



**Pesticide
Action
Network**
Europe

Brussels, 14 April 2022

Pesticide Action Network Europe's comment on the "Harmonised classification and labelling targeted consultations" of the substance glyphosate by ECHA.

We would like to focus on the "Aquatic Chronic 2" classification. Some of the studies submitted in the second round of consultation, sponsored by Bayer, Monsanto and Syngenta focusing on aquatic plants are outdated. Apart from studies on aquatic plants there are several independent fish and crustacean studies supporting the 'Aquatic Chronic 2, H411' classification. Some studies even indicate the need of 'Aquatic Chronic 1' classification of glyphosate.

Independent scientists often did not had an access to pure glyphosate substance, and they used different glyphosate based herbicide products with adjuvants. However glyphosate is not effective without surfactant adjuvant, such co-formulants are always being used in the products. Several of these studies showed that most of the surfactants applied in the glyphosate based herbicides makes the GBH product harmful to fish, crustacean and algae/aquatic plants.

We would like to drive your attention to 53 independent studies, published since 2010 investigating the effect of pure glyphosate or GBH products on fish and crustacean species.

Since industry submitted studies from the 1990s we consider ECHA should give priority to more state of the art studies.

Furthermore we identified 22 studies underlying that glyphosate and glyphosate based herbicides harm amphibians, especially tadpoles of amphibians in aquatic environment, at field-relevant concentrations.

From beforehand, thank you for your consideration.

Best regards,

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Studies on fish and crustacean species

Title	Author, source	Species	Substance (Glyphosate, GBH, etc.)	Summary/main findings	link
Effects of low-concentration glyphosate and aminomethyl phosphonic acid on zebrafish embryo development	Zhang Et. Al., Ecotoxicol Environ Saf. 2021	Zebrafish embryos	GLY and AMPA	With increasing exposure dose, heart rates of both embryos and larvae showed a rising trend and obvious arrhythmia appeared. Defects in cardiac development and function of zebrafish juveniles may be related to altered transcription levels of cardiac development genes (TBX5, NKX2.5, BMP4) and apoptosis genes (Bcl-2, Bax). In addition, pericardial edema and bone deformation of zebrafish embryos may be caused by inhibition of Na ⁺ /K ⁺ -ATPase and Ca ²⁺ -ATPase after exposure to GLY and AMPA. The present results demonstrated that at typical environmental residual concentrations of GLY and AMPA had similar developmental toxicity in zebrafish embryos.	https://pubmed.ncbi.nlm.nih.gov/34619474/
Acute toxicity and morphology alterations of glyphosate-based herbicides to <i>Daphnia magna</i> and <i>Cyclops vicinus</i>	Gustinasi Et Al. , Toxicol Res. 2020 Jul	aquatic environment / <i>Daphnia magna</i> and <i>Cyclops vicinus</i>	GBHs	"The mortality data indicated that GBHs at higher concentration had a detrimental effect on the survival of <i>D. magna</i> and <i>C. vicinus</i> ." - "There were no significant differences of the alteration of spin length, body length, and head length of <i>D. magna</i> to exposure of GBHs, except the head width. While body length alteration of <i>C. vicinus</i> was significantly different towards the increase in concentration."	https://www.researchgate.net/publication/343238510_Acute_toxicity_and_morphology_alterations_of_glyphosate-based_herbicides_to_Daphnia_magna_and_Cyclops_vicinus
Low Concentrations of Glyphosate-Based Herbicide Affects the Development of <i>Chironomus xanthus</i>	Ferreira-Junior Et. AL., Water, Air, & Soil Pollution, 2017	<i>Chironomus xanthus</i>	GLY	48 h LC50 for glyphosate to <i>C. xanthus</i> was 251.5 mg a.e./L. Larval growth of <i>C. xanthus</i> was reduced under glyphosate exposure. Low concentrations of glyphosate caused delayed emergence of females (at 1.53 mg/L) and induced fast emergence of males (at 0.49 mg/L), compared to control treatment. The deleterious effects of environmental relevant concentrations of glyphosate (0.7 mg/L) observed in terms of <i>C. xanthus</i> growth and development suggest that glyphosate-based herbicides can have negative consequences for aquatic non-target invertebrates such as <i>Chironomus</i> .	https://link.springer.com/article/10.1007%2Fs11270-017-3536-9
Sub-lethal effects of a glyphosate-based commercial formulation and adjuvants on juvenile oysters (<i>Crassostrea gigas</i>) exposed for 35 days	Séguin Et. Al., Marine Pollution Bulletin, 2017	juvenile oysters (<i>Crassostrea gigas</i>)	Roundup® and POEAs	Both pollutants had harmful effects on shell but not on flesh growth and tissues. However, when comparing the biomarker's temporal variations, some different patterns (e.g. condition index, reproduction, parameters of oxidative stress) were observed depending on the molecules and concentrations. These results suggest that a longer exposure to an environmental concentration (0.1 µg L ⁻¹) of REX and POEAs could induce harmful effects on oysters.	https://www.sciencedirect.com/science/article/abs/pii/S0025326X1730142X?via%3Dihub

Effects of glyphosate-based herbicides on embryo-larval development and metamorphosis in the Pacific oyster, <i>Crassostrea gigas</i>	Mottier Et. Al., Aquatic Toxicology, 2013	Pacific oyster, <i>Crassostrea gigas</i>	Roundup Express® (REX) and Roundup Allées et Terrasses® (RAT)	Above a threshold, which varied according to the chemical used, the gradient of herbicide concentrations correlated with a gradient of severity of abnormality ranging from normal larvae to arrested development (an "old embryo" stage). The EC50 values were 28,315 and 40,617 µg L ⁻¹ for glyphosate and its metabolite, respectively, but much lowered values of 1133 and 1675 µg L ⁻¹ for REX and RAT, respectively. Metamorphosis tests also revealed a significant difference between molecules, as the EC50 values exceeded 100,000 µg L ⁻¹ for glyphosate and AMPA but were as low as 6366 and 6060 µg L ⁻¹ for the commercial formulations, which appeared relatively more toxic.	https://www.sciencedirect.com/science/article/abs/pii/S0166445X12003219?via%3Dihub
In vivo and in vitro effects of the herbicide Roundup® on developmental stages of the trematode <i>Echinostoma paraensei</i>	Monte Et. Al. Experimental Parasitology, 2016	<i>Echinostoma paraensei</i>	Roundup®	There was a significant difference in the hatching miracidia rate only for the newly embryonated eggs. The mortality of specimens and excystment rate of metacercariae were concentration-dependent. There was a significant difference in the miracidia mortality with respect to concentration until 56.3 mg/L. The same effect was observed for cercariae, and mortality was observed from 15 min onwards at concentrations of 225–900 mg/L. At low concentrations, mortality was detected after 30 min. The effects of the herbicide concentration on NEL and on helminths at seven and fourteen days showed a significant difference after 24 h. All developmental stages of the trematode <i>E. paraensei</i> were affected by Roundup® exposure under experimental conditions.	https://www.sciencedirect.com/science/article/abs/pii/S0014489416301345?via%3Dihub
Effects of Glyphosate and its Formulation, Roundup, on Reproduction in Zebrafish (<i>Danio rerio</i>)	Webster Et. Al., Environ. Sci. Technol. 2014,	Zebrafish (<i>Danio rerio</i>)	glyphosate & Roundup	Both 10 mg/L Roundup and glyphosate increased early stage embryo mortalities and premature hatching. Transcript profiling of the gonads revealed 10 mg/L Roundup and glyphosate induced changes in the expression of <i>cyp19a1</i> and <i>esr1</i> in the ovary and <i>hsd3b2</i> , <i>cat</i> , and <i>sod1</i> in the testis.	https://pubs.acs.org/doi/10.1021/es404258h
Effect of glyphosate on the sperm quality of zebrafish <i>Danio rerio</i>	Evaluation of the genotoxic, mutagenic, and histopathological hepatic effects of polyoxyethylene amine (POEA) and glyphosate on <i>Dendropsophus minutus</i> tadpoles	Zebrafish (<i>Danio rerio</i>)	Gly	Glyphosate causes damage on sperm membrane and DNA. Reduction of mitochondrial functionality and sperm motility was observed. Glyphosate would reduce <i>D. rerio</i> male fertility.	https://www.sciencedirect.com/science/article/abs/pii/S0166445X14002422?via%3Dihub
Changes in Ultrastructure and Expression of Steroidogenic Factor-1 in Ovaries of Zebrafish <i>Danio rerio</i> Exposed to Glyphosate	Armiliato Et. Al., Journal of Toxicology and Environmental Health, 2014	Zebrafish (<i>Danio rerio</i>)	GLY	A significant increase in diameter of oocytes was observed after exposure to glyphosate. When ovarian ultrastructure was examined the presence of concentric membranes, appearing as myelin-like structures, associated with the external membranes of mitochondria and with yolk granules was found. After glyphosate exposure, immunohistochemistry and immunoblotting revealed greater expression of SF-1 in the oocytes, which suggests a relationship between oocyte growth and SF-1 expression. These subtle	https://www.tandfonline.com/doi/abs/10.1080/15287394.2014.880393

