Finding Funding and Writing a Successful Proposal

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What is a grant? A grant is a way the government funds your ideas and projects to provide public services and stimulate the economy. Grants support critical recovery initiatives, innovative research, and a litany of other programs. On Grants.gov you will find all the funding opportunities from the 26 federal agencies that award grants.
Grant Funding Resources

- **Grants.gov** is designed to enable Federal grant-making agencies to create funding opportunities and applicants to find and apply for these Federal grants.

- The **Grants.gov Online User Guide** provides explanations and step-by-step instructions for both applicants and grantors to complete these processes.
Get Started

Grants.gov is designed to enable Federal grant-making agencies to create funding opportunities and applicants to find and apply for these Federal grants. The Grants.gov Online User Guide provides explanations and step-by-step instructions for both applicants and grantees to complete these processes.

Each page of information is referred to as a “help article,” which you can access by clicking the 🛡️ icons throughout Grants.gov and by using the navigation features listed below. For general information about the grants lifecycle and policies, refer to the Learn Grants tab on Grants.gov.

Navigation in the Online User Guide

Table of Contents: The primary navigation tool is the table of contents, which is designed to follow the Grants.gov system structure. The help instructions in the Register, Login, and Search Grants sections are for all users. The Applicants, Grantors, and Administrators sections pertain specifically to those types of users.

Glossary: The online user guide also features a glossary of terms pertinent to using the Grants.gov system. If you are new to grants or would like a broader range of grant-related terms, the Grant Terminology page on Grants.gov may also be helpful.

Search: Type the name of an action, a page title, or any other questions you may have into the search bar. Click the Search icon in the search bar or select the Enter key on your keyboard to return results with the instructions and help you need.

Index: The online user guide also features an index of all the terms and associated topics used throughout the guide. The index sorts these terms alphabetically, then connects them with other terms and topics used in relation to each other.
Grants.Gov - Navigation

- **Home** - The Grants.gov home page, which provides quick access to the latest funding opportunities, system updates, and other resources.

- **Learn Grants** - Links users to the Grants Learning Center (GLC), which provides grants lifecycle, policy, and other grants management-related information.

- **Search Grants** - Directs users to the Search page. Here, you can perform keyword or faceted search for funding opportunities. Using the Search Grants tab, grantors can confirm that their agency’s new or modified opportunities are successfully displayed. The screen default will display all open opportunities sorted by posted date in descending order.

- **Applicants** - Contains the applicant-specific functionality and resources within the Grants.gov website. The Applicants tab directs users to the Applicant Center, which lists the different Applicant Actions and Resources links depending on the user’s roles and login status.

- **Grantors** - Contains the functionality and resources for federal grant-making agencies and its users. The Grantors tab directs users to the Grantor Center, which lists different Grantor Actions and Resources links depending on the user’s roles and login status. Within the Grantors tab, users post grant opportunities, publish and retrieve grant applications, and access resources specifically for grant-making agencies.
Stay Connected and Manage Subscriptions

- Grants.gov offers several ways to stay up to date with grant opportunities and system updates. Use the features that best serve your needs.

- **Manage Subscriptions**: The Manage Subscriptions link directs users to the Manage Subscriptions page to subscribe to RSS feeds or email notifications. User may also unsubscribe from this page.

- **Email Notifications**: Grants.gov is designed to allow users to register (i.e., subscribe) to receive email notifications of new grant postings that meet specific criteria. To register to receive grant postings that meet the needs of you or your organization, click the Manage Subscriptions link in the upper right corner of the Grants.gov banner. Once on the Manage Subscriptions page, there are several options to select from. You may also choose to unsubscribe from receiving email notifications on this page.

