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14th International HCH and Pesticides Forum

TOXICOLOGICAL EVALUATIONS OF GLYPHOSATE IN ZEBRAFISH EARLY-LIFE STAGES

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GLYPHOSATE

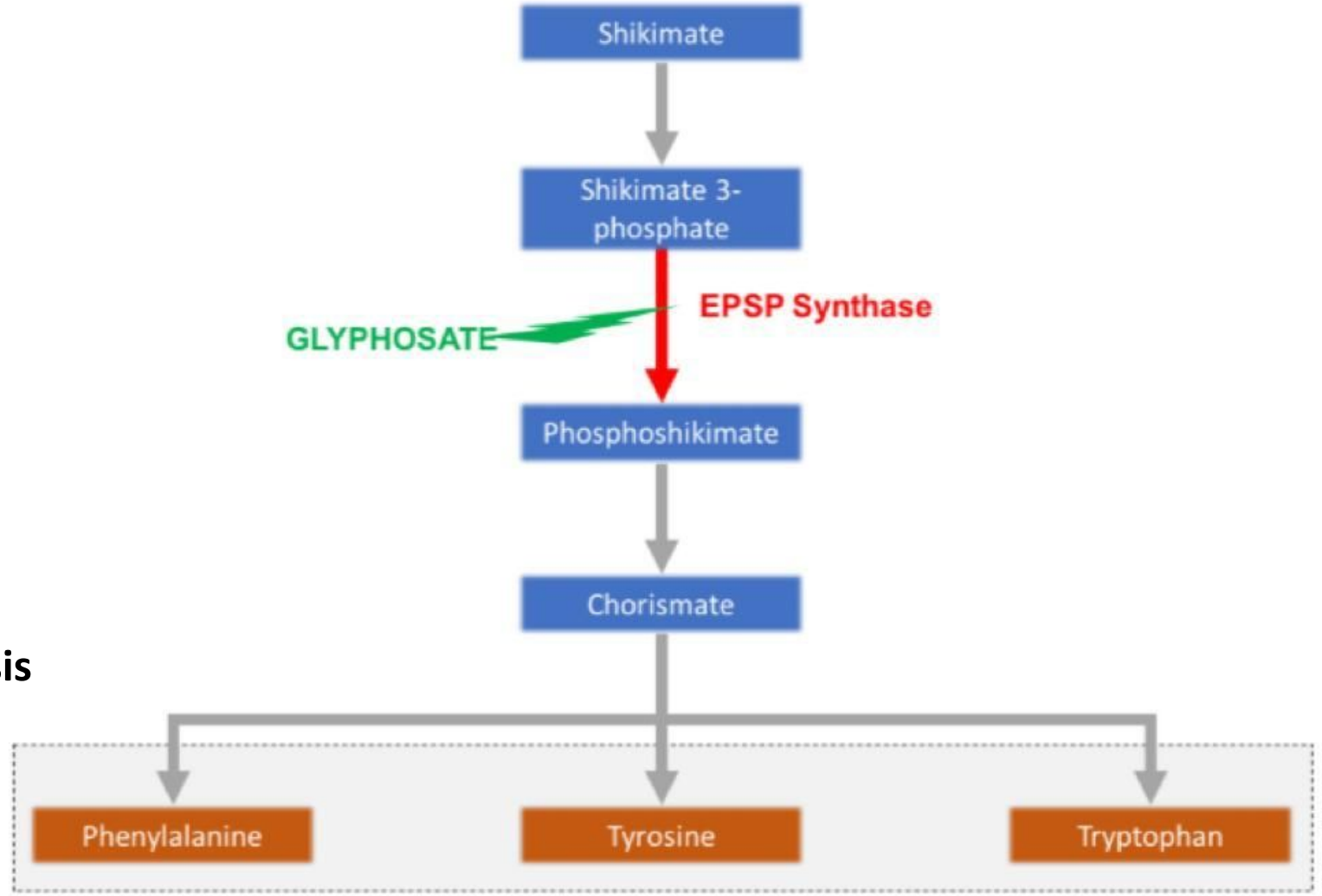
Is a systemic and non-selective post emergence foliar herbicide.

