

The Effect of Glyphosate and AMPA on Behavioral and Neurological Disorders

Environmental Toxin and Genomics Conference
Hershey, PA Nov 2019

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UbiquiTox
Environmental Medicine

UBIQUITOUS EXPOSURES

+

MULTIPLE PATHWAYS

=

ADVERSE OUTCOMES

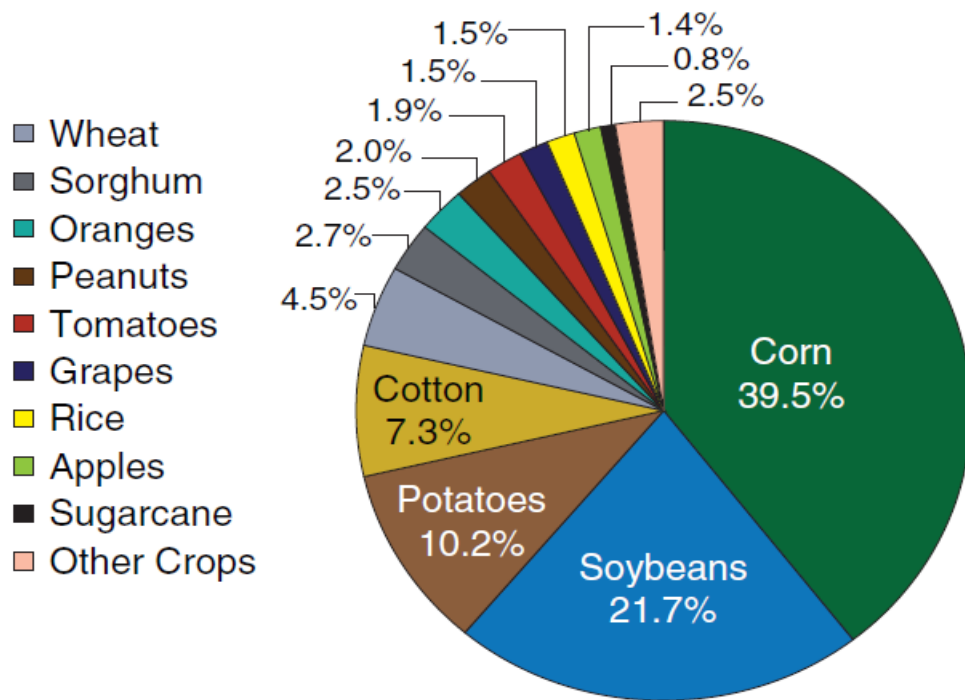
Background

- Roundup is the Most Widely Used Herbicide on the Planet
- Glyphosate is the active ingredient in Roundup
- AMPA is the primary metabolite of glyphosate

Annual worldwide use is over
1.5 billion lbs

U.S. agriculture uses over
250 M lbs/yr

Pesticide use by crop, 21 selected crops, 2008, percent total pounds of active ingredient applied



Note: "Other Crops" include: lettuce, pears, sweet corn, barley, peaches, grapefruit, pecans, and lemons.

Sources: Economic Research Service with USDA and proprietary data. See Appendix 2.

Glyphosate is used on over 90% of the soybeans and 60% of the corn grown in the U.S.*

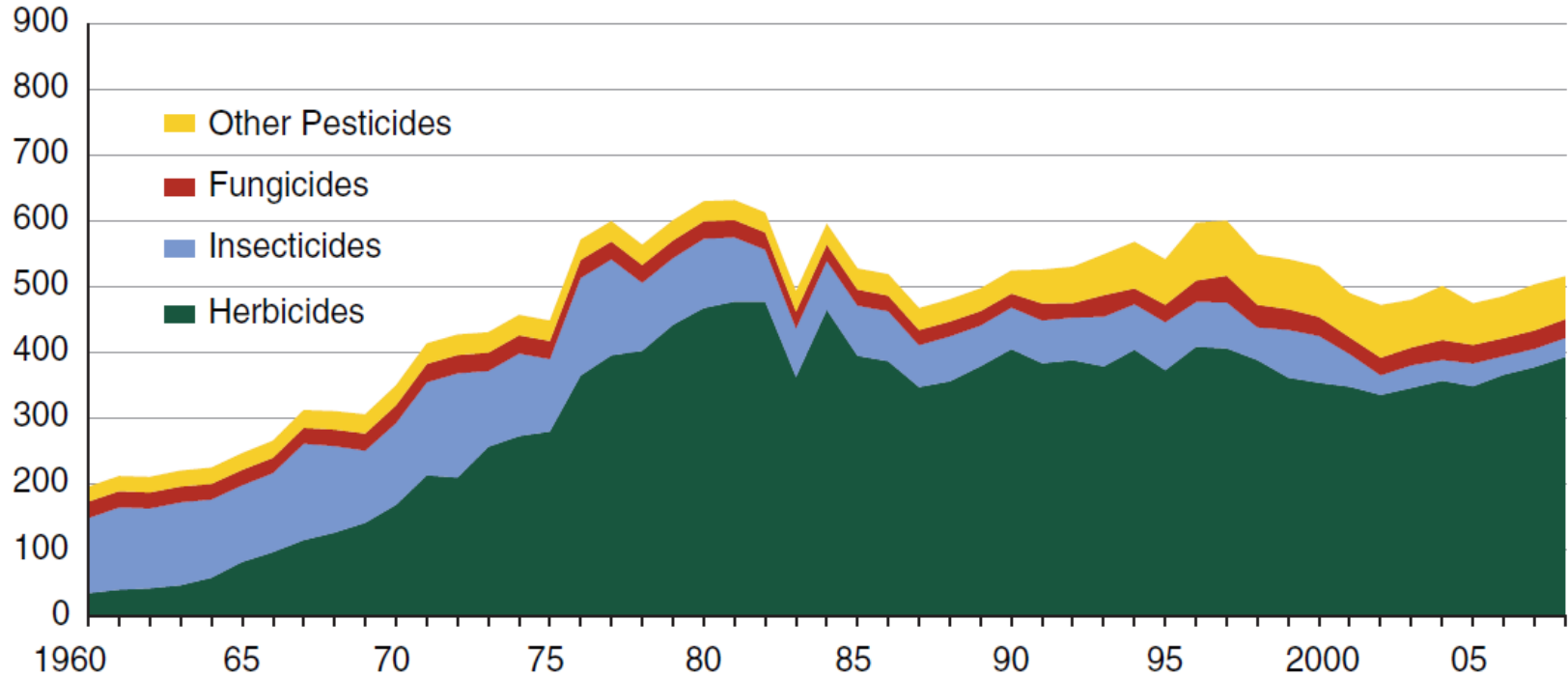
USDA testing showed residues of glyphosate and its primary metabolite AMPA in over 90% of the soybean samples tested

