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MULTI-STRESSORS IN FRESHWATER AND TRANSITIONAL ENVIRONMENTS: FROM LEGACY POLLUTANTS TO EMERGING ONES

Effects of glyphosate and a commercial formulation Roundup® exposures on maturation of *Xenopus laevis* oocytes

Sylvain Slaby[1,2,3] · Pauline Titran[1] · Guillaume Marchand[1] · Julie Hanotel[1] · Arlette Lescuyer[1] · Alain Leprêtre[3] · Jean-François Bodart[1] · Matthieu Marin[1] · Sébastien Lemiere[3]

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Abstract
Pesticides are often found at high concentrations in small ponds near agricultural field where amphibians are used to live and reproduce. Even if there are many studies on the impacts of phytopharmaceutical active ingredients in amphibian toxicology, only a few are interested in the earlier steps of their life cycle. While their populations are highly threatened with extinction. The aim of this work is to characterize the effects of glyphosate and its commercial formulation Roundup® GT Max on the *Xenopus laevis* oocyte maturation which is an essential preparation for the laying and the fertilization. Glyphosate is an extensively used herbicide, not only known for its effectiveness but also for its indirect impacts on non-target organisms. Our results showed that exposures to both forms of glyphosate delayed this hormone-dependent process and were responsible for spontaneous maturation. Severe and particular morphogenesis abnormalities of the meiotic spindle were also observed. The MAPK pathway and the MPF did not seem to be affected by exposures. The xenopus oocyte is particularly affected by the exposures and appears as a relevant model for assessing the effects of environmental contamination.

Keywords Amphibian toxicology · Glyphosate · Roundup · Pesticide · Oocyte · Maturation · *Xenopus*

Introduction

Since the 1970s, glyphosate (*N*-(phosphonométhyl) glycine, $C_3H_8NO_5P$) is recognized as a potent herbicide, but it was initially patented for its metal chelator properties. Its first commercialization was in 1974 under the name of Roundup® (Duke and Powles 2008). The glyphosate is a broad-spectrum systemic herbicide, which acts through leaves and inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase in the shikimate pathway essential for the amino acid synthesis (Martinez et al. 2018). Commercial formulations of glyphosate contain also surfactants, as the polyethoxylated tallow amine (POEA), adding to improve the efficiency of treatment (Székács and Darvas 2012; Mesnage et al. 2015). POEA is also often referenced as a harmful compound of the herbicide formulation. In fact, in different amphibian toxicology studies, it has been proved that commercial formulations containing POEA are more toxic than glyphosate alone (Howe et al. 2004; Moore et al. 2012). Thus, it appears highly essential to compare the impact of glyphosate in pure form and in complex formulations. The International Agency for Research on Cancer (IARC) has classified glyphosate in the group 2A as a probable carcinogen to humans (IARC 2017). There are also major concerns on the impacts of this herbicide substance in the environment. It is highly soluble in water, and it is the third pesticide found in the surface water in France, knowing that the first is the aminomethylphosphonic acid (AMPA) (Ministère de la Transition écologique et solidaire 2017)—its metabolite (Mesnage et al. 2015). In aquatic environments, the concentrations vary according to the area and

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✉ Sébastien Lemiere
sebastien.lemiere@univ-lille.fr

[1] CNRS, INRA, UMR 8576-UGSF-Unité de Glycobiologie Structurale et Fonctionnelle, University Lille, F-59000 Lille, France

[2] Present address: URAFPA, Unité de Recherche Animal et Fonctionnalités des Produits Animaux, University de Lorraine, INRA, 2 Avenue de la Forêt de Haye, F-54500 Vandoeuvre-lès-Nancy, Nancy, France

[3] EA 4515-LGCgE-Laboratoire Génie Civil et géo-Environnement, Cité scientifique, SN3, University Lille, F-59655 Villeneuve d'Ascq, France

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the season. For example, glyphosate concentration was detected at 1.082 μg L^{-1} in Boële River (France) (Botta et al. 2009), 8.7 μg L^{-1} in Midwestern United States streams (Battaglin et al. 2005), 40.8 μg L^{-1} in surface waters of Southern Ontario (Struger et al. 2008), but can reach approximatively 0.1 to 0.7 mg L^{-1} in Argentina culture areas (Peruzzo et al. 2008).

Amphibian is uncontestably the group most threatened with 41% of known species concern by the risk of extinction (Monastersky 2014). Pollution, destruction of habitats, climate change, invasive species, human uses, diseases, and parasitism are known to be primary or interacting causes of the decline (Blaustein and Wake 1990, 1995; Alford and Richards 1999; Blaustein et al. 2003; Alford 2010). Due to their particular life cycle, amphibians are especially affected by environmental contaminations. Their biological particularities, as aquatic external fertilization, no shell-eggs, and metamorphosis, make them very vulnerable and dependent on water ecosystem quality (Sparling et al. 2010). Moreover, small ponds or ditches, likely to receive in agricultural or industrial areas high levels of xenobiotics, are known to support their life and reproduction (Bridges and Boone 2003).

Various effects have been described in amphibian species exposed to glyphosate or its commercial formulations. Serious skin morphology changes and reduced oxygen uptakes were observed in *Lithobates catesbeianus* tadpoles exposed 96 h to 1 mg L^{-1} of glyphosate (pure or acid equivalent (a.e.) in Roundup Original® or in Roundup Transorb R®) (Rissoli et al. 2016). *Xenopus laevis* tadpoles exposed from the stage 8 to 46 (Nieuwkoop and Faber 1967) to glyphosate (> 30 mg L^{-1}) showed cardiac edemas while those exposed to Roundup® Power 2.0 at a range of concentrations between 1 and 25 mg [a.e.] L^{-1} exhibited in a dose-dependent manner critical morphological alterations as disappearance of the upper and lower jaws, edemas, or gut miscoiling (Bonfanti et al. 2018). Acetylcholinesterase, butyrylcholinesterase, glutathione S-transferase, and carboxylesterase activities decreased in *Rhinella arenarum* tadpoles exposed to different glyphosate-based formulations C-K Yuyos FAV®, Glifoglex®, Infosato® or Roundup® Ultra-Max at concentrations higher than 1.85 mg [a.e.] L^{-1} for 48 h (Lajmanovich et al. 2011).

