Examining a Possible Link between Glyphosate Exposure and Health Status among Racehorses

Stephanie Seneff¹, Anthony Samsel² and Gregory Bennett³

1. Computer Science and Artificial Intelligence Laboratory, MIT, Cambridge MA 01890 USA. Email: seneff@csail.mit.edu
2. Samsel Environmental and Public Health Services, Deerfield, NH 03037, USA. Email: anthony.samsel@acoustictracks.net
3. ???

1 Introduction

The recent deaths of up to 11 young racehorses on the Del Mar, CA, race track is cause for alarm. This is part of a much larger problem of failing health among racehorses, as there is now general agreement that horses today are suffering from more debilitating health issues than was the case two or three decades ago. In a 2006 study investigating over 1000 horses, veterinary problems were found in 62% of the two-year olds and 50% of three-year olds [1]. The most commonly reported problems included sore shins, inflammatory airway disease, joint problems, and fractures.

While it has been suggested that unreasonable performance demands under harsh conditions and/or the overuse of certain drugs such as Lasix may be contributing to this increase, we highly suspect a toxic chemical exposure mainly from food sources as the primary cause of their overall deteriorating health. In fact, we hypothesize that the pervasive herbicide, Roundup®, may be a significant causal factor, as there is strong evidence from the known toxicological effects of Roundup® to implicate a possible causative role. If we succeed in showing a cause-and-effect relationship between Roundup® and health status of racehorses, it will be good news, because a straightforward solution is to switch racehorses to a 100% certified organic diet. The higher food costs will be more than offset by the reduced medical bills as well as the superior athletic performance of the horses.

Glyphosate is the active ingredient in Roundup®. Although Monsanto, its
manufacturer, has claimed that glyphosate is nearly nontoxic to mammals, much evidence from the independent research literature is proving otherwise [2, 3, 4, 5, 6, 7]. Many of the conditions that racehorses suffer from today can be explained by the known toxicological effects of glyphosate. Perhaps the most troubling one is cardiac arrest following arrhythmias at the conclusion of a race. This is remarkably similar to cardiovascular effects of glyphosate on exposed rats in a recently published study [8]. Reproductive issues are likely also in part attributable to glyphosate exposure. Glyphosate’s adverse effects on the male reproductive system are well characterized [4]. Glyphosate has also been shown to cause severe health problems, including congenital deformities and failure to thrive, among piglets in Denmark [9].

A consideration of the standard diet of racehorses reveals some likely sources of glyphosate exposure. Alfalfa [10, 11] and sugar beets (beet pulp) [12] are among the short list of crops that have been engineered to be glyphosate resistant. As a consequence, glyphosate application to control weeds results in significant uptake by the crops. The use of glyphosate has risen sharply over the past decade or more, as a consequence of the widespread appearance of glyphosate-resistant weeds. Very little monitoring has been conducted by the government, so there is insufficient knowledge to date of the degree of exposure from food sources. However, a recent study found glyphosate residues in several samples of various brands of cat and dog food, including premium brands [13].

In this research, we propose to systematically measure glyphosate levels in the urine of a large number of racehorses, and to look for correlations of high levels, if found, with health status. We also want to replace the diet with organic alternatives, and to observe whether this results in health improvements.

2 Evidence Linking Glyphosate to Health Issues

Racehorses suffer from multiple health problems. The most common issues relate to fractures and other musculoskeletal injury [14] and respiratory problems [15, 16]. Neurological, gastrointestinal, ophthalmic, and reproductive problems, as well as infection, are also relatively common. As in humans, there is a condition called “equine metabolic
syndrome” that parallels the human version of metabolic syndrome, which is associated with obesity and diabetes in horses [17]. Laminitis is a crippling condition often linked to insulin resistant diabetes [17, 18]. Diabetes in humans is associated with increased risk to osteoporosis and fragility fractures [19]. In this section, we discuss several different health issues facing racehorses and explain how glyphosate could be causal in these conditions.