USDA.GOV

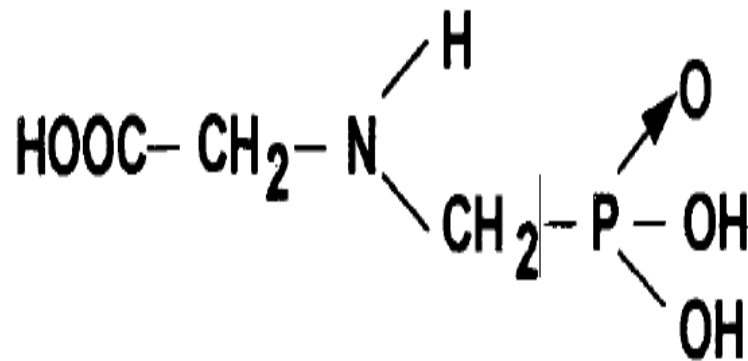
*mostly ends up in animal and poultry feed

Pesticide use in U.S. agriculture, 21 selected crops, 1960-2008

Million pounds of pesticide active ingredient

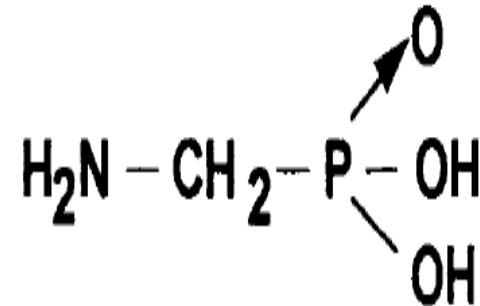


Source: Economic Research Service with USDA and proprietary data. See Appendix 2.



N-(Phosphonomethyl)glycine

GLYPHOSATE



aminomethylphosphonic acid (AMPA)

METABOLITE

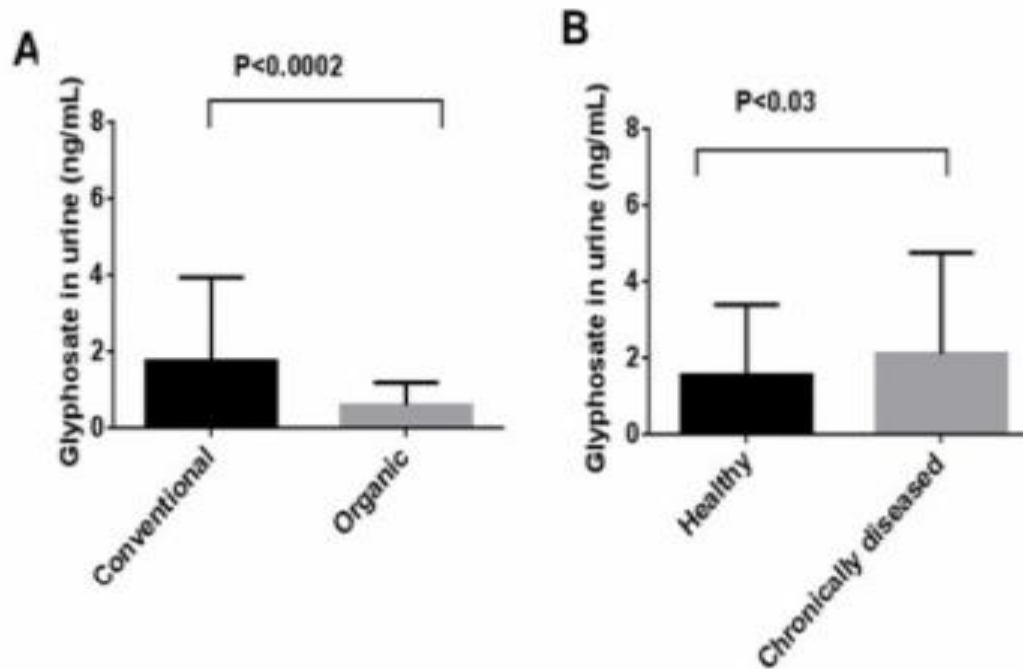




Why Is Glyphosate Sprayed on Crops Right Before Harvest?

Glyphosate, the main ingredient in [Monsanto's Roundup](#) herbicide, is recognized as the world's most widely used weed killer. What is not so well known is that farmers also use glyphosate on crops such as wheat, oats, edible beans and other crops right before harvest, raising concerns that the herbicide could get into food products.

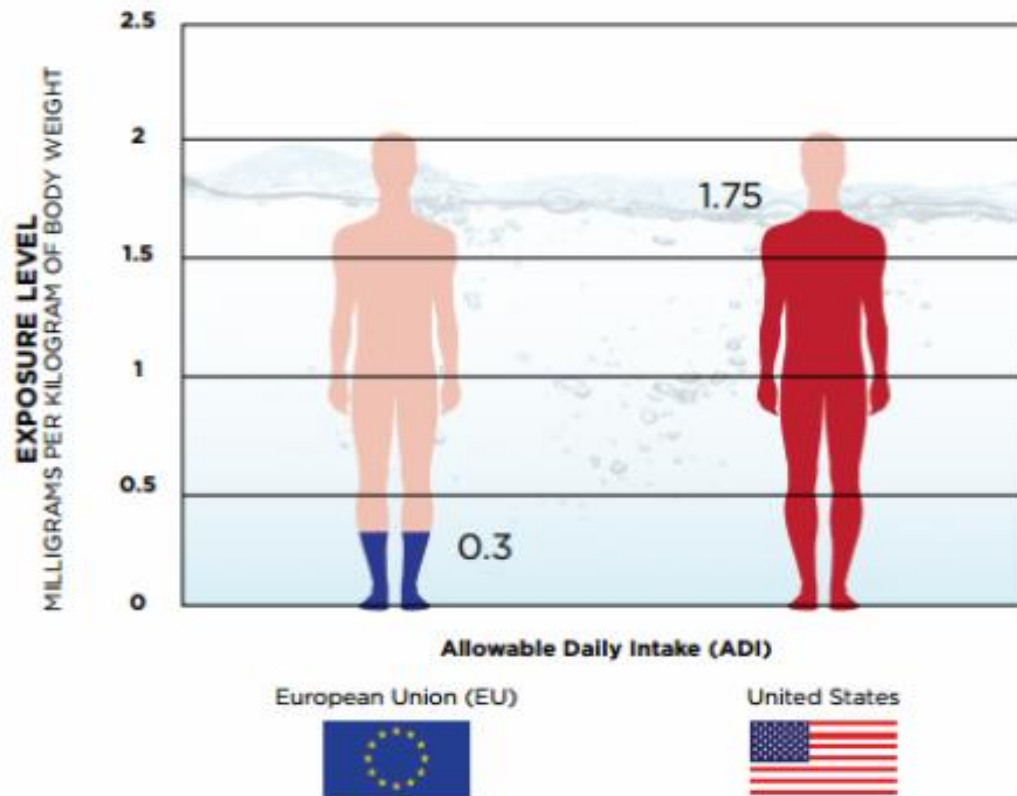
Glyphosate Exposure Levels in Humans: Healthy and Chronically Diseased



Glyphosate in humans.