Grant Funding Resources ...Continued

- National Institute of Health
- NIH Grant Writing Tips Sheets
- Department of Defense
- National Science Foundation
Any Questions on Finding Funding?
Writing a Successful Grant Proposal

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Applying for Grant Funding

IDEA
- Novel
- Exciting
- Addresses a need
- Well-developed

FUNDING SOURCE
- Federal, state
- Foundations, societies
- Talk to Joy

GRANT WRITING
- Research plan
- Environment
- Budget

SUBMISSION
- Meet all internal and sponsor requirements
- Submit on time (early is better)
- Sponsored Programs Administration
Developing your idea

Identify the problem/need
- Will my idea address the problem?
- Is the idea important to someone other than me?

Research
- Has this been done before?
- Is there evidence to suggest that my idea will actually work?

Hypothesis
- \( A \rightarrow B, B \rightarrow C \). Based on this data, my hypothesis is that \( A \rightarrow C \)
- Is it testable?
Layout of the research proposal (NIH, R)

Project Summary

Project Narrative

Specific Aims

Research Strategy
- Significance
- Innovation
- Research Plan
Project Summary

• Should convey the broad, long-term objectives and specific aims
• Brief description of research design
• Background, gap in knowledge, problem, rationale for the study, central hypothesis, specific aims
• Plain language, even a non-scientist should be able to understand the importance of the project
• 30 line limit
Project Narrative

- For lay audience
- Max 3 sentences long
Specific Aims

• Objectives of the project that will allow you to address your overall hypothesis
• One page, the “make” or “break” page
• After reading this page, the reviewer will already have preconceived notion of the quality of the proposal
• Restate your Project Summary and expand on each Aim
Specific Aims: General Structure

Paragraph 1: Background

Paragraph 2: Gap in knowledge, problem

Paragraph 3: Rationale for the study, central hypothesis

**Specific Aim 1.** To determine......
Hypothesis:
Rationale:

**Specific Aim 2.** To determine.....
Hypothesis:
Rationale:

Closing Paragraph: how the study will contribute to the science, future projects, how the project addresses the mission of the sponsor/foundation
**IMPORTANT:** Your aims should be interconnected but not dependent on the successful outcome of another aim.

BAD: Aim 2 cannot proceed until the studies in Aim 1 are completed.

GOOD: Aim 2 proceeds in parallel with Aim 1 and findings from Aim 1 might direct future studies in Aim 2 or 3.

*Each aim relates back to the overall hypothesis.*
Research Strategy

Significance

• Convey the importance of your project to the field
• How novel is it? How will this change treatment/diagnosis? How will it contribute to the gap in knowledge? Potential to contribute to future studies?
• About ¾ of a page

Innovation

• Highlight the new techniques or approaches that you will use to address the problem
• About ¼ of a page

Research Plan

• How you are going to do it
• About 5-11 pages
Research Plan: General Structure

Start with a summary paragraph of what you are proposing and why.

**Background** – expand on Specific Aims page

**Preliminary studies** – data that supports your line of thinking, feasibility

**Rationale for the study** – expand on Specific Aims page, use diagrams

  - **Specific Aim 1.** Verbatim what is written in the specific aims page.
  - **Specific Aim 2.** Verbatim what is written in the specific aims page.

**Experimental Approach** - Methods, interpretation of results, alternative hypotheses, timeline
Putting it all together

Research Plan

Introduction:

Glyphosate is the most commonly used pesticide worldwide and has been so for the past 40 years. Due to increasing resistance to glyphosate, as well as wide acceptance of genetically modified crops, in addition to the use of pesticides for desiccation of crops, increasing amounts of glyphosate have been used each decade since its introduction in 1974. Small amounts of glyphosate are present in most foods available today (Webster, 2015; Kruger, 2014).

Glyphosate likely is magnified through the food chain, making humans especially vulnerable. Individuals at either end of our life span are especially vulnerable to its harmful effects. It has been implicated as a likely cause of Alzheimer’s disease, Parkinson’s disease, and Autism Spectrum Disorder. Surprisingly, although glyphosate has been found in the majority of urine and blood samples of humans across the globe, its effects in the human body have never been studied pathologically.

Although formerly glyphosate has been considered relatively safe for humans (Huang et al., 2004; Thompson et al., 2014), new evidence suggests that it may have devastating effects on human as well as other species as diverse as mammals, amphibians, and insects/honeybees. Glyphosate appears to have effects on learning, memory, nutrition, GI flora, and immune functioning.

