FULL PROTOCOL TITLE

Peppermint Oil Pharmaco-Kinetics/Dynamics and Novel Biological Signatures in Children with Functional Abdominal Pain

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PRÉCIS

Study Title

Peppermint Oil (**PMO**) Pharmacokinetics (**PK**) and Pharmacodynamics (**PD**) and Novel Biological Signatures in Children with Functional Abdominal Pain (**FAP**)

Objectives

In children 7-12 years of age with FAP (n=30) determine:

Aim 1 - PK of PMO (menthol)

Sub-Aim: YP2A6 and UGT2B7 genotyping (exploratory)

Aim 2 – PD of PMO as assessed by gut:

a) Microbiome composition (16S RNA sequencing)

b) Transit rate/contractile activity (using the SmartPill®)

Design and Outcomes

An initial single-dose PK study of PMO in 30 children with FAP will be carried out using a 3-fold dosing range (180, 360, and 540 mg - 10 children per dose).

Following the single-dose PK study, a PD study will be carried out wherein the subjects will receive their assigned dose of PMO for 7 days administered thrice daily.

In a subset of subjects, on day 7 steady state menthol concentrations will be measured for the purpose of assessing steady state PK and PK/PD relationships.

Gut microbiome composition and a SmartPill® study (gut contractility and transit time) will be carried out before and after administration of the PMO during the PD study.

Interventions and Duration

Randomization to one of three doses of PMO administered orally and PK measured over 24 hours along with *CYP2A6* and *UGT2B7* genotyping.

Administration of PMO for 7 days using the dose given during the PK study administered thrice daily.

Measurement of gut microbiome composition and gut contractility and transit time.

Sample Size and Population

Children 7-12 years of age with FAP (n=30).

1. STUDY OBJECTIVES

1.1 Primary Objective

Define the PK of PMO in children with FAP ages 7-12 years.

1.2 Secondary Objectives

Carry out a pilot study to determine the PD of PMO based on changes in gut microbiome composition and gut contractility and transit time.

2. BACKGROUND AND RATIONALE

2.1 Background on Condition, Disease, or Other Primary Study Focus

1) Importance of the Problem – Worldwide, 10-15% of children and adults up through the geriatric age range experience chronic intermittent abdominal pain without a clear etiology, classifying them as functional abdominal pain gastrointestinal (**GI**) disorders (**FGID**).[9-15] FGIDs may be sub-classified with the most common of these in children being irritable bowel syndrome (IBS) and functional abdominal pain (**FAP**).[16,17] Despite the subcategories (e.g., FAP vs. IBS) it is well established that in children and adults, symptoms may overlap between subcategories of FGIDs and/or patients may transition from one subcategory to another over time.[18,19] FAP is associated with variable levels of symptoms and distress in adults and children directly related to decreased quality of life.[20,21] For example, we reported that children with FAP have abdominal pain a mean of 7 times/wk.; these pain episodes significantly affect activities 24% of the time.[16] A large body of data shows that up to 60% of children have similar pain as adults.[22-24] The public health impact of adult IBS care alone in the US is enormous with total costs reaching $30 billion yearly.[25]

2) Barriers to Progress – Despite the public health importance of FGIDs, there are few effective pharmacologic treatments. In part because of the paucity of effective treatments, at least 15-50% of patients with FGIDs employ complementary and alternative treatments such as botanicals like peppermint oil (**PMO**).[26-28] A previous PMO study in children with IBS in fact also recruited children with FAP (J. Kline, personal communication 5/2007). Unfortunately this study had issues with lack of clarity of: what defined significant improvement; how diaries were analyzed; and lacked presentation of data on symptoms.[29] Importantly, there are no studies of appropriate dosing of PMO in pediatric patients. Thus, future clinical trials need to be based on pharmaco-kinetic/dynamic (PKD) data and the relationship between drug concentration and biological effects (signatures) to increase the likelihood of treatment benefit while reducing side effects**.** In previous PMO studies in adults only half of patients respond to treatment.[30,31] Data are needed to clarify what factors affect treatment response; specifically whether this may be dose related.

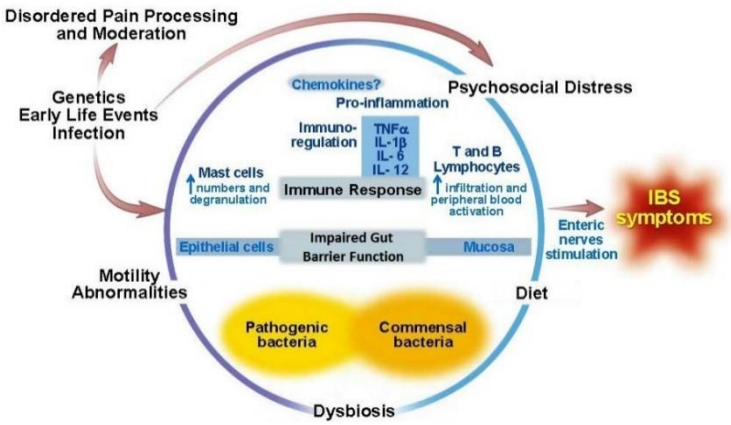
Different pathophysiologic changes are likely responsible for symptom generation in different patients with FAP (see below). Thus, PMO may or may not be effective depending on its effects on the GI tract and the specific GI abnormalities in an individual patient. Despite the long history of PMO use in treating GI disorders, few data are available in humans regarding its effects on gut function. Improving scientific knowledge of the pharmacokinetics (**PK)** and pharmacodynamics (**PD**) and their relationships to biological signatures (e.g., effects on gut microbiome composition, gut transit time, gut contractility) will allow treatment to be tailored to individual patients. For example, PMO would be anticipated to be effective in a patient with gut dysbiosis if it normalizes the gut microbiome composition but is unlikely to be effective in a patient in whom psychosocial distress is primarily operative.

3) Emerging View of the Etiologies of FAP: With the launch of the Human Microbiome Project, attention has increasingly focused on the **Microbiota-Brain-Gut Axis model** to illuminate the pathobiology of FGIDs such as

FAP.[32] Studies suggest interplay between abnormalities in the composition of the GI bacterial population (dysbiosis), motility disturbances, impaired gut barrier function (as reflected by increased GI permeability), altered mucosal and systemic immune responses, and psychosocial distress leading to the symptoms of FAP (Figure 1). In an individual patient, one or more factors may be primarily contributory.

- **Altered GI Bacterial Population in children with FGIDs** –

Figure 1. Modification of Rodríguez-Fandiño[1] et al.'s updated version of Drossman's[2-4] biopsychosocial model ofFAP takes into account recent investigations of pathobiological markers. These disorders can develop from centrally dominant factors (e.g., stress) or luminal factors (e.g., alterations in the GI microbial composition) triggering impaired gut barrier function and low grade gut inflammation. Gut barrier function also may be influenced by genetics.[5] Stress can alter GI motility thereby modifying the GI microbiota and symptom generation/exacerbation. All these factors contribute to the visceral hyperalgesia commonly found in IBS/FAP.[6-8]



Our recent work and that of others suggest that the composition of the GI microbiome (defined as bacteria, their genomes and interaction with the host) differs between children with FGIDs and healthy individuals.[33-37] We made the novel observation that the composition was related to severity of abdominal pain symptoms and stooling characteristics.[33,36] Gammaproteobacteria are increased in abundance in children with FGIDs and this class contains known pathogens.[33,38] Visceral hyperalgesia is a common finding in children and adults with FAP which may be related to GI microbiome composition.[39-42] Some commensal GI microbes are responsible for inflammation-related gut hyperalgesia whereas others can downregulate afferent pain from the gut.[43-45] Indeed, visceral hypersensitivity, can be induced in a mouse by transplanting fecal material from a patient with a FGID (but not from a healthy individual).[46]

A variety of bacteria directly up- and/or down-regulate cytokine and innate immune responses, some by binding to Toll-like receptors (Figure 1).[47-49] These pro- or anti-inflammatory changes can impair gut barrier function (increase GI permeability). Inflammation also can affect the function of gut smooth muscle and enteric nerves resulting in clinical symptoms of dysmotility (see below).[50,51] The ability of microbes to alter gut pain perception and function underlies the sometimes successful use of probiotics to treat FAP but the effects often are temporary.[52,53] Recent data show that altering the composition of gut bacteria can affect behavior and anxiety levels in mice; effects mediated via the vagus nerve.[54,55] These data fit with observations that adults with IBS-like symptoms have higher Beck depression scores than those without malabsorption and that administration of probiotics alters brain processing in humans based on magnetic resonance imaging.[56,57] These data underscore the potential contribution of the GI microbiome not only to abdominal pain but also the psychosocial distress often seen in FAP.

- **Dysmotility in FAP** - Some adult and pediatric patients with FAP have gastric, small bowel, and/or colonic dysmotility.[58,59] [60] Abdominal pain may be associated with colonic rapid transit whereas bloating may be associated with rapid or slow colonic transit.[42,60] Importantly, dysmotility is present only in some patients with FAP and, as noted, may affect different parts of the GI tract. Therefore, it is critical to determine if dysmotility exists and if so, what type, in a specific patient and thus, whether a treatment (e.g., PMO) is likely to correct (or worsen) the physiological abnormality. Such data are critical to designing clinical trials and ultimately, indications for treatments.

2.2 Study Rationale

Describe the scientific and medical data (e.g., results of observational studies and early clinical trials) that justifies the study, its design, and the intervention groups. Include any data from animal and human studies relevant to mechanism of action, effect size, and possible effects of the intervention on selected outcomes.

Name and describe the intervention regimen(s) and justify why the intervention(s) have been chosen. Describe and justify the route of administration, dosage regimen, intervention period, frequency and intensity, etc. Summarize the known and potential risks of the interventions.

Peppermint is a perennial flowering plant that grows throughout Europe and North America. Peppermint (Mentha × piperita) is a (usually) sterile hybrid mint, a cross between Water mint (*Mentha aquatica*) and Spearmint (*Mentha spicata*) that is believed to have arisen naturally. Mint plants have a long history of medicinal use, dating to ancient Egypt, Greece, and Rome where they were used as stomach soothers. PMO is on the GRAS list as determined by the FDA.

The main constituent and active ingredient of PMO is menthol.[61,62] Studies in both rats and humans demonstrate that PMO is rapidly absorbed and excreted primarily in bile as menthol glucuronide.[61,63] However, when taken in capsule form designed for delayed release approximately 70% reaches the colon.[63 30,31,64]

The use of peppermint oil is ubiquitous, with nearly 6 million pounds produced in the US in 2014 (www.statista.com), Mint (active ingredient menthol) has been used to treat abdominal ailments dating to ancient civilizations.[65] A number of meta-analyses have shown that PMO is efficacious in the treatment of functional gastrointestinal pain disorders such as irritable bowel syndrome.[30,31,66-70] One randomized, double blind trial has shown it to be effective in children with irritable bowel syndrome and functional abdominal pain (personal communication with the investigator revealed that both types of conditions were studied).[29] PMO also has been shown to be effective in treating functional dyspepsia in adults.[71-73] There are a number of potential mechanisms whereby PMO may be effect in treating functional abdominal pain disorders.

