

RESEARCH MONOGRAPH . COMPOUND PROFILE

Tributyryn

A glycerol tributyrate ester functioning as a stable, orally bioavailable prodrug of butyric acid, distinguished from other butyrate delivery forms by its resistance to gastric degradation, intracellular lipase-mediated release of three butyrate equivalents, and HDAC-inhibitory activity supporting applications in oncology chemoprevention, intestinal barrier maintenance, hepatoprotection, metabolic inflammation, and skeletal muscle differentiation.

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RESEARCH USE ONLY

ABSTRACT

Tributylin (glycerol tributyrate, CAS 60-01-5) is the 1,2,3-tributanoyl ester of glycerol and the principal triglyceride prodrug of butyric acid studied in human clinical pharmacology and preclinical oncology. Classified by the United States Food and Drug Administration as Generally Recognized as Safe (GRAS) for food use, tributyrin is a naturally occurring lipid constituent of bovine milk fat and honey that has attracted sustained research interest as a vehicle for delivering pharmacologically active butyrate concentrations to systemic circulation and to the colonic epithelium, overcoming the rapid first-pass hepatic clearance, objectionable odor, and poor oral bioavailability that limit the therapeutic application of free butyric acid and its sodium salt.

The molecular pharmacology of tributyrin is mediated through its hydrolytic product, butyrate, a four-carbon short-chain fatty acid that operates through at least three characterized molecular mechanisms: inhibition of class I and class II histone deacetylases (HDACs), particularly HDAC1, HDAC2, and HDAC3; agonism at the G-protein-coupled receptor GPR109A (also designated HCAR2 or HM74a), the niacin receptor expressed on colonocytes, adipocytes, and immune cells; and agonism at the free fatty acid receptors GPR41 (FFAR3) and GPR43 (FFAR2) on enteroendocrine cells and immune cells. The HDAC-inhibitory activity produces histone hyperacetylation, chromatin remodeling, and consequent transcriptional activation of tumor suppressor genes (p21WAF1/CIP1, BAX, p53), cell cycle arrest proteins, and differentiation-associated gene programs in neoplastic cells. The GPR109A agonism mediates anti-inflammatory signaling through

suppression of NF-kappaB-dependent proinflammatory cytokine production and through promotion of regulatory T-cell and M2-macrophage phenotypes in adipose tissue and intestinal lamina propria.

Two Phase I clinical trials in patients with advanced solid tumors have characterized the human pharmacology of tributyrin. The Conley et al. (1998) study administered tributyrin at 50 to 400 mg/kg/day orally to 13 patients and demonstrated dose-proportional plasma butyrate concentrations reaching 0 to 0.45 mM, with no dose-limiting toxicity. The Edelman et al. (2003) study administered tributyrin at 150 to 200 mg/kg three times daily to 20 patients with advanced solid tumors and achieved median plasma butyrate concentrations of 52 micromolar with considerable interpatient variability; escalation was halted at 200 mg/kg three times daily owing to the large number of capsules required rather than to toxicity. Both trials confirmed that tributyrin is well tolerated, with mild gastrointestinal complaints (nausea, eructation, diarrhea) as the principal adverse events.

Preclinical pharmacology spans oncology, gastroenterology, hepatology, metabolic disease, and muscle biology. In oncology, tributyrin induces differentiation, growth arrest, and apoptosis in human prostate cancer (PC3, LNCaP, TSU-Pr1), colon cancer (HT-29, Caco-2), breast cancer (MCF-7), and leukemia cell lines at concentrations of 0.1 to 4 mM, and suppresses hepatocarcinogenesis in the resistant hepatocyte rat model through p53 acetylation and activation of the p53 apoptotic signaling pathway. In gastroenterology, tributyrin maintains intestinal tight junction integrity, reduces antibiotic-induced intestinal injury in combination with *Lactobacillus* GG, and mitigates chronic-binge ethanol-induced intestinal barrier

disruption and liver injury. In metabolic disease, tributyrin attenuates obesity-associated inflammation, improves insulin responsiveness, and reduces hepatic triglyceride accumulation through a GPR109A-dependent mechanism. In muscle biology, dietary tributyrin promotes satellite cell terminal differentiation through HDAC inhibition and epigenetic priming, producing measurable increases in muscle fiber cross-sectional area in neonatal piglet models.

This monograph reviews the chemistry, synthesis, and physicochemical properties of tributyrin; the three-arm molecular pharmacology (HDAC inhibition, GPR109A agonism, FFAR agonism); the human pharmacokinetic record from Phase I oncology trials; the preclinical evidence base across oncology, gastrointestinal, hepatoprotective, metabolic, and muscle biology applications; sourcing and quality verification for research use; reconstitution and handling; stack-interaction considerations; adverse-event signal; and a comparative assessment of five alternative butyrate delivery forms against tributyrin on five competency standards (bioavailability, effect size, delivery specificity, tolerability profile, and overall validation). The compound is not approved as a drug by any regulatory authority. It is sold as a dietary supplement and as a research-grade preparation; investigators should obtain analytical confirmation of identity and purity on every lot.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Common name: Tributyrin.

Systematic synonyms: Glycerol tributyrate, glyceryl tributyrate, butyrin, 1,2,3-tributanoylglycerol.

Chemical Abstracts Service registry number: 60-01-5.

International Union of Pure and Applied Chemistry name: 2,3-bis(butanoyloxy)propyl butanoate. The compound is the triester of glycerol with three equivalents of butyric acid (butanoic acid, C4:0), placing it in the class of simple triglycerides with identical acyl chains.

European Community number: 200-451-5.

FEMA GRAS number: 2223.

Codex Alimentarius: Listed as a permitted food flavoring agent.

PubChem CID: 6050.

DrugBank accession: DB13959.

1.2 Structural class and chemistry

Tributyrin is a short-chain triglyceride, specifically the symmetric triacylglycerol in which all three hydroxyl groups of glycerol are esterified with butyric acid (n-butanoic acid, C₄H₈O₂). It belongs to the broader class of glycerolipids and is the simplest naturally occurring triglyceride of pharmacological interest.

Molecular formula: C₁₅H₂₆O₆.

Exact mass: 302.1729 g/mol (monoisotopic).

Molecular weight: 302.36 g/mol.

Physical properties: Colorless to pale yellow oily liquid at room temperature. Density approximately 1.032 g/mL at 20 degrees Celsius. Boiling point approximately 305 to 310 degrees Celsius at atmospheric pressure. Refractive index approximately 1.435 at 20 degrees Celsius. Practically insoluble in water (approximately 0.01 g/100 mL at 25 degrees Celsius); freely soluble in ethanol, diethyl ether, chloroform, and most organic solvents. The compound has a characteristic rancid butter odor attributable to trace free butyric acid from hydrolysis.

