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Formulants of glyphosate-based herbicides have more deleterious impact than glyphosate on TM4 Sertoli cells

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ABSTRACT

Roundup and Glyphogan are glyphosate-based herbicides containing the same concentration of glyphosate and confidential formulants. Formulants are declared as inert diluents but some are more toxic than glyphosate, such as the family of polyethoxylated alkylamines (POEA). We tested glyphosate alone, glyphosate-based herbicide formulations and POEA on the immature mouse Sertoli cell line (TM4), at concentrations ranging from environmental to agricultural-use levels. Our results show that formulations of glyphosate-based herbicides induce TM4 mitochondrial dysfunction (like glyphosate, but to a lesser extent), disruption of cell detoxification systems, lipid droplet accumulation and mortality at sub-agricultural doses. Formulants, especially those present in Glyphogan, are more deleterious than glyphosate and thus should be considered as active principles of these pesticides. Lipid droplet accumulation after acute exposure to POEA suggests the rapid penetration and accumulation of formulants, leading to mortality after 24 h. As Sertoli cells are essential for testicular development and normal onset of spermatogenesis, disturbance of their function by glyphosate-based herbicides could contribute to disruption of reproductive function demonstrated in mammals exposed to these pesticides at a prepubertal stage of development.

1. Introduction

Roundup® Bioforce (R) or Glyphogan (Gan) commercial formulations are non-selective herbicides containing 360 g/L of glyphosate (G) and formulants such as polyethoxylated detersive petroleum compounds like polyethoxylated alkylamines (POEA) (Benachour et al., 2007; Mesnage et al., 2013). At certain concentrations, G inhibits the shikimic acid pathway involved in aromatic amino acid biosynthesis, and consequently induces plant death. Formulants may exert herbicidal activity in their own right, and assist with G solubilization, penetration in plants and stability (Cox, 1998, 2004; Seralini, 2015). G and its metabolite aminomethylphosphonic acid (AMPA), as well as formulants, are major contaminants of surface waters (IFEN, 2007; ANSES, 2013) and are found in air, feed and food (Takahashi et al., 2001; Acquavella et al., 2004; Cox and Surgan, 2006; Székács and Darvas, 2012). G has also been detected in the tissues, blood and urine of either humans or animals exposed directly or indirectly *via* food, water or air to herbicides (Acquavella et al., 2004; Curwin et al., 2007; Aris and Leblanc, 2011; Mesnage et al., 2012; Niemann et al., 2015).

G and/or R can induce apoptosis or necrosis in mammalian cells (Richard et al., 2005; Benachour and Seralini, 2009; Clair et al., 2012; Liz Oliveira Cavalli et al., 2013; Mesnage et al., 2013; Cattani et al., 2014). G and R are responsible for oxidative damage, enzymatic disorders and lipid peroxidation (Gehin et al., 2005; El-Shenawy, 2009; Gasnier et al., 2010; Liz Oliveira Cavalli et al., 2013). Studies have shown that formulations of glyphosate-based herbicides are more cytotoxic than G alone and suggest that formulants aggravate cell damage (Liz Oliveira Cavalli et al., 2013). Formulants of glyphosate-based herbicides are declared as inert diluents but some are more toxic than G, such as the family of POEA compounds (Adam et al., 1997; Tsui and Chu, 2003; Marc et al., 2005; Mesnage et al., 2013; Defarge et al., 2016). These formulants are ethoxylated adjuvants, which can insert

Abbreviations: AMPA, aminomethylphosphonic acid; cDH, complemented DMEM/HamF12; CDNB, 1-chloro-2,4-dinitrobenzene; DMEM, Dulbecco's Modified Eagle's Medium; DMSO, Dimethyl sulfoxide; DTT, Dithiothreitol; EDTA, Ethylenediaminetetraacetic acid; G, glyphosate; Gan, glyphogan; GSH, reduced glutathione; GST, Glutathione-S-transferase; LC50, lethal concentration 50 (50 % of mortality); MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; PBS, Phosphate Buffer saline; POEA, polyethoxylated alkylamine; POE-15, POE (15) tallowamine; R, Roundup® Bioforce; SD, succinate dehydrogenase

