



Glyphosate toxicity for animals

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Received: 24 October 2017 / Accepted: 7 December 2017
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Abstract

Pesticides and herbicides gained popularity due to a strong need to curb the starvation of billions of humans. Glyphosate is the most commonly used herbicide and was considered to be non-toxic. But its use in excess in agricultural lands has polluted soils and waters. Nowadays, glyphosate residues are found in soil, water and food. As a result glyphosate causes severe acute and chronic toxicological effects. We review toxicological effects of glyphosate and metabolites on organisms of the kingdom animalia, both unicellular and multicellular organisms. Adverse effects on unicellular organisms have been established in many experiments. For instance, glyphosate has reduced the rate of photosynthesis in *Euglena*, has decreased the radial growth of mycorrhizal fungal species and is also reducing the profusion of certain bacteria present in rhizospheric microbial communities. Glyphosate poses serious threat to multicellular organisms as well. Its toxicological effects have been traced from lower invertebrates to higher vertebrates. Effects have been observed in annelids (earthworms), arthropods (crustaceans and insects), mollusks, echinoderms, fish, reptiles, amphibians and birds. Toxicological effects like genotoxicity, cytotoxicity, nuclear aberration, hormonal disruption, chromosomal aberrations and DNA damage have also been observed in higher vertebrates like humans.

Keywords Glyphosate · Toxicity · Excessive use · Herbicide · Environmental contamination

Introduction

Agrochemicals have become global necessity to increase crop productivity in agricultural fields. Nowadays, they play a pivotal role in controlling not only the pests and rodents but also many microbial infections. There are several types of herbicides, insecticides and pesticides that are in use in the modern cultivation of lands. Sadly, the surge in human needs and the greed for enhanced production of food yields has resulted in excessive consumption of these agrochemicals. Astonishingly, the initial use of pesticides began along

with the “agricultural evolution” of mankind. According to definition of US Environmental Protection Agency, pesticide is any substance proposed for repelling, destroying, preventing, regulating or controlling pests (Taylor et al. 2007). Originally, natural and organic pesticides were used for pest control. However, after World War II, there was starvation all around and in order to boost the fight against hunger and malnutrition there was an urgent need to augment the crop productivity. This excessive demand from the contemporary agricultural infrastructure motivated the scientific fraternity to invent many synthetic chemicals which could shoot up the crop productions manifolds. The need of hour and the accomplishment of modern pesticide industry persuaded the widespread recognition of these synthetic chemicals around the world, and it led to subsequent dependence on them (Fishel and Ferrell 2013). Soon after its discovery in 1970, glyphosate (N-(phosphonomethyl) glycine) was initially accepted as an herbicide in 1974. Since then, it has globally become the most prominent herbicide. Looking into history, it was synthesized by Henri Martin of Swiss Pharmaceutical Company (Cilag). But its herbicidal properties were analyzed later on by John. E. Franz of Monsanto Company (Gill et al. 2017). Glyphosate is a broad spectrum, post-emergent,

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systemic and non-selective herbicide. It is used to kill several annual and perennial plants (Tu et al. 2001). Glyphosate-based herbicides are used to kill unwanted weeds from farmlands, but along with them, they also quell all the plants which are not genetically resistant to them. Glyphosate alone is not used as an herbicide; in fact it is always blended with different surfactants to increase its perforation into plant cells which adds toxicity to it (Monsanto International and Monsanto Europe 2010). It is used to repress superfluous plants (mostly weeds) and clear the space for the growth of vegetation in fields apart from enhancing the plantation in the parks, forests, railway lines, public streets and gardens. In spite of its tremendous benefits in controlling the weeds and having a reputation of being least toxic pesticide (Franz et al. 1997), its overuse has severely affected other nontarget organisms present in soil biota (Friends of Earth Europe 2013).