Initial industry sponsored studies of glyphosate concluded that glyphosate could not accumulate in human or animal tissue. This, however, has been disproven with a 2014 paper by Kruger which represented one of the few studies of glyphosate in mammalian tissue. We plan to extend that study to human tissue and hope to show an association of glyphosate levels with various human diseases, beginning with Autism Spectrum Disorder.

Specific Aims: To evaluate the relationship between glyphosate and other related pesticides and the development of Autistic Spectrum Disorder

Research Strategy:

Pending the approval from the Regents of the University of California (UC), in the period of one year, the listed UC Davis investigators will

1. Coordinate the effort to obtain frozen, post-mortem human brain samples from 100 individuals (cases) (age 8 or younger) with the diagnosis of Autistic Spectrum Disorder and neurologically normal age matched controls. These samples will be drawn from the UC Davis Brain Bank Repository and associated tissue repositories.
2. Dissect pieces of frozen tissue of appropriate sizes from 6 anatomic regions of each case (Wernicke’s area, Broca’s area, the auditory cortex, insula, angular gyrus, and hippocampus), totally approximately 600 pieces (samples) from 100 cases.
3. Homogenize the frozen pieces according to published procedures and obtain soluble fractions that are suitable for glyphosate determination.
4. Determine the glyphosate level of each sample using the ELISA method in triplicate.
5. Determine the glyphosate level of each sample using UPLC/MS. The feasibility of measuring metabolites of glyphosate will also be explored.
6. Perform statistical analysis of the data and if necessary, power calculations for adequate sample size
7. Write manuscripts for publication under the discretion of the investigators after discussion with the sponsor

Significance:

Remarkably, glyphosate has never been studied in human tissue, aside from assessment of blood and urine specimens. This research will be significant, regardless of its findings, for this very reason.

Innovation:

Research involving long-term effects of pesticides/herbicides in humans is sorely lacking. As above, no prior pathologic studies have been done regarding glyphosate in human tissue. No studies have ever correlated levels of glyphosate in diseased tissue vs. controls.

Approach:

Include preliminary studies- no prior studies of this type have been attempted, although DDT derivatives have been investigated in brain tissue of patients with Alzheimer's disease.

Bibliography:

1.
PROJECT SUMMARY/ABSTRACT

Autism spectrum disorder (ASD) is characterized by deficits in social communication and causes significant impairment in day-to-day functioning in developing children. The number of ASD cases continues to rise and the cause of ASD is still not known. The link between pesticide exposure and ASD is of particular interest because of the widespread and increasing use of pesticides (insecticides and herbicides) and the high risk of direct and indirect (in utero) chronic exposure. Recent studies suggest that women with higher levels of organophosphate pesticides exposure were 2 to 6 times more likely to have a child with ASD. However, there is no direct evidence in the literature to suggest that pesticide exposure causes autism.

The broad spectrum herbicide glyphosate (N-phosphonomethylglycine or Roundup®) is the most common pesticide used worldwide and its use has been increasing due to the development of glyphosate-resistance, acceptance of genetically modified crops, and the desertification of crops. Recent in vitro and in vivo animal studies of glyphosate exposure demonstrate its ability to illicit toxic effects in various tissues, including genotoxicity, oxidative stress and neurotoxicity. In March 2015, the International Agency for Research on Cancer classified glyphosate as “probably carcinogenic to humans” (category 2A). Kroger, et al. demonstrated that glyphosate could be detected in mammalian tissue, including liver and intestine, but the group did not assess if glyphosate could be detected in brain. Thus, humans at either end of the developmental spectrum are especially vulnerable to the harmful effects of glyphosate and exposure to pesticides has been implicated as a risk factor for neurodegenerative and neurocognitive disorders, such as Alzheimer’s disease (AD), Parkinson’s disease (PD), and ASD. Glyphosate exposure has never been studied pathologically in the brain.

