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Evidence that glyphosate is a causative agent in chronic sub-clinical metabolic acidosis and mitochondrial dysfunction

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Abstract: Many types of chemicals, including pesticides and pharmaceutical drugs, cause metabolic acidosis and mitochondrial disorder. We provide evidence from the scientific literature that glyphosate can be metabolized by humans, that it disrupts the intestinal microbiota, causes severe metabolic acidosis when ingested in high doses and leads to mitochondrial dysfunction by uncoupling of phosphorylation. The symptoms and diseases associated with metabolic acidosis and mitochondrial dysfunction compare well with those attributed to glyphosate. Taken together, this evidence suggests that glyphosate, in the doses equivalent to allowed residues in food ingested over a long period of time, causes a low-grade, chronic acidosis as well as mitochondrial dysfunction. We also provide evidence from the literature supporting the biochemical pathways whereby this occurs. We then extract the reports for symptoms and diseases associated with glyphosate from the U.S. Food and Drug Administration's Adverse Event Reporting System database. These are compared to the symptoms and diseases reported in the database for drugs that are known to cause mitochondrial dysfunction. The results are startlingly consistent. Finally, we hypothesize that many modern diseases are primarily acquired mitochondrial disorders caused by chemical pesticides, pharmaceutical drugs, food additives and industrial chemicals. Keywords: **glyphosate, pesticides, mitochondrial disorder, acidosis**

1. Introduction

We propose that humans who ingest low doses of glyphosate over long periods develop an acid-base imbalance and subsequent sub-clinical, low-grade metabolic acidosis, with production of lactic and formic acid at the cellular level. These events appear to be critical upstream precursors to acquired mitochondrial errors of metabolism, which then lead to a plethora of diseases. We argue that the widely-held perception and oft-repeated dogma that "the dose makes the poison" is inaccurately applied to long-term glyphosate exposure, such as occurs among millions of people via daily intake through food, air, and water. We present evidence that glyphosate's likely induction of low-grade metabolic acidosis is being entirely overlooked in toxicology evaluations and public policy.

Well-established is the fact that ingesting large amounts of glyphosate causes metabolic acidosis and other pathophysiologic changes. Clinical signs of acute glyphosate poisoning include severe acidosis determined by low blood pH, hyperkalemia, hypernatremia, raised creatinine and blood urea levels, hypotension, hypoxemia and reduced serum bicarbonate. Severe poisoning causes dehydration, pneumonitis, oliguria, altered level of consciousness, hepatic dysfunction, pulmonary edema and dysrhythmias. [1,2,3] We submit by logical extension that ingesting low levels of glyphosate on a continuous basis can contribute to sub-clinical, low-grade acidosis. We have identified several mechanisms by which glyphosate can cause metabolic acidosis and acquired errors of metabolism and have presented the data and citations in the Background section of this paper.

Lactic acidosis is a high-ion gap metabolic acidosis caused by overproduction and/or underutilization of lactic acid. Lactic acid is overproduced when tissues are deficient in oxygen, forcing the conversion of pyruvate to lactate in anaerobic glycolysis (fermentation). Pyruvate, the sole precursor of lactic acid, is utilized by the mitochondria in aerobic cellular respiration. Lactic acid can be converted to glucose via pyruvate dehydrogenase (requires thiamine and other nutrients that are commonly deficient) or oxidized. Lactate is oxidized in the liver via bicarbonate. The kidneys also dispose of lactate, albeit to a lesser extent. The possible causes of lactic acidosis are oxygen deficit (tissue hypoxia) resulting from pulmonary or circulatory problems, thiamine deficiency, liver disease, renal failure, and uncoupling of the oxidative phosphorylation (OxPhos) step in the Krebs cycle. Prolonged acidosis leads to multiple organ failure and death.[4]

High-ion gap metabolic acidosis is common in the critically ill. Malignant cells produce much more lactate than normal cells. Circulatory disturbances lead to tissue hypoxia and can cause metabolic acidosis in extreme cases such as sepsis. Certain drugs and environmental toxins interfere with cellular metabolism by uncoupling of OxPhos, causing metabolic acidosis. However, in many cases, only a small portion (~15-20%) of the ion gap can be accounted for by lactic acid.

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Much of the ion gap is caused by unknown ions. Forni et al.[5] measured ions principally associated with the Krebs cycle in the generation of the ion gap. Their findings suggest that the mitochondria are clearly one possible source of ions and although these ions did not account for the total ion gap, their contribution to lactic acidosis and acidosis of "unknown cause" may be greater than previously thought.[6] D-Lactate also appears to be an important and under-appreciated contributor to metabolic acidosis.[7,8]

Glyphosate is listed in PubChem[9] as an enzyme inhibitor and as a chemical agent that uncouples oxidation from phosphorylation in the mitochondrial electron transport chain (ETC) so that adenosine triphosphate (ATP) synthesis does not occur with normal efficiency. Possible mechanisms are disruption of electron transfer via short-circuiting the proton gradient across mitochondrial membranes; binding to cytochrome c oxidase, thus competitively inhibiting the protein from functioning resulting in chemical asphyxiation of the cell; or inhibition of mitochondrial protein synthesis.[10]

In the *Background* section of this paper, we provide evidence that glyphosate can cause metabolic acidosis by both primary and secondary routes and that it also causes acquired errors in metabolism by uncoupling of OxPhos. We then present evidence that these disorders lead to multiple chronic diseases. In the *Method* section, we describe how we probed the U.S. Food and Drug Administration's (FDA's) Adverse Event Reporting System (FAERS) database to match these symptoms/diseases with certain drugs known to disrupt mitochondrial respiration. We present these data and show how they are remarkably consistent with both acute and chronic glyphosate poisoning.

2. Background

Acidosis is the condition of low serum pH resulting from excess positive charge, particularly ionized hydrogen (H+). Many processes in the body are mediated by electric charge. An imbalance of electric charge (electrolytes) will affect cell membrane permeability,[11] enzyme processes (binding, catalysis), mitochondrial metabolism, blood flow, protein synthesis, stability and folding, to name but a few.[12] Metabolic acidosis develops when the rate of H+ production exceeds the rate of H+ removal/buffering, caused by:

a. consumption of substances that are metabolized to acids,
b. increased acid production,
c. decreased acid consumption (by intestinal microbes) or excretion (lungs, kidneys, liver),
d. decreased production or loss of buffers (plasma proteins, phosphates, bicarbonates).

In this section, we show that glyphosate can be implicated in all of the above. Cole reported that the toxic effects of glyphosate on nematodes was "primarily a pH effect."[13] Glyphosate, a patented chelator, antibiotic and biocide,[14,15,16] is being ingested by Americans in the food and water every day for life. The staggering array of diseases and symptoms associated with metabolic acidosis and mitochondrial disorder is remarkably similar to that reported for chronic glyphosate poisoning.[17,18,19,20]

Antibiotics are known to cause metabolic acidosis through several mechanisms. One mechanism by which antibiotics can cause acidosis is by selectively killing bacteria in the intestines thereby causing an imbalance in the microbiota. One type of imbalance results in an over-growth of D-lactate-producing *Lactobacillus acidophilus*, causing an overproduction of lactic acid.[21] Another type of imbalance results in a lack of lactate-consuming microbes. A second possible mechanism is through inhibition of mitochondrial protein synthesis,[10] or otherwise disrupting the mitochondrial metabolism process and driving the conversion of pyruvate to lactate and anaerobic metabolism.[22,23] Finally, antibiotics can cause renal tubular dysfunction resulting in hyponatremia, hypokalemia, hyperkalemia, renal tubular acidosis, and nephrogenic diabetes insipidus resulting in improper excretion of H+ and recovery of minerals and bicarbonate.[24,25,26] Unlike glyphosate, people only take antibiotics for a limited time and buffers are added to help the body maintain pH balance.

2.1. Mechanisms for glyphosate causing metabolic acidosis

a. Consumption of substances that are metabolized to acids

Metabolic acidosis can be caused by direct consumption of an acid, or by consumption of a substance that is metabolized to an acid.

