

## Glyphosate Neurotoxicity

### Description

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<https://www.cognovo.eu/people/research-fellows/christopher-germann.php>

? Primary goals of research and innovation policy as defined by the European Commission: [Open Innovation, Open Science, Open to the World](#).

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## Glyphosate – A neurotoxic, genotoxic, and carcinogenic chemical which is ubiquitous in our food and environment

*Let food be thy medicine and medicine be thy food. ? Hippocrates*

[su\_spoiler title="Domain details" style="fancy" icon="folder-2?"] [su\_qrcode data="http://glyphosate-neurotoxicity.de" title="http://glyphosate-neurotoxicity.de" size="100? align="left" link="http://glyphosate-neurotoxicity.de"] REST-style URL: [glyphosate-neurotoxicity.ga](http://glyphosate-neurotoxicity.ga)

Permanent URL: [cognitive-liberty.online/glyphosate-neurotoxicity/](http://cognitive-liberty.online/glyphosate-neurotoxicity/)

New domain: [glyphosate-neurotoxicity.de](http://glyphosate-neurotoxicity.de)

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### Abstract

Glyphosate (IUPAC name: *N*-(*phosphonomethyl*)*glycine*) is a [su\_tooltip style="bootstrap" position="north" shadow="yes" rounded="yes" title="Definition:" content="Glyphosate is a non-selective herbicides (sometimes called 'total weedkillers' in commercial products) which kills all plant material with which it comes into contact. "[non-selective broad-spectrum systemic herbicide[/su\_tooltip] and crop desiccant. It is primarily used to "kill weeds", especially annual broadleaf weeds and grasses that compete with crops. Furthermore, it is utilized massively by the agricultural industry on many food crops which people consume on a daily basis (e.g., fruits, vegetables, grains, etc.).

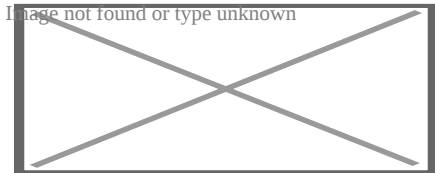


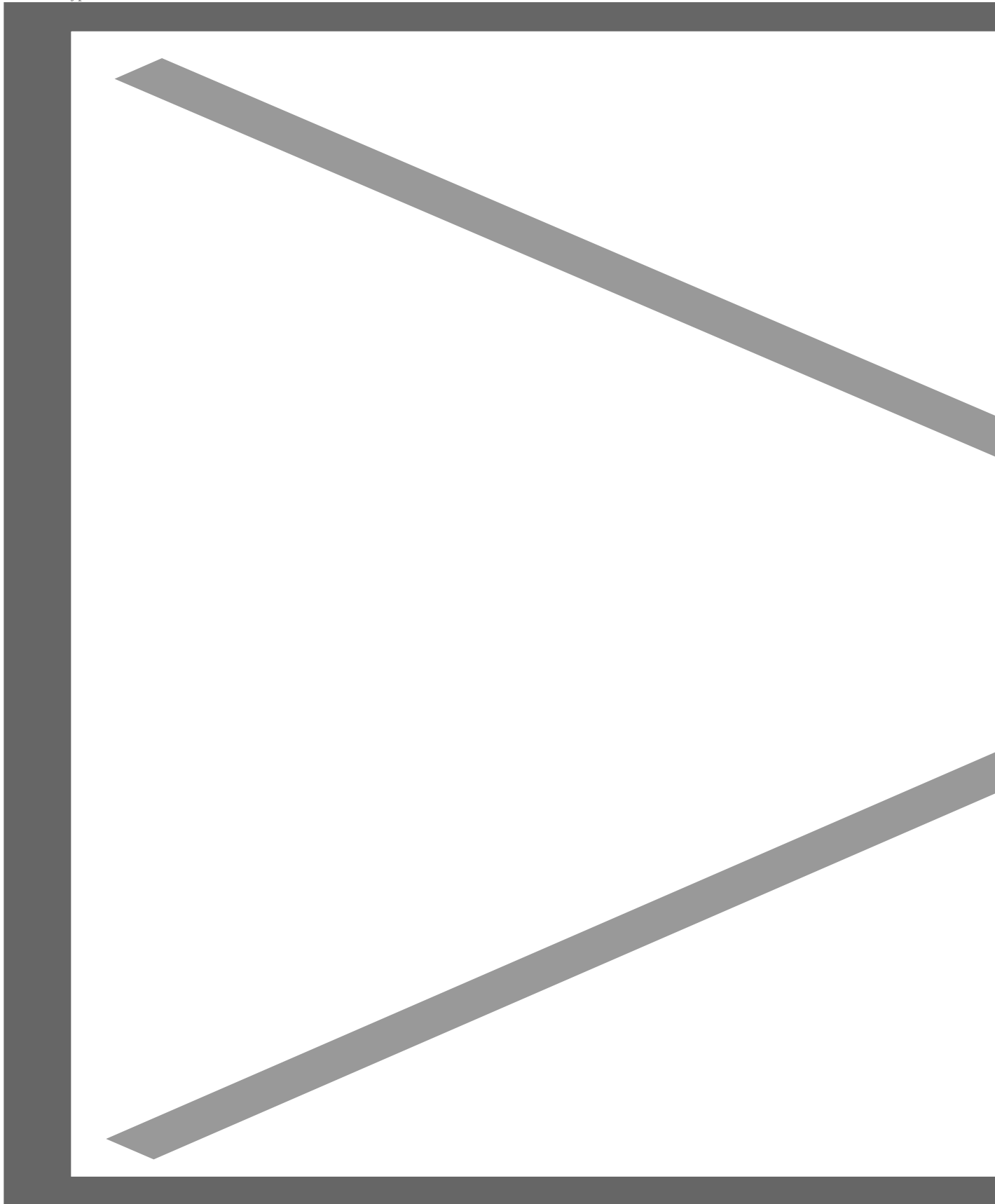
Figure 1. Chemical structure of Glyphosate (chemical formula:  $C_3H_8NO_5P$ ).

[su\_spoiler title="Effects of glyphosate on the ovarian function of pregnant mice, the secretion of hormones and the sex ratio of their fetuses" style="fancy" icon="folder-2?"]