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				adverse effects of glyphosate on oocytes raised a potential concern for fish reproduction.	
Ovarian growth impairment after chronic exposure to Roundup Ultramax® in the estuarine crab <i>Neohelice granulata</i>	Canosa Et. Al., Environmental Science and Pollution Research, 2018	estuarine crab (<i>Neohelice granulata</i>)	Roundup Ultramax®	At both concentrations tested (same as those used in vivo), a decrease in the ovarian vitellogenin content was observed, whereas the ovarian protein synthesis was significantly inhibited by glyphosate at 0.2 mg/L in the Roundup formulation used.	https://link.springer.com/article/10.1007%2Fs11356-017-0581-2
Effects of Glyphosate on Somatic and Ovarian Growth in the Estuarine Crab <i>Neohelice granulata</i> , During the Pre-Reproductive Period	Avigliano Et. Al., Water, Air, & Soil Pollution, 2018	Estuarine crab (<i>Neohelice granulata</i>)	Gly	A decrease in the body weight gain was observed by effect of pure glyphosate, at all concentrations assayed. The obtained results indicate that glyphosate is able to harm, in the studied species, both somatic and the ovarian growth.	https://link.springer.com/article/10.1007%2Fs11270-018-3698-0
A fluorescence-based hydrolytic enzyme activity assay for quantifying toxic effects of Roundup® to <i>Daphnia magna</i>	Ørsted & Roslev, Environmental Toxicology, 2015	<i>Daphnia magna</i>	Roundup®	Exposure of <i>D. magna</i> to lethal and sublethal concentrations of Roundup resulted in loss of whole body enzyme activity and release of cell constituents, including enzymes and DNA. Roundup caused comparable inhibition of mobility and alkaline phosphatase activity with median effective concentration values at 20 °C of 8.7 mg active ingredient (a.i.)/L to 11.7 mg a.i./L. Inhibition of alkaline phosphatase activity by Roundup was lowest at 14 °C and greater at 20 °C and 26 °C.	https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.2997
Effects of glyphosate on immune responses and haemocyte DNA damage of Chinese mitten crab, <i>Eriocheir sinensis</i>	Hong Et. Al., Fish & Shellfish Immunology, 2017	Chinese mitten crab, <i>Eriocheir sinensis</i>	Gly	Glyphosate could induce different levels of immune inhibition and haemocyte DNA damage by different concentration of exposure. The THC, phagocytic activity, Comet ratio, %DNA in tail, and β-GD activity, were considered more sensitive biomarkers.	https://www.sciencedirect.com/science/article/abs/pii/S1050464817305818?via%3Dihub
Impact of chronic exposure of rainbow trout, <i>Oncorhynchus mykiss</i> , to low doses of glyphosate or glyphosate-based herbicides	Du-Carrée Et. Al., Aquatic Toxicology, 2020	rainbow trout, <i>Oncorhynchus mykiss</i>	Gly & Roundup Innovert® & Viaglif Jardin®	Exposure were analyzed over a period of 4 months covering spawning. Significant changes were observed two months before spawning with a 70% decrease of the proportion of macrophages in trout exposed to Viaglif only and a reduction of 35% of the phagocytic activity in fish exposed to the two GBHs. Trends towards lower levels of expression of tumor necrosis factor-α (between 38% and 66%) were detected one month after the spawning for all contaminated conditions but without being statistically significant.	https://enx-mol.com/paper/article/1332169134617829376?recommendPaper=1449652889145212928
Immunological and metabolic effects of acute sublethal exposure to glyphosate or glyphosate-based herbicides on juvenile rainbow	Du-Carrée Et. Al., Science of the Total Environment, 2020	rainbow trout, <i>Oncorhynchus mykiss</i>	Gly & Roundup Innovert® & Viaglif Jardin®	Hematological analysis revealed significant increases of 30% for RBCC for Roundup at S1, and of 22% for WBCC at S2.	https://enx-mol.com/paper/article/1384732444079448064?recommendPaper=1449652889145212928

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trout, Oncorhynchus mykiss					
Clone- and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in Daphnia magna	Cuhra Et. Al., Ecotoxicology, 2012	Daphnia magna	glyphosate & Roundup	Roundup showed slightly lower acute toxicity than glyphosate IPA alone. Significant reduction of juvenile size was observed even in the lowest test concentrations of 0.05 mg a.i./l, for both glyphosate and Roundup. D. magna was adversely affected by a near 100 % abortion rate of eggs and embryonic stages at 1.35 mg a.i./l of Roundup. The results indicate that aquatic invertebrate ecology can be adversely affected by relevant ambient concentrations of this major herbicide. We conclude that glyphosate and Roundup toxicity to aquatic invertebrates have been underestimated and that current European Commission and US EPA toxicity classification of these chemicals need to be revised.	https://link.springer.com/content/pdf/10.1007%2Fs10646-012-1021-1.pdf
Gender-specific histopathological response in guppies Poecilia reticulata exposed to glyphosate or its metabolite aminomethylphosphonic acid	Antunes Et. Al., Applied Toxicology, 2017	Guppies, Poecilia reticulata	Gly & AMPA	Both fish organs exposed to sublethal concentrations of GLY (35 mg l ⁻¹) and AMPA (82 mg l ⁻¹) for 96 h showed a tissue- and gender-specific histopathological response. The liver response was different between the genders. The hepatic inflammatory changes were more common in males. The increase in the area of hepatocyte vacuoles is gender dependent with higher values in the male compared to the female guppies exposed to GLY and AMPA. Multiparametric analysis indicated that the male guppies are more sensitive than females, particularly in the presence of AMPA.	https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/jat.3461 https://www.academia.edu/33209787/Gender-specific-histopathological-response-in-guppies-Poecilia-reticulata-exposed-to-glyphosate-or-its-metabolite-aminomethylphosphonic-acid?email_work_card=view-paper
Effects of glyphosate on early life stages: comparison between Cyprinus carpio and Danio rerio	Fiorino, Environmental Science and Pollution Research, 2018	common carp (Cyprinus carpio) and zebrafish (Danio rerio)	Gly	Hatching retardation (p < 0.05) was observed in experimental groups of common carp exposed to glyphosate with significant statistical difference especially at the highest concentration after 72, 96, and 120 hpf. The significantly highest cumulative mortality at concentration of 50 mg/l was observed. In contrast, hatching stimulation was observed in embryos of zebrafish exposed to the highest concentration of glyphosate. The significantly highest cumulative mortality for zebrafish was observed only at concentration of 50 mg/l. Based on our results, early life stages of common carp are more sensitive in comparison to zebrafish to the toxic action of glyphosate.	https://www.researchgate.net/publication/322321008_Effects_of_glyphosate_on_early_life_stages_comparison_between_Cyprinus_carpio_and_Danio_rerio
An approach to clarify the effect mechanism of glyphosate on body malformations during embryonic development of zebrafish (Danio rerio)	Sulkan Et. Al., Chemosphere, 2017	zebrafish (Danio rerio)	Gly	Glyphosate treatment inhibited CA activity, caused production of ROS especially branchial regions, triggered cellular apoptosis and caused several types of malformations including pericardial edema, yolk sac edema, spinal curvature and body malformation in a dose-dependent manner.	https://www.sciencedirect.com/science/article/abs/pii/S0045653517305416?via%3Dihub