Direct exposure through ingestion

The chemical structure for glyphosate (*N*-[phosphonomethyl]glycine) is $C_3H_8NO_5P$. Most glyphosate formulations in current use are in the form of the potassium salt, $C_3H_7KNO_5P$, where one of the hydrogen atoms in the phosphate group is replaced with potassium. Some formulations are glyphosate isopropylammonium, $C_6H_{17}N_2O_5P$, where the isopropylamine molecule is attached to the carboxylate group. Glyphosate and glyphosate potassium salt molecules are shown in Figure 1. Glyphosate is a zwitterion, meaning that the molecule contains both negative and positive ions with dissociation constants that are pH dependent. Glyphosate has eight hydrogen atoms, of which three protons (H^+) will dissociate in any solution with a pH greater than six. Proteins are zwitterions whose dissociation constants (pK_a values) are within the normal operating pH of the body. The

charged groups on proteins are acids and bases that exchange protons with water; therefore they are natural buffering agents in dynamic equilibrium with their environment. The pK_a values for glyphosate are out of normal physiological range. Table 1 shows the various values that have been measured for glyphosate. Table 2 shows the normal range of pH in various locations throughout the body.

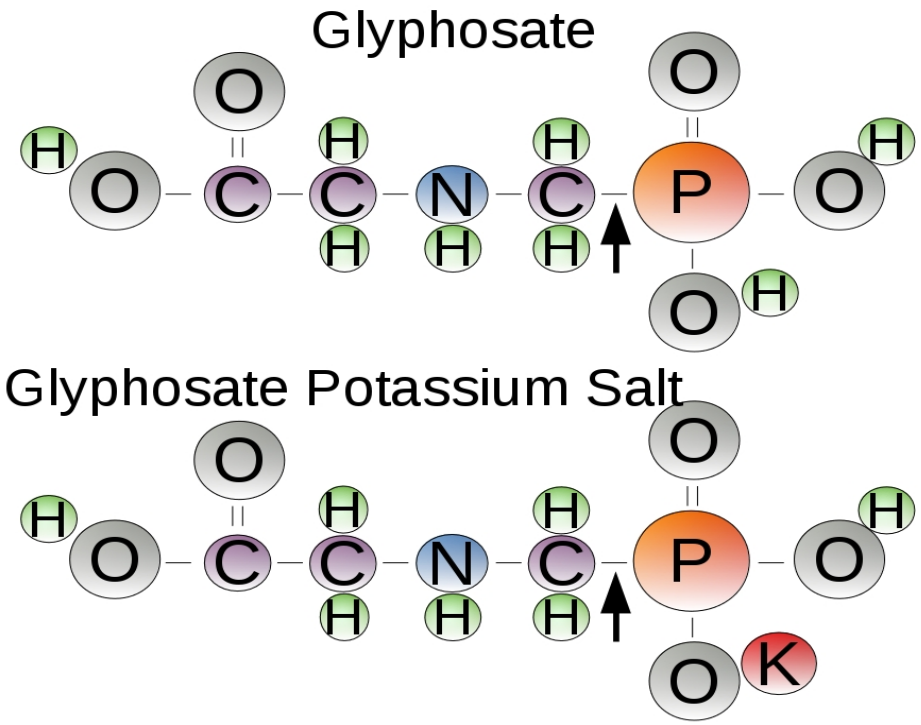

Figure 1. Glyphosate and glyphosate potassium salt molecules.

Table 1. Dissociation constants for glyphosate per pH, with citations.

Author/Editor	pK_a1 (1st phosphonic)	pK_a2 (carboxylate)	pK_a3 (2nd phosphonic)	pK_a4 (amine)
MacBean[27] (2008)		2.34	5.73	10.2
Wauchope[28] (1976)		2.32	5.86	10.86
Caceres-Jensen[29] (2009)	2.0	2.6	5.6	10.6
Wollerton & Husband[30] (1997)	2.0	2.25	5.50	10.34
Tomlin[31] (1997)	0.8	2.3	6.0	11

When glyphosate is ingested or inhaled, immediately three positively charged protons are released, or, in the case of the potassium salt formulation, two protons and one potassium atom. We propose that these excess positive charges lower the pH in the mouth, stomach and intestines causing more acidity, contributing to the metabolic acidosis noted with glyphosate poisoning. In the stomach, the chyme will be more acidic, resulting in over-stimulation of the pancreas and raising the pH as the chyme passes from the stomach to the small intestine. This is the likely cause of metabolic acidosis reported for a large quantity of glyphosate ingested in suicide attempts.[1-3]

Table 2. Normal pH values

Saliva	Esophagus	Stomach (empty)	Stomach (full)	Intestines	Lungs	Blood	Interstitial fluids	Urine (AM)	Urine (PM)
6.5-7.5	4-6	1-3	4-5	6.4-7.5	7.38-7.42	7.35-7.45	7.34-7.4	6-7	7.5-8

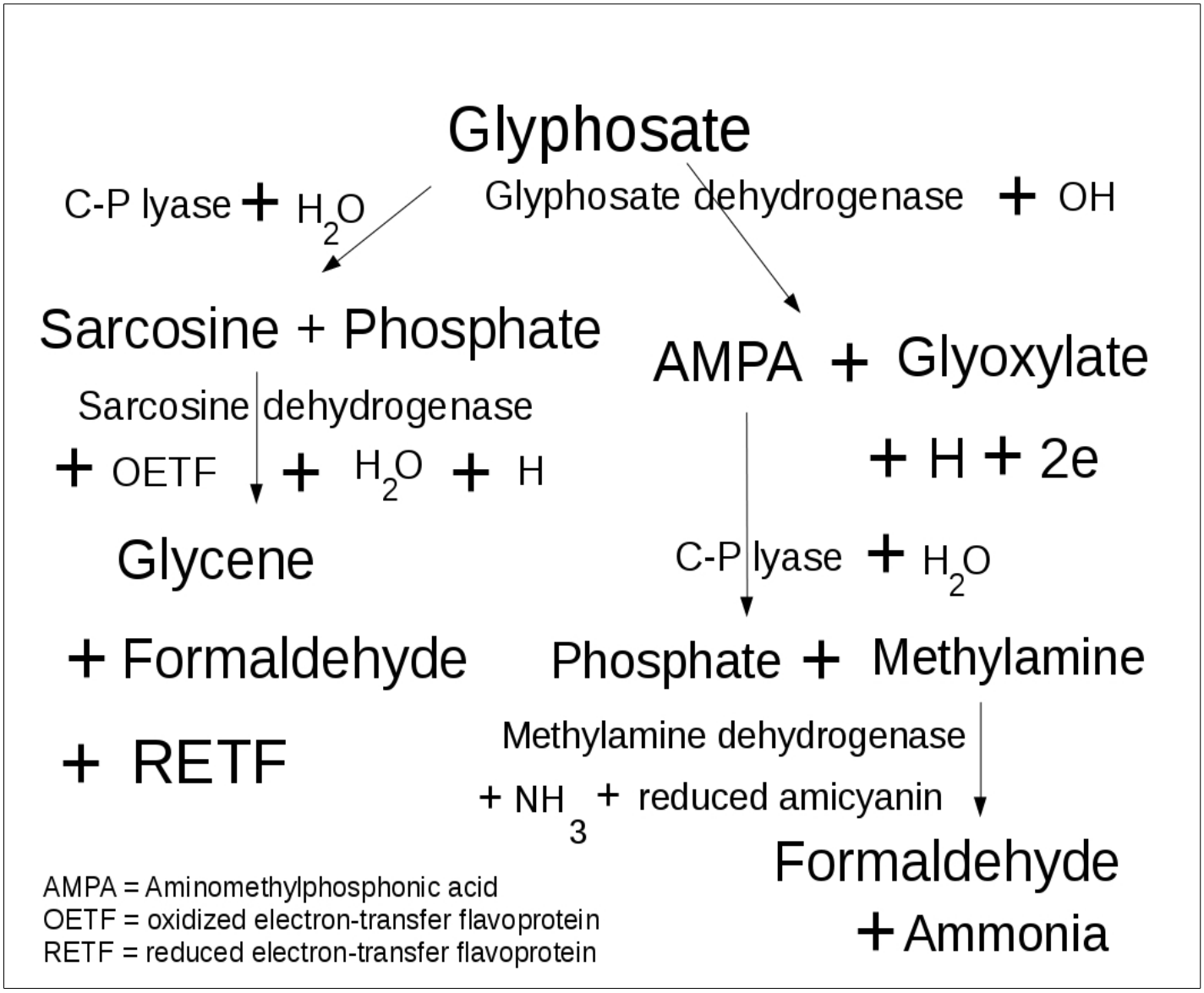

***Figure 2**. Possible metabolic pathways for glyphosate.*

After releasing the three protons, the glyphosate molecule is left with three negatively charged oxygen atoms that are highly reactive (See Fig. 1). In particular, they will bind to any available metals, including Zn, Ca, Mg, Cu, Co, Fe, Cr, and Al, to form stable metallic compounds.[32] This property has been linked to chronic kidney disease in agricultural workers in Sri Lanka.[33,34]