- A) Comparison of glyphosate excretion with urine of humans with conventional (N=99) and predominantly organic (N=41) feeding
- B) B) Glyphosate in healthy (N=102) and chronically (N=199) diseased human (Krüger M,2014).

U.S. Government Allows Nearly 6 Times More Glyphosate in Our Food than Europe



Comparison of EU and US ADI levels of G in food.



Glyphosate/Roundup® Damage by the Numbers (ppb)

0.1 ppb: Roundup® (0.05 ppb glyphosate) altered the gene function of over 4,000 genes in the livers and kidneys of rats. (Mesnage, et al, 2015).

0.1 ppb: Roundup® (0.05 glyphosate) severe organ damage in rats (Seralini, et al, 2014).

0.1 ppb: Permitted level for glyphosate and all other herbicides in EU tap water (Uren, et al, 2015).

10 ppb: Toxic effects on the livers of fish.

700 ppb: Alterations of Kidneys and livers in rats (Larsen, et al, 2012).

700 ppb: Permitted level for glyphosate in U.S. tap water (EPA)

1,125.3 ppb (1.1253 mg/kg): Level found in General Mills Cheerios®.



Standard operating procedure in
the Ag industry is:

“Don’t ask, don’t tell”

Federal Government

US Congressman (D)
US Senator (D)
Dep Dir FDA, HFS (Bush Sr, Clinton)
White House Senior Staff (Clinton)
Sec of Commerce (Clinton)
WH CSA, Gore's SDR (Clinton)
White House Communications (Clinton)
Gore's Chief Dom Policy Adv (Clinton)
WH-Appointed Consumer Adv (Clinton)
Deputy Admin EPA (Clinton, Bush)
USDA, EPA (Clinton, Bush, Obama)
Dep Commissioner FDA (Obama)
US Sen (D), Sec of State (Obama)
Dir, USDA NIFA (Obama)
Ag Negotiator Trade Rep (Obama)

Monsanto

Toby Moffett
Dennis DeConcini
Margaret Miller
Marcia Hale
Mickey Kantor
Virginia Weldon
Josh King
David Beler
Carol Tucker-Foreman
Linda Fisher
Lidia Watrud
Michael Taylor
Hillary Clinton
Roger Beachy
Islam Siddiqui

Monsanto Consultant
Monsanto Legal Counsel
Chemical Lab Supervisor
Director, Int'l Government Affairs
Board Member
VP, Public Policy
Director, Int'l Government Affairs
VP, Government & Public Affairs
Monsanto Lobbyist
VP, Government & Public Affairs
Manager, New Technologies
VP, Public Policy
Rose Law Firm, Monsanto Counsel
Director, Monsanto Danforth Center
Monsanto Lobbyist

GEKE.US

This bureaucratic reality is graphically illustrated above.

<http://geke.us/Monsanto.001.jpg>

Regulatory Environment

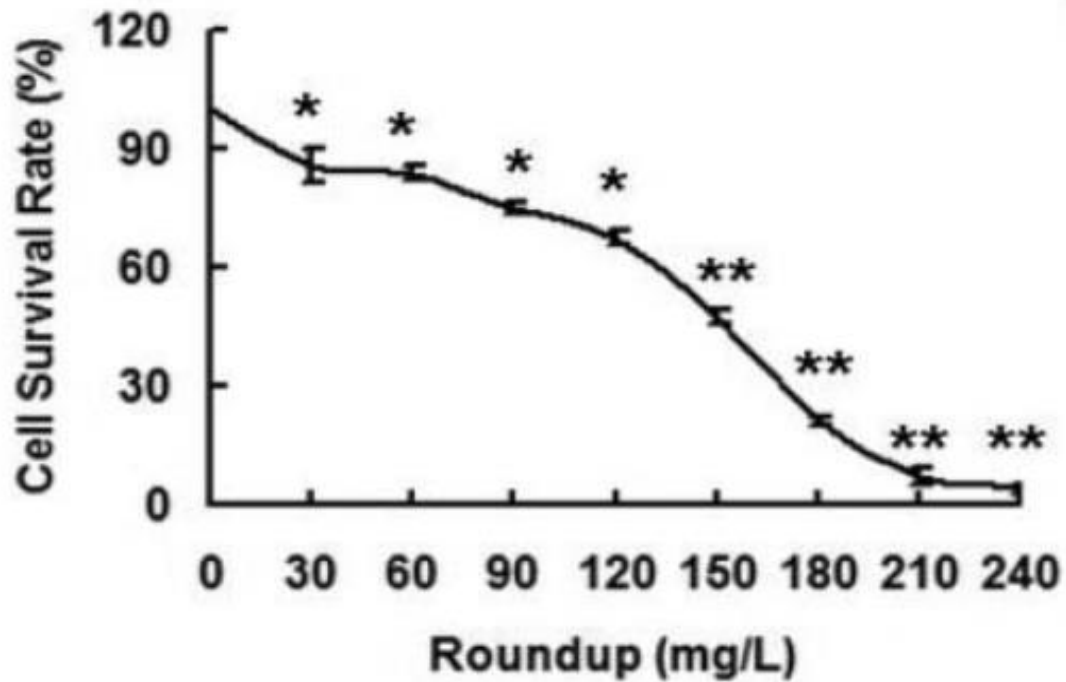
- IARC vs EFSA
- IARC- The International Agency for Research on Cancer – designated glyphosate as a probable human carcinogen.
- EFSA – The European Food Safety Authority – has suggested that there is insufficient evidence to support the IARC position.

