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Glyphosate Exposure Associated with Human Neurodegenerative Disorders: A Scoping Review

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Abstract

Chemically engineered agricultural products such as pesticides, insecticides, and herbicides, although used considerably for both industrialized and personal agricultural use, have recently been associated with a number of serious human health disorders. This rapid literature review aims to accumulate and analyze research from the last ten years, focusing specifically on the effects of exposure to glyphosate-based herbicide products such as Roundup as associated with the formation of various neurological disorders. Specifically, this review focuses on laboratory research using animal models or human cell cultures as well as human population-based epidemiological studies. It associates exposure to glyphosate or glyphosate-based products with the formation or exacerbation of neurological disorders such as Parkinson's disease, Alzheimer's disease, seizures, and autism spectrum disorder. In addition, it examines the correlation between the gut-brain axis, exposure to glyphosate, and neurodegeneration.

Keywords

Herbicide, Glyphosate, Roundup, Neurodegeneration, Neurodegenerative Disorder, Parkinson's Disease, Alzheimer's Disease, Seizures, Autism Spectrum Disorder, Gut-Brain Axis

1. Introduction

The use of chemically engineered products for the commercial growth and maintenance of crops is essential to agricultural research and industry. In addition, these agricultural products are consumed extensively for personal use in lawns and gardens. Such products include a wide variety of different herbicides,

fungicides, pesticides, insecticides, and fertilizers. The United States consumes pesticides at the second highest rate in the world behind China, and herbicides are among the most widely used of these products [1]. However, recent research has begun demonstrating serious health consequences associated with exposure to various types of these widely used products. One of the products that has raised significant concerns is Roundup and associated products (e.g., Glyphomax Plus, Glyphos, Touchdown IQ, Touchdown 5) containing its active ingredient glyphosate.

N-(phosphonomethyl) glycine chemical, known by its common name glyphosate, is an amino acid substitute, and its action in destroying plant life stems from its ability to inhibit the EPSPS enzyme in plant metabolism [2]. This creates severe consequences for the function of the shikimic acid pathway [3]. While it is intended that the toxic action of glyphosate-containing agricultural products be selectively aimed at the pathways in plant systems, there has been developing concern that glyphosate may also interfere with pathways in mammals, including humans who are exposed to and consumed products containing trace amounts of these substances. In addition, those who work in the agricultural industry would experience a greater degree of exposure, leading to an increase in complications associated with such exposure. Recent research conducted by Connolly *et al.* [4] demonstrates that the half-life of glyphosate in the human body, as calculated from human urine samples, is about 3.5 to 14.5 hours. Soares *et al.* [5] demonstrated that glyphosate residues existed in popular food items, such as honey, fruit juice, wheat products (cereals and bread), vegetables, beans, meats, and fish.

Research has demonstrated various diseases and disorders that have been suggested to be associated with glyphosate-containing herbicide exposure, such as exposure to Roundup. For example, glyphosate exposure in children and adolescents is associated with liver inflammation, which has been further linked with cancer, diabetes, and heart disease formation [6]. Other studies have directly correlated glyphosate and glyphosate-containing herbicide exposure with cancer, such as non-Hodgkins lymphoma and reproductive issues [7] [8]. Thus, it has been established that glyphosate is of substantial concern to human health.

This systematic review seeks to investigate the neurological effects of exposure to agricultural herbicides containing the chemical glyphosate as their primary ingredient. Numerous studies have demonstrated that herbicides containing the chemical glyphosate can be implicated in the formation of a variety of different disorders associated with neurotoxicity and neurodegeneration [9]-[12]. One of the well-documented neurologically-based associations is between glyphosate-containing Roundup herbicide and the formation of Parkinson's disease [11]. Other research, however, has begun to surface, investigating the association between glyphosate exposure and the formation of other neurological disorders, such as Alzheimer's disease, seizures, and autism spectrum disorder. In addition, the connection between the gut microbiome, the brain, and exposure to glyphosate has recently been analyzed, with implications of the gut-brain axis as being a

potential pathway through which glyphosate acts in the human body.

2. Rapid Literature Review Methodology

This rapid literature review seeks to consolidate and analyze articles examining the correlation of both pure glyphosate and glyphosate-based herbicide exposure with the formation and/or exacerbation of neurological disorders. This review concentrates on the correlation between glyphosate and glyphosate-based herbicide exposure with the formation of Parkinson's disease, Alzheimer's disease, seizures or convulsive behavior, and autism spectrum disorder. Pathogenesis of each disorder is briefly addressed, followed by a review of literature investigating glyphosate or glyphosate-containing herbicide exposure in animal models or population cohorts. For particular neurological disorders, the animal models expressed the pathogenic behavior associated with the target disease, and population cohorts were previously diagnosed with the disorder. In addition to this, the literature review discusses the gut-brain axis and the influence of alterations to the gut microbiota on the formation of neurological disorders as associated with exposure to glyphosate or glyphosate-containing herbicides. The literature cohort spans research conducted over the last ten years and includes articles that collected research through animal models or human cell cultures as well as through epidemiological studies. The primary animal models investigated were mice, rats, *C. elegans*, lambs and poultry. Laboratory research articles were conducted in the United States as well as in international locations in Europe, Asia, and South America including South Korea, Japan, Mexico, Brazil, Argentina, Spain, France, Finland, and Italy. Epidemiological studies were conducted both in the United States and in South Korea. This review excludes research published before January 2014, as well as literature reviews, case studies, and computational models.