## Study Highlights

- Prenatal exposure to pure glyphosate affected sex ratios of litters.
- Glyphosate caused ovarian histopathological alterations in pregnant mice.
- Glyphosate disrupted the secretion of progesterone and estrogen in pregnant mice.
- Glyphosate disrupted expressions of steroidogenesis-related genes in pregnant mice.
- Glyphosate induced oxidative stress in ovary and serum of pregnant mice.

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## Visual summary

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## Abstract

Glyphosate is the active ingredient of the commercial formulation Roundup®, which is used worldwide. This study aimed to investigate the toxic effects of pure glyphosate or Roundup® on pregnant mice and their fetuses during pregnancy. From gestation days (GDs) 1–19, ICR mice were orally administered distilled water, 0.5% glyphosate solution or 0.5%-glyphosate Roundup® solution. The ovaries and serum were collected at GD19. The results showed decreases in body weight gain and, ovary and liver weight in glyphosate-treated mice. Additionally, histopathological alterations in the ovary including increased atretic follicles, interstitial fibrosis and decreased mature follicles were observed in the groups treated with glyphosate. The serum concentrations of both progesterone and estrogen were markedly altered after glyphosate exposure, and there were also changes in the expression of GnRH, LHR, FSHR, 3 $\beta$ -HSD and Cyp19a1 genes at the hypothalamic-pituitary-ovarian axis. Furthermore, oxidative stress was observed in the treated mice, increasing the activity of T-AOC, CAT and GSH-Px, as well as the MDA content in both the serum and ovary. With regard to litters, the sex ratio was significantly altered by pure glyphosate. These results show that glyphosate is able to cause several effects on pregnant mice, such as ovarian failure, interference with hormone secretion by affecting the steroidogenesis-related gene expression, and oxidative stress. The sex ratio of litters was also influenced by prenatal exposure to pure glyphosate.

URL: [www.sciencedirect.com/science/article/pii/S0269749118330197](http://www.sciencedirect.com/science/article/pii/S0269749118330197)

[/su\_spoiler] [su\_spoiler title="Glyphosate and Roundup® alter morphology and behavior in zebrafish" style="fancy" icon="folder-2?"]

## Study Highlights

- Glyphosate or Roundup® altered locomotion and aversive behavior in zebrafish larvae.
  - Glyphosate or Roundup® reduced the locomotion in adult zebrafish.
  - Glyphosate decreased ocular distance in zebrafish larvae.
  - Roundup®-exposed adult zebrafish demonstrated impairment in memory.
  - Glyphosate and Roundup® reduced aggressive behavior in adult zebrafish.
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## Abstract

Glyphosate has become the most widely used herbicide in the world, due to the wide scale adoption of transgenic glyphosate resistant crops after its introduction in 1996. Glyphosate may be used alone, but it is commonly applied as an active ingredient of the herbicide Roundup®. This pesticide contains

several adjuvants, which may promote an unknown toxicity. The indiscriminate application poses numerous problems, both for the health of the applicators and consumers, and for the environment, contaminating the soil, water and leading to the death of plants and animals. Zebrafish (*Danio rerio*) is quickly gaining popularity in behavioral research, because of physiological similarity to mammals, sensitivity to pharmacological factors, robust performance, low cost, short spawning intervals, external fertilization, transparency of embryos through larval stages, and rapid development. The aim of this study was evaluate the effects of glyphosate and Roundup® on behavioral and morphological parameters in zebrafish larvae and adults. Zebrafish larvae at 3 days post-fertilization and adults were exposed to glyphosate (0.01, 0.065, and 0.5 mg/L) or Roundup® (0.01, 0.065, and 0.5 mg/L) for 96 h. Immediately after the exposure, we performed the analysis of locomotor activity, aversive behavior, and morphology for the larvae and exploratory behavior, aggression and inhibitory avoidance memory for adult zebrafish. In zebrafish larvae, there were significant differences in the locomotor activity and aversive behavior after glyphosate or Roundup® exposure when compared to the control group. Our findings demonstrated that exposure to glyphosate at the concentration of 0.5 mg/L, Roundup® at 0.065 or 0.5 mg/L reduced the distance traveled, the mean speed and the line crossings in adult zebrafish. A decreased ocular distance was observed for larvae exposed at 0.5 mg/L of glyphosate. We verified that at 0.5 mg/L of Roundup®-treated adult zebrafish demonstrated a significant impairment in memory. Both glyphosate and Roundup® reduced aggressive behavior. Our data suggest that there are small differences between the effects induced by glyphosate and Roundup®, altering morphological and behavioral parameters in zebrafish, suggesting common mechanisms of toxicity and cellular response.

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[su\_youtube\_advanced url="https://www.youtube.com/watch?v=mB4YR-O5X5M" showinfo="no" autoplay="yes" loop="yes" rel="no" modestbranding="yes" theme="light" https="yes" playsinline="yes" width="300?]

Glyphosate was first synthesized in 1950 as a potential pharmaceutical compound. It was discovered to be an herbicide by Monsanto chemist John E. Franz in 1970. Unfortunately glyphosate is found in almost all non-organic food crops (think about it when you buy 'non-organic food' in the supermarket next time) and the chemical is now ubiquitous in our environment (it has even been detected in the [umbilical cord blood of newborns](#)) because it has been used in agriculture for a long time and has spread throughout the environmental systems. The analogy of a drop of ink in a glass of water (cf. osmosis) is appropriate to communicate the concept of [chemical dispersion](#) throughout the environment. Its interactions and synergies with other chemical compounds are currently only poorly understood as the majority of studies focus on isolated compounds. However, since the industrial revolution our environment has been bombarded with countless synthetic chemicals an only time will tell what the cumulative effects on biology and life are. Currently nobody knows how all these chemical compounds interact. In other words, humanity is currently conducting a large scale biochemical experiment without any control group (and without ethical permission or consensus by the participants).

[su\_spoiler title="Analysis of concentrations of Glyphosate in pregnant versus nonpregnant women (i.e., in maternal and fetal cord blood)" style="fancy" icon="folder-1?]