Overall in amphibian toxicology, the effects of xenobiotic exposures on gametic stages are still scarce. Incidences at this level would affect consequently the reproduction, future individual fitness and thus population viability.

This work proposed to characterize impacts of glyphosate and its commercial formulation Roundup® GT Max on the oocyte maturation process of *X. laevis*. After a hormonal induction, females produce mature oocytes which are blocked in metaphase of meiosis II and prepared for ovulation and fertilization (Ferrell 1999). Then, at the animal pole of the cell, a mature oocyte will exhibit a white spot (WS) caused by the Germinal Vesicle Breakdown (GVBD) and the formation of the meiotic spindle supporting condensed chromosomes lined up on the metaphasic plate. The oocyte maturation is enhanced by different signaling pathways. The activation of the mitogen-activated protein kinases (MAPKs) cascade involves successive phosphorylations of protein kinases such extracellular signal-regulated kinase 2 (ERK2) and p90 kDa ribosomal S6 kinases (RSK) (Ferrell 1999; Frödin and Gammeltoft 1999). This phosphorylation cascade is associated with the activation of the M-promoting factor (MPF) which promotes the M-phase entry. The MPF is a complex composed of a catalytic subunit (cyclin-dependent kinase 1; Cdc2) and a regulatory unit (cyclin B) and its activation requires of the dephosphorylation of Cdc2 (Norbury and Nurse 1990; Ferrell 1999). The serine 10 histone H3 is one of the principal targets of the MPF. Its phosphorylation is a conserved histone modification essential to the condensation of chromosomes (Hendzel et al. 1997; Van Hooser et al. 1998; Hans and Dimitrov 2001). In the present work, the oocyte maturation was studied by time-courses experiments, cytological analyses of the meiotic spindle morphogenesis, and assessments phosphorylation states of ERK2, RSK Cdc2, and H3, as described in our previous work with cadmium and lead as contaminants (Slaby et al. 2017).

Material and methods

Chemicals

All chemical compounds were obtained from Sigma-Aldrich Chimie® (France), except tricaine mesylate (MS222; Honeywell Fluka®, France), glyphosate (GLY; Molekula Ltd., UK), and Roundup® GT Max (Rup; Monsanto Europe, Belgium; French local reseller). Rup contains 588 g L^{-1} (43.78% *w*/*w*) of potassium salt of glyphosate, equivalent to 480 g L^{-1} of glyphosate acid (35.74% *w*/*w*). Tested contaminants were daily diluted in the weekly prepared control medium ND96 (Nathan Dascal 96: 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM $MgCl_2$, 5 mM HEPES-NaOH, pH 7.5). Concentrations of GLY and Rup used in the experiments are expressed in acid equivalent: 0.148, 1.48, 14.8, 148, and 1480 μM. This concentration range frames the environmental quality standards (EQS) defined in France in the Water Framework Directive context (INERIS 2014). These values are 28 μg L^{-1} (0.166 μM) and 70 μg L^{-1} (0.414 μM). They correspond respectively for freshwater to the annual average concentration-EQS and to the Maximal Acceptable Concentration-EQS.