Metabolism of glyphosate to formic acid

We have identified two possible metabolic pathways for glyphosate in the human body (Figure 2). Both pathways lead eventually to formaldehyde. At least six different enzymes capable of catalyzing the conversion of formaldehyde to formic acid are present in animal tissue: aldehyde dehydrogenase, xanthine oxidase, glyceraldehyde-3-phosphate dehydrogenase, catalase, peroxidase, and aldehyde oxidase.[22] Formic acid is a well-known cause of metabolic acidosis and mitochondrial disorder.[22, 23, 35]

Metabolic pathway #1: In this pathway, the first step is to cleave the carbon-phosphate bond as depicted by the

arrow in Figure 1. Because phosphorus is an essential bacterial nutrient, many bacteria have evolved to cleave the carbon-phosphorus bond, releasing a phosphate molecule from phosphonate compounds.[36,37,38,39,40] Certain gram-negative bacteria found in animal tissue, including *Escherichia coli*, *Pseudomonas sp.*, *Ochrobactrum sp.*, *Enterobacter sp*., *Arthrobacter sp.* and *Burkholderia* utilize the enzyme carbon phosphorus (C-P) lyase to perform this function.[41,42] The polycyclic aromatic hydrocarbon catabolism (phn) genes encode the enzymes for this pathway[38, 39, 41] and it has recently been shown that these genes are upregulated in the presence of glyphosate.[43]

The very property that some bacteria are resistant to glyphosate is the underlying premise for the use of bacterial genes in the production of genetically modified/manipulated crops, wherein a bacterial gene is transferred into the plant genome. In particular, the gene cp4 5-enolpyruvylshikimate-3-phosphate (esps) (aroA:CP4) from *Agrobacterium tumefaciens* strain CP4 is used to convey glyphosate resistance to crops. Because of the rise in glyphosate-resistant weeds, there is active research in identifying other bacteria with this property. In particular, strains of *E. coli* and *Pseudomonas sp* with resistance to glyphosate have been identified.[42,44] It is therefore highly probable that glyphosate can be metabolized by bacteria in the human gut.

The metabolic path on the left side of Figure 2 is: glyphosate —> sarcosine + phosphate (via C-P lyase); sarcosine —> glycine + formaldehyde + CO_2 (via sarcosine dehydrogenase). This has been identified as the primary metabolic pathway for glyphosate by *Pseudomonas sp*.[45,46] A notorious pathogen that causes pulmonary infections and is associated with the triggering of neuronal autoimmunity, the *Pseudomonas* thrive at low (~4.5) pH,[47] switching from aerobic to anaerobic metabolism as the amount of glyphosate is increased.[42] The glyphosate molecule is COOH-CH_2-NH-CH_2-PHOSPHONATE. C-P lyase takes water and breaks off the phosphonate group leaving sarcosine, COOH-CH_2-NH-CH_3.

Excess sarcosine results in sarcosinemia or hypersarcosinemia. This is thought to be an inherited metabolic disorder caused by impairment of sarcosine dehydrogenase. If glyphosate is being consumed at every snack and meal, and if it is being metabolized through this pathway, this could cause an excess of sarcosine on a sub-clinical and long-term basis.

There is a positive feedback loop in effect whereby glyphosate disrupts the distribution of gut bacteria by selectively killing some and not affecting others.[48] Some of these others are proficient at metabolizing glyphosate; therefore, the more glyphosate is consumed, the more gram-negative bacteria there will be in the gut, and the more glyphosate will be metabolized.[47] Thus the glyphosate consumption common in the American diet could cause a vicious cycle of microbial imbalance which further enhances glyphosate metabolism by microbes.

Metabolic pathway #2: The second metabolic pathway, shown on the right side of Figure 2, is through the metabolite, aminomethylphosphonic acid (AMPA): glyphosate —> AMPA + glyoxylate + H^+ + $2e^-$ (via glyphosate dehydrogenase or glyphosate acetyltransferase); AMPA —> phosphate + methylamine (via C-P lyase and H_2O); methylamine —> NH3 + reduced amicyanin + formaldehyde (via methylamine dehydrogenase + H_2O + amicyanin).

It has been shown that a number of bacterial species are able to use AMPA as a phosphorus source, including *E. coli, Arthrobacter sp*., and *Pseudomonas sp*.[41] The mechanism is the same as in path 1 (via C-P lyase); however, the phosphonate is cleaved from the secondary product, AMPA, rather than the primary glyphosate molecule.

Metabolic pathway #2 has been identified as the primary degradation pathway for glyphosate in soils. Bacteria that produce glyphosate dehydrogenase are *Geobacillus caldoxylosilyticus* T20 (found in soil and water) and *Flavobacterium sp*., which are ubiquitous, including in the human body.

The manufacturer of glyphosate makes the claim that it is not metabolized by mammals. This is based on experiments with rodents administered a *single dose*.[49] Because the majority of the glyphosate was excreted via urine or feces after four days and because only small amounts of AMPA were measured (0.2-0.6% of initial glyphosate dose),[50] they make this claim. About 30% of ingested glyphosate is absorbed and distributed throughout the body with low residues occurring in all tissues. Generally, accumulation is below 1% after seven days.[51]

Other researchers have measured higher percentages of AMPA (6.49%)[52] after a single dose. One would reasonably expect higher amounts of both glyphosate and AMPA when glyphosate is consumed continuously. Clearly there must be a route for glyphosate metabolism or there would be no detectable AMPA. Friends of the Earth, Europe measured glyphosate and AMPA in the urine of European city dwellers and found 0.15 - 1.82 ppb of glyphosate and 0.15 - 2.63 ppb of AMPA.[53] The manufacturer still claims that glyphosate cannot be metabolized and that the AMPA found in these Europeans must have come from some other source. Monika Krüger could not account for the much lower than expected amount of glyphosate recovered from the urine and feces of the cows in her experiment based on what they were eating.[54] She hypothesized that either some of the glyphosate was being metabolized to AMPA or that the amount of glyphosate in the feed was much lower than expected. Another possibility is that glyphosate accumulated in the tissues.

b. Increased acid production

Imbalance in gut biota

It is well-known that glyphosate causes microbial dysbiosis in soils[55] and animals.[48,56,57] There is a delicate balance in the intestinal microbiota wherein some species prevent the overgrowth of other species and also some species metabolize the waste products of others. It is becoming increasingly clear that this balance is essential to health, not just in the intestines, but also the liver, kidneys and even the neurological system.[58] When this balance is disturbed, overgrowth of some species occurs along with loss of other species. If the overgrowth continues unchecked, the imbalance increases in a vicious cycle as more beneficial colonies are damaged, making the imbalance more pronounced. The waste products of the overgrown colonies increase and the body becomes overburdened. If this goes unchecked long enough, a pervasive and chronic imbalance between colonies will set in. We propose that continual ingestion of residual amounts of glyphosate in our food causes a chronic imbalance in our intestinal microbiota, which ultimately results in multiple chronic diseases.