[farmlandbirds.net/sites/default/files/BTInpregnantwomen.pdf](http://farmlandbirds.net/sites/default/files/BTInpregnantwomen.pdf)

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Cattani, D., de Liz Oliveira Cavalli, V. L., Heinz Rieg, C. E., Domingues, J. T., Dal-Cim, T., Tasca, C. I., ... Zamoner, A.. (2014). Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: Involvement of glutamate excitotoxicity. *Toxicology*

Plain numerical DOI: 10.1016/j.tox.2014.03.001

[DOI URL](#)

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### Show/hide publication abstract

"Previous studies demonstrate that glyphosate exposure is associated with oxidative damage and neurotoxicity. therefore, the mechanism of glyphosate-induced neurotoxic effects needs to be determined. the aim of this study was to investigate whether roundup®(a glyphosate-based herbicide) leads to neurotoxicity in hippocampus of immature rats following acute (30min) and chronic (pregnancy and lactation) pesticide exposure. maternal exposure to pesticide was undertaken by treating dams orally with 1% roundup®(0.38% glyphosate) during pregnancy and lactation (till 15-day-old). hippocampal slices from 15 day old rats were acutely exposed to roundup®(0.00005-0.1%) during 30min and experiments were carried out to determine whether glyphosate affects  $Ca^{2+}$  influx and cell viability. moreover, we investigated the pesticide effects on oxidative stress parameters, 14c-?-methyl-amino-isobutyric acid (14c-meaiB) accumulation, as well as glutamate uptake, release and metabolism. results showed that acute exposure to roundup®(30min) increases  $Ca^{2+}$  influx by activating nmda receptors and voltage-dependent  $Ca^{2+}$  channels, leading to oxidative stress and neural cell death. the mechanisms underlying roundup®-induced neurotoxicity also involve the activation of camkii and erk. moreover, acute exposure to roundup®increased 3h-glutamate released into the synaptic cleft, decreased gsh content and increased the lipoperoxidation, characterizing excitotoxicity and oxidative damage. we also observed that both acute and chronic exposure to roundup®decreased 3h-glutamate uptake and metabolism, while induced  $Ca^{2+}$  uptake and 14c-meaiB accumulation in immature rat hippocampus. taken together, these results demonstrated that roundup®might lead to excessive extracellular glutamate levels and consequently to glutamate excitotoxicity and oxidative stress in rat hippocampus. © 2014 elsevier ireland ltd."

Roy, N. M., Carneiro, B., & Ochs, J.. (2016). Glyphosate induces neurotoxicity in zebrafish. *Environmental Toxicology and Pharmacology*, 42, 45–54.

Plain numerical DOI: 10.1016/j.etap.2016.01.003

[DOI URL](#)

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### Show/hide publication abstract

"Glyphosate based herbicides (gbh) like roundup® are used extensively in agriculture as well as in urban and rural settings as a broad spectrum herbicide. its mechanism of action was thought to be specific only to plants and thus considered safe and non-toxic. however, mounting evidence suggests that gbhs may not be as safe as once thought as initial studies in frogs suggest that gbhs may be

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teratogenic. here we utilize the zebrafish vertebrate model system to study early effects of glyphosate exposure using technical grade glyphosate and the roundup® classic formulation. we find morphological abnormalities including cephalic and eye reductions and a loss of delineated brain ventricles. concomitant with structural changes in the developing brain, using in situ hybridization analysis, we detect decreases in genes expressed in the eye, fore and midbrain regions of the brain including pax2, pax6, otx2 and epha4. however, we do not detect changes in hindbrain expression domains of epha4 nor exclusive hindbrain markers krox-20 and hoxb1a. additionally, using a retinoic acid (ra) mediated reporter transgenic, we detect no alterations in the ra expression domains in the hindbrain and spinal cord, but do detect a loss of expression in the retina. we conclude that glyphosate and the roundup® formulation is developmentally toxic to the forebrain and midbrain but does not affect the hindbrain after 24h exposure."

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**[icon name="paperclip" class="" unprefixd\_class=""]For further peer-reviewed scientific studies on the numerous reported neurotoxic effects of Glyphosate in the brain see bibliography below**

[su\_spoiler title="Glyphosate and neurodegenerative disorders" style="fancy" icon="folder-1?"]  
[people.csail.mit.edu/seneff/2018/Roundup\\_and\\_Neurodegenerative\\_Disorders\\_RosemaryMason.pdf](http://people.csail.mit.edu/seneff/2018/Roundup_and_Neurodegenerative_Disorders_RosemaryMason.pdf)

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[/su\_spoiler] [su\_spoiler title="Further relevant websites on Glyphosate" style="fancy" icon="folder-1?"]

- [www.epa.gov/pesticides](http://www.epa.gov/pesticides)
- [www.sciencedirect.com/search/advanced?qs=glyphosate%20neurotoxic&show=25&sortBy=relevance](http://www.sciencedirect.com/search/advanced?qs=glyphosate%20neurotoxic&show=25&sortBy=relevance)
- [www.theguardian.com/environment/2017/mar/07/un-experts-denounce-myth-pesticides-are-necessary-to-feed-the-world](http://www.theguardian.com/environment/2017/mar/07/un-experts-denounce-myth-pesticides-are-necessary-to-feed-the-world)

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In the USA there are currently 7580 lawsuits pending against Monsanto (now BAYER). The lead case in The USA is 3:16-md-02741-VC. The first trial in the federal court is set for Feb. 25, 2019 in the U.S. District Court in San Francisco. In the UK Glyphosate is unfortunately still completely unregulated and readily available in regular supermarkets where it is advertised as an effective weed killer for gardeners (*inter alia*). Moreover, it is applied to almost all non-organic food crops (fruits, vegetables, grains, etc.) we all consume on a daily basis. It is impossible to "wash off" Glyphosate because it penetrates deep into the organism after it is topically applied. Moreover, the human body and brain has great difficulty to detox from Glyphosate and it [accumulates in the human body and brain](#) over time. The exact longitudinal effects of Glyphosate are hitherto unknown and therefore pose a significant risk to the environment and human health.

URL: [usrtk.org/pesticides/mdl-monsanto-glyphosate-cancer-case-key-documents-analysis/](http://usrtk.org/pesticides/mdl-monsanto-glyphosate-cancer-case-key-documents-analysis/)

## Investigative documentary: The world according to Monsanto

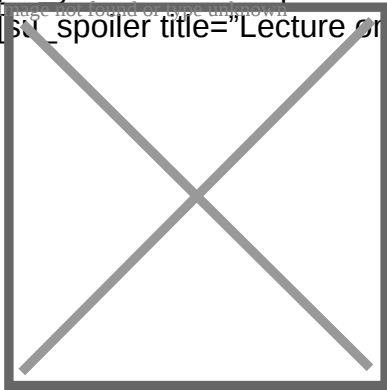
[su\_youtube url="https://www.youtube.com/watch?v=hxb0KTShHwY" width="500" responsive="no"]

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### The Monsanto papers – The secret tactics Monsanto used to protect Glyphosate

[su\_youtube url="https://www.youtube.com/watch?v=JszHrMZ7dx4" width="500" responsive="no"]

[su\_spoiler title="Lecture on glyphosate by Dr. Seneff" style="fancy" icon="folder-1?"]