It is today known as the most widely used herbicide worldwide.

MECHANISM OF ACTION:

Glyphosate operates via 5-enolpyruvylshikimate-3-phospha te synthase (EPSPS) enzyme, <u>inactivating him.</u>

- Loss of essential amino acids
- Interferes with chlorophyll photosynthesis
- Accumulation of toxic intermediates



THE AIM OF THE STUDY

Investigate the glyphosate potential effects on the development of zebrafish early-life stages.

Embryo
- Rapid development
- Optical clarity
- Genetic malleability
- External development

Larva
- Optical clarity
- Genetic malleability
- High throughput screening

Adult
- Vertebrate neural structure
- Low cost, easy maintenance
- Genome comprises of several orthologs of human genes mutated in FAD

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MATERIALS AND METHODS:

Zebrafish early-life stages used in the experiments were obtained from the University of Teramo facility. Adult wild type AB strain zebrafish were breed in a recirculating water system with a light/dark cycle

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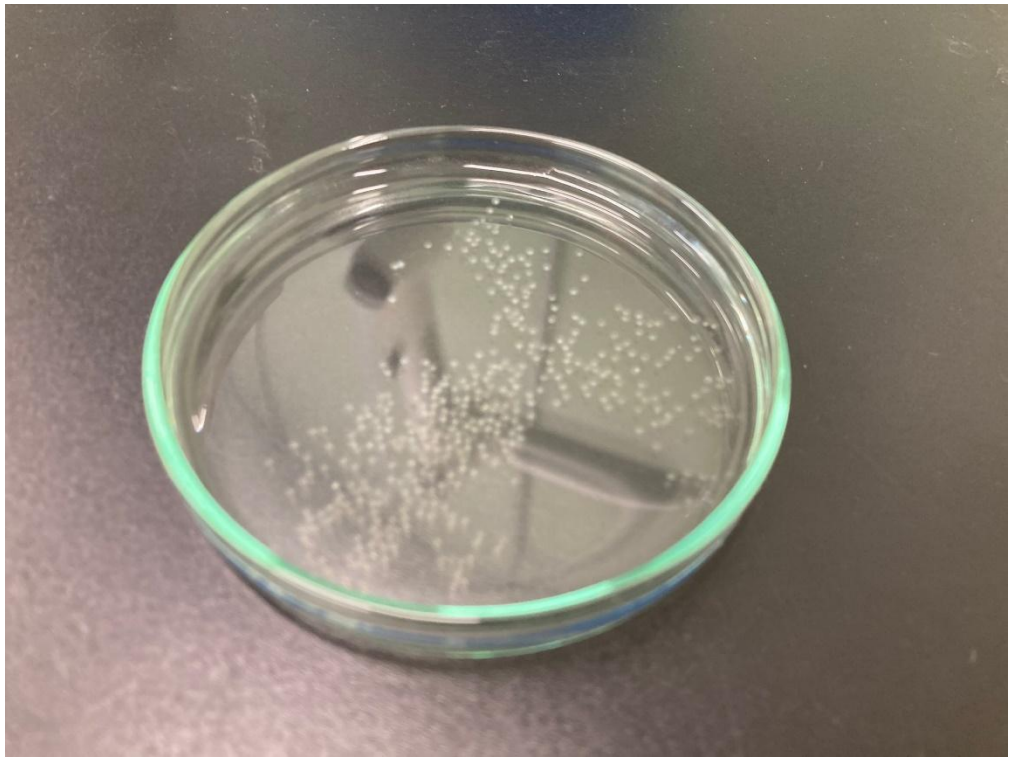
MATERIALS AND METHODS:

Breeding tanks



Collected eggs

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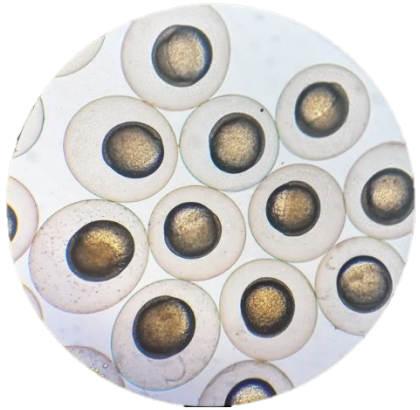
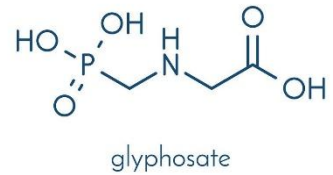
MATERIALS AND METHODS:

Fish Embryo Acute Toxicity Tests (FET)

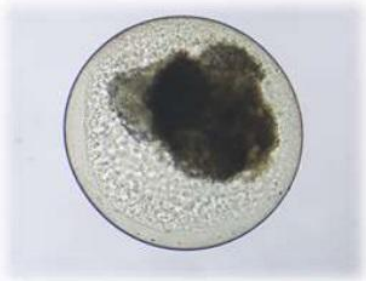
Tested concentrations: 25, 50, 100, 110, 125 mg/L
Negative control (NK) and positive control



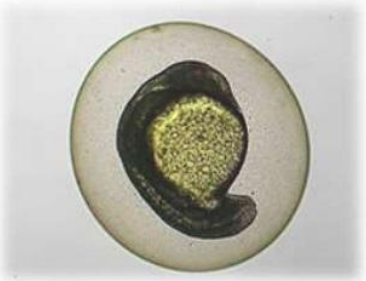


MATERIALS AND METHODS:

Zebrafish early-life stages were daily observed up to 96 h with the inverted optical microscope considering four lethal alterations:

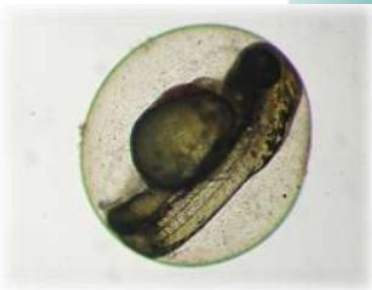


Coagulation of embryos



Lack of detachment of the tail from the yolk sac

Lack of the somite formation



Abstance of heartbeat

At the end of the exposure period were evaluated:

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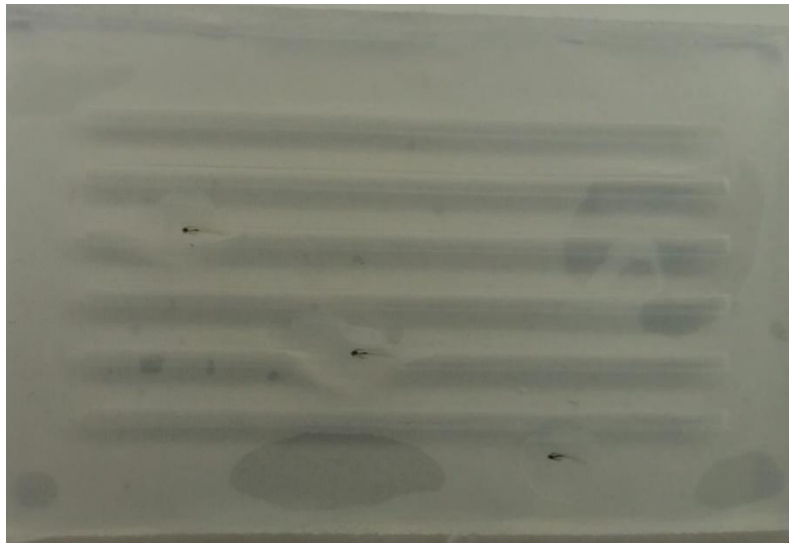
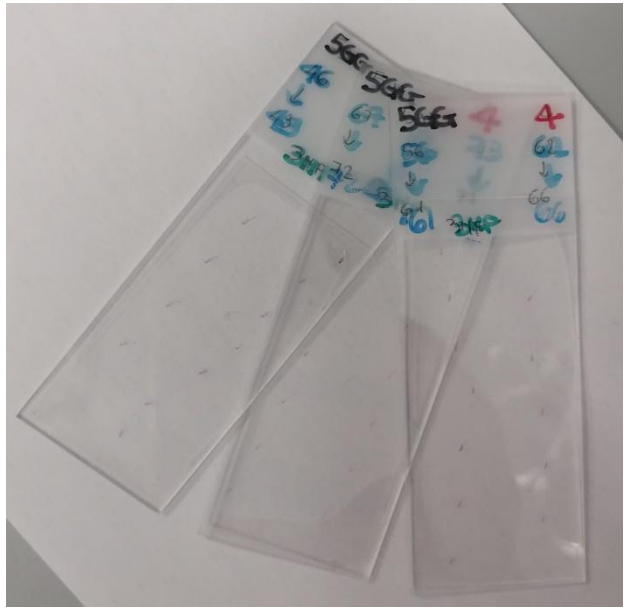
→

