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Benzoate and Sorbate Salts: A Systematic Review of the Potential Hazards of These Invaluable Preservatives and the Expanding Spectrum of Clinical Uses for Sodium Benzoate

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Abstract: Sodium benzoate and potassium sorbate are extremely useful agents for food and beverage preservation, yet concerns remain over their complete safety. Benzoate can react with the ascorbic acid in drinks to produce the carcinogen benzene. A few children develop allergy to this additive while, as a competitive inhibitor of D-amino acid oxidase, benzoate can also influence neurotransmission and cognitive functioning. Model organism and cell culture studies have raised some issues. Benzoate has been found to exert teratogenic and neurotoxic effects on zebrafish embryos. In addition, benzoate and sorbate are reported to cause chromosome aberrations in cultured human lymphocytes; also to be potently mutagenic toward the mitochondrial DNA in aerobic yeast cells. Whether the substantial human consumption of these compounds could significantly increase levels of such damages in man is still unclear. There is no firm evidence that it is a risk factor in type 2 diabetes. The clinical administration of sodium benzoate is of proven benefit for many patients with urea cycle disorders, while recent studies indicate it may also be advantageous in the treatment of multiple sclerosis, schizophrenia, early-stage Alzheimer's disease and Parkinson's disease. Nevertheless, exposure to high amounts of this agent should be approached with caution, especially since it has the potential to generate a shortage of glycine which, in turn, can negatively influence brain neurochemistry. We discuss here how a small fraction of the population might be rendered—either through their genes or a chronic medical condition—particularly susceptible to any adverse effects of sodium benzoate.

Keywords: DNA damage, food preservatives, potassium sorbate, sodium benzoate, urea cycle disorder therapy

Introduction

Until recently, the extensive use of benzoate and sorbate salts for large-scale food and drink preservation was regarded as completely safe. Claims to this effect are often still issued by the organizations that represent the soft drink industry. Such statements reflect the long history of apparently safe use of these preservatives and a safety testing that has largely focused on the maximum levels of these compounds that could be tolerated without adverse effects in the diet of laboratory animals (Final report on the safety assessment of sorbic acid and potassium sorbate 1988; Andersen 2001; Nair 2001). The maximum levels of benzoate and sorbate permitted in food and drinks are based on these studies. Ever since these original safety tests, there have been dramatic advances in the technologies available to investigate damage to cells and tissues, providing opportunities for much deeper investigation of the effects of these additives and the consequences of their long-term, large-scale dietary consumption. Indeed, we are now aware of mechanisms of damage to biological systems that were completely unknown at the time of much of the original safety testing of these preservatives.

Given the considerable value of benzoate and sorbate salts as simple practical preservatives and the very substantial levels of these compounds consumed by world populations, it was inevitable that diverse effects of these compounds would be investigated by the wider academic community. We have sought to overview the relevant peer-reviewed literature for 2 reasons. First, it is given scant attention in the literature of regulatory bodies such as the U.S. Food and Drug Administration (FDA) and European Food Standards Authority (EFSA). Second, as discussed later, there is an ever-increasing need to evaluate the safety of sodium benzoate (SB) in view of a rapidly expanding body of medical research, which indicates that this compound may provide inexpensive therapy for a number of diseases, in addition to its well-established use in the treatment of urea cycle disorders (UCDs).

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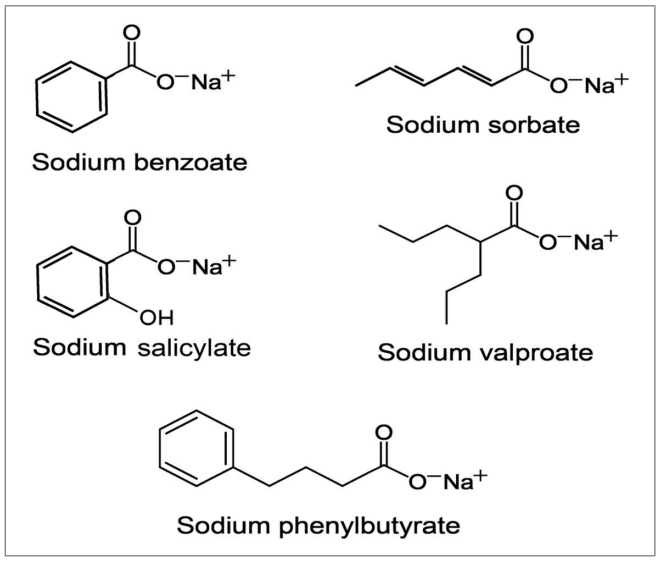

Figure 1–The monocarboxylate compounds discussed in this article, shown as their sodium salts.

It is important to appreciate that a low exposure to benzoate will be inevitable for most individuals, as this is a compound that is both generated by the actions of our gut microbes (Beyoğlu and Idle 2012) and present—usually in relatively low concentrations <40 mg/kg—in many berries and milk products (Rangan and Barceloux 2009a, 2009b; Vandevijvere and others 2009). Benzoate can arise from the hippuric acid naturally occurring in milk at concentrations of up to 50 mg/kg, and forms during cheese ripening (Sieber and others 1995). However, many individuals are consuming considerably larger amounts of this preservative (it has been reported that Coca Cola, lemonade, and iced tea can contain 112 to 146 mg/L benzoate, just under the regulatory level of 150 mg/L; while prepared salads, emulsified sauces, and dressings can have 500 to 850 mg/kg benzoate (Vandevijvere and others 2009)). One issue is whether there should be any serious concerns over this much higher exposure to SB or potassium sorbate (PS). As this article will elaborate, certain medical conditions might render individuals particularly susceptible to any adverse effects of consuming foods and drinks containing the maximum permitted levels of these preservatives.

In this review, reference will also be made to 3 other small monocarboxylate compounds whose properties may overlap with, and yield insights into, the possible biological actions of PS and SB. These are sodium salicylate (the active metabolite of the widely used oral analgesic and anti-inflammatory drug aspirin [acetylsalicylic acid] (Baltazar and others 2011)); sodium valproate (widely used in the clinical treatment of epilepsy (Loscher 2002)); and sodium phenylbutyrate (used in the clinical treatment of UCDs (Husson and others 2016); Figure 1).