A recent report showed that exposure of pregnant and immature rats to glyphosate caused dysregulated neuronal calcium (Ca++) influx in the hippocampus of the offspring, leading to decreased glutamate uptake and metabolism, and increased oxidative stress and neuronal cell death. Given that altered glutamate metabolism is implicated in both glyphosate exposure and ASD, and that glutamate and altered excitability are important for the development of the human brain, our working hypothesis is that the accumulation of glyphosate (and its metabolites) in brain is correlated with ASD. To address our hypothesis and study goals, we propose the following Specific Aims: 1) To determine if glyphosate and other pesticides accumulate in human brain tissue and 2) To examine the degree of glyphosate and pesticide accumulation in brain tissue from ASD patients. We will perform enzyme-linked immunosorbent assay and ultra-performance liquid chromatography/mass spectrometry to detect glyphosate in normal and ASD brain regions. We will perform regression analysis to determine the association between glyphosate accumulation and the development of ASD.

PROJECT NARRATIVE

The link between pesticide (insecticide and herbicide) exposure and autism spectrum disorder is of particular interest because of the widespread and increasing use of pesticides and the high risk of direct and indirect (in utero) chronic exposure to humans. The broad spectrum herbicide glyphosate (Roundup®) is the most common pesticide used worldwide and has been implicated in cancer and is damaging to developing brain cells. We will study how glyphosate in the brain and autism spectrum disorder are related.
RESEARCH STRATEGY

(a) SIGNIFICANCE.

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders that can cause social, communication, and behavioral challenges. The prevalence of ASD has increased exponentially from 1 in 150 children reported in 1980 to 1 in 60 children in 2010 (1). Autism and ASD are complex disorders with no single cause. Various theories are explored in the literature. (2) What causes ASD and how does it affect individuals with ASD is still unknown. There is growing evidence of genetic contributions in the etiology of ASD. Genetic factors influence the susceptibility to ASD, with an increased risk observed in families of individuals with ASD. (3) Studies have shown that some genetic factors are associated with specific traits and behaviors observed in individuals with ASD.

(b) INNOVATION.

This proposed study is innovative because: (1) it will be quantifying glymphatic levels in brain tissue from normal subjects and subjects diagnosed with ASD, which has not been attempted before; (2) we will employ both validated and enzyme-linked immunosorbent assay (ELISA) and ultra-performance liquid chromatography/mass spectrometry (UPLC-MS) to accurately detect glymphatic levels; and (3) we will be utilizing the existing University of California Pediatric Neuropsychology Consortium brain bank. Dr. Lee-Way Jin is the University of California Davis Principal investigator to access a large number of samples to ensure the power of the study.

(c) RESEARCH PLAN.

Background

The role of the environmental pesticide exposure in the rise of ASD.

Autism spectrum disorder (ASD) is characterized by persistent deficits in social communication and interaction, and restricted, repetitive patterns of behavior that present early in development and cause significant impairment in day-to-day functioning. (1) The number of ASD cases continues to rise (800% from 1990 to 2001 in California alone). (2) Partially due to the fact that the cause of ASD is still unknown. Research points to a combination of genetic risk (27) and environmental exposures. (28, 29) The organs that play a significant role in the development of ASD. The link between pesticide exposure and ASD is of particular interest because of the widespread and increasing use of pesticides (insecticides and herbicides) and the high risk of direct and indirect exposure to the mother. (29) Recent studies have suggested that certain pesticides (DDT, organochlorine compounds, etc.) may cause neurodevelopmental disorders and may be responsible for an increased risk of ASD. (29)

A growing body of evidence suggests that early-life exposure to pesticides may play a role in the development of ASD. The effects of pesticides on brain function are complex and may involve multiple pathways and mechanisms.

Glymphatic and human health.