Systemic Effects

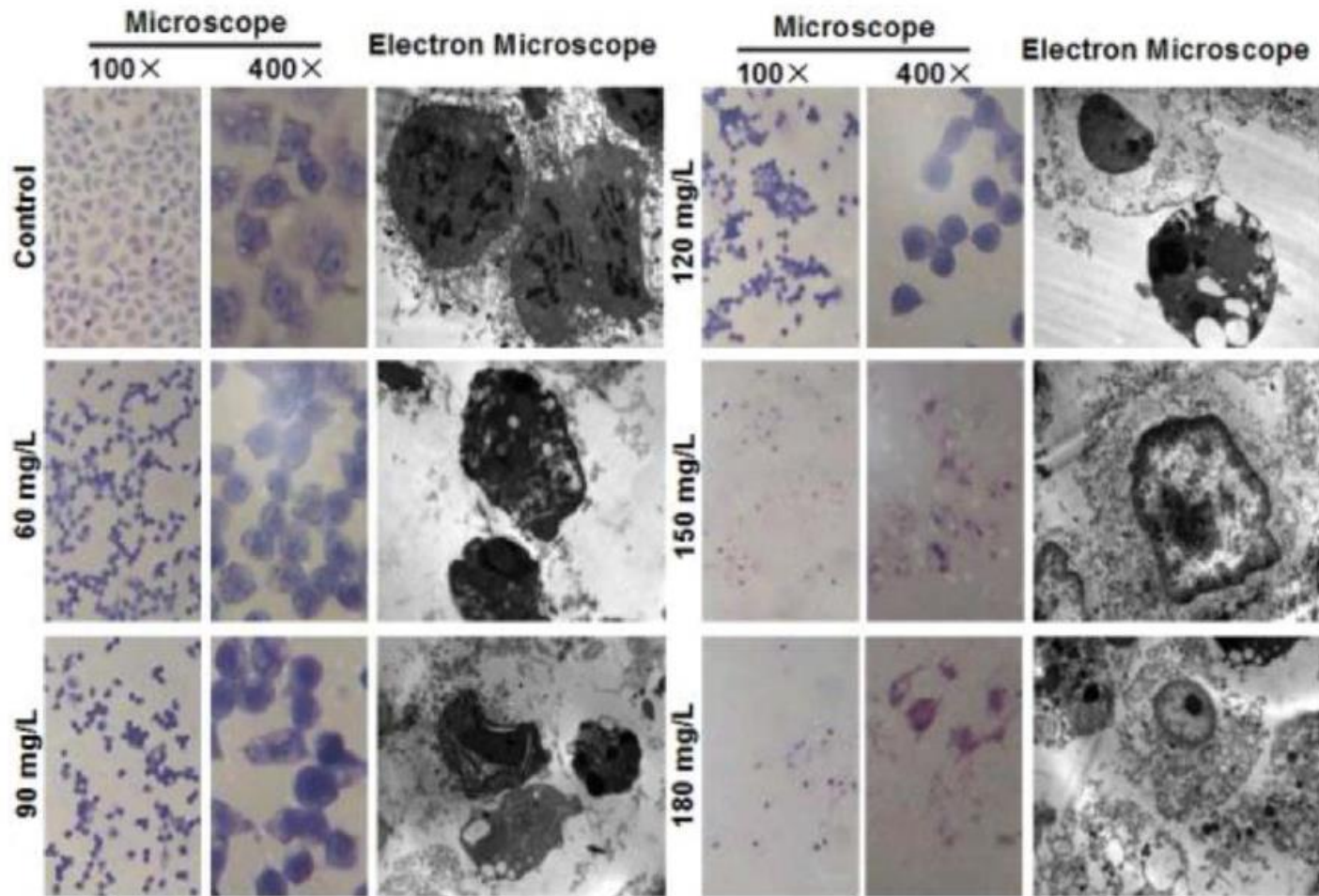
- Cancer
- Endocrine disruption
- Perturbation of the microbiome
- DNA damage
- Birth defects and reproductive problems
- Neurological disorders

Mechanisms of action

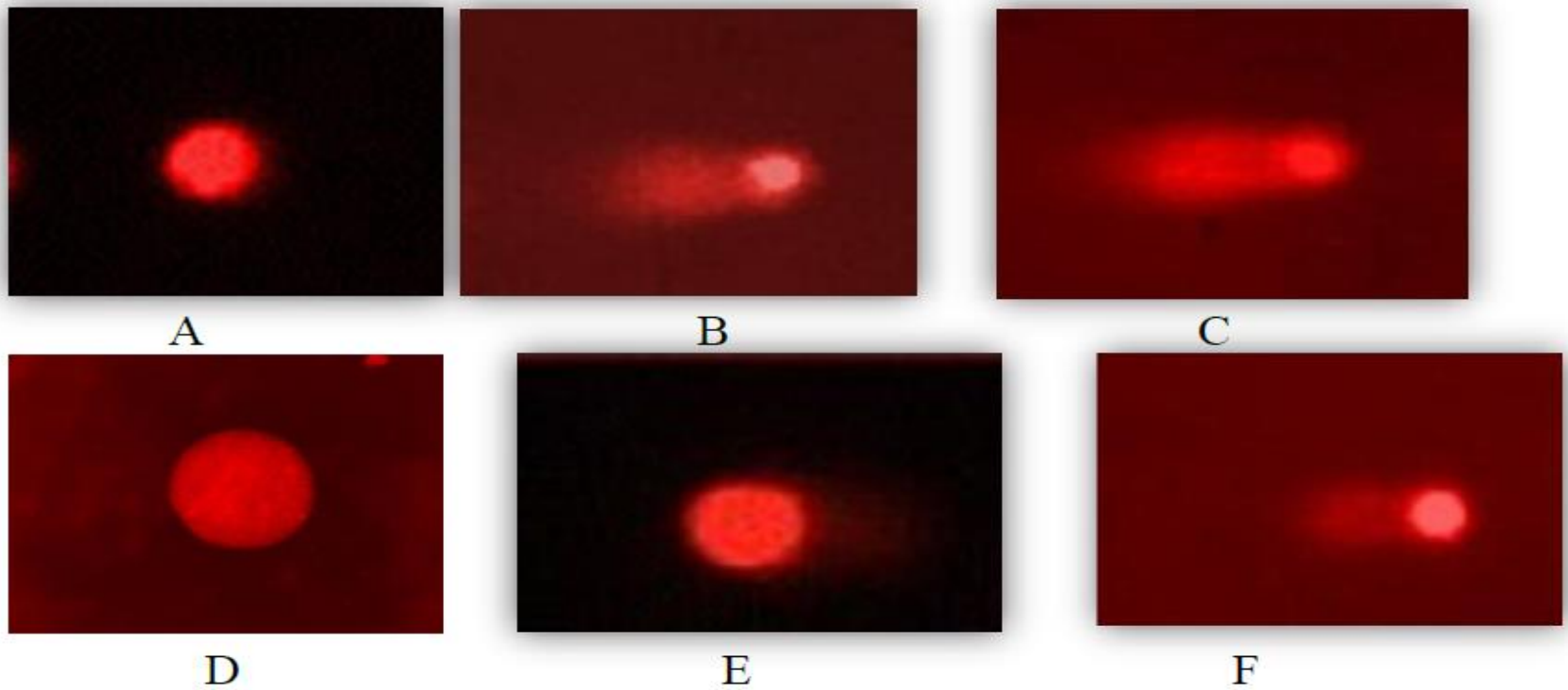
- Oxidative stress
- Chelation of metals
- Antibiotic
- Analog of glycine
- AMPA is an agonist for glutamate receptors in the brain