The Capacity of Sorbate and Benzoate Preservatives to Promote the Generation of Mutagenic and Carcinogenic Compounds

Sorbate can promote the formation of mutagenic or carcinogenic compounds, notably in the presence of nitrite

Sorbic acid and sorbates have a very low mammalian toxicity. There is a general consensus that they are intrinsically devoid of carcinogenic activity, but have the potential to undergo a conversion to potential mutagens. In tests on Syrian hamster embryo fibroblasts, Chinese hamster ovary cells, or bone marrow cells, no genotoxic or cell-transforming activity was detected with freshly prepared sodium sorbate solution. However, products of sodium sorbate with genotoxic and cell-transforming properties were formed under conditions of heating and storage (Münzner and others 1990; Schiffmann and Schlatter 1992). Sorbate can undergo oxidation to 4,5-oxohexanoate (Jung and others 1992) and oxidized PS can react with ascorbic acid in the presence of ferrous iron (Kitano and others 2002). Much attention has been focused on the reactions between sorbate and nitrite at pH2-4.2, conditions that mimic the gastric environment. Among the products of such reactions are the mutagenic agents 1,4-dinitro-2-methylpyrrole and ethylnitrolic acid (Hayatsu and others 1975; Namiki and others 1980; Hartman 1983; Binstok and others 1998; Perez-Prior and others 2008).

There is 1 report of hepatoma arising from the feeding of mice on a diet of very high (15% w/v) sorbic acid, this being correlated with a depletion of the levels of reduced glutathione (GSH) in the mouse liver (Tsuchiya and Yamaha 1984). Causation of this hepatoma was attributed to the oxidative stress caused by the depleted GSH pool, together with the gradual production of various mutagens in the intestine, mutagens that following their absorption were transferred to the liver where they were, in turn, metabolically activated to carcinogenic compounds (Tsuchiya and Yamaha 1984; Nishimaki-Mogami and others 1991).

The potential of benzoate to undergo decarboxylation, thereby generating benzene

Certain beverages containing benzoate salts and ascorbic or erythorbic acids have been found to contain low (ng/g) levels of the carcinogen benzene (Gardner and Lawrence 1993). This benzene is thought to form during storage through decarboxylation of the benzoate by hydroxyl radicals. Elevated temperatures and ultraviolet light can accelerate, while sugar and metal ion-chelating agents can inhibit, such hydroxyl radical formation catalyzed by trace levels of metal ions.

Since the 1990s, food safety organizations have conducted surveys to determine the levels of benzene in retail beverages. Many companies have, in turn, responded to this benzene problem by reformulating those products that were found to contain benzene, substituting PS for the SB in soft drinks or—where possible—eliminating the preservative altogether. There may be additional benefits of this use of sorbate, as compared to benzoate, notably a prevention of the allergic response or altered cognitive function effects of benzoate described in more detail later in this article. A few cases of children having benzoate allergy were recently confirmed (Jacob and others 2016), while benzoate has been cited as a food additive that might be a contributory factor to hyperactivity in children (Eigenmann and Haenggeli 2007). However, the usage of benzoate in cosmetics does appear to be on the increase (Jacob and others 2016).

It is important to see this benzene contamination in perspective. Benzene can occur naturally in small amounts in a number of fruits, including mangoes, cranberries, prunes, greengages, and cloudberries, as well as fruit juices with naturally occurring benzoic and ascorbic acids. Second, our major exposure to benzene is from the atmosphere. On average, most people inhale 220 μg benzene every day from exhaust emissions, whereas cigarette smokers may be exposed to up to 7900 μg/d (Lindner and others 2011; Falzone and others 2016). While, as described below, benzene is potentially very harmful, it is improbable that the low levels of

benzene in soft drinks are leading to any appreciable increase in benzene exposure for most individuals.

The mechanistic basis of toxicity to benzene. Are there similarities to the possible weak genotoxic effects of sorbate and benzoate?

The mechanistic basis of benzene toxicity has been under investigation for a number of years, this after all being a chemical that is used in many industrial processes, as well as present in cigarette smoke and vehicle exhaust fumes. Individuals can differ greatly in their susceptibility to this pollutant. The uptake of benzene into the body and the subsequent excretion of S-phenylmercapturic acid in the urine is dependent on glutathione S-transferase T1, an enzyme that differs appreciably between and among African American, White, and Japanese American cigarette smokers and which is correlated with their lung cancer risks (Haiman and others 2016). Furthermore minimizing community-level exposure to benzene may be especially important for the prevention of insulin resistance (IR) in older adults, in view of the finding of an association of IR with urinary benzene levels in the urban elderly, independent of traditional risk factors (Choi and others 2014).

In the liver, benzene can also be metabolized to trans,trans-muconic acid (Grotz and others 1994) or partially oxidized to hydroquinone (HQ). Multiple detoxification systems, including NAD(P)H:quinone oxidoreductase 1 (NQO1) and glutathione, protect us against benzene metabolite-induced toxicity (Moran and others 1999). NQO1, a 2-electron reductase, detoxifies quinones derived from the oxidation of phenolic metabolites of benzene. Mutations in the gene for NQO1 are associated with an increased risk of hematotoxicity after exposure to benzene, as well as susceptibility to various forms of cancer (Moran and others 1999). Neither benzene nor its hepatic metabolites would appear to lead to the production of the highly electrophilic metabolites that chemically modify DNA *in vivo*. Instead, there is now substantial evidence that they mainly cause chromosome aberrations, notably aneuploidy (Dean 1985; Smith and others 1998; Zhang and others 1998, 2007; Snyder 2002; McHale and others 2008; Shiga and others 2010), as opposed to point mutations, such as base substitutions or frameshifts. It has long been known that occupational exposure to benzene is hemotoxic and leukemogenic (Snyder 2002), causing chromosome aberrations and aneuploidy in blood lymphocytes (Smith and others 1998; Zhang and others 1998, 2007; McHale and others 2008). Benzene has also been shown to cause mutagenicity in a number of mouse tissues, including the lung and spleen (Mullin and others 1995, 1998); mutations at the glycophorin A locus of exposed humans (Rothman and others 1995); also to modulate signal transduction pathways activated by oxidative stress that are involved in cell proliferation and apoptosis (Fenga and others 2016).

The chromosome aberrations that are induced in human lymphocytes by benzene may be similar to those reported when lymphocytes (Mpountoukas and others 2008; Zengin and others 2011; Mamur and others 2012; Pongsavee 2015), Chinese hamster cells (Abe and Sasaki 1977; Hasegawa and others 1984), or the bone marrow cells of mice (Mukherjee and others 1988) are exposed to sorbate and/or benzoate. However, not all such investigations of this nature have identified genotoxicity (Münzner and others 1990), and those that do detect it do so only when the sorbate or benzoate is used in high concentrations, or when sorbate is present together with nitrate (Mukherjee and others 1988). It is apparent, therefore, that sorbate and benzoate can only be exerting weak genotoxic effects. However, to firmly establish whether these involve the same underlying processes as those caused by benzene clearly needs further study. Equally the teratogenic and neurotoxic effects of SB reported for larval development of zebrafish embryos (Tsay and others 2007; Chen and others 2009) have yet to be confirmed in other model organism systems. SB was actually found to be protective against experimentally induced neurodegeneration in the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* (Stavinoha and others 2015).