3. Areas of Research Focus & Relevant Findings

3.1. Parkinson's Disease

3.1.1. Pathogenesis

Parkinson's disease is a major neurocognitive disorder categorized by motor symptoms such as tremors, stiffness, and reduced coordination [13]. Parkinson's disease pathology has been associated with the death of dopaminergic neurons as well as with the accumulation of α-Synuclein (α-Syn) protein structures within Lewy bodies protein deposits [14]. Degradation of neurons in the pathological process of Parkinson's disease can also lead to dementia symptoms, which may be associated with the presence of Lewy bodies [15].

1) Dopaminergic Neurons

Dopaminergic neurons are essential in the brain for emotional processes, such as those involved in mood and stress regulation as well as for voluntary movement processes [16]. Death of dopaminergic neurons, particularly those located in the substantia nigra, has been associated with the formation of Parkinson's

disease [16]. Damier *et al.* [17] investigated this phenomenon using brain samples of five patients who died of Parkinson's disease as compared with brain samples from individuals with no neuropsychiatric disease of any kind. Based on charts created of the dopaminergic neurons in both the diseased and the healthy patients, it was determined that there was a 64% decrease in dopamine neurons in the diseased patients. These were concentrated in several areas, including the substantia nigra [17].

2) Alpha-Synuclein (*α*-Syn) Protein Structures and Lewy Bodies

α-Syn accumulation has also been implicated in the formation of Parkinson's disease [18]. Alpha-synuclein is a presynaptic protein, whose formation has been associated with a mutation in the *SNCA* gene [18]. The accumulations of *α*-Syn are partial constituents of Lewy bodies, which are neuronal inclusions in the brain associated with the development of Parkinson's disease [19] [20]. Gruden *et al.* [21] exposed adult male C57Bl/6 mice to either a saline solution control, an *α*-Syn oligomeric aggregate solution, or an *α*-Syn oligomeric aggregate and fibril combined solution and were subsequently administered behavioral tests that measured Parkinsonian symptoms. Their results demonstrated that although the saline solution control and the *α*-Syn oligomeric aggregate solution demonstrated no significant difference in performance on the behavioral tests, the *α*-Syn oligomeric aggregate and fibril combined treatment demonstrated a significant reduction in locomotor activity [21].

3.1.2. Glyphosate Exposure

The association between glyphosate exposure and the formation of Parkinson's disease is one of the most well-documented correlations among neurodegenerative disorders (e.g., [11] [12]). Various studies have demonstrated glyphosate to be associated with the formation or exacerbation of Parkinsonian pathology.

For example, Hernández-Plata *et al.* [22] used male Sprague-Dawley rats to demonstrate the effect of acute glyphosate exposure on dopaminergic neurons and on the formation of visible symptoms associated with locomotion. Glyphosate was administered to the rats using six injections over two weeks at 50, 100, or 150 mg, as compared to a purely saline solution. Their results demonstrated significant decreases in locomotion immediately and extending into the two days following glyphosate exposure as compared with the saline solution [22]. It was also demonstrated that the D1 dopamine receptors had reduced binding in the nucleus accumbens as associated with decreased locomotion. The final significant discovery was that glyphosate exposure effects were absent at sixteen days following exposure, demonstrating a short-term effect [22]. However, this study did not investigate what impact repeated glyphosate exposure may have on the formation of long-term effects.

Pu *et al.* [23] examined adult male C57BL/6 mice who were exposed to Roundup Maxload at a 0.098% concentration or pure water control through their drinking water. Mice were also administered either MPTP, which produces dopaminergic neurotoxicity, or saline control, leading to a total of four study

groups: water and saline, water and MPTP, glyphosate and saline, and glyphosate and MPTP. Their results demonstrated that, as expected, MPTP resulted in reduced dopamine transporter immunoreactivity as compared with the saline control. In addition to this, mice which had been exposed both to MPTP and glyphosate demonstrated a significant exacerbation in reduced dopamine transporter immunoreactivity [23].

Additional research combined investigations of pure glyphosate and glyphosate herbicide exposure on formation of Parkinsonian abnormalities by using adult female Sprague-Dawley rats exposed both to 98% pure glyphosate as well as Roundup herbicide containing glyphosate [24]. The target for investigation in this study was dopaminergic release in the rat striatum. Behavior and motor performance were assessed following exposure, as well as striatal dopamine release. The investigator's results demonstrated that rats treated with glyphosate exhibited significantly poorer performance than their control counterparts on rotarod and Bonferroni motor tests up to 24 hours following treatment [24]. In addition to this, an unusual finding was discovered in that a significant increase in release of dopamine in the rat striatum was noted for both the pure glyphosate and the Roundup treatment groups [24]. While this may seem contradictory, a study conducted by Zhang *et al.* [25], who used electrophysiological recordings of a Parkinsonian primate, demonstrated dopamine overflow similar to the finding of Costas-Ferreria *et al.* [24]. In addition to this, Costas-Ferreria *et al.* [26] conducted a further study investigating this unusual phenomenon and, using adult female Sprague-Dawley rats exposed to glyphosate intrastriatally, demonstrated that glyphosate's influence on increasing dopamine levels through modification of the dopaminergic transporter is mediated by voltage-dependent calcium levels.