Stereochemistry: The glycerol backbone of tributyrin is prochiral; the sn-1 and sn-3 positions bear identical butanoyl groups, and the molecule is therefore achiral and optically inactive. No stereochemical verification is required for research-grade material.

Stability: Tributyrin is chemically stable under anhydrous conditions at room temperature and at refrigerated storage. Hydrolysis in aqueous or high-humidity environments liberates free butyric acid, producing the characteristic odor and reducing potency. The compound is stable at gastric pH (pH 1 to 3) in the absence of lipase activity, which is the physicochemical basis for its resistance to premature gastric hydrolysis and its utility as a colonic butyrate delivery vehicle.

1.3 Synthesis

Tributyrin is synthesized by Fischer esterification of glycerol with three equivalents of butyric acid under acid catalysis (sulfuric acid, p-toluenesulfonic acid, or acidic ion-exchange resin) with azeotropic removal of water, typically using toluene or cyclohexane as an entrainer [1]. The reaction is driven to completion by the use of excess butyric acid (typically 4 to 6 equivalents relative to glycerol) and continuous water removal. The crude product is washed with aqueous sodium bicarbonate to remove residual butyric acid and catalyst, dried over anhydrous sodium sulfate or magnesium sulfate, and purified by vacuum distillation or by column chromatography on silica gel. Industrial synthesis achieves yields of 85 to 95 percent and produces material of greater than 97 percent purity by gas chromatography. Enzymatic synthesis using immobilized lipases (*Candida antarctica* lipase B, *Thermomyces lanuginosus* lipase) in solvent-free or low-water systems has been reported as a green chemistry alternative and produces tributyrin of comparable purity with milder reaction conditions [2].

The compound is also isolated from natural sources (bovine milk fat, where it constitutes approximately 3 to 4 percent of total triglyceride by mass) by molecular distillation or supercritical carbon dioxide extraction, though natural-source material is not the predominant supply for research or supplement applications.

2. Abstract

Tributyryn (glycerol tributyrate) is a short-chain triglyceride prodrug that delivers three equivalents of butyric acid upon hydrolysis by pancreatic and intracellular lipases. The compound is distinguished from free butyric acid and its sodium, calcium, and magnesium salts by superior oral bioavailability, resistance to premature gastric degradation, reduced odor, and the capacity to achieve sustained plasma butyrate concentrations at pharmacologically relevant levels [3, 4].

The pharmacological activity of tributyrin is mediated entirely through its hydrolytic product, butyrate, which exerts biological effects through three principal molecular mechanisms. First, butyrate is a potent inhibitor of class I histone deacetylases (HDAC1, HDAC2, HDAC3, and HDAC8) and to a lesser extent class IIa HDACs, producing histone hyperacetylation and consequent reactivation of silenced tumor suppressor genes, cell cycle regulators (p21WAF1/CIP1), and proapoptotic effectors (BAX, BAK) [5, 6]. Second, butyrate is an agonist at GPR109A (HCAR2), the G-protein-coupled niacin receptor expressed on colonocytes, adipocytes, macrophages, and dendritic cells, through which it suppresses NF-kappaB-mediated proinflammatory cytokine release and promotes anti-inflammatory immune cell phenotypes [7, 8]. Third, butyrate activates the free fatty acid receptors GPR41 (FFAR3) and GPR43 (FFAR2) on enteroendocrine L-cells and immune cells, stimulating glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) secretion and modulating intestinal motility and mucosal immune tone [9].

Two Phase I clinical trials in advanced solid tumor patients established the human pharmacology of oral tributyrin. Conley et al. (1998) administered tributyrin at 50 to 400 mg/kg/day to 13 patients and reported dose-proportional peak plasma butyrate concentrations of 0 to 450 micromolar with no dose-limiting toxicity [3]. Edelman et al. (2003) administered tributyrin at 150 to 200 mg/kg three times daily to 20 patients and achieved median plasma butyrate of 52 micromolar; escalation was halted at the highest dose owing to the capsule burden rather than toxicity [4]. The plasma butyrate concentrations achieved in both trials are within the range required for HDAC inhibition in vitro (50 to 500 micromolar), supporting the translational relevance of oral tributyrin for epigenetic modulation.

The preclinical evidence base is extensive and spans oncology (differentiation and apoptosis induction in prostate, colon, breast, and hematologic malignancies; chemoprevention of hepatocarcinogenesis), gastroenterology (intestinal barrier maintenance, antibiotic-induced injury mitigation, ethanol-induced gut-liver axis protection), metabolic disease (obesity-associated inflammation attenuation, insulin sensitization through GPR109A), hepatology (ethanol-induced steatosis and hepatic injury attenuation through epigenetic restoration of CPT-1A expression), and muscle biology (satellite cell terminal differentiation and muscle fiber hypertrophy through HDAC-mediated epigenetic priming) [10, 11, 12, 13, 14].

A clinical trial (NCT05446168) investigating tributyrin supplementation in Parkinson's disease patients to assess brain short-chain fatty acid metabolism has been registered, reflecting expanding clinical interest in the neurological applications of butyrate delivery [15].

Tributyrin is classified as GRAS by the FDA for food flavoring use. It is commercially available as a dietary supplement (typically 150 to 1000 mg capsules) and as a research-grade chemical from multiple suppliers. The compound is not approved as a drug by any regulatory authority.

3. Discovery and Development History

3.1 Origin as a food constituent

Tributyryn has been recognized as a naturally occurring milk fat component since the early characterization of bovine butter triglycerides in the nineteenth century. The compound constitutes approximately 3 to 4 percent of total triglyceride mass in bovine milk fat and is the principal short-chain triglyceride in dairy lipids. Its name derives directly from the Latin butyrum (butter), reflecting this historical association. The compound was listed as a GRAS food flavoring agent (FEMA 2223) in the United States prior to its investigation as a pharmacological agent [1].

3.2 Identification as a butyrate prodrug

The recognition that tributyrin could serve as a pharmacologically useful butyrate prodrug emerged in the early 1990s from the convergence of two research streams: the identification of butyric acid as an HDAC inhibitor and cell differentiation agent in cancer cell lines, and the recognition that free butyric acid had severely limiting pharmacokinetic properties (rapid hepatic clearance, offensive odor, poor oral bioavailability, inability to achieve sustained plasma concentrations above the HDAC-inhibitory threshold of approximately 50 micromolar) [3, 5]. Tributyrin was proposed as an alternative delivery vehicle because it is chemically stable, orally bioavailable, resistant to gastric acid, and hydrolyzed by pancreatic and intracellular lipases to release butyrate at controlled rates.