Dr. Stephanie Seneff is a Senior Research Scientist at the MIT Computer

Science and Artificial Intelligence Laboratory. She received the B.S. degree in Biophysics in 1968, the M.S. and E.E. degrees in Electrical Engineering in 1980, and the Ph.D degree in Electrical Engineering and Computer Science in 1985, all from MIT. For over three decades, her research interests have always been at the intersection of biology and computation – developing a computational model for the human auditory system, understanding human language so as to develop algorithms and systems for human computer interactions, as well as applying natural language processing (NLP) techniques to

gene predictions. She has published over 170 refereed articles on these subjects, and has been invited to give keynote speeches at several international conferences. She has also supervised numerous Master's and PhD theses at MIT.

In recent years, Dr. Seneff has focused her research interests back towards biology. She is concentrating mainly on the relationship between nutrition and health. Since 2011, she has published over 30 papers, together with colleagues, in various peer-reviewed medical and health-related journals on topics such as modern day diseases (e.g., Alzheimer, autism, cardiovascular diseases), analysis and search of databases of drug side effects using NLP techniques, and the impact of nutritional deficiencies and environmental toxins on human health.

It is noteworthy how Wikipedia editors try to discredit her valid concerns by using cheap *ad hominem* arguments which do not address the crux of the problem. It is very difficult to add objective information to her Wikipedia entry (this known as "Wikipedia [edit wars](#)").

But see: [en.wikipedia.org/wiki/Stephanie\\_Seneff](https://en.wikipedia.org/wiki/Stephanie_Seneff)

Cf. [www.globalresearch.ca/monsanto-accused-of-hiring-army-of-trolls-to-silence-online-dissent-court-papers/5588396](http://www.globalresearch.ca/monsanto-accused-of-hiring-army-of-trolls-to-silence-online-dissent-court-papers/5588396)

[su\_youtube url="https://www.youtube.com/watch?v=qYC6oyBglZI" width="300? responsive="no"]  
[/su\_spoiler] The Guardian stated the following:

"The internal correspondence noted by Johnson could support a jury finding that Monsanto has long been aware of the risk that its glyphosate-based herbicides are carcinogenic ... but has continuously sought to influence the scientific literature to prevent its internal concerns from reaching the public sphere and to bolster its defenses in products liability actions," Karnow wrote. "Thus there are triable issues of material fact." Monsanto "championed falsified data and attacked legitimate studies" that revealed dangers of its herbicides, and led a "prolonged campaign of misinformation" to convince government agencies, farmers and consumers that Roundup was safe, according to Johnson's lawsuit.

URL: [www.theguardian.com/business/2018/may/22/monsanto-trial-cancer-weedkiller-roundup-dewayne-johnson](http://www.theguardian.com/business/2018/may/22/monsanto-trial-cancer-weedkiller-roundup-dewayne-johnson)

"Biotech giant Monsanto is being accused of hiring, through third parties, an army of Internet trolls to counter negative comments, while citing positive "ghost-written" pseudo-scientific reports which downplay the potential risks of their products."

On a larger scale, Monsanto allegedly

"quietly funnels money to 'think tanks' such as the 'Genetic Literacy Project' and the 'American Council on Science and Health'— organizations intended to shame scientists and highlight information helpful to Monsanto and other chemical producers," according to the plaintiffs."

URL: [www.globalresearch.ca/monsanto-accused-of-hiring-army-of-trolls-to-silence-online-dissent-court-papers/5588396](http://www.globalresearch.ca/monsanto-accused-of-hiring-army-of-trolls-to-silence-online-dissent-court-papers/5588396)

From a meta-analytic perspective the scientific evidence against the utilisation of Glyphosate is very strong. In addition, synergistic toxicity needs to be taken in account. Such synergies are unfortunately rarely addressed in the scientific literature (and even less frequently in public/political debates). We know that 1 + 1 is not always 2. Specifically, in the domain of chemistry and neurochemistry. Currently, nobody really knows how Glyphosate interacts with the numerous other chemical we unfortunately find in our environments. Even less is known about the longitudinal effects of such synergies. What we do know is that Monsanto has *de facto* been found guilty for crimes against nature and humanity in the past.

We should be specifically concerned about the potential genotoxic & mutagenic effects (i.e., damage to the human genome is irreversible). That is, the problem does not only concern this generation but the evolution of the species *homo sapiens* as a whole (the Latin binomial ironically translates into "the wise/rational man"). Cross-generational responsibility is an important factor of governmental decision-making and it should be weighted accordingly.

As stated above, we are all exposed to Glyphosate on a daily basis (it is sprayed on almost all non-organic crops and it is widely distributed in our environment). Children are specifically susceptible to the detrimental effects because their blood-brain barrier (which filters exogenous substances) is less developed and because their bodies/brains are much smaller than those of adults.

A comparative historical perspective is very informative in this context. Remember that it took decades for the government to respond to the early warnings about tobacco, asbestos, and X-Rays.