From various surveys conducted to determine the quantum of usage of glyphosate, it has been found that by 2014, annual consumption of glyphosate has increased to 240 million pounds. The annual consumption of glyphosate in last two decades has increased substantially (Fig. 1), and it is the most commonly used herbicide in USA (Myers et al. 2016). In other countries like Germany and Denmark, 35–39% of the agriculture depends on glyphosate (Steinmann et al. 2012). In Argentina, as well, glyphosate is the most frequently used herbicide, with annual usage of 180–200 million liters (Nedelkoska and Low 2004). However, with the evolution of glyphosate-resistant crops, the farmers all around the world have been forced to increase the use of this herbicide manifold (CCM Information International Report 2011; Sansom 2012).

Earlier, it was contemplated to be non-carcinogenic in nature (Duke and Powles 2008). Ironically, a latest report by World Health Organisation (WHO) and Food and Agriculture Organisation (FAO) proved that glyphosate is responsible for non-Hodgkin's lymphoma in some case-control studies. However, large sample sizes have not yet shown any positive verification for this statement. The report also manifests that glyphosate is non-carcinogenic at lower doses, but it could not negate the possibility of cancer in rats at higher doses (Gill et al. 2017). Toxicity mechanisms of glyphosate are quite complicated and vary with its various formulations. Empirically, glyphosate has also shown very low oral and dermal toxicity, whereas, its toxicity by intraperitoneal route is very much evident (Bradberry et al. 2004).

Mode of action

Glyphosate kills the plant by hampering the biosynthesis of essential aromatic amino acids required for its growth. It hinders the production of enzyme

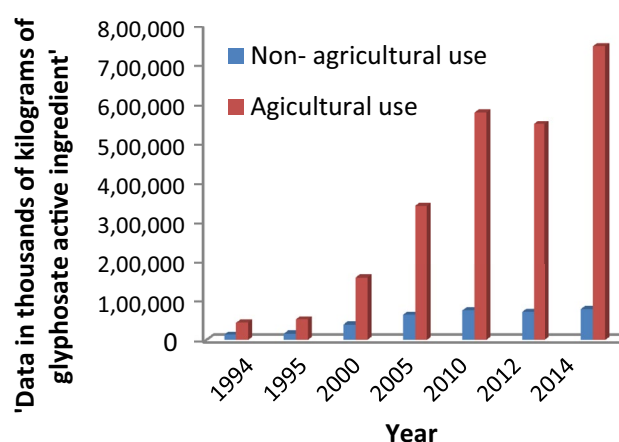


Fig. 1 The annual consumption of glyphosate throughout the world in the last two decade (from 1994 to 2014). Values shown are for global agricultural and non-agricultural use of glyphosate. Data from Benbrooke (2016)

5-enolpyruvylshikimate-3-phosphate synthase of shikimate pathway. Shikimate pathway is a metabolic pathway present in plants for the biosynthesis of aromatic amino acids (Gill et al. 2017). 5-Enolpyruvylshikimate-3-phosphate synthase is liable for the biogenesis of chorismate. Chorismate is the intermediate in the synthesis of aromatic amino acids (tryptophan, phenylalanine and tyrosine) (Williams et al. 2000). Glyphosate acts as antagonistic analog of phosphoenolpyruvate which acts as active substrate for 5-enolpyruvylshikimate-3-phosphate synthase. Scarcity of the enzyme leads to the deficiency of aromatic amino acids, which affects various metabolic functions of the plant and hence destroys the plant (Tu et al. 2001).

Glyphosate translocation and uptake by plants

Glyphosate is promptly taken up by plant surfaces (Kirkwood et al. 2000). It is diffused through plant cuticle. The rate at which leaf imbibes glyphosate varies from species to species. Glyphosate has unique physicochemical properties due to which it is transferred from leaf, by means of phloem, to the tissues like roots, tubers and bulbs which are also the metabolic sinks of sucrose in plants (Siehl 1997). The phytotoxicity of glyphosate accomplishes meristems, storage organs, young roots, leaves and other growing tissues of the plant. Glyphosate shows its efficiency due to its excellent uptake by the plant, brilliant translocation to meristems, partial degradation and slow mode of action (Geiger et al. 1999).

