimer's disease (AD)-associated symptoms of dementia, including increased immobility and memory and learning deficits. In the other model, dosing with scopolamine, a nonselective muscarinic receptor antagonist, produces cholinergic hypofunction and amnesia-mimicking cognitive impairments and brain changes seen in elderly humans. Acute administration of thymol (0.5-1.0 mg/kg, i.p.) and carvacrol (1-2 mg/kg, i.p.) 30 min prior to testing lessened the learning deficits and cognitive impairments following amyloid-β treatment and scopolamine dosing (91), compared to controls. This occurred without changes in locomotion or sensorimotor coordination. In another model in which diabetes-associated cognitive deficits in male rats are produced by STZ, carvacrol treatment (25-100 mg/kg, i.p.) for 7 weeks resulted in a substantial and significant improvement in cognitive function at all doses, compared to controls (92). Remarkably, carvacrol dosing improved blood glucose levels and body weights of the diabetic rats, and also suppressed oxidative stress, inflammation (brain TNFα and IL-1β) and apoptosis (brain caspase 3), compared to controls, suggesting that it alleviated hyperglycemia-associated brain toxicity.

Carvacrol demonstrated neuroprotective actions in an investigation (93) using a mouse model of traumatic brain injury. Mice were subjected to closed head injury (CHI). One hour later they were evaluated for damage by the neurological severity score (NSS), and then injected with a single dose of carvacrol (75 mg/kg, i.p.) or vehicle. At 21 days post-dosing carvacrol (75 mg/kg. i.p.) significantly improved NSS from closed head injury (CHI), compared to controls. The authors additionally evaluated whether TRP channels of the TRPC subfamily could influence carvacrol's actions. Specifically, 3 groups of mice genetically lacking the ion channels TRPC1, C3 or C5 were treated with carvacrol (75 mg/kg, i.p.) for 21 days after CHI. The authors found that inhibition of the TRPC1 channel in combination with carvacrol dosing synergistically improved recovery from traumatic brain injury (93). The authors suggested that a strategy to disrupt TPC1 along with carvacrol treatment could have rapid translation to the clinic. Additionally, carvacrol was evaluated in a mouse middle cerebral artery occlusion model (94). When carvacrol (25 and 50 mg/kg, i.p.) was administered 2 hr before cerebral ischemia and reperfusion (I/R), it significantly decreased cerebral infarct volume and improved neurological deficits in a dose-depenedent manner, compared to controls. It was determined as well that carvacrol treatment reduced apoptotic neurons induced by cerebral I/R and that the PI3K/Akt pathway participated in carvacrol's protective effects in the brain. The therapeutic window for effectiveness of carvacrol (50 mg/kg, i.p.) when given after injury was fairly short, about 2 hr, with 4 hr post-injury administration having no effect. The authors suggested that carvacrol has high potential for translation as a therapeutic agent in clinical trials of stroke.

**Gastrointestinal (GI) Actions:**

**In Vitro**

As assessed *in vitro* (95) in preparations of chicken duodenal tissue, addition of thyme oil (0.4%) lowered trans-epithelial electrical resistance (TEER) indicative of a negative effect on intestinal integrity. In an experiment with guinea pig taenia coli tissue samples, addition of thyme-derived thymol ($\geq 0.1$ mM) to the organ bath reduced membrane electrical resistance in a dose-dependent manner (96). In three other *in vitro* investigations thymol was shown to alter intestinal secretions. Specifically, in Ussing chamber preparations of piglet jejunal epithelium, thymol treatment (25-100 μM), compared to controls, did not alter TEER but did induce $Cl^-$ and $HCO_3^-$ secretion by activating enteric nerve nicotinic receptors (97). It is known that this increased water and electrolyte secretion can be used by the intestinal mucosa to flush out detrimental bacteria and antigens. However, despite the potentially beneficial changes in water and electrolytes, thymol treatment had no effect on bacterial adhesion. Similarly, in human ascending colon and rat colon tissue preparations, addition of thymol (0.1-1.0 mM) stimulated anion secretion in a reversible, dose-dependent manner, compared to controls, an action in part involving activation of the TRPA1 channel (98). Thymol also increased paracellular nonelectrolyte permeability. In contrast, thymol treatment significantly weakened short chain fatty acid (SCFA) secretion, suggesting that thymol may be a competitive antagonist of the SCFA receptors FFA2 and FFA3. The authors suggested that thymol's capacity to affect the chemical receptors, and the TRP and anion secretion and channels could influence intestinal homeostasis. However, whether these intestinal effects are beneficial or detrimental likely would depend on luminal monoterpenoid concentrations and the resultant outcomes. In another experiment, normal

enterochromaffin cells (EC) were isolated from human ileum. These cells are the major neuroendocrine cells of the small intestine and colon, contain a majority of the body's serotonin, and contribute substantially to the control of secretion, motility and visceral pain. Exposure of EC cultures to thymol stimulated serotonin secretion ($EC_{50}$=0.29μM) through a mechanism involving $Ca^{++}$ influx and ERK signaling (99). The authors noted that thymol and other spice components can be detected by these EC cells which act as luminal sensors. Whether these thymol-associated changes in intestinal secretion, permeability, and motility observed *in vitro* translate into similar effects *in vivo* and how these might affect gut function are not known.