Global transcriptomic profiling demonstrates induction of oxidative stress and of compensatory cellular stress responses in brown trout exposed to glyphosate and Roundup	Webster & Santos, BMC Genomics, 2015	brown trout (Salmo trutta)	Gly & Roundup	Both glyphosate and Roundup caused many variations in the complex interacting signaling pathways that control cellular stress response particularly in apoptosis. It was found that at all the three test concentrations of Roundup and at the lowest concentration of glyphosate there was a common mechanism of toxicity and cellular response. Also both these herbicides increase cell proliferation and cellular turnover and an up-regulation of metabolic processes	https://www.researchgate.net/publication/271648808_Global_transcriptomic_profiling_demonstrates_induction_of_oxidative_stress_and_of_compensatory_cellular_stress_responses_in_brown_trout_exposed_to_glyphosate_and_Roundup
Glyphosate as an acetylcholinesterase inhibitor in Cnesterodon decemmaculatus	Menéndez-Helman et al., Bull Environ Contam Toxicol. 2012	Cnesterodon decemmaculatus	Gly	Significant inhibitory effect on AChE activity was recorded even for the lowest herbicide concentration tested (1 mg L ⁻¹), in the homogenates corresponding to the anterior body section. The inhibition ranged from 23 to 36%.	https://pubmed.ncbi.nlm.nih.gov/22002176/
Exposure to different glyphosate formulations on the oxidative and histological status of Rhamdia quelen	Murussi et al. Fish Physiol Biochem, 2016	silver catfish (Rhamdia quelen)	three different formulations of glyphosate 48% (Orium®, Original® and Biocarp®)	Thiobarbituric acid-reactive substances were found to increase and the amount of catalase produced in the liver was decreased in all the treatments at all the test concentrations. Superoxide dismutase activity was increased at 2.5 mg/L concentration of Orium® and Original®. Its activity was also found to increase at 5 mg/L concentration of Orium® and Biocarp®. Glutathione-S-transferase activity was increased at 2.5 mg/L concentration of Orium® and decreased at the same concentration of Biocarp® in comparison with the control. Analysis of plasma also recorded certain alterations in the enzymatic processes of the fish. Alanine aminotransferase was decreased after exposure of fish to 2.5 mg/L concentration of Biocarp®. Similarly the amount of aspartate aminotransferase increased at 2.5 mg/L of Orium® and Original® and 5.0 mg/L of Biocarp® in comparison with the control. Histopathological studies on the liver of fish showed certain changes in hepatic tissue. vacuolization, leukocyte infiltration, the degradation of cytoplasm and melanomacrophage were noticed in the hepatocytes of the fish	https://pubmed.ncbi.nlm.nih.gov/26508170/
Metabolic and Behavior Changes in Surubim Acutely Exposed to a Glyphosate-Based Herbicide	Sinhorin et al., Arch Environ Contam Toxicol, 2014	hybrid fish surubim	Roundup Original	Glyphosate exposure altered the glucose level in the plasma of the fish. It reduced the level of glucose in the plasma, but increased it in the liver of the fish. Also the lactate level in both plasma and liver was increased, but it gets reduced in the muscles. Protein and glycogen levels were decreased in both plasma and muscles. Cholesterol was also decreased in the plasma of the fish at all the test concentrations. Apart from the metabolic alterations, certain changes were also noticed in the enzymatic activity of the fish. Alanineaminotransferase was found to increase in the plasma, but no significant change was noticed in the case of aspartate aminotransferase levels. Certain behavioral changes were also noticed in the fish. The ventilatory frequency was increased after glyphosate exposure for 5 min, but it finally gets decreased after 96-h exposure. Also the swimming activity of the fish was altered at the test concentration of 7.5 mg/L	https://www.researchgate.net/publication/264986108_Metabolic_and_Behavior_Changes_in_Surubim_Acutely_Exposed_to_a_Glyphosate-Based_Herbicide

Toxicological Responses of <i>Cyprinus carpio</i> Exposed to a Commercial Formulation Containing Glyphosate	Cattaneo et al. Bull Environ Contam Toxicol, 2011	<i>Cyprinus carpio</i>	Roundup®	The activity of AChE was inhibited in the brain and in the muscle after exposure. However, after recovery period brain and muscle AChE activity increased. Brain thiobarbituric acid reactive species (TBARS) were measured as an indicator of oxidative stress. Increased TBARS levels were observed with all concentrations tested of the glyphosate formulation, and remained increased after the recovery period.	https://link.springer.com/article/10.1007/s2Fs00128-011-0396-7
Mutagenicity and genotoxicity in gill erythrocyte cells of <i>Poecilia reticulata</i> exposed to a glyphosate formulation	De Souza Filho et al. Bull Environ Contam Toxicol 2013	<i>Poecilia reticulata</i>	Roundup Transorb®	The number of micronucleus and comets had increased in the gill erythrocyte cells. This indicates that herbicide's exposure resulted in the increase in the number of damaged cells.	https://link.springer.com/article/10.1007/s00128-013-1103-7
Exposure to a Commercial Glyphosate Formulation (Roundup®) Alters Normal Gill and Liver Histology and Affects Male Sexual Activity of <i>Jenynsia multidentata</i> (Anablepidae, Cyprinodontiformes)	Hued et al. Archives of Environmental Contamination and Toxicology, 2011	<i>Jenynsia multidentata</i>	Roundup	Roundup induced different histological alterations in a concentration-dependent manner. In subchronic-exposure tests, Roundup also altered normal histology of the studied organs and caused a significant decrease in the number of copulations and mating success in male fish exposed to the herbicide.	https://www.researchgate.net/publication/51194040_Exposure_to_a_Commercial_Glyphosate_Formulation_RoundupR_Alters_Normal_Gill_and_Liver_Histology_and_Affects_Male_Sexual_Activity_of_Jenynsia_multidentata_Anablepidae_Cyprinodontiformes
Microplastics modify the toxicity of glyphosate on <i>Daphnia magna</i>	Zocchi et al. <i>Science of Total Environment</i> 2019	<i>Daphnia magna</i>	Glyphosate acid, Roundup Gran, Glyphosate-IPA	The mortality in the Roundup Gran formulation also increased when combined with the two microplastics, though the effect was less pronounced. In all experiments, the effect of the treatments and time was significant, though there was no significant interaction between them. In most treatments, negative effects were not observed after 48h or later. The change in toxicity of the glyphosate formulations caused by microplastics can be linked to the different sorption properties of the glyphosate-based chemicals formulations. The outcome of this study highlights that beside the potential direct negative effects of microplastics, they can modify the toxicity of pollutants, such as herbicides.	https://www.researchgate.net/publication/335490413_Microplastics_modify_the_toxicity_of_glyphosate_on_Daphnia_magna
The Effect of Cypermethrin, Chlorpyrifos, and Glyphosate Active ingredients and formulation on <i>Daphnia magna</i> (Straus)	Demetrio et al. Bulletin of Environmental Contamination and Toxicology 2014	<i>Daphnia magna</i>	Roundup Max, glyph active ingredient	The results of toxicity tests with <i>D. magna</i> (LC1 through LC99) for F (expressed as a.i.) and a.i.. The following order of potency was detected: chlorpyrifos F> chlorpyrifos a.i.>cypermethrin F> cypermethrin a.i.> glyphosate F>glyphosate a.i. Three to five orders of magnitude differences between the potencies (µg/L to mg/L) of insecticides and the herbicide for both F and a.i. were observed. The difference between insecticides and the herbicide can be explained by their distinct modes of action and the test organism.	https://pubmed.ncbi.nlm.nih.gov/25056515/