- **Antimicrobial/Antifungal Actions** - Multiple studies have shown that PMO (menthol) is one of the most potent antimicrobial/antifungal/antiviral botanicals.[74] Representative studies are reviewed here. PMO is active against obligate and facultative anaerobes.[75] It also is bactericidal to at least 20 common enteric pathogens including *Helicobacter pylori, Escherichia coli, Staphylococcus aureus*, Klebsiella sp., *Salmonella typhi*, *Shigella boydii*, and *Shigella flexneri*.[76-79]

- **Effects on GI Tract Motor Function** - Evidence suggests that PMO can act as a smooth muscle relaxant.[61] Hawthorn et al. showed in guinea pig ileal smooth muscle in vitro that both PMO and its constituent menthol were capable of blocking calcium channels.[80] In vitro studies using guinea pig colon and rabbit jejunum smooth muscle suggest PMO reverses acetylcholine induced contraction and antagonizes serotonin-induced contraction through calcium channel blockade.[81] In humans the GI response to PMO remains unclear, likely related to the wide range in doses used (>6-fold). For example, in two studies PMO accelerated gastric emptying in adults, in one study had no effect, and in another decreased the gastric motility index.[71,82-84] Conflicting data also exist regarding PMO's effects on small intestinal motility, again likely related to differences in dosing.[83,85,86]

- **Effects on GI Tract Visceral Sensation** - Visceral hyperalgesia contributes to the symptoms of functional gastrointestinal pain disorders in adults and children. Although PMO (via menthol) is a well-known topical analgesic, rodent studies show that PMO can decrease visceral pain when administered orally or intraperitoneally.[87-89] Recent studies suggest that the reduction in visceral pain is mediated through the TRPM8 and/or TRPA1 receptor of the transient receptor potential (TRP) cation channel superfamily located in the gut.[90-93]

- **Effects on Inflammation** - Studies demonstrate that PMO (menthol) possesses anti-inflammatory activity. Xylene induced inflammation in mice is prevented by oral administration of PMO.[87] In vitro, menthol suppresses the production of inflammatory mediators from human monocytes at clinically relevant concentrations.[94] Immune cells also contain transient receptor potential (TRP) cation channels and it is believed that the anti-inflammatory effects of PMO may be mediated, in part, via TRPM8 as activation downregulates chemically induced colitis in mouse models.[95,96] Patients with functional gastrointestinal pain disorders often show evidence of low grade gastrointestinal inflammation. Thus, PMO may alleviate this inflammation to some degree.

- **Effects on Behavior** - Studies in humans demonstrated that inhalation of peppermint aroma improves attention but whether it improves mood remains unclear.[97-99] Studies in rodents, which suggest menthol has dose dependent anxiolytic effects, implicate involvement of dopamine pathways.[100-102] Given the potential role of psychosocial distress in the expression of functional gastrointestinal pain disorders symptoms, this may be another potential mechanism of action.

Despite its widespread use and what is known about the clinical pharmacology of menthol, there have been no studies evaluating its PKD in pediatric patients. Thus, there is no scientific basis upon which to derive effective dosing regimens for PMO in pediatric patients which will generate local and/or systemic exposure of menthol previously associated with putative beneficial effects in adults with FAP or IBS. Only an old, small (n=6) study in adults examined the pharmacokinetics alone of the enteric coated form of PMO and only urine menthol was measured.[63] Other data do show, however, the importance of CYP2A6 and UGT2B7 expression in menthol clearance[103,104] and thus, the potential for pharmacogenomics to alter the dose vs. concentration vs. effect relationship for PMO.

Although clinical (albeit imperfect) data support the use of PMO in IBS, only one study has evaluated whether PMO is effective for treating FAP.[105] Asgarshirazi et al. randomized children with FAP to treatment with PMO, a synbiotic, or placebo.[105] Per protocol results (n=88) showed those in the PMO group to have the greatest reduction in abdominal pain symptoms compared with the other groups. However, interpretation of the study is limited by the same issues present in PMO IBS studies (see above).[105] That said, if PMO works via an antispasmodic effect, it is conceivable it would be effective in FAP as some data (albeit imperfect) suggest potential efficacy for other antispasmodics in FAP in children and adults.[106-108] Thus, as in the case of IBS, well designed, rigorous clinical trials are needed to determine the efficacy of PMO in FAP based on PKD data and the response to biological signatures of the disorder.

We propose to carry out our studies in - children – a population who frequently are overlooked in determining therapeutic dosing. Our results are likely to move science forward significantly because we will be able to dose PMO based on PKD rather than extrapolating from adult dosing as is done now. For the PK study we will administer the PMO in the doses (180, 360, 540 mg) used for many years to treat patients, including those with irritable bowel syndrome and FAP. We propose to study a total of 30 subjects assigned to three different (3-fold) doses (180, 360, 540 mg; 10 children per dose). This “three dose” approach in light of the anticipated range of body weights in the study cohort would produce a dynamic, > 3-fold range in systemic exposure to PMO (e.g., AUC of menthol). For the PD study the subjects will continue on the same dose (180 once, twice or three times daily) for a total of 7 days.

Potential Risks

Menthol is listed as generally regarded as safe by the US Food and Drug Administration (**FDA**). The European Medicines Agency (**EMA**) recently released their assessment and recommendations regarding PMO: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/07/WC500211079.pdf

Few adverse events have been reported in PMO trials.[30,67] In those studies reporting adverse events, no differences were noted between PMO and placebo groups except in the study by Nash et al. in which heartburn was more common in the PMO vs placebo group; however the efficacy of the enteric coating used in

this study of 30 years ago is unknown. In theory, enteric coated formulations of PMO allow for release of PMO distal to the stomach – thereby minimizing the risk of gastroesophageal reflux. [30,67]

The safety of PMO also has been reviewed by the Cosmetic Ingredient Review Expert Panel.[109] In rat studies cystlike spaces have been identified in the cerebellum in some rat studies but not others at doses of ≥ 40 $mg \cdot kg^{-1} \cdot d^{-1}$.[109] Subsequently it was shown that the cystlike spaces were artifacts of poor tissue fixation.[110]

Pulegone, and its metabolite, menthofuran which are present in PMO have been considered to be potentially toxic in high doses. In a rat study, ≥ 80 $mg \cdot kg^{-1} \cdot d^{-1}$ of pulegone was associated with vacuolization of hepatocytes.[109] In a more recent series of studies from the National Toxicology Program, liver necrosis and cytoplasmic vacuolization were only seen in rats given ≥150 $mg \cdot kg^{-1} \cdot d^{-1}$ of pulegone for 2 weeks.[111] In a 3-month administration study in rats, bile duct hyperplasia, hepatocyte focal necrosis and hypertrophy and renal glomerulopathy were only seen at doses ≥75 $mg \cdot kg^{-1} \cdot d^{-1}$ of pulegone.[111] In contrast, mice were more resistant to the effects than were rats with doses of 300 $mg \cdot kg^{-1} \cdot d^{-1}$ of pulegone required to see hepatic injury at 2 weeks.[111] The EMA statement points out that "prevailing opinion is that no certain cases of liver toxicity in humans are associated with the use of peppermint oil or mint oil." In a case report of suspected to have ingested PMO in a suicide attempt, although comatose on arrival, she recovered without evidence of hepatic or renal injury.[112]

In a 2-year study in rats, pulegone was associated with an increased risk of bladder cancer at a 150 $mg \cdot kg^{-1} \cdot d^{-1}$ dose. In contrast, in a 2-year study of mice, an increased risk for hepatoblastoma was found at the 75 $mg \cdot kg^{-1} \cdot d^{-1}$ dose but not at the 150 $mg \cdot kg^{-1} \cdot d^{-1}$ dose in males with no increased risk in females.[111] The EMA statement posits that "non-relevance of rodent neoplasms to human carcinogenesis seems probable" in part, because of the long term sustained exposure required and doses which are not relevant in human situations.

The amount of pulegone in PMO can be reduced depending on how the peppermint is grown, when it is harvested, and how it is processed.[113] PMO normally contains a maximum of 0.1% pulegone and its metabolite menthofuran according to the European Pharmacopoeia (and more commonly 0.03-0.07%). As likely would be used to treat FGIDs in adults, the EMA statement proposes for adults a maximum intake of 1.5 $mg \cdot kg^{-1} \cdot d^{-1}$ of pulegone and menthofuran combined up to a maximum of 75 mg/day. The maximum usual dose of PMO used to treat FGIDs such as IBS is 540 mg/d which would deliver 54 mg of pulegone and menthofuran combined. Pharmacokinetic data are needed from studies in children to understand how these recommendations might apply to the pediatric population.

3. STUDY DESIGN

- **Primary Outcome**

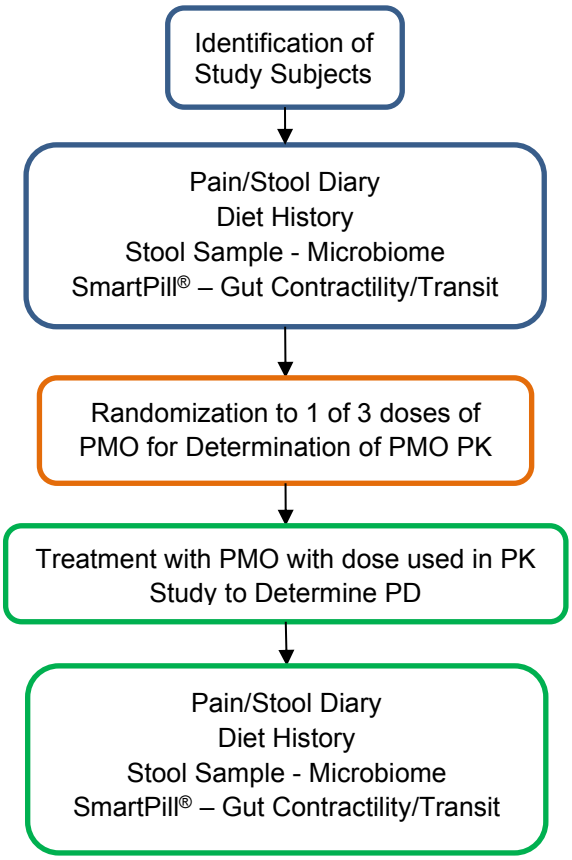
  PK of PMO (menthol) determined in children with FAP (n=30)

- **Secondary Outcomes –** changes in the following as a result of PMO administration
  - Gut microbiome composition measured by 16S DNA sequencing
  - Gut contractility and transit time measured by SmartPill®

Children with FAP will be recruited through the Texas Children's Hospital (**TCH**) Pediatric Gastroenterology, Hepatology, and Nutrition Service and Texas Children's Hospital Pediatrics (**TCP**) which is the nation's largest group of general pediatricians. Thus, the profile of the local population will be reflected in the study sample. IRB approval will be obtained to allow us to recruit children as described in the following sections. Subjects will be identified by reviewing billing records for ICD-10 codes. All medical charts will be reviewed by the principal investigator. Families will be invited to participate in a letter sent out by their nurse practitioner, pediatrician, or pediatric gastroenterologist. All health care providers are in the same academic practice so there is full participation in referring children to the study. Once the child has been identified and the letter sent out, the research coordinators will call the family for additional screening using a previously validated questionnaire updated by the Pediatric Rome IV Working Group.[114]

The overall design is outlined in the Figure 2 below.

