Impacts of three glyphosate formulations on gonadal development of *Xenopus laevis*

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Abstract
The emergence of widespread morphological malformations in the reproductive system of wildlife is generating increasing concerns. This concern is because the observed malformities may be linked to pollution by pesticides and other chemicals. The amphibian declines, for example, have been linked to pesticide pollution among other factors. Using an extended Xenopus Metamorphosis Assay protocol, until the tadpoles metamorphosed, the exposure impacts of three glyphosate formulations, namely, Roundup, Kilo Max and Enviro Glyphosate, were assessed on the reproductive system of *Xenopus laevis*, vis-a-vis the body mass, sex ratios and morphological malformations as endpoints. The exposure concentrations ranged between 0.2–0.6 mg/L, 0.9–28 mg/L and 90–280 mg/L for Roundup, Enviro Glyphosate, and Kilo Max, respectively. Both Kilo Max and Enviro Glyphosate formulations significantly reduced the body mass of the metamorphs compared to the control. In sex ratios, only Kilo Max altered the percentage sex ratio of the treated frogs at a ratio of 68:32 (F:M) compared to 50:50 ratio in the control. In reproductive malformations, the three formulations showed abnormality index range of 22.3–49%, 17.5–37.5% and 20–30% for the Kilo Max, Enviro Glyphosate and Roundup formulations, respectively, compared to 7.5% in the control. Observed reproductive malformations include mixed sex, translucence, aplasia, segmented hypertrophy and segmented aplasia and translucence. This result indicates that some of the glyphosate formulations have the capacity to cause widespread reproductive malformations in a way that could reduce the reproductive fitness of the amphibian. Care must therefore be taken to reduce the application rate of these formulations, particularly in aquatic environments.

Keywords
Glyphosate Anura, malformations, intersex, Kilo Max, Enviro Glyphosate

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Introduction
There are increasing concerns about many of the anthropogenic chemicals in the environment with potential modulating effects on the reproductive system of organisms.¹–⁴ This is because several studies are linking environmental chemical exposure to reproductive disorders in wildlife, particularly in fish,⁵–⁸ amphibians,⁹ ¹⁰ ¹¹ ¹² ¹³ reptiles,¹⁴ ¹⁵ ¹⁶ birds and mammals.¹⁷ ¹⁸ These substances can adversely affect the reproductive biology of animals in many ways including inhibition of spermatogenesis, induction of sex reversal and disruption of gonadal development,¹ ¹⁸ as well as numerous other reproductive abnormalities.¹⁷ ¹⁸ These...

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widespread reproductive malformations being observed in wildlife provide considerable evidence that environmental contaminants can play a critical role in reproductive and developmental dysfunction.\(^{18}\)

The global amphibian population have been declining,\(^{19}\) with increasing data pointing towards pesticides pollution,\(^{20,21}\) among other factors as potential causes. The amphibians are important components of aquatic ecosystems, especially in tropical regions of the world.\(^{22}\) Extinctions as well as population declines in the amphibians is considered as a critical threat to the global biodiversity.\(^{23}\) According to Egea-Serrano et al.\(^{24}\) chemical pollution has been identified as one of the major threats faced by amphibians and, consequently, may play a role in their present global decline status. However, in spite of these documentations of amphibian declines, there are only few reports on their use as model for chemical-induced reproductive abnormalities.\(^{21}\)

Glyphosate (\(N\)-(phosphonomethyl) glycine), with a molecular formula \(\text{C}_3\text{H}_8\text{NO}_5\text{P}\), is a broad-spectrum, non-selective, post-emergence herbicide.\(^{25,26}\) Glyphosates are used for plant control, in no-till farming practices, as well as for ‘glyphosate-ready’ genetically modified crops.\(^{27}\) The herbicide is also approved and widely used for aquatic weed management through direct application to water-bodies. It is perhaps the most important and best-selling herbicide ever produced.\(^{28-31}\) The increasing global use of this herbicide has generated widespread concern regarding the adverse effects on non-target aquatic organisms.\(^{31-34}\) However, there is still lack of in depth understanding of how different types of pollutants affect amphibians, especially their reproductive system. There are also still numerous contradictory and controversial points of views, particularly on amphibians.\(^{35}\) For example, there is a huge gap regarding the exposure impacts of glyphosate formulations on amphibian reproductive systems.