3.3 Phase I clinical development in oncology

The National Cancer Institute and academic collaborators at Johns Hopkins University and the University of Pittsburgh advanced tributyrin into Phase I clinical trials in the late 1990s. The Conley et al. (1998) Phase I trial in Clinical Cancer Research enrolled 13 patients with refractory solid tumors and established dose-proportional pharmacokinetics at doses of 50 to 400 mg/kg/day [3]. The Edelman et al. (2003) follow-up study in Cancer Chemotherapy and Pharmacology enrolled 20 patients at doses of 150 to 200 mg/kg three times daily and confirmed tolerability and plasma butyrate concentrations in the HDAC-inhibitory range [4]. Neither trial

was designed to assess antitumor efficacy; both were pharmacokinetic and safety studies intended to support the development of tributyrin as a cancer differentiation agent.

3.4 Expansion into non-oncology applications

Beginning approximately 2005, the research literature on tributyrin expanded substantially beyond oncology into gastrointestinal physiology, hepatology, metabolic disease, and muscle biology. The Cresci laboratory at the Cleveland Clinic and subsequently at Vanderbilt University published a series of studies (2014 to 2019) demonstrating that tributyrin protects against ethanol-induced intestinal barrier disruption and hepatic steatosis through maintenance of tight junction protein expression and through epigenetic restoration of fatty acid oxidation gene transcription [12, 16, 17]. The Vinolo laboratory in Brazil characterized the GPR109A-dependent anti-inflammatory and insulin-sensitizing activity of tributyrin in diet-induced obesity models [8]. The Murray and Velleman laboratories characterized the HDAC-inhibitory activity of dietary tributyrin on skeletal muscle satellite cell differentiation in neonatal piglet and poultry models [13, 14]. These non-oncology applications have driven the more recent commercial interest in tributyrin as a dietary supplement for gut health, metabolic support, and body composition.

3.5 Current clinical trajectory

Tributyrin is not in active pharmaceutical development by any commercial sponsor for a drug indication. The compound is the subject of an active clinical trial (NCT05446168) investigating short-chain fatty acid metabolism in Parkinson's disease patients receiving tributyrin supplementation [15]. The commercial trajectory is principally as a dietary supplement, where it is marketed under several branded forms (CoreBiome, ProButyrate, and others) for gut health and microbiome support applications. Research-grade tributyrin continues to be used in preclinical oncology, gastroenterology, and metabolic disease investigations.

4. Mechanism of Action

Tributyrin is a prodrug; its biological activity is mediated entirely through its hydrolytic product, butyrate (n-butanoic acid, $C_4H_8O_2$). The mechanisms described below are therefore the mechanisms of butyrate action, with tributyrin serving as the delivery vehicle that determines the pharmacokinetic profile (site, rate, and magnitude of butyrate release).

4.1 Hydrolytic activation

Tributyrin is hydrolyzed sequentially by lipases to release butyrate. In the gastrointestinal tract, pancreatic lipase cleaves the sn-1 and sn-3 ester bonds preferentially, producing 2-monobutyrin (sn-2 monoacylglycerol) and two equivalents of free butyrate. The 2-monobutyrin is absorbed intact and is subsequently hydrolyzed intracellularly by monoacylglycerol lipase to release the third butyrate equivalent and free glycerol [3]. In systemic circulation and in tissues, intracellular lipases (including hepatic lipase and lysosomal acid lipase) hydrolyze intact tributyrin that has been absorbed as the parent compound, releasing butyrate directly at the intracellular site of action. This two-compartment hydrolysis (luminal and intracellular) is the mechanistic basis for the sustained and distributed butyrate release that distinguishes tributyrin from free butyrate salts.

4.2 Histone deacetylase inhibition

Butyrate is a competitive inhibitor of class I histone deacetylases (HDAC1, HDAC2, HDAC3, and HDAC8) with IC_{50} values in the range of 50 to 500 micromolar, depending on the assay system and the specific HDAC isoform [5, 6]. At these concentrations, butyrate occupies the zinc-containing active site of the HDAC enzyme and prevents deacetylation of epsilon-amino-acetyllysine residues on histone H3 and H4 tails, resulting in histone hyperacetylation, chromatin relaxation, and transcriptional activation of genes whose promoters are subject to HDAC-mediated silencing.

The principal transcriptional consequences of HDAC inhibition by butyrate include:

Upregulation of p21WAF1/CIP1, producing G1 cell cycle arrest in neoplastic cells. Upregulation of BAX and BAK, promoting the intrinsic (mitochondrial) apoptotic pathway. Acetylation and stabilization of p53 protein, enhancing its transcriptional activity at pro-apoptotic target genes and its nuclear retention [10, 18]. Upregulation of differentiation-associated gene programs in cancer cell lines (cytokeratins, alkaline phosphatase, carcinoembryonic antigen in colonic carcinoma; prostate-specific antigen and androgen receptor modulation in prostate carcinoma) [19, 20]. Downregulation of HDAC3 and HDAC4 gene expression, producing a feedforward loop that amplifies histone acetylation [10].

The HDAC-inhibitory mechanism is the molecular basis for the anticancer differentiation and apoptosis activity observed in preclinical models and for the chemoprevention of hepatocarcinogenesis in the resistant hepatocyte rat model.

4.3 GPR109A (HCAR2) agonism

GPR109A (hydroxycarboxylic acid receptor 2, HCAR2, also designated HM74a in humans and PUMA-G in mice) is a Gi/Go-coupled receptor that was originally identified as the molecular target of the lipid-lowering agent niacin (nicotinic acid) and was subsequently found to be activated by butyrate at low-millimolar concentrations [7, 8]. The receptor is expressed on colonocytes (apical membrane), adipocytes, macrophages, dendritic cells, and neutrophils.

Butyrate activation of GPR109A produces several downstream effects. In colonocytes, GPR109A signaling promotes epithelial barrier function and mucin production. In adipose tissue macrophages and dendritic cells, GPR109A activation suppresses NF-kappaB nuclear translocation and reduces transcription of proinflammatory cytokines including tumor necrosis factor alpha (TNF-alpha), interleukin-1 beta (IL-1beta), and interleukin-6 (IL-6) [7, 8]. GPR109A activation in the colonic lamina propria promotes regulatory T-cell (Treg) differentiation and M2-macrophage polarization, producing a net anti-inflammatory tissue environment [8]. In adipocytes, GPR109A signaling suppresses lipolysis through inhibition of adenylyl cyclase, an effect shared with niacin.