Inhibition of the survival rate of L-02 hepatocyte by roundup. * $P < 0.05$, ** $\bar{P} < 0.01$ compared with control.



Morphologic changes of L-02 hepatocyte induced by roundup.



Comet images by florescent microscope (400X)

(A) Undamaged DNA (control) (B) Damaged DNA (treated with normal dose of glyphosate) (C) Damaged DNA (treated with double dose of glyphosate) (D) Undamaged DNA (control "feeding") (E) Damaged DNA (treated with normal dose of glyphosate by feeding) (F) Damaged DNA (treated with double dose of glyphosate by feeding).

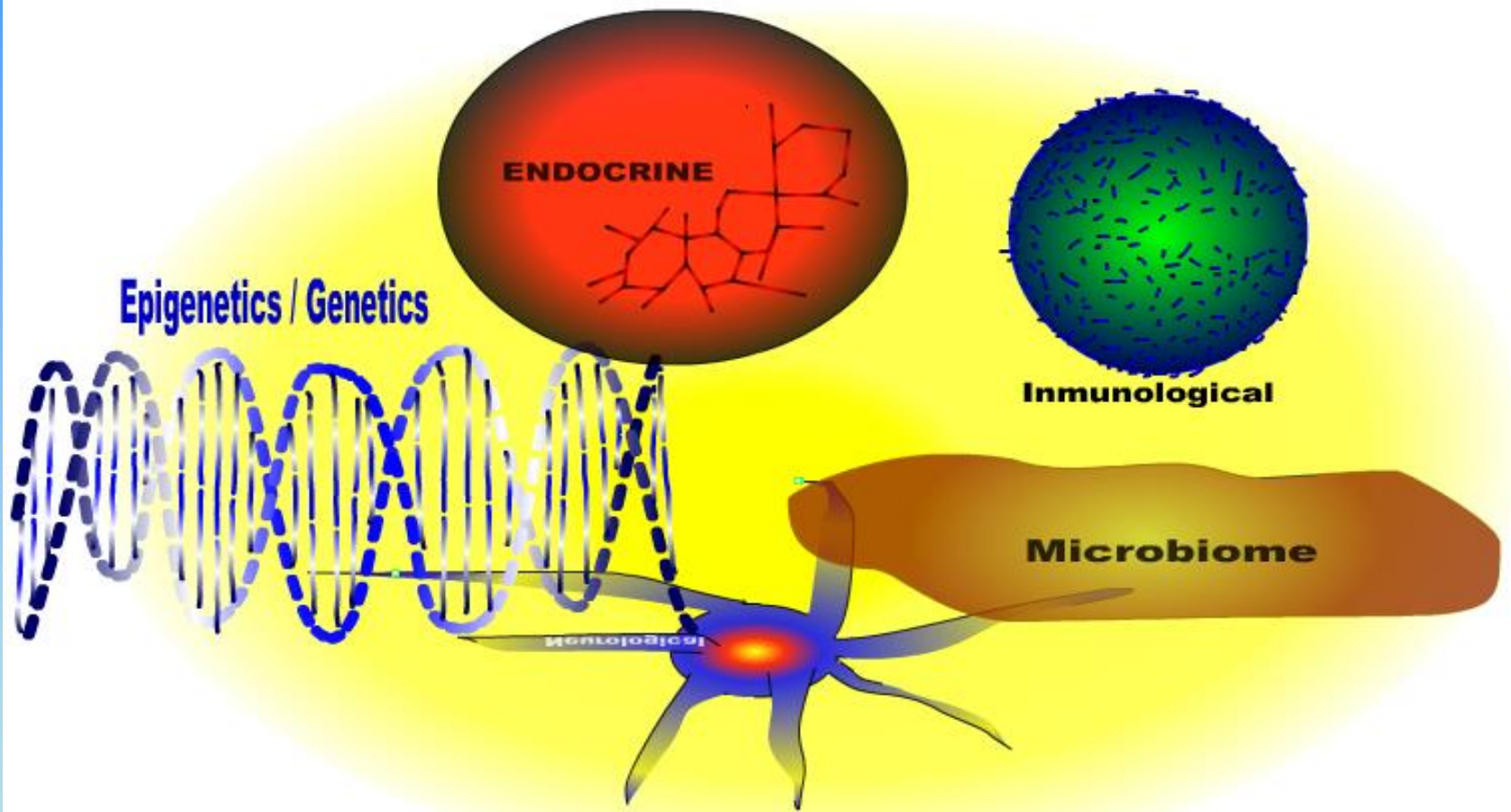
DNA Damage vs. Repair Rates

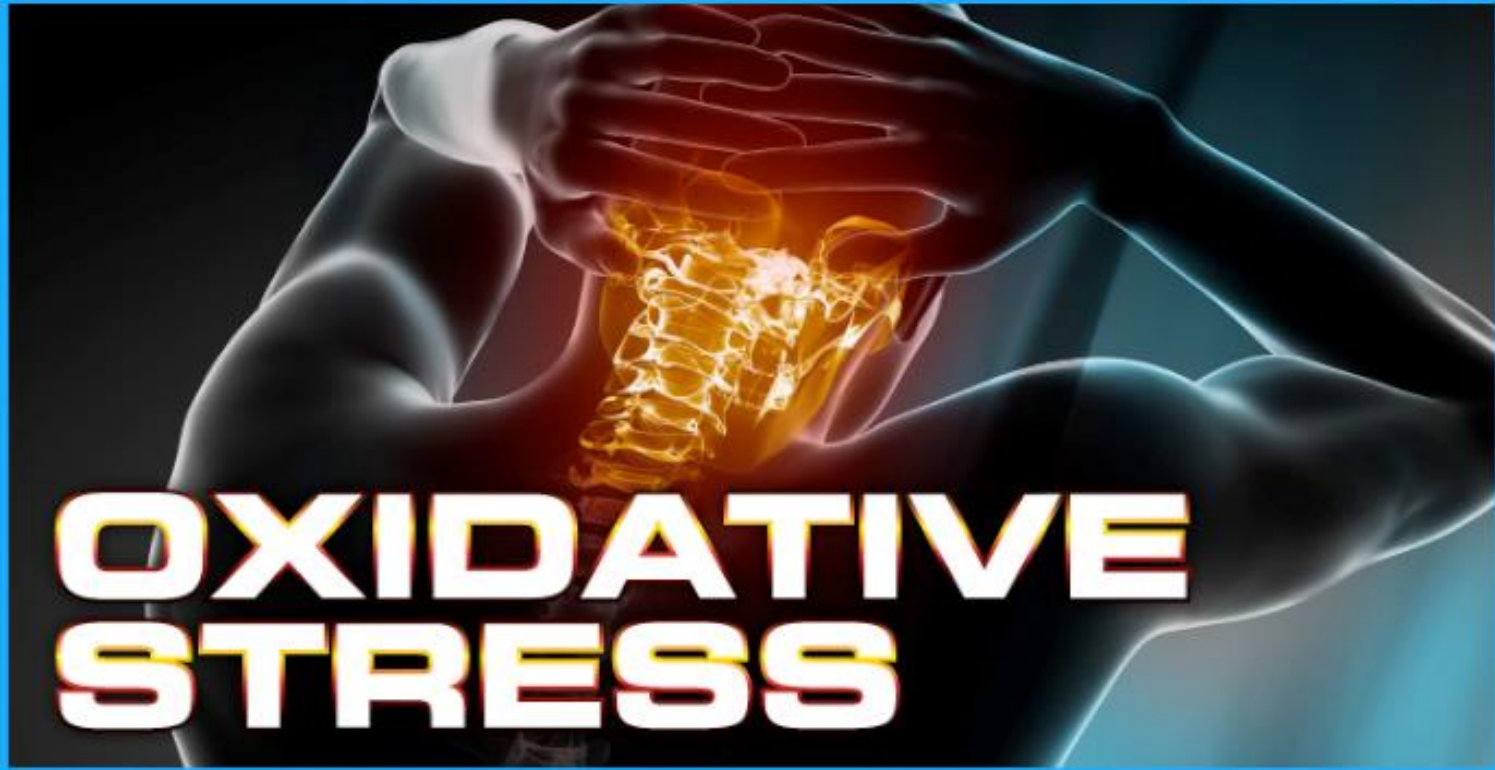
Estimates of Endogenous DNA Damage and Repair Processes in Human Cells in Vivo

TYPE OF DAMAGE	ESTIMATED OCCURRENCES OF DAMAGE PER HOUR PER CELL	MAXIMAL REPAIR RATE, BASE PAIRS PER HOUR PER CELL
Depurination	1000	10^4
Depyrimidination	55	10^4
Cytosine deamination	15	10^4
Single-stranded breaks	5000	2×10^5
<i>N</i> ⁷ -methylguanine	3500	Not reported
<i>O</i> ⁶ -methylguanine	130	10^4
Oxidation products	120	10^5

SOURCE: Modified from data from the National Academy of Science (1989).

ENERGETIC



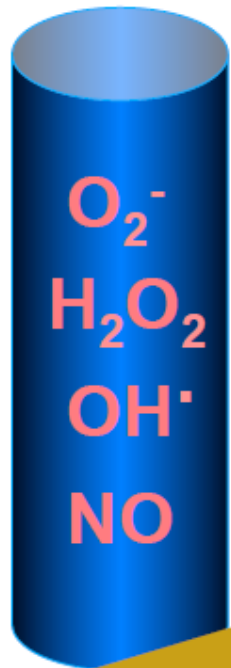


Adverse effects occur when the generation of Reactive Oxygen Species (ROS) in a system exceeds the system's ability to neutralize and eliminate them, excess ROS can damage a cell's lipids, protein or DNA.

Oxidative Stress



Mitochondria: the major energy sources for all cells of the body



Oxidants



Antioxidants

- Cellular stress
 - Energy production by mitochondria generates free radicals (oxidants)
 - As mitochondria overwork, malfunction or age, they **make more oxidants and become porous to leak oxidants**
- Defense against stress
 - Cells produce their own antioxidants (GSH)