An epidemiological study of deceased individuals in Washington state, where residential pesticide exposure was analyzed using spatial analyses and a crop-exposure matrix, was informative as well [27]. Here, mapping demonstrated the correlation between Parkinson's disease and spatial land-use data of glyphosate and Paraquat. Results demonstrated that Paraquat exposure was not correlated significantly with premature death. Conversely, glyphosate was correlated with Parkinson's disease premature death, with the link directly correlated with an increase in glyphosate exposure [27].

Diverse literature exists that correlates Parkinson's disease formation with exposure to glyphosate and glyphosate-containing herbicides [22]-[24] [26] [27]. This literature ranges from laboratory research to epidemiological studies investigating pure glyphosate exposure and glyphosate-containing Roundup exposure. Both factors demonstrated significant symptoms in test subjects. In addition to this, impacts were implicated in the death of dopaminergic neurons. Research had previously been conducted investigating the correlation between α-Syn associated with Parkinsonian symptoms and glyphosate exposure, demonstrating mixed results; however, these studies did not meet the criteria for this review as they were published before 2014.

3.2. Alzheimer's Disease

3.2.1. Pathogenesis

Alzheimer's disease is another major neurocognitive disorder characterized by detrimental physiological and psychological abnormalities. These include death of the neurons in the brain as well as severe loss of memory and cognition ([28], pp. 602-605). Ultimately, brain damage becomes so severe that it results in fatality. The primary brain abnormalities that have been demonstrated to be associated with the formation of amyloid-beta plaques leading to the formation of Alzheimer's disease include the amyloid-beta precursor protein, the *APOe4* mutation of the *APOE* gene, and tumor necrosis factor *α* (TNF*α*) [29]-[31].

1) Amyloid Precursor Protein (APP)

The APP is essential in the proper functioning of neurons, including the formation of synapses and cellular regulation [29]. The irregular functions of this protein can cause disruption in protein transport and neurotoxic effects on neuronal cells [29]. One abnormality in the APP associated with the formation of Alzheimer's disease is a particular mutation of the protein called the amyloid-beta precursor protein [32]. This leads to the formation and deposition of extracellular amyloid-beta peptide plaques by giving rise to changes in amyloid-beta cleavage [32] [33]. These changes result in cleavage by *β*- and *γ*-secretases rather than the normal *α*- and *γ*-secretases [33]. Masuda *et al.* [34] examined the toxicity of the APP using the mouse model App-KI. Their findings were consistent with behavioral abnormalities associated with individuals suffering from Alzheimer's disease. As compared with the wild-type mice, the APP-KI mouse model demonstrated deficits in spatial learning and attention as well as place avoidance [34].

2) Apolipoprotein Gene (*APOE*)

In addition to the APP, the apolipoprotein gene (*APOE*) has also been linked to the formation of Alzheimer's disease. Certain mutations of the *APOE* gene have been implicated in exacerbated formation of the amyloid-beta precursor protein [30]. The *APOe4* mutation of this gene has been associated with increased deposition of amyloid-beta peptide plaques, indicating regulatory dysfunction associated with the APP [30]. This occurs due to alterations in lipid transportation, as seen in the *APOe4* mutation but not the *APOe3* and *APOe2* mutations [35]. In another investigation [36], the impact of the e4 allele on the *APOE* gene associated with cognitive decline in mice that mimicked Alzheimer's disease pathogenesis was explored. The results demonstrated significantly poorer performance in *APOe4* mice on the radial arm maze as associated with the use of working memory, but not on the Morris Water Maze [36]. Similarly, Schmitt *et al.* [37] examined the role of the *APOe4* gene in causing deterioration of cognitive function in knock-in *APOe4* mice compared to knock-in *APOe3* mice. Their results demonstrated a significant decrease in cognitive flexibility of *APOe4* mice at the 6-month-old mark compared to *APOe3* mice.

3) Tumor Necrosis Factor *α* (TNF-*α*)

In addition to the APP and the *APOe4* gene, TNF-*α* has been associated with

the formation of Alzheimer's disease [31]. TNF-α has been implicated in the formation and deposition of amyloid-beta plaques as well as inflammation in the brain and the formation of neurofibrillary tangles, all of which have been implicated in Alzheimer's disease pathogenesis [31]. For example, McAlpine *et al.* [38] investigated the impact of blocking TNF-α in 3xTgAD mice models that demonstrated Alzheimer's disease pathogenesis. Their results demonstrated that blocking of the TNF-α reduced the number of amyloid-beta plaques detected. Thus, it has been recognized that TNF-α is another major contributor to Alzheimer's disease pathogenesis.

3.2.2. Glyphosate Exposure

The association between glyphosate exposure and Alzheimer's disease has only recently been evaluated, and thus, it is not currently well documented. However, it is important to note that a few recent studies have found correlations implicating the potential for glyphosate exposure to impact the formation of Alzheimer's disease risk factors.

For example, Winstone *et al.* [39] conducted a study in which non-transgenic C57BL/6J mouse models were orally administered varying levels of pure glyphosate (125, 250, and 500 mg/kg/day) prepared at 0.107 g/L in 1.89 M sodium hydroxide. Glyphosate levels were measured in urine samples collected from the mice as well as in brain tissue using UPLC-MS and in blood plasma to assess an increase in TNF-α. The results of their study demonstrated that glyphosate could cross the blood-brain barrier, making Winstone *et al.* the first to use an *in vivo* model to demonstrate this phenomenon. In addition to this, a significant increase in the expression of TNF-α in the APP/PS1 primary cortical neurons was demonstrated, a phenomenon associated with cellular death as well as the increase in $A\beta_{40\text{-}42}$ as associated with the formation of Alzheimer's disease [39].