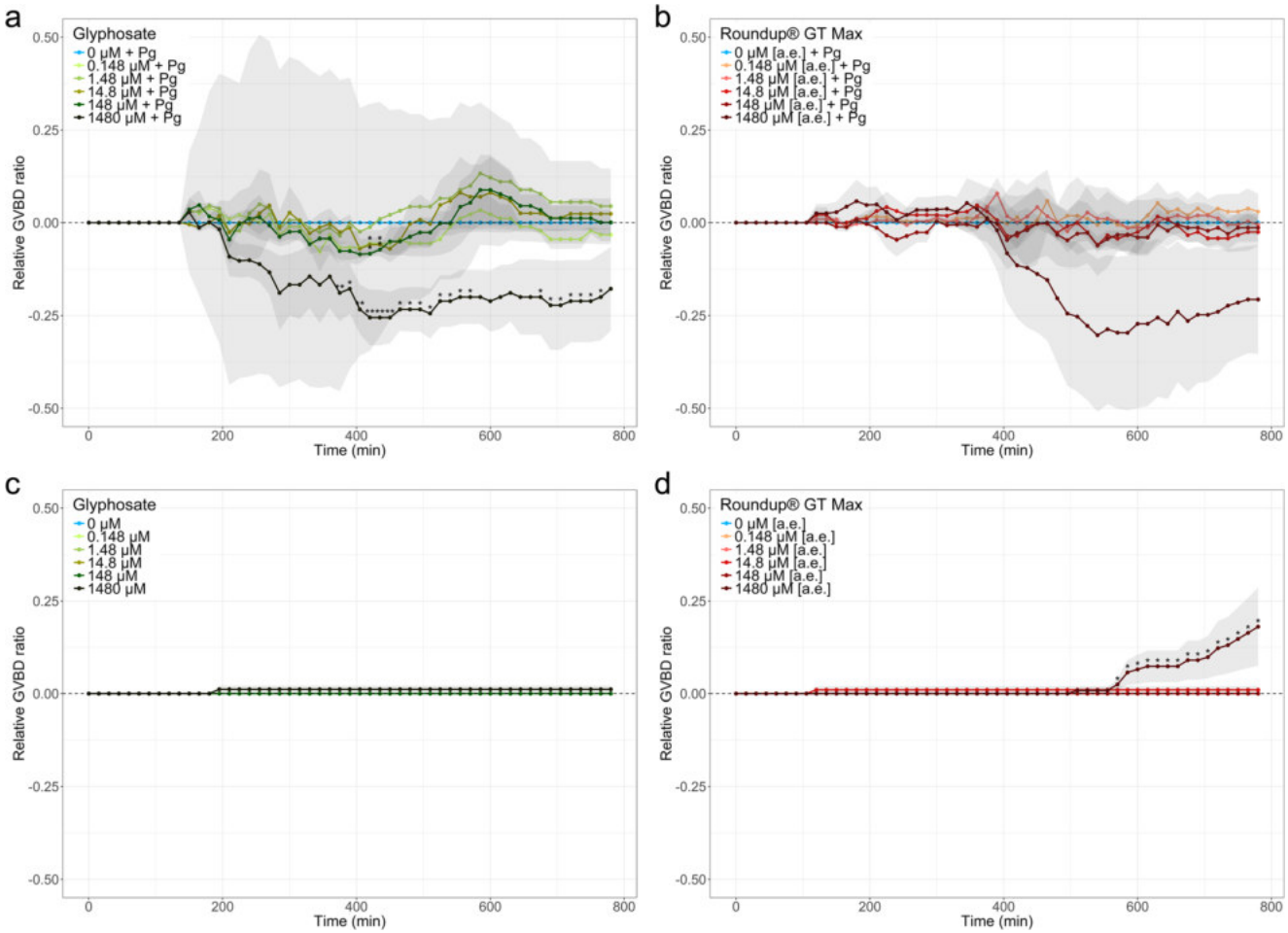

Fig. 1 Oocyte maturation time courses under GLY and Rup exposures. WS appearances were assessed every 15 min for 13 h. GLY (green lines) or Rup (red lines) exposures were conducted at 0 (blue line), 0.148, 148, 14.8, 148, and 1480 µM [a.e.] in the presence (**a**, **b**) or absence (**c**, **d**) of Pg. Results are expressed as mean ratios relative to the control ± SEM (gray areas). Significant differences were assessed by Friedman's tests and post hoc multiple paired comparisons between conditions (*$p < 0.05$, **$p < 0.01$)

Handling of *Xenopus laevis* oocytes

Animals used came originally from the University of Rennes 1 and were maintained in control tanks (XenopLus−Amphibian Housing System from Tecniplast) in the animal house of our campus (ULille Sciences et Technologie, Villeneuve d'Ascq, France). Adults were fed with floating expanded pellets (Aquatic 3, Special Diets Services) *ad libitum*. Water quality parameters were controlled, and daily water removal was done. All animal experiments were conducted at the campus animal facility according to the rules of the European Community Council guidelines (86/609/EEC) for laboratory animal experimentation. The animal protocol was approved by the local institutional review board (Comité d'Ethique en Experimentation Animale Nord-Pas-de-Calais, CEEA 07/2010). Collection of *xenopus* oocytes were weekly realized by ovariectomy after the anesthesia of the female in a bath of tricaine mesylate (1 g L^{-1}) for 45 min. Collected oocytes were treated with collagenase solution (1 g L^{-1}) for 45 min. Remaining follicular cells were then removed manually with tweezers. Only stage VI oocytes (Dumont 1972) were selected and stored in ND96 medium at 14 °C before the experiments begin. Oocyte maturation was in vitro induced by progesterone (Pg; 4 mg L^{-1}).

GVBD time courses

Kinetics of the GVBD was conducted monitoring the appearance of the WS at the top of the exposed gametes. For each concentration of GLY or Rup, 30 stage VI oocytes (Dumont 1972) were exposed with or without Pg (4 mg L^{-1}) at 19 °C. The GVBD ratios were assessed every 15 min for 13 h from the beginning of the exposures. Time-courses were repeated three times for GLY and four times for Rup exposures.