The GPR109A-dependent mechanism is the molecular basis for the anti-inflammatory and metabolic effects of tributyrin in obesity, ethanol-induced intestinal injury, and colonic inflammation models.

4.4 Free fatty acid receptor agonism (GPR41/GPR43)

Butyrate is an agonist at GPR41 (FFAR3, EC50 approximately 100 micromolar) and GPR43 (FFAR2, EC50 approximately 500 micromolar), G-protein-coupled receptors for short-chain fatty acids expressed on enteroendocrine L-cells, immune cells, sympathetic ganglia, and adipocytes [9]. Activation of GPR43 on colonic L-cells stimulates secretion of glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), contributing to satiety signaling, improved glucose homeostasis, and reduced intestinal transit. Activation of GPR41 on sympathetic ganglia modulates energy expenditure and heart rate. The FFAR signaling pathway contributes to the metabolic and enteroendocrine effects of butyrate but is less extensively characterized than the HDAC and GPR109A mechanisms in the context of tributyrin-specific pharmacology.

4.5 Colonocyte energy metabolism

Butyrate is the preferred oxidative substrate for colonocytes, providing approximately 60 to 70 percent of the energy supply to the colonic epithelium through mitochondrial beta-oxidation [21]. This metabolic role is distinct from the signaling mechanisms described above and is relevant to the maintenance of colonocyte viability, barrier function, and oxygen consumption that establishes the hypoxic luminal environment favorable to obligate anaerobic commensal bacteria. Tributyrin, by delivering butyrate to the colonic epithelium, supports this metabolic function in states of butyrate deficiency (antibiotic-induced dysbiosis, low-fiber diets, inflammatory bowel disease).

4.6 Composite pharmacology

The composite pharmacology of tributyrin at oral doses studied in human Phase I trials (150 to 200 mg/kg three times daily) produces median plasma butyrate concentrations of approximately 52 micromolar [4]. At this concentration, partial HDAC inhibition is achieved (IC50 approximately 50 to 500 micromolar, depending on the isoform), GPR109A activation is submaximal (the receptor responds to butyrate at low-millimolar concentrations in most assay systems), and FFAR activation is minimal at systemic concentrations but is likely substantial at luminal concentrations in the colon where butyrate accumulates after oral tributyrin administration. The principal pharmacological activity at clinically studied doses is there-

fore HDAC inhibition in accessible tissues (particularly colonocytes and hepatocytes that receive portal venous butyrate) and GPR109A-mediated anti-inflammatory signaling in the intestinal and hepatic compartments.

5. Pharmacokinetics and Routes of Administration

5.1 Absorption

Tributyryn is absorbed from the gastrointestinal tract after oral administration through two pathways. The predominant pathway involves luminal hydrolysis by pancreatic lipase, principally in the duodenum and jejunum, releasing free butyrate and 2-monobutyryn, which are absorbed across the intestinal epithelium by passive diffusion and by the monocarboxylate transporter MCT1 (SLC16A1) [3, 4]. A secondary pathway involves absorption of intact tributyrin across the intestinal epithelium, particularly in the jejunum and ileum, with subsequent intracellular hydrolysis. The relative contribution of each pathway to systemic butyrate exposure has not been precisely quantified in humans.

Tributyryn is more resistant to gastric acid hydrolysis than to pancreatic lipase hydrolysis, and a portion of the administered dose transits the stomach intact and reaches the small intestine and potentially the proximal colon before complete hydrolysis [22]. This resistance to premature degradation is the principal pharmacokinetic advantage over sodium butyrate, which dissociates immediately in aqueous solution and is absorbed primarily in the stomach and upper small intestine.

5.2 Distribution

After oral administration, butyrate released from tributyrin enters the portal venous circulation and is substantially extracted by the liver on first pass. Portal venous butyrate concentrations are therefore higher than systemic venous concentrations, and the liver is a primary target organ for butyrate-mediated HDAC inhibition and metabolic effects. Systemic plasma butyrate concentrations after oral tributyrin administration in Phase I trials reached median values of 52 micromolar (Edelman et al. 2003) with peak values in the range of 0 to 450 micromolar (Conley et al. 1998) [3, 4]. Considerable interpatient variability was observed, likely reflecting differences in gastric emptying, pancreatic lipase activity, intestinal transit, and hepatic extraction.

Butyrate is not substantially protein-bound in plasma. The volume of distribution has not been formally characterized in humans but is expected to be moderate, consistent with the distribution of a small polar molecule. Butyrate crosses cell membranes readily by passive diffusion at physiological pH (where approximately 50 percent is in the protonated, membrane-permeable form at pH 4.8, the pKa of butyric acid) and by active transport through MCT1 at neutral pH where the ionized form predominates.

5.3 Metabolism

Butyrate is metabolized rapidly by beta-oxidation in mitochondria, primarily in colonocytes (where it is the preferred oxidative substrate), hepatocytes, and skeletal muscle. The metabolic pathway proceeds through butyryl-CoA synthetase activation, beta-oxidation to acetyl-CoA, and entry into the tricarboxylic acid cycle. The hepatic first-pass extraction is substantial; systemic plasma butyrate concentrations represent a small fraction of the total butyrate absorbed from the gastrointestinal tract [3, 4].

The plasma elimination half-life of butyrate after oral tributyrin administration is approximately 40 minutes to 4 hours, depending on the dose and the analytical method used [4, 22]. The longer estimates (approximately 4 hours) for tributyrin-derived butyrate compared to free butyrate (approximately 2 to 3 hours) reflect the sustained release from intracellular hydrolysis of absorbed intact tributyrin and from continued luminal hydrolysis of tributyrin transiting the distal small intestine.

5.4 Elimination

Butyrate is oxidized to carbon dioxide and water; there is no appreciable renal excretion of intact butyrate or of tributyrin. The elimination is entirely metabolic (beta-oxidation) rather than renal or biliary clearance.

5.5 Routes of administration

Oral administration is the only route used in human clinical trials and dietary supplement applications. Tributyrin is supplied as soft gelatin capsules or as liquid-filled hard capsules for oral use. Intraperitoneal administration has been used in rodent preclinical studies (typically at 2 g/kg body weight in the hepatocarcinogen-

esis chemoprevention model) [10]. Intravenous, subcutaneous, and other parenteral routes have not been studied in humans. Tributyrin has been added directly to cell culture media at concentrations of 0.1 to 4 mM for in vitro studies; the oily liquid is typically dissolved in dimethyl sulfoxide or ethanol as a vehicle before dilution into aqueous media.