One of the consequences of a lower pH in the intestines is overproduction of lactate. There are lactate-producing bacteria and lactate-metabolizing bacteria in the intestine. Lower pH does not change the production of lactate, but rather inhibits the metabolism rate of lactate. At low pH, the lactate-metabolizing bacteria die, leaving an over-abundance of lactate-producing bacteria, and thus causing an accumulation of lactate leading to metabolic acidosis.[47]

People with inflammatory bowel disease have both an excess of lactate and lower pH in their feces.[59] In a study of microbial populations as a function of pH in human feces, Belenguer et al.[59] reported an accumulation of both L-lactate and D-Lactate at pH 5.2. Some bacterial groups were directly probed, others identified by their known by-products: lactate, acetate, butyrate, and propionate. The latter three are products of lactate-utilizing bacteria. At the highest pH of 6.4, the dominant lactate-producing bacteria were *Bacteroides spp*. and *Bifidobacterium spp.* The propionate-producing bacteria (*Veillonella sp.* and *Megasphaera elsdenii*), butyrate-producing (*Eubacterium hallii* and *Anaerostipes caccae*), and the acetate-producing bacteria were all present at pH 6.4. As the pH was lowered to 5.2, detection of butyrate and propionate drastically decreased while the acetate and lactate increased. *Bacteroides spp*. and *Bifidobacterium spp*. accounted for 77 to 87% of the initial bacteria present. Bacteroides numbers decreased during incubation at the lower pH values of 5.9 and 5.2. In contrast, *Bifidobacterium spp*. increased sixfold or more. At the lowest pH of 5.2, *Bifidobacterium spp*. became the dominant group with *Lactobacilli* also detected. Lactate utilization was small or negligible at pH 5.2; butyrate and propionate formation were nearly zero, with accumulations of acetate and lactate. D-lactate was also detected at pH 5.2 which the authors ascribe to activity of *Lactobacillus spp*., *Faecalibacterium prausnitzii*, or *Bacteroides spp.* because *Bifidobacteria* can produce only L-lactate. The lactate accumulated at pH 5.2 because production was maintained, but utilization was reduced markedly.

It is well-known that low pH in the rumen of cows and sheep causes disruption in the microbe population leading to metabolic acidosis from overproduction of lactic acid.[60,61] Mackie et al.[61] reported that, as the pH decreased in the rumen of sheep, the acid-sensitive, lactate-utilizing bacteria, *Veillonella* and *Selenomonas*, were superseded by the more acid-tolerant, *Anaerovibrio* and *Propionibacterium*. The lactate-producing bacteria, *Bacteroides*, were superseded by the more acid-tolerant, *Lactobacillus* and *Eubacterium*. If the pH changes were gradual, the lactate-producing/utilizing microbe balance was maintained, though by different species.

According to Hernandez et al.,[60] a drop in pH in the rumen of cows results in the disappearance of many gram-negative bacteria, including lactate-consuming bacteria like *Megasphaera elsdenii* and *Selenomonas ruminantium,* which convert lactate to pyruvate. At the same time, there is an increase in the population of some gram-positive bacteria, especially *Streptococcus bovis*, a lactate-producing bacteria. This causes a further drop in pH from an increase in L-lactic acid production and resulting in another ruminal bacterial population change, specifically, *Lactobacilli spp.* The *Lactobacilli* are "great lactate producer bacteria, especially for D-lactate, which will conduct a new drop of ruminal pH, up to 3.8, an isoelectric point for this acid, and, in this moment, acid will be undissociated, crossing the ruminal wall to the bloodstream and provoking a metabolic acidosis."[62] The decrease in ruminal pH and changes in the ruminal microbiota population are both responsible for the metabolic acidosis in a synergistic way.

It has recently been shown that the high ion gap present in diabetic ketoacidosis is not accounted for completely by ketones and that D-Lactic acid accounts for a large component.[7,8] D-lactic acidosis can be caused by fermentation of incompletely digested carbohydrates by anaerobic bacteria, by an overpopulation of *Lactobacilli spp*. as described above, or, as these authors suggest, is generated by degradation of methylglyoxal. Methylglyoxal, also called pyruvaldehyde, is an intermediate glucose metabolite that is associated with the development of diabetic complications.

Inducing mitochondrial dysfunction

The cellular metabolic process, known as the Krebs cycle, is the oxidation of nutrients to produce usable chemical energy in the form of ATP. The entire cellular metabolic process is critically dependent on electric charge and charge transfer. Since changes in pH are actually changes in total electric charge, it is easy to see how pH changes can alter metabolic processes.

Glycolysis, the first step in energy production, occurs in the cytoplasm of the cell. The reaction is:

$$\text{Glucose} + 2\ NAD^+ + 2\ ADP + 2\ Pi \longrightarrow 2\ \text{Pyruvate} + 2\ NADH + 2\ H^+ + 2\ ATP + 2\ H2O,$$

where NAD^+ and NADH are the reduction oxidation (redox) pair, nicotinamide adenine dinucleotide. NAD^+ is an

electron acceptor (oxidizing agent) and NADH is an electron donor (reducing agent). If hydrogen ions accumulate in the cell the pH drops rapidly. The NAD^+ catalyzes the reaction of the $2H^+$ with oxygen to form H_20. Both of these reactions deplete the cell of NAD^+. The balance between the oxidized and reduced forms (the NAD^+/NADH ratio) reflects the metabolic activities and the health of the cells. In the presence of oxygen, NAD^+ is regenerated in aerobic respiration in the mitochondria through the Krebs cycle. If there is insufficient oxygen, the NAD^+ instead attaches the hydrogen to the pyruvate to form lactate in anaerobic respiration. Anaerobic respiration commonly occurs in the muscles during vigorous exercise where oxygen is quickly depleted. The burning sensation is caused by the buildup of H^+ in the cells and a lowering of the pH.

The cell can generate 2 molecules of ATP per reaction through anaerobic glycolysis in the intercellular matrix. In the mitochondria, a series of interactions occur to generate thirty four molecules of ATP. In the presence of oxygen, pyruvate is transported into the mitochondria, which are like batteries, with an inner and outer membrane. ATP and NAD^+ are generated by OxPhos, an aerobic process where electrons are exchanged between proteins (electron transport) with the final electron acceptor being oxygen and the final products water and ATP. The charge transfer is dependent on a voltage gradient between the inner and outer membrane. As the proteins embedded in the membrane pass electrons from donors to acceptors within the membrane, the electrons provide the energy necessary to transport protons (H^+) across the membrane.

Two processes by which excess hydrogen can build up in cells are hypoxia or a short-circuit in the electron transport chain, decreasing the redox ratio of NAD^+/NADH and forcing the conversion of pyruvate to lactate and anaerobic metabolism. ATP depletion and abnormal changes in the redox ratio lead to oxidative stress, causing further toxic effects through the production of peroxides and free radicals that damage all components of the cell. The effects of oxidative stress depend upon the size of these changes, with a cell being able to overcome small perturbations and regain its original state. More severe oxidative stress can cause cell death through apoptosis, while even more severe or prolonged stresses cause necrosis. Glyphosate, in combination with surfactants, has been shown to cause mitochondrial damage and induce apoptosis and necrosis.[63]

Cytochrome c oxidase is the last enzyme in the respiratory electron transport chain of mitochondria. It contains two copper atoms while cytochrome c has one iron atom within a heme group. There are also iron-sulfur clusters along the electron transport chain. The presence of the copper and iron are critical to the exchange of electrons. If glyphosate binds the available copper and iron, causing a deficiency, this could impact the production of ATP via OxPhos. Alternatively, glyphosate could bind directly to cytochrome c oxidase, thus competitively inhibiting the protein from functioning, which results in chemical asphyxiation of cells. Alternatively, glyphosate could simply deplete cytochrome c, since it has been shown to inhibit the synthesis of all compounds containing porphyrin rings, which include the cytochromes.[64]

Uncoupling of mitochondrial OxPhos by glyphosate has been proposed as an explanation for reduction in respiratory control ratios obtained in experiments on rat liver and corn mitochondria. A 50% reduction was reported for rat liver mitochondria at a concentration of 1.25 mM of the isopropylamine salt of glyphosate administered *in vitro*.[65] The same effect was reported for glyphosate isopropylamine administered *in vitro* to corn mitochondria at a concentration of 10 mM.[66] Glyphosate isopropylamine injected interperitoneally into rats *in vivo* resulted in a 27% reduction in the respiratory control ratio at a dose of 15 ppm and a 46% reduction at the highest dose of 120 ppm.[67] This, along with measured changes in enzyme activity, points to disruption in the OxPhos stage of energy production. A 25% reduction in Krebs cycle enzymes in corn mitochondria was reported for glyphosate at 10 mM concentration.[68]

In a more recent study, multiple parameters associated with the Krebs cycle were measured after administration of Roundup™ and glyphosate to rat liver mitochondria *in vitro*.[69] This author reported null results for glyphosate in concentrations of 0-15 mM. Roundup™, on the other hand, collapsed the mitochondrial membrane potential, increased the membrane permeability, inhibited enzyme activity and caused osmotic swelling of the mitochondria. The author suggests that the difference in his results from those of Olorunsogo et al.[67] are due to the difference in measurements *in vitro* vs. measurements *in vivo*. "For in vitro assays, higher concentrations or a longer incubation period should probably be used in order to obtain a better correlation between in vivo and in vitro results. However, the assays with viable mitochondria cannot be longer than a few hours, which means that only acute effects can be studied."[70]

This does not, however, explain the discrepancy between the *in vitro* measurements of Peixoto[69] and Bababunmi,[65] who reported uncoupling effects at 1.25 mM. We propose that the differences lie in the different formulations used. Peixoto was using glyphosate and Roundup™, whereas Bababunmi and Oloronsogo were using isopropylamine salt of glyphosate. Roundup™ formulations change, but it was almost certainly a salt formulation containing surfactants. The toxicity would therefore be greatest for the Roundup™, somewhat less for the isopropylamine glyphosate and least for glyphosate alone.