Similarly, the effects of glyphosate exposure on producing oxidative stress in dopaminergic human neuroblastoma SH-SY5Y cells were evaluated [40]. Within this study, the effect of glyphosate exposure on changing the folding of various proteins was assessed, demonstrating a modification of several genes associated with normal neuronal development and functioning [40]. Among the proteins evaluated was the APP, but it was not found to undergo a protein alteration following exposure and thus demonstrated no potential for gene alteration. However, it was discovered that pro-inflammatory IL6 and TNF-α were significantly upregulated [40]. This, as previously demonstrated, can be correlated with the potential for formation of Alzheimer's disease.

Recently, an epidemiological study was conducted to evaluate glyphosate exposure in humans and assessed the presence of behaviors associated with Alzheimer's disease using an Alzheimer's disease memory test. In this study, Hsiao *et al.* [41] conducted a human subjects investigation in which a representative sample of the population had urinary samples analyzed for glyphosate. Subsequently, participants 60 years and older were subjected to a variety of tests in-

cluding the Consortium to Establish a Registry for Alzheimer's Disease Word List Memory Test (CERAD-WLT) [41]. The results of their study demonstrated a significant negative correlation between urinary glyphosate levels and the test scores obtained on the CERAD-WLT, suggesting a relationship between cognitive decline and greater long-term exposure to glyphosate. Ultimately, this finding indicates a possible implication for the association of glyphosate exposure with formation of Alzheimer's disease pathogenesis in humans.

In summary, research on the impact of exposure to glyphosate and glyphosate-containing products in the formation of Alzheimer's disease is clearly still deficient. Recent studies have implicated a possible correlation between exposure and the upregulation of TNF*a*, an Alzheimer's disease pathogenesis factor. However, no literature was found that examined the correlation between glyphosate exposure and the mutation of *APOe4* or between glyphosate exposure and APP. Since a potential correlation to Alzheimer's disease has been suggested by the limited sources available, a greater body of literature needs to be accumulated that investigates the correlation between factors influencing amyloid-beta plaque formation (APP, *APOe4*, & TNF-*a*) and glyphosate exposure, with the goal of eliminating irrelevant factors.

3.3. Convulsive Disorder and Seizure

3.3.1. Pathogenesis

Convulsions and seizures have much broader pathological causes as compared with Parkinson's and Alzheimer's disease. Seizures are most often associated with the condition of epilepsy [42]. However, other triggers have been associated with the formation of seizures, including high fever, infection or illness, certain medications or drugs, and a head trauma or brain injury [42]. One of the major neurotransmitter systems in the brain associated with seizure behavior is the GABAergic system.

1) GABAergic System

Gamma-aminobutyric acid (GABA) is one of the primary neurotransmitters in the body and serves as an inhibitory neurotransmitter both in the brain and the spinal cord [43]. It has been associated with a variety of different neurologic and psychiatric disorders, including seizures and epilepsy [43]. According to Macdonald *et al.* [44], mutations in the GABA-A receptor have been correlated with the formation of epilepsy. In addition to this, Petroff *et al.* [45], who investigated seizure control in patients with complex partial seizures, correlated low seizure control with low levels of GABA neurotransmitter in the brain.

3.3.2. Glyphosate Exposure

The correlation between glyphosate exposure and seizures is incredibly novel. Several case studies have previously investigated the correlation between glyphosate and seizure behavior, with unclear results. Naraine *et al.* [46] were the first to demonstrate a glyphosate and seizure correlation in the laboratory using the *C. elegans* model.

Recently, a study was conducted investigating seizures in 464 individuals who ingested one or more mouthfuls of pesticide with the intention of committing suicide [47]. These pesticides included the following categories: glufosinate ammonium, pyrethroid, glyphosate, paraquat, organophosphate, and others. The highest number of seizures was seen in those who ingested glufosinate ammonium (31.5% instance of seizures), while only a 5.4% instance of seizures was demonstrated in those who ingested glyphosate [47]. Thus, it was unclear from this study whether a strong correlation existed between glyphosate exposure and the formation of seizures.

In a population epidemiological study conducted by Requena *et al.* [48], people living with epilepsy, as compared with control subjects, were investigated for pesticide exposure, including pesticides such as paraquat, diquat, glyphosate, glufosinate, and lufenuron. Their results demonstrated that areas with high pesticide concentrations were correlated with epilepsy incidence and risk. However, it is important to note that the study needed to elaborate on which pesticides contribute more than others. Thus, it cannot be determined if glyphosate was a prominent contributor to this result.

In a recent study [46], the goal was to examine convulsant behavior in *C. elegans* models following exposure to glyphosate-containing Roundup using an electroshock convulsion assay. Results from this study demonstrated that a 0.1 mM concentration of glyphosate followed by the electroshock assay resulted in convulsions in the *C. elegans* models and a non-recovery phenotype. In addition, it was noted that introducing a GABA-A antagonist with a subeffective glyphosate dose resulted in a threefold increase in nonrecovery [46]. Of note, when the *C. elegans* were administered the antiepileptic drug sodium valproate; however, it was noted that the duration of seizures was significantly reduced, and every worm demonstrated recovery. The GABAergic mechanism, which has been implicated in seizure pathology of humans, was thus implicated as the potential pathway affected by the glyphosate exposure and subsequent convulsion assay [46].