The broad spectrum of organophosphorus and carbamate pesticides (e.g., chlorpyrifos, diazinon) has been associated with neurodevelopmental and neurobehavioral disorders, such as autism spectrum disorder. (30) Glycerophosphoryl choline (GPC) levels have been shown to be decreased in the brains of individuals with ASD. (31) However, the role of GPC in the development of ASD is not well understood. Recent evidence suggests that the glymphatic system, which is responsible for the clearance of waste products and the delivery of nutrients to the brain, may play a role in the development of ASD. (32) The glymphatic system is a network of small blood vessels that lies adjacent to the brain and spinal cord and is responsible for the clearance of waste products and the delivery of nutrients to the brain. (33) The glymphatic system is composed of the periventricular space, the Virchow-Robin space, and the interstitial space, which are interconnected by a network of microvascular channels. (34) The glymphatic system is involved in the clearance of waste products, such as amyloid beta peptides, from the brain. (35) The glymphatic system is also involved in the delivery of nutrients to the brain, such as glucose, oxygen, and amino acids. (36)

In a recent study, we found that individuals with ASD had reduced glymphatic function compared to controls. (37) This reduced glymphatic function may be responsible for the accumulation of waste products in the brain, leading to the development of neurodevelopmental and neurobehavioral disorders, such as autism spectrum disorder.
indicating uptake of the herbicide into plant tissue. A German research group found glyphosate in the urine of 70% of adults and 50% of children in the United States, in particular in those exposed to glyphosate residues from glyphosate-resistant crops. (12) This finding is significant because it suggests that glyphosate exposure is widespread and may be occurring at levels that could be harmful to human health.

Therefore, glyphosate has the potential to affect immune function, gastrointestinal flora, nutrition, learning, and memory. Given that glyphosate is the world's most widely used herbicide, it is reasonable to expect that glyphosate exposure may be harmful to humans in multiple ways.

A recent study of US government databases for genetically modified corn, glyphosate application, and epidemiology revealed a positive correlation (Pearson correlation > 0.1) between glyphosate applications and hyperactivity, diabetes, obesity, breast cancer, lung cancer, and thyroid cancer and others. (13) Evidence that glyphosate interferes with many metabolic processes in plants and animals and that such studies have been conducted in both, glyphosate exposure may impact the physiology and function of the gut and the balance of gut bacteria. (15) This may lead to the development of new diseases such as autism, Alzheimer's disease, and Parkinson's disease.

Recent press has focused on the potential carcinogenicity of glyphosate in humans. A number of studies demonstrate associations between pesticides and non-Hodgkin lymphoma (NHL) subtypes. (30, 37) A recent systematic review showed that cell lines were positively associated with postmenopausal and glyphosate and phytoglyphosate. (37) Moreover, a number of epidemiological studies of the potential carcinogenicity of glyphosate in humans (22) prompted the International Agency for Research on Cancer, an arm of the World Health Organization, to classify glyphosate as "probabilistic carcinogenic to humans" (category 2A) in March 2015. However, there is skepticism regarding this assessment of the carcinogenicity of glyphosate in humans because the conclusion is largely based on animal studies. For example, a study of Roundup in drinking water (90 mg/L), which is half of the level permitted in drinking water in the European Union (38) and 1.4 mg/L recommended by the World Health Organization (39) resulted in liver and kidney damage and a trend of increased incidence of mammary tumors in female animals over a 2-year period. (40) However, this study was recently retracted because it was scientifically flawed and the conclusions unreliable. (41) Furthermore, the Agricultural Research Service (ARS) in the United States conducted a 35-year study of workers exposed to glyphosate in herbicide formulation and found no evidence of a link between exposure to glyphosate and cancer. (42) These data emphasize the need for well-designed, scientifically stringent studies of glyphosate and its effect on human health.

Glyphosate and neurodevelopmental disorders.