One study indicated that potassium benzoate can induce teratogenic effects during mouse fetal eye development (Afshar and others 2013). In contrast, SB was not found to affect neural tube development in the chicken embryo (Emon and others 2015); neither was PS observed to cause teratogenic effects in pregnant mice and rats (Final report on the safety assessment of sorbic acid and potassium sorbate 1988).

Are the Preservatives in Soft Drinks a Risk Factor in Diabetes?

Large-scale consumption of soft drinks, rather than pure fruit juice, has frequently been linked with an increased risk of developing Type 2 Diabetes Mellitus (T2DM). It is still unresolved whether the preservatives in these beverages are a significant factor in this risk. In cultured murine adipocytes, SB was found to inhibit leptin release (Ciardi and others 2012). Should this study *in vitro* reflect the situation *in vivo*, such lowering of leptin levels should contribute to increases in obesity (Mangge and others 2013), increases that are one of the contributory factors in T2DM. Another randomized, controlled, crossover study of 14 overweight subjects found that the oral administration of SB does not have adverse effects on insulin or glucose homeostasis (Lennerz and others 2015). This would appear to be in stark contrast to the effects of the more hydrophilic sodium salicylate (Figure 1). Salicylates (not used as preservatives) have been known for over a century to lower glucose in T2DM (Rumore and Kim 2010). Furthermore, sodium salicylate, an agent with well-known antioxidant properties (Baltazar and others 2011), may be useful in retarding the manifestation and progression of diabetes since it protects human islets against the detrimental effects of high glucose (Zeender and others 2004; Fernandez-Real and others 2008). Iron homeostasis might also be important in these events (Guo and others 2016), in view of the evidence that iron removal improves insulin sensitivity and delays the onset of T2DM, whereas an elevation of body iron stores exerts a detrimental effect on the clinical course of obesity-related conditions (Datz and others 2013).

Actions of Sorbate and Benzoate Preservatives on Mitochondria

Benzoate has the potential to induce the mitochondrial permeability transition, a feature of the pathogenesis of Reye's syndrome

As described later, benzoate does not persist in the body but is largely excreted in the urine in the form of hippurate, the latter being the product of its conjugation with glycine in the mitochondria of the liver and kidneys. While hippurate in the diet can be converted back to benzoate by gut microbes, the hippurate synthesis in the liver and kidneys is irreversible and critically dependent on mitochondrial function (Beyoğlu and Idle 2012). Mitochondria provide most of the energy for liver cell function via oxidative phosphorylation fed by the tricarboxylic acid cycle and β oxidation of long-chain fatty acids. Mitochondria are also closely involved in cell death processes such as the mitochondrial permeability transition (MPT). MPT is the opening of a cyclosporin-sensitive

pore in the mitochondrial inner membrane, leading to mitochondrial swelling, depolarization, the uncoupling and failure of oxidative phosphorylation, formation of reactive oxygen species (ROS), and cell death by apoptosis or necrosis (Halestrap 2009).

Micromolar levels of several small monocarboxylate compounds, including salicylate, valproate, and benzoate (Figure 1) have been shown to initiate the MPT in isolated liver mitochondria (Trost and Lemasters 1996). They induce in these mitochondria the changes characteristic of the pathogenesis of Reye's syndrome, a childhood disorder that sometimes manifests following viral infection and which is characterized by hyperammonemia, microvesicular steatosis, and encephalopathy (Glasgow 2006). An inherited deficiency in isovaleryl coenzyme A dehydrogenase, leading to the accumulation of isovaleric acid, also generates an acute Reye's-like syndrome (Trost and Lemasters 1996).

Studies on salicylate and valproate (Figure 1), monocarboxylate compounds that when administered to patients severely aggravate Reye's syndrome (Glasgow 2006), provide a note of caution for the use of benzoate since all 3 of these compounds initiate the MPT in isolated liver mitochondria (Trost and Lemasters 1996). In addition, salicylate causes uncoupling and swelling in isolated mitochondria (Gutknecht 1992) and is known to be an inhibitor of cardiac respiration (Nulton-Persson and others 2004). There is therefore a distinct possibility that severe mitochondrial damage might ensue should a rapid metabolism of benzoate not occur in liver mitochondria, a situation that might arise with a shortage of glycine (hypoglycinemia). Impaired energy metabolism, onset of the MPT and mitochondrial swelling, may be the pathophysiologic mechanism causing toxic injury to liver mitochondria in Reye's syndrome (Glasgow 2006).

Sorbate and benzoate are mutagenic toward the mitochondrial genome through their ability to enhance mitochondrial ROS production

Benzoic acid (pKa 4.16) and sorbic acid (pKa 4.76) are substantially dissociated to the anion at physiological pH. These amphiphilic forms have a strong propensity to partition into the phospholipid bilayer and thereby influence membrane structure (Stratford and others 2013). The study of artificial phospholipid bilayer membranes has revealed that both benzoate and salicylate cause significant increases in membrane conductance and proton permeability, mainly by acting as lipid soluble anions at neutral pH and as proton carriers when the pH approximates to the pK of the acid (Gutknecht 1992). The actions of benzoate and sorbate on membranes are thought to be exerted mainly through a disruptive effect on membrane structure (Stratford and others 2013). In contrast, salicylate (pKa 2.97) is known to act partly as a classical uncoupling agent (Norman and others 2004; Nulton-Persson and others 2004). However, all of these weak acids have a pronounced effect on mitochondrial function, generating a decreased electron flow from substrate dehydrogenases to ubiquinone that in turn increases free electron "leakage" from the respiratory chain, electrons that then combine with molecular oxygen to produce superoxide ($O_2^{\bullet -}$). A number of other moderately lipophilic compounds of food and drink relevance, notably ethanol (Ibeas and Jiminez 1997) and certain plant essential oils (Bakkali and others 2008), including the widely used menthol (Ferreira and others 2014), generate ROS in a similar manner.