This is verified by Lee and Guo[71] who reported on the results of intravenous infusions of glyphosate and its adjuvants in piglets. In addition to the active ingredient, glyphosate, herbicide formulations also contain so-called "inert" ingredients such as oxalates[72] and the surfactants such as polyoxyethylene amine POEA. The role of a

surfactant in herbicides is to improve adherence to the hydrophobic surface of plant leaves for maximum coverage and to aid penetration through the plant surface. It seems obvious that an aid to penetration would also degrade membranes. Lee and Guo[71] infused saline, glyphosate, isopropylamine, POEA and isopropylamine salt of glyphosate into the piglets at a rate of 10 ml/hour for one hour or until the mean arterial blood pressure was reduced by 50%. Results are summarized in Table 3.

This table shows that POEA by itself is more lethal than glyphosate, but there is some synergistic effect between the glyphosate and the isopropylamine, which, alone, do not have much effect. It was also noted in the study that, after 48 hours, glyphosate was barely detectable in the glyphosate-only group, which is consistent with unpublished, industry-sponsored toxicology reports for a single dose administered to rats and mice. In sharp contrast, 148.74 ± 73.36 ppm was measured in the glyphosate iso-salt group. All groups showed an initial increase in arterial oxygen due to the anesthesia, which was retained in the control (saline) group. The groups receiving glyphosate, POEA and glyphosate iso-salt all showed clinical signs of high ion gap acidosis, with glyphosate being the least toxic of the three. The greatest drop in pH was for the glyphosate iso-salt, which also had the biggest drop in arterial CO_2, indicating a rapid depletion in the bicarbonate buffering agent. It is not difficult to understand that Roundup™, containing all three, would be the most toxic, which is consistent with published studies.[69, 73, 74]

Table 3. Arterial blood gas analysis after administration of saline, glyphosate, isopropylamine, POEA and glyphosate iso-salt injection (Derived from Table 5 in Lee and Guo[71]).

			Percent change			
Substance	Survival ratio	Average dose mg/kg BW	pH	P_{O2} (arterial oxygen)	P_{CO2} (arterial CO_2)	lactate
Saline	6/6		-0.13	10	-1.2	-16.3
Glyphosate	6/6	238.47	-0.54	-7.8	2.9	19
Isopropylamine	6/6	75.24	0.40	4.2	8.8	8.8
POEA	2/6	0.094	-0.33	-14.7	0	354
Glyphosate Iso-salt	2/6	159.8	-0.94	-33.2	-16.4	184

In an effort to discover where in the electron transport chain the uncoupling occurs, Oloronsogo et al.[75] designed an experiment to measure the permeability of mitochondrial membranes to protons and Ca^{2+} ions in the presence of glyphosate. A critical part of the ATP generation process is electron exchange through the inner mitochondrial membrane where a potential difference across the internal membrane must be maintained, with the inner matrix being more negative (alkaline) and the inter-membrane volume being more positive (acid). The authors thought that perhaps the uncoupling occurred as protons were transferred across the inner mitochondrial membrane, resulting in the collapse of the electric potential. A proton translocator, or an ionophore that moves protons across lipid bilayers is known as a protonophore.

In this experiment, the action of glyphosate was compared to a known protonophore, carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP). It took 5,000 times the concentration of glyphosate and 3 times as long to obtain the same effect as that of FCCP in the mitochondrial membrane. The addition of glycine had no effect and the addition of Ca^{2+} or Mg^{2+} ions to the reaction media only slightly diminished the effect of glyphosate on proton translocation and on Ca^{2+} accumulation. Thus, the authors concluded that glyphosate does not seem to act like a true protonophore, and the observed uncoupling effect may be due to its ability to act both as a chelator and a mild protonophore.

Finally, the metabolite of glyphosate, formic acid, also inhibits the OxPhos system. Formic acid is highly reactive, readily binds to tissue proteins, and is known to interfere with oxidative metabolism through inhibition of the cytochrome oxidase system.[22, 23]

c. Decreased acid excretion (lungs, kidneys, liver)

Maintaining a narrow operating pH is critical to all bodily functions. The most rapid buffering system is via the lungs. During respiration each molecule of CO_2 that is expelled by the lungs eliminates one H^+ from the

system, leaving only water. The reaction, requiring bicarbonate (HCO_3) as the buffer, is: $H^+ + HCO_3^- <-> H_2CO_3 <-> H_2O + CO_2$.

Failure of the lungs to eliminate CO_2 as fast as it is produced is known as respiratory acidosis. If there is excess H+ in the system, for whatever reason, and the lungs are compromised, respiratory acidosis will ensue. If the compromised lungs cannot supply adequate oxygen, tissue hypoxia results, forcing anaerobic metabolism, which produces lactic acid. Pulmonary edema and bleeding have been reported for acute glyphosate poisoning,[2,71] and pulmonary edema, inflammation and bleeding have been associated with glyphosate use.[20]

In the meantime, the kidneys are overloaded since it is the kidneys that ultimately remove H^+ ions and other components of the pH buffers that build up in excess. The liver converts ammonia (NH_3 a by-product of protein metabolism) to either urea or ammonium (NH_4^+). If the pH is too low (acidosis), production of ammonium increases and ammonium and hydrogen are excreted by the kidneys. If the kidneys begin to fail, the ammonia instead gets converted to urea, resulting in increased blood urea, a classic sign of kidney failure. It is well-documented that glyphosate causes both acute[1-3] and chronic kidney disease[33,34] and both liver and kidney damage.[76] The histopathological findings of the chronic kidney disease (CKD) found in rice paddy workers in Sri Lanka[33] have shown tubular interstitial nephritis associated with mononuclear cell infiltration, glomerular sclerosis and tubular atrophy. Similar reports of CKD are coming in from sugar cane workers in Central America,[77,78] where glyphosate is routinely sprayed as a pre-harvest desiccant.

d. Decreased production or loss of buffers (plasma proteins, phosphates, bicarbonates)

The most important way that the pH is kept relatively constant is by buffers. The body has a huge buffering capacity, and this system is essentially immediate in effect. Body buffers are primarily bicarbonate, ammonium, phosphates, plasma protein and haemoglobin. If the kidneys fail to filter and recycle bicarbonate, stores become too low, the body exchanges H^+ in the blood and inter-cellular fluid for Ca^{2+}, Na^+ and K^+ in the tissues causing hypercalcemia, hyperkalemia and hypernatremia. The imbalance in these minerals leads to a change in osmolality and results in edema, among other things. If a low level of chronic acidosis persists, Ca^{2+}, Na^+ and K^+ is exchanged for H^+ in the bones and teeth.