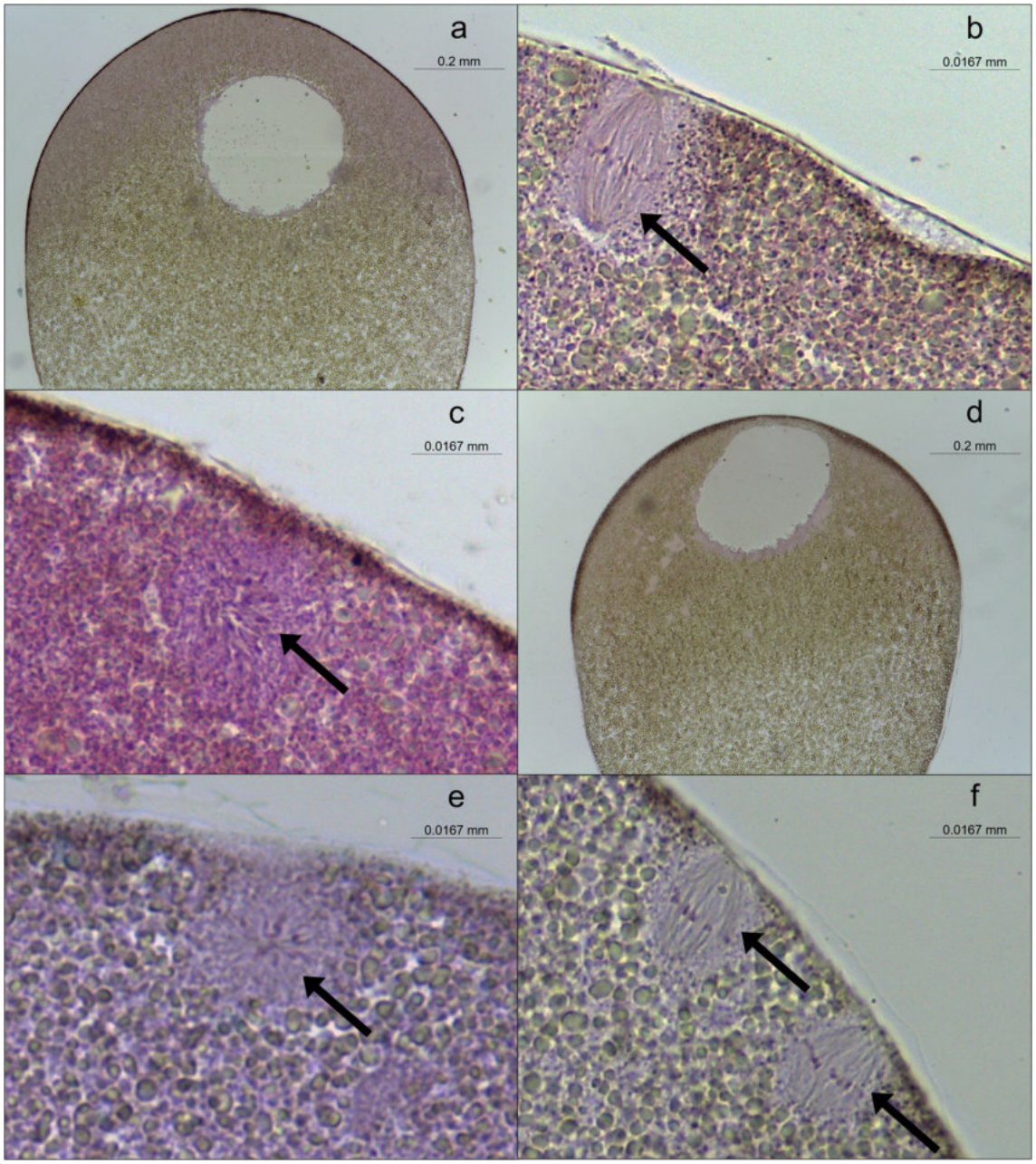

Fig. 2 Optical microscopy typical photographs after glyphosate-based form exposures of *X. laevis* oocytes. **a** Control immature oocyte. **b** Control mature oocyte (normal). **c** dS + dC + E. **d** GV. **e** A. **f** 2S

Meiosis spindle formation analyses

Oocytes were exposed to GLY or Rup in the presence of Pg overnight at 19 °C. Only germ cells exhibiting the WS were sampled. Fixation was conducted overnight in Smith reagent (80% Smith A: Potassium Bichromate 17 mM, 20% Smith B: formol and acetic acid) in the dark at room temperature. Then, oocytes were dried, paraffin-embedded, and sliced with a microtome (7-μm slice thickness). Nuclear structures, chromosomes, and cytoplasmic structures were revealed thanks to nuclear red (0.1 g of nuclear red QSP in 100 ml 5% aluminum sulfate) and picro-indigo-carmine (0.25 g of picro-indigo-carmine QSP in 100 ml saturated picric acid) stainings. Slices mounted on slides were analyzed by optical microscopy.

MAPK and MPF activity investigations

Oocytes exposed overnight to GLY or Rup with or without Pg at 19 °C were used. For each concentration, WS oocytes and non-WS oocytes were sampled separately. Electrophoresis and western blotting were conducted

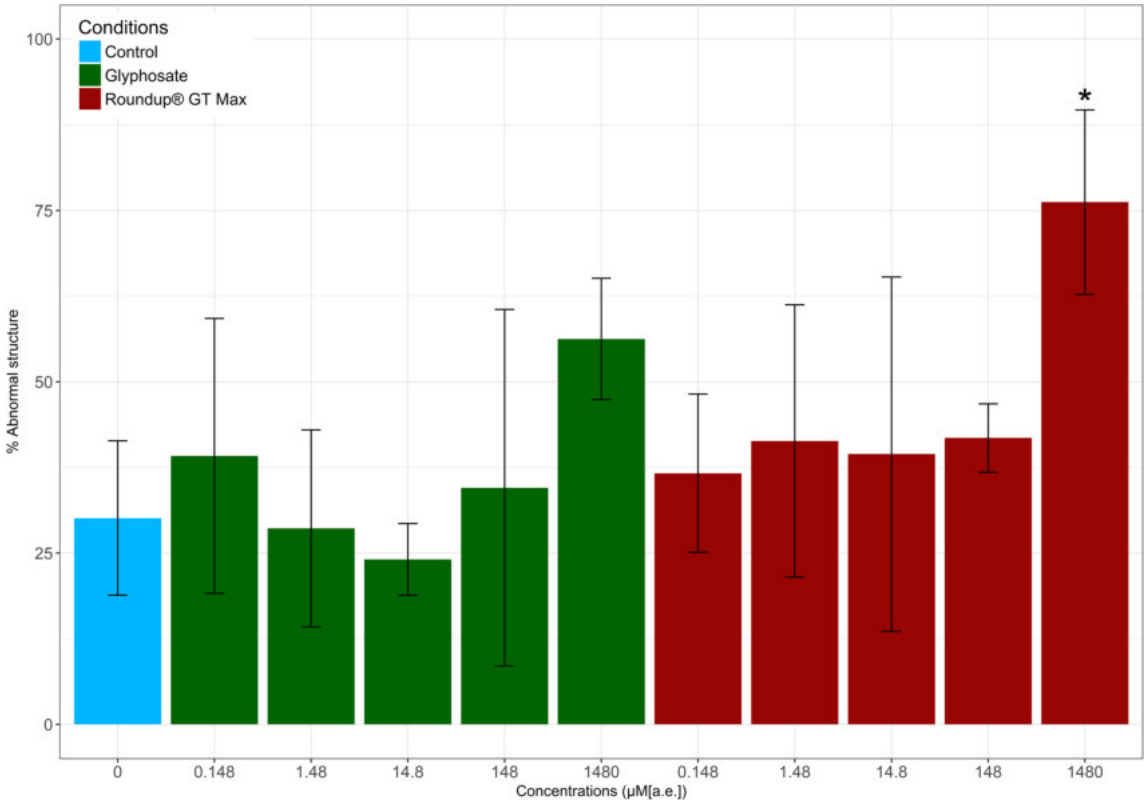

Fig. 3 Percentages of cytological abnormalities after oocyte GLY or Rup exposures. Results are expressed as average percentages (±SD) per condition. Significant differences were assessed by Kruskal-Wallis tests and post hoc multiple paired comparisons between conditions (*$p < 0.05$)

according to the protocol previously described by our lab (Gelaude et al. 2015). Briefly, after the lysis of pooled oocytes in the homogenization buffer followed by a centrifugation (13.8×*g*, 4 °C, 15 min), the proteic part was extracted. Supernatants were added to one volume of Laemmli 2× buffer with 4% beta-mercaptoethanol. Samples were heated at 75 °C for 5 min and stored at − 20 °C before SDS-PAGE (15% Chesnel modified). Then, transfer was done onto a nitrocellulose membrane (Hybond, Amersham Pharmacia Biotech, UK). Immunoblots were saturated with 5% low fat dry milk and incubated overnight with specific antibodies. Rabbit polyclonal antibodies were used to detect $p90^{Rsk}$ (p90Rsk-1 C-21 sc-231 antibody; Santa Cruz Biotechnology®, USA), pCdc2 (Phospho-Cdc2 (Tyr15) antibody; Cell Signaling Technology®, Netherlands), and pH3 (Phospho-Histone H3 (Ser10) antibody; Cell Signaling Technology®, Netherlands). Mouse monoclonal antibody was used to detect ERK2 (Erk2 D-2 sc-1647 antibody; Santa Cruz Biotechnology®, USA). After incubation with the appropriate secondary antibodies (Sigma-Aldrich®, France), chemiluminescent revelations (Clarity™ Western ECL Substrate; Bio-Rad®, USA) were conducted according to the manufacturer's instructions. Every phosphorylation states were assessed at least three times with pools of oocyte from different females.