Mitochondrial ROS levels can be enhanced not just by the above opening of the MPT pore, but also by this increased electron "leakage" from the respiratory chain. Antioxidant molecules such as glutathione exit mitochondria through the open MPT pore, reducing the organelles' ability to neutralize ROS, while the electron transport chain itself suffers the loss of components such as cytochrome *c*. Studies on yeast as a model organism have revealed that both SB and PS cause a dramatic enhancement to mitochondrial ROS formation in living cells, ROS that then damage the mitochondrial DNA (mtDNA) (Piper 1999, 2011). While ROS can be a positive force for life through their apoptosis-inducing role, we generally fear them for their ability to irreversibly damage key proteins and nucleic acid molecules. Superoxide produced by the respiratory chain is not particularly reactive in itself, mostly becoming dismutated to hydrogen peroxide (H_2O_2) in a reaction catalyzed by the enzyme superoxide dismutase. While this hydrogen peroxide is, in turn, largely decomposed by catalases and peroxidases, it can also readily diffuse across membranes to sites where it encounters free, or weakly liganded, ferrous (Fe^{2+}) iron. There, it engages in reactions leading to the generation of the very dangerous hydroxyl radical (OH•) via the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\bullet + HO^-$). Superoxide itself can also react with ferric iron in the Haber-Weiss reaction ($O_2^{\bullet -} + Fe^{3+} \rightarrow O_2 + Fe^{2+}$) so as to again produce Fe^{2+}, thereby effecting a redox cycling process in which the free iron is catalytic. It is this combination of poorly liganded Fe^{2+} or Fe^{3+} in the presence of peroxide/superoxide, leading to the formation of the highly reactive hydroxyl radical, which is potentially so damaging to living systems. Cells of normal, healthy individuals maintain very low levels of free, unliganded Fe^{2+} in their cytosol as part of their defense against oxidative damage (Halliwell and Gutteridge 1998). Hence, their major pools of unliganded Fe^{2+} or Fe^{3+} are confined to the lysosome.

Functional mitochondria are essential for our survival, the assembly of the mitochondrial respiratory apparatus requiring proteins encoded by both the nuclear genome and the mtDNA. However, the small yet vital mtDNA is highly susceptible to oxidative damage since it lies in immediate proximity to the site of the above ROS production by the respiratory chain. Mitochondrial ROS production leading to the progressive accumulation of mutations in mtDNA is thought to be a significant contributor to the normal events of aging and cell death via apoptosis (Mammucari and Rizzuto 2010). Furthermore, because mitochondria that are dysfunctional generate more ROS (Indo and others 2007), a feed-forward loop is set up whereby the ROS-mediated oxidative damage to mitochondria favors more ROS generation, leading to more damage—a so-called "vicious cycle" (Indo and others 2007). In this way, mtDNA damage and mitochondrial dysfunction serve not only as important downstream markers of mitochondrial ROS production but are themselves involved in exacerbating the excessive ROS scenario (Indo and others 2007; Mammucari and Rizzuto 2010). In yeast (where, unlike in man, the mtDNA is nonessential), this damage to the mtDNA is readily apparent as the generation of respiration-deficient ("petite") colonies, cells that carry either a rearranged (rho-) or no (rho^0) mtDNA. Such formation of respiration-deficient cells is dramatically enhanced in the presence of benzoate, sorbate (Piper 1999), ethanol (Ibeas and Jiminez 1997), and certain plant essential oils (Bakkali and others 2008; Ferreira and others 2014).

Could sorbate and benzoate accelerate this "vicious cycle" of mitochondrial damage in man?

Whether a long-term, substantial consumption of SB and PS in the human diet might significantly increase the levels of mitochondrial damage in man is not an easy subject to address, partly because enhanced mitochondrial dysfunction leading to an increased ROS

production is part of the normal process of aging (López-Otín and others 2013). It is, however, clear that increased mtDNA mutation and dysfunction would not have been detected in the earlier animal testing of these compounds. Not only did such testing largely predate the ability to analyze detrimental effects on mtDNA, but most of it also predates the proper appreciation of how mutation of the mtDNA—leading to defects in the electron transport chain—can generate diseases with severe pathologies. In man, 1 marked consequence of mtDNA defects is neuronal energy failure, leading to encephalopathy (Tzoulis and Bindoff 2012), neurodegenerative diseases (notably Parkinson's disease, Alzheimer's disease [AD], and Huntington disease) (Schapira 2006; Blanch and others 2016), as well as premature neural aging (Keogh and Chinnery 2015). Defective mtDNA can also have a variety of other systemic manifestations, such as cardiac myopathy and diabetes (Zeviani and Di Donato 2004; Finsterer 2016). Devising effective treatments for these disorders of progressive mtDNA dysfunction presents one of the major challenges now facing medical research (Schapira 2006; Viscomi 2016).

It is possible to study the effects of an increased mtDNA mutation rate—such as might be expected with high levels of endogenous ROS production—by engineering mice to have a genetic defect in POLG, the gene encoding the catalytic subunit of the mtDNA polymerase γ. This defect was found to cause accelerated aging and a reduced lifespan (Trifunovic and others 2005, 2008). Mutations in POLG have been identified in a small number of human individuals. They cause multiple deletions in, or depletion of, the mtDNA in affected tissues and are associated with a spectrum of disease phenotypes (Schapira 2006), including autosomal dominant and recessive forms of progressive external ophthalmoplegia, spino-cerebellar ataxia, and epilepsy (Tzoulis and Bindoff 2012); also the relentlessly progressive and ultimately fatal neurometabolic disorder known as Alpers-Huttenlocher syndrome (Baruffini and others 2006; Spinazzola and others 2009). Remarkably, Alpers-Huttenlocher syndrome is associated with an increased risk of fatal hepatotoxicity in response to treatment with sodium valproate (Figure 1) (Finsterer 2016). Sodium valproate is widely used to treat epilepsy, migraine, chronic headache, and bipolar disorder and it is usually well tolerated (Loscher 2002; Stewart and others 2009, 2011). Nevertheless, valproate generates an increased mitochondrial ROS production in cultured HepG2 cells (Komulainen and others 2015), mirroring the effects of PS and SB in yeast cells (Piper 1999). While pronounced sodium valproate toxicity as a consequence of POLG defects is rare, its hepatotoxicity profile reveals that a small, moderately lipophilic monocarboxylate compound can have the capacity to exacerbate a disorder of mtDNA maintenance in man. It is increasingly being recognized that certain antiepileptic drugs can influence mitochondrial function in both beneficial and detrimental ways, the actions of sodium valproate in this regard being mainly detrimental (Finsterer 2016).