Multigenerational Toxic Effects on <i>Daphnia magna</i> Induced by Silver Nanoparticles and Glyphosate Mixture	Silva <i>et al.</i> Environmental Toxicology 2020	<i>Daphnia magna</i>	gly-acid, Roundup Max	The acute toxicity results obtained for the organisms exposed to Gly and AgNP. For the organisms exposed to AgNP the average EC50 value was 0.1799 (0.1682 to 0.1924) µg/L. However, Gly produced effects on the age at first brood at 14.86 mg/L, with the hatching of eggs two days later. Also, Gly presented serious adverse effects when considering reproduction and from the lowest (2.92 mg/L) to the highest (14.86 mg/L) concentration tested there were statistically significant decreases when compared with the control (p<0.05). Studies involving Gly have demonstrated that at Roundup concentrations of 0.45 mg/L, the microcrustacean <i>D. magna</i> shows a reduction in fertility, an increase in the egg abortion rate of neonates with morphological changes and a reduction in the size of released neonates, probably caused by genotoxic effects that may affect the viability of progenies	https://setac.onlinelibrary.wiley.com/doi/abs/10.1002/etc.4952
Effect of Glyphosate Herbicide on Some Hematological and Biochemical Parameters in <i>Carassius auratus</i> L	Dumitru <i>et al.</i> Revista de Chimie, 2019	<i>Carassius auratus</i> L	gly-acid	Literature on the field suggests that glyphosate, considered to be non-toxic to humans and animals so far, can bioaccumulate in animal organisms leading to intoxication, various degrees of disease and types of multiple diseases. Moreover, the excessive use of glyphosate-based pesticides in agriculture has led to the disruption of the health of the aquatic environment, the increase in the bioaccumulation rate being due to the high solubility of this compound in water.	https://www.revistadechimie.ro/pdf/32%20DUMITRU%20G%202%2019.pdf
Toxic effects of chlorpyrifos, cypermethrin and glyphosate on the non-target organism <i>Selenastrum capricornutum</i> (Chlorophyta)	Fernández <i>et al.</i> Anais da Academia Brasileira de Ciências 2021	Algae (<i>Selenastrum capricornutum</i>)	Roundup	After 48 h all tested concentrations of the three pesticides reduced significantly the population growth. The 96 h effective concentration (EC50) was 15.60 mg/l for glyphosate. Cells exposed to the three pesticides showed an increase in the cellular size related to the increase in pesticide concentration and exposure time. Regarding to glyphosate, we found an EC50 of 15.6 mg L ⁻¹ for the commercial formulation Roundup®.	https://pubmed.ncbi.nlm.nih.gov/34378759/
Evaluation of Behavioral Changes and Tissue Damages in Common Carp (<i>Cyprinus carpio</i>) after Exposure to the Herbicide Glyphosate	Yalsuyi <i>et al.</i> Vet Sci . 2021	common carp (<i>Cyprinus carpio</i>)	Glyphosate Aria 41% SL, Tehran Iran	There were significant differences between the swimming pattern of treated individuals and control ones during both steps. The sublethal concentration of glyphosate led to hypertrophy, hyperplasia and hyperemia in the gill of fish. However, changes were obvious only after sampling. The exposed fish also displayed clinical signs such as darkening of the skin and increasing movement of the operculum.	https://pubmed.ncbi.nlm.nih.gov/34679048/
Glyphosate and Roundup® alter morphology and behavior in zebrafish	Bridi <i>Et. Al.</i> , Toxicology , 2017	zebrafish (<i>Dainoreio</i>)	glyphosate & Roundup	Glyphosate or Roundup® altered locomotion and aversive behavior in zebrafish larvae; reduced the locomotion in adult zebrafish & reduced aggressive behavior in adult zebrafish. Glyphosate decreased ocular distance in zebrafish larvae. Roundup®-exposed adult zebrafish demonstrated impairment in memory.	https://www.sciencedirect.com/science/article/pii/S0300483X17303128?via%3Dihub
Understanding the influence of glyphosate on the structure and function of freshwater microbial community in a microcosm.	Lu <i>Et. Al.</i> Environmental Pollution, 2020	freshwater microbial community	glyphosate	Transcriptions of some cyanobacteria were significantly influenced by glyphosate. Addition of glyphosate in our artificial microcosms did not strongly affect the aquatic microbial community composition but did alter the community's transcription levels, which might be potentially explained by that some microbes could alleviate glyphosate's toxicity by utilizing glyphosate as a P source.	https://en.x-mol.com/paper/article/1220089269107838976?recommendPaper=1449652889145212928

Multistressor negative effects on an experimental phytoplankton community. The case of glyphosate and one toxigenic cyanobacterium on Chlorophycean microalgae.	Hernandez-Garcia Et. Al. Science of the Total Environment, 2020	microalgae Ankistrodesmus falcatus, Chlorella vulgaris, Pseudokirchneriella subcapitata, and Scene desmus incrasatulus.	Faena®	The simultaneous presence of glyphosate and toxigenic cyanobacteria increases the stress on microalgae, jointly affecting their growth and development. The joint action of both stressors affected growth rate and population dynamics, macromolecule content, and led to increased CAT and GPx levels.	https://en.x-mol.com/paper/article/1227702482968268800?recommendedPaper=1449652889145212928
New findings on the effect of glyphosate on autotrophic and heterotrophic picoplankton structure: A microcosm approach.	Sabio Y Garcia Et. Al., Aquatic Toxicology, 2020	picoplankton cytotometric populations	glyphosate	85 % decrease in the abundance of the whole autotrophic picoplankton. Glyphosate also changed the structure of the heterotrophic fraction by means of changing bacterial dominant OTUs fingerprinting patterns in both systems and by shifting the relative abundances of cytometric groups in the clear scenario.	https://pubmed.ncbi.nlm.nih.gov/32172181/
Ammonium haloacetates – an alternative to glyphosate?	Turek et al. Chemosphere 2018	Vibrio fischeri, Heterocypris incongruens	Ammonium haloacetates	The synthesized QAS are generally less (phyto)toxic than glyphosate, while their herbicidal activity is stronger or almost equal against some weeds, and therefore these compounds may cause a less risk to the aquatic and benthic environments	https://pubmed.ncbi.nlm.nih.gov/29241140/
Glyphosate Herbicide Induces Changes in the Growth Pattern and Somatic Indices of Crossbred Red Tilapia (O. niloticus x O. mossambicus)	Muhammad et al. MDPI Animals 2021	O. niloticus x O. mossambicus	glyphosate	The results also indicate that the bodyweight index is the most sensitive toxicity parameter in that a reduction in body weight was observed at 25 mg/L of glyphosate. Negative correlations between the glyphosate concentration and toxicity parameters such as specific growth rate (SGR), hepato-somatic index (HIS), and gonado-somatic index (GSI) were observed.	https://pubmed.ncbi.nlm.nih.gov/33922293/
Determination of the Ecotoxicity of Herbicides Roundup® Classic Pro and Garlon New in Aquatic and Terrestrial Environments	Tajnaiová et al. MDPI Plants 2020	duckweed Lemna minor and green algae Desmodesmus subspicatus	Roundup Classic Pro, AMPA	IC50Roundup = 267.3 µg/L, IC50AMPA = 117.8 mg/L	https://www.mdpi.com/2223-7747/9/9/1203