→

MATERIALS AND METHODS:

Histological analyses





Survived larvae at 96 hpf treated with 50, 100, 110 an 125 mg/L

→

Were fixed in 10% neutral-buffered formalin for 2h

→

5 µm thickness sections were stained with hematoxylin and eosin and then visualized by light microscope

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RESULTS:

Fish Embryo Acute Toxicity Tests (FET)

Critical Doses [mg		0-24 h	0-48 h	0-72 h	0-96 h
Survival					
	LD10	88,096	86,990	86,693	85,683
95%-CL	lower	n.d.	n.d.	n.d.	n.d.
	upper	n.d.	n.d.	n.d.	n.d.
	LD20	101,452	98,571	97,671	96,974
95%-CL	lower	n.d.	n.d.	n.d.	n.d.
	upper	n.d.	n.d.	n.d.	n.d.
	LD50	132,904	125,194	122,696	122,888
95%-CL	lower	n.d.	n.d.	n.d.	n.d.
	upper	n.d.	n.d.	n.d.	n.d.
Survival	LOED	>125,000	>125,000	>125,000	>125,000
	NOED	>=125,000	>=125,000	>=125,000	>=125,000

RESULTS:

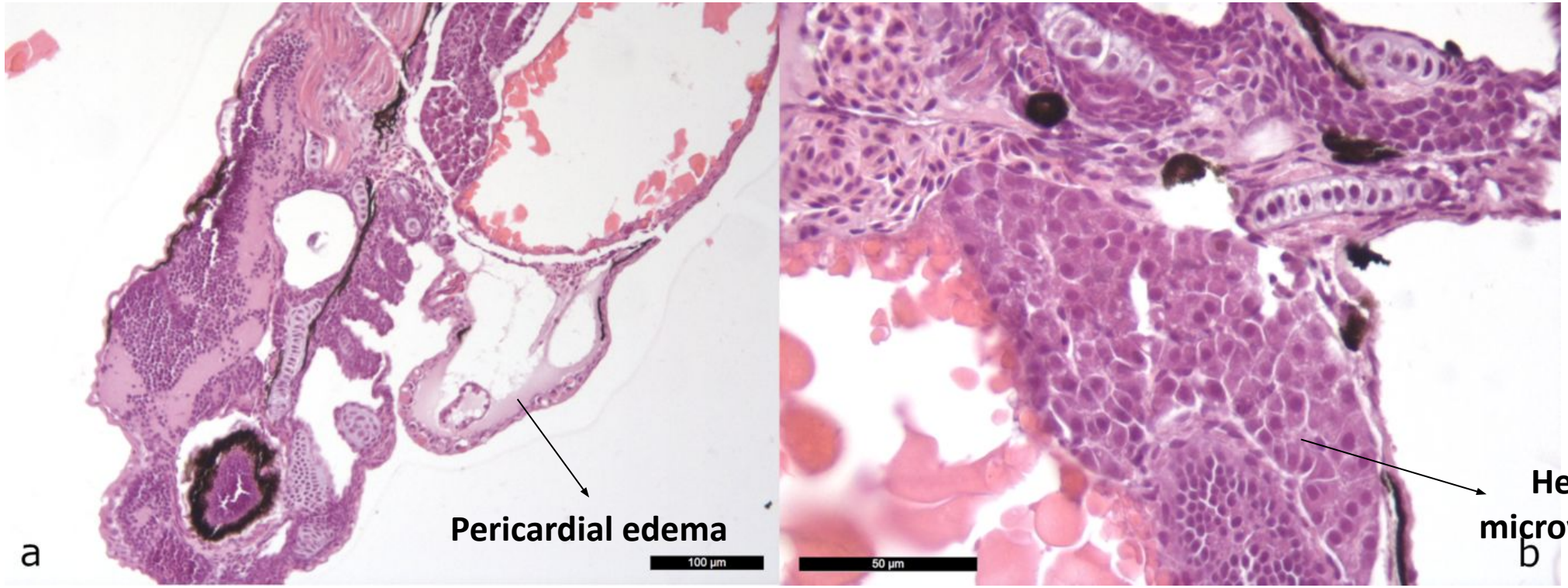
Sublethal alterations:

a-b)From the concentration of 100 mg/L at 48 hpf, the larvae showed pericardial edema and impaired blood flow and blood stasis.
In addition, a great number of zebrafish larvae showed a smaller head then NK, suggesting a possible neurological involvement

c) Zebrafish larva exposed to negative control (NK)

RESULTS:

Histological analyses



Histological observation confirmed the results of FET test in fact 80% of embryos treated with glyphosate 100 mg/ L showed high pericardial edema and diffuse hepatic microvacuoles.
In addition, changes in head size were also observed

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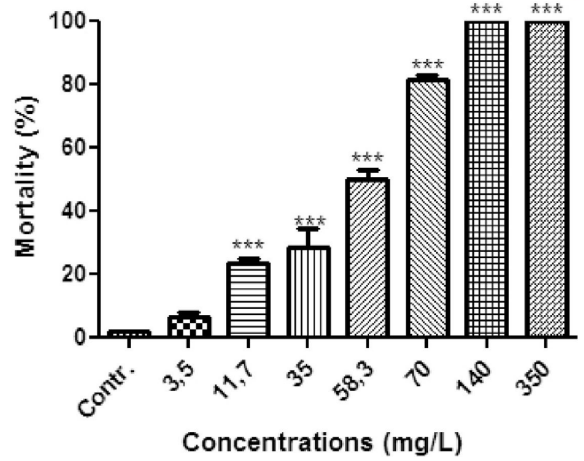
DISCUSSION:

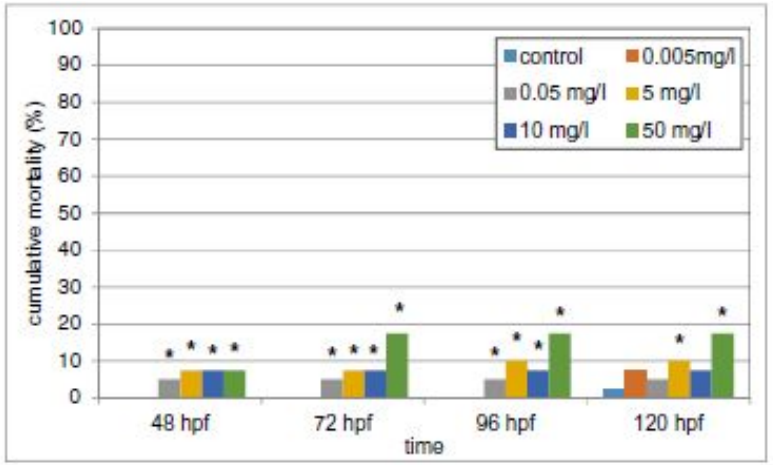
TOXICOLOGIAL ENDPOINTS

Panetto, S., Gomes, F., Fraga Gomes, D.S., Campos, Eldo., Romeiro, N.C., Costa, E.P., do Carmo, P.R.L., Feitosa, N.M., & Moraes, J. (2019). **The effects of Roundup® in embryo development and energy metabolism of the zebrafish (*Danio rerio*).** *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, volume 222*. doi:0.1016/j.cbpc.2019.04.007.



Rodrigues, L., Gonçalves Costa, G., Lundgren Tha, E., da Silva, L.R., de Oliveira, R., Morais Leme, D., Cestari, M.M., Grisolia, C.K., Campos Valadares, M., & de Oliveira, G.A.R. (2019**). Impact of the glyphosate-based commercial herbicide, its components, and its metabolite AMPA on non-target aquatic organisms**. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, volume 842.* https://doi.org/10.1016/j.mrgentox.2019.05.002

Fiorino, E., Sehonova, P., Plhalova, L., Blahova, J., Svobodova, Z., & Faggio, C. (2018). **Effects of glyphosate on early life stages: comparison between Cyprinus carpio and Danio rerio.** *Environmental science and pollution research, 25(9):8542-8549.* doi: 10.1007/s11356-017-1141-5



DISCUSSION:

CARDIOTOXICITY

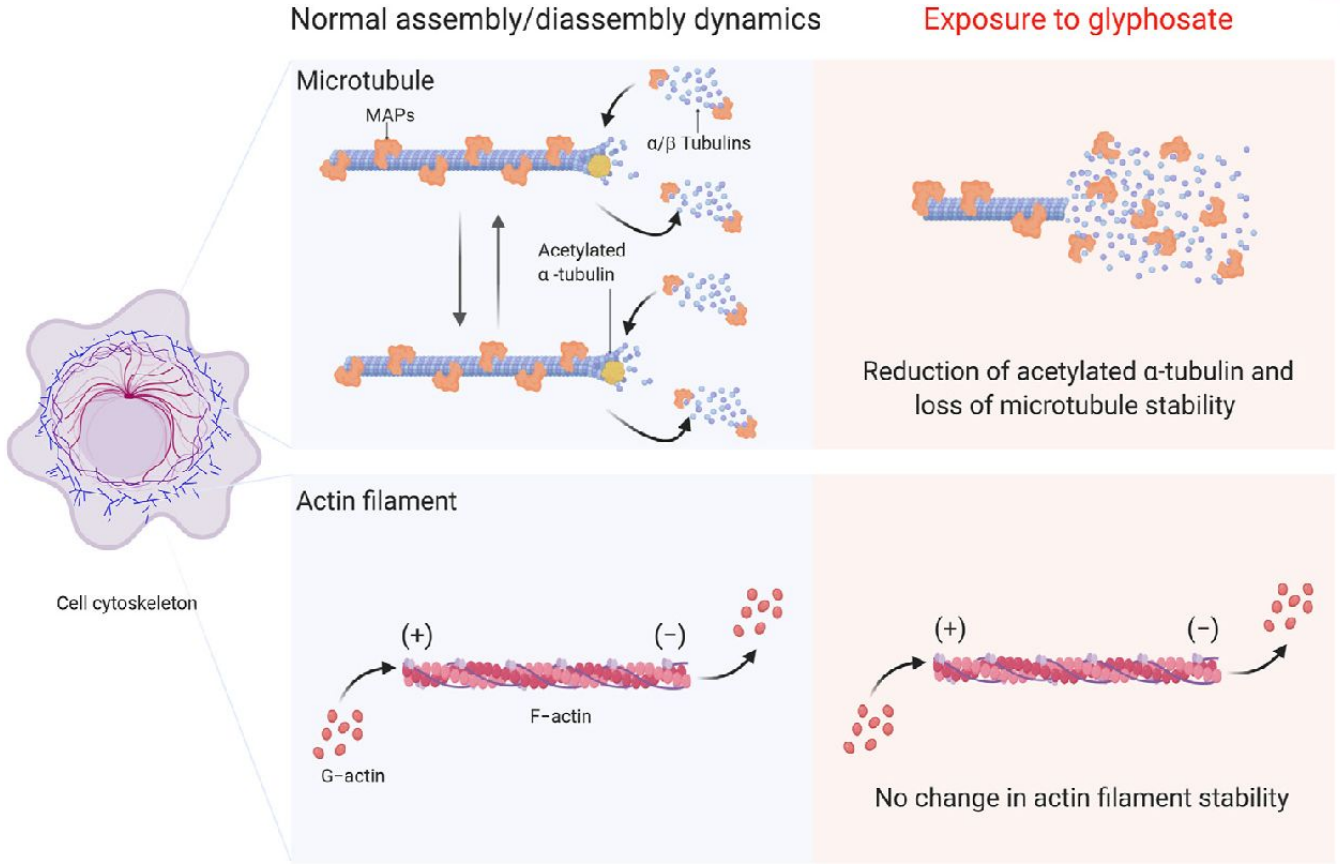
Lu, J., Wang, W., Zhang, C., Xu, W., Chen, W., Tao, L., Li, Z., Cheng, J., & Zhang, Y. (2022). **Characterization of glyphosate-induced cardiovascular toxicity and apoptosis in zebrafish**. *Science of the Total Environment, volume 851, Part 2.* https://doi.org/10.1016/j.scitotenv.2022.158308