5.6 Special populations

No formal pharmacokinetic studies in renal impairment, hepatic impairment, pediatric, or geriatric populations have been published. The Phase I oncology trials enrolled adults with advanced solid tumors and did not stratify by hepatic or renal function. Given the metabolic (beta-oxidation) rather than renal elimination pathway, renal impairment is not expected to substantially alter butyrate pharmacokinetics. Hepatic impairment may reduce first-pass extraction and increase systemic butyrate exposure, but this has not been characterized.

6. Clinical Evidence Base

6.1 Phase I oncology trials

The clinical evidence for tributyrin in humans is limited to two Phase I trials in advanced solid tumor patients, both designed to characterize pharmacokinetics and safety rather than efficacy.

The Conley et al. (1998) Phase I study enrolled 13 patients with refractory solid tumors and administered oral tributyrin at escalating doses from 50 to 400 mg/kg/day, given once daily after an overnight fast, for 3 weeks followed by a 1-week rest period [3]. Peak plasma butyrate concentrations occurred between 0.25 and 3 hours after dosing, increased with dose, and ranged from undetectable to 450 micromolar. No dose-limiting toxicity was observed. The principal adverse events were mild nausea and gastrointestinal discomfort. The study demonstrated that oral tributyrin could achieve plasma butyrate concentrations in the range associated with in vitro HDAC inhibition and differentiation induction.

The Edelman et al. (2003) study enrolled 20 patients with advanced solid tumors and administered tributyrin at 150 to 200 mg/kg three times daily [4]. The three-times-daily regimen was adopted to sustain plasma butyrate concentrations above the HDAC-inhibitory threshold for a greater proportion of the dosing interval. Median plasma butyrate concentration was 52 micromolar, with substantial inter-patient variability. No dose-limiting toxicity was observed. Dose escalation was halted at 200 mg/kg three times daily because of the large number of capsules required (up to 40 capsules per dose for a 70 kg patient), not because of safety concerns. The study confirmed tolerability and pharmacokinetic feasibility but did not demonstrate antitumor responses, consistent with the Phase I design.

6.2 Combination oncology studies

Tributyrin has been studied in combination with all-trans retinoic acid (ATRA) for acute myelogenous leukemia in elderly patients, on the basis of preclinical evidence that butyrate and retinoids produce synergistic differentiation induction in leukemia cell lines [5]. The clinical data from this combination are limited to early-phase investigations and have not produced registration-quality efficacy evidence.

6.3 Parkinson's disease

A clinical trial (NCT05446168) registered at ClinicalTrials.gov is investigating tributyrin supplementation in Parkinson's disease patients to assess brain short-chain fatty acid metabolism using magnetic resonance spectroscopy [15]. The rationale is that Parkinson's disease is associated with gut microbiome dysbiosis, reduced fecal butyrate concentrations, and intestinal barrier dysfunction, and that exogenous butyrate delivery may address these deficits. The trial is exploratory and is not powered for clinical efficacy endpoints.

6.4 Dietary supplement applications

Tributylin is marketed as a dietary supplement for gut health, microbiome support, and intestinal barrier maintenance. Clinical evidence supporting these supplement applications is limited to a small pilot human study (Smith et al. 2024) that assessed tolerability and potential health benefits of a novel butyrate-generating supplement containing tributyrin in healthy adults [23]. The study reported that tributyrin was well tolerated by all individuals, with no adverse effects related to the supplement. Formal randomized controlled trials supporting specific health claims for tributyrin supplementation in healthy or clinical populations have not been published.

7. Reported Research Dose Ranges

7.1 Human clinical doses

Phase I oncology trials: 50 to 400 mg/kg/day (Conley et al. 1998), administered once daily; 150 to 200 mg/kg three times daily (Edelman et al. 2003), corresponding to approximately 10.5 to 14 g/day for a 70 kg patient at the highest dose levels [3, 4].

Dietary supplement doses: Commercially available tributyrin supplements are typically dosed at 150 to 2000 mg per day, divided into one to three doses. These doses are substantially lower than the Phase I oncology doses and are intended for gastrointestinal and general health support rather than systemic HDAC inhibition.

7.2 Preclinical doses

Hepatocarcinogenesis chemoprevention (rat): 2 g/kg body weight daily by oral gavage for 8 weeks [10].

Obesity and metabolic inflammation (mouse): 2 g/kg body weight daily by oral gavage in high-fat diet models [8].

Intestinal barrier and ethanol injury (mouse): 5 mM tributyrin by oral gavage, daily, in acute and chronic-binge ethanol models [12, 16, 17].

Muscle satellite cell differentiation (piglet): Dietary supplementation at 0.5 percent of total feed for 21 days [13, 14].

In vitro cell culture: 0.1 to 4 mM tributyrin in culture medium, with 0.5 to 2 mM being the most commonly used concentration range for differentiation and apoptosis studies [19, 20, 24].

7.3 Dose-response considerations

The dose-response relationship for tributyrin is complex because the pharmacologically active moiety (butyrate) has distinct concentration thresholds for its three principal mechanisms. HDAC inhibition requires sustained butyrate concentrations of approximately 50 to 500 micromolar; GPR109A activation requires low-mil-

limolar concentrations; and colonocyte metabolic effects occur at luminal concentrations that are typically in the millimolar range and are achieved by both dietary fiber fermentation and oral tributyrin supplementation. The clinical dose required to achieve a specific pharmacological effect therefore depends on the target mechanism and the target tissue compartment.

8. Sourcing and Quality Verification

Tributyryn is commercially available from multiple sources in food-grade, supplement-grade, and research-grade purities.

Food-grade and supplement-grade: Tributyrin is manufactured by several ingredient suppliers (Compound Solutions, Inc. under the CoreBiome brand; Healthy Origins; and others) for incorporation into dietary supplement capsules. Supplement-grade material is typically supplied at greater than 95 percent tributyrin by gas chromatography, with residual components being dibutyryn, monobutyryn, glycerol, and free butyric acid. The FDA GRAS classification (FEMA 2223) applies to tributyrin used as a food flavoring agent; supplement manufacturers rely on the GRAS classification and on the Dietary Supplement Health and Education Act (DSHEA) framework for regulatory authorization.

Research-grade: Tributyrin is supplied by Sigma-Aldrich (catalog W222322 and others), TCI America (catalog T0364), Cayman Chemical, Selleckchem, Santa Cruz Biotechnology, and other chemical suppliers at greater than 97 percent purity. Research-grade material is suitable for in vitro cell culture, in vivo animal studies, and analytical reference applications.