Behavioral Analysis of <i>Folsomia Candida</i> (Collembola) with Herbicide Using Electronic and Computational Instrumentation: Bioassays	Ximenes <i>et al.</i> Soil and Sediment Contamination: An International Journal 2020	<i>Folsomia candida</i>	glyphosate 815g/l a.i.	It was possible to identify organism's preference for sites not contaminated with systemic herbicide. When the 1:50 dilution was used, it can be seen that the organism moved in the whole medium, however with expressive preference for the uncontaminated area. When 1:100 dilution was used, it can be seen that the organism moved in the whole medium, however with slight preference for the uncontaminated area. In the tests performed with the 1:50 dilution, it was possible to verify more consistently that the organism avoided contaminated area, due to the greater concentration of the herbicide.	https://www.researchgate.net/publication/340791637_Behavioral_Analysis_of_Folsomia_Candida_Collembola_with_Herbicide_Using_Electronic_and_Computational_Instrumentation_Bioassays
Proteomic and histopathological response in the gills of <i>Poecilia reticulata</i> exposed to glyphosate-based herbicide	Roche <i>et al.</i> , Environmental toxicology and pharmacology, 2015	<i>Poecilia reticulata</i>	GBHs	Guppies exposed to GBH at 1.82mg GLI L-1 showed time-dependent histopathological response in different epithelial and muscle cell types. The histopathological indexes indicate that GBH cause regressive, vascular and progressive disorders in the gills of guppies	https://www.academia.edu/14720856/Proteomic_and_histopathological_response_in_the_gills_of_Poecilia_reticulata_exposed_to_glyphosate_based_herbicide_email_work_card=int-raction-paper
Alterations in the general condition, biochemical parameters and locomotor activity in <i>Cnesterodon decemmaculatus</i> exposed to commercial formulations of chlorpyrifos, glyphosate and their mixtures	Bonifacio <i>et al.</i> Ecological Indicators, 2016	<i>Cnesterodon decemmaculatus</i>	Clorfox® and Roundup Max®	Clorfox affected the fish K, behavior, AST/ALT, AChE and CAT activity. Roundup Max affected AST/ALT, ALP and GST activity. The mixture of both products affected the fish K, GST and ALP activity.	https://www.sciencedirect.com/science/article/abs/pii/S1470160X16300267
Roundup® exposure promotes gills and liver impairments, DNA damage and inhibition of brain cholinergic activity in the Amazon teleost fish <i>Colossoma macropomum</i>	Braz-Mota <i>et al.</i> , Chemosphere, 2015	<i>Colossoma macropomum</i>	Roundup	Both RD concentrations affected the biotransformation process in gills of tambaqui negatively. Instead, liver responses suggest that a production of reactive oxygen species (ROS) occurred in fish exposed to RD, particularly in the animals exposed to 75% RD, as seen by imbalances in biotransformation and antioxidant systems. The increased DNA damage observed in red blood cells of tambaqui exposed to RD is in agreement with this hypothesis. Finally, both tested sub-lethal concentrations of RD markedly inhibited the cholinesterase activity in fish brain.	https://www.academia.edu/34258117/Roundup_exposure_promotes_gills_and_liver_impairments_DNA_damage_and_inhibition_of_brain_cholinergic_activity_in_the_Amazon_teleost_fish_Colossoma_macropomum_email_work_card=title
Roundup Effects on Oxidative Stress Parameters and Recovery Pattern of <i>Rhamdia quelen</i>	de Menezes <i>et al.</i> Arch Environ Contam Toxicol (2011)	<i>Rhamdia quelen</i>	Roundup	Glutathione S-transferase (GST) levels decreased at both concentrations. Then non-protein thiols levels decreased at the 0.95 mg/l concentration. During the recovery period, some of the parameters that had altered, such as protein carbonyl content, later recovered. However, some enzymes reacted during this period, e.g., GST increased its activity, possibly indicating a compensatory response against the toxic conditions. In contrast, CAT and SOD activities decreased during the recovery period, indicating herbicide toxicity. Oxidative stress that occurred during the exposure period was likely due to the increased lipid peroxidation and protein carbonyl content.	https://www.academia.edu/12778690/Roundup_Effects_on_Oxidative_Stress_Parameters_and_Recovery_Pattern_of_Rhamdia_quelen_email_work_card=title

Roundup causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish <i>Prochilodus lineatus</i> .	Modesto & Martinez, <i>Chemosphere</i> , 2010	<i>Prochilodus lineatus</i>	Roundup	Fish exposed to RD for 24h showed reduction on superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities, and increased glutathione (GSH) content. After 24 and 96h, fish of RD group showed increased glutathione-S-transferase (GST) activity and lipid peroxidation. AChE activity was inhibited in brain after 96h and in muscle after 24 and 96h of exposure. Thus, acute exposure to RD stimulated the biotransformation pathway, with increased GST, but interfered on the antioxidant defenses, with reduction of SOD and GPx activity, leading to the occurrence of lipid peroxidation. Inhibition of AChE showed that RD acts as a contaminant with anti-AChE action.	https://www.semanticscholar.org/paper/Roundup-causes-oxidative-stress-in-liver-and-in-and-Modesto-Martinez/c04f1f4586d7039d1b87a663c6280ae5df61b519
Changes in defense capacity to infectious hematopoietic necrosis virus (IHNV) in rainbow trout intergenerationally exposed to glyphosate	Le Duc-Carrée et al, <i>Fish Shellfish Immunol.</i> 2022 Jan	rainbow trout, <i>Oncorhynchus mykiss</i>	pure glyphosate, Roundup Innovert®, and Viaglif Jardin®	Pure glyphosate did not induce higher mortality but reduced interleukin-1 β . Glyphosate co-formulations modulated fish viral susceptibility. Chemical contamination with GBHs strongly modulated viral trout susceptibility. Pure glyphosate induced a cumulative mortality of 35.8%, comparable to the control (37.0%), which was significantly reduced with Roundup Innovert® (-9.9%) and increased (+14.8%) with Viaglif Jardin®. No modification was observed for the biomarkers analysed for any conditions. These results demonstrate that the nature of the co-formulants associated to glyphosate in GBHs can modulate the susceptibility of fish to pathogens.	https://www.sciencedirect.com/science/article/abs/pii/S1050464821004381
Inflammatory, Oxidative Stress, and Apoptosis Effects in Zebrafish Larvae after Rapid Exposure to a Commercial Glyphosate Formulation	Lanzarin et al. <i>Biomedicine</i> . 2021 Nov	Zebrafish Larvae	Roundup® Flex (RF)	None of the RF concentrations tested showed changes in the number of neutrophils and NO. However, the concentration of 10 μ g a.i. mL ⁻¹ was able to induce an increase in ROS levels and cell death. The activity of antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)), the biotransformation activity, the levels of reduced (GSH) and oxidised (GSSG) glutathione, lipid peroxidation (LPO), lactate dehydrogenase (LDH), and acetylcholinesterase (AChE) were similar among groups. Overall, the evidence may suggest toxicological effects are dependent on the concentration of RF, although at concentrations that are not routinely detected in the environment.	https://pubmed.ncbi.nlm.nih.gov/34944599/
Long-term exposure to polyethylene microplastics and glyphosate interferes with the behavior, intestinal microbial homeostasis, and metabolites of the common carp (<i>Cyprinus carpio</i> L.)	Chen et al. <i>Sci Total Environ</i> . 2021 Dec	carp (<i>Cyprinus carpio</i> L.)		These findings illustrate that exposure to PE-MPs or GLY alone is toxic to fish and results in physiological changes to the brain-gut axis.	https://pubmed.ncbi.nlm.nih.gov/34973326/
Alterations at biochemical, proteomic and transcriptomic levels in liver of tilapia (<i>Oreochromis niloticus</i>) under chronic exposure to environmentally relevant level of glyphosate	Jia et al, <i>Chemosphere</i> . 2022 Jan	tilapia	Gly	Transcriptomic analysis revealed that Gly exposure changed dramatically the expression of 225 genes in liver, including 94 up-regulated genes and 131 down-regulated genes. GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analyses showed that these genes were predominantly enriched in ion transport, lipid metabolism and PPAR (peroxisome proliferator-activated receptor) signaling pathway. Meanwhile, at proteomic level, long-term Gly exposure resulted in alteration of 21 proteins, which were principally related to hepatic metabolism function. In conclusion, our data	https://pubmed.ncbi.nlm.nih.gov/35114268/