Figure 2. Study Design



Study Detail (see also Table 1 below)

Prior to Study Visit 1 (Study Day -120 to -1)

Prior to Study Visit 1, subjects will complete a phone screen/parent survey. Subjects who meet the phone screening criteria will be sent the informed consent and assent form to review and sign. Signed consent forms will be returned by mail or in person.

Once the signed consent forms are returned, subjects will be given instructions on how to complete a 2-week pain/stooling diary, a 3-day Diet History, and will receive a kit to collect a stool sample for microbiome/metabolomics analyses at home. We will send a courier to pick up the stool sample.

Subjects will be receive a call to schedule Study Visit 1, and to complete the SmartPill® Checklist. They will receive information on medications to stop before the SmartPill® Test, and information on the Smart Meal or an alternative meal if the subject is unable to eat the Smart Meal.

One day prior to Study Visit 1, subjects will be instructed to fast 8 hours before the SmartPill® Test.

Study Visit 1

Subjects will visit the Children's Nutrition Research Center (**CNRC**) for Study Visit 1 (see Table 1. Study Timeline, below). Informed consent and assent will be obtained and they will complete questionnaires (demographics, medical history, modified pediatric Rome III questionnaire (or Rome IV if available and validated), the BASC-3,the CSI, FDI and the PCS).
Subjects will swallow a SmartPill® during the visit immediately after ingesting a standardized meal (SmartBar®) to measure gut contractility and transit time. If the subject is unable to swallow the SmartPill, they still may be allowed to continue in the study as the discretion of the PI. The SmartPill® capsule has been used previously in this age range in studying children with abdominal pain in addition to other disorders. We have previously used a similar sized pill (PillCam®) in similarly aged children with functional gastrointestinal pain disorders without issue in our previous study (RC2 NR011959). Subjects will wear a recorder on a lanyard or belt clip for 5 days to record SmartPill® data. They will keep a 5-day Event Dairy while wearing the recorder. The recorder and any other study materials will be returned by courier.

Study Visit 1 to Study Visit 2 (60 days allowable)

Prior to Study Visit 2, subjects will receive a call to schedule the overnight study. The subjects will receive information on medications to stop 5-10 days before the 2nd SmartPill®Test, and SmartBar® Meal or alternative meal.

Subjects will be asked to discontinue all PMO products at least 72 hours before the study visit. Subjects also will be instructed to fast 8 hours before Study Visit 2.

Subjects who do not complete the Baseline procedures within 8 weeks of Study Visit 2 will be required to repeat them prior to Study Visit 2.

Study Visit 2

At Study Visit 2, subjects they will come to the CNRC. A general physical examination will be performed by either the PI or co-investigator. Height, weight, and vital signs (pulse and respiratory rates, and blood pressure) will be obtained. After application of a topical anesthetic to the site chosen for study-related blood sampling, a cannula will be inserted to obtain repeated blood samples. A maximum of three attempts will be made to place the cannula. A maximum of two attempts will be made to replace the cannula should it become dislodged. After obtaining a 2 mL baseline blood sample, children will be randomized to receive either 180 (83mg menthol equivalent), 360, or 540 mg of PMO.

A 0 time point (pre-dose) blood sample (2 mL) will be obtained to measure total menthol concentration and for isolation of genomic DNA that will be used to determine *CYP2A6* and *UGT2B7* genotyping. The PMO used is a commercially available, proprietary, nonprescription enteric coated product available over the counter (Peptogest®, Nature's Way Products, LLC, Lehi, UT) and deemed GRAS (generally regarded as safe) by the FDA.

At approximately 0900, subjects will receive their assigned single oral dose of PMO. Ingestion of the dose will be followed by ingestion of up to 120 mL of water. Subjects who completed the 1st SmartPill® Test, will be given the 2nd SmartPill® Test after ingesting the assigned PMO dose. Repeated venous blood samples (1.5 mL each) will be obtained at the following time points: 1, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 18 and 24 hours post dosing. Based upon our previous pilot PK data obtained in pediatric patients, the aforementioned sampling scheme will enable us to capture both the absorption phase of menthol and also, adequately characterize the apparent elimination of the drug from plasma (e.g., > 4 times average menthol elimination half-life of 3.0 hours).[115] It is also consistent with the approach outlined in the current FDA guidance (General Clinical Pharmacology Considerations for Pediatric Studies for Drugs and Biological Products: Guidance for Industry, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research; December 2014). Namely, for drugs that do not have extensive pharmacokinetic data in pediatric patients, to utilize "rich" sampling schemes sufficient to accurately describe the pharmacokinetics of an active pharmaceutical ingredient after a single dose so as to facilitate understanding of the variability in the dose vs.

systemic exposure relationship. As described in this FDA Guidance, a non-compartmental approach will be used to derive important pharmacokinetic parameter estimates (e.g., AUC, apparent elimination rate constant, apparent oral plasma clearance) for menthol.

Six hours after SmartPill® Test administration, participants will be given a standardized meal and will eat ad libitum thereafter. The subjects will be restricted from any strenuous physical exercise.

After completion of the final (24-hour) blood sample, vital signs will be reassessed, the venous cannula removed, and the insertion site evaluated for redness, swelling and/or bruising. At this point, all subjects will continue taking the PMO at their randomized dose for 7 days. The subjects will be given a 14-day supply of the assigned randomized dose of PMO with instructions on use. Although administration of the PMO ideally is designated to be for 7 days, the additional days (up to 14 days total) are provided recognizing that not all subjects may be able to return for Study Visit 3 at exactly 7 days. The SmartPill® test, stool collection, diet history, pain/stool diary, (i.e., baseline studies) will be repeated while on the PMO (see below - *Study Visit 2 to Study Visit* 3 and Table 1). Subjects also will receive instructions on filling out the Adverse Event form.

Arrangements will be made to set up for Study Visit 3. It is recognized that not all subjects may be willing to participate in Study Visit 3. Subjects then will be sent home.

Study Visit 2 to Study Visit 3

Subjects will take the PMO capsules at the same dose they were initially randomized to for the Day 0 PK study evaluation (i.e., 180 mg once daily, 180 mg twice daily, 180 mg thrice daily). Ideally,

Subjects will again keep the pain/stooling diary and also be instructed to report any potential adverse effects or healthcare utilization using a designated 24-hour study phone line. Subjects will keep a daily adverse events diary during the time they take the PMO capsules. They will also keep a daily record of any other medications they take during the study.

On the day following the pharmacokinetic study the research coordinator will contact the subjects to inquire about any adverse events post the 24-hour overnight study and while they are receiving the PMO capsules. If any subject withdraws from the study or is withdrawn by the investigator, a study contact will be done as planned post-withdrawal.

Subjects will again keep the SmartPill® Event Diary 2 while wearing the recorder.

Subjects will again collect a stool sample and complete a 3-Day Diet History. A courier will be sent to up the recorder and the stool sample.

All participants will be encouraged to complete all study measures while taking PMO through day 7 and provide a blood sample during study visit 3 after 6-14 days of PMO ingestion (at the randomized dosage). At the end of Study Visit 2, staff will document whether the parent and participant are willing to have blood collected at study visit 3. Every effort will be made to collect blood samples from all participants that are willing for the Study Visit 3 pharmacokinetic data collection. Any participant who chooses to opt out of this blood collection will be documented as participant choice to opt-out of blood collection.

Between 6 and 14 days after starting the PMO daily dosing, at Study Visit 3 in those subjects who are willing, the subjects on the 180 mg twice daily dose will take their doses 12 hours apart and those subjects on the 180 mg thrice daily dose will take their doses 8 hours apart. Subjects will have blood drawn (1.5 mL) immediately prior to their dose and then again either 3 or 4 hours after their dose to assess steady state pharmacokinetics of menthol (e.g., to assess whether there is unexpected accumulation in the dose vs. plasma concentration profile) and to enable a linked PK/PD analysis of menthol at steady state conditions. This approach is consistent with that recommended in the current FDA draft guidance describing clinical pharmacology considerations for pediatric studies (see aforementioned citation to December 2014 FDA draft guidance document).

A phone call will be made to the subjects to inquire about any potential adverse effects following completion of the PMO dosing.

The PK data will be analyzed by Dr. Kearns at Arkansas Children's Hospital. The PD data will be analyzed by Dr. Shulman at Baylor College of Medicine and Texas Children's Hospital.

Table 1. Detailed Study Timeline

Assessments	Allowable Timeframe Between Visits: -120 to -1 days Pre-Visit 1	Visit 1	60 days Post-Visit 1/Pre-Visit 2	Visit 2	7-14 days Post-Visit 2/Pre-Visit 3	Visit 3
Phone Screen/Parent Survey	X					
Informed Consent Signed by mail	X					
Baseline 2-wk Pain/stool diary	X					
Baseline Stool Collection	X					
Baseline 3-d Diet History	X					
Smart Pill Checklist	X					
Schedule Visit-1	X					
Phone contact - Smart Pill Medication Washout / Meal vs Alternative Meal / 8-hr Fast	X					
SmartPill Medication Washout	X					
8-hr Fast	X					
Informed Consent		X				
Inclusion/Exclusion Criteria		X				
Enrollment		X				
Demographics		X				
ROME III questionnaires		X				
BASC-3		X				
CSI		X				
PCS		X				
FDI		X				
Medical History		X				
Current Medications		X				
Baseline Smart Pill Administration		X				
SmartPill Event Diary 1			X			
Schedule Visit 2			X			
SmartPill Medication Washout			X			
72-hr off PMO Products			X			
8-hr Fast			X			
Physical Examination				X		
Vital Signs				X		
Randomization				X		
PMO Administration				X		
PMO Pharmacokinetics				X		

CYP2A6 and UGT2B7 genotyping				X		
Post PMO SmartPill Administration				X		
Adverse Event Call					X	
PMO Stool Collection					X	
PMO 3-Day Diet History					X	
PMO Administration					X	
SmartPill Event Diary 2					X	
Adverse Event Diary					X	
8-hr Fast					X	
Vital Signs					X	
Current Medications					X	
2-wk Pain/stool diary					X	
Optional Menthol Blood Draw with 24-h Followup Call						X

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

Subjects must meet all of the following inclusion criteria to participate in this study.