Glyphosate’s toxicity arises due to multiple distinct physical and chemical properties of this unusual synthetic molecule. In [7], arguments were advanced that glyphosate’s ability to strongly chelate multiple minerals makes them unavailable to the microbiome, leading to an imbalance in gut microbes and subsequent gastrointestinal disease. In fact, glyphosate has been patented as an antimicrobial agent. Chronic idiopathic inflammatory bowel disease is a relatively common condition in horses today that was first observed in 1974 [20], which is when glyphosate was first introduced on the market. Glyphosate also disrupts the activity of cytochrome P450 (CYP) enzymes in the liver, which has multiple effects on liver function including impairing the ability to detoxify multiple other environmental toxic chemicals. Vitamin D is activated by CYP enzymes in the liver and kidney, so deficient CYP activity could be causal in vitamin D deficiency.

Glyphosate is an amino acid analogue of the coding amino acid, glycine, and it likely derives much of its toxicity from its ability to disrupt glycine’s multiple roles in the body. Most significantly, it is highly likely that glyphosate can substitute by mistake for glycine during protein synthesis [21], and this ability would lead to an insidious accumulation of glyphosate in multiple proteins throughout the tissues, causing grave structural and functional damage and easily explaining the strong correlations between glyphosate and the alarming rise we are observing today in multiple diseases among humans.

2.1 Sudden Death

Multiple cases of sudden death during high profile horse race competitions have alerted the general public to this serious health threat for racehorses. Possible causes include rupture of an aortic aneurism and arrhythmias leading to cardiac arrest. Both of these can easily be
explained by chronic exposure to glyphosate.

A recent paper by Gress et al. [5] involved exposing Sprague-Dawley male rats to Roundup® and specifically addressing cardiovascular effects. Roundup® caused conduction blocks, long QT syndrome, and arrhythmias in the exposed rats. Interestingly, the effects were substantially blocked through administration of ouabain, a drug known to suppress the activity of the sodium-potassium pump.

Humans exposed to toxic levels of glyphosate through suicide attempts also exhibit similar cardiovascular effects, including long QT syndrome, atrioventricular conduction block, arrhythmias and cardiac arrest [22]. In this study, patients who died were more likely to show a prolonged QT interval. Long QT syndrome is also a feature of racehorses that is linked to sudden death [23].

The sodium-potassium pump is tightly regulated. Multiple proteins are involved in either suppressing the gain or triggering clearance of the protein based on appropriate signaling cascades. We have discovered that at least three of the proteins involving the pump contain highly conserved glycine residues that could be disrupted by glyphosate. One of these, phospholemman, is normally attached to the pump in the membrane, and it suppresses its activity level by about a factor of two. Disruption of its two highly conserved glycines by glyphosate will prevent it from anchoring in the membrane, essentially disabling its function altogether [24]. The second protein is a kinase which phosphorylates and inactivates phospholemman. Glyphosate’s substitution for glycine in the active site of the kinase can be predicted to increase its activity [21], thus disabling phospholemman’s suppression of the pump even when the phospholemman is not itself contaminated with glyphosate. The third protein is the glycine-rich ZNRF2, which controls the degradation of the Na-K pump. Disruption of its conserved glycines will cause the pump to linger longer than it should, thus exhausting ATP supplies and causing the cell to reach a hyperpolarized state, with too much internal potassium and too little internal sodium [25]. In the situation of a race horse at the sudden cessation of movement at the end of a race, it can be predicted that the pump will not be able to turn off sufficiently fast, the potassium levels in the blood will decline sharply, and this will initiate the arrhythmias and subsequent cardiac arrest. Marfan syndrome is a relatively common
genetic disease among humans that leads to a high risk for ruptured aorta, particularly if they choose an athletic career [26, 27, 28, 29]. A well-known case is the US Olympic volleyball star Flo Hyman, who died at the age of 31 from a ruptured aorta [27]. The disease typically involves one of a large number of mutations in the gene for the protein fibrillin, an elastic fiber found in connective tissue. A study examining six distinct mutations, all involving a substitution of a bulkier amino acid for an essential glycine in fibrillin, showed that each of these mutations resulted in Marfan syndrome and a strong risk of aortic rupture among multiple family members sharing the gene [29].

Collagen, a dominant protein in connective tissue, contains huge amounts of glycine. About 25% of the amino acid residues in collagen are glycine molecules. Osteogenesis imperfecta is a generalized disorder of connective tissue characterized by fragile bones that are easily susceptibility to fracture. Marini et al. have tabulated 832 distinct mutations in collagen linked to osteogenesis imperfecta, and the overwhelming majority of these involve point substitutions for glycine [30]. Therefore, it becomes evident that glyphosate substitution for glycine in collagen can lead to a similar defective condition. Random glyphosate substitutions for glycine in both collagen and fibrillin during protein synthesis leads to a weakened collagen matrix which is particularly dangerous in the aorta which faces considerable stress under high pressure.