Statistical analysis

All statistical analyses and graphical representations were conducted with R software (version 3.3.2; The R Foundation for Statistical Computing, 2016). The GVBD ratios from the time-courses were compared performing Friedman's tests and post hoc multiple paired comparisons between conditions. Cytological data were analyzed using Kruskal Wallis' tests and post hoc multiple paired comparisons. For all experiments, N refers to the number of replications (used females) and n to the number of oocytes per exposure concentrations.

Results

GVBD time courses

Kinetics of the maturation process was assessed by determining GVBD ratios every 15 min for 13 h. Figure 1a presents the GVBD time-courses under GLY exposures in presence of Pg. Proportions of oocytes with a WS significantly decreased ($p < 0.05$) after 420 and 435 min of exposure to 14.8 and 148 µM of GLY, but these delays quickly disappeared and were not observed again until the end of the experiments. At 1480 µM of GLY, more important delays were recorded. GVBD ratios were

Table 1 Observed abnormalities in WS oocytes after the GLY or Rup exposures

Conditions	(µM [a.e.])	*N*	*n*	Normal (%)	dS (%)	dC (%)	dS + dC (%)	E (%)	dS + E (%)	dC + E (%)	dS + dC + E (%)	nS + nC (%)	2S (%)	A (%)	2A (%)	GV (%)
Control	0	6	60	68.4	1.7	10	8.3	3.3	1.7	–	3.3	3.3	–	–	–	–
GLY	0.148	3	28	60.8	–	7.1	7.1	3.6	3.6	3.6	7.1	7.1	–	–	–	–
	1.48	3	35	70.36	–	3.7	7.4	7.4%	–	–	3.7	7.4	–	–	–	–
	14.8	3	38	76	–	–	6.9	–	–	–	10.3	–	–	3.4	–	3.4
	148	3	38	66.7	–	3.7	3.7	11.1	–	3.7	–	7.4	–	–	–	3.7
	1480	2	20	44.4	–	16.7	11.1	11.1	–	–	11.1	5.6	–	–	–	–
Rup	0.148	3	28	64.4	–	10.7	3.6	7.1	–	–	7.1	–	–	7.1	–	–
	1.48	3	27	62.7	–	8.6	11.4	–	–	2.9	8.6	2.9	–	2.9	–	–
	14.8	3	29	57.9	–	10.5	5.3	7.9	–	–	5.3	–	2.6	7.9	2.6	–
	148	3	27	57.9	2.6	13.2	13.2	–	–	–	5.3	2.6	2.6%	–	2.6	–
	1480	2	18	20	–	35	10	–	–	–	–	15	–	5	10	5

N number of females, *n* number of oocytes, *dS* disorganized spindle, *dC* disorganized chromosomes, *E* ectopic spindle, *nS* no spindle, *nC* no chromosomes, *2S* double spindle, *A* aster, *2A* double aster, *GV* germinal vesicle

significantly lower after 375 ($p < 0.01$), 390 ($p < 0.05$), 405 to 450 ($p < 0.01$), 465 to 570, and 695 to 785 ($p < 0.05$) minutes. The last ratio that measures at 800 min was not significantly different from the control condition. Rup exposures with Pg induced approximatively the same response profiles, but no significant difference was found after statistical analyses (Fig. 1b). Without Pg, spontaneous GVBD were observed in GLY and Rup conditions and especially for the concentration 1480 µM [a.e.] of Rup where GVBD ratios were significantly higher from 570 min to the end of the exposure (Fig. 1c, d).

Meiosis spindle formation analyses

Control immature oocytes show at the cytological level a large germinal vesicle (Fig. 2a). After the GVBD, in a control mature oocyte, there is the formation of the meiotic spindle. This barrel-shaped structure close to the cell membrane supports the condensed chromosomes lined up on the metaphase plate (Fig. 2b). Abnormal structures of the meiotic spindle formation can be observed in the control mature oocytes (30.10%). For the Rup concentration of 1480 µM [a.e.], this percentage significantly increased to 76.19% ($p < 0.05$; Fig. 3). A similar increase was observed for the same concentration of GLY (56.25%) but did not differ significantly from the control condition ($p = 0.0572$; Fig. 3). Table 1 reports the proportion of the different kinds of abnormality found in WS oocytes after the exposures. The following categories were defined: disorganized spindle (dS), disorganized chromosomes (dC), ectopic spindle (E), no spindle (nS), no chromosomes (nC), double spindles (2S), aster (A), double asters (2A), and germinal vesicle (GV). As indicated in Table 1 by the symbol "+," many abnormalities of the previous list could be observed in one oocyte, for example, dS + dC + E (Fig. 2c). It appeared that specific structures never found in control mature oocytes were formed after GLY or Rup exposures, i.e., GV (Fig. 2d) and A (Fig. 2e). Moreover, the double structures 2S (Fig. 2f) and 2A were only observed in oocyte exposed to Rup.

MAPK and MPF activity investigations

No particular abnormality in the phosphorylation states of ERK2, RSK, Cdc2, and H3 was detected on the immunoblots (Fig. 4). Only one experiment exhibited an aberrant phosphorylation state in pooled white spotted oocytes of Cdc2 (GLY 1480 µM) and H3 (Rup 1480 µM [a.e.]) (data not shown). This suggested that the MAPK pathway and also the activation and the activity of the MPF were not impacted by GLY or Rup exposures.