- Children ages 7-12 years who are able to assent to the procedures
- Able to complete the diaries which have been validated for use in this age range
- The history and medical evaluation reveal no organic reason for the abdominal pain
- The child has abdominal pain that meets the definition of FAP according to pediatric Rome III criteria (Rome IV if available and validated by the time of the start of the study)
- Ability to understand study procedures and to comply with them for the entire length of the study

4.2 Exclusion Criteria

Subjects who meet any of the following exclusion criteria at Screening/Baseline will be excluded from study participation. Children with:

- Past bowel surgery
- Documented GI disorders (e.g., Crohn's disease)
- A serious chronic medical condition (e.g., diabetes)
- A weight and/or height < 2 SD for age
- Chronic conditions with GI symptoms (e.g., cystic fibrosis)
- Autism spectrum disorder, significant developmental delay, psychosis, depression, or a history of bipolar disorder
- Antibiotic/probiotic treatment within 2 mo.
- Allergy/sensitivity to PMO or its ingredients
- Inability to swallow the PMO capsule or the SmartPill®
- Inability to speak English - testing materials are available only in this language
- Unable to discontinue laxative, prokinetic, or neuromodulator from 3 wk prior to Visit 1 through the end of the study

4.3 Study Enrollment Procedures

Children with FAP will be recruited through the Texas Children's Hospital (**TCH**) Pediatric Gastroenterology, Hepatology, and Nutrition Service and Texas Children's Hospital Pediatric Associates (**TCPA**). Subjects will be identified by reviewing billing records for ICD-10 codes. Families will be invited to participate in a letter sent out by their nurse practitioner, pediatrician, or pediatric gastroenterologist. Once the child has been identified and the letter sent out, the research coordinators will call the family for additional screening using which will include a previously validated questionnaire updated by the Pediatric Rome Working Group. Potentially eligible children who receive their routine care at TCH or TCPA will be sent a letter briefly outlining the study. A follow-up call will be made by the study staff following the letter. If the child and parent/legal guardian are interested in participating and the child qualifies after screening, the research coordinator will invite the family to come to the Children's Nutrition Research Center for a visit. In addition, we will advertise the study in the Texas Children's Hospital Newsletter, Children's Nutrition Center Newsletter, and on the Baylor College of Medicine Research website.

A **Screening Log** will be kept:

- All subjects screened will be recorded: subject initials, date of birth, date of screen, results of screen (qualify or do not qualify and reason for disqualification)
- A subject will be disqualified if they do not meet the inclusion/exclusion criteria
- Subjects declining participation will be considered disqualified

Informed consent procedures:

All aspects of the protocol will be explained in depth to the subjects and parent/guardian. Subjects will receive a written copy of the consent form. Assent will be obtained from the child and consent from the parent/guardian. The research coordinator will explain the protocol to the parent/guardian and then explain the protocol to the child. The procedures will be explained in terms that a 7-year-old child can understand. Adequate time will be provided for questions from both the parent/guardian and the child. Questions will be answered in terms that a 7-year-old child can understand.

Randomization procedure for assigning a participant to an intervention group:

- Prior to randomization for the PK study, all inclusion and exclusion criteria will be verified to ensure the potential participant qualifies for study enrollment
- A dosing randomization scheme from www.randomization.com and stratified by gender and age will be used
- The subject will be assigned a participant ID number and a PMO dose as determined by the randomization scheme
- Participant ID number will consist of the three letters "PMO" followed by a 3-digit number that will be assigned sequentially as participants are enrolled. For example: The first participant enrolled will be assigned PMO-001 and enrollees will be assigned numbers sequentially thereafter.
- The participant will retain their unique participant ID number throughout the study
- The study coordinator will complete the "PMO Randomization Assignment List Form denoting whether the subject is male or female
- The information on the Randomization Assignment List Forms will include: participant initials, ID number, birthdate, date dose assigned, and the assigned dose
- The completed PMO Assignment List Forms will be kept in the Essential Documents/Regulatory Binder in the study coordinator's locked file cabinet
- Once a participant is randomized, the ID number will not be reassigned or reissued to a new participant. If a subject drops out or withdraws before the randomized PMO dose is administered, the same dose will be given to the next sex-appropriate participant.

STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

Aims 1 and 2

PMO capsules (Peptogest™) will be obtained from the manufacturer using a single lot number. The manufacturer will provide the investigators with an assessment of potency and active ingredient (PMO/menthol) content in the product. An IND will be approved by the FDA prior to starting the study.

Aim 1 – PK Study

Study Visit 2 will be carried out in the Metabolic Unit of the CNRC. After obtaining a 2 mL baseline blood sample, at approximately 0900, subjects will receive a single oral dose of delayed release PMO as a

commercially available, proprietary, nonprescription enteric coated product (Peptogest™, Nature's Way Products, LLC, Lehi, UT). Children will be randomized to receive one dose of either 180 (83 mg menthol equivalent), 360, or 540 mg of PMO. Ingestion of the dose will be followed by ingestion of 120 mL of water.

Immediately prior to and after PMO administration, repeated blood samples (1.5mL each) will be obtained directly into green top glass tubes contain sodium heparin (Vacutainer®, Becton Dickinson, East Rutherford, NJ) at the following time points: 0 (pre-dose),1, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 18 and 24 hours. Based upon our pilot PK data obtained in pediatric patients,[115] the aforementioned sampling scheme will enable us to capture both the absorption phase of menthol and also, adequately characterize the apparent elimination of the drug from plasma (e.g., > 4 times average menthol elimination half-life of 3.0 hours).

Two hours after PMO administration, participants will be given a standardized meal and will eat ad libitum thereafter. The study participant will be restricted from any strenuous physical exercise.

After completion of the final (24-hour) blood sample, vital signs will be reassessed, the venous cannula removed, and the insertion site will be evaluated for redness, swelling and/or bruising. Parents will receive a followup call from the research coordinator the following day to access/evaluate if any adverse effects were experienced.

At Study Visit 3 subjects will be requested to have 1.5 mL of blood drawn prior to and either 3 or 4 hours after a dose of PMO at the end of the study (final PMO doses). These blood samples will be obtained by venipuncture. This will be the last PK study blood draw taken during day 6-14 of PMO dosing.

Aim 2 – PD Study

Following completion of the PK study described in Aim 1, children will be sent home to continue on their assigned dose of PMO for 7 days. Children who received 180 mg of PMO will take that dose once daily 30 minutes before breakfast. Those who received 360 mg will take 180 mg twice daily 30 minutes before breakfast and dinner and those who received 540 mg will take 180 mg thrice daily (30 minutes prior to breakfast, lunch, and dinner).

5.2 Handling of Study Interventions

The PMO capsules will be sent to the investigator in sealed bottles by the manufacturer and stored at room temperature (between 68 - 77°F) as recommended by the manufacturer. The PMO capsules will be placed in a locked cabinet with access available only to the investigator and study staff. The PMO capsules will be dispensed by the study coordinator. For the PK study the capsules will be placed in a small plastic bag labeled with the randomization number, the subject ID number, the subject initials, date dispensed, and the number of capsules assigned. For the PD study the capsules for the 14 days will be placed in a box labeled with the randomization number, the subject ID number, the subject initials, date dispensed, the number of capsules assigned, and directions for administration.

Subject Compliance

- Subjects will be advised how to store and administer their daily capsules
- Subjects will be asked to complete the **PMO-Daily Use Form** by recording the day and time the capsules were taken
- If the capsules were not taken, the subject will record the reason
- The subjects will be asked to return the bottle with any unused capsules at the end of the study

Study Product Accountability

Inventory will be recorded as will capsules dispensed. Data will be kept as to who dispensing the capsules, the number of capsules dispensed, to whom they are dispensed, date, and time. The study coordinator will complete the **"PMO Investigator Study Product Accountability Form"** and **"PMO Patient Study Product Accountability Form**" and the "**PMO Capsule Inventory Log** for each subject.

Study Documentation Product Returned/Destruction

The study coordinator will complete the "**Study Product Destroyed or Return Product Form** "at the end of the study. The number of remaining capsules returned by the study subjects will be documented. All unused and subject returned study products will be destroyed at the study site.

5.3 Concomitant Interventions

5.3.1 Allowed Interventions

- Over the counter medications for symptomatic relief of pain (e.g., acetaminophen)
- Medications for nasal congestion
- Medications for FAP that normally are taken by the subject but that do not completely relieve symptoms
- Drugs prescribed for an adverse event

5.3.2 Required Interventions

None

5.3.3 Prohibited Interventions

- Any food or drink products with peppermint oil
- Antibiotics
- Probiotics
- Over the counter drug not approved by the PI

Medications prohibited related to the use of the SmartPill:

- Dexilant - dexlansoprazole
- Nexium - esomeprazole
- Prevacid - lansoprazole
- Prilosec - omeprazole
- Protonix - pantoprazole
- AcipHex - rabeprazole
- Zegerid – omeprazole/sodium bicarbonate
- Tagamet - cimetidine
- Pepcid - famotidine
- Axid - nizatidine
- Zantac - ranitidine
- Alka-Seltzer
- Alka-2, Surpass Gum, Titralac, TUMS
- Milk of Magnesia
- AlternaGEL, Amphojel
- Bisacodyl
- Gaviscon, Gelusil, Maalox, Mylanta, Rolaids
- Imodium
- Lactulose
- Lomotil
- Pepto-Bismol
- Polyethylene Glycol 3350
- Senna
- Benzamide
- Bethanechol
- Cisapride

- Domperidone
- IBgard
- Metoclopramide
- Mirtazapine
- Metamucil
- Narcotics (codeine, hydrocodone, etc.)
- Ondansetron
- Pyridostigmine

5.4 Adherence Assessment

- Subjects will be asked to complete a PMO capsule daily use compliance diary
- Subjects will be asked to return all remaining capsules for study staff to check for adherence
- All subjects will be included in the analysis
- The data will be analyzed using the total dose taken over the week as a covariate
- Sensitivity analysis can be considered to assess the impact of compliance on outcome

6. STUDY PROCEDURES

6.1 Schedule of Evaluation

See Table 1.

6.2 Description of Evaluations

Forms for each of the following have been prepared:

- Informed consent
- Enrollment
- Demographics
- Medical history
- Current medications
- 3-Day diet history
- Pain/stool diary
- General physical examination
- Vital Signs Height /Weight
- 24 Hour Blood Sample Collection
- Adverse events
- Vital signs
- PMO administration
- Phone call
- Plasma menthol pre and post dosing

6.2.1 Screening Evaluation

Consenting Procedure

The study will include a single informed consent form that describes the study procedures. The research coordinator will conduct the consent process and how it will implemented. Consent will be obtained from the parent or guardian and assent from the subject. The risks, benefits, alternatives, purpose, and study procedures will be reviewed in a developmentally appropriate language so that when feasible the child may also understand. If the child does not either assent or understand the study or does not agree to participate in the research study, we will not enroll the child even if parent or legal guardian informed consent is obtained.