**In Vivo**

*In vivo* studies indicate that thyme and some of its constituents are active in both gastric and intestinal environments. For example, a thyme oil-supplemented diet (0.5g/kg diet) provided to rabbits for 42 days enhanced the integrity of the intestinal barrier as measured by transepithelial electrical resistance (TEER) *ex vivo*, compared to controls. Blood phagocytic activity also significantly increased in the animals consuming the oil as measured by direct counting of microspheric hydrophilic particles ingested by polymorphonuclear cells (8). It was determined that the diet contained p-cymene, thymol and carvacrol at levels of 0.1, 0.08, and 0.01 mg/kg diet, respectively. No significant differences in *Enterococcus* sp., *Staphylococcus* sp., lactic acid bacteria, or coliform bacteria, between thyme-oil treated and control rabbits were observed. However, a small decrease in potentially pathogenic *Clostridium* sp was noted in the oil treated group. The authors speculated that thyme oil may improve intestinal health even without substantially improving the population of beneficial gut microbes. The inability of this

dose of thyme oil to modify the microbial ecosystem of the GI tract underscores the need to evaluate multiple doses of thyme oil. Individual thyme constituents also affect gut health *in vivo*. When an equal mixture of thymol and carvacrol was fed to chickens (60 to 200 mg/kg diet) the activities of intestinal and pancreatic trypsin, lipase, and protease increased in young but not older birds, compared to controls (21). The basis for this increased activity in young birds and its effect on digestion was not determined. In studies of weanling piglets fed diets supplemented with carvacrol or thymol (500 and 2000 mg/kg diet) for 28 days, the villus/crypt (v/c) ratio at the distal small intestine was significantly higher, compared to controls. For animals fed the 2000 mg/kg diet of carvacrol, the mean carvacrol concentration in the proximal small intestine was 5 mg/kg digesta and for those fed the same amount of thymol its concentration was between 13-24 mg/kg digesta. In thymol-fed animals there were reduced numbers of intraepithelial lymphocytes (IEL), compared to controls (100). The changes in v/c and IEL were viewed to reflect improved gut functionality. Decreased IEL reflects lower mucosal inflammatory responses, and the higher v/c ratio is associated with improved small intestine digestion and absorption functions. However, carvacrol and thymol supplementation had no effect on the number of gut bacteria. Lack of effect of these monterpenoids on gut microbes have been reported in other studies. For example, dietary thyme (10 g/kg diet) and thyme oil (1 g/kg diet) fed to chickens for 21 days had no effect on intestinal microbial populations. Similarly, 10 g thyme/kg diet fed to piglets did not affect the population of pathogenic, hemolytic *Escherichia coli* (101). Thymol, when fed to growing swine at levels of 0.0067 and 0.0201%, had no effect on rectal numbers of the pathogen *Campylobacter* (102). The authors suggested that poor bioavailability

contributed to this lack of effect. In another study, weaned pigs fed a diet supplemented with thymol (1% w/w) for 21 days did not show any significant changes in density and overall diversity of dominating bacteria, compared to controls. However, limited changes in select intestinal microbial populations were noted (103). Specifically, there was a reduction in *Actinobacillus minor* and an increase in *Citrobacter freundii* populations for animals fed the thymol-containing diet, compared to controls. *C. freundii* is known to be a common intestinal bacterium that may be an opportunistic pathogen in humans but its pathogenicity in pigs is not known. *A. minor* has low virulence for pigs. Overall, the impact of these differential changes in microbiota on GI tract function is not known and the basis for these shifts in bacterial groups was not determined. Although thyme constituents appear to have no major effects on gut microbes in these investigations, the effect of thyme phytochemicals on the gut microflora needs to be better characterized, particularly in light of the growing importance of the gut microbiome as a factor modifying risk for infectious and chronic diseases that impact human health (104,105).

In the stomach thyme constituents demonstrated beneficial effects. Compared to controls, carvacrol administered orally (10 ml/kg of 8.3-33.3 mM solutions) to rats prior to induction of acute gastric lesions inhibited damage to the gastric epithelium (106). This protection was evident even when 3 methods of inducing gastric damage (by ethanol, ibuprofen, and I/R) were used. Carvacrol significantly increased gastric mucus content, but did not alter gastric juice volume or total acidity. The carvacrol-associated benefits in part were related to opening of $K_{ATP}$ channels, activation of the NOS pathway, stimulation of mucus production, and alteration of endogenous prostaglandin production. Also, carvacrol given at oral doses of 25-100 mg/kg reduced the severity of chemically-

induced gastric lesions in rodents after 14 days of treatment, compared to controls (34). Similarly, α-terpineol was gastroprotective when administered orally (10 to 50 mg/kg) prior to dosing of either of two ulcer-inducing agents. This effect apparently was not mediated by inhibition of gastric acid secretion or changes in prostaglandin synthesis (107). A patent application for the use of a thyme extract for treatment of ulcerative colitis and Crohn's disease has been submitted in the European Union (publication number EP 1080727 A1). In a study involving young pigs, thymol was orally administered (50 mg/kg) after the morning meal (108). At 12 hr post-dosing, tissue samples from the oxyntic gland and pyloric mucosa of the stomach were collected. RNA was then isolated and changes in gene expression were determined by Affymetrix (Santa Clara, CA) microarray analysis. Numerous genes were differentially expressed in response to thymol. However, a central outcome was that acute thymol dosing activates genes associated with mitosis and mitosis regulation and induced genes involved in the digestive function of the stomach. Although the authors contend that these changes could potentially influence gastric maturation and function, additional chronic testing of multiple thymol doses, not only on gene expression but also on several gastric functions, is needed before any beneficial actions of thymol can be established.

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