The carbonate and phosphate salts in bone act as a long-term supply of buffer especially during prolonged metabolic acidosis. The important role of bone buffers is often omitted from discussions of acid-base physiology. Bone consists of a matrix composed of organic [collagen and other proteins] and inorganic [hydroxyapatite crystals; $Ca_{10}(PO_4)6(OH)_2$] components. The hydroxyapatite crystals make up two-thirds of the total bone volume but they are extremely small and consequently have a huge total surface area. The crystals contain a large amount of carbonate (CO_3^{-2}). Bone, the major CO_2 reservoir in the body, contains bicarbonate and carbonate. The bicarbonate makes up a readily exchangeable pool because it is present in the bone water, which makes up the 'hydration shell' around each of the hydroxyapatite crystals. The carbonate is present in the crystals and its release requires dissolution of the crystals. This is a much slower process but the amounts of buffer involved are much larger. Chronic metabolic acidosis is associated with significant loss of bone mineral such as in osteomalacia and osteoporosis.[79]

2.2 Consequences

Prolonged acidosis and metabolic dysfunction leads, over time, to multiple chronic illnesses. The risk is not equal for all people, just as has been noted in cows.[60] The individual response will vary depending on the overall health, health history and hereditary predisposition. Short-term exposure to environmental toxins and drugs is easily overcome by the body's buffering system. However, long-term exposure will eventually tax the system to the point of failure, though it may take a long time.

It's unclear which situation is more serious: mitochondrial dysfunction or acidosis. One actually causes the other and once the system gets out of balance it continues to get more and more out of balance. There are several feedback loops that cause the situation to worsen. Imbalance in intestinal microbiota leads to more imbalance, producing more lactic acid. Compromised organ function in removing excess acids and retaining bicarbonate causes more organ damage and more acidosis. Failure of mitochondria to perform aerobic respiration forces the system to anaerobic respiration, generating more lactic acid.

a. Metabolic acidosis

While acute acidosis is a life-threatening condition, a low-level, chronic acidosis is expected and treated in diabetics and it is not generally considered a disorder with associated symptoms. Nevertheless, chronic metabolic acidosis has been implicated in bone disorders,[79,80,81] cancer,[82,83,84] pancreatitis and liver failure,[85] decreased thyroid function,[86] endocrine and metabolic alterations,[87] osteonecrosis of the jaw,[88] gastrointestinal disorders[89] and inflammation.[90]

The significant increase in the incidence of athletes dying suddenly of cardiac arrest while engaging in heavy exercise has been attributed primarily to heart defects.[91] This was reported as early as 1986, 12 years after

Roundup™ was first registered for use.[92] Congenital cardiovascular disease and congenital coronary artery anomalies are reported as the most common causes.[93] The majority of cases of sudden cardiac death in people under the age of 35 appears to be caused by congenital structural heart abnormalities and defects in the heart muscles. Heart defects are now widely recognized as the most common birth defect among newborn children, and approximately 95% of those with noncritical heart anomalies survive to age 18 or over.[94] Heavy exercise results in tissue hypoxia, forcing the cells into anaerobic respiration, which produces lactic acid and excess H^+, quickly acidifying the tissues. This situation is not dangerous for healthy individuals as the body buffering system is more than adequate to handle it. However, if a sub-clinical, low-grade acidosis already exists, in combination with a congenital heart defect, this is deadly.

There has simultaneously been an increase in sudden death of race horses and show horses, with primary causes being cardiac failure, apparent pulmonary failure, pulmonary haemorrhage, haemorrhage associated with pelvic fractures or with idiopathic blood vessel rupture, and spinal cord injury.[95] Race horses are directly exposed to glyphosate by ingesting grain, hay and sugar beet pulp that have been sprayed with Roundup™. Alfalfa and sugar beets are genetically modified to withstand direct application of glyphosate and grain and hay crops are routinely sprayed as a pre-harvest desiccant.

These reports are consistent with reports from Western Montana. In 1994, there was a significant increase, by many millions of pounds in use of Roundup™ in Montana and in states directly upwind.[96] Beginning in 1995, white-tailed deer (*Odocoileus virginianus*), elk (*Cervis canadensis*), mule deer (*Odocoileus hemionus*), beef calves (*Bos taurus*), domestic goats (*Capra aegagrus hircus*), individuals of multiple species of bird and other animals were necropsied. These showed previously uncommon characteristics of the heart, especially an enlarged right ventricle.[20] From industry-sponsored studies, glyphosate was shown to cause dilated heart in rabbit fetuses, and the percentage of rabbit fetuses with dilated heart was significantly elevated at all dose levels.[97] Additional birth defects, including skeletal defects similar to those reported on ruminants in Western Montana were found on the rabbit fetuses exposed to glyphosate.

In adult animals necropsied in summer of 2006 and in all ages between spring of 2007 and 2011, enlarged right heart ventricle increased dramatically and was found on approximately one third of animals necropsied, with nearly all newborns having this symptom. In the same time period, most necropsied newborn ruminants had severely dilated lymphatic vessels on the surface of the heart.[20] This dilation was not nearly as severe in adults as that observed on the hearts of newborns. Severely dilated lymphatic vessels on the heart surface were not observed prior to 2007, which coincides with many of the farmers switching to herbicides with salt formulations.

Acidosis is common in calves with perinatal weak calf syndrome.[98] All dead newborn calves with veterinarian-diagnosed weak calf syndrome necropsied by Hoy in Western Montana had an underdeveloped, misshapen thymus, which would make them susceptible to infections. Newborns of other ungulate species, including the wild ruminants, often have the same symptoms as those listed for weak calf syndrome, including underdeveloped thymus. Other common symptoms besides weakness and inability to stand or walk are: bone malformations, especially brachygnathia superior, lethargy, inability to maintain body temperature and failure to thrive if they survive more than two days. Underdeveloped and misshapen thymus, along with congenital facial malformations in wild ungulates have been increasing in prevalence in Western Montana as documented by Hoy et al.[20, 99]

b. Mitochondrial dysfunction

Most of the energy needed by the body to sustain life and support growth is generated in the mitochondria. When they fail, less energy is generated within the cell and cell injury or death will result. If this process is repeated throughout the body, whole systems begin to fail: brain, heart, liver, skeletal muscles, kidney, endocrine and respiratory systems. Symptoms of mitochondrial dysfunction may include loss of motor control, muscle weakness and pain, gastro-intestinal disorders and swallowing difficulties, poor growth, cardiac disease, liver disease, diabetes, pancreatic failure, respiratory complications, seizures, visual/hearing problems, lactic acidosis, developmental delays and susceptibility to infection.[100] Pieczenik and Neustadt[101] have linked mitochondrial dysfunction with schizophrenia, bipolar, dementia, Alzheimer's, Parkinson's, epilepsy, migraines, strokes, neuropathic pain, ataxia, cardiomyopathy, coronary artery disease, chronic fatigue, fibromyalgia, retinitis pigmentosa, diabetes, hepatitis C, and primary biliary cirrhosis.

Prion protein misfolding has been recently associated with dramatic reductions of intracellular NAD^+ followed by decreased ATP production.[102] Decreases in NAD^+ indicate that it is not being replenished in the mitochondria in the final stages of the Krebs cycle, resulting in ATP depletion. The protein misfolding is therefore associated with mitochondrial failure. Protein misfolding is a hallmark of neurodegenerative diseases such as Alzheimer's and Parkinson's. Mutations or defects in the mitochondrial protein synthesis can lead to neurological disorders, cardiomyopathy, congestion of the liver, lactic acidosis and renal failure.[103]

The increased susceptibility to infection is particularly

interesting. According to the U.S. Centers for Disease Control, the number of times people were in the hospital with septicemia increased 84% from 2000 to 2008 (621,000 to 1,141,000). In addition, "in 70% of cases of sepsis, the offending pathogen could not be identified although infection seemed to be the only plausible initiating agent."[104] There is a systemic, inflammatory response in all tissues, yet no pathogens can be found.

c. Sarcosinemia

Sarcosinemia is a metabolic disorder characterized by an increased concentration of sarcosine in blood plasma and urine (sarcosinuria). It can result from severe folate deficiency since folate is required for the conversion of sarcosine to glycine. Folate is produced for the host by the gut microbiota as a product of the shikimate pathway, which glyphosate disrupts. We include sarcosinemia because elevated sarcosine is a biomarker for mitochondrial dysfunction and it is also a metabolite of glyphosate though Metabolic Path 1 (above). If glyphosate is being metabolized through this path, an excess of sarcosine could result. Sarcosinemia also has a symptom profile similar to that of mitochondrial disorder; indeed, elevated sarcosine is a biomarker for mitochondrial disorder.