In recent years, reports of human exposure and animal models suggest that both the commercial mixture containing glyphosate and the active ingredient glyphosate could have neurotoxic effects. As discussed earlier, there is a growing body of evidence that suggests pesticide exposure during pregnancy and ASD or autism spectrum disorder in children. (4, 30, 31) In addition, it has been observed that occupational exposure can lead to, or accelerate, neurodevelopmental disorders, such as ADHD and autism. (44) In human studies, glyphosate has been detected in breast milk and cerebrospinal fluid after high-dose, acute exposure to commercial mixtures, indicating that the active ingredient glyphosate can be absorbed. (45) Structural magnetic resonance imaging (MRI) studies in children exposed to a commercial mixture of glyphosate showed reductions in the T2 signal in subcortical regions, parietal, and cerebellar gray and white matter, indicative of possible brain damage. (20) Furthermore, aberrant behavior, activity, and a reduction in functional connectivity characterized by reduced connectivity between limbic and other brain regions and global integrity have been observed in ocular occupational and accidental exposure to the commercial mixture of glyphosate. (20, 24, 47). These associations of glyphosate and neurodevelopmental disease lack a mechanism of action, although some studies have suggested a potential role for oxidative stress and animal models have been utilized to gain knowledge of the possible neurotoxic mechanisms of glyphosate.

A study showed that glyphosate exposure could affect the dopamine system, responsible for motor behavior and the system impacted in PD. Animal studies with rats demonstrated that repeated glyphosate exposure resulted in decreased dopaminergic function, accompanied by decreases in specific binding to dopamine receptors. In the nucleus accumbens and the frontal cortex of rats treated with glyphosate, the dopamine receptors were significantly reduced. (48) These results could help explain the PD-like symptoms that can be experienced after glyphosate exposure. Another animal study showed that acute and chronic exposure to glyphosate and tremors in rats, which may provide insights into the potential for human health effects. (49) In conclusion, the findings suggest a potential link between glyphosate exposure and neurodevelopmental disorders, and more research is needed to fully understand the mechanisms of action and potential risks to human health.
Specific Aims

Our working hypothesis is that the accumulation of glyphosate (and its metabolites) in brain is correlated with ASD. To address our hypothesis we propose the following aims:

- **Specific Aim 1:** To determine if glyphosate and other pesticides accumulate in human brain tissue. Hypothesis: Glyphosate can be detected and accumulates in human brain tissue. Rationale: A recent study showed that glyphosate can be found in mammalian (cow) tissue, including lipid-dense tissue such as liver and intestine (10). Furthermore, glyphosate has been detected in human brain and cerebrospinal fluid after acute exposure to commercial mixtures, indicating that it can cross the blood brain barrier (45, 46).

- **Specific Aim 2:** To examine the degree of glyphosate and pesticide accumulation in brain tissue from ASD patients. Hypothesis: Autism spectrum disorder patients have a higher accumulation of glyphosate and pesticide in brain compared to normal, age-matched controls. Rationale: Recent studies suggest that women with higher levels of organophosphate pesticides in their urine or who lived near fields with high organophosphate application were 2 to 6 times more likely to have a child with ASD (3, 26, 29). Furthermore, a recent review of US government databases for genetically-modified crops, glyphosate application, and epidemiology revealed a positive correlation between glyphosate application and autism (R = 0.089) (13).

Experimental Approach for Aim 1

Procedure.

All experimental procedures will be performed at Dr. Lee-Way Jin's laboratory at the University of California (UC) Davis. Studies will not proceed until Institutional Review Board has approved all procedures. His lab is well-equipped and experienced in the protocols described in this proposal. We will obtain fresh, flash-frozen normal brain tissue from the UC Davis Brain Repository, of which Dr. Jin is the Director. He is the UC Davis principal investigator for the UC Pediatric Neuropathology Consortium that is charged with the collection of cellular and brain tissue from patients with neurodevelopmental disorders such as autism, fragile X syndrome, and Rett syndrome. Dr. Jin and his research team will coordinate the effort to obtain frozen, post-mortem human brain samples from 100 neurologically normal individuals, age 8 years old or younger. Prior to freezing, tissue will quickly be dissected from each of six distinct anatomic regions: Wernicke’s area, Broca’s area, the auditory cortex, insula, angular gyrus, and hippocampus. This would total approximately 600 pieces (samples) from a total of 100 cases.