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				displayed a potential toxicity, mainly manifested as redox imbalance and dysregulation of metabolism function, in the liver of tilapia after long-term Gly exposure at 2 mg/L.	
Differential impact of <i>Limnoperna fortunei</i> -herbicide interaction between Roundup Max® and glyphosate on freshwater microscopic communities	Gattás et al. Environ Sci Pollut Res Int. 2016	mussel <i>Limnoperna</i>	Roundup Max	glyphosate (G), Roundup Max® (R), 100 mussels (M), the combination of mussels and herbicide either in the technical-grade or formulated form (MG and MR, respectively), Herbicides significantly increased total phosphorus in water; R and MR showed greater initial total nitrogen and ammonium. R increased picoplankton abundance and caused an eightfold increase in phytoplankton, with high turbidity values; G had a lower effect on these variables. Herbicide-mussel combination induced an accelerated dissipation of glyphosate in water (MG 6.36 ± 0.83 mg G g DW(-1) day(-1) and MR 5.16 ± 1.26 mg G g DW(-1) day(-1)). A synergistic effect on ammonium was observed in MR but not in MG. MR and MG had an antagonistic effect on phytoplankton, which showed a drastic reduction due to grazing, as revealed by M.	https://pubmed.ncbi.nlm.nih.gov/27324498/
DNA damage and oxidative stress modulatory effects of glyphosate-based herbicide in freshwater fish, <i>Channa punctatus</i>	Nwani et al. Environmental Toxicology and Pharmacology, 2013	<i>Channa punctatus</i>	Roundup®	Exposure to glyphosate induced DNA damage in <i>Channa punctatus</i> in dose-dependent manner. •At sublethal test concentrations, LPO elevated and ROS production increased. •CAT, SOD and GR were suppressed after exposure to sublethal test concentrations. •LPO level and DNA damage was higher in gills than blood cells at all concentrations.	https://www.science-direct.com/science/article/pii/S1382668913001336
Metabolomic and Transcript Analysis Revealed a Sex-Specific Effect of Glyphosate in Zebrafish Liver	Giommi et al, Int J Mol Sci. 2022	Zebrafish	Gly	Exposure to GLY also caused a decrease of UMP levels in the pyrimidine metabolism pathway. In male, GLY exposure decreased the amino adipic acid within the lysine degradation pathway. Transcript analysis of genes involved in stress response, oxidative stress and the immune system were also performed. Results demonstrated an increased stress response in both sexes, as suggested by higher nr3c1 expression. However, the hsp70.2 transcript level was increased in female but decreased in male. The results demonstrated reduced sod1, sod2, and gpx1a in male following exposure to GLY, indicating an impaired oxidative stress response. At the same time, an increase in the cat transcript level in female was observed. mRNA levels of the pro-inflammatory interleukins litaf and cxcl8b.1 were increased in female. Taken together, the results provide evidence of disrupted nucleotide hepatic metabolism, increased stress inflammatory response in female and disruption of oxidative stress response in male.	https://pubmed.ncbi.nlm.nih.gov/35269866/
Developmental toxicity of glyphosate on embryo-larval zebrafish (<i>Danio rerio</i>)	Liu et al. Ecotoxicol Environ Saf. 2022	Zebrafish	Gly	GLY treatment induced developmental toxicity in the fish, including premature hatching, reduced heartbeats, pericardial and yolk sac oedema, swim bladder deficiency, and shortened body length, which was possibly due to a significantly decreased triiodothyronine (T3)/thyroxine (T4) ratio and the abnormal expression patterns of hypothalamic-pituitary-thyroid and growth hormone/insulin-like	https://pubmed.ncbi.nlm.nih.gov/35398647/

				growth factor (GH/IGF) axis-related genes in larvae exposed to GLY.	
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Studies on amphibian species

Title	Author, source	Species	Substance (Glyphosate, GBH, etc.)	Summary/main findings	link
New effects of Roundup on amphibians: predators reduce herbicide mortality; herbicides induce antipredator morphology	Rick A Relyea, Ecol Appl., 2012 Mar	(wood frogs leopard frogs [R. pipiens or L. pipiens], and American toads	Roundup Original MAX - with POAE	Predators reduce herbicide mortality; herbicides induce antipredator morphology. Moreover, the data suggest that the herbicide might be activating the tadpoles' developmental pathways used for antipredator responses. Collectively, these discoveries suggest that the world's most widely applied herbicide may	https://pubmed.ncbi.nlm.nih.gov/22611860/
Effects of glyphosate on hepatic tissue evaluating melanomacrophages and erythrocytes responses in neotropical anuran Leptodactylus latinasus.	Pérez-Iglesias Et. Al. Environ Sci Pollut, 2016	Frog - Leptodactylus latinasus	Gly	Use of glyphosate enhanced the melanin area in melanomacrophage clusters of the frogs. It also altered the presence of hepatic catabolism pigments into melanomacrophages along with the alterations in the nucleus of erythrocytes	https://pubmed.ncbi.nlm.nih.gov/26856864/ https://repositorio.unesp.br/bitstream/handle/11449/172514/2-s2.0-84957716764.pdf?sequence=1
Can environmental concentrations of glyphosate affect survival and cause malformation in amphibians? Effects from a glyphosate-based herbicide on Physalaemus cuvieri and P. gracilis (Anura: Leptodactylidae)	Herek Et. Al. Environmental Science and Pollution Research, 2020	Physalaemus cuvieri and P. gracilis	Roundup original® DI	Glyphosate had lethal effects on both studied species. Tadpoles showed shorter lengths and lower masses; that is, those that survived suffered chronic effects on growth and weight. The GBH maximum acceptable toxicant concentration for mortality and malformation was lower than the allowed level for Brazilian waters. The GBH tested in this study presented a high environmental and acute risk for the two studied species.	https://enx-mol.com/paper/article/1257932450765381632?recommendPaper=1449652889145212928
Developmental and lethal effects of glyphosate and a glyphosate-based product on Xenopus laevis embryos and tadpole	Turhan Et. Al. Bulletin of Environmental Contamination and Toxicology, 2020	Xenopus laevis	Gly & GBH	The 96h LC50 values for the commercial herbicide, in contrast, were 32.1 and 35.1 mg active ingredient L-1 for embryos and tadpoles, respectively. Since pure glyphosate has no effect on the selected biomarkers, it is thought that developmental toxic effects caused by glyphosate-based products are increased mainly due to formulation additives.	https://www.researchgate.net/publication/338562804_Developmental_and_lethal_effects_of_glyphosate_and_a_glyphosate-based_product_on_Xenopus_laevis_embryos_and_tadpoles