All research prior to the study reported above had been conducted to investigate the correlation between seizures and glyphosate exposure but were unable to demonstrate distinct results. As with any epidemiological study, studies attempting to draw correlations from neighborhood or work-related glyphosate exposure to subsequent population increases in seizure formation could have been subject to confounding variables associated with other toxins or lifestyle choices relevant to the area studied. With the research conducted by Naraine *et al.* [46], more unambiguous evidence now exists for a correlation between seizures and glyphosate-containing herbicide exposure. Further research should seek to confirm this correlation, using multiple different animal models. In addition, research should be conducted on pure glyphosate to determine whether the glyphosate or another component in the Roundup herbicide resulted in the seizure behavior.

3.4. Autism Spectrum Disorder

3.4.1. Pathogenesis

Unlike the previously discussed neurological disorders, the pathology of autism spectrum disorder is still not well known. However, it has been suggested that a combination of genetic and environmental factors, especially ones implicating prenatal exposure, can be associated with the formation of autism. In addition, associations have been made with biological mechanisms, including the Wnt/Ca^{2+} pathway, corpus callosum agenesis, and proinflammatory cytokines.

1) Wnt/Ca^{2+} Pathway

The Wnt/Ca^{2+} pathway plays critical roles in the development of humans, ranging from effects on the formation of the head and neuron patterns as well as different bodily organs [49]. Abnormalities in the Wnt/Ca^{2+} system could cause major effects on neuronal signaling and have been implicated in the formation of autism spectrum disorder [49]. For example, Sowers *et al.* [50] demonstrated that *Prickle2* mutant mice, a mutation that leads to a mutation in the Wnt signaling protein, displayed abnormalities associated with autism spectrum disorder including alterations in learning and social behavior. In addition, the investigators reported a decrease in the size of synaptic currents.

2) Agenesis of the Corpus Callosum

Agenesis of the corpus callosum is a congenital cerebral malformation in which there is either a complete or a partial deficiency in corpus callosum brain matter [51]. This malformation has been associated with a variety of different factors, including maternal alcohol use, phenylketonuria, Chiari II malformation, and several genetic and chromosomal factors [51]. Past research has demonstrated that among individuals with brain malformations, including agenesis of the corpus callosum, autism screening was positive for 45% of children, 35% of adolescents, and 18% of adults in the study [52].

3) Proinflammatory Cytokines

Proinflammatory cytokines are found in the human immune system following the activation of macrophages [53]. Further, they include IL-1β, IL-6, and TNF-α and are released to increase the inflammatory reaction of the body [53]. Last, both IL-6 and TNF-α, previously discussed in correlation with amyloid-beta plaques and Alzheimer's disease, are located within the nervous system [53]. More recently, research discovered that patients with autism spectrum disorder demonstrated upregulation of both IL-6 and TNF-α [54]. In addition to this, maternal stress, including exposure to infections and pollution, has been associated with prenatal complications leading to the upregulation of proinflammatory cytokines and the formation of autism spectrum disorder in the child [55].

3.4.2. Glyphosate Exposure

Since autism spectrum disorder has been associated with environmental factors in conjunction with genetic abnormalities (see, [50]), glyphosate has been investigated as a potential exacerbator of autism. Several studies have been conducted which link glyphosate exposure with various autism risk factors.

For example, research was conducted to investigate glyphosate exposure associated with the formation of autism spectrum disorder in both mother mice and juvenile offspring [56]. In the study, pregnant mice were administered 1% Roundup in drinking water over the span of E5 to P21 (pup weaning). The results demonstrated that prenatal exposure to Roundup mimics the behavior of autism spectrum disorder in newborn pups. In addition, it was discovered that the sHE protein, which has been associated with autism spectrum behavior, was upregulated in juveniles exposed to Roundup prenatally as compared with the control group. Finally, a decrease in parvalbumin immunoreactivity was noted in the prenatally exposed juveniles compared to the control group [56].

A follow-up study by the same research group was conducted but Roundup was replaced with pure glyphosate and examined in pregnant mice [57]. A formula of 0.098% glyphosate was administered to the pregnant ddY mice (embryo 9 - 10 weeks old) between E5 and P21 (weaning). When this study was compared to the previous report [56], the results suggested similarities between the two, in which juveniles exhibited autism spectrum behaviors following exposure as compared with the control group. Thus, based on the combined results from both studies, it is likely that the glyphosate itself, and not any other ingredient in the Roundup herbicide contributed to the formation of autism-like behaviors in the juveniles.

Conducted in California, a relevant population epidemiological study investigated individuals who were born in the area under consideration and had been diagnosed while residing there [58]. Using an open-source geocoder called CA-PUR in combination with a land use survey, the investigation included examination of the application of such pesticides as glyphosate, chlorpyrifos, diazinon, acephate, malathion, permethrin, bifenthrin, methyl bromide, imidacloprid, avermectin, and myclobutanil both individually and in multi-pesticide models. Results demonstrated a significantly greater number of males with autism spectrum disorder as compared with females, and the mothers of those individuals were relatively older [58]. In addition, it was demonstrated that the odds of children developing autism spectrum disorder in the first year were significantly increased for most of the pesticides, including glyphosate. Last, for the combination of autism spectrum disorder and intellectual disability, increased odds were significantly marked in individuals exposed to glyphosate and permethrin [58].