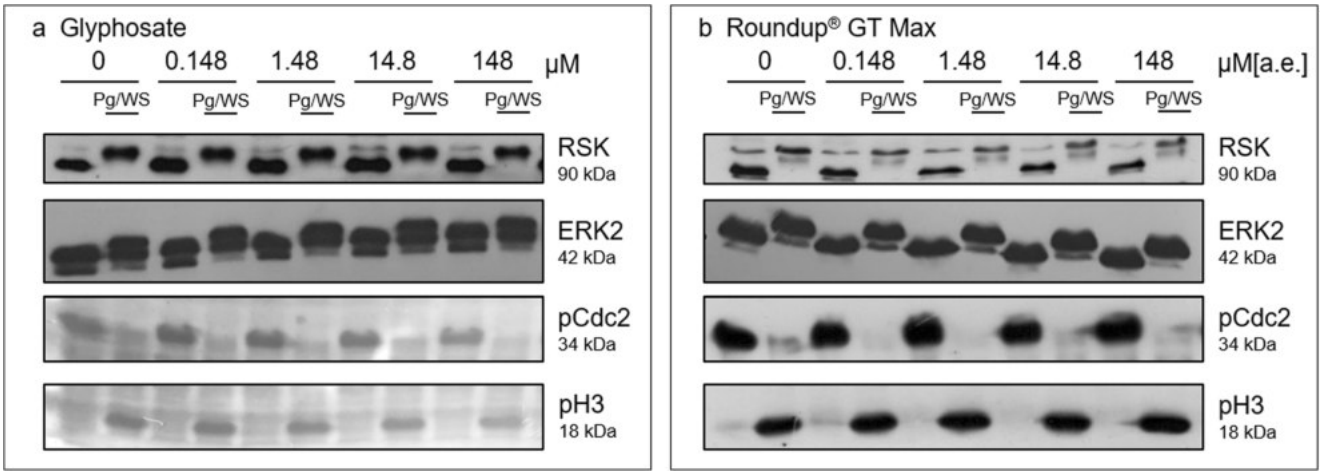

Fig. 4 Effects of GLY (**a**) or Rup (**b**) exposures on the phosphorylation states of RSK and ERK2 of the MAPK cascade and of the catalytic subunit Cdc2 of the MPF and H3, a target of the MPF. No bar, non-WS oocytes not exposed to Pg; Pg/WS, WS oocytes exposed to Pg.

Discussion

This work proposed to investigate mechanisms of action of glyphosate and one of its commercial formulation on an essential and early step of the amphibian reproduction. To this purpose, *X. laevis* oocyte maturation was examined under exposure conditions. Main results and especially cytological analyses showed that this preparation to the fertilization was seriously disturbed by both forms of glyphosate (pure and in formulation). Concerning the time-course experiments under progesterone influence, we observed that glyphosate alone at 1480 µM significantly delayed the maturation process. At the same dose (a.e.), similar but not significant delay was also induced by Roundup® GT Max. Others' toxicological studies showed disturbances of this parameter. In the presence of progesterone, cadmium also delayed the maturation process at 136 µM (Slaby et al. 2017) but in opposite atrazine and malathion alone or in the mixture at 50 and 100 µg L^{-1} each shortened the time of appearance of the WS in a dose-dependent manner (Ji et al. 2016). Interestingly, the last measure in glyphosate kinetic experimentations at 1480 µM did not differ from the control condition, suggesting that maturation timing impairment would have been missed if only final GVBD ratio was recorded. That underlines the interest of time-course approaches to assess *X. laevis* oocyte maturation.

In the absence of progesterone, spontaneous maturation occurred after Roundup® GT Max or glyphosate exposures. Even if significant results were only detected at the highest concentration of the commercial formulation, any WS oocytes not induced by a hormone stimulation is abnormal. In previous works, we observed that cadmium, cobalt, and zinc ions similarly induced this biological abnormality (Marin et al. 2015; Slaby et al. 2017). However, different mechanisms seem to imply the different tested contaminants. The first WS oocyte observed in cadmium conditions without progesterone appeared largely later than the first under progesterone stimulation (Slaby et al. 2017). Here, pesticide exposures induced spontaneous maturation at around the same moment as in hormone presence conditions. These results are associated with no particular MAPK pathway or MPF disorder in contrary to abnormalities of the phosphorylation states of the same protein studied observed after metal ion exposures (Slaby et al. 2017). Further experiments are needed to understand how glyphosate induces such spontaneous GVBD.

Cytological analyses underlined an increase in the occurrence of abnormalities during spindle morphogenesis (1480 µg [a.i.] L^{-1} for the two tested forms of glyphosate). Some abnormalities were only detected in herbicide-exposed oocytes as asters, double asters, double spindles, and germinal vesicles in WS oocytes. This last cytological anomaly corresponded to an ascent of the germinal vesicle without its breakdown, but the pigments located at the surface of the animal pole have been displaced. Similar effects were observed after 2,4-D exposure (10 mM for 10 h). Actin-dependent modifications of the cell shape and the spontaneous WS appearance without GVBD and depolymerization of microtubules were induced (Stebbins-Boaz et al. 2004). For the other abnormalities (2S, A, 2A), such structures were never reported in oocytes exposed to cadmium and lead ions (Slaby et al. 2017). Double spindle formations were also observed in co-exposed oocytes to Pregnyl® (Human chorionic gonadotropin; 150 IU mL^{-1}) and hydroxyurea (1 mg L^{-1}) for 30 min (Brachet et al. 1970). The authors hypothesized a splitting of the meiotic spindle affecting the chromosomes and also cytoplasmic constituents, possibly the centrioles, to explain such structures. For our exposures, double structures seemed to be specific anomalies, never observed in previous control or exposed oocytes historically in our lab and could correspond to signature of glyphosate (in both forms) exposures.

Several authors observed genotoxicity after glyphosate exposures of aquatic species. For example, *Euphlyctis cyanophlyctis* exposed for 24 to 96 h to glyphosate-based herbicide (concentrations range from 1 to 3 mg [a.e.] L^{-1}) exhibited an increased incidence of micronucleated erythrocytes (Yadav et al. 2013). From these results, the authors reaffirmed the genotoxic potential of glyphosate is rather associated with clastogenicity. Similar conclusions have been drawn from exposure data of *Anguilla anguilla* to 18 and 36 μg L^{-1} of glyphosate contained in Roundup® solution (Guilherme et al. 2010). These authors studied DNA breaks by means of comet assay and erythrocyte nuclear aberrations for chromosomal and cytological damage. They hypothesized the oxidative stress contribution as a mechanism of genetic damage. In the case of our work, cytological results suggest genotoxic effects associated with the spindle morphogenesis instead of DNA breaks.