Abnormal concentrations of sarcosine have been associated with Alzheimer's and dementia,[105] neurodevelopmental disability, dystonia, developmental delay and cognitive impairment,[106] "failure to thrive," hypotonia, mental retardation, ataxia, feeding problems and saliva problems.[107,108]

An elevated level of sarcosine has also been identified as a biomarker for certain cancers. Elevated sarcosine not only indicates the presence of prostate[109] and breast cancer,[110] but their aggressiveness as well.[111,112] Injection of 225 mg/kg of nitrosylated sarcosine into mice at days 1, 4 and 7 of life led to the development of metastasizing liver carcinomas in later life in 8 out of 14 exposed animals.[113]

3. Method

The US Food and Drug Administration's (FDA's) Adverse Event Reporting System (FAERS) database is a large collection of drug side effect reports dating back to 2004. FAERS, containing information on both adverse events and medication errors, is a central part of the FDA's post-marketing safety surveillance program for drugs and biological products. The system is voluntary for healthcare professionals and consumers, but mandatory for regulated industry and user facilities. The data are made available on the Web for free download. Each report is a structured entry containing the date of the incident, the age, gender and race of the person, a list of drugs that were taken and a list of side effects that were experienced. It is widely acknowledged that spontaneous reporting systems substantially under-represent the actual number of cases of adverse reactions that occur, estimated at only 6% of actual events.[114]

If a condition of acidosis exists through ingestion of toxic substances in the food, the first affected system will be the mouth: the teeth, salivary glands and jaw. Indeed, excess salivation, cellular changes in, and enlargement of the salivary glands were often reported in the industry-sponsored safety studies on glyphosate.[49] A separate study was even undertaken to determine the cause of the salivary issues and it was found to be dependent on pH.[115]

The primary secretion from the salivary glands is a plasma-like fluid rich in Na+ and Cl-. Signaling by neurotransmitters and hormones activates release of Ca^{+} and K^{+}. The pH is maintained by balancing the ion exchange between these electrolytes.[116] The salivary glands are the first line of defense in maintaining the acid-base balance in the body. Furthermore, osteonecrosis of the jaw (ONJ) has been associated with acidosis.[88] We therefore hypothesize that ONJ will capture symptoms related to acidosis and mitochondrial dysfunction in the FAERS database.

We began by finding all cases where ONJ was mentioned as a side effect in the FAERS database during the time window from 2002 to 2012. We then identified the top-10 other side effects that were most commonly associated with ONJ, and downloaded all the cases where one or more of these ten side effects occurred in the database over the same time span. These top ten were: pain, anxiety, osteomyelitis, bone disorder, back pain, osteoarthritis, anaemia, injury, arthralgia and depression. We then had a larger database of all instances where any of these ten symptoms were listed as a side effect which we then divided into two distinct sets, those with ONJ (the target dataset) and those without ONJ as a side effect (the comparison dataset). The frequency of the occurrence of a particular drug or symptom in the two datasets could then be compared.We devised a score for the bias in the distribution of each symptom class between the ONJ and NOT ONJ subsets. The score was computed as follows:

F1 = Count1/N1 = Frequency of drug-class or symptom in subset 1 (target).
F2 = Count2/N2 = Frequency of drug-class or symptom in subset 2 (comparison).
Score = $1000 * (F1/(F1 + F2))$ (1)

The scores capture skewed distributions of each symptom over our contrastive datasets. The score ranges from 0 to 1000, with 500 denoting that a symptom occurs with equal frequency in the two datasets. Any score over 800 is highly skewed towards the target set: 800 means that the symptom is four times more frequent in the target score set than in the comparison set (e.g., F1 = 4*F2). Armed with this method, it was then possible to define a lower cutoff for the score and focus on all symptoms that exceeded this cutoff.

We consider that a symptom is significantly over-represented in the target data set if the counts correspond to non-overlapping frequency distributions in the two datasets. In order to determine this, we calculated the 95% interval assuming a Gaussian distribution. With a fixed value for Count1, we varied Count2 until its 95% interval was close, but did not overlap with the interval of Count1. We then calculated the score on the basis of these two counts, choosing an appropriate range of values for the fixed Count1, from 20 to 450. This yields the minimum score that can be considered significant at the 95% confidence interval, as a function of Count1. Results are shown in Table 4 over the range from 20-450. Table 4 shows that for count1>450, any score over 530 is significantly over-represented in the target set; i.e. the distributions in counts for the two datasets do not overlap. There were a total of 10,580 counts in the Top_10+ONJ target set and 762,002 in the Top_10-ONJ comparison set.

In order to probe these datasets, we then sorted the symptoms associated with mitochondrial dysfunction and acidosis into classes as shown in Supplementary Table 1. Each dataset was then searched for all instances of the symptom listed in each class. A string in quotes must be an exact match, whereas a string preceded by % collects all events that include that string. For example, "ACIDOSIS" will only collect a match for acidosis, whereas "%ACIDOSIS" will collect not only acidosis, but metabolic acidosis, lactic acidosis, respiratory acidosis. The number of counts for a symptom class can exceed the number of records in the dataset because each record contains many symptoms.

Finally, we explored our symptom classes against drugs that are known to cause mitochondrial disorders. There are several classes of drugs that are known to cause acidosis and/or mitochondrial dysfunction.[10,25,26,117,118,119,120,121] These studies document a connection between liver disease and mitochondrial disorder,[121] renal tubular dysfunction and acidosis,[25,26] along with electrolyte imbalance,[26] acidoses,[10,117,118] mitochondrial damage,[119,120] by uncoupling of OxPhos,[118,121] and the drugs that are known to cause them.

In order to investigate the effects of drugs that cause mitochondrial dysfunction on the subgroup that are diagnosed with ONJ, we reverted to the original dataset including all instances of ONJ (14055 events) and divided that into two subsets: those events where a drug known to cause mitochondrial disorder was listed among the drugs administered and those where these drugs were not listed. We started with the drugs documented to cause mitochondrial disorder given in Table 5 of Neustadt & Pieczenik.[120] We then used a web search to identify all of the trade names or aliases for each drug in the list. The results for all of the drugs we identified are shown in Supplementary Table 2. The significant scores for 95% confidence levels for this dataset are also given in Table 4. There were a total of 14055 records in the ONJ dataset with 5734 in the target set (with drugs) and 8320 in the comparison set (without drugs). Table 4 shows that for Count1>450, any score over 542 is significantly over-represented in the target set.

Table 4: Score required to obtain 95% confidence intervals for count1 in the target dataset. In the case of Top 10+ONJ there were 10,579 target cases and 762,001 comparison cases. For DRUGS, there were 5734 target cases and 8320 comparison cases.

Count1	>450	>270	>170	>75	>50	>35	>25	>20
95% CI score for Top 10+ONJ	>530	>536	>546	>575	>590	>612	>634	>658
95% CI score for DRUGS	>542	>558	>574	>615	>645	>679	>721	>744

4. Results

The results for the Top_10+ONJ and Top_10-ONJ datasets are shown in Table 5. Results for the ONJ_with_DRUGS and ONJ without_DRUGS is shown in Table 6.

Table 5: Counts and scores for the Top_10+ONJ target set compared to Top_10-ONJ comparison set for various symptoms linked to mitochondrial disorder, as defined in Table 5. The scores are computed as defined in Eqn. (1).

SYMPTOM CLASS	C1 (target)	C2 (compare)	SCORE
Neurological disorder	3929	64749	813
Neurological symptoms	8163	125063	824
Mood disorder	11747	242842	776
Loss of consciousness	1622	29248	799
Headache	2457	69460	718
Acidosis	363	8758	749
Acidosis symptoms	4329	73565	809

Mineral imbalance	2257	13841	921
Protein problem	737	10577	833
Weight regulation	3256	52416	817
Temperature regulation	910	30435	682
Hearing problems	2128	11110	932
Eye problems	1222	28255	756
Muscle problems	7018	131636	793
Fatigue	5072	84046	812
Heart failure	3167,	25047	901
Arrhythmia	4950	62934	849
Cardiovascular disease	2356	47453	781
Lung	15831	151269	882
Liver failure	2631	33262	850
Renal tubular dysfunction	116	2817	747
Intestinal problems	5675	82400	832
Joint problems	11654	118401	876
Bone disorder	63274	222685	953
Bone marrow oedema	430	872	972
Mucosa oedema	687	5972	892
Jaw, mouth and throat disorder	32228	50429	978
Acid reflux	11634	150602	847
Pancreas issues	528	10264	787
Thyroid disorder	1136	7819	912
Infection	16073	126242	901
Cancer	7941	32271	946
Diabetes	2201	23408	871
Oedema	6097	56392	886
Allergy	868	5762	915
Chemical sensitivity	80	2098	733

Table 6: Counts and scores for the ONJ_with_ DRUGS target set, compared to ONJ_without_DRUGS for various symptoms linked to mitochondrial disorder, as defined in Table 5. The scores are computed as defined in Eqn. (1).