Sorption of glyphosate in soil

When glyphosate combines with soil, that is if applied directly to the soil surface, released from plant roots or emitted from decomposed plant, it undergoes various chemical and physical changes which control its retention, transport and degradation. Retention of glyphosate in soil plays an important role, as it commands the accessibility of glyphosate for degradation, plant uptake and offsite transfer (Duke et al. 2012). Sorption of glyphosate to the soil is very large as compared to other pesticides, as it is a polyprotic molecule with three polar functional groups (amino, phosphate and carboxyl group) (Gill et al. 2017). Its sorption occurs on minerals, organic matter, variable charged surfaces (such as iron and aluminum oxides, aluminum silicates) and goethite. The rate of sorption increases with the increase in the surface area of the minerals and decreased pH (Duke et al. 2012). Once the glyphosate is sorbed in the soil, it is not easily desorbed. Desorption and sorption are inversely proportional to each other (Mamy and Barriuso 2007). Desorption rate of glyphosate from the soil is very less and depends on the type of soil. About 5–24% of initially sorbed glyphosate is desorbed and the rest remains bounded in the soil (Al-Rajab et al. 2008). Due to this, only a slight amount of glyphosate is left available in the soil for plant uptake, degradation and interaction with metal cations (Gill et al. 2017). Glyphosate molecule has many active donor sites, so it can form chelates and complexes with metal ions present in the soil. Cu and Zn ions get strongly complexed with glyphosate, whereas Fe, Ca, Mg and Mn ions get complexed to lesser amount (Vereecken 2005).

Degradation of glyphosate in soil

Glyphosate is degraded by microorganisms present in the soil. Biological degradation pathway involves the cleavage of glyphosate to glyoxylate and aminomethylphosphonic acid by the enzyme glyphosate oxidoreductase. It also gets degraded to methylamine and inorganic phosphate in the presence of enzyme C-P lyase. Further, both methylamine and glyoxylate are consumed by the microorganisms. Glyphosate can also be converted to aminomethylphosphonic acid and glyoxylate in the presence of glycine oxidase (Pollegioni et al. 2011). Another degradation pathway involves the cleavage of glyphosate to inorganic phosphate and sarcosine by enzyme C-P lyase. Sarcosine is further degraded to formaldehyde and glycine which are utilized by microorganisms present in soil (Dick and Quinn 1995). Glyphosate is strongly adsorbed by the soil, so its degradation by the microorganisms is quite slow. Its average half life in soil is 2 months. Degradation rate of glyphosate is

affected by the type of microbial community present in the soil (Tu et al. 2001). It is easily degraded by the enzymes released by microbes, which help in the cleavage of C-P bond of glyphosate molecule. Similar type of metabolic processes was investigated in a *Pseudomonas* PG2982 strain that break glyphosate into phosphorous (Jacob et al. 1985; Moore et al. 1983). Other microorganisms like *Rhizobium meliloti*, *Arthrobacter* GLP-1 strain, *Agrobacterium radiobacter* and *Rhizobium* strains also show analogous pathway for glyphosate degradation (Dick and Quinn 1995; Liu et al. 1991; McAuliffe et al. 1990; Pipke et al. 1987). Another bacterial strain *Arthrobacter* GLP-1/Nit-1 utilizes glyphosate as nitrogen source (Pipke and Amrhein 1988). *Streptomyces* spp. consumes glyphosate for both phosphorus and nitrogen (Obojska et al. 1999). The presence of phosphorus in glyphosate is responsible for its microbial degradation, as phosphorus is required by the microorganisms for their metabolic functions (Lane et al. 2012).