 - As cells age, they produce **fewer antioxidants**
- Nasty imbalance: **OXIDATIVE STRESS!**

Blame Oxidative Stress



Toxicology. 2014 Jun 5;320:34-45. doi: 10.1016/j.tox.2014.03.001. Epub 2014 Mar 15.

Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: involvement of glutamate excitotoxicity.

[Cattani D1](#), [de Liz Oliveira Cavalli VL1](#), [Heinz Rieg CE1](#), [Dominques JT1](#), [Dal-Cim T1](#), [Tasca CI1](#), [Mena Barreto Silva FR1](#), [Zamoner A2](#).

Results showed that acute exposure to Roundup(®) (30min) increases (45)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 (3)H-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 (3)H-glutamate uptake and metabolism, while induced (45)Ca(2+) uptake and (14)C-MeAIB accumulation in immature rat hippocampus.

Toxicology. 2017 Jul 15;387:67-80. doi: 10.1016/j.tox.2017.06.001. Epub 2017 Jun 13.

Developmental exposure to glyphosate-based herbicide and depressive-like behavior in adult offspring: Implication of glutamate excitotoxicity and oxidative stress.

[Cattani D](#)¹, [Cesconetto PA](#)¹, [Tavares MK](#)², [Parisotto EB](#)¹, [De Oliveira PA](#)³, [Rieg CE](#)¹, [Leite MC](#)⁴, [Prediger RDS](#)³, [Wendt NC](#)², [Razzera G](#)², [Filho DW](#)⁵, [Zamoner A](#)⁶.

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 45Ca^{2+} 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- κ 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.

**Glyphosate → AMPA → Glutamate agonist →
Exitotoxicity → Adverse neurological outcome**

Degeneration of spinal motor neurons by chronic AMPA-induced excitotoxicity in vivo and protection by energy substrates

Citlalli Netzahualcoyotzi and Ricardo Tapia*

Chronic progressive excitotoxicity due to AMPA receptors overactivation results in MN death and astrogliosis, with consequent motor deficits and paralysis.