Few studies deal with the impacts of glyphosate on aquatic species gametes. In male, sperm quality of zebra fish exposed in vivo for 24 and 96 h was reduced (Lopes et al. 2014). For both concentrations (5 and 10 mg L^{-1}), sperm motility and motility period decreased, and only for the highest dose the authors observed mitochondrial functionality and DNA damage. These concentrations are largely superior to those tested in vitro on oyster spermatozoa (Akcha et al. 2012). No cytotoxicity nor DNA damage has been observed after glyphosate or Roundup express® exposures (1 h) to concentrations ranging from 0.5 to 5 μg [a.i.] L^{-1}. For oocytes, we did not find any study. However, our results highlighted that oocyte maturation could be critically affected by contaminant exposures. Any effect as abnormal spindle morphogenesis, during this process, may prevent the fertilization.

Conclusions

This work presents new information on the impacts of glyphosate and one of its commercial formulation on the oocyte maturation. It appears that this essential step of the reproduction can be seriously altered by these herbicide exposures. The *X. laevis* oocyte seems to be an efficient model to assess the quality of aqueous solutions in environmental toxicology.

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References

Akcha F, Spagnol C, Rouxel J (2012) Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos. Aquat Toxicol 106–107: 104–113. https://doi.org/10.1016/j.aquatox.2011.10.018

Alford RA (2010) Declines and the global status of amphibians. In: Sparling DW, Linder G, Bishop CA, Krest SK (eds) Ecotoxicology of amphibians and reptiles, 2nd edn. CRC Press, Boca Raton, USA, pp 13–45

Alford RA, Richards SJ (1999) Global amphibian declines: a problem in applied ecology. Annu Rev Ecol Syst 30:133–165. https://doi.org/10.1146/annurev.ecolsys.30.1.133

Battaglin WA, Kolpin DW, Scribner EA, Kuivila KM, Sandstrom MW (2005) Glyphosate, other herbicides, and transformation products in midwestern stream, 2002. J Am Water Resour Assoc 41:323–332. https://doi.org/10.1111/j.1752-1688.2005.tb03738.x

Blaustein AR, Romansic JM, Kiesecker JM, Hatch AC (2003) Ultraviolet radiation, toxic chemicals and amphibian population declines. Divers Distrib 9:123–140. https://doi.org/10.1046/j.1472-4642.2003.00015.x

Blaustein AR, Wake DB (1990) Declining amphibian populations: a global phenomenon? Trends Ecol Evol 5:203–204. https://doi.org/10.1016/0169-5347(90)90129-2

Blaustein AR, Wake DB (1995) The puzzle of declining amphibian populations. Sci Am 272:52–57

Bonfanti P, Saibene M, Bacchetta R, Mantecca P, Colombo A (2018) A glyphosate micro-emulsion formulation displays teratogenicity in *Xenopus laevis*. Aquat Toxicol 195:103–113. https://doi.org/10.1016/j.aquatox.2017.12.007

Botta F, Lavison G, Couturier G, Alliot F, Moreau-Guigon E, Fauchon N, Guery B, Chevreuil M, Blanchoud H (2009) Transfer of glyphosate and its degradate AMPA to surface waters through urban sewerage systems. Chemosphere 77:133–139. https://doi.org/10.1016/j.chemosphere.2009.05.008

Brachet J, Hanocq F, Van Gansen P (1970) A cytochemical and ultrastructural analysis of in vitro maturation in amphibian oocytes. Dev Biol 21:157–195. https://doi.org/10.1016/0012-1606(70)90067-9

Bridges CM, Boone MD (2003) The interactive effects of UV-B and insecticide exposure on tadpole survival, growth and development. Biol Conserv 113:49–54. https://doi.org/10.1016/S0006-3207(02)00348-8

Duke SO, Powles SB (2008) Glyphosate: a once-in-a-century herbicide. Pest Manag Sci 64:319–325. https://doi.org/10.1002/ps.1518

Dumont JN (1972) Oogenesis in *Xenopus laevis* (Daudin) I Stages of oocyte development in laboratory maintained animals. J Morphol 136:153–179. https://doi.org/10.1002/jmor.1051360203

Ferrell JE (1999) *Xenopus* oocyte maturation: new lessons from a good egg. BioEssays 21:833–842. https://doi.org/10.1002/(SICI)1521-1878(199910)21:10<833::AID-BIES5>3.0.CO;2-P

Frödin M, Gammeltoft S (1999) Role and regulation of 90 kDa ribosomal S6 kinase (RSK) in signal transduction. Mol Cell Endocrinol 151: 65–77. https://doi.org/10.1016/S0303-7207(99)00061-1

Gelaude A, Marin M, Cailliau K, Jeseta M, Lescuyer-Rousseau A, Vandame P, Nevoral J, Sedmikova M, Martoriati A, Bodart JF (2015) Nitric oxide sonor s-nitroso-n-acetyl penicillamine (SNAP) alters meiotic spindle morphogenesis in *Xenopus* oocytes. J Cell Biochem 116:2445–2454. https://doi.org/10.1002/jcb.25211

Guilherme S, Gaivão I, Santos MA, Pacheco M (2010) European eel (*Anguilla anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup®—a glyphosate-based herbicide. Mutagenesis 25:523–530. https://doi.org/10.1093/mutage/geq038

Hans F, Dimitrov S (2001) Histone H3 phosphorylation and cell division. Oncogene 20:3021–3027. https://doi.org/10.1038/sj.onc.1204326

Hendzel MJ, Wei Y, Mancini MA, van Hooser A, Ranalli T, Brinkley BR, Bazett-Jones DP, Allis CD (1997) Mitosis-specific phosphorylation

of histone H3 initiates primarily within pericentromeric heterochromatin during G2 and spreads in an ordered fashion coincident with mitotic chromosome condensation. Chromosoma 106:348–360. https://doi.org/10.1007/s004120050256