Environmental profile of glyphosate

Glyphosate binds the soil constituents firmly; hence, it has no soil activity; thus it is foliar-applied and is post-emergent herbicide. It has very little seepage to groundwater and causes minimum contamination. It has fairly short half life, due to its degradation by the microorganisms. It is nonvolatile and does not contaminate atmosphere (Duke and Powles 2008). When used in recommended dose, glyphosate has little or no effect on nontarget organisms except some species of fungi (Franz et al. 1997). It is soluble in water and does not accrue in food web (Lane et al. 2012). Glyphosate changes the activity of various enzymes present in the soil. For example, it decreases the activity of enzyme phosphomonoesterase by 40–70%. In the case of urease and β -glucosidase, the activity was reduced by 5–40%, whereas for the enzyme dehydrogenase the activity was reduced up to 70% at soil pH 6.9. However, it does not affect the activity of enzymes (fluorescein diacetate hydrolase and arylsulfatase) present in soil at pH 6.6 (Riah et al. 2014).

Development of glyphosate-immune crops

Low animal toxicity and high herbicidal activity (Henderson et al. 2010) are the main aspects for the widespread use of glyphosate throughout the world (Sansom 2012; Steinmann et al. 2012; Garthwaite et al. 2010; CCM Information International report CCM International 2011). Excessive use of glyphosate in the farming led to the evolution

of glyphosate-resistant crops in 1996 (Johnson et al. 2009). Thereafter, glyphosate-resistant crops like cotton, soybean and corn were cultivated throughout the world (Duke et al. 2012). With the progression in the field of biotechnology, *Agrobacterium sp.* gene (CP4) was used to conceal glyphosate-resistant 5-enolpyruvylshikimate-3-phosphate synthase. Similarly genes from *Ochrobactrum anthropi* were used to assimilate glyphosate resistance in canola plants (Padgett et al. 1996). Also genetic mutations were performed on maize genes to introduce glyphosate resistance in maize plants (Vande Berg et al. 2008). With the commencement of these tailored transgenic plants in agriculture the use of glyphosate has increased manifold. This resulted in the deterioration of farming systems. Also weed species had become more and more immune to this herbicide (Cerdeira et al. 2011). Consequently, it becomes intricate to stifle them. To overcome the rise of glyphosate-resistant weeds, genetically engineered crops were cultivated that were resistant to more than one kind of herbicide.

Evolution of glyphosate-resistant crops was a major breakthrough in the field of agriculture. Hence, the farmers became more casual and started using glyphosate with the loose hand. Such an over use of glyphosate resulted in the presence of glyphosate residues and its metabolite aminomethylphosphonic acid in many food crops at their harvesting as well as in their processed food (Myers et al. 2016). The overuse of glyphosate is not only causing the development of resistant crops, passing over of residues in food materials but is also creating a significant toxic impact over a wide plethora of organisms in the environment.

Various formulations of glyphosate are available in the market worldwide. Table 1 shows the list of different glyphosate formulations available in the market.

Toxicity of glyphosate over a wide range of organisms

Gratuitous use of glyphosate is not only distressing the weed species but is also causing severe threat to several other nontarget organisms found in the environment (Alliance 1996). It affects the growth and other metabolic functions of many unicellular as well as multicellular organisms found in both soil and water (<http://www.national-toxic-encephalopathy-foundation.org/roundup.pdf>).

Unicellular organisms

(a) *Euglena gracilis* Glyphosate also shows its adverse effects on many single-celled organisms like *Euglena gracilis*. It has been found that the use of glyphosate (at concentration 3×10^{-3} M) decreases the chlorophyll content from 21 to 69%. It also reduces photosynthesis and respiration at levels below 1.2×10^{-4} M by 20% (Richardson et al. 1979).

(b) Mycorrhizal fungal species Glyphosate reduced the radial growth of all ectomycorrhizal fungal species like (*Cenococcum geophilum* Fr., *Pisolithus tinctorius* (Pers.) Coker and Couch and *Hebeloma longicaudum* (Pers.)) at concentrations ≥ 1000 ppm, and their growth was completely inhibited at concentrations ≥ 5000 ppm. *Cenococcum geophilum* Fr. species was least sensitive to the glyphosate (Estok et al. 1989).