Diaz-Martin, R.D., Valencia- Hernández, J.D., Betancourt-Lozano, M., & Yáñez-Rivera, B. (2021). **Changes in microtubule stability in zebrafish (Danio rerio) embryos after glyphosate exposure.** *Biochemistry, Molecular and Cell Biology, Volume 7, Issue 1.* https://doi.org/10.1016/j.heliyon.2021.e06027

Sulukan E., Köktürk M., Ceylan H., Beydemir Ş., Işik M., Atamanalp M., & Ceyhun S.B. (2017). **An approach to clarify the effect mechanism of glyphosate on body malformations during embryonic development of zebrafish (Daino rerio).** *Chemosphere, 180:77-85.* doi: 10.1016/j.chemosphere.2017.04.018.

ood flow

Dysregulation myocyte enhancer factor-2 (MEF2)



DISCUSSION:

NEUROTOXICITY

Roy, N. M., Carneiro, B., & Ochs, J. (2016). **Glyphosate induces neurotoxicity in zebrafish.** *Environmental Toxicology and Pharmacology, 42, 45–54.* https://doi.org/10.1016/j.etap.2016.01.003

Diaz-Martin, R.D., Carvajal-Peraza, A., Yáñez-Rivera, B., & Betancourt-Lozano, M. (2021). **Short exposure to glyphosate induces locomotor, craniofacial, and bone disorders in zebrafish (*Danio rerio*) embryos.** *Environmental Toxicology and Pharmacology, volume 87.* https://doi.org/10.1016/j.etap.2021.103700

Faria, M., Bedrossiantz, J., Ramirez Rosas, J.R., Mayol, M., Garcia Heredia, G., Bellot, M., Prats, E., Reyero-Garcia, N., Gomez-Canela, C., Gomez-Olivan, L.M., & Raldua D. (2021). **Glyphosate targets fish monoaminergic systems leading to oxidative stress and anxiety.** *Environment International, volume 146.* https://doi.org/10.1016/j.envint.2020.106253

Reduction of essential genes for the normal development of the central nervous system, such as pax2, pax6, Otx2, ephA4

CONCLUSION:

Does Glyphosate Affect the Human Microbiota?

Future studies

Long-terms effects of glyphosate

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THANK YOU FOR YOUR ATTENTION

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