Effects of a glyphosate-based herbicide and predation threat on the behaviour of agile frog tadpoles.	Mikó Et Al., Biology, Medicine Ecotoxicology and environmental safety, 2017	amphibians/frog (Rana dalmatina) tadpoles	Glyphogon® Classic, containing 41.5 w/w% glyphosate and 15.5 w/w% POEA	We found that at the higher herbicide concentration tadpoles decreased their activity and more tadpoles were hiding, and at least at the lower concentration their vertical position was closer to the water surface than in tadpoles of the control treatment. Tadpoles also decreased their activity in the presence of dragonfly larvae, but did not hide more in response to either predator, nor did tadpoles avoid predators spatially. Further, exposure to the herbicide did not significantly influence behavioural responses to predation threat. Our study documents a definite influence of glyphosate-based herbicides on the behaviour of agile frog tadpoles and indicates that some of these changes are similar to those induced by dangerous predators. This may suggest that the underlying physiological mechanisms or the adaptive value of behavioural changes may similar.	http://evolecol.hu/wp-content/uploads/2017/02/Miko_et_al_2017_EES.pdf
Toxicity of four herbicide formulations with glyphosate on <i>Rhinella arenarum</i> (Anura: Bufonidae) tadpoles: B-esterases and glutathione S-transferase inhibitors.	Lajmanovich Et. Al. Archives of Environmental Contamination and Toxicology, 2011	Common Toad <i>Rhinella arenarum</i> (Anura: Bufonidae)	Roundup Ultra-Max (ULT), Infosato (INF), Glifoglex, and C-K YUYOS FAV.	Acetylcholinesterase (AChE), butyrylcholinesterase (BChE), carboxylesterase (CbE), and glutathione S-transferase (GST) activities were measured among tadpoles sampled from those treatments that displayed survival rates >85%. Forty-eight-hour LC(50) for <i>R. arenarum</i> tadpoles exposed to CF-GLY in the static tests ranged from ULT = 2.42 to FAV = 77.52 mg ae/L. For all CF-GLY, the LC(50) values stabilized at 24 h of exposure. Tadpoles exposed to all CF-GLY concentrations at 48 h showed decreases in the activities of AChE (control = 17.50 ± 2.23 nmol/min/mg/protein; maximum inhibition INF 30 mg ae/L, 71.52%), BChE (control = 6.31 ± 0.86 nmol/min/mg/protein; maximum inhibition INF 15 mg ae/L, 78.84%), CbE (control = 4.39 ± 0.46 nmol/min/mg/protein; maximum inhibition INF 15 mg ae/L, 81.18%), and GST (control = 4.86 ± 0.49 nmol/min/mg/protein; maximum inhibition INF 1.87 mg ae/L, 86.12%).	https://www.researchgate.net/publication/45407365_Toxicity_of_Four_Herbicide_Formulations_with_Glyphosate_on_Rhinella_arenarum_Anura_Bufonidae_Tadpoles_B-esterases_and_Glutathione_S-transferase_Inhibitors
Harmful Effects of the Dermal Intake of Commercial Formulations Containing Chlorpyrifos, 2,4-D, and Glyphosate on the Common Toad <i>Rhinella arenarum</i> (Anura: Bufonidae)	Lajmanovich Et. Al. Water Air and Soil Pollution, 2015	Common Toad <i>Rhinella arenarum</i> (Anura: Bufonidae)	Gly & other pesticides	Toads were exposed to nominal concentrations of all the pesticides. Results confirmed that toad's exposure to these pesticides endured neurotoxicity, oxidative stress and immunological depression	https://www.researchgate.net/publication/284275576_Harmful_Effects_of_the_Dermal_Intake_of_Commercial_Formulations_Containing_Chlorpyrifos_24-D_and_Glyphosate_on_the_Common_Toad_Rhinella_arenarum_Anura_Bufonidae
Genotoxic effect of a binary mixture of dicamba- and glyphosate-based commercial herbicide formulations on <i>Rhinella arenarum</i> (Hensel, 1867) (Anura, Bufonidae) late-stage larvae	Soloneski Et. Al., June 2016, Environmental Science and Pollution Research	amphibians/frog (<i>Rhinella arenarum</i>)	48 % glyphosate (GLY)-based Credit® & 57.71 % dicamba (DIC)-based Banvel®	Lethality studies revealed LC50/96 h values of 358.44 and 78.18 mg L ⁻¹ DIC and GLY for Banvel® and Credit®, respectively. SCGE assay revealed, after exposure for 96 h to either 5 and 10 % of the Banvel® LC50/96 h concentration or 5 and 10 % of the Credit® LC50/96 h concentration, an equal significant increase of the genetic damage index (GDI) regardless of the concentration of the herbicide assayed. The binary mixtures of 5 % Banvel® plus 5 % Credit® LC50/96 h concentrations and 10 % Banvel® plus 10 % Credit® LC50/96 h concentrations induced equivalent significant increases in the GDI in regard to GDI values from late-stage larvae exposed only to Banvel® or Credit®. A synergistic effect of the mixture of GLY and DIC on the induction of primary DNA breaks on circulating blood cells of <i>R. arenarum</i> late-stage larvae could be demonstrated.	https://link.springer.com/article/10.1007%2Fs11356-016-6992-7

Effects of glyphosate and its commercial formulation, Roundup® Ultramax, on liver histology of tadpoles of the neotropical frog, Leptodactylus latrans (amphibia: Anura).	Bach, Chemosphere, 2018	amphibians/frog (Leptodactylus latrans)	Glyphosate & Roundup® Ultramax	GBH increased the number of liver MMc and MMCs in L. latrans tadpoles. Liver damages were present on L. latrans larvae exposed to pure and formulated. First evidence of adverse effects of glyphosate and RU formulation on the liver of anuran larvae at concentrations frequently found in the environment.	https://www.sciencedirect.com/science/article/abs/pii/S0045653518305277?via%3Dihub https://www.academia.edu/37040774/Effects_of_glyphosate_and_its_commercial_formulation_Roundup_Ultramax_on_liver_histology_of_the_neotropical_frog_Leptodactylus_latrans_amphibia_Anura?email_work_card=view-paper
Effects on growth, development and abnormalities induced by a glyphosate commercial formulation and its active ingredient on two developmental stages of the South-American common frog, Leptodactylus latrans.	Bach, Environ Sci Pollut Res, 2016	amphibians/frog (Leptodactylus latrans)	Glyphosate & Roundup® Ultramax	No lethal effects were observed on larvae exposed to GLY during either Gs-25 or Gs-36. The concentrations inducing 50% lethality in RU-exposed larvae at different exposure times and Gss ranged from 3.26 to 9.61 mg a.e./L. Swimming activity was affected by only RU. Effects on growth and development and the induction of morphologic abnormalities like oral abnormalities and edema were observed after exposure to either GLY or RU. Gs-25 was the most sensitive stage to both forms of the herbicide. The commercial formulation was much more toxic than the active ingredient on all the endpoints assessed.	https://www.academia.edu/30997980/Effects_on_growth_development_and_abnormalities_induced_by_a_glyphosate_commercial_formulation_and_its_active_ingredient_on_two_developmental_stages_of_the_South_American_common_frog_Leptodactylus_latrans?email_work_card=title
Toxic, cytotoxic, and genotoxic effects of a glyphosate formulation (Roundup® SL–Cosmoflux®411F) in the direct-developing frog Eleutherodactylus johnstonei	Meza-Joya Et. Al., Environ. Mol. Mutagen. 2013	amphibians/frog Eleutherodactylus johnstonei	Roundup® SL–Cosmoflux®411F	Toxicity results indicated that the application rate [37.4 µg acid equivalent (a.e.)/cm ²] equivalent to that used in aerial spraying (3.74 kg a.e./ha) is not lethal in male and female adult frogs, whereas neonates are highly sensitive. Glyphosate formulation at application rates above 5.4 µg a.e./cm ² (in vivo) and concentrations above 95 µg a.e./mL (in vitro) showed clear evidence of cytotoxicity. In vivo and in vitro exposure of E. johnstonei erythrocytes to the glyphosate formulation induced DNA breaks in a dose-dependent manner with statistically significant values (P < 0.05) at all doses tested.	https://onlinelibrary.wiley.com/doi/10.1002/em.21775
Effects of glyphosate and the glyphosate based herbicides Roundup Original® and Roundup Transorb® on respiratory morphophysiology of bullfrog tadpoles.	Rissoli Et. Al., Chemosphere, 2016	amphibians/bullfrog tadpoles	Roundup Original® and Roundup Transorb®	Glyphosate and Roundup formulations cause distinct skin alterations. Glyphosate and Roundup formulations differently altered the respiratory function. Bullfrog tadpoles' skin is very sensitive to glyphosate and Roundup formulations. In summary, GLY, RO and RTR exert different effects in bullfrog tadpoles, in particular the surfactants and inert compounds appear to influence oxygen uptake.	https://www.sciencedirect.com/science/article/abs/pii/S0045653516305690?via%3Dihub
Toxic and genotoxic effects of Roundup on tadpoles of the Indian skittering frog (Euflectis cyanophlyctis) in the presence and	Yadav Et. Al., Aquat. Toxicol., 2013	amphibians/Indian skittering frog (Euflectis cyanophlyctis)	Roundup	Roundup at environmentally relevant concentrations has lethal and genotoxic impact on E. cyanophlyctis; which may have long-term fitness consequence to the species. Presence of predator stress apparently increased the toxicity and genotoxicity of Roundup; but these effects were not statistically significant.	https://www.sciencedirect.com/science/article/abs/pii/S0166445X1300026X?via%3Dihub