Toxic effects of glyphosate were also studied on some of the common mycorrhizal fungal species like *Hebeloma crustuliniforme*, *Laccaria laccata*, *Thelephora americana*, *T. terrestris* and *Suillus tomentosus*. It has been found that glyphosate reduced the growth of these fungal microorganisms at concentrations above 10 ppm (Chakravarty and Sidhu 1987).

(c) Rhizospheric microbial communities Glyphosate shows negative impact on the growth of certain rhizospheric microbial communities. Bacteria like *Fusarium*, fluorescent *pseudomonads*, Mn-transforming bacteria, and indoleacetic acid-producing bacteria present in the rhizosphere soils of soybean were treated with glyphosate. Glyphosate increased the profusion of *Fusarium spp.* while it reduced the profusion of fluorescent *pseudomonads*, Mn-reducing bacteria and indole acetic acid-producing rhizobacteria (Zobiolo et al. 2011).

Another research group also studied the effects of glyphosate on soil rhizosphere-associated communities and found that the application of glyphosate increases the relative abundance of *proteobacteria* (particularly *gammaproteobacteria*). But the excessive use of glyphosate on glyphosate-resistant crops like corn and soybean decreases the relative abundance of *Acidobacteria*. Since *Acidobacteria* are also implicated in biogeochemical processes, the decline in the profusion of these bacteria could lead to considerable changes in nutrient status of the rhizosphere and would affect plant growth (Newman et al. 2016).

(d) Poultry microbiota Further the effects of glyphosate on some common pathogens and useful members of poultry microbiota were studied in vitro, and it had been found that extremely pathogenic bacteria like *Salmonella gallinarum*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Clostridium perfringens* and *Clostridium botulinum* are resistant to

Table 1 Common formulations of glyphosate used worldwide

S. No	Brand name	Active ingredients
1.	Roundup® Renew	360 g/L Glyphosate
2.	Watson weed killer 360 concentrate	Glyphosate isopropyl amine salt (360 g/L)
3.	McGregor's Weedout	Glyphosate isopropylamine salt (48%)
4.	Agpro Glyphosate 360	Glyphosate isopropylammonium salt (360 g/L)
5.	Dow Glyphosate 360	Glyphosate isopropylamine salt (360 g/L)
6.	Clearout 180	Glyphosate isopropyl ammonium salt
7.	Clearout 360	Glyphosate isopropyl ammonium salt
8.	Cobra	Glyphosate isopropyl ammonium salt
9.	Erase	Glyphosate isopropyl ammonium salt
10.	Glygran SG	Glyphosate ammonium salt
11.	Glyphofix	Glyphosate isopropyl ammonium salt
12.	Glyphogan	Glyphosate isopropyl ammonium salt
13.	Glyphosate WSG	Glyphosate-sodium
14.	Kalach	Glyphosate-sodium
15.	Mamba	Glyphosate isopropyl ammonium salt
16.	Mamba MAX	Glyphosate isopropyl ammonium salt
17.	Nexus Glyphosate	Glyphosate isopropyl ammonium salt
18.	Rondo	Glyphosate-sodium
19.	Roundup	Glyphosate isopropyl ammonium salt
20.	Roundup Bio-dry	Glyphosate-sodium
21.	Roundup CT	Glyphosate isopropyl ammonium salt
22.	Roundup Turbo	Glyphosate isopropyl ammonium salt
23.	Roundup Ultra	Glyphosate isopropyl ammonium salt
24.	Slash Turbo	Glyphosate isopropyl ammonium salt
25.	Sting	Glyphosate isopropyl ammonium salt
26.	Touchdown	Glyphosate + trimesium
27.	Touchdown plus	Glyphosate + trimesium
28.	UAP Glyphosate	Glyphosate-sodium
29.	Back draft	Glyphosate + imazaquin
30.	Expert	Glyphosate + S-metolachlor + atrazine
31.	Extreme	Glyphosate + imazethapyr
32.	Flexstar GT	Glyphosate + fomesafen
33.	Sequence	Glyphosate + S-metolachlor