#### **The tobacco time line:**

- The health effects of tobacco were first debated in 1856 in the medical journal The Lancet
- Dr. Isaac Adler suggested lung cancer was related to smoking in 1912
- A British medical journal published a study in 1950 finding that smokers were 50 times more likely to get lung cancer
- It wasn't until 1997 that tobacco companies agreed to fund healthcare costs from smoking

[cognitive-liberty.online/tobacco-timeline/](http://cognitive-liberty.online/tobacco-timeline/)

#### **The X-Ray time line:**

- Thomas Edison noted injuries from X-Rays in 1896
- Edison's assistant died from X-Ray exposure in 1904
- Fluoroscopes were used in shoe stores to see through shoes to aid proper fitting in 1930
- The deaths of over 200 radiologists from radioactive cancer were published in 1934
- Radiation levels of fluoroscopes were questioned in 1949
- In 1990 the risk of cancer from radiation was found to be five times greater than previously thought

**The Asbestos time line:**

- A British factory inspector warned of asbestos harm in 1898
- Rat studies raised questions about harmful effects of asbestos dust in 1911
- U.S. insurers refused to cover asbestos worker's claims in 1918
- From 1935 to 1949 lung cancer was reported in asbestos workers
- Asbestos was finally banned in the U.S. in 1989

*Summa summarum*, there is robust and substantial scientific evidence that Glyphosate is potentially neurotoxic, genotoxic, & cancerogenic. As can be seen from the timelines above (viz., tobacco, X-Rays, asbestos) it took many decades and untold casualties before those carcinogens were ever properly addressed. Glyphosate is a much bigger toxin than tobacco, X-Rays or asbestos due to the sheer numbers of people who are exposed to this chemical. As pointed out before, there is strong evidence that it has potentially highly detrimental irreversible effects on human genes and their expression. Genetic effects are by definition irreversible and molecular biology is in no position to correct such influences *post festum* any time soon (despite CRISPR/Cas9 and other gene editing technologies). The human "gene-pool" should be seen as a public good which needs to be protected. Otherwise future generation will pay a very high price for our irresponsible and irrational short-sighted and profit oriented behaviour (which dominates the current climate of profit-oriented neoliberalism). Further, Glyphosate possesses a very stable chemical structure and it lingers in the environment (and the human body/brain) for a very long time. The numbers of people now being affected is enormous (not just in the UK but worldwide). There isn't enough time for us to wait for our government or industry to come to the realization that a serious problem exists. We know that they will be reluctant to make such an admission (for various reasons which go beyond the scope of this email). Reasonable precautions must be taken now! The evidence is before us. Research studies tell us there is a problem. We cannot afford to wait until industry and government are forced to admit that Glyphosate is dangerous. We did that with tobacco, X-Rays and asbestos.

## References

Cattani, D., Cesconetto, P. A., Tavares, M. K., Parisotto, E. B., De Oliveira, P. A., Rieg, C. E. H., ... Zamoner, A.. (2017). Developmental exposure to glyphosate-based herbicide and depressive-like behavior in adult offspring: Implication of glutamate excitotoxicity and oxidative stress. *Toxicology*

Plain numerical DOI: 10.1016/j.tox.2017.06.001

[DOI URL](#)

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### Show/hide publication abstract

"We have previously demonstrated that maternal exposure to glyphosate-based herbicide (gbh) leads to glutamate excitotoxicity in 15-day-old rat hippocampus. the present study was conducted in order to investigate the effects of subchronic exposure to gbh on some neurochemical and behavioral parameters in immature and adult offspring. rats were exposed to 1% gbh in drinking water (corresponding to 0.36% of glyphosate) from gestational day 5 until postnatal day (pnd)-15 or pnd60.

results showed that gbh exposure during both prenatal and postnatal periods causes oxidative stress, affects cholinergic and glutamatergic neurotransmission in offspring hippocampus from immature and adult rats. the subchronic exposure to the pesticide decreased l-[14c]-glutamate uptake and increased 45ca2+ influx in 60-day-old rat hippocampus, suggesting a persistent glutamate excitotoxicity from developmental period (pnd15) to adulthood (pnd60). moreover, gbh exposure alters the serum levels of the astrocytic protein s100b. the effects of gbh exposure were associated with oxidative stress and depressive-like behavior in offspring on pnd60, as demonstrated by the prolonged immobility time and decreased time of climbing observed in forced swimming test. the mechanisms underlying the gbh-induced neurotoxicity involve the nmda receptor activation, impairment of cholinergic transmission, astrocyte dysfunction, erk1/2 overactivation, decreased p65 nf- $\kappa$ b phosphorylation, which are associated with oxidative stress and glutamate excitotoxicity. these neurochemical events may contribute, at least in part, to the depressive-like behavior observed in adult offspring."

Cattani, D., de Liz Oliveira Cavalli, V. L., Heinz Rieg, C. E., Domingues, J. T., Dal-Cim, T., Tasca, C. I., ... Zamoner, A.. (2014). Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: Involvement of glutamate excitotoxicity. *Toxicology*

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Cattani, D., Lúcia, V., Oliveira, D. L., Elise, C., Rieg, H., Domingues, J. T., ... Zamoner, A.. (2014). Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: Involvement of glutamate excitotoxicity. *Toxicology*

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"Previous studies demonstrate that glyphosate exposure is associated with oxidative damage and neurotoxicity. therefore, the mechanism of glyphosate-induced neurotoxic effects needs to be determined. the aim of this study was to investigate whether roundup®(a glyphosate-based herbicide) leads to neurotoxicity in hippocampus of immature rats following acute (30min) and chronic (pregnancy and lactation) pesticide exposure. maternal exposure to pesticide was undertaken by treating dams orally with 1% roundup®(0.38% glyphosate) during pregnancy and lactation (till 15-day-old). hippocampal slices from 15 day old rats were acutely exposed to roundup®(0.00005-0.1%) during 30min and experiments were carried out to determine whether glyphosate affects  $Ca^{2+}$  influx and cell viability. moreover, we investigated the pesticide effects on oxidative stress parameters, 14C-methyl-amino-isobutyric acid (14C-meaiB) accumulation, as well as glutamate uptake, release and metabolism. results showed that acute exposure to roundup®(30min) increases  $Ca^{2+}$  influx by activating nmda receptors and voltage-dependent  $Ca^{2+}$  channels, leading to oxidative stress and neural cell death. the mechanisms underlying roundup®-induced neurotoxicity also involve the activation of camkii and erk. moreover, acute exposure to roundup®increased 3h-glutamate released into the synaptic cleft, decreased gsh content and increased the lipoperoxidation, characterizing excitotoxicity and oxidative damage. we also observed that both acute and chronic exposure to roundup®decreased 3h-glutamate uptake and metabolism, while induced  $Ca^{2+}$  uptake and 14C-meaiB accumulation in immature rat hippocampus. taken together, these results demonstrated that roundup®might lead to excessive extracellular glutamate levels and consequently to glutamate excitotoxicity and oxidative stress in rat hippocampus. © 2014 elsevier ireland ltd."