Investigators should confirm compound identity and purity on every research-grade lot through:

- Gas chromatography or high-performance liquid chromatography for tributyrin content and for dibutyryn and monobutyryn impurities
- Acid value determination (a measure of free butyric acid from hydrolysis; acceptable limits are typically less than 1 mg KOH/g)
- Karl Fischer titration for residual water content
- Refractive index measurement as a rapid identity confirmation (expected approximately 1.435 at 20 degrees Celsius)
- Infrared spectroscopy for functional group confirmation (ester carbonyl stretch at approximately 1740 cm⁻¹)

Third-party analytical confirmation is advisable for any investigation where dose-response precision is important, particularly for in vivo studies where the butyrate delivery rate depends directly on the purity and hydrolytic state of the starting material.

9. Reconstitution and Handling

Tributylin is a liquid at room temperature and does not require reconstitution for most applications.

Storage: Store at 2 to 8 degrees Celsius under inert atmosphere (nitrogen or argon) for long-term storage. The compound is stable at room temperature for short periods (days to weeks) but is susceptible to hydrolysis in the presence of moisture, producing free butyric acid and an increasingly offensive odor. Protect from moisture; use desiccant in the storage container. Protect from light for long-term storage, although the compound is not acutely photosensitive.

In vitro use: Tributyrin is practically insoluble in water and must be dissolved in an organic vehicle for cell culture applications. Dimethyl sulfoxide (DMSO) is the standard vehicle; prepare a stock solution at 100 to 500 mM in DMSO, filter-sterilize through a 0.22-micron syringe filter, and dilute into culture medium to the desired final concentration (typically 0.1 to 4 mM). Final DMSO concentration in culture medium should not exceed 0.1 to 0.5 percent to avoid vehicle toxicity. Ethanol may be substituted as a vehicle where DMSO interference with the assay is a concern. Stock solutions in DMSO are stable at minus 20 degrees Celsius for at least 6 months.

In vivo use (preclinical): For oral gavage in rodent studies, tributyrin is typically administered neat (undiluted) or dissolved in corn oil or olive oil as a vehicle, at volumes of 5 to 10 mL/kg body weight. The oily consistency is compatible with standard gavage needles. For intraperitoneal injection, tributyrin is dissolved in a biocompatible vehicle (corn oil, olive oil, or polyethylene glycol 400) and administered at the study dose.

Supplement capsules: Commercial tributyrin supplements are supplied as liquid-filled soft gelatin capsules or as powder-filled capsules containing tributyrin adsorbed onto a solid carrier (silicon dioxide, medium-chain triglyceride powder). No reconstitution is required; capsules are taken orally with water.

Handling precautions: Tributyrin has low acute toxicity (oral LD50 in rats is greater than 5 g/kg). Standard laboratory personal protective equipment (gloves, lab coat, eye protection) is sufficient. The compound and its hydrolysis product (bu-

tyric acid) have an offensive rancid butter odor; work in a well-ventilated area or fume hood. Avoid prolonged skin contact, as the oily consistency impedes removal and the butyric acid released on hydrolysis is a mild skin irritant.

10. Stack Interaction Considerations

Tributyryn interacts with several compound classes through the pharmacological activity of its butyrate metabolite and through physicochemical compatibility considerations. Investigators conducting research with tributyrin in combination regimens should consider the following:

Other HDAC inhibitors: Concurrent use with pharmaceutical HDAC inhibitors (vorinostat, romidepsin, panobinostat, entinostat, tucidinostat) produces additive or synergistic histone hyperacetylation. The combination has been studied in pre-clinical models and is therapeutically rational for oncology applications, but the additive HDAC inhibition may increase the risk of gastrointestinal adverse events (nausea, diarrhea) and of hematologic toxicity characteristic of the pharmaceutical HDAC inhibitor class.

Retinoids: All-trans retinoic acid (ATRA) and tributyrin produce synergistic differentiation induction in acute myelogenous leukemia and hepatocellular carcinoma cell lines [5]. The combination was the basis for early clinical investigation in elderly AML patients. The retinoid-butyrate synergy operates through complementary transcriptional mechanisms (retinoid receptor activation plus HDAC-mediated chromatin remodeling) and is among the best-characterized tributyrin combination pharmacologies.

Vitamin D analogs: 1,25-dihydroxycholecalciferol (calcitriol) and tributyrin produce synergistic antiproliferative effects in colon cancer cell lines [24]. The combination enhances vitamin D receptor-mediated transcription of differentiation genes through butyrate-mediated chromatin remodeling at vitamin D-responsive promoter elements.

Probiotics: Lactobacillus GG and tributyrin produce additive protection against antibiotic-induced intestinal injury in preclinical models [25]. The combination is mechanistically rational (probiotic restoration of commensal community plus direct butyrate supplementation of the colonocyte energy substrate) and is compatible with the supplement market positioning of tributyrin.

Fiber and prebiotics: Dietary fiber (inulin, fructooligosaccharides, resistant starch) is fermented by colonic microbiota to produce endogenous butyrate. Concurrent tributyrin and prebiotic supplementation produces additive colonic butyrate concentrations and may be synergistic for intestinal barrier maintenance. The combination is widely used in functional nutrition and supplement formulations.

CYP enzyme interactions: Butyrate is not metabolized by cytochrome P450 enzymes and does not inhibit or induce major CYP isoforms at pharmacologically relevant concentrations. Tributyrin is therefore not expected to produce clinically significant CYP-mediated drug-drug interactions.

Niacin (nicotinic acid): Butyrate and niacin are both GPR109A agonists. Concurrent administration produces additive receptor activation and potentially additive flushing (the niacin-associated prostaglandin-mediated cutaneous vasodilation mediated through GPR109A on Langerhans cells). The interaction is pharmacologically predictable but not well characterized clinically.

Anticoagulants and antiplatelet agents: No characterized interaction. Butyrate does not have known anticoagulant or antiplatelet properties at pharmacologically relevant concentrations.

11. Adverse Events and Safety

11.1 Regulatory status

Tributyryn is classified as GRAS (Generally Recognized as Safe) by the United States FDA for use as a food flavoring agent (FEMA 2223). This classification reflects a long history of consumption as a natural milk fat component and the absence of identified safety concerns at food-use levels.

11.2 Common adverse events

The principal adverse events observed in Phase I oncology trials and in supplement tolerability studies are gastrointestinal in nature:

- Nausea (dose-dependent; the most commonly reported event in Phase I trials at doses above 100 mg/kg) [3, 4]
- Eructation with unpleasant taste (reflecting the release of butyric acid in the stomach; more common with liquid formulations and higher doses)
- Diarrhea (mild; consistent with the osmotic and motility effects of butyrate on colonocytes)
- Abdominal discomfort or bloating (typically self-limiting within the first few days of supplementation)

These events are generally mild (Grade 1 on the Common Terminology Criteria for Adverse Events), self-limiting, and manageable by dose titration (starting at lower doses and gradually increasing over 1 to 2 weeks).