It is essential to characterize the strength of association between the presently used aquatic herbicides like glyphosates and various morphological malformations that are currently observed in the wild. This study therefore assessed the potential impacts of selected glyphosate formulations on gonadal and reproductive development in developing \(X.\) \(\text{laevis}\) tadpoles using extended Xenopus Metamorphosis Assay protocol. Three glyphosate formulations including Roundup, Kilo Max and Enviro Glyphosate, that are utilized to manage aquatic weeds as part of the South African Working for Water Project, were tested.

**Materials and Methods**

**Test Chemicals.** The glyphosate formulations tested include Roundup (360 g/acid equivalent (a.e.)/L Monsanto South Africa Ltd), Enviro Glyphosate (360 g a.e./L, Enviro Industries Ltd, South Africa) and Kilo Max (700 g a.e/kg, Volcano Agro-science Ltd, South Africa). The Roundup formulation contains 360g glyphosate/litre and poly-ethoxylated tallow amine as surfactant. The Kilo Max formulation contains 700 g/L glyphosate sodium salt with an undisclosed surfactant. The Enviro Glyphosate formulation contains 480 g/L isopropyl ammonium salt of glyphosate and polyethylene alkylamine as surfactant.

**Test Organisms**

**Care of Xenopus laevis and Breeding of Tadpoles.** Two mature males and two females were selected from our in-house breeding stock of \(X.\) \(\text{laevis}\). They were separately maintained in 15 L glass tanks containing buffered (2.5 g sea salt/10 L) reverse osmosis water and fed three times per week with fish pellets (Aqua-Nutro, South Africa). Following the international breeding protocol,\(^{36}\) the breeding induction was performed, where the males and females were first primed with 100 IU human chorionic gonadotropin (Merck Ltd Germany), injected into their dorsal lymph sac. The males and females were given the second treatment of 100 IU and 300 IU, respectively, on the third day, just prior to the mating. A breeding pair was then allocated to a 15 L exposure tank, which was lined with plastic netting (in order to separate the adults from the eggs). The whole breeding tank set-up was then placed in a well-ventilated dark corner away from disturbance. The adults were then removed from the tanks and the eggs adequately aerated. By the second day, the new tadpoles were distributed among different 15 L tanks to prevent overloading, which could disrupt their growth. The tadpoles were fed with Sera Micron (Sera Heinsberg, Germany) from free swimming NF-stage 47-48. Tadpole staging was done following the Nieuwkoop and Faber developmental atlas.\(^{37}\)

**Test Procedure**

**Exposure Set-Up.** At NF stage 51, twenty (20) healthy tadpoles were selected from holding tanks and transferred into new 15 L exposure tanks containing 10 L of buffered RO water. Each of the selected concentrations was represented by two exposure tanks. Using the extended XEMA protocol, the study was carried out under regulated climatic conditions including the following: water temperature of 24 ± 1°C, pH ranging between 7.5 and 8.5, dissolve oxygen concentration of >6.5 mg/L and a 12 h of light and dark photoperiod (\(\text{L}\)\(_{12}\)\(_{12}\)).\(^{38}\) Following initial toxicity exposure studies at NF-stage 48,\(^{39}\) the exposure concentrations in this study were 15, 30 and 45% of 96 h \(\text{LC}_{50}\) at NF 48 of the glyphosate herbicide formulations against \(X.\) \(\text{laevis}\) (Table 1). The exposure medium was completely changed every 3 days. The
mixture of Sera Micron food and water has been tested for both oestrogenicity and androgenicity using yeast oestrogen screen and yeast androgen screen. At the end of the exposure, the young metamorphs were weighed, dissected and assessed for various reproductive malformations, before the carcasses were preserved for future reference.

**Nominal Concentration Test.** Random samples from each of the exposure groups were taken and analysed to confirm the experimental concentrations. In brief, 100 mL of water sample was taken from each of the selected exposure tanks into 100 mL glass bottles. In the case of replicates, 100 mL was taken from each and pooled together, from where a single 100 mL was obtained. The water samples were then placed in an ice pack and transported to the Synexa Life Sciences certified laboratory (Cape Town, South Africa), for analysis within 2 hours after collection. The analysis and quantification were done using liquid chromatography coupled with mass spectrometry. The results of the analysis showed no major difference to the predicted nominal concentrations during the exposure study.