Coullery *et al.* [59] examined the development of hippocampal neurons associated with exposure to glyphosate, particularly in the Wnt5a pathway, which, as previously discussed, has been implicated in the formation of autism spectrum disorder. As the authors noted, the Wnt5a pathway is particularly associated with axonal branching, sympathetic neuron growth, as well as other types of neuronal development. Hippocampal pyramidal neuron cell cultures were prepared from Wistar rats followed by exposure to glyphosate, and these cultures contained recombinant protein Wnt5a. Results demonstrated that the glypho-

sate did not kill any of the neurons but did affect the polarization of the neurons and neuronal development such as delayed axon differentiation and dendrite growth in the Wnt5a pathway [59].

In 2020, Coullery *et al.* conducted a second study which also investigated the association of glyphosate exposure in the Wnt5a-CaMKII pathway [60]. This study investigated the ingestion of glyphosate as compared with administration directly to the neuronal cells, and it combined assessment of the Wnt5a-CaMKII pathway with behavioral tests. Pregnant female Wistar rats were administered either glyphosate in 25 mg/kg or 35 mg/kg as compared to a control, and pups were subjected either to a behavioral test, the Morris Water Maze, or the Conditioned Fear Test [60]. In addition, the activity of the Wnt5a-CaMKII pathway was analyzed from hippocampal homogenates using electrophoresis and Western Blot. Their results demonstrated that glyphosate-exposed pups spent less time in the target quadrant of the probe trial of the Morris Water Maze and displayed impaired contextual fear. In addition, it demonstrated that exposure to the higher dose of glyphosate resulted in decreased expression of Wnt5a, which forms synaptic structures, and an overall downregulation of Wnt-CaMKII signaling [59]. Since Wnt is correlated with the formation of autism spectrum disorder, these two studies conducted by Coullery *et al.* suggest that glyphosate plays a significant role in altering the Wnt pathway and associated neurons, which can be further correlated with the potential of glyphosate to impact autism spectrum disorder formation.

Other research models have also been utilized. For example, Alarcón *et al.* investigated the Wnt system by studying glyphosate exposure in prepubertal ewe lambs [61]. Female Friesian lambs were exposed to either a saline solution or a commercial glyphosate formula, with no exposure in the pasture and drinking water as controlled variables. Blood samples and uterine transverse sections were collected from the lambs and analyzed. Their results revealed that the ewe lambs who ingested glyphosate demonstrated that Wnt5a and Wnt7a expression were significantly decreased [61]. As mentioned previously, the Wnt pathway has been associated with the formation of autism spectrum disorder, and Alacrón *et al.* [61] demonstrated that glyphosate not only affected the Wnt5a pathway, as demonstrated by Coullery *et al.* [60] but also caused alterations to the Wnt7a pathway.

Thus, a growing body of literature is being accumulated that correlates glyphosate and glyphosate-containing herbicide exposure with autism spectrum disorder. In particular, the research mentioned above has suggested that prenatal exposure to glyphosate is significant in determining the impact of glyphosate on the formation of autism in an individual. Glyphosate was positively correlated with alterations in the Wnt pathway and upregulation of proinflammatory cytokines, which are both implicated in prenatal development as associated with pregnant mother exposure. No research has been found that links glyphosate exposure with agenesis of the corpus callosum.

3.5. Gut-Brain Axis and Gut Microbiome

More recently, the association between the gut microbiome and the brain system has become a significant focus in research regarding neurodegenerative disorders. This system, known as the gut-brain axis, connects the enteric nervous system and central nervous system physically but also includes connections with other systems, including the endocrine system and the immune system [62]. The gut-brain axis has been associated with influences both on emotional health, including state and regulation, as well as on physical brain health, including neuronal growth and cognitive function [62]. In addition to this, the gut-brain axis is associated with the release of metabolites including neurotransmitters and hormones which have been further associated with neurodegenerative disorders such as Parkinson's and Alzheimer's disease [62]. A growing body of literature has been released that has begun to examine the association of the gut-brain axis with the ingestion of herbicides such as those containing glyphosate.

A relevant example can be found in the report of a study investigating the microbiome, brain plasticity and behavior of pregnant female rats following exposure to pure glyphosate and Roundup [63]. The pregnant Sprague-Dawley rats were examined for changes in behavior, and brain, synapse, and fecal microbiome analyses were conducted. The results demonstrated that the rats who ingested both the pure glyphosate and the glyphosate formula in Roundup experienced changes in maternal licking behavior and neurogenesis, with effects including a greater number of immature neurons in the dentate gyrus and alterations to synaptophysin [63]. In addition to this, results showed that Roundup exposure caused a significant decrease in *Firmicutes* bacteria and a significant increase in *Bacteroidetes* bacteria. However, this result was not demonstrated for the formulation of pure glyphosate, which indicates that either another chemical besides glyphosate or the interaction between glyphosate and the other chemicals in the formulation contributed to this result [63]. As these investigators noted, it also shows the potential for Roundup to contribute to neurodegenerative diseases as well as psychiatric disorders, as any dysregulation of the microbiota can result in significant changes in the function of the central nervous system.