Coullery, R. P., Ferrari, M. E., & Rosso, S. B.. (2016). Neuronal development and axon growth are altered by glyphosate through a WNT non-canonical signaling pathway. *NeuroToxicology*

Plain numerical DOI: 10.1016/j.neuro.2015.12.004

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## Show/hide publication abstract

"The growth and morphological differentiation of neurons are critical events in the establishment of proper neuronal connectivity and functioning. the developing nervous system is highly susceptible to damage caused by exposure to environmental contaminants. glyphosate-containing herbicides are the most used agrochemicals in the world, particularly on genetically modified plants. previous studies have demonstrated that glyphosate induces neurotoxicity in mammals. therefore, its action mechanism on the nervous system needs to be determined. in this study, we report about impaired neuronal development caused by glyphosate exposure. particularly, we observed that the initial axonal differentiation and growth of cultured neurons is affected by glyphosate since most treated cells remained undifferentiated after 1 day in culture. although they polarized at 2 days in vitro, they elicited shorter and unbranched axons and they also developed less complex dendritic arbors compared to

controls. to go further, we attempted to identify the cellular mechanism by which glyphosate affected neuronal morphology. biochemical approaches revealed that glyphosate led to a decrease in wnt5a level, a key factor for the initial neurite development and maturation, as well as inducing a down-regulation of camkii activity. this data suggests that the morphological defects would likely be a consequence of the decrease in both wnt5a expression and camkii activity induced by glyphosate. additionally, these changes might be reflected in a subsequent neuronal dysfunction. therefore, our findings highlight the importance of establishing rigorous control on the use of glyphosate-based herbicides in order to protect mammals' health."

Gallegos, C. E., Baier, C. J., Bartos, M., Bras, C., Domínguez, S., Mónaco, N., ... Minetti, A.. (2018). Perinatal Glyphosate-Based Herbicide Exposure in Rats Alters Brain Antioxidant Status, Glutamate and Acetylcholine Metabolism and Affects Recognition Memory. *Neurotoxicity Research*

Plain numerical DOI: 10.1007/s12640-018-9894-2

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## Show/hide publication abstract

"Changes in biogenic amine content in the substantia nigra and in both forms of monoamine oxidase in substantia nigra and striatum of the rat during postnatal development (15-180 days) have been studied. dopamine and serotonin had the same levels at day 15, however, each monoamine showed a different developmental profile. dopamine levels and their metabolites (except 3-methoxytyramine) decreased during postnatal development. serotonin levels and their main metabolite, 5-hydroxyindolacetic acid, underwent an increase during all stages studied. there were no statistically significant changes in noradrenaline levels until day 180 when they increased with respect to day 15. the highest activity of the monoamine oxidase-a in substantia nigra coincided with the highest 5-hydroxyindolacetic acid:serotonin ratio. monoamine oxidase-a in the striatum did not change contrary to that which happened in substantia nigra. the monoamine oxidase-b:monoamine oxidase-a ratio increased during development both in the substantia nigra and the striatum. the significance of these changes is discussed."

Ji, H., Xu, L., Wang, Z., Fan, X., & Wu, L.. (2018). Differential microRNA expression in the prefrontal cortex of mouse offspring induced by glyphosate exposure during pregnancy and lactation. *Experimental and Therapeutic Medicine*

Plain numerical DOI: 10.3892/etm.2017.5669

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## Show/hide publication abstract

"Glyphosate is the active ingredient in numerous herbicide formulations. the role of glyphosate in neurotoxicity has been reported in human and animal models. however, the detailed mechanism of the role of glyphosate in neuronal development remains unknown. recently, several studies have reported evidence linking neurodevelopmental disorders (ndds) with gestational glyphosate exposure. the current group previously identified micrornas (mirnas) that are associated with the etiology of ndds, but their expression levels in the developing brain following glyphosate exposure have not been

characterized. in the present study, mirna expression patterns were evaluated in the prefrontal cortex (pfc) of 28 postnatal day mouse offspring following glyphosate exposure during pregnancy and lactation. an mirna microarray detected 55 upregulated and 19 downregulated mirnas in the pfc of mouse offspring, and 20 selected deregulated mirnas were further evaluated by quantitative polymerase chain reaction (pcr). a total of 11 targets of these selected deregulated mirnas were analyzed using bioinformatics. gene ontology (go) terms associated with the relevant mirnas included neurogenesis (go:0050769), neuron differentiation (go:0030182) and brain development (go:0007420). the genes cdkn1a, numbl, notch1, fosl1 and lef1 are involved in the wnt and notch signaling pathways, which are closely associated with neural development. pcr arrays for the mouse wnt and notch signaling pathways were used to validate the effects of glyphosate on the expression pattern of genes involved in the wnt and notch pathways. nr4a2 and wnt7b were downregulated, while dkk1, dixdc1, runx1, shh, lef-1 and axin2 were upregulated in the pfc of mice offspring following glyphosate exposure during pregnancy and lactation. these results indicated abnormalities of the wnt/beta-catenin and notch pathways. these findings may be of particular interest for understanding the mechanism of glyphosate-induced neurotoxicity, as well as helping to clarify the association between glyphosate and ndds." Lajmanovich, R. C., Junges, C. M., Attademo, A. M., Peltzer, P. M., Cabagna-Zenklusen, M. C., & Basso, A.. (2013). Individual and mixture toxicity of commercial formulations containing glyphosate, metsulfuron-methyl, bispyribac-sodium, and picloram on rhinella arenarum tadpoles. Water, Air, and Soil Pollution

Plain numerical DOI: 10.1007/s11270-012-1404-1

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## Show/hide publication abstract

"We investigated the effects of four commercial formulations of herbicides (glyphosate [gly], metsulfuron-methyl [met], bispyribac-sodium [bis], and picloram [pic]) individually, and in three 50:50 mixtures (gly-met, gly-bis, gly-pic) on the common toad rhinella arenarum (anura: bufonidae) tadpoles. enzymatic parameters such as, glutathione s-transferase (gst), butyrylcholinesterase (bche) and acetylcholinesterase (ache) activities, as well as erythrocyte nuclear abnormalities (ena) were studied. interactions between herbicides in mixtures were evaluated and classified as additive, synergistic, or antagonistic. toxicity results (48-h lc50) showed that pic was the most toxic herbicide, followed by bis, gly, and met, while gly-pic was the most toxic mixture, followed by gly-bis, and gly-met. all commercial herbicide formulations and their mixtures significantly inhibited bche activity in exposed tadpoles. the ache activity was also inhibited by all herbicides and their mixtures, except by gly-bis. the inhibition of gst activity was only significant for gly, met, pic, and gly-met. a significant increase in the frequency of ena was found for tadpoles exposed either to commercial herbicide formulations or to mixtures, except for gly. all the mixtures showed synergism for bche activity while for ache only the gly-met and gly-pic mixtures acted synergistically. gly-met showed synergism for gst, whereas for ena, the mixture gly-bis was antagonistic. this study with r. arenarum tadpoles demonstrates that the interactions between three of the most intensively used herbicides in soybean crops results in synergistic effects on mortality and neurotoxicity and synergistic or additive effects in genotoxicity."