Once the information is presented, an immediate response will not be required or requested to enter the study. The family will have the right to review the consent form in advance and the parent or the child will not be pressured into participating the study. All alternatives to participating will be presented during the inform consent process.

The volunteer signed consent forms will be kept within locked cabinets only accessible by the PI and his research coordinator. The cabinets and the room with all study records will be locked at all times the PI or the study

Screening

- TCH will generate a list of potential subjects using ICD-10 codes for abdominal pain
- The primary care physician's declination of permission to review the potential subject's chart must be received within 2 weeks of notification being submitted by the PI's research team
- If the request is not disapproved by the patient's primary care physician, the research coordinator will contact TCH to request a TCP clinic "Patient List" for chart review - the request will be submitted within one month of permission being granted by the primary care physician
- The research coordinator will review the potential subject's record to determine whether they qualify based on the study inclusion/exclusion criteria within one month of receiving the records
- If the subject potentially qualifies, the study coordinator will create letters inviting the subjects to participate. The PI or Co-I will sign the letters.

- The study coordinator will send these letters within 3 weeks to the clinics for a signature from the child's PCP or GI doctor
- After receiving the letters from the clinic, the study coordinator will mail the signed letters to the qualified subjects within 3 weeks
- One to two weeks after mailing the letters the study coordinator will followup with a call to the families and inquire regarding their potential interest and provide additional information about the study
- If necessary, the study coordinator will place a followup call once a week for up to three total contacts (or more if requested by the family once contact is made).
- If the family is interested, the "**PMO Phone Screening Questionnaire"** will be completed
- If the child qualifies after the phone screening, the study coordinator will complete the **"PMO Medical Chart Review Form**
- The study coordinator will submit the medical chart to the PI (or Co-I) for review and approval
- After the PI or Co-I has reviewed and approved the subject to participate in the study, the study coordinator will contact the family to let them know their child qualifies for the study within 2 weeks
- The study coordinator will schedule the PK study visit within the next 3 months (i.e., at the convenience of the family in relationship to availability within the CNRC Metabolic Unit. The date/time confirmed will be sent in a **"PMO Pre Study Visit Letter"** to the family with a copy of the informed consent form for their review.

6.2.2 Enrollment, Baseline, and/or Randomization

Enrollment

The subject will be considered enrolled once they have passed screening and the consent/assent form has been signed. Enrollment will be documented on the Enrollment Log.

Baseline Assessments

- Demographics
- Medical history
- Physical examination
- Vital Signs
- Height and weight
- Concomitant medications
- 3-Day diet history
- Pain/stooling diary
- Gut microbiome composition
- Gut contractility/transit time via SmartPill
- Rome III Questionnaire
- BASC-3
- CSI
- FDI
- PCS

Randomization

After completing the baseline assessment the subjects will undergo the PK study at which time they will be randomized to one of three doses of PMO: 180, 360, or 540 mg.

6.2.3 Blinding

This is an open label study. However, the investigators responsible for analyzing the PK data (Drs. Kearns and Garg) will be blinded to the dose assignment of the subject. Similarly, the investigator responsible for analyzing the gut microbiome and contractility/transit time data will be blinded to the dose assignment of the subject as well as the timing of the measurements (i.e., pre- versus post-treatment with PMO). Data only will be identified by study code so that dosing data and time of assessments will not be known when samples/data are analyzed.

Only the PI and DSMB will be authorized to break the blind prior to the end of the study. The code will be broken once the study analyses are complete. The blind may be broken should data be required to investigate an adverse event. The blind will be broken by opening the randomization form for the particular patient in question.

6.2.4 Followup Visits

There are no followup visits as such. Subjects who do not participate in the steady state studies (Study Visit 3) will have their last visit to the CNRC at the time of the PK study. Those subjects willing to participate in the steady state studies at Study Visit 3 will return to the CNRC for the study. Activities to be carried out after Study Visit 2 are shown in Table 1. These will include a followup call to assess for AE and pickup of the recorder and stool sample by courier.

6.2.5 Completion/Final Evaluation

See above (6.2.4)

Potential reasons for early termination of subject from the study

- If any of the exclusion criteria develop in the course of the study
- If a serious adverse event occurs during the study
- If the subject withdraws from the study or is withdrawn by the investigator

7. SAFETY ASSESSMENTS

Given the safety profile of PMO, it is not anticipated that (serious) adverse events directly related to the PMO will occur. Theoretically the following are possible following PMO ingestion:

- Allergic reactions – will be managed by discontinuation of the product if the reaction is deemed to be related to the product
- Anal/perianal burning or discomfort – will be managed by making sure that the capsule is not chewed, administering the PMO closer to meal time, use of skin barriers such as petroleum jelly, or if severe enough/unresponsive to the previous manipulations, discontinuation of the product
- Heartburn - will be managed depending on severity, by making sure that the capsule is not chewed, advising that the subject should not lay down for an hour after PMO administration, administering the PMO closer to meal time, the addition of antacids, or if severe enough/unresponsive to the previous manipulations, discontinuation of the product
- Nausea - will be managed depending on severity, by making sure that the capsule is not chewed,

advising that the subject should not lay down for an hour after PMO administration, administering the PMO closer to meal time, the addition of antacids, or if severe enough/unresponsive to the previous manipulations, discontinuation of the product

7.1 Specification of Safety Parameters

No laboratory testing is involved. Adverse events will be based upon subject report.

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

Subjects will have the opportunity to report adverse events and intercurrent illness at any time during the study. In addition, there is a 24-hour telephone call line established for subjects to leave messages for the principal investigator and research coordinator. The consent form will include the paging operator number for the pediatric gastroenterology physicians (as well as the principal investigator). The paging service is available 24-hours a day, 7-days a week. Adverse Events will be recorded and reported by the research coordinator on the Baylor College of Medicine IRB Forms and forwarded to the FDA and Data Safety Monitoring Board and IRB as required by regulations.

Menthol is listed as generally regarded as safe by the US Food and Drug Administration (**FDA**). The European Medicines Agency (**EMA**) recently released their assessment and recommendations regarding PMO (see below).[116]

Few adverse events have been reported in PMO trials. In those studies reporting adverse events (see Table below), no differences were noted between PMO and placebo groups except in the study by Nash et al. in which heartburn was more common in the PMO vs placebo group; however the efficacy of the enteric coating used in this study of 30 years ago is unknown. In theory, enteric coated formulations of PMO allow for release of PMO distal to the stomach – thereby minimizing the risk of gastroesophageal reflux.

The safety of PMO also has been reviewed by the Cosmetic Ingredient Review Expert Panel.[109] In rat studies cystlike spaces have been identified in the cerebellum in some rat studies but not others at doses of ≥ 40 $mg \cdot kg^{-1} \cdot d^{-1}$.[109] Subsequently it was shown that the cystlike spaces were artifacts of poor tissue fixation.[110]

Pulegone, and its metabolite, menthofuran which are present in PMO have been considered to be potentially toxic in high doses. In a rat study, ≥ 80 $mg \cdot kg^{-1} \cdot d^{-1}$ of pulegone was associated with vacuolization of hepatocytes.[109] In a more recent series of studies from the National Toxicology Program, liver necrosis and cytoplasmic vacuolization were only seen in rats given ≥150 $mg \cdot kg^{-1} \cdot d^{-1}$ of pulegone for 2 weeks.[111] In a 3-month administration study in rats, bile duct hyperplasia, hepatocyte focal necrosis and hypertrophy and renal glomerulopathy were only seen at doses ≥75 $mg \cdot kg^{-1} \cdot d^{-1}$ of pulegone.[111] In contrast, mice were more resistant to the effects than were rats with doses of 300 $mg \cdot kg^{-1} \cdot d^{-1}$ of pulegone required to see hepatic injury at 2 weeks.[111] The EMA statement points out that “prevailing opinion is that no certain cases of liver toxicity in humans are associated with the use of peppermint oil or mint oil.”[116] In a case report of a woman suspected to have ingested PMO in a suicide attempt, although comatose on arrival, she recovered without evidence of hepatic or renal injury.[112]

In a 2-year study in rats, pulegone was associated with an increased risk of bladder cancer at a 150 $mg \cdot kg^{-1} \cdot d^{-1}$ dose. In contrast, in a 2-year study of mice, an increased risk for hepatoblastoma was found at the 75 $mg \cdot kg^{-1} \cdot d^{-1}$ dose but not at the 150 $mg \cdot kg^{-1} \cdot d^{-1}$ dose in males with no increased risk in females.[111] The EMA statement posits that “non-relevance of rodent neoplasms to human carcinogenesis seems probable” in part, because of the long term sustained exposure required and doses which are not relevant in human situations.

The amount of pulegone in PMO can be reduced depending on how the peppermint is grown, when it is harvested, and how it is processed.[113] PMO normally contains a maximum of 0.1% pulegone and its metabolite menthofuran according to the European Pharmacopoeia (and more commonly 0.03-0.07%). The EMA statement proposes a life-long exposure acceptable dose of 0.75 $mg \cdot kg^{-1} \cdot day^{-1}$.[116] As likely would be used to treat FGIDs (oral use for less than one year or intermittently over several years) in adults, the EMA statement proposes an

acceptable exposure of pulegone plus menthofuran to be 75 mg/day.[116] The maximum usual dose of PMO used to treat FGIDs such as IBS is 540 mg/d which would deliver 0.54 mg of pulegone and menthofuran combined.

Thus, in summary, PMO has an excellent safety profile backed up by years of study (Table 2).

Table 2. Safety Profile of Peppermint Oil (PMO) Used in Clinical Trials in Irritable Bowel Syndrome

Reference	Design	Adverse Events
Cash (2016)[117]	• Adults with Rome III IBS-diarrhea or IBS-mixed (n=72) • Randomized double blind trial PMO 180 mg TID vs placebo X 4 wk	• No differences between groups
Asgarshirazi (2015)[105]	• Children with FGIDs (n=120); 32 excluded because they didn't complete the trial • Randomized to PMO (Colpermin®) 187 mg TID (BID for children < 45 kg) vs. Lactol® tablet (*Bacillus coagulans* and fructooligosaccharides) or placebo (1 mg folic acid) X 1 month • Unblinded	• No differences between groups
Merat (2010)[118]	• Adults with Rome II IBS (n=90); 30 withdrawals • PMO (Colpermin®) 187 mg (0.2 mL) TID vs. placebo X 8 weeks • Double-blind parallel group study	• No differences between groups
Kline (2001)[29]	• Childhood FGIDs (IBS and FAP; n=50); 8 withdrawals • PMO 187 mg (Colpermin®) or 0.1mL/capsule • 2 capsules TID (>45kg) or 1 capsule TID (30-45 kg) vs. placebo X 2 weeks • Double-blind parallel group study	• No differences between groups
Weiss (1988)[119]	• Adults with IBS (n=60) • PMO (Colpermin®) 1 cap TID vs. placebo x 3 weeks	• No differences between groups
Lech (1988)[120]	• Adults with FGIDs • PMO 50 mg (Mintoil®) 4 caps TID (n=19) vs. placebo (n=23) X 4 weeks • Double blind parallel group study	• No differences between groups
Nash (1986)[121]	• Adults with IBS (n=41); 8 withdrawals	• 6 in PMO group had heartburn

	• PMO 0.2 mL (Colpermin®) 2 caps TID vs placebo X 2 weeks treatment each • Double blind crossover study	but did not withdraw vs none in the placebo group*
* The efficacy of the enteric coating used in this study of 30 years ago is unknown		

7.3 Adverse Events and Serious Adverse Events

ICH-E2A defines an AE as “any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.”