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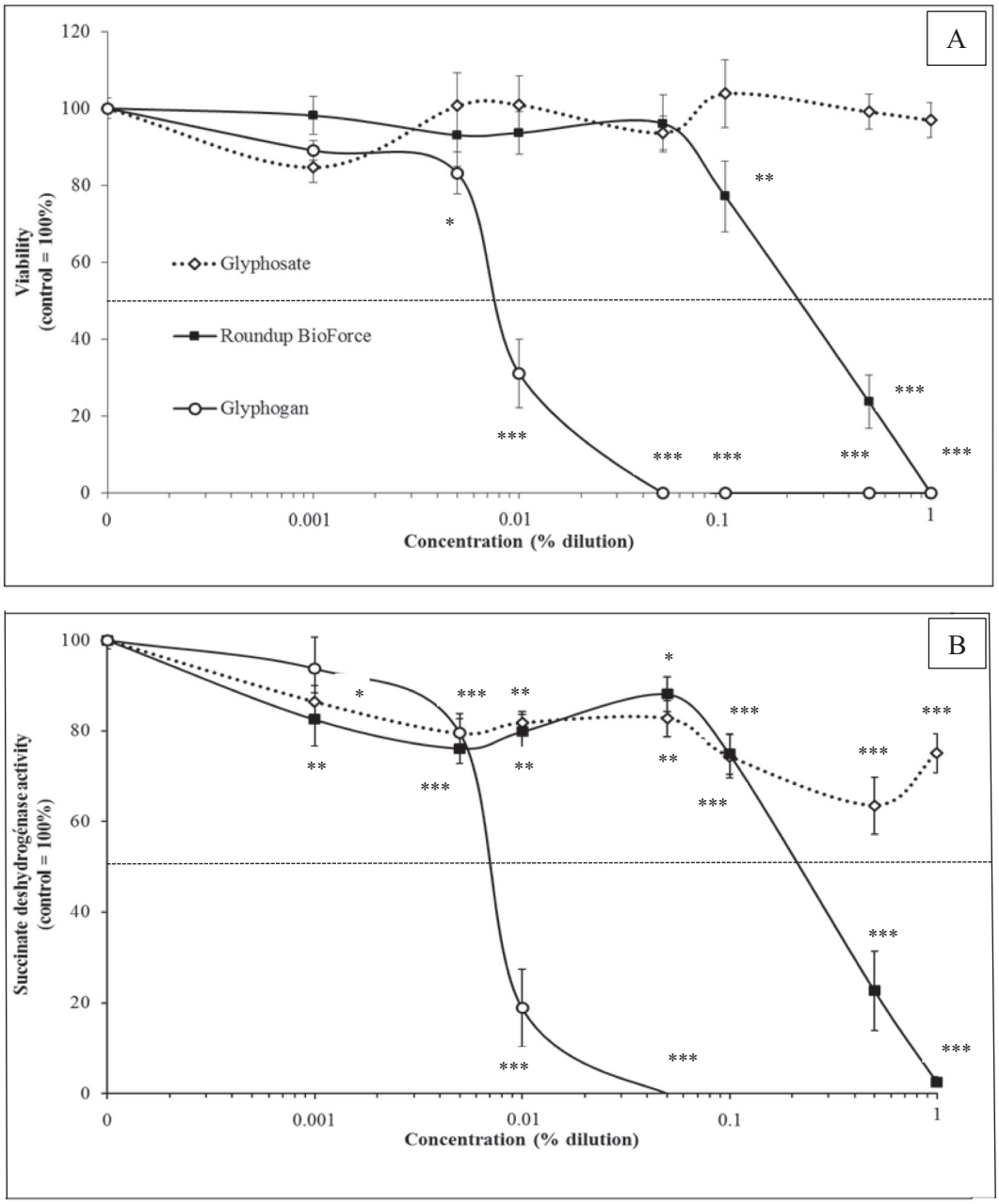

Fig. 1. Effects of Roundup, Glyphogan or Glyphosate on TM4 viability after 24 h of treatment. Cells were grown at 37 °C (% CO_2, 95% air) in complemented DMEM/Ham F12 medium (cDH) for 24 h until 80% confluent. Then, cells were exposed to different dilutions of glyphosate formulations (Roundup Bioforce® or Glyphogan) or equivalent doses of Glyphosate in cDH for 24 h. Cytotoxicity of Roundup Bioforce®, Glyphogan or Glyphosate alone were evaluated using the Crystal violet (A) and MTT (B) assays. A value of 0% of succinate dehydrogenase activity reveals total cell death (B). SEM are shown in all instances (Anova test $p < 0.05^{*}$, $p < 0.01^{**}$ and $p < 0.001^{***}$). The LC50 of Roundup Bioforce and Glyphogan are indicated by the empty square above the curves.

into cell membranes, disrupting their structure and function (Nobels et al., 2011) and have the ability to penetrate into cells (Mesnage et al., 2013).

In mammals, and rats in particular, the respiratory, hepatic, renal, cardiovascular and brain systems can be altered by R (Adam et al., 1997; Daruich et al., 2001; Beuret et al., 2005; Seralini et al., 2014; Gress et al., 2015; Larsen et al., 2016; Mesnage et al., 2017). Sperm production, sperm quality and libido (Yousef et al., 1995; Dallegrave et al., 2007; Romano et al., 2012; Abarikwu et al., 2014; Cassault-Meyer et al., 2014; Lopes et al., 2014), pregnancy (Savitz et al., 1997; Daruich et al., 2001; Beuret et al., 2005), and fetal development (Chan and Mahler, 1992; Yousef et al., 1995; Dallegrave et al., 2003) including reproductive development (Romano et al., 2012), are affected by this herbicide. Alterations of the structure of the testis and/or epididymis have also been demonstrated (Oliveira et al. 2007, Romano et al., 2010). Studies have shown that R affects reproduction in animals by endocrine disruption (Oliviera et al., 2007; Romano et al., 2010; Abarikwu et al., 2014), which is known to have an impact on survival and physiological function of testicular cells (Carreau and Hess, 2010).

Prepubertal exposure of male rats to R alters testicular morphology (reduction of seminiferous epithelium height) and serum testosterone concentration (Romano et al., 2010). Acute R administration at low doses induces oxidative stress and activates multiple stress-response pathways, leading to cell death in prepubertal rat testis including Sertoli cells (de Liz Oliveira Cavalli et al., 2013). Mature rat Sertoli cells, as Leydig and germ cells, are also sensitive to R. Their insensitivities to G