**Survival and Developmental Disruption**

At 96-h post-hatching (NF-stage 46), embryonic survival rate was determined. Disruption in growth (size) was assessed by determining body mass (to the nearest gram) at prometamorphic (NF-stage 55) and at metamorphosis (NF-stage 66). Sex ratios of treated metamorphs were statistically compared to the sex ratios of the control groups. The metamorphs were dissected at the gastrointestinal cavity, which opened up the gonads for malformation assessment (Figure 1). Using a dissecting stereo microscope, the gonads were assessed for gross morphological abnormalities and categorized as either testes or ovaries, or recorded as improper formation of part(s) of the organs using the following characteristics (Table 2).

**Histological Sex Validation**

Histological examination of 10 selected males and females was used to validate the macroscopic views of testes and ovaries. The identification of males was based on the absence of ovarian cavities as well as the growth of germ cells (in seminiferous tubules) in the medullar region (Figure 2(a)). The female identifications were based on the presence of ovarian cavity and the growth of germ cell in the cortical region (Figure 2(b)).

### Table 1. The selected exposure concentrations (based on the 15, 30 and 45% of 96 h LC₅₀ at NF stage 48) for the three glyphosate formulations.

| Formulation       | Concentration (mg a.e./L) |
|-------------------|---------------------------|
| Roundup           | 0, 0.2, 0.4 and 0.6       |
| Enviro Glyphosate | 0, 9, 19 and 28           |
| Kilo Max          | 0, 90, 190 and 280        |

### Table 2. Gonad abnormality index characteristics as reported by Lutz et al.

| Identifier          | Description                                                                 |
|---------------------|-----------------------------------------------------------------------------|
| Adhesion           | Gonads that join to other abdominal tissues                                 |
| Aplasia (agenesis)  | Total lack of gonad development                                             |
| Segmented aplasia   | Longitudinal discontinuous gonads (e.g. tissue separation and extraneous gonadal tissue) |
| Bifurcation         | Division of gonads oriented longitudinally                                   |
| Angular deformity   | Gonads that bend to an excessive degree (e.g. gonad folded)                 |
| Displaced gonads    | Gonads or section of it at an atypical position (e.g. lateral or medially)  |
| Fused               | The fusion of left and right gonads to a varying degree                      |
| Hypertrophy         | Enlargement of gonads (e.g. wider, thickened or enlarged)                    |
| Segmented hypertrophy| Excessive enlargement of one or more areas of the gonads (e.g. mass enlargement, pearling and partly thick) |
| Hypoplasia          | Decrease in gonad’s size (e.g. narrow, slightly narrow and truncated, slightly truncated, thin and margin entire) |
| Segmented hypoplasia| Gonads where one or more parts are excessively reduced, attenuated, or poorly developed but not separated (including partly narrowed, partly thinned, margin slightly, margin partially entire and pearling) |
| Intersex            | Gonads where ovary and testicular tissues are present in separate structures |
| Mixed sex           | Gonads where ovary and testicular tissues are present in the same structure  |
| Translucent         | Gonads that appear to have a low density (e.g. slightly translucent)         |
| Segmental translucent| Section(s) of the gonads appears not so dense                                |
| Malformation index  | The percentage of the observed abnormality                                  |
Data Analysis
Normality and homogeneity of variance was evaluated using Shapiro–Wilk’s and Levene’s tests. Using one way ANOVA, the mean body mass (MBM) at prometamorphosis and at the completion of metamorphosis were compared to the respective control. This was followed by Tukey’s HSD post hoc test for parametric data and Kruskal–Wallis ANOVA and Dunn’s multiple comparison of mean rank test for non-parametric data. Differences in sex ratio at each of the concentrations and the control were analysed with chi square test ($X^2$) using GraphPad online software (GraphPad Software Inc., USA).

Results
Survival
The 96-h post-hatching survival rate of tadpoles was 96%.

Body Mass Effects Results
In the exposure to Kilo Max formulation, the MBM at prometamorphic stage (NF-stage 55) reduced in a dose-dependent manner across the exposure concentrations (Table 3). This reduction in mean body mass was statistically significant ($p < 0.05$) at all exposure concentrations compared to the control exposure. At metamorphosis (NF-stage 66), the MBM was also reduced in a dose-dependent manner, which was statistically significant ($p < 0.05$) at concentrations of 190 and 280 mg/L compared to the control. For the exposure to Roundup formulation, the MBM of the Roundup-treated $X. laevis$ at prometamorphic stage fluctuated with increased concentrations (Table 3). The reduction in body mass was statistically significant ($p < 0.05$) at concentrations of 0.4 and 0.6 mg/L relative to the control treatment (Table 3). At metamorphosis, the MBM reduction was not significant at all exposure concentrations (Table 3). In the exposure to the Enviro Glyphosate formulation, the mean body mass at prometamorphic stage was significantly reduced ($p < 0.05$) at a concentration of 28 mg/L compared to the control. At metamorphosis, the MBM reduced in a concentration-dependent manner and was statistically significant ($p < 0.05$) at 19 and 28 mg/L concentrations compared to the control treatment (Table 3).
Sex ratio effects