The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor. Subjects will use a daily “Adverse Events Diary” to capture events to report. This will be kept while the PMO is being given. All adverse events will be recorded on the attached Adverse Event Record. Information to be collected includes event description, time of onset, clinician’s assessment of severity, relationship to study product, and time of resolution/stabilization of the event. All AEs occurring while on study will be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution. Any medical condition that is present at the time that the patient is screened will be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it will be recorded as an AE. All AEs will be graded for severity and relationship to study product. See attached Adverse Event Record.

Grading of Adverse Events

- Mild: usually transient, requiring no special treatment
- Moderate: usually ameliorated by simple therapeutic measures
- Severe: requires vigorous therapeutic intervention. Hospitalization may or may not be required.

Abdominal Pain

- Mild: No interference with activity
- Moderate: Some interference with activity not requiring medical intervention
- Severe: Prevents daily activities and requires medical intervention
- Potentially Life-threatening: ER visit or hospitalization

Diarrhea

- Mild: 2-3 loose stools per 24 hours
- Moderate: 4-5 stools or 400-800 grams of stool/24 hours
- Severe: 6 or more watery stools or greater than 800 grams of stool/24 hours OR requires outpatient IV hydration.
- Potentially Life Threatening: ER visit or hospitalization

Vomiting

- Mild: No interference with activity or 1-2 episodes/24 hours
- Moderate: Some interference with activity or more than 2 episodes/24 hours
- Severe: Prevents daily activities, requires outpatient IV hydration
- Potentially Life-threatening: ER visit or hospitalization for hypotensive shock

Bloating

- Mild: No interference with activity
- Moderate: Some interference with activity not requiring medical intervention
- Severe: Prevents daily activities and requires medical intervention
- Potentially Life-threatening: ER visit or hospitalization

Constipation

- Mild: No interference with activity
- Moderate: Some interference with activity
- Severe: Prevents daily activities and requires medical intervention
- Potentially Life-threatening: ER visit or hospitalization

Grading of fever also will be used as an adverse event as noted below:

- Mild: 38.0°C – 38.5°C
- Moderate: 38.6°C – 39.0°C
- Severe: > 39°C

Relationship to Study Product:

The investigator's assessment of an AE's relationship to test article is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event will be reported. All AEs will have their relationship to study product assessed using the terms: Not Related, Possibly/ related, Related/associated, Research Related.

Serious Adverse Event (SAE)

ICH-E2A defines Serious Adverse Event (SAE) or Reaction as untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect

Subjects will keep a daily log of any adverse effects; hence, they will have the opportunity to report AEs and intercurrent illness at any time during the study. In addition, there is a 24-hour telephone call line established for subjects to leave messages for the principal investigator and research coordinator. The consent form will include the paging operator number for the pediatric gastroenterology physicians (as well as the principal investigator). The paging service is available 24-hours a day, 7-days a week. Adverse Events will be recorded and reported by the research coordinator on the Baylor College of Medicine IRB Forms and forwarded to the FDA and Data Safety Monitoring Board and IRB as required by regulations.

No specific laboratory studies will be done to assess safety for the subject's as a whole. Where clinically indicated, a laboratory or other test may be ordered to assess a reported adverse event, in which case, the laboratory normal value will be used an interpreted in a clinical context (i.e., an elevation immediately outside the normal range (but within 3 SD of normal) may be interpreted as normal.

As noted above, AEs will be collected throughout the 7 days of the study and at day 14.

7.4 Reporting Procedures

Subjects who report AEs of moderate severity or higher during the study will be asked to be seen by the subject's primary healthcare provider, pediatric gastroenterologist, and or the principal investigator within 48 hours for evaluation of the event.

Data related to the event will be recorded in the subject's record.

With the subject's permission, information on adverse events will be communicated to the primary care healthcare provider for additional followup.

Followup through resolution of the adverse event and for one month after completion of the study protocol will be provided in person or by phone by study personnel.

AEs judged by the investigator to be Possibly/related, Related/associated, or Research Related will be reported the Data Safety Monitoring Board as well as the IRB for review.

We will report any serious AEs to the Data Safety Monitoring Board within 2 working days if research-related and 5 working days if not related. All SAEs will be reported to the Baylor College of Medicine IRB according to institutional policy.

The principal investigator as sponsor of the IND will report events that are both serious and unexpected and that are associated with study product(s) to the Food and Drug Administration (FDA) within the required timelines as specified in 21 CFR Part 312.32: fatal and life-threatening events within 7 calendar days (by phone or fax) and all other SAEs in writing within 15 calendar days. All serious events designed as "not associated" to study product(s), will be reported to the FDA at least annually in a summary format.

7.5 Follow up for Adverse Events

Subjects who report adverse events of moderate severity or higher during the study will be asked to be seen by the subject's primary healthcare provider, pediatric gastroenterologist, and or the principal investigator within 48 hours for evaluation of the event. Data related to the event will be recorded in the subject's record. With the subject's permission, information on adverse events will be communicated to the primary care physician for additional followup. Followup through resolution of the adverse event will be provided in person or by phone by study personnel. The principal investigator, Dr. Robert Shulman and the study coordinator will review every adverse event and evaluate the severity of such events.

All SAEs will be:

- Recorded on the appropriate Adverse Event Report form
- Followed through resolution by the principal investigator
- Reviewed and evaluated by the principal investigator

7.6 Safety Monitoring

The Texas Medical Center Digestive Diseases Center (DDC) will provide a Data Safety Monitoring Board of 3 reviewers to monitor the trial data and participant safety (Drs. Marc Rhoads, Craig Jensen, and Moreshwar Desai). The members of the Data Safety Monitoring Board have no potential personal, professional, or financial conflicts of interest related to the proposed study and its investigators. The DDC is a NIH-funded digestive disease center and consists of clinical and basic science investigators working in and around the Texas Medical Center in Houston who meet weekly for the DDC sponsored seminar. There is active collaboration among members of the DDC. Members of the Data Safety Monitoring Board were chosen who are familiar with the methodology proposed and the condition of FAP. The Data Safety Monitoring Board will review baseline and

telephone followup and final visit data at least annually and whenever any adverse event occurs to an enrolled subject. The plan will include review by the principal investigator, the research coordinator, and the IRB.

8. INTERVENTION DISCONTINUATION

Abdominal pain, bloating, and/or constipation may be, by definition, a part of FAP. In order to have better discrimination between normal changes in a subject's symptoms and an adverse reaction, an individual subject will discontinue PMO if any of the following occurs:

- Diarrhea: 6 or more watery stools or greater than 800 grams of stool/24 hours OR requires outpatient IV hydration
- Vomiting: Prevents daily activities, requires outpatient IV hydration
- Bloating: Prevents daily activities and requires medical intervention
- Constipation: Prevents daily activities and requires medical intervention
- Abdominal pain: Interference with activity and requiring medical intervention

These criteria meet a severity grade of 3 based on National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE). The study also will be stopped in an individual for an allergic reaction to PMO. The study will be stopped in all individuals if ≥ 2 subjects on drug develop the same CTCAE Grade 3 or if 1 patient develops a CTCAE Grade 4 or higher.

An individual also can be removed from the study for poor adherence to study product. If a subjects misses > 4 doses of medication in a 7 day period of time, they will be considered for removal from the study for non-compliance. Followup by phone will be continued for one week after removal from study.

PMO may be discontinued temporarily if an AE occurs and cessation and reinstitution of the medication would clarify whether the AE was related to administration of PMO. Discontinuation would be for ≤ 24 hrs.

9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

Hypothesis: A relationship exists between systemic menthol exposure and GI function. The design is predicated on the clinical need to define appropriate dosing for the use of PMO in the treatment of children with functional abdominal pain (**FAP**). Thus, children are first randomized to the three doses of PMO typically used in clinical practice in order to determine first the pharmacokinetics (**PK**). Then, children continue on their assigned dose to determine the effect of that dose on gastrointestinal (**GI**) function. As an exploratory hypothesis, we propose that systemic menthol exposure after PMO reflects a genotype-phenotype relationship in subjects with allelic variants of CYP2A6 and/or UGT2B7.

9.2 Sample Size and Randomization

A total of 30 subjects will be assigned to three different (3-fold) doses (10 children per dose). This "three dose" approach in light of the anticipated range of body weights in the study cohort would produce a dynamic, > 3-fold range in systemic exposure to PMO (e.g., AUC of menthol). While the limited size of the participant

groups would not support (i.e., with statistical validity) therapeutic dose selection for PMO in children with FAP, it will enable us to assess and explore important PK (e.g., dose proportionality) and PD (e.g., concentration vs. effect) relationships. The planned 3-fold range in PMO dose used in the proposed study (180, 360 and 540 mg) is associated with an ability to detect a difference in the three dose groups (n=10 per group) with a power of > 90% (assuming alpha = 0.05 and fixed standard deviation in dose-normalized [mg/L*hr per 1 mg/kg of menthol derived from PMO] AUC).

The following biological signatures (microbiome composition, motility, transit time) will be assessed to determine if giving peppermint oil (PMO) to humans results in a clinically meaningful change in the measure. A change in any of the following will be considered to be meaningful. Given the limited information regarding the physiologic effects of PMO, particularly in children, it is recognized that the direction of change in the biological signatures following PMO treatment may not be predictable. Because the following biological signatures are independent of each other, there will be no contradictory changes.

A clinically meaningful change for:

Microbiome is defined for this study as a statistically significant change from baseline after treatment with PMO, correcting for multiple testing (q value). Such changes may occur with respect to gut microbiome diversity and/or composition (e.g., phylum, family, genera). We anticipate there will be an increased bacterial diversity, as well as increases in the abundance of organisms associated with a healthy microbiome, in response to PMO administration. Using a paired study design, with n=30, a presumed mean difference of 0.17 in the Simpson diversity index, and a standard deviation of 0.25 (Hollister et al. Microbiome 2016;3:36), we will have a power of 0.96 to detect a significant (P= 0.05) difference.