SYMPTOM CLASS	C1 (target)	C2 (compare)	SCORE
Neurological disorder	3102	839	842
Neurological symptoms	5910	2307	788
Mood disorder	7911	3839	749
Loss of consciousness	1196	444	796
Headache	1738	763	767
Acidosis	265	102	790
Acidosis symptoms	3320	1033	823
Mineral imbalance	1829	451	854
Protein problem	548	196	802
Weight regulation	2284	998	768
Temperature regulation	663	249	794
Hearing problems	1490	660	766
Eye problems	959	271	836

Muscle problems	4968	2113	773
Fatigue	3834	1270	814
Heart failure	2459	724	831
Arrhythmia	3585	1426	784
Cardiovascular disease	1709	659	790
Lung	11661	4279	798
Liver failure	1987	657	814
Renal tubular dysfunction	88	28	820
Intestinal problems	3986	1786	764
Joint problems	5872	2804	752
Bone disorder	41050	26695	690
Bone marrow oedema	370	62	896
Mucosa oedema	483	230	752
Jaw, mouth and throat disorder	18649	18057	599
Acid reflux	7961	3833	750
Pancreas issues	401	128	819
Thyroid disorder	702	461	688
Infection	10352	6088	711
Cancer	5769	2420	775
Diabetes	1513	705	756
Oedema	4734	1391	831
Allergy	430	464	573
Chemical sensitivity	61	19	823

5. Discussion

All of the symptom classes are over-represented in both Top_10+ONJ and ONJ_with_DRUGS as we hypothesized. All of the symptom classes associated with mitochondrial dysfunction are over-represented by a factor of 2 or more in the Top_10+ONJ dataset. Discounting the jaw problems, which is used to define the set, 86% are over-represented by a factor of 3 or more (score = 750); 69% by a factor of 4 or more (score = 800); and 26% by a factor of 9 (score=900) or more.

In the ONJ_with_DRUGS dataset, 89% of the symptoms are over-represented by a factor of 3 or more and 42% by a factor of 4 or more. This is a remarkable result, as it is on top of the strong discrimination between ONJ and NOT_ONJ shown in Table 5. The distinction here is those cases where a person with a diagnosis of ONJ is or is not taking at least one drug known to cause mitochondrial damage. Note that the renal tubular dysfunction class only includes renal tubular problems and does NOT include any of the following: "%RENAL FAILURE" "%NEPHRO" %KIDNEY" "%BLADDER" or "%RENAL". It is striking that renal tubular problems are 4.6 times more likely to occur in the ONJ_with_DRUGS than in the ONJ_without_DRUGS dataset. This is consistent however, with the kidney problems of the rice paddy workers in Sri Lanka which was reported as tubular interstitial nephritis.[33, 34]

It is perhaps not surprising that diabetes, neurological disorders, heart problems, cancer, infection and mood disorders are over-represented in the ONJ_with_DRUGS dataset since these drugs are used to treat these disorders. The remainder of the symptoms are not so easily explained unless you accept the assertion that these patients suffer from metabolic acidosis and associated mitochondrial dysfunction. This could even have been the case before treatment with the drugs that then made the situation worse.

We noted also a striking resemblance to symptoms for Lyme disease in our symptom classes. Lyme disease is a tick-borne infection of the bacterium, *Borrelia burgdorferi* that apparently is an epidemic in the US with more than 300,000 new cases reported per year.[122] There is a great deal of controversy surrounding the test for the *Borrelia burgdorferi* antibody, with many false negatives. Thus, diagnosis of Lyme disease is primarily made by clinical observation. We propose that what is being diagnosed and reported as Lyme disease is, in fact, chronic glyphosate poisoning. Even a positive antibody test could be due to an overactive immune system in response to toxins.

6. Conclusion

We have shown that liberally spraying our food crops with glyphosate is cause for grave concern. With the

steep increase in the number of confirmed cases of glyphosate-resistant weeds,[123] the manufacturers are already looking for alternatives to glyphosate. One alternative under consideration is 2,4-D (2,4-dinitrophenol), but 2,4-D has long been known to uncouple OxPhos.[124] In fact, a wide variety of agricultural pesticides are known to uncouple OxPhos or otherwise interfere with mitochondrial respiratory chain functions.[125] The solution is not to exchange one toxic chemical for another and wait 20 years or more for people to discover its toxicity.

The allowed residues for pesticides are based on industry-sponsored animal studies. In these studies the dose is increased until adverse effects are observed. The highest dose where no adverse effects are observed is divided by 100 to obtain a "safe" daily intake value. This is done for each agricultural pesticide but there is no accounting for adjuvants, multiple pesticides, interactions between them or total residues from all. These toxicity tests are based on the assumption that "the dose makes the poison." We are asked to believe that known poisons are safe in low doses, though they are consumed at every meal for life.

We have given direct evidence from the literature that glyphosate: causes acidosis and acidosis symptoms; causes kidney and liver damage; causes disruption of microbiota in the human gut; can be metabolized by microbes residing in the human gut; and disrupts mitochondrial function. We have given circumstantial evidence from the FAERS database that the symptoms of chronic glyphosate poisoning due to chronic low-level acidosis and mitochondrial dysfunction overlap with symptoms of mitochondrial disorder and the drugs that are known to cause it.

We have given direct evidence from the literature that acidosis/mitochondrial dysfunction can cause a multitude of symptoms and diseases. These diseases and symptoms are remarkably consistent with reports of correlations between the rise in neurological diseases, diabetes, obesity, chronic and acute kidney failure, infections and cancers of the liver, kidney, thyroid, bladder and pancreas along with glyphosate applications to US corn and soy crops.[19] These diseases and symptoms are remarkably consistent with reports of correlations between pesticide usage in the US and heart, lung, liver, skin, eye, genitourinary problems, metabolic disorders and congenital facial anomalies reported in newborns and wildlife.[20]

How much evidence is needed? The rate of chronic disease in the entire US population has been dramatically increasing, with an estimated 25% of the US population suffering from multiple chronic diseases.[126] Furthermore, the onset of serious illness is appearing in increasingly younger cohorts.[127] The US leads the world in the increase of deaths due to neurological diseases between 1979-81 and 2004-06 for the 55-65 age group. These mental disorder deaths are more typical of the over-65 age group. There have been similar findings for obesity, asthma, chronic disease, and behavior and learning problems in children and young adults.[128] Type II diabetes in youth is being called an epidemic.[129]

All of the body's signaling and regulation systems depend on charge and charge transfer. The body's mechanism for generating usable energy is critically dependent on charge transfer. This is why the body pH must be maintained within a narrow band. It should be obvious that any substance disrupting this critical balance will cause problems and, if allowed to continue over a long period, will eventually cause catastrophic harm. Otto Warburg was awarded the Nobel Prize in Physiology in 1931 for his work in the area of cellular metabolism and respiration. Warburg hypothesized that cancer growth is caused by tumor cells generating energy by anaerobic respiration instead of through the aerobic Krebs cycle within the mitochondria.[130] Cancer is therefore, primarily, a mitochondrial dysfunction.

We would go one step further and hypothesize that all modern diseases are primarily mitochondrial disorders. The mitochondrial dysfunction is caused by chemicals: pesticides, pharmaceutical drugs, food preservatives and additives, environmental and industrial chemicals. Our solution is to give the patient drugs, many of which themselves also cause mitochondrial dysfunction. The drugs may alleviate a symptom in the short-term, but cause irreparable harm in the long-term. When the patient develops cancer or kidney failure, the medical professionals shrug and say it is just bad luck. The reason this has not been revealed is because these are huge, multi-billion dollar industries. How much is enough?

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