11.3 Dose-limiting considerations

No dose-limiting toxicity was identified in either Phase I oncology trial [3, 4]. Dose escalation was halted at 200 mg/kg three times daily in the Edelman et al. (2003) study owing to the impractical number of capsules required (approximately 40 capsules per dose for a 70 kg patient), not owing to toxicity. The maximum tolerated dose of tributyrin in humans has not been formally established.

11.4 Preclinical toxicology

The oral LD50 of tributyrin in rats is greater than 5 g/kg body weight, placing it in the lowest toxicity category (category 5 or "unclassified" by Globally Harmonized System criteria). Subchronic toxicology studies in rats at oral doses of 2 g/kg/day for 8 weeks (the hepatocarcinogenesis chemoprevention dose) have not reported treatment-emergent organ toxicity beyond the expected gastrointestinal effects [10].

11.5 Reproductive and developmental toxicology

No formal reproductive or developmental toxicology studies of tributyrin have been published. Butyric acid is a normal physiological metabolite present in the colonic lumen and in portal venous blood; no teratogenic or reproductive toxicity signal has been identified for butyrate at physiological or moderately supra-physiological concentrations. Pregnant and lactating individuals should consult their healthcare provider before using tributyrin supplements.

11.6 Drug interaction safety

Butyrate is not metabolized by cytochrome P450 enzymes and is not expected to produce CYP-mediated drug interactions. The principal interaction concern is additive HDAC inhibition with pharmaceutical HDAC inhibitors (see Section 10), which is relevant only in oncology research settings and not in typical dietary supplement use.

11.7 Supplement market considerations

Tributyrin supplements are marketed under the DSHEA regulatory framework in the United States and under equivalent food supplement frameworks in other jurisdictions. They are not subject to premarket drug approval. Consumers should be aware that manufacturing quality varies across supplement brands; independent third-party testing (NSF International, USP Verified, ConsumerLab) provides assurance of identity, purity, and label accuracy.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five alternative butyrate delivery forms against tributyrin, evaluated on five competency standards: bioavailability (the fraction of administered butyrate that reaches the target compartment), effect size (magnitude of biological or clinical effect), delivery specificity (capacity to deliver butyrate to the colon and to systemic circulation selectively), tolerability profile (adverse events and patient acceptability), and overall validation (depth and quality of supporting evidence). The five comparators were selected as the principal butyrate delivery vehicles with substantive research and commercial presence.

12.1 Sodium butyrate

Structure and delivery: Sodium butyrate ($\text{NaC}_4\text{H}_7\text{O}_2$, MW 110.09) is the sodium salt of butyric acid. It dissociates immediately and completely in aqueous solution, releasing free butyrate and sodium ion. In an uncoated capsule, essentially 100 percent of the butyrate content is released in the stomach and upper small intestine, with minimal delivery to the colon in the absence of enteric coating or microencapsulation [22].

Bioavailability: Systemic butyrate bioavailability from sodium butyrate is low and transient, with rapid absorption in the proximal gastrointestinal tract and extensive hepatic first-pass extraction. Peak plasma butyrate concentrations after oral sodium butyrate are brief and variable. Colonic delivery is poor without formulation technology to protect the compound from proximal absorption.

Effect size: In vitro, sodium butyrate is the reference standard for butyrate-mediated HDAC inhibition, differentiation induction, and apoptosis in cancer cell lines. In vivo, the effect size is limited by the pharmacokinetic constraints described above. Clinical studies using sodium butyrate enemas for ulcerative colitis have demonstrated modest benefit.

Delivery specificity: Low for colonic delivery in the absence of enteric coating. High for proximal gastrointestinal and hepatic exposure. Microencapsulated sodium butyrate formulations (enteric-coated, polymer-matrix) improve colonic delivery but add formulation complexity and cost.

Tolerability profile: The principal limitations are the offensive odor (butyric acid is released immediately on dissolution) and the unpleasant taste. These reduce patient compliance, particularly for chronic supplementation. Enteric-coated formulations mitigate the odor and taste.

Overall validation: High for in vitro applications; moderate for in vivo and clinical applications. Sodium butyrate is the most extensively studied butyrate form and the reference standard against which other delivery forms are compared.

Comparison to tributyrin: Tributyrin is superior to uncoated sodium butyrate in colonic delivery specificity, odor control, and sustained plasma butyrate exposure. Well-formulated microencapsulated sodium butyrate may approach tributyrin in colonic delivery but at greater formulation complexity. Sodium butyrate is lower in cost and is more extensively validated in clinical studies (particularly in inflammatory bowel disease enema studies).

12.2 Calcium/magnesium butyrate

Structure and delivery: Calcium butyrate ($\text{Ca}(\text{C}_4\text{H}_7\text{O}_2)_2$, MW 214.26) and magnesium butyrate ($\text{Mg}(\text{C}_4\text{H}_7\text{O}_2)_2$, MW 198.50) are divalent cation salts of butyric acid. They dissociate in aqueous solution, releasing two butyrate equivalents and the cation.

Bioavailability: Similar to sodium butyrate; the butyrate is released and absorbed primarily in the proximal gastrointestinal tract. The divalent cation salts may provide marginally slower dissolution than sodium butyrate, but the difference is not pharmacokinetically significant without additional formulation technology.

Effect size: Comparable to sodium butyrate in vitro. In vivo evidence is limited; most preclinical and clinical butyrate studies have used sodium butyrate or tributyrin rather than calcium or magnesium salts.

Delivery specificity: Low for colonic delivery without enteric coating, similar to sodium butyrate.

Tolerability profile: Comparable to sodium butyrate with respect to odor and taste. The cation (calcium or magnesium) is a minor nutritional contribution. Some consumers prefer these salts to reduce sodium intake.

Overall validation: Low to moderate. The calcium and magnesium butyrate salts have substantially less published research support than either sodium butyrate or tributyrin. They are primarily marketed as dietary supplements.

Comparison to tributyrin: Tributyrin is superior in delivery specificity, odor control, and depth of supporting research. Calcium/magnesium butyrate salts offer the practical advantage of mineral co-supplementation but do not provide pharmacokinetic advantages over tributyrin.

12.3 Sodium phenylbutyrate

Structure and delivery: Sodium phenylbutyrate (C₁₀H₁₁NaO₂, MW 186.19) is the sodium salt of 4-phenylbutyric acid, an aromatic butyrate analog approved by the FDA as Buphenyl for the treatment of urea cycle disorders. It is a prodrug of phenylacetate, not of butyrate; its mechanism of action in urea cycle disorders is as a nitrogen scavenger (phenylacetate conjugates with glutamine to form phenylacetylglutamine, which is renally excreted).