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absence of predator stress		cyano phlyctis)			
Questions concerning the potential impact of glyphosate-based herbicides on amphibians.	Wagner, <i>Et. Al., Environ. Toxicol. Chem.</i> 2013	summary article	GBH	The impact on amphibians depends on the herbicide formulation, with different sensitivity of taxa and life stages. Effects on development of larvae apparently are the most sensitive endpoints to study.	https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.2268
A glyphosate micro-emulsion formulation displays teratogenicity in <i>Xenopus laevis</i>	Bonfanti <i>et al.</i> Aquatic Toxicology 2017	<i>Xenopus laevis</i> , African clawed frog	Roundup® Power 2.0, Rodeo®	Conversely, Roundup® Power 2.0 exhibited a 96 h LC50 of 24.78 mg a.e./L and a 96 h EC50 of 7.8 mg a.e./L. In conclusion, the differences in GBH formulations should be carefully considered by the authorities, since sublethal and/or long-term effects (e.g. teratogenicity) can be significantly modulated by the active ingredient salt type and concentration of the adjuvants. Finally, the mechanistic toxicity of glyphosate and GBHs are worthy	https://www.sciencedirect.com/science/article/abs/pii/S0166445X17303764
Lethal toxicity of the herbicides acetochlor, ametryn, glyphosate and metribuzin to tropical frog larvae	Daam <i>et al.</i> Ecotoxicology 2019	<i>Physalasmus cuvieri</i> and <i>Hypsiobas pardalis</i>	gly a. i. 99.2%	The greatest part of the total tadpole mortality throughout the 96 h tests were elicited within the first 24 h: 95–97% (glyphosate), for <i>P. cuvieri</i> and <i>H. pardalis</i> , respectively. The calculated 96 h-LC50 (median lethal concentration; in mg a.s./L) values for <i>P. cuvieri</i> and <i>H. pardalis</i> were 115mg/l and 106mg/l (glyphosate).	https://link.springer.com/article/10.1007/s10646-019-02067-5
DNA damage exerted by mixtures of commercial formulations of glyphosate and imazethapyr herbicides in <i>Rhinella arenarum</i> (Anura, Bufonidae) tadpoles	Carvalho <i>et al.</i> Ecotoxicology 2019	Rhinella arenarum	Credit® is a GBH commercial formulation containing 48% of isopropylamine salt	In mortality experiments, LC50 values for GLY of 89.44 mg/L (82.68–96.36 mg/L), 85.96 mg/L (65.02–113.66 mg/L), 82.08 mg/L (80.16–92.20 mg/L) and 78.18 mg/L (75.77– 81.22 mg/L) were reported after 24, 48, 72 and 96 h of exposure, respectively. Results revealed a significant time-dependent increase in lethality when the time of exposure increased from 24 to 96 h.	https://pubmed.ncbi.nlm.nih.gov/30826955/
Comparative Early Life Stage Toxicity of the African Clawed Frog, <i>Xenopus laevis</i> Following Exposure to Selected Herbicide Formulations Applied to Eradicate Alien Plants in South Africa	Babalola and Wyk Archives of Environmental Contamination and Toxicology, 2018	<i>Xenopus laevis</i> , African clawed frog	three glyphosate formulations (Roundup, Kilo Max, and Environ Glyphosate)	For Roundup, the 96-h LC50 of 0.89 mg/L at NF-stage 48 was lower relative to both NF-stage 8–11 and NF-stage 60 at concentrations of 1.05 and 2.75 mg/L, respectively, For Kilo Max formulation, the 96-h LC50 of 58.1 mg/L at NF-stage 48 was far lower compared with both corresponding 96-h LC50s of 207 mg/L at NF-stage 8–11 and 455 mg/L at NF-stage 60. In Environ glyphosate exposure, the 96-h LC50 of 134.6 mg/L at NF stage 48 was equally lower relative to the corresponding 96-h LC50s of 466 mg/L at NF-stage. 8–11 and 5257 mg/L at NF-stage 60.	https://pubmed.ncbi.nlm.nih.gov/29051999/
Evaluation of Genotoxic and Mutagenic Effects of Glyphosate Roundup Original® in <i>Dendropsophus minutus</i> Peters, 1872 Tadpoles	Carvalho <i>et al.</i> South American Journal of Herpetology 2018	<i>Dendropsophus minutus</i> Peters (<i>lesser treefrog</i>)	Roundup Original®	At lower concentrations of glyphosate Roundup Original®, the DNA damage, evaluated by comet assay, demonstrated increased levels, differing from the results of the micronucleus test. We also detected that physicochemical changes did not influence micronucleus frequency or DNA damage, except that the temperature variation caused an increase in the frequency of binucleate cells.	https://www.researchgate.net/publication/327976270_Evaluation_of_Genotoxic_and_Mutagenic_Effects_of_Glyphosate_Roundup_Original_R_in_Dendropsophus

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Evaluation of the genotoxic, mutagenic, and histopathological hepatic effects of polyoxyethylene amine (POEA) and glyphosate on <i>Dendropsophus minutus</i> tadpoles	Lopes et al. Environmental Pollution, 2021	<i>Dendropsophus minutus</i> (lesser treefrog)	Gly, POEA	On average, 174 % more genomic damage was observed in the tadpoles exposed to all concentrations of POEA in comparison with the control, while up to seven times more micronuclei were recorded, on average, at a concentration of 5 µg/l of POEA. All the individuals exposed to 10 µg/L of POEA died. The tadpoles exposed to GLY presented 165 % more DNA damage than the control, on average, at the highest concentrations (260 and 520 µg/l), and up to six times more micronuclei at 520 µg/l.	https://pubmed.ncbi.nlm.nih.gov/34365244/
Impact of glyphosate-based herbicide on early embryonic development of the amphibian <i>Xenopus laevis</i>	Flach et al. Aquat Toxicol. 2022 Mar	South African clawed frog <i>Xenopus laevis</i>	Roundup® LB plus	GBH treatment with sublethal concentrations resulted in a reduced body length and mobility of embryos. Furthermore, incubation with GBH led to smaller eyes, brains and cranial cartilages in comparison to untreated embryos. GBH incubation also resulted in shorter cranial nerves and had an effect on cardiac development including reduced heart rate and atrium size. On a molecular basis, GBH treatment led to reduced expression of marker genes in different tissues and developmental stages.	https://pubmed.ncbi.nlm.nih.gov/35074614/
Effects of glyphosate-based herbicides on survival, development, growth and sex ratios of wood frogs (<i>Lithobates sylvaticus</i>) tadpoles. I: chronic laboratory exposures to VisionMax®	Navarro-Martin et al. Aquat Toxicol. 2014	tadpoles (<i>Lithobates sylvaticus</i>)	VisionMax®	Chronic exposures to VisionMax(®) had direct effects on the metamorphosis of <i>L. sylvaticus</i> tadpoles by decreasing development rates, however, there was a decrease in survival only in the group exposed to the highest dose of VisionMax. There was a decrease in the number of tadpoles reaching metamorphic climax, from 78% in the control group to 42% in the VisionMax(®) (2.9 mg a.e./L) group, and a 7-day delay to reach metamorphic climax in the same treatment group. There was a clear dose-response effect for VisionMax(®) to increase thyroid hormone receptor β in tadpole brain.	https://pubmed.ncbi.nlm.nih.gov/24878356/