Another study, reported by Del Castilo *et al.* [64], investigated low-dose glyphosate-containing herbicide exposure on the gut microbiome and behavior of mice. In order to demonstrate the effects of chronic exposure as compared with acute exposure, the mice were exposed to Roundup from pregnancy to adulthood. Analyses were conducted which analyzed gut microbiome, intestinal alterations, and behavioral changes. The relevant results demonstrated an alteration in behavior with Roundup exposure, including increased behavioral repetitions and decreased social interest, especially in male mice [64]. This finding suggests a correlation with autism spectrum disorder, as previously discussed, since these behavioral alterations are consistent with the formation of autism. Of considerable import, significant alterations in bacterial levels such as *Proteobac-*

teria, *Desulfobacterota*, and *Bacteroideta*. In addition, other gut changes were demonstrated, including increased mucus cells, lymphocyte differentiation, and alterations of adhesion molecules [64]. Thus, this research indicates a correlation between glyphosate-containing herbicide exposure, alterations to the gut microbiome, and the formation of autism spectrum disorder, and it could also, as previously suggested by Dechartres *et al.* [63], indicate the pathway through which individuals develop other neurodegenerative disorders.

In a similar study conducted using an avian model the impact of glyphosate-containing herbicides on gut microbiome and reproductive hormones was assessed [65]. Japanese quails fed either Roundup-treated organic feed or just the organic feed as a control were analyzed for glyphosate residue in the liver as well as subjected to fecal microbiome analysis and oxidative stress analysis. The research also included consideration of the impact of long-term exposure, subjecting the poultry to Roundup over the course of 52 weeks, as compared to an acute exposure model. Results showed no evidence of damage associated with oxidative stress and no effect associated with acetylcholinesterase activity, which was novel compared to rodent models. However, dysbiosis of the gut microbiome was significantly noted, with a particular decrease in the growth of bacteria such as *Firmicutes* and an increase in *Actinobacteria* [65]. Further, the change was especially notable with increases in age and in the female poultry [65]. Therefore, although this study did not discover any correlation with the neurological system directly, nor was it able to link Roundup exposure with alterations to neurotransmitter systems such as that which is associated with acetylcholinesterase, it did demonstrate, as with previously listed studies, a significant alteration to the gut microbiome as associated with the Roundup exposure, which has been further associated with central nervous system alterations.

In another study of low-dose glyphosate-based herbicide exposure as well as pure glyphosate exposure, the impact of such exposure on Sprague-Dawley female rats and both male and female pups [66]. Females were exposed to glyphosate-based herbicide or pure glyphosate through drinking water from gestation to weaning, and male and female pups were exposed to the herbicide in utero and through drinking their mother's milk. Metabolomics was analyzed through urine and feces samples. Results demonstrated that glyphosate-based herbicide exposure and the pure glyphosate exposure resulted in alterations in metabolomic biomarkers in both the females and the pups as investigated in urine samples [66]. This included an increase in homocysteine which suggests a reduction in the activity of *Prevotella* bacteria. This finding is significant because a reduction in microbiota such as *Prevotella* has been implicated in the formation of autism spectrum disorder [66].

Last, Mesnage *et al.* [67] investigated the impact of glyphosate or Roundup exposure on the gut microbiota system and serum metabolome, investigating in particular the shikimate pathway. Sprague-Dawley female rats were treated for 90 days with pure glyphosate, Roundup, or control and were subsequently inves-

tigated with metabolomics, shikimic acid, and bacterial growth analysis, as well as shotgun metagenomics. Their results demonstrated through metabolomic studies that glyphosate exposure resulted in metabolite changes, which implicated an inhibitory reaction with the shikimate pathway of the gut microbiome [67]. This, as previously mentioned, further suggests a correlation between glyphosate exposure and microbiome alterations that can have greater impacts on the nervous systems and ultimately the formation of neurodegenerative disorders.

The research discussed above demonstrates correlations between Roundup exposure and alterations to the gut microbiome, suggesting significant implications for the gut-brain axis and the formation of neurologic disorders. The most well-documented association between the gut-brain axis and the formation of a previously mentioned neurological disorder, as discussed in the above literature, was the correlation with autism spectrum disorder. The association between the gut-brain axis, glyphosate exposure, and neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease was less direct. However, since changes in the gut microbiome were demonstrated in correlation with glyphosate and glyphosate-containing herbicides and gut microbiome alterations have been associated with changes in the central nervous system, this suggests the gut microbiome is a potential pathway through which glyphosate and glyphosate-containing herbicides impact the formation of neurodegenerative disorders.

4. Literature Analysis

The body of research considered for this literature review suggests a significant correlation between the formation of human neurodegenerative disorders and exposure to glyphosate or glyphosate-containing herbicide products. This research suggests that exposure can occur both through dermal absorption and through ingestion, with implications for interactions with both individual neurological systems, such as TNF-α as associated with the formation of Alzheimer's disease or the Wnt pathway as associated with the formation of autism spectrum disorder. In addition, the research considered here suggests a potential correlation between exposure to glyphosate and glyphosate-containing herbicides with the recently investigated gut-brain axis and alterations to the gut microbiome. It is important to note, however, that the literature alludes to larger associations for some neurodegenerative disorders as compared with others, and significant limitations are evident in the existing body of literature. These limitations should be addressed in further research in order to establish more concrete correlations between the formation of these disorders and exposure to glyphosate or glyphosate-containing herbicides.