2.2 Botulism

Botulism is a serious threat to horses fed silage contaminated with Clostridium botulinum [31, 32]. The condition is manifested by neuromuscular paralysis and is often fatal. Enterococcus species thriving in the gut can suppress the growth of pathogens such as Clostridium. In vitro experiments have shown that glyphosate at low concentrations inhibits the growth of Enterococcus facailis in cattle and horse feces, which leads to increased risk to botulism due to Clostridium overgrowth in the gut [33]. More generally, disruption in the gut microbiota is a major potential risk of chronic exposure to environmental toxins, including glyphosate [34].

2.3. Liver Disease
Diagnosis of liver disease in horses can be difficult because the clinical signs are highly variable and often nonspecific [35]. Common causes of liver disease in the horse include biliary obstruction secondary to intestinal displacement or biliary stones [36], chronic active hepatitis and fatty liver disease. Elevation in the serum level of gamma glutamyl transpeptidase (GGT) is a good indicator of liver disease and is a strong risk factor for multiple other diseases, including cardiovascular disease, diabetes, metabolic syndrome (MetS), and all-cause mortality [37]. Increased GGT levels have been reported in young Thoroughbred racehorses even without obvious liver dysfunction.

There has been a recent upward trend in the serum levels of GGT across the human population in the past two decades, particularly in the United States and Korea, two countries where glyphosate exposure is high [37]. GGT is an enzyme that breaks down glutathione, a tripeptide sequence containing glycine, glutamate and cysteine. It is possible that glyphosate, acting as a glycine analogue, is substituting for glycine in glutathione, disrupting its function and necessitating clearance.

A postmortem case study of a nine-year-old Thoroughbred racehorse that died of liver disease revealed a marked elevation in GGT levels, along with a markedly enlarged liver (3.6% of body weight), hepatic lipidosis, severely obstructed bile flow due to cholelithiasis and severe portal fibrosis [35].

### 2.4 Musculoskeletal Problems

Glyphosate’s ability to replace glycine in collagen could result in weaker collagen and predispose to breakdown injuries of tendons and ligaments. In addition, incorporation into the bone matrix may predispose to fractures. Tissue sample collection of surgical procedures and postmortem samples could be analyzed for the presence of glyphosate. Glyphosate is also neurotoxic: glyphosate’s known ability to chelate minerals, especially cobalt and manganese [38], can lead to deficiencies in blood and tissues. Chondroitin sulfate synthesis depends on manganese and is critical for bone development. Furthermore, attachment of chondroitin sulfate, heparan sulfate and keratan sulfate to collagen depends on critical glycine residues that could be displaced by glyphosate [30].
2.5 Pulmonary Disease

Exercise-induced pulmonary hemorrhage (EIPH) - bleeding that occurs from the lungs during intense exercise – is a serious issue with race horses that is believed to be related in part to the widespread practice of administering furosemide (Lasix) [39]. However, glyphosate’s ability to interfere with the function of CYP (cytochrome p450) enzymes, especially endothelial nitric oxide synthase (eNOS) [7], could also be a factor in EIPH by its expected adverse effects on vascular resistance and blood pressure regulation. Furthermore, the potential of glyphosate to substitute for glycine in collagen and elastin could weaken the vascular-respiratory interface at the alveoli, disturbing the elasticity of the lung and predisposing to EIPH. Autopsy studies to characterize pathology specimens of lung may reveal a weakened lung tissue identified by the replacement of collagen with a more inelastic protein structure, and by a deficiency in attached sulfated proteoglycans such as heparan sulfate. By enzymatic analyses of lung tissue, glyphosate residues could possibly be discovered [40].

Infectious respiratory disease is common in racehorses, particularly young Thoroughbred racehorses [41]. Chronic obstructive pulmonary disease (COPD) is also a common disease of horses, not especially seen in young thoroughbred racehorses, but presented more often in older horses. As glyphosate is able to incorporate in place of glycine in collagen and elastin, it could lead to some of the clinical signs of COPD.