Use of SB in the Clinical Treatment of Patients with UCDs and Other Conditions Associated with Hyperammonemia

Sorbate, benzoate, and valproate do not persist in the body. Dietary sorbic acid is generally metabolized by the same oxidation pathway as the 5-carbon saturated fatty acid caproic acid. The branched-chain valproate (Figure 1) mainly undergoes β-oxidation using the isoleucine catabolic pathway and enzymes. Benzoate accumulates in the matrix of liver and kidney mitochondria to about 50-fold over its extra-mitochondrial concentration (Gatley and Sherratt 1977), where it is conjugated with glycine—the most abundant free amino acid in the adult human liver. It is thereby converted to hippurate, a compound that can be efficiently removed from the body by the kidneys (Gatley and Sherratt 1977; Beyoğlu and Idle 2012). This process acts not only as a mechanism of detoxification, increasing the water solubility of this organic acid in order to facilitate its urinary excretion, but also as a means of regulating systemic levels of amino acids utilized as neurotransmitters in the central nervous system (Badenhorst and others 2014).

This conversion of benzoate to hippurate is exploited in the clinical treatment of UCDs, inborn errors of metabolism that usually manifest initially as life-threatening emergencies in the newborn (Husson and others 2016). These are inherited deficiencies of 1 of the 5 enzymes (carbamylphosphate synthetase, ornithine transcarbamylase [OTC], argininosuccinate synthetase, argininosuccinate lyase, arginase) of the urea cycle, the metabolic pathway by which ammonia is converted to urea. Defects in OTC are the most common. In normal individuals, the ammonia generated when amino acids are broken down is converted rapidly through the urea cycle to urea, which is then excreted in the urine. However in UCD patients, this conversion to urea is compromised, the reduced nitrogen flux causing an accumulation of ammonia and glutamine together with a disordered metabolism of other amino acids. This build-up of ammonia (the condition of hyperammonemic encephalopathy, hereafter referred to as hyperammonemia) is highly toxic to nerve cells, causing a wide spectrum of neuropsychiatric abnormalities and motor disturbances.

Treatments of UCD primarily involve either the lowering of ammonia production and/or increasing ammonia removal. They include a low-protein diet, supplementation of arginine or citrulline, and the administration of SB and/or sodium phenylbutyrate. Administration of high doses of SB facilitates the excretion of this excess nitrogenous waste from the body as benzoate conjugates, mainly hippurate. The sparse-fur (spf) mutant mouse with OTC deficiency provides an animal model system for investigating this process, as well as the detrimental effects of hyperammonemia on neurotransmitter function (Qureshi and others 1989).

Hyperammonemia sometimes arises as the consequence of drug therapy (Beyoğlu and Idle 2012). It can result during intravenous therapy with asparaginase, a standard treatment for acute lymphoblastic leukemia and non-Hodgkin lymphoma in childhood, whereupon it, in turn, can be counteracted by a therapy consisting of protein restriction, together with the administration of SB and glucose/insulin (Jörck and others 2011). Hyperammonemia can also arise in patients medicated on the anticonvulsant sodium valproate (Bega and others 2012; Amanat and others 2013). Under normal metabolic conditions, acyl-CoA is transported into the mitochondria via a carnitine transport system. It is then converted to acetyl-CoA via β-oxidation and eventually to *N*-acetyl glutamate. However, valproate, by causing reductions in free coenzyme A, acetyl-CoA, and carnitine, can decrease the availability of the cofactors necessary for the functioning of the urea cycle and thereby cause a serious elevation in serum ammonia levels. SB has been used to treat potentially fatal valproate poisoning (Cooke and others 2009). The often fatal disorder Reye's syndrome, mentioned above in connection with MPT, is also associated with hyperammonemia (Trost and Lemasters 1996). This condition of liver mitochondrial dysfunction is severely aggravated by salicylates (Glasgow 2006), which are—like benzoate—substantially metabolized through their conjugation with glycine (to form salicylglycine, or salicyluric acid (Beyoğlu and Idle 2012)).

Increasingly, the administration of SB to UCD patients is being replaced by treatment with the proprietory drug sodium phenylbutyrate (Figure 1). Sodium phenylbutyrate is a prodrug, rapidly converted in the body to phenylacetate. This phenylacetate combines, in turn, with a glutamine molecule to form phenylacetylglutamine. The latter is then removed from the body by the kidneys. Oral administration of phenylbutyrate presents a distinct advantage over that of benzoate since a benzoate molecule that reacts with glycine removes from the body just 1 molar equivalent of nitrogen, whereas a phenylbutyrate molecule that conjugates with glutamine effectively removes from the body 2 molar equivalents of nitrogen (Komatsuzaki and others 2009). Sodium phenylbutyrate also appears to be more effective than SB in generating an increased protein tolerance without hyperammonemia in UCD patients (Albers and others 1996; Komatsuzaki and others 2009). One attraction of SB though is that it is a relatively inexpensive treatment of UCD for resource constrained countries.

SB therapy has now been used in the clinical treatment of UCD for nearly 40 y (Beyoğlu and Idle 2012). The monitoring of UCD patients on prolonged SB administration clearly shows this to be a highly beneficial treatment of the metabolic disorder (Enns and others 2007; Ferenci 2007; Komatsuzaki and others 2009; Husson and others 2016; NeSmith and others 2016). However, it is not without risk. The significant dose-dependent sodium content of this therapy means it may not be appropriate for patients with significant fluid retention or kidney dysfunction (Misel and others 2013). Less sodium can be administered if sodium phenylbutyrate glyceryltri(4-phenylbutyrate) is used as the nitrogen scavenger in place of SB (Beyoğlu and Idle 2012). Another potential problem is that the glycine conjugation of the benzoate can generate a shortage of glycine, causing hypoglycinemia—a condition that has the potential to negatively influence brain neurochemistry (Badenhorst and others 2014). Hypoglycinemia is one of the characteristics of aspirin overdose (since salicylic acid is, like benzoate, largely eliminated from the body as a glycine conjugate), the psychosis of aspirin intoxication being associated with these low levels of glycine in the central nervous system (Beyoğlu and Idle 2012). Furthermore, studies in mice have revealed that very high dietary intake of SB can potentiate ammonia toxicity and inhibit urea synthesis by decreasing the intramitochondrial levels of *N*-acetyl glutamate, causing a mortality that is rescuable with pretreatments with either L-carnitine or carbamyl glutamate (O'Connor and others 1987, 1989).