Bioavailability: Sodium phenylbutyrate is well absorbed orally and is extensively metabolized to phenylacetate by beta-oxidation. It does not release butyrate; its pharmacology is fundamentally different from that of tributyrin despite the structural similarity in the name.

Effect size: In the context of HDAC inhibition, phenylbutyrate has been studied as an HDAC inhibitor in oncology with IC₅₀ values in the low-millimolar range, higher than butyrate. Clinical oncology trials of phenylbutyrate have produced modest and inconsistent results. In the context of urea cycle disorders, it is the standard of care.

Delivery specificity: Not applicable for colonic butyrate delivery; the compound does not release butyrate.

Tolerability profile: Well characterized; the principal adverse events are the offensive body odor from phenylacetate metabolites, nausea, and transient amenorrhea in premenopausal women.

Overall validation: High for urea cycle disorders (FDA-approved). Moderate for HDAC inhibition in oncology (Phase I/II data). Not validated for butyrate-specific mechanisms.

Comparison to tributyrin: Sodium phenylbutyrate is mechanistically distinct from tributyrin and is not a true butyrate delivery vehicle. It is included in this comparison because the structural name similarity leads to frequent confusion. Investigators seeking butyrate-specific pharmacology (HDAC inhibition by butyrate, GPR109A agonism, colonocyte energy substrate) should use tributyrin or butyrate salts, not phenylbutyrate.

12.4 Butyrate-producing probiotics

Structure and delivery: Certain commensal bacteria (*Faecalibacterium prausnitzii*, *Roseburia intestinalis*, *Clostridium butyricum*, *Eubacterium rectale*) produce butyrate as a fermentation end-product from dietary fiber. Probiotic formulations containing these organisms deliver butyrate indirectly by colonizing the colon and fermenting available substrates.

Bioavailability: Variable and dependent on successful colonization, substrate availability (dietary fiber), and host microbiome composition. Butyrate concentrations achieved in the colonic lumen are in the physiological range (5 to 20 mM) when colonization is successful, but are unpredictable and patient-specific.

Effect size: *Clostridium butyricum* (MIYAIRI 588) has demonstrated clinical benefit in antibiotic-associated diarrhea, irritable bowel syndrome, and ulcerative colitis in randomized trials, though the contribution of butyrate production specifically (versus other probiotic mechanisms) is difficult to isolate.

Delivery specificity: High for colonic delivery (butyrate is produced in situ). Negligible for systemic delivery; probiotic-derived butyrate is consumed locally by colonocytes and contributes minimally to portal or systemic butyrate concentrations.

Tolerability profile: Generally well tolerated. Rare cases of bacteremia have been reported with *Clostridium butyricum* in immunocompromised patients.

Overall validation: Moderate for colonic applications; low for systemic applications. The butyrate-specific contribution of probiotic therapy is difficult to quantify separately from other probiotic mechanisms (competitive exclusion, immune mod-

ulation, mucin stimulation).

Comparison to tributyrin: Tributyrin provides predictable, dose-dependent butyrate delivery to both the colonic and systemic compartments. Butyrate-producing probiotics provide physiological colonic butyrate delivery but are variable in magnitude and do not contribute to systemic butyrate exposure. The two approaches are complementary and are commonly combined in functional nutrition formulations.

12.5 Resistant starch and prebiotic fibers

Structure and delivery: Resistant starch (RS2, RS3, RS4 types), inulin, fructooligosaccharides, and other prebiotic fibers are fermented by colonic microbiota to produce short-chain fatty acids including butyrate, propionate, and acetate. The butyrate yield depends on the fiber type, the microbiome composition, and the fermentation kinetics.

Bioavailability: Colonic butyrate concentrations from prebiotic fiber fermentation are in the physiological range (5 to 20 mM) and are the principal source of endogenous butyrate. Systemic butyrate concentrations from fiber fermentation are minimal (low-micromolar portal venous, negligible peripheral venous).

Effect size: Epidemiological data consistently associate high-fiber diets with reduced colorectal cancer risk, improved glycemic control, and reduced markers of systemic inflammation. Interventional studies with resistant starch and other prebiotic fibers have demonstrated increased fecal butyrate concentrations and improved markers of colonic health.

Delivery specificity: High for colonic delivery. Negligible for systemic delivery.

Tolerability profile: Generally well tolerated. The principal adverse events are gas, bloating, and abdominal distension, which typically resolve with gradual dose titration. Prebiotic fibers are broadly acceptable for chronic use.

Overall validation: High for colonic butyrate delivery and for association with health outcomes. The mechanistic attribution of health benefits specifically to butyrate (versus other short-chain fatty acids and fiber effects) is incomplete.

Comparison to tributyrin: Tributyrin provides direct, dose-quantifiable butyrate delivery independent of microbiome composition. Prebiotic fibers provide indirect butyrate delivery that is dependent on the microbiome and that produces a mixture of short-chain fatty acids. The two approaches are complementary. Tributyrin is preferable when a defined butyrate dose is required (pharmacological or research applications); prebiotic fibers are preferable for long-term dietary health support and for overall microbiome nourishment.

12.6 Comparator summary

The five comparators occupy distinct positions in the butyrate delivery landscape. **Sodium butyrate** is the most extensively studied form and the reference standard for in vitro work but has poor colonic delivery specificity and objectionable odor in uncoated formulations. **Calcium/magnesium butyrate** salts are practical supplement alternatives with mineral co-supplementation but limited pharmacokinetic advantage. **Sodium phenylbutyrate** is a mechanistically distinct compound that does not deliver butyrate and is included only to clarify nomenclature confusion. **Butyrate-producing probiotics** provide physiological colonic butyrate but are variable and do not contribute to systemic exposure. **Prebiotic fibers** provide indirect, microbiome-dependent butyrate delivery with extensive epidemiological support.

Tributyrin occupies a unique position as the only delivery form that combines gastric acid resistance, sustained and dose-proportional plasma butyrate exposure, both luminal and intracellular butyrate release, reduced odor relative to free butyrate salts, GRAS regulatory status, and a human Phase I clinical pharmacokinetic dataset. Its principal limitation is the small and incomplete clinical evidence base (two Phase I oncology trials, no Phase II or Phase III data, no registration-quality efficacy trials in any indication). The preclinical pharmacology is extensive and spans multiple therapeutic areas, providing a strong scientific foundation that has not yet been translated into late-phase clinical development.

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