In exposure to the Kilo Max formulation, the percentage sex ratio of *X. laevis* shifted towards female-bias in a dose-dependent manner. The ratio increased from 47.5:52.5 (F: M) at 90 mg/L concentration to ratio of 52.5:47.5 (F: M) at concentration of 190 mg/L and 68:32 (F: M) at concentration of 280 mg/L compared to 50:50 (F: M) at the control exposure. The ratio was only significant ($X^2$ test, $p < 0.05$) at 280 mg/L compared to the control (Figure 3).

In the exposure to Roundup formulation, the percentage sex ratio of *X. laevis* showed a U-shaped pattern but were not significantly different at all exposure concentrations compared to the control (Figure 3(b)). For the Enviro Glyphosate formulation, the percentage sex ratio showed a little bias towards the female phenotype. However, none of the sex ratios at all the exposed Enviro Glyphosate concentrations were significantly different compared to the control (Figure 3(c)).

Reproductive malformation effects

For the Kilo Max formulation, numerous abnormalities in a concentration-dependent manner were observed in the treated juveniles. The abnormality indexes (expressing the proportion of malformed relative to normal gonads) ranged from 25 to 45% at concentrations of 90–280 mg/L relative to 10% in the control (Table 4; Figure 4). The observed abnormalities included mixed sex (been the most widespread), aplasia, segmented hypertrophy, gonad folded, tissue separation, protuberance, translucence, segmented hypoplasia, segmented bifurcation and segmented aplasia. In Roundup exposure, various abnormalities were observed

### Table 3. The exposure impacts of the Kilo Max, Roundup and Enviro Glyphosate formulations on MBM at NF-stage 55 and NF-stage 66 of *X. laevis*. Asterisks indicate significant difference relative to the control.

| Herbicide Formulation | Conc. (mg/L) | MBM (g) ± SD @ Prometamorphic (NF-stage 55) | MBM (g) ± SD @ Metamorphosis (NF-stage 66) |
|------------------------|--------------|--------------------------------------------|--------------------------------------------|
| Kilo Max               | 0            | 0.96 ± 0.22                                | 0.84 ± 0.11                                |
|                        | 90           | 0.72 ± 0.13*                               | 0.72 ± 0.10                                |
|                        | 190          | 0.62 ± 0.12*                               | 0.66 ± 0.11*                               |
|                        | 280          | 0.57 ± 0.19*                               | 0.64 ± 0.12*                               |
| Roundup                | 0            | 0.96 ± 0.22                                | 0.84 ± 0.11                                |
|                        | 0.2          | 0.94 ± 0.17                                | 0.82 ± 0.12                                |
|                        | 0.4          | 0.78 ± 0.15*                               | 0.81 ± 0.11                                |
|                        | 0.6          | 0.83 ± 0.22*                               | 0.79 ± 0.11                                |
| Enviro Glyphosate      | 0            | 0.90 ± 0.12                                | 0.82 ± 0.10                                |
|                        | 9            | 0.93 ± 0.12                                | 0.76 ± 0.09                                |
|                        | 19           | 0.80 ± 0.17                                | 0.75 ± 0.10*                               |
|                        | 28           | 0.68 ± 0.16*                               | 0.68 ± 0.16*                               |

Abbreviation: MBM, mean body mass.

Figure 3. Sex ratio of *X. laevis* juveniles exposed to the Kilo Max (a), Roundup (b) and Enviro Glyphosate formulations (c), all compared to their negative control. Asterisks indicate significant difference from the control treatment ($X^2$ square test, $p < 0.05$). The horizontal line indicates a 1:1 50% sex ratio.
on the treated juveniles. The abnormality indexes recorded ranged between 20 and 30% at concentrations of 0.2–0.6 mg/L relative to 10% in the control (Table 4), with abnormalities that included translucence segmented aplasia, narrow hypoplasia, partly narrow hypoplasia, segmented hypoplasia, gonad folded, adhesion, angular deformity and displaced (Table 4; Figure 4). In the case of the Enviro Glyphosate formulation, abnormality indexes of 20–40% at 9–28 mg/L concentrations relative to 10% in control were observed. Just like the other two formulations, Enviro Glyphosate showed very similar abnormalities on the treated juveniles, including slightly translucence, aplasia, and hypoplasia folded gonadal and narrow hypoplasia (Figure 4).