Because of the notable protection against these effects exerted by pyruvate and β HB, which are well established mitochondrial energy substrates, we conclude that deficits in mitochondrial energy metabolism are an important factor in the mechanisms of this slowly developed excitotoxic MN death, while the lack of protective effect of the antioxidants indicates that oxidative stress seems to be less significant factor. Because excitotoxicity may be involved in MN degeneration in ALS, these findings suggest possible preventive or therapeutic strategies for the disease.

Neurotoxicology. 2016 Mar;53:20-28. doi: 10.1016/j.neuro.2015.11.015. Epub 2015 Nov 26.

Exposure to a glyphosate-based herbicide during pregnancy and lactation induces neurobehavioral alterations in rat offspring.

[Gallegos CE1](#), [Bartos M2](#), [Bras C2](#), [Gumilar F2](#), [Antonelli MC3](#), [Minetti A2](#).

...Pups exposed to a **glyphosate**-based herbicide showed early onset of cliff aversion reflex and early auditory canal opening. A decrease in locomotor activity and in anxiety levels was also observed in the groups exposed to a **glyphosate**-containing herbicide. ...early **exposure** to a **glyphosate**-based herbicide affects the central nervous system in rat offspring probably by **altering mechanisms or neurotransmitter systems that regulate locomotor activity and anxiety.**

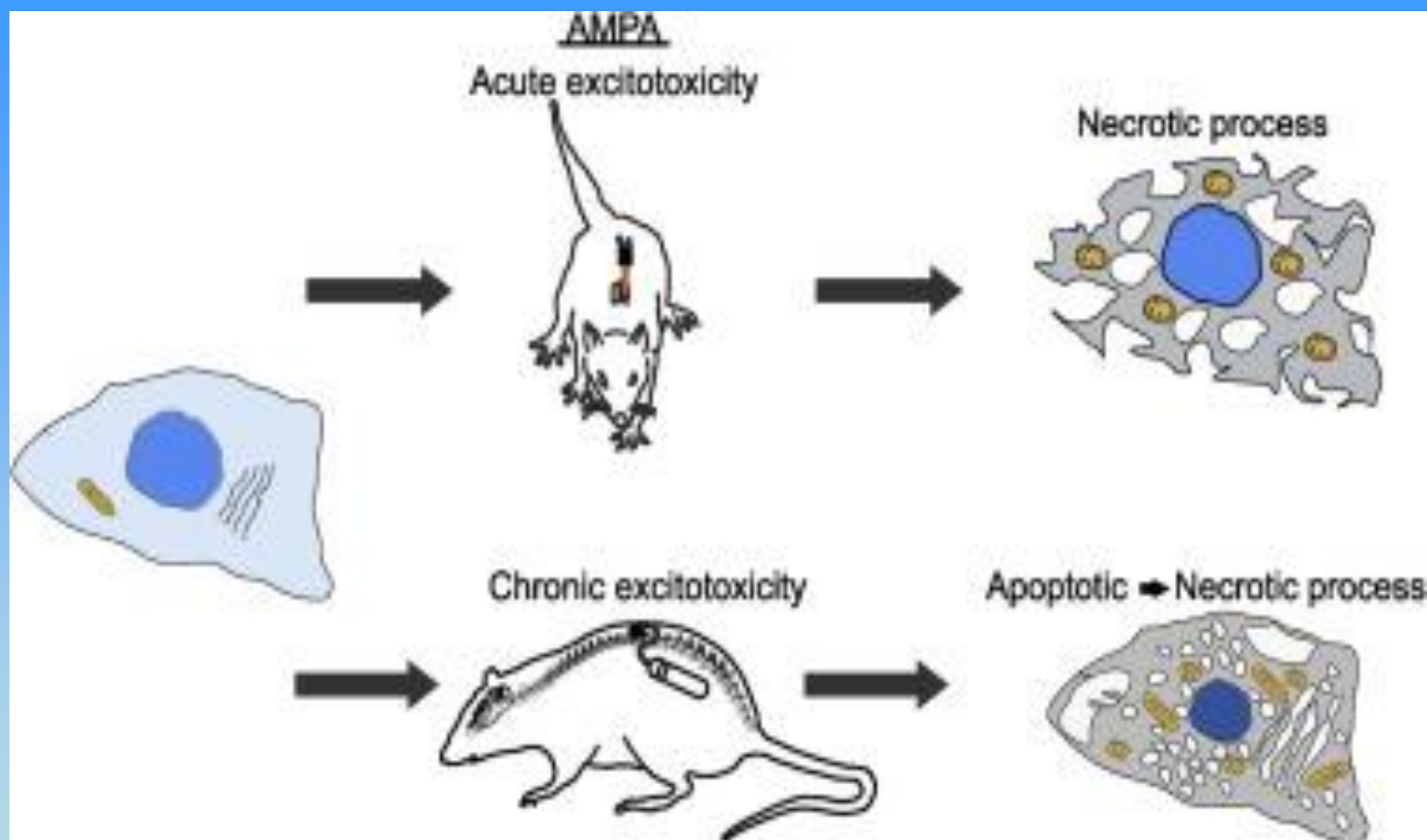
Brain Res. 2011 Apr 22;1386:1-14. doi: 10.1016/j.brainres.2011.01.059. Epub 2011 Jan 26.

Tumor necrosis factor- α (TNF- α) augments AMPA-induced Purkinje neuron toxicity.

[Bliss RM1](#), [Finckbone VL](#), [Trice J](#), [Strahlendorf H](#), [Strahlendorf J](#).

It is well recognized that **exposure** of neurons to excessive levels of the excitatory neurotransmitter glutamate, termed glutamate excitotoxicity, contributes to the damage and degeneration seen in many acute and chronic **neurological** diseases.

However, it is becoming increasingly evident that inflammation also can play a role in certain neurodegenerative diseases and inflammatory mediators, such as tumor necrosis factor- α (TNF- α), may directly interact with excitotoxic processes. In a postnatal rat cerebellar slice model, we found that **TNF- α exacerbated AMPA-induced excitotoxicity** in Purkinje neurons in a dose-dependent manner beyond the toxicity caused by **AMPA** alone. It also was shown that combinations of TNF- α and **AMPA** increased the mean intracellular activity of calpains, calcium-activated cysteine proteases that are known to contribute to cell death in Purkinje neurons.