2.6 Retinoic Acid Overexpression

A seminal study involving exposing frog embryos to glyphosate revealed considerable disruption of development consistent with excess exposure to retinoic acid [3]. This is plausibly due in part to glyphosate’s known effect to suppress CYP enzyme activity [42], since retinoic acid is metabolized by CYP enzymes. A retinoic acid analogue used to treat acne, Tretinoin, has many side effects that correspond to some of the known common health issues in racehorses [43, 44]. Respiratory side effects that signify retinoic acid syndrome include upper respiratory tract disorders, dyspnea, respiratory insufficiency, pleural effusion, pneumonia, rales and expiratory wheezing. Excessive retinoic acid also has multiple effects on the cardiovascular system, including arrhythmias, hypotension, cardiac
arrest, pericarditis, pulmonary hypertension, and enlarged heart.

Intracranial abscesses due to intracranial hypertension are relatively common among horses, and are often fatal [45]. A causal factor could be glyphosate’s induction of excessive levels of retinoic acid. Retinoic acid treatment for leukemia has caused intracranial hypertension in both children [46] and adults [47].

2.7 Laminitis and Insulin Resistance

Laminitis (also known as founder) results from a failure of the hoof lamellar-distal phalangeal attachment apparatus, and it is a significant cause of lameness in horses. While the exact causal mechanism remains unknown, laminitis is clearly linked to diabetes and insulin resistance in horses [18]. In fact, a controlled experiment conclusively demonstrated that insulin and glucose infusion can induce laminitis in non-insulin resistant horses. Equine metabolic syndrome is a growing problem in horses and is directly tied to both insulin resistance and laminitis [17]. Metabolic syndrome and associated diabetes in humans has reached epidemic proportions in the modern world [48], and the rise in diabetes incidence in the US is strongly correlated with the rise in glyphosate usage on core crops [49]. In [21], it was shown that glycine plays an essential role in the insulin receptor’s ability to bind to the plasma membrane. Glyphosate substitution for glycine in the insulin receptor would therefore directly lead to insulin resistance.

3 Research Plan

Based on initial samples of the racehorse feed collected and analyzed for the presence of glyphosate, it was determined that three commonly used feeds have glyphosate residues present. The initial feed collection study was based on clinical signs of high liver enzymes (particularly GGT), weight loss and inappetence in a racing thoroughbred filly. Upon cessation of feeding the tested feed, the filly showed marked improvement in appetite within five days. Serial blood analysis showed significant improvement (lowering) of GGT within thirty days, with return to normal values within ninety days.

Based on evidence presented in this paper, we propose a more thorough
study analyzing feed samples and blood/urine samples from a larger population of racehorses. Introduction of organic feeds in selected horses would be beneficial to test the clearance of glyphosate from blood/urine samples and to see whether symptoms improve following the switch to an organic diet.

Due to glyphosate’s ability to substitute for glycine in many proteins, especially collagen [21], further investigation of select tissue may reveal a weakened collagen structure, which could further potentially lead to MSK injuries.

Studies into necropsy specimen tissues may reveal more information regarding damaged structural integrity of collagen tissue, which could influence breakdown injury and cardiovascular events. We will also subject tissue specimens to analysis for glyphosate contamination, both before and after extensive enzymatic proteolysis, in order to assess whether glyphosate is embedded in the peptides present in the tissues.

4 Conclusion

As veterinarians and researchers, we have an obligation to protect the welfare and health of animals we care for. Any potential sources of feeds, supplements or pharmaceuticals that could adversely affect our patients should be vigorously investigated. Much data exists in humans and other animal species that indicate that glyphosate could adversely affect many organ systems in the horse. Further investigation is warranted based on finding glyphosate in very high amounts in commercial horse feeds. Toxicity is magnified many times as glyphosate is formulated with additional adjuvants that enhance its toxicity [50]. As racehorses are stabled indoors more frequently and are exposed less to sunlight, vitamin D deficiency could result. Racehorses that train in very early sets in the morning may get very little sun exposure especially in the winter months in the northeast U.S.A. Reduced sun exposure could also cause reduced cholesterol and sulfate metabolism and transport, and negatively affect overall health [51].

References


Roundup ready alfalfa. Penn State Extension. 


[21] Samsel A, Seneff S. Glyphosate, pathways to modern diseases V:


2010;31:E1021-E1042.