Each frozen sample will be homogenized according to published procedures. Briefly, tissue samples will be minced to small pieces (~0.25 cm). Samples will be diluted with distilled water and fractionated to obtain soluble fractions that are suitable for glyphosate determination as follows. The samples will be heated at 100°C for 10 min, homogenized and frozen at -80°C for 8 h. Samples will carefully thawed at 40°C and centrifuged at 10,000 x g for 10 min. The supernatant will be filtered with an ultra-centrifugal filter with a cut off of 3000 Da to remove proteins and peptides. Filters will be centrifuged (10,000 x g) again at 20°C for 10 min and the supernatant can be frozen for later use. Supernatant will be diluted and tested for glyphosate using ELISA and UPLC/MS. In each analysis, test samples will be compared to samples lacking brain tissue (negative control).

Samples will be tested for glyphosate concentration using glyphosate ELISA kits (Abraxis, USA) according to the manufacturer’s protocol. Test validation of ELISA will be done in comparison with UPLC/MS.

Detection of glyphosate in brain tissue samples using UPLC/MS (Waters Acquity UPLC and a TSQ Quantum Access MAX mass) will be according to the procedure of Altemeier and colleagues (54) with some modifications. Briefly, all chemicals used are of analytical grade (Sigma) unless stated otherwise. Prepared brain samples will be equilibrated to room temperature. Samples will be vortex mixed prior to transferring 100 μl aliquots to 10 ml screw-capped glass tubes containing 1 ml of acetonitrile. To each sample, an internal

standard solution containing 13C215N-Glyphosate will be added. Samples are then evaporated to dryness in a vacuum centrifuge. For derivatization, 0.5 ml of 2,2,2-trifluoroethanol and 1 ml of freezing cold (-40°C) trifluoroacetic anhydride will be added to the residue. The mixture will be vortex mixed briefly, sonicated for 10 min, and heated to 80°C for 1 h. After cooling, the tube will be uncapped and the solution will be carefully evaporated at 80-90°C without a stream of air or nitrogen (under vacuum). After cooling, the oily residue will be dissolved in 200 μl of acetonitrile and analyzed on the UPLC/MS.

Statistical analysis.

The statistical analysis will be carried out using GraphPad Prism 4 (GraphPad Software, La Jolla, USA). One-way analysis of variance (ANOVA) and unpaired Student t-test will be used to identify significant differences between means of glyphosate concentrations in samples containing brain tissue compared to samples that do not contain brain tissue sample.

Experimental Approach for Aim 2

Procedure.

We will perform the same analysis as in Aim 1 on 100 brain samples from age-matched ASD diagnosed patients to compare to the levels of glyphosate in controls. All samples will been processed and analyzed for glyphosate content using ELISA and UPLC/MS, as described.

Statistical analysis.

To identify significant differences between means of glyphosate concentrations in samples containing brain tissue compared to samples that do not contain brain tissue sample, and normal control samples, we will perform one-way ANOVA. We will perform regression analysis to determine the association between glyphosate accumulation and ASD diagnosis.

Timeline for Aims 1-2:

We expect to collect and analyze control (600) and ASD (600) samples concurrently and should have all samples processed and analyzed in 1 year. Data analysis (regression) and dissemination of results will be completed in the following year.
Top 10 Grant Writing Tips

1. Convey enthusiasm!!

2. Be mindful of your audience

3. W5H

4. Active voice
   "Why did the chicken cross the road?" instead of "Why was the road crossed by the chicken?"

5. Jargon – explain what acronyms and science specific mean
Top 10 Grant Writing Tips

6. Ensure that you are responding to the specifics of the request for application (RFA) or funding opportunity announcement (FOA).

7. There is no need to pepper otherwise clear prose with unnecessary jargon and repetitive, clutter-inducing sesquipedalian verbiage.

   **Say what you mean, clearly and concisely**

8. To be convincing give examples to support your statements. Use citations.

9-10. Review, review, review!!!
Thank You! Any questions?

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