Landrigan, P. J., & Belpoggi, F.. (2018). The need for independent research on the health effects of glyphosate-based herbicides. Environmental Health: A Global Access Science Source

Plain numerical DOI: 10.1186/s12940-018-0392-z

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## Show/hide publication abstract

"BACKGROUND glyphosate, formulated as roundup, is the world's most widely used herbicide. glyphosate is used extensively on genetically modified (gm) food crops designed to tolerate the herbicide, and global use is increasing rapidly. two recent reviews of glyphosate's health hazards report conflicting results. an independent review by the international agency for research on cancer (iarc) found that glyphosate is a 'probable human carcinogen'. a review by the european food safety agency (efsa) found no evidence of carcinogenic hazard. these differing findings have produced regulatory uncertainty. regulatory actions reflecting this regulatory uncertainty, the european commission on november 27 2017, extended authorization for glyphosate for another 5 years, while the european parliament opposed this decision and issued a call that pesticide approvals be based on peer-reviewed studies by independent scientists rather than on the current system that relies on proprietary industry studies. ramazzini institute response the ramazzini institute has initiated a pilot study of glyphosate's health hazards that will be followed by an integrated experimental research project. this evaluation will be independent of industry support and entirely sponsored by worldwide crowdfunding. the aim of the ramazzini institute project is to explore comprehensively the effects of exposures to glyphosate-based herbicides at current real-world levels on several toxicological endpoints, including carcinogenicity, long-term toxicity, neurotoxicity, endocrine disrupting effects, prenatal developmental toxicity, the microbiome and multi-generational effects."

Martínez, M. A., Ares, I., Rodríguez, J. L., Martínez, M., Martínez-Larrañaga, M. R., & Anadón, A.. (2018). Neurotransmitter changes in rat brain regions following glyphosate exposure. Environmental Research

Plain numerical DOI: 10.1016/j.envres.2017.10.051

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## Show/hide publication abstract

"The effects of glyphosate oral exposure (35, 75, 150 and 800 mg/kg bw, 6 days) on brain region monoamine levels of male wistar rats were examined. glyphosate-treated rats (35, 75, 150 and 800 mg/kg bw, 6 days), had no visible injury, i.e., no clinical signs of dysfunction were observed. after last dose of glyphosate, serotonin (5-ht), dopamine (da) and norepinephrine (ne) and its metabolites levels were determined in the brain regions striatum, hippocampus, prefrontal, cortex, hypothalamus and midbrain, by hplc. glyphosate caused statistically significant changes in the 5-ht and its metabolite 5-hydroxy-3-indolacetic acid (5-hiaa), da and its metabolites 3,4-hydroxyphenylacetic acid (dopac) and homovanillic acid (hva), and ne and its metabolite 3-metoxi-4-hydroxyphenylethyleneglycol (mhp) levels in a brain regional- and dose-related manner. moreover, glyphosate, dose-dependent, evoked a statistically significant increase in 5-ht turnover in striatum and hypothalamus and in da turnover in prefrontal cortex and hippocampus, and a statistically significant decrease in ne turnover in prefrontal cortex and hypothalamus. the present findings indicate that glyphosate significantly altered central

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nervous system (cns) monoaminergic neurotransmitters in a brain regional- and dose-related manner, effects that may contribute to the overall spectrum of neurotoxicity caused by this herbicide."

Menéndez-Helman, R. J., Ferreyroa, G. V., Dos Santos Afonso, M., & Salibián, A.. (2012). Glyphosate as an acetylcholinesterase inhibitor in *Cnesterodon decemmaculatus*. Bulletin of Environmental Contamination and Toxicology

Plain numerical DOI: 10.1007/s00128-011-0423-8

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## Show/hide publication abstract

"The toxic effect of sublethal concentrations (1, 17.5 and 35 mg l<sup>-1</sup>) of pure glyphosate was evaluated on acetylcholinesterase (ache) activity in the fish species, *Cnesterodon decemmaculatus*. acute bioassays (96 h) under laboratory conditions were conducted and homogenates for each specimen corresponding to the anterior, middle and posterior body sections were performed. fish survival was 100%, even at the highest concentration tested (35 mg l<sup>-1</sup>), in accordance with the low lethal toxicity reported for glyphosate. however, a significant inhibitory effect on ache activity was recorded even for the lowest herbicide concentration tested (1 mg l<sup>-1</sup>), in the homogenates corresponding to the anterior body section. the inhibition ranged from 23 to 36%. the analytical determination of glyphosate in assay media by ion chromatography, was used to verify its stability. these results indicate that ache- a neurotoxicity biomarker-in *C. decemmaculatus* may be affected by exposure to environmentally relevant concentrations of glyphosate."

Negga, R., Rudd, D. A., Davis, N. S., Justice, A. N., Hatfield, H. E., Valente, A. L., ... Fitsanakis, V. A.. (2011). Exposure to Mn/Zn ethylene-bis-dithiocarbamate and glyphosate pesticides leads to neurodegeneration in *Caenorhabditis elegans*. NeuroToxicology

Plain numerical DOI: 10.1016/j.neuro.2011.02.002

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## Show/hide publication abstract

"Epidemiological evidence suggests positive correlations between pesticide usage and the incidence of parkinson's disease (pd). to further explore this relationship, we used wild type (n2) *Caenorhabditis elegans* (*C. elegans*) to test the following hypothesis: exposure to a glyphosate-containing herbicide (td) and/or a manganese/zinc ethylene-bis-dithiocarbamate-containing fungicide (mz) may lead to neurotoxicity. we exposed n2 worms to varying concentrations of td or mz for 30min (acute) or 24h (chronic). to replicate agricultural usage, a third population was exposed to td (acute) followed by mz (acute). for acute td exposure, the lc50=8.0% (r<sup>2</sup>=0.6890), while the chronic lc50=5.7% (r<sup>2</sup>=0.9433). acute mz exposure led to an lc50=0.22% (r<sup>2</sup>=0.5093), and chronic lc50=0.50% (r<sup>2</sup>=0.9733). the combined treatment for td+mz yielded an lc50=12.5% (r<sup>2</sup>=0.6367). further studies in nw1229 worms, a pan-neuronally green fluorescent protein (gfp) tagged strain, indicated a statistically significant (p<0.05) and dose-dependent reduction in green pixel number in neurons of treated worms following each paradigm. this reduction of pixel number was accompanied by visual neurodegeneration in photomicrographs. for the dual treatment, bliss analysis suggested synergistic interactions. taken

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together, these data suggest neuronal degeneration occurs in *c. elegans* following treatment with environmentally relevant concentrations of td or mz. © 2011 elsevier inc."