**Discussion**

Glyphosate herbicide formulations currently dominate the agricultural and environmental weed management landscape of the world. Their exposure impact on non-target organisms has therefore continued to generate global interest. This concern is premised on the alleged potential of some of the formulations to exhibit teratogenicity in aquatic organisms, the high toxicity on many critically endangered wildlife species as well as their potential impacts on reproductive organs. This current study therefore assessed the potential reproductive impacts of three glyphosate formulations including Roundup, Kilo Max and Enviro Glyphosate on the gonadal development of amphibians using *X. laevis* as model.

The results showed that these three selected glyphosate formulations have differential negative exposure impacts on the reproductive system of the *X. laevis* causing abnormalities.

### Impacts on Body Mass

All three formulations affected the body mass of the treated *X. laevis*. Although all three formulations reduced the body mass of the treated *X. laevis* at prometamorphic stage, the reduction was sustained till metamorphic stage in both the Kilo Max and Enviro Glyphosate treatments but not in Roundup treatment, all relative to the control. The reduction in body mass of the treated *X. laevis* is consistent with several other scientific results. As noted in a previous study, significant reduction in body size was observed in *X. laevis*, leopard frogs and green frogs after 96 h exposure to a glyphosate herbicide. Low growth rate and smaller body size at metamorphosis would affect the survival, immune-competence and territorial defence of amphibians. The small size organisms are also at risk of decreased egg clutch size and fewer potential breeding attempts although life, which could all have larger impacts on growth and sexual development, and the population dynamics of the amphibian.

### Sex Ratio

The significant shift towards female-biased sex ratios in the Kilo Max formulation of 68:32 (F:M) relative to a ratio of

### Table 4. Types of reproductive malformations, abnormality index and % sex ratio at graded concentrations of the Kilo Max, Roundup and Environ Glyphosate formulations.

| Herbicide       | Conc. (mg/L) | No of Morphological Malformations                                                                 | Malformation Index (%) | % Sex Ratio, F:M |
|-----------------|--------------|--------------------------------------------------------------------------------------------------|------------------------|------------------|
| Kilo max        | 0            | Narrow hypoplasia (1) and hypoplasia (1)                                                        | 10                     | 50:50            |
|                 | 90           | Aplasia (1), segmented aplasia (1), segmented hypoplasia (1) and slightly translucence (2)       | 25                     | 47.5:52.5        |
|                 | 190          | Tissue separation (1), mixed sex (3), protuberances (1), translucence (1), segmented aplasia (1) and segmented hypoplasia (1) | 25                     | 52.5:47.5        |
|                 | 280          | Aplasia (2), segmented bifurcation (1), segmented hypertrophy (1), translucence (4), segmental aplasia (1) and mixed sex (5) | 45                     | 68:32            |
| Roundup         | 0            | Aplasia (1) and hypoplasia (1)                                                                  | 10                     | 50:50            |
|                 | 0.2          | Aplasia (1), segmented aplasia (1), segmented hypoplasia (1) and folded gonadal (1)            | 20                     | 45:55            |
|                 | 0.4          | Aplasia (1), segmented aplasia (1), narrow hypoplasia (1), segmented hypoplasia (1) and folded gonadal (1) | 25                     | 45:55            |
|                 | 0.6          | Translucence (2), segmented hypoplasia (1), narrow hypoplasia (1), aplasia (1) and adhesion (1) | 30                     | 47.5:52.5        |
| Enviro Glyphosphate | 0            | Hypoplasia (2)                                                                                  | 10                     | 52.5:47.5        |
|                 | 9            | Slightly translucence (1), segmental aplasia (1), segmented hypoplasia (1) and aplasia (1)       | 20                     | 52.5:47.5        |
|                 | 19           | Slightly translucence (1), hypoplasia (1), segmental aplasia (1) and aplasia (2)                | 25                     | 52.5:47.5        |
|                 | 28           | Folded gonadal (3), segmented hypoplasia (1), aplasia (2), segmental aplasia (1) and narrow hypoplasia (1) | 40                     | 55:45            |
Figure 4. Various morphological abnormalities in reproductive gonads of *X. laevis* juveniles exposed to Roundup, Kilo Max and EnviroGlyphosate formulations. Description of abnormalities was based on Lutz et al. 