Contractile activity will be defined as a significant change from baseline for the group. We anticipate that contractile activity will decrease significantly as a function of PMO administration. Using a paired study design, with n=30, presuming a mean difference (baseline vs post-PMO) of 20% in contractile activity, with a standard deviation of 26 pressure waves per hour (Rao et al. Neurogastroenterol Motil 2010;22: 640), we will have a power of 0.932 to detect a significant (P=0.05) difference.

Transit time will be defined as a significant change from baseline for the group. We anticipate that transit time will decrease significantly as a function of PMO administration. Using a paired study design, with n=30, presuming a mean difference (baseline vs post-PMO) of 7 hours in transit, with a standard deviation of 12.5 hours (Haase et al. Neurogastroenterol Motil 2014;26:1783), we will have a power of 0.842 to detect a significant (P=0.05) difference.

Since this is not a randomized trial other than by dose, there is no intention to treat. The data will be analyzed on a per protocol basis. Consequently, the data will provide the greatest amount of information regarding the effects of PMO on the three outcomes. Should 30 subjects initially not complete the trial, additional subjects may be recruited if warranted scientifically.

Treatment Assignment Procedures

The PI and research coordinator will plan and implement the randomization using www.randomization.com. Subjects will be stratified by sex and ages 7-9 and 10-12. The samples will be coded so that the investigator analyzing the data (menthol, GI studies) will not know to which dose group the subject had been randomized. The data will be analyzed using the total dose taken over the week as a covariate. Sensitivity analysis can be considered to assess the impact of compliance on outcome.

Should there be an AE in which knowing the dose assigned is critical, the PI or research coordinator may provide that information to the relevant individual (e.g., primary care physician, DSMB). Thus, the blind will only be broken if need be on an individual subject.

9.3 Definition of Populations

As noted above, the study does not employ an intention to treat design; rather the data will be analyzed per protocol.

9.4 Interim Analyses and Stopping Rules

There will be no interim analysis unless in the extremely unlikely situation of multiple instances of similar types of AEs in which case halting or stopping of the study may be considered (see below).

Abdominal pain, bloating, and/or constipation may be, by definition, a part of FAP. In order to have better discrimination between normal changes in a subject's symptoms and an adverse reaction, an individual subject will discontinue PMO if any of the following occurs:

- Diarrhea: 6 or more watery stools or greater than 800 grams of stool/24 hours OR requires outpatient IV hydration
- Vomiting: Prevents daily activities, requires outpatient IV hydration
- Bloating: Prevents daily activities and requires medical intervention
- Constipation: Prevents daily activities and requires medical intervention
- Abdominal pain: Interference with activity and requiring medical intervention

These criteria meet a severity grade of 3 based on National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE). The study will be stopped in all individuals if ≥ 2 subjects on drug develop the same CTCAE Grade 3 or if 1 patient develops a CTCAE Grade 4 or higher. The study also will be stopped in an individual for an allergic reaction to PMO.

9.5 Outcomes

9.5.1 Primary Outcome

State and define the primary outcome measure and specify at which study visit the outcome assessments will be performed.

There are two primary outcomes which are independent of each other:

- The pharmacokinetics of menthol after oral administration of 180 mg, 360 mg or 540 mg of PMO
- The effects of PMO after daily administration of 180 mg, 360 mg or 540 mg of PMO as reflected by gut microbiome composition, gut contractile activity, and gut transit time.

9.5.2 Secondary Outcomes

A secondary exploratory outcome is the potential relationships between systemic menthol exposure after PMO and allelic variants of CYP2A6 and/or UGT2B7.

9.6 Data Analyses

PK/PD Data Analyses

Concomitant assessment of drug disposition (PK) and effect (PD) is possible using a PK-PD link model. This approach entails characterizing the concentration (generally plasma) vs. time relationship for the analyte(s) of interest in each study participant. Model dependent (classical PK) or independent (based on statistical moment theory) can be used to derive relevant PK parameters (e.g., elimination half-life, mean absorption time, apparent volume of distribution, apparent total plasma clearance) for each subject. Similarly, the PD approach entails repeated measurement of functionally relevant effect endpoints (e.g., those reflecting motility and/or intensity of contraction) as a function of time after the administration of the test article. In most instances, the apparent time of peak effect lags the time of the peak plasma concentration. In this case, the Sigmoid E-max model (based on the Hill equation) is used to derive PD parameter estimates of interest (e.g., EC_{50}, Emax, E_0). The descriptive assessment of certain physiologic data captured by the SmartPill technology (e.g., change in motility) also will enable us to define an area under the effect curve (AUEC) that is associated with a given degree of systemic exposure (i.e., AUC) for menthol from a given dose of PMO. With the simultaneous determination of relevant PK and PD parameters, these can be evaluated as a function of age (i.e., to assess potential developmental differences in either PK and/or PD) or disease severity. Most importantly, the PK/PD approach enables characterization of the clinical pharmacology of a given drug/xenobiotic in a specific patient population. It does not require that the exposure-response relationship first is well characterized in adults as the approach per se creates the exposure-response relationship in the population of interest (i.e., target population).

Three different doses were selected for the initial PK evaluation so as to purposefully create a dynamic range of systemic menthol exposures (i.e., range of AUC values). We anticipate that this range will not only verify dose proportionality in PK but also, provide a real dynamic range for a careful exposure-response analysis (i.e., the PK/PD link model). For the effect (PD) biomarkers, we will be able to discern from our pharmacokinetic modeling the menthol AUC that appears to correspond to specific changes from baseline for the activity of a given PD marker. Once the PK/PD link model is built and the exposure-response relationships identified, we will use standard modeling/simulation techniques to assess the PMO dose (mg/kg) needed to produce a target menthol AUC (corrected per 1 mg/kg dose of menthol) associated with a given (desirable) effect on the gut. These data will be used to inform PMO dose selection for a subsequent study of PMO.

In the steady state assessment carried out on Day 7, concurrent exposure vs. response data will be collected in subjects willing to undergo two additional blood draws. Response data (i.e., pharmacodynamic surrogate endpoints) will consist of the physiologic measurements captured after the collection of the stool for microbiome analysis and administration of the SmartPill®. A population-based pharmacokinetic approach will be used to determine menthol PK parameters. This particular sparse sampling approach (also known as an opportunistic sampling design) has been previously used by one of our investigative team (Dr. Kearns) to assess the PK of antimicrobial agents in pediatric patients.[122,123] The linked PK/PD analysis will be focused on exposure (i.e., plasma menthol AUC) vs. response data and will be accomplished using subroutines available in a widely used, proprietary software program (NONMEM v 7.2, Icon Development Solutions, San Antonio, TX).

Finally, menthol plasma PK data from both the single-dose and steady-state evaluation will be used to parameterize a final model that will be used to project PMO dose for the second phase of our research program. Specifically, Monte Carlo simulations will be performed using the aforementioned parameter estimates to define a PMO dosing regimen that will produce systemic exposures (e.g., 5th to 95th percentile) previously associated with safety and desired effect in adults and, as informed by the PK/PD data from the first phase of our program, a “target” level of exposure that appears to be associated with PD properties of menthol that based on objective (i.e., SmartPill) and subjective (i.e., reports of clinical improvement) data, are considered as desirable. In the second phase of our research program, we will select a subset from our study cohort to serve as the “test population” that will be used for the purposes of validation of PMO dose selected from our initial Monte Carlo simulations.

Paired t testing will be used to compare baseline and post-PMO changes in gut microbiome composition, transit time, and contractility after we establish that each parameter of interest is normally distributed.

Gender and racial/ethnic subgroups will be used as covariates to explore if they impact the study results.

TRPM8, CYP2A6, and *UGT2B7* Genotyping Analyses

TRPM8 is a membrane transport protein located in the small intestine. It is also known as the cold-receptor for menthol. To our knowledge, there are no studies which examine the in vivo ontogeny of TRPM8. CYP2A6

and UGT2B7, the hepatic drug metabolizing enzymes primarily responsible for catalyzing the biotransformation of menthol are polymorphically expressed in humans and show an apparent ontogenic pattern.

The pharmacogenomic aspect of this study is exploratory as the size of the study population is not large enough, given the frequency of functionally important allelic variants of the drug metabolizing enzymes associated with menthol biotransformation, to incorporate genotype as a covariate in the PK/PD analysis. The value of the genotyping data would be expected to reside with being able to explain a PK "outlier" (e.g., a disproportionately increased AUC in a patient with a genotype known to correspond to a slow metabolizer phenotype).

If our data analysis reveals either an apparent developmental or pharmacogenomic association between menthol exposure and response of the gastrointestinal tract, we will use the data for hypothesis generation for future investigations. Specifically, the data generated from our proposed study will be sufficient to construct larger, mechanism-based trials sufficient to further characterize, at a tissue and cellular level, the effect of menthol on the bowel in children FAP.

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

Data forms specific to this study have been prepared and submitted to NCCIH. Data collection will be the responsibility of the research coordinator under the supervision of the principal investigator. During the study, the investigator will maintain complete and accurate documentation for the study. Data with identifiers will be kept in a locked cabinet in the research coordinator's locked office in the Children's Nutrition Research Center at 1100 Bates Ave, Houston, Texas, 77030. Only the principal investigator and principal investigator staff will have access to research data with identifiers. The research coordinator will enter the data collected directly in the study electronic records developed in a REDCap database.

10.2 Data Management

Data forms specific to this study have been prepared and submitted to NCCIH. Data entered into the study database will include a subject study identification number; names will not be linked with subject data in the database. Study staff will maintain records linking the subject initials with the identification number assigned for the study in a secure area. All source documents and laboratory reports will be reviewed by the clinical team and data entry staff who will ensure that they are accurate and complete.

Several levels of security will be employed to ensure privacy and integrity of the study data, including the following:

- Study access will require use of assigned unique user names and passwords that are modified at specified time intervals
- Individual roles and access levels will be assigned
- Passwords will be changed regularly
- Data will not store on laptop computers

Study Records Retention

Study documents will be retained for a minimum of 2 years after the study completion date

10.3 Quality Assurance

10.3.1 Training

Research staff will be trained on the study Manual of Operation and the use of all electronic data forms in the REDCap database.

10.3.2 Quality Control Committee

Not applicable.

10.3.3 Metrics

Review of the data on each individual subject will be performed to insure that blood samples for menthol sampling were obtained at the appropriate time.

Analysis of total (conjugated and un-conjugated) plasma menthol will be measured using a gas chromatography mass spectrometry (GC-MS) method which we have validated and adapted using a previously published analytical method.[115,124,125] UGT2B7 genotyping (UGT2B7-900A>G) will be performed using commercially available TaqMan assays (Applied Biosystems, Foster City, CA) and CYP2A6 genotyping will be performed using a validated PCR-RFLP assay using standard quality control methods.[126]

Quality of microbiome analysis will be insured using our previously vetted and published pipeline.[127-132] Parents and children will receive detailed instructions regarding stool collection and storage prior to courier pickup.