SB and sodium phenylbutyrate are sometimes used for treating the hyperammonemia common in adult patients with advanced liver disease (estimated to occur in 30% to 45% of patients with liver cirrhosis and in 10% to 50% of patients with transjugular intrahepatic portosystemic shunts). While they have been administered to patients with acute liver cirrhosis (De Las Heras and others 2017), as well as postliver transplantation (Aggarwal and others 2015), the complex nature of advanced liver disease generally makes this therapy less effective than when it is applied to UCD. Thus, it is recognized that SB may not always be beneficial for cirrhosis treatment (Efrati and others 2000). However, SB and sodium phenylbutyrate have proven beneficial in treating the hyperammonemia that results from certain forms of chemotherapy (Del Rosario and others 1997; Doloy and others 2002; De Las Heras and others 2017).

While inherited UCD is rare in neonates (1 in about 8000), it is vital that it is diagnosed quickly before it can lead to appreciable brain damage (Häberle 2011; Häberle and others 2012). Once diagnosed, ammonia-scavenging medication is often of considerable benefit for the pediatric intensive care of these UCD infants, and its subsequent continuous use can facilitate the normal development of these children (Häberle 2011; Go and others 2012; Abily-Donval and others 2015). It is a treatment that can produce substantial increases in free bilirubin concentrations in jaundiced neonates (Green and others 1983). Initial results indicate that SB may have potential for the treatment of fetuses with a prenatal diagnosis of inherited UCD, the benzoate then being administered prenatally via the placenta by infusing their mothers with benzoate (Das and others 2009; Wilnai and others 2014).

SB therapy has also provided appreciable rescue of the neurocognitive development defects in children with arginase deficiency (Hewson and others 2003) or argininosuccinate lyase deficiency (together with an arginine supplementation) (Ioannou and Augoustides-Savvopoulou 2013; Jackson and others 2014). SB (together with dichloroacetate supplementation) can similarly treat the congenital lactic acidosis caused by a partial deficiency of the E1 component of pyruvate dehydrogenase (McCormick and others 1985). Both positive (Fiori and others 2016) and negative (Mercimek-Mahmutoglu and others 2014) results have been reported for SB in treating a disorder of creatine metabolism generated by lack of the enzyme guanidinoacetate methyltransferase. A further use of SB, in conjunction with an *N*-methyl-D-aspartate (NMDA) receptor antagonist, is for the treatment of infants with nonketotic hyperglycinemia. This is an autosomal recessive disorder caused by mutations in the mitochondrial glycine cleavage system in which abnormally high glycine levels cause a devastating neurological condition, probably resulting from an overstimulation of the NMDA receptors in the brain. Use of SB only partially normalized the high plasma glycine levels in these children, and while—generally used in conjunction with the NMDA receptor antagonist ketamine—it eliminated seizures and allowed for a degree of developmental progress in some of them, but did not prevent a poor long-term outcome (Hamosh and others 1998; Chien and others 2004; Rossi and others 2009; Bjoraker and others 2016). In 1 child with psychomotor retardation and epilepsy, due to a less severe, attenuated nonketotic hyperglycinemia, treatment with SB proved beneficial (Neuberger and others 2000). The symptoms of nonketotic hyperglycinemia are severely aggravated by sodium valproate treatment (Dhamija and others 2011; Tsuyusaki and others 2012).

Much of the current interest in sodium phenylbutyrate (Figure 1) stems from the fact that the oral administration of this compound might potentially benefit treatments of a number of other diseases, in addition to UCDs. This monocarboxylate compound has the ability to act as a chemical "chaperone" within the endoplasmic reticulum (ER). By counteracting ER stress, it can help alleviate the symptoms of a number of diseases generated through the misfolding of soluble and transmembrane proteins that transverse the exocytic pathway, diseases such as T2DM, hypertension, and cystic fibrosis. In cystic fibrosis bronchial epithelial cells, sodium phenylbutyrate has been shown to provide a substantial rescue of the defective folding of the cystic fibrosis transmembrane conductance regulator chloride channel (Singh and others 2008). In the leptin-deficient mouse model of severe obesity and IR, administration of sodium phenylbutyrate alleviates a number of the disease-associated traits (Ozcan and others 2006). In other mouse studies, sodium phenylbutyrate was shown to reduce cardiac damage and improve vascular function in hypertension (Kassan and others 2012; Luo and others 2015). In rats, sodium phenylbutyrate was found to counteract the most common end stages of renal disease (Liu and others 2016).

The Immunomodulatory Actions of Benzoate

While 1 recent study reported that PS and SB can contribute to the activation of inflammatory pathways (Raposa and others 2016), other studies indicate that SB is primarily anti-inflammatory *in vivo* and acts to downregulate many immune signaling pathways responsible for inflammation, glial cell activation, switching of T-helper cells, modulation of regulatory T cells, cell-to-cell contact, and migration (Brahmachari and others 2009; Maier and others 2010; Pahan 2011). These latter, anti-inflammatory effects of SB would appear to resemble those of sodium salicylate (Figure 1), the active metabolite of the well-known nonsteroidal anti-inflammatory drug aspirin (acetylsalicylic acid) (Baltazar and others 2011). In mouse microglia, SB inhibits NF-kappaB activation, modulates the mevalonate pathway, and suppresses the activation of $p21^{ras}$ (Brahmachari and others 2009). Recently, SB administration was shown to induce the expression of TGF-beta in splenocytes and also to upregulate regulatory T cells during experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis (MS) (Kundu and others 2016). Interleukin 4 (IL-4) is known to improve the clinical manifestations in this animal model of MS, and the administration of SB to human subjects has been shown to induce IL-4 production in their peripheral blood mononuclear cells (Rezaei and others 2016). SB might therefore deserve consideration as a useful candidate for conjunctive therapy in treating MS, the most common human demyelinating disease of the central nervous system.

The Neuroprotective Actions of Benzoate

Benzoate rapidly traverses the blood–brain barrier and is now attracting increased attention as an agent for the treatment of certain brain disorders, partly because it presents the advantages of a ready oral administration and an existing approval for the treatment of UCD. Cinnamon—the most consumed spice worldwide—is also of interest in this regard, since the oral feeding of cinnamon (Cinnamonum verum) powder is known to generate benzoate in the blood and brain of mice (Jana and others 2013; Khasnavis and Pahan 2014) and to protect memory and learning in a transgenic mouse model of AD (Modi and others 2015). SB and a number of other cinnamon-derived compounds have also been found to protect against experimentally induced neurodegeneration in model organisms such as the nematode *C. elegans* and fruit fly *D. melanogaster* (Stavinoha and others 2015).