A- Normal testis  
B- Normal ovary  
C- Fused gonad  
D- Translucence  
E- Slight translucence  
F- Segmented aplasia  
G- Narrow hypoplasia  
H- Narrow  
I- Angular deformity  
J- Segmented aplasia  
K- Truncated  
L- Aplasia  
M- Tissue separation  
N- Narrow hypoplasia  
O- Mixed sex  
P- Mixed sex
formulation accounted for the majority of the exposure
studies have shown that the surfactants in the Roundup
increase the contribution of their different surfactants. Several
formulations relative to that of the Kilo Max may be the
increasing exposure to surfactants. This further showed the contri-
butions of the different surfactants in each of the formulations.tions tested.

Even though the possible underlining mechanisms be-
hind the reproductive organs’ malformations are not clear,
yet, disruption in sex hormone signalling may have been
involved. This is because a previous study has demon-
strated that Roundup and glyphosate disrupts repro-
duction by altering the expression of the mRNA for
different steroidogenic enzymes in male and female
zebrafish, also causing decreased egg production. This
decrease in egg production could be a function of a
disruption in sex hormone synthesis and part of the cause
of the observed morphological disruption in the present
study. Reddy et al. also pointed out that glyphosate
exposure decreased testosterone and increased oestra-
diol, in a way that leads to a slight increase in oestriadiol
to testosterone (E:T) ratio, potentially due to altered
aromatase activity. The observed morphological dis-
ruptions as observed in this study may therefore in part be
related to abnormal aromatase activity, but further in-
vestigation is required.

In general, the observed gonadal abnormalities as ob-
erved in this study could lead to critical reproductive dys-
function in exposed population that will invariably reduce
their reproductive fitness and the breeding success. The
long-term exposure impacts of these reproductive
malformations could lead to population decline, not only
locally but also on a global scale.

Conclusions

This study showed the potential capacities of glyphosate
formulations (Roundup, Kilo Max and Environ Glyphosate)
to negatively impact the reproductive fitness of X. laevis.
Given the array of gonadal malformations observed in re-
sponse to exposure to these formulations, it is very im-
portant to understand the physiological and reproductive
impacts of these malformations, particularly whether it
affects reproductive fitness and success within the larger
population. The observed effects include reduced growth
rate as demonstrated following exposures to the three formulations, the feminization following exposure to the Kilo Max formulation, the sex ratio modulation by Kilo Max, as well as differential impaired gonadal development observed for all three formulations. The observed impaired gonadal development in this study is possibly associated with hormonal disruption in the treated metamorphs, and an indication of potential endocrine disruption by the glyphosate formulations.

It is difficult to define the physiological impacts of the gonadal abnormalities observed in the present study on the amphibians and influence on reproductive success. Further research assessing the influence of glyphosate herbicide exposure on reproductive success within and across generations is needed. More importantly, more attention should be given on other aquatic herbicides because it is clear that they may have more negative impacts on animal reproductive potential than previously thought. The fact that several exposure impacts of many of these herbicide formulations have been linked to their surfactants suggests that effort must be made to reduce the use of the currently toxic surfactants, and less toxic, and wildlife friendly formulations should be developed.

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Authors contribution

JH supervised the whole research and project; JH and OO conceptualized and designed the experiment; funding acquisition was by JH; OO carried out the laboratory work; JC did the data analysis and OO wrote the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

Xenopus laevis used for this study were collected, cared for and treated under strict compliance with all ethical practices and law. The breeding of tadpoles and all other procedures were approved by the Animal Research Ethical Committee of the Stellenbosch University (Approval No: SU-ACUM 12-00015).

Data accessibility

The data are available online in the doctoral thesis of the lead author.

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Table A.1. The nominal herbicide concentrations, using LC/MSMS (Synexa laboratory in Cape Town). The limit of detection is 0.001 μg/L.

| Roundup | Introduced | Detected | Kilo Max | Introduced | Detected | Environ Glyphosate | Introduced | Detected |
|---------|------------|----------|----------|------------|----------|-------------------|------------|----------|
| 0       | 0          | 0        | 0        | 0          | 0        | 0                 | 0          | 0        |
| 0.2     | 0.18       | 90       | 90       | 188        | 9        | 8.8               | 19         | 18.7     |
| 0.4     | 0.39       | 190      | 188      | 277        | 28       | 27.4              | 18.7       |          |
| 0.6     | 0.6        | 280      | 277      | 28         | 24       |                    |            |          |