A common limitation noted in the laboratory experiments discussed in this literature review, which correlate glyphosate and glyphosate-containing herbicide exposure directly with the formation of neurological disorders, is the lack of establishment of specific chemical pathways contributing to the interaction of

the glyphosate chemical with the targeted brain system. This is especially true of glyphosate-containing herbicides, where it is unclear whether glyphosate or another chemical compound in the herbicide contributes to alterations in the targeted brain systems. Pu *et al.* [23], combined with Pu *et al.* [57] as well as Costas-Ferreira *et al.* [24] [26] are an exception in that they investigated both Roundup and pure glyphosate in correlation with the targeted neurodegenerative disorder, demonstrating similar results for both formulations. A similar limitation exists for epidemiological studies, where it is unclear whether the glyphosate-containing herbicide as an environmental factor was the sole contributor to pathogenesis. Other contributors could exist including other pesticides, herbicides, and insecticides as well as additional environmental pollutants or toxins, both chemically engineered and naturally formed. The glyphosate chemical may become altered as it interacts with other chemicals in the formulation of glyphosate-containing herbicides, which could alter the toxicity and function of glyphosate in the human body. For more established bodies of literature, such as that which exists for Parkinson's disease and autism spectrum disorder, these limitations are a less significant issue. This is because the combined body of literature examines both pure glyphosate and glyphosate-containing herbicides, demonstrating significant correlations to neurodegenerative risk factors for both formulations. This suggests a greater likelihood that the chemical glyphosate itself may be a contributing factor. By comparison, the literature investigating Alzheimer's disease and especially the literature investigating seizures have not accumulated to a significant extent. In these areas, research is either limited or nonexistent which investigates pure glyphosate exposure and associated outcomes compared with glyphosate-containing herbicides. Therefore, it is unknown whether another chemical or changes in the glyphosate chemical during formulation may contribute to the formation of the pathogenesis observed in the research.

Among the disorders examined in this review, it was noted that the greatest body of literature currently exists that correlates glyphosate and glyphosate-containing herbicide exposure with the formation and/or exacerbation of Parkinson's disease as well as autism spectrum disorder. The correlation between glyphosate and Alzheimer's disease has been investigated to a lesser extent, with critical pathways such as *APOe4* not yet examined and limited research existing for potential mutations of apolipoprotein (APP). Seizure behavior associated with glyphosate exposure is an incredibly novel discovery, with the first significant research study conducted only recently [46]. Other previous research conducted which investigated seizure behavior associated with glyphosate exposure were epidemiological studies, and it was unclear whether the glyphosate-containing herbicide or another environmental factor contributed to the reported seizures. Based on the research discussed in this review, it is essential that further research directions investigate, to a greater extent, the association between seizures or convulsive behavior and glyphosate or glyphosate-containing

herbicide exposure, with a deeper examination of the pathways that might underly such a finding. In addition to this, a greater body of literature needs to be collected for Alzheimer's disease-related abnormalities, specifically those associated with the *APO4* gene and mutations of the apolipoprotein (APP) which lead to the formation of amyloid-beta plaques in the brain.

An increasing body of literature has associated gut health and the gut microbiome with neurological and psychiatric alterations. Since this literature review demonstrates a correlation between glyphosate-containing herbicide exposure and alterations to the gut microbiome, this suggests a potential pathway through which these glyphosate-containing herbicides alter the human neurological system. It is important to note, however, that unlike the studies that individually discussed correlations with particular neurological disorders, several studies investigating both pure glyphosate and glyphosate-containing herbicides, such as Roundup, have demonstrated that only the Roundup causes alterations to the gut microbiome. The pure glyphosate in these studies showed no change. Thus, it is possible that a different chemical in the herbicide or the formulation of glyphosate with the other chemicals in the herbicide results in a microbiome change. This may also suggest that the gut-brain axis is being impacted by glyphosate-containing herbicide exposure in a manner independent of the action of the glyphosate chemical for individual disorders such as the alterations found in TNF-α associated with Alzheimer's disease or the Wnt pathway associated with autism spectrum disorder. Further research should be conducted to investigate the potential for other chemicals in glyphosate-based herbicides or the modifications to glyphosate in the herbicide formulation process to cause significant alterations to the gut microbiome.

5. Conclusion

Due to the widespread popularity of the use of glyphosate-containing herbicide products such as Roundup and the associated health concerns recent research has established, it has become imperative that such conditions be investigated and pathways be targeted. Literature has demonstrated that Roundup has the potential to be carcinogenic and cause diseases such as non-Hodgkin's lymphoma. In addition, it has recently been associated with the formation of major neurodegenerative disorders. The primary of these which was previously studied was Parkinson's disease, but a growing body of literature now demonstrates implications for the formation of other such neurological disorders. This rapid literature review sought to accumulate and analyze current studies which investigate the potential for formation of Parkinson's disease, Alzheimer's disease, seizures, and autism spectrum disorder following exposure to glyphosate or glyphosate-containing herbicides such as Roundup. It was discovered that literature exists which suggests glyphosate as an environmental trigger for particular pathways implicated in Parkinson's disease, Alzheimer's disease, and autism spectrum disorder pathogenesis. The only significant literature that exists to date

addressing seizures as a potential factor was a study conducted by Naraine *et al.* [46], thus making seizures the most important factor for continued study to this point. The final aspect investigated in this review was the gut-brain axis, glyphosate exposure, and implications for the formation of neurological disorders, which was suggested by the literature reviewed in this paper as a potential pathway for glyphosate's action on the brain. A timely and more comprehensive review can be found elsewhere [68]. At any rate, since discovering a link between glyphosate and neurodegenerative disorders has crucial implications for the agricultural industry and human health, it is essential that the literature on this topic, which is increasingly becoming substantial, be investigated to a greater extent.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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