The neuromodulatory actions of SB are generally attributed to the capacity of benzoate to act as a competitive inhibitor of D-amino acid oxidase (DAAO), one of the enzymes that regulate levels of the endogenous ligand (D-serine) for the glycine modulatory binding site on the NR1 subunit of the NMDA receptor in various brain areas. Zinc benzoate, an environmental contaminant derived from polystyrene, acts in a similar manner (Egashira and others 2003). Because NMDA receptor dysfunction is implicated in both the positive (psychotic) and negative (impairment of normal emotional responses, thought processes, and behavior) symptoms of schizophrenia, there has been much interest in developing potent and selective DAAO inhibitors for the treatment of this condition. Increasing the function of NMDA receptors, by inhibiting the DAAO-induced degradation of D-serine has the potential to alleviate many of these symptoms in schizophrenic patients (Smith and others 2010; Sacchi and others 2013; Chue and Lalonde 2014; Hashimoto 2014). Increased levels of DAAO expression and enzyme activity have been found in postmortem brain tissue samples from patients with schizophrenia compared to healthy controls. In a double-blind randomized controlled trial, SB therapy was reported to significantly improve a variety of symptoms and neurocognition in patients with chronic schizophrenia (Lane and others 2013). In another randomized, double-blind, placebo-controlled trial, a combined treatment of SB with sarcosine (the latter a glycine transporter I inhibitor) was found to both enhance NMDA receptor-mediated neurotransmission and improve the cognitive functioning of patients with schizophrenia (Lin and others 2015). In mice, SB administration was found to attenuate the behavioral (prepulse inhibition deficits and hyperlocomotion) abnormalities induced in response to a single administration of the NMDA receptor antagonist phencyclidine (Matsuura and others 2015). Despite this, the latter authors did not find any associated increase in the levels of D-serine in the brains of these mice. Ground cinnamon and SB both caused increased levels of neurotrophic factors in primary human neurons and astrocytes, while oral feeding of either of these compounds increased the levels of these neurotrophic factors *in vivo* in the mouse central nervous system (Jana and others 2013). SB has been reported to induce anxiety and motor impairment in rats (Noorafshan and others 2014).

NMDA receptor-mediated neurotransmission is also vital for learning and memory, loss of NMDA receptor function being implicated in the early stages of AD. In 1 double-blind randomized controlled trial, SB was studied as a potential agent for the treatment of amnesic mild cognitive impairment and mild AD. It was reported to substantially improve cognitive and overall functions in patients with early-phase AD, indicating a promise for DAAO inhibition as a novel approach for tackling early dementia (Lin and others 2014). Furthermore, in a mouse model of AD the oral administration of cinnamon was shown to both generate benzoate in the hippocampus, as well as to protect memory and learning. The latter effects were correlated with an attenuation of hippocampal oxidative stress via the suppression of $p21^{rac}$, thereby protecting hippocampal neurons, suppressing Tau phosphorylation, and reducing Aβ fibril formation (Modi and others 2015).

Treatment with SB leads to an upregulation of DJ-1, a protein that is protective against oxidative stress in primary mouse and human astrocytes, as well as human neurons (Khasnavis and Pahan 2012). DJ-1 is also thought to be neuroprotective, since loss-of-function mutations in this protein have been linked to a familial form of early onset Parkinson's disease. In mouse astrocytes, SB appears to abrogate the proinflammatory cytokine IL-1beta-induced downregulation of proteins that counteract Parkinson's disease such as Parkin and DJ-1 (Khasnavis and Pahan 2014). Administrations of the structurally similar sodium salicylate (Thakur and Nehru 2014) and sodium phenylbutyrate (Roy and others 2012) (Figure 1) also confer neuroprotection in various models of Parkinson's disease.

In addition to its occurrence in the brain, DAAO also exists within the peroxisomes of kidney tubular epithelial cells, the site of D-serine reabsorption. The initial onset of the nephrotoxicity caused by D-serine administration in the rat is prevented by a prior SB administration (Williams and Lock 2005). This would appear to be yet another reflection of the capacity of benzoate to act as a potent inhibitor of DAAO, in this case by an action on the kidney enzyme.

Should We be Concerned about High Benzoate Intake, Either from the Diet or in the Clinical Treatment of Hyperammonemia?

The governmental regulatory bodies use the "highest dose at which no adverse effects are observed in the most susceptible

Table 1–Studies that have uncovered potentially hazardous effects of benzoate and/or sorbate in cell culture, model organisms, or isolated mitochondria

Effect	Comments	References
Whole organism studies		
Growth retardation and organ injury (SB)	Excess dietarybenzoate leads to growth retardation, hematological abnormality, and organ injury of piglets; also Necrotic and cirrhotic changes in the livers of mice.	(Kaboglu and Aktac 2002; Shu and others 2016)
Teratogenic and neurotoxic effects (SB)	Significant developmental defects on the motor neuron axons and neuromuscular junctions in zebrafish larvae; also teratogenic effects during mouse fetal eye development. Reported to downregulate the expression of tyrosine hydroxylase and dopamine transporter in dopaminergic neurons.	(Tsay and others 2007; Chen and others 2009; Afshar and others 2013).
In cell culture studies		
Genotoxic effects (sorbate and benzoate)	Mutagenic to human lymphocytes *in vitro*; also bone marrow chromosomes of mice *in vivo,* causing enhanced micronucleus formation, and/or chromosome aberrations.	(Abe and Sasaki 1977; Banerjee and Giri 1986; Yilmaz and others 2009; Zengin 2011; Mamur 2012; Pongsavee 2015)
Lowering of leptin (SB)	Inhibition of leptin release in cultured murine adipocytes	(Ciardi and others 2012)
Oxidative stress, mutagenic to mtDNA (PS and SB)	In yeast enhances oxidative stress, causing damage to mtDNA readily identified as an increase in respiratory deficient cells. In human erythrocytes benzoic acid induces oxidative stress, causing enhanced malondialdehyde formation, symptomatic of lipid peroxidation. In cultured rat hepatocytes potassium sorbate causes decreased glutathione, another characteristic of oxidative stress.	(Kinderlerer and Hatton 1990; Piper 1999; Yetuk and others 2014; Baş and others 2014)
Loss of cell viability (SB)	Cell death, increases in intracellular Ca(2+) concentration and loss of mitochondrial transmembrane potential in cultured rat cortical neurons and in HeLa cells.	(Park 2011)
In isolated mitochondria		
Induction of the mitochondrial permeability transition in isolated mitochondria (SB)	Opening of a high-conductance, cyclosporin-sensitive pore in the mitochondrial inner membrane, causing mitochondrial swelling, depolarization, and uncoupling of oxidative phosphorylation.	(Trost and Lemasters 1996)