Excitotoxicity Theory

Once glutamate binds to the receptor, Glutamate “excites” the cells by causing positive ions to flow into the cell, increasing the cell’s electrical charge.

At high concentrations the increased cellular activity caused by glutamate results in over-excitation of nerve cells, which eventually leads to cell death.

About Glutamate Toxicity

By Stephanie Liou 26 Jun, 2011 Glutamate Toxicity

Molecular Effects

- **ROS +** reactive oxygen species
- **GHS --** glutathione
- **SOD –** super oxide dismutase
- **MDA +** malondialdehyde -indicates lipid peroxidation
- **Mitochondria damage +**
- **DNA damage +**
- **Cell membrane integrity –**
- **MMP-** mitochondrial membrane potential
- **PTP +** permeability transition pore
- **p53 +** tumor suppression, apoptosis
- **AIF +** apoptosis inducing factor
- **Cyt C +** indicator of apoptosis
- **ALT/AST +** alanine/ aspartate aminotransferase
- **Apoptosis +**
- **Necrosis +**

Roundup exposure induces

- ROS accumulation and antioxidant system imbalance
- Cell membrane lipid peroxidation and permeability increase
- Cellular oxidation damage
- DNA damage/fragmentation
- Intracellular Ca² overload and mitochondrial dysfunction
- Liver pathology
- Apoptosis and necrosis

Neurotoxicol Teratol. 2018 May - Jun;67:44-49. doi: 10.1016/j.ntt.2018.04.002. Epub 2018 Apr 7.

Glyphosate based-herbicide exposure affects gut microbiota, anxiety and depression-like behaviors in mice.

[Aitbali Y1](#), [Ba-M'hamed S1](#), [Elhidar N2](#), [Nafis A2](#), [Sora N3](#), [Bennis M4](#).

Subchronic and **chronic exposure** to GBH induced an increase of anxiety and depression-like behaviors. GBH significantly altered the GM composition in terms of relative abundance and phylogenetic diversity of the key microbes.

Toxicology. 2019 Mar 1;415:18-25. doi: 10.1016/j.tox.2019.01.010. Epub 2019 Jan 22.

Learning and memory impairments associated to acetylcholinesterase inhibition and oxidative stress following glyphosate based-herbicide exposure in mice.

[Bali YA](#)¹, [Kaikai NE](#)¹, [Ba-M'hamed S](#)¹, [Bennis M](#)².

This study demonstrates that **GBH induced numerous cognitive abnormalities** referred to different forms of memory likely associated with a significant inhibition of AChE activity and oxidative stress induction.

The role of glutamate and its receptors in autism and the use of glutamate receptor antagonists in treatment

Donald C. Rojas

Department of Psychology Colorado State University

Glutamate is the major excitatory neurotransmitter in the brain and may be a key neurotransmitter involved in autism. Interest in glutamatergic dysfunction in autism is high due to increasing convergent evidence implicating the system in the disorder from peripheral biomarkers, neuroimaging, protein expression, genetics and animal models.

This review presents evidence in support of glutamate abnormalities in autism and the potential for translation into new treatments for the disorder.

Biochem Biophys Res Commun. 2018 Jul 2;501(4):838-845. doi: 10.1016/j.bbrc.2018.04.200. Epub 2018 May 19.

Circular RNA expression profiles in hippocampus from mice with perinatal glyphosate exposure.

[Yu N1](#), [Tong Y1](#), [Zhang D2](#), [Zhao S2](#), [Fan X2](#), [Wu L3](#), [Ji H4](#).

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.

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.**

Brain Res. 2015 Aug 7;1616:1-9. doi: 10.1016/j.brainres.2015.04.042. Epub 2015 May 2.

AMPA receptor activation causes preferential mitochondrial Ca²⁺ load and oxidative stress in motor neurons.

[Joshi DC](#)¹, [Tewari BP](#)¹, [Singh M](#)¹, [Joshi PG](#)¹, [Joshi NB](#)².

It is well established that motor neurons are highly vulnerable to **glutamate induced excitotoxicity**. The selective vulnerability of these neurons has been attributed to **AMPA** receptor mediated excessive rise in cytosolic calcium and consequent mitochondrial Ca(2+) loading.

ACS Chem Neurosci. 2016 Jul 20;7(7):886-96. doi: 10.1021/acschemneuro.6b00032.
Epub 2016 Apr 29.

Mitochondrial Dysfunction during the Early Stages of Excitotoxic Spinal Motor Neuron Degeneration in Vivo.

[Santa-Cruz LD1](#), [Guerrero-Castillo S1](#), [Uribe-Carvajal S1](#), [Tapia R1](#).

Glutamate **excitotoxicity** and mitochondrial **dysfunction** are involved in motor neuron degeneration process during **amyotrophic lateral sclerosis (ALS)**.

These mechanisms could be involved in ALS motor neuron degeneration.

Neurotoxicology. 2018 Dec;69:23-28. doi: 10.1016/j.neuro.2018.08.008. Epub 2018 Aug 31.

AMPA-induced extracellular Zn²⁺ influx into nigral dopaminergic neurons causes movement disorder in rats.

[Tamano H1](#), [Morioka H1](#), [Nishio R1](#), [Takeuchi A1](#), [Takeda A2](#).

...the rapid influx of extracellular Zn²⁺ into nigral dopaminergic neurons causes dopaminergic neurodegeneration...

J Comp Neurol. 2009 Oct 1;516(4):277-90. doi: 10.1002/cne.22118.

Excitotoxic motoneuron degeneration induced by glutamate receptor agonists and mitochondrial toxins in organotypic cultures of chick embryo spinal cord.

[Brunet N1](#), [Tarabal O](#), [Esquerda JE](#), [Calderó J](#).

Glutamate receptor-mediated **excitotoxicity and mitochondrial dysfunction** appear to play an important role in motoneuron (MN) degeneration in amyotrophic lateral sclerosis (ALS).

The globally used herbicide glufosinate-ammonium (GLA) is structurally analogous to the excitatory neurotransmitter glutamate, and is known to interfere with cellular mechanisms involved in the glutamatergic system.

Future research topics

Neurological effects of long term chronic
AMPA exposure

The chelating effects on zinc finger binding
proteins

Effects of herbicide/pesticide combinations

Disruption of the microbiome

Conclusions

Glyphosate and AMPA exposure have been found to be associated with multiple adverse neurological Outcomes

Residues allowed in food crops are well in excess of ppm found to have adverse effects on human health.

Given the regulatory environment and the relative lack of meaningful enforcement, personal awareness and vigilance are the best defenses.



Questions ????