Negga, R., Stuart, J. A., Machen, M. L., Salva, J., Lizek, A. J., Richardson, S. J., ... Fitsanakis, V. A. (2012). Exposure to glyphosate-and/or Mn/Zn-ethylene-bis-dithiocarbamate-containing pesticides leads to degeneration of c-aminobutyric acid and dopamine neurons in *caenorhabditis elegans*. *Neurotoxicity Research*

Plain numerical DOI: 10.1007/s12640-011-9274-7

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## Show/hide publication abstract

"Previous studies demonstrate a positive correlation between pesticide usage and parkinson's disease (pd), which preferentially targets dopaminergic (daergic) neurons. in order to examine the potential relationship between two common pesticides and specific neurodegeneration, we chronically (24 hours) or acutely (30 min) exposed two *caenorhabditis elegans* (*c. elegans*) strains to varying concentrations (lc25, lc50 or lc75) of touchdown® (td) as per cent active ingredient (glyphosate), or mancozeb® (mz) as per cent active ingredient (manganese/zinc ethylene-bis-dithiocarbamate). furthermore, to more precisely model environmental exposure, worms were also exposed to td for 30 min, followed by 30-min incubation with varying mz concentrations. previous data from our lab suggested general neuronal degeneration using the worm strain nw1229 (pan-neuronal::green fluorescent protein (gfp) construct). to determine whether distinct neuronal groups were preferentially affected, we specifically used eg1285 (gabaergic neurons::gfp construct) and bz555 (daergic neurons::gfp construct) worms to verify gabaergic and daergic neurodegeneration, respectively. results indicated a statistically significant decrease, when compared to controls (cn), in number of green pixels associated with gabaergic neurons in both chronic (\*p < 0.05) and acute (\*p < 0.05) treatment paradigms. analysis of the bz555 worms indicated a statistically significant decrease (\*p < 0.05) in number of green pixels associated with daergic neurons in both treatment paradigms (chronic and acute) when compared to cn. taken together, our data suggest that exposure to td and/or mz promotes neurodegeneration in both gabaergic and daergic neurons in the model organism *c. elegans*." Roy, N. M., Carneiro, B., & Ochs, J.. (2016). Glyphosate induces neurotoxicity in zebrafish. *Environmental Toxicology and Pharmacology*

Plain numerical DOI: 10.1016/j.etap.2016.01.003

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## Show/hide publication abstract

"Glyphosate based herbicides (gbh) like roundup® are used extensively in agriculture as well as in urban and rural settings as a broad spectrum herbicide. its mechanism of action was thought to be specific only to plants and thus considered safe and non-toxic. however, mounting evidence suggests that gbhs may not be as safe as once thought as initial studies in frogs suggest that gbhs may be teratogenic. here we utilize the zebrafish vertebrate model system to study early effects of glyphosate exposure using technical grade glyphosate and the roundup® classic formulation. we find

morphological abnormalities including cephalic and eye reductions and a loss of delineated brain ventricles. concomitant with structural changes in the developing brain, using in situ hybridization analysis, we detect decreases in genes expressed in the eye, fore and midbrain regions of the brain including pax2, pax6, otx2 and epha4. however, we do not detect changes in hindbrain expression domains of epha4 nor exclusive hindbrain markers krox-20 and hoxb1a. additionally, using a retinoic acid (ra) mediated reporter transgenic, we detect no alterations in the ra expression domains in the hindbrain and spinal cord, but do detect a loss of expression in the retina. we conclude that glyphosate and the roundup® formulation is developmentally toxic to the forebrain and midbrain but does not affect the hindbrain after 24h exposure."

Yu, N., Tong, Y., Zhang, D., Zhao, S., Fan, X., Wu, L., & Ji, H.. (2018). Circular RNA expression profiles in hippocampus from mice with perinatal glyphosate exposure. Biochemical and Biophysical Research Communications

Plain numerical DOI: 10.1016/j.bbrc.2018.04.200

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## Show/hide publication abstract

"Glyphosate is the active ingredient in numerous herbicide formulations. the roles of glyphosate in embryo-toxicity and neurotoxicity have been reported in human and animal models. recently, several studies have reported evidence linking neurodevelopmental disorders (ndds) with gestational glyphosate exposure. however, the role of glyphosate in neuronal development is still not fully understood. our previous study found that perinatal glyphosate exposure resulted in differential microrna expression in the prefrontal cortex of mouse offspring. however, the mechanism of glyphosate-induced neurotoxicity in the developing brain is still not fully understood. considering the pivotal role of circular rnas (circrnas) in the regulation of gene expression, a circrna microarray method was used in this study to investigate circrna expression changes in the hippocampus of mice with perinatal glyphosate exposure. the circrna microarrays revealed that 663 circrnas were significantly altered in the perinatal glyphosate exposure group compared with the control group. among them, 330 were significantly upregulated, and the other 333 were downregulated. furthermore, the relative expression levels of mmu-circrna-014015, mmu-circrna-28128 and mmu-circrna-29837 were verified using quantitative real-time polymerase chain reaction (qrt-pcr). gene ontology (go) and kyoto encyclopedia of genes and genomes (kegg) pathway analyses demonstrated that stress-associated steroid metabolism pathways, such as aldosterone synthesis and secretion pathways, may be involved in the neurotoxicity of glyphosate. these results showed that circrnas are aberrantly expressed in the hippocampus of mice with perinatal glyphosate exposure and play potential roles in glyphosate-induced neurotoxicity."

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Keywords:

- public engagement
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