animal species—the No Observed Adverse Effect Level (NOAEL)" as their basis for setting the safety standards for the human consumption of food additives. NOAEL is measured in milligrams per kilogram of body weight per day. The acceptable daily intake, originally introduced by the Joint FAO/WHO Expert Committee on Food Additives in 1961, was postulated to be the amount of an additive in food that could be ingested orally on a daily basis over a lifetime without an appreciable health risk. Organizations such as the U.S. FDA and EFSA regularly update their criteria for the approval of food additives, making these increasingly more stringent (Authority EFS 2012). However, for those additives already approved over a long period, a testing to these latest guidelines is seldom performed. The reports of potentially adverse effects of benzoate in cell culture and in model organisms (Table 1) have not prompted a retesting of this compound according to modern standards. This reflects the history of substantially safe, large-scale, long-term use of this preservative, both in the human diet and for UCD treatment; also the lack of any definitive evidence that the benzoate in soft drinks can increase the risk of T2DM. Indeed, it would be extremely challenging for clinical studies to distinguish cellular damages due to a prolonged exposure to such an additive over many years from the natural events of aging, since aging involves the progressive accumulation of DNA damage in the nucleus and mitochondria. The increased ROS production with mitochondrial dysfunction leads to decreased production of adenosine triphosphate (ATP), oxidative damage to proteins and other macromolecules in cells, the induction of proinflammatory cytokines, telomere shortening, and cell senescence (López-Otín and others 2013). All of these events will impact on mitotically active tissues (such as intestinal stem cells, hematopoietic stem cells, mesenchymal stem cells) over time by triggering senescence and apoptosis; also postmitotic tissues (muscle, heart, and brain) by causing cellular dysfunction and loss. While a high SB administration has been reported to cause necrotic and cirrhotic changes in the livers of mice (Kaboglu and Aktac 2002), such damages have not so far been reported in UCD patients on prolonged SB therapy. Nevertheless, the risks of high benzoate consumption should not be underestimated. Glycine conjugation of benzoate can exacerbate the effects of a glycine deficiency in humans, lack of glycine having negative impact both on brain neurochemistry as well as the synthesis of collagen, nucleic acids, porphyrins, and other important metabolites (Badenhorst and others 2014).

The U.S. FDA and EFSA criteria for the approval of food additives notably give little consideration to the possibility that certain individuals may be rendered, either through their genes or a chronic medical condition, hypersensitive to what are, in most people, readily repairable and relatively innocuous effects. With regard to the dietary consumption of SB and PS, this may be an especially pertinent consideration. Despite concerns not having been raised in large-scale human irritation testing (Walters and others 2015), it does appear that a small number of children do develop asthma (Petrus and others 1996) or allergy (Jacob and others 2016) in response to dietary SB. Also, despite benzoate being a known DAAO inhibitor, there has been little consideration to date of whether it, like valproate (Loscher 2002), acts to influence neuronal excitation. If so, this might reinforce the suspicions that a high dietary SB may influence child hyperactivity disorders (Eigenmann and Haenggeli 2007). Benzoate—like aspirin—might not be safe in children with the devastating encephalopathy Reye's syndrome (Glasgow 2006) since, like salicylate which aggravates this condition, it can induce the MPT and mitochondrial damage (Trost and Lemasters 1996). Furthermore, hippurate may accumulate to high, even dangerously high, levels

in patients with renal insufficiency, since this—the product of benzoate conjugation with glycine in the liver—is a metabolite that is renally cleared. Hippurate is reported to impair basal and insulin-stimulated glucose uptake into cells in culture (Spustova and Dzurik 1991). Individuals with genetic defects in the genes of iron homeostasis (hereditary hemochromatosis) (Gozzelino and Arosio 2016; Guo and others 2016) or with Alpers-Huttenlocher syndrome (Komulainen and others 2015) are especially susceptible to ROS-induced damages. For them, the capacity of sorbate and benzoate to enhance mitochondrial ROS production may be a concern, as shown for genetic defects in the maintenance of human mtDNA integrity causing a sensitization to sodium valproate (Stewart and others 2009, 2011). Already POLG DNA testing is advocated before commencing valproic acid therapy for pediatric seizure disorders (Saneto and others 2010). In due course, it may be considered desirable to advise these and certain other individuals on whether they might have sensitivity to food additives, as an adjunct to linking DNA profiling to their personalized medication.

As indicated above, there are a number of situations where SB appears to be anti-inflammatory (Brahmachari and others 2009; Maier and others 2010), or exerts more of an antioxidant than a prooxidant action (Khasnavis and Pahan 2012; Modi and others 2015). Furthermore, prolonged oral administration of SB to UCD patients is clearly a very effective therapeutic intervention, with no reports to date of major adverse effects (Husson and others 2016). As described above, SB is now attracting increased interest as a potentially promising agent for use in conjunctive therapy during the treatment of liver failure (De Las Heras and others 2017), MS (Kundu and others 2016), schizophrenia (Lane and others 2013; Matsuura and others 2015), early-stage AD (Lin and others 2014), and Parkinson's disease (Khasnavis and Pahan 2012).

Conclusions

Safe exploitation of the above, existing and potential therapeutic benefits of SB will require a more complete understanding of the biological actions of this agent. As highlighted in this article, issues remain as to: (1) the effects of its long-term dietary consumption (reflecting the large range of daily intake for different individuals, both from the diet and as a food preservative); (2) the potential for SB to display increased toxicity or cause altered neuromodulatory (mood, learning, and personality) effects in those individuals with specific medical conditions or genetic defects; and (3) the possibility that very high dosages of SB might exacerbate either the neuromodulatory effects of glycine deficiency, or the mitochondrial damage linked to diverse disease mechanisms, accelerated aging, and damages caused by ROS. Despite these uncertainties, it can be anticipated that SB will find an increased clinical use, especially in resource-constrained countries, not just as a relatively inexpensive treatment for UCD but also in future therapies for neurodegenerative and other disorders.

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Author Contributions

Joseph D. Piper performed systematic searches of the EMBASE, MEDLINE (including in-process and other nonindexed citations), Web of Science, and CENTRAL databases. A total of 14633 citations of benzoate/benzoic acid and sorbate/sorbic acid up until December 2016 were retrieved, and duplicates removed. The remaining 11766 citations were then filtered by title. Joseph D. Piper and Peter W. Piper then analyzed by title and abstract 1539 citations, those screened for prespecified inclusion criteria of biological effects of benzoate or sorbate (and related compounds), both beneficial and harmful, also the clinical uses of SB. Papers were then selected for this review, together with additional references concerning the actions of sodium salicylate, sodium valproate, and sodium phenylbutyrate that discussed mechanisms of biological action that may be relevant to the reported effects of SB or PS.

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