10.3.4 Protocol Deviations

Protocol deviations will be identified and reviewed during regularly scheduled reviews of all data entry into the source documents. Protocol deviations reports will be sent to the IRB per their guidelines.

10.3.5 Monitoring

The research coordinator will be responsible for coordinating monitoring visits.

Prior to the monitoring visit:

- Notification: The research coordinator will be notified of a visit by the study monitor at least 2 weeks before the site visit. Urgent visits may be accommodated when needed
- Scheduling: The study coordinator will schedules the monitoring visit after the availability of the Principal Investigator (PI) is confirmed

Regulatory binder preparation:

- The Regulatory Coordinator will ensure that the regulatory binders are organized
- The Regulatory Coordinator will document for completeness and accuracy, including the availability of any outstanding items from the previous monitoring visit

Research charts:

- The Research Coordinator will organize and flag subject research charts, if needed. The following research documents will include: consent/assent forms, eligibility checklist and source documentation, pre-study evaluations, on study evaluations, study product administration records and serious adverse events.
- Case report forms (CRFs) will be reviewed by the research coordinator and the data manager for accuracy and completeness
- Outstanding items from previous monitoring visits will be addressed by the Study Coordinator and the Data Manager

Study Product Record Forms:

The Research Coordinator will review study product supplies and study product accountability records for completeness and accuracy.

Day of the monitoring visit

- The Research Coordinator ensure that subject research charts and regulatory binders are available
- The Research Coordinator will set up a study folder for the study monitor with passwords to access electronic case report forms, fax, copier, as needed
- The Research Coordinator, and the Lab Staff will be available to respond to queries
- Patient identifiers will be replaced with study identifiers on copies provided to the monitor
- A record of copies of documents provided to the study monitor will be filed in the regulatory binder or the subject research chart, as applicable

Exit Interview:

- The Study Coordinator will meet with the study monitor to discuss issues or outstanding queries, if needed
- The PI will meet with the study monitor, if requested. If the PI is unable to meet due to unforeseen circumstances, the Clinical Research Manager (CRM) may meet the study monitor.
- A copy of the monitoring report will be filed in the regulatory binder
- Findings will be reviewed for accuracy
- Relevant findings will be addressed/corrected by the appropriate research staff

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Institutional Review Board (IRB) Review

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB or ethics committee responsible for oversight of the study. The consent form should be separate from the protocol document.

Institutional Review Board

Prior to enrollment of subjects into this trial, the approved protocol and the informed consent form will be reviewed and approved by the appropriate IRB.

The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this trial. Any amendments to the protocol will be submitted to the IRB and approval obtained prior to implementation.

11.2 Informed Consent Forms

A signed consent form will be obtained from each subject (consent from the parent/guardian and assent from the child). The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy will be given to each participant or legal guardian and this fact will be documented in the participant's record.

Informed Consent Process

Informed consent/assent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible

benefits of this therapy will be provided to the subjects and their parent/guardian. Consent forms describing in detail the study interventions/products, study procedures, and risks are given to the subject and their parent/guardian and written documentation of informed consent/assent is required prior to starting intervention/administering study product.

Potential subjects will be contacted and will be interviewed regarding basic inclusion and exclusion criteria to determine if they may be eligible. If they meet basic entry criteria the potential subject will be offered the opportunity for study participation and consent/assent will be obtained by the principal investigator or research coordinator. Subjects who choose to participate will be consented prior to data collection at the first research visit. All study personnel obtaining consent have received "Human Subjects Compliance Training" from the Baylor College of Medicine IRB. Information provided to subjects during recruitment include the purpose of the study, procedures, withdrawal procedures, subject termination, risk/discomforts, benefits, costs, compensation, and alternatives to participation. Informed consent will be documented on the Baylor College of Medicine IRB-approved consent forms. All signed consent forms will be kept in a locked cabinet in the research coordinator's locked office in the Children's Nutrition Research Center at 1100 Bates Ave, Houston, Texas, 77030.

11.3 Participant Confidentiality

Subject Confidentiality

Subject confidentiality is strictly held in trust by the principal investigator, his staff, and the sponsor(s) and their agents.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The FDA, study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

11.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the NCCIH, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research participants are protected.

12. COMMITTEES

As noted above, a DSMB will be established consisting of three members who will review study progress, data, and AEs at least yearly.

13. PUBLICATION OF RESEARCH FINDINGS

Research findings will be disseminated via oral/poster presentation, abstract, or manuscript with the material provided to NCCIH.

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15. SUPPLEMENTS/APPENDICES

APPENDIX A: ADVERSE EVENTS RECORD

SUBJECT ID NO.: _____ Adverse Events Record 1

Were there any adverse events reported by the study participant? Yes No

Adverse Event No.	Specify Event	Onset Date Check box if AE is continuing from previous	Severity Grade	Seriousness	Action Taken	Outcome	Resolution Date Enter date OR check box if AE is continuing	Causality
__ __		__ __/__ __/__ __ ☐					__ __/__ __/__ __ ☐	
__ __		__ __/__ __/__ __ ☐					__ __/__ __/__ __ ☐	
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__ __		__ __/__ __/__ __ ☐					__ __/__ __/__ __ ☐	

Grading of Adverse Events	Seriousness	Action Taken	Outcome	Causality
1 - Mild: usually transient, requiring no special treatment 2 - Moderate: usually ameliorated by simple therapeutic maneuvers 3 - Severe: requires vigorous therapeutic intervention. Hospitalization may or may not be required.	0 – Not serious 1 – Serious	0 - None 1 - Symptomatic OTC medications 2 - Remedial therapy 3 - Evaluation by PI or other physician 4 - Hospitalization	1 - Resolved 2 - Resolved with sequela 3 - Ongoing 4 - Unknown/lost to follow-up	1 - Not related 2 - Possibly related 3 - Related/Associated 4 - Research related

Principal Investigator's Signature _____ Date: __ __/__ __/__ __

Severity Grade	Adverse Events	Action Taken	Outcome	Causality
0 – None		0 - none		
1 – Mild	• Diarrhea: 2-3 loose stools per 24 hours • Vomiting: No interference with activity or 1-2 episodes/24 hours • Bloating: No interference with activity • Abdominal pain: No interference with activity	1- Symptomatic OTC medications	1 - Resolved 2 - Resolved with sequelae 3 - Ongoing 4 - Unknown/lost to followup	1 - Not related 2 - Possibly related 3 - Probably related 4 - Related/Associated 5 - Research-related
2 – Moderate	• Diarrhea: 4-5 stools or 400-800 grams of stool/24 hours • Vomiting: Some interference with activity or more than 2 episodes/24 hours • Bloating: Some interference with activity not requiring medical intervention • Constipation: Some interference with activity • Abdominal pain: Some interference with activity not requiring medical intervention	2 - Remedial therapy		
3 – Severe	• Diarrhea: 6 or more watery stools or greater than 800 grams of stool/24 hours OR requires outpatient IV hydration • Vomiting: Prevents daily activities, requires outpatient IV hydration • Bloating: Prevents daily activities and requires medical intervention • Constipation: Prevents daily activities and requires medical intervention • Abdominal pain: Interference with activity and requiring medical intervention	3 - Discontinue study treatment 3 - Evaluation by PI or other physician 3 - Consider hospitalization		
4 – Life threatening or disabling	• Diarrhea: ER visit or hospitalization • Vomiting: ER visit or hospitalization for hypotensive shock • Bloating: ER visit or hospitalization • Constipation: ER visit or hospitalization	4 - Inpatient hospitalization		
5 – Fatal	• Death			

An individual also can be removed from the study for poor adherence to study medication and/or study visits. If a subjects misses > 4 doses of medication in a 7 day period of time they may be considered for removal from the study for non-compliance. Follow up by phone will be continued for one week after removal from study.

Adverse Event SOP

Responsibility

- The principal investigator (PI), research coordinators, and designated members of the PI's research staff are responsible for Adverse Event recording and reporting to the Sponsor, Data Safety Monitoring Board, the FDA and the IRB.
- The PI will determine the causality of AEs or SAEs.
- The PI/Sub-I/Research Staff are responsible for follow-up information on all SAEs until resolution or an endpoint is reached.

Definitions

Adverse Event: any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

Serious Adverse Event: untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect

Expected Adverse Event: Described in the "Risks" section of the Consent Form.

Unexpected Adverse Event: An adverse event for which the nature or severity is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product. Not described in the "Risks" section of the Consent Form

Causality, Suggested Guidelines for:

1. Not related: no temporal association, or the cause of the event has been identified, or the drug cannot be implicated
2. Possibly related: temporal association, other etiologies are possible
3. Probably related: temporal association, other etiologies are possible but unlikely
4. Related/Associated: established temporal or other association for event not reasonably explained by the subject's known clinical state or any other factor
5. Research-related: related to the study entity *or* to a procedure or intervention conducted for the purposes of the research study

Procedure

1. All clinical events and symptoms are recorded by the clinical staff on progress notes or other forms supplied for the study. A copy of the progress note containing details of adverse events will be placed in the research record.
2. For each event or symptom, start date, severity, treatment or action taken will be recorded on the Adverse Event Record. A stop date or determination of ongoing status will be noted.
3. The research coordinator must notify the PI immediately of any serious or life-threatening medical or laboratory event.
4. The PI will review Adverse Event Records for each subject and assign causality and sign.

5. SAEs (including description, notifications, intervention, and resolution) are reported on appropriate source documents. These may include: report sheets, copies of lab reports, medical orders, medication administration records, progress notes, etc.
6. The PI will report any serious AEs to the Data Safety Monitoring Board within 2 working days if research-related and 5 working days if not related.
7. All SAEs will be reported to the Baylor College of Medicine IRB according to institutional policy.
8. The PI will report events that are both serious and unexpected and that are associated with study product(s) to the Food and Drug Administration (FDA) within the required timelines as specified in 21 CFR Part 312.32: fatal and life-threatening events within 7 calendar days (by phone or fax) and all other SAEs in writing within 15 calendar days.
9. All serious events designed as "not associated" to study product(s), will be reported to the FDA at least annually in a summary format.
10. Only the Data Safety Monitor Board may determine the causality of all SAEs.
11. The PI must follow all subjects who have experienced an SAE until there is resolution of the SAE or satisfactory stabilization of the subject's condition
12. A summary of all SAEs is reported to the IRB and FDA in Annual Reports and in the Final Report.

References

1. Baylor College of Medicine IRB Data and Safety Monitoring: http://intranet.bcm.tmc.edu/?tmp=/research/oor/home
2. Code of Federal Regulations, Title 21, Volume 5; Revised as of April 1, 2005: http://www.access.gpo.gov/cgi-bin/cfrassemble.cgi?title=200521
3. Guidance for Industry - E6 Good Clinical Practice: http://www.fda.gov/cder/guidance/959fnl.pdf
4. Guidance for Industry – Clinical Safety Data Management: http://www.fda.gov/cder/guidance/iche2a.pdf