Howe CM, Berrill M, Pauli BD, Helbing CC, Werry K, Veldhoen N (2004) Toxicity of glyphosate-based pesticides to four north American frog species. Environ Toxicol Chem 23:1928–1938. https://doi.org/10.1897/03-71

IARC (2017) Some organophosphate insecticides and herbicides - IARC monographs on the evaluation of carcinogenic risks to humans, vol 112. WHO Press, Lyon, France

INERIS (2014) INERIS-Valeur Guide Environnementale : Glyphosate

Ji Q, Lee J, Lin Y-H, Jing G, Tsai LJ, Chen A, Hetrick L, Jocoy D, Liu J (2016) Atrazine and malathion shorten the maturation process of *Xenopus laevis* oocytes and have an adverse effect on early embryo development. Toxicol in Vitro 32:63–69. https://doi.org/10.1016/j.tiv.2015.12.006

Lajmanovich RC, Attademo AM, Peltzer PM, Junges CM, Cabagna MC (2011) Toxicity of four herbicide formulations with glyphosate on *Rhinella arenarum* (Anura: Bufonidae) tadpoles: B-esterases and glutathione S-transferase inhibitors. Arch Environ Contam Toxicol 60:681–689. https://doi.org/10.1007/s00244-010-9578-2

Lopes FM, Junior ASV, Corcini CD et al (2014) Effect of glyphosate on the sperm quality of zebrafish *Danio rerio*. Aquat Toxicol 155:322–326. https://doi.org/10.1016/j.aquatox.2014.07.006

Marin M, Slaby S, Marchand G, Demuynck S, Friscourt N, Gelaude A, Lemière S, Bodart JF (2015) *Xenopus laevis* oocyte maturation is affected by metal chlorides. Toxicol in Vitro 29:1124–1131. https://doi.org/10.1016/j.tiv.2015.04.016

Martinez DA, Loening UE, Graham MC (2018) Impacts of glyphosate-based herbicides on disease resistance and health of crops: a review. Environ Sci Eur 30:2. https://doi.org/10.1186/s12302-018-0131-7

Mesnage R, Defarge N, de Vendômois JS, Séralini GE (2015) Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. Food Chem Toxicol 84:133–153. https://doi.org/10.1016/j.fct.2015.08.012

Ministère de la Transition écologique et solidaire (2017) Les pesticides les plus rencontrés dans les eaux continentales. In: Les Pestic. dans les eaux. http://www.statistiques.developpement-durable.gouv.fr/lessentiel/ar/246/0/pesticides-plus-rencontres-eaux-continentales.html. Accessed 26 Jun 2018

Monastersky R (2014) Life—a status report. Nature 516:158–161. https://doi.org/10.1038/516158a

Moore LJ, Fuentes L, Rodgers JH et al (2012) Relative toxicity of the components of the original formulation of Roundup® to five north American anurans. Ecotoxicol Environ Saf 78:128–133. https://doi.org/10.1016/j.ecoenv.2011.11.025

Nieuwkoop PD, Faber J (1967) Normal table of *Xenopus laevis* (Daudin): a systematical and chronological survey of the development from the fertilized egg till the end of metamorphosis, 2nd edn. Garland Science, Amsterdam, Netherlands

Norbury C, Nurse P (1990) Controls of cell proliferation in yeast and animals. In: Bock G, Marsh J (eds) Ciba Foundation Symposium 150-proto-oncogenes in cell development. John Wiley & Sons, Ltd., Chichester, pp 168–183

Peruzzo PJ, Porta AA, Ronco AE (2008) Levels of glyphosate in surface waters, sediments and soils associated with direct sowing soybean cultivation in north pampasic region of Argentina. Environ Pollut 156:61–66. https://doi.org/10.1016/j.envpol.2008.01.015

Rissoli RZ, Abdalla FC, Costa MJ, Rantin FT, McKenzie DJ, Kalinin AL (2016) Effects of glyphosate and the glyphosate based herbicides Roundup Original® and Roundup Transorb® on respiratory morphophysiology of bullfrog tadpoles. Chemosphere 156:37–44. https://doi.org/10.1016/j.chemosphere.2016.04.083

Slaby S, Hanotel J, Marchand G, Lescuyer A, Bodart JF, Leprêtre A, Lemière S, Marin M (2017) Maturation of *Xenopus laevis* oocytes under cadmium and lead exposures: cell biology investigations. Aquat Toxicol 193:105–110. https://doi.org/10.1016/j.aquatox.2017.10.009

Sparling DW, Linder G, Bishop CA, Krest SK (2010) Recent advancements in amphibian and reptile ecotoxicology. In: Sparling DW, Linder G, Bishop CA, Krest SK (eds) Ecotoxicology of amphibians and reptiles, 2nd edn. CRC Press, Boca Raton, USA, pp 1–11

Stebbins-Boaz B, Fortner K, Frazier J, Piluso S, Pullen S, Rasar M, Reid W, Sinclair K, Winger E (2004) Oocyte maturation in *Xenopus laevis* is blocked by the hormonal herbicide, 2,4-dichlorophenoxy acetic acid. Mol Reprod Dev 67:233–242. https://doi.org/10.1002/mrd.10396

Struger J, Thompson D, Staznik B, Martin P, McDaniel T, Marvin C (2008) Occurrence of glyphosate in surface waters of southern Ontario. Bull Environ Contam Toxicol 80:378–384. https://doi.org/10.1007/s00128-008-9373-1

Székács A, Darvas B (2012) Forty years with glyphosate. In: Hasaneen MN (ed) Herbicides-properties, Synthesis and Control of Weeds. InTech, Rijeka, HR, pp 247–284

Van Hooser A, Goodrich DW, Allis CD et al (1998) Histone H3 phosphorylation is required for the initiation, but not maintenance, of mammalian chromosome condensation. J Cell Sci 111:3497 LP–3493506

Yadav SS, Giri S, Singha U, Boro F, Giri A (2013) Toxic and genotoxic effects of Roundup on tadpoles of the Indian skittering frog (*Euflictis cyanophlyctis*) in the presence and absence of predator stress. Aquat Toxicol 132–133:1–8. https://doi.org/10.1016/j.aquatox.2013.01.016