glyphosate. But the vulnerability of glyphosate toward the beneficial bacteria of poultry like *Enterococcus faecalis*, *Enterococcus faecium*, *Bacillus badius*, *Bifidobacterium adolescentis* and *Lactobacillus spp.* varies from species to species. Some of these microorganisms are highly susceptible, while some are moderate. Out of these, *Campylobacter spp.* (which are responsible for gastrointestinal diseases in humans) are highly prone to glyphosate. Intake of glyphosate by the poultry reduces the beneficial bacteria present in the gastrointestinal tract, which could perturb the normal functions of bacterial community present in the gut of these birds (Shehata et al. 2013).

(e) Periphyton communities Runaway of glyphosate from soil to water has also affected the unicellular organisms present in the periphyton communities present in the lakes and rivers. Goldsborough and brown found that glyphosate suppresses the photosynthetic rates in the lentic periphyton

systems at concentrations of 89 and 890 mg/L (Goldsborough and brown 1988). Also, it stimulates certain structural changes in the diatom community present in lotic systems (Sullivan et al. 1981). Furthermore, glyphosate can act as potential phosphorus source for certain microorganisms, which could increase the detrimental eutrophication in water systems. This enhances the algal growth which could indirectly affect the aquatic life (Austin et al. 1991).

Multicellular organisms

Glyphosate's toxicity is not only observed in unicellular organisms but also shows its toxic effects on many multicellular organisms found on both soil and water.

(a) Algae Algae are diverse group of aquatic organisms that have ability to conduct photosynthesis. Wide varieties

of algae are found in aquatic systems. Glyphosate has also shown its toxic effects on various algal species.

Saenz et al. verified the toxicological effects of glyphosate and its commercial formulation Ron-do on two fresh water green algal species, *Scenedesmus acutus* and *Scenedesmus quadricauda*. It was found that the concentration of glyphosate in its commercial formulation (Ron-do) found in water systems does not cause acute toxicity to both these algal species. But this could cause long-term chronic and sublethal effects on *Scenedesmus quadricauda* population. Significant decrease in chlorophyll “a” content was also observed when *S. quadricauda* was exposed to 50 mg/L concentration. However, this herbicide does not signify any harmful chronic effects in the case of *Scenedesmus acutus* (Saenz et al. 1997).

Another research group also studied the effect of glyphosate on different macroalgae and seagrasses species of marine and anchialine aquatic systems (*Gayralia oxysperma*, *Rhizoclonium riparium*, *Ulva intestinalis*, *Pterocladia capillacea*, *Myriophyllum aquaticum* and *Ruppia maritima*). Glyphosate reduces the chlorophyll content in the tested species even at the lowest concentration of 0.45 g L⁻¹. *Pterocladia capillacea* showed maximum sensitivity, while *Gayralia oxysperma* was least sensitive toward this herbicide (Kittle and McDermid 2016).

Negative effects of glyphosate, Roundup and aminomethylphosphonic acid (main degradation product of glyphosate) on macroalgae (*Nitella microcarpa* var. *wrightii*) were studied by Oliveira et al. Three different concentrations (0.28, 3.5 and 6 mg/L) of glyphosate and Roundup were used. It has been found that glyphosate when applied in association with Roundup hampered the photosynthesis process. The rate of inhibition depends on the concentration of the herbicide used and the exposure time. In contrary to glyphosate, aminomethylphosphonic acid stimulates the photosynthesis process in algae. It degrades into phosphorus which provides nutrients to the algae. Excessive use of glyphosate-based herbicides affects both spatial and temporal distribution of *Nitella microcarpa* var. *wrightii* in the ecosystem (Oliveira et al. 2016).

Acute toxicity of glyphosate, isopropyl salt of glyphosate, Roundup (commercial formulation of glyphosate) and its common adjuvant polyoxyethyleneamine (POEA) on two common algal species *Selenastrum capricornutum* and *Skeletonema costatum* were checked by Tsui and Chu. It was found that out of these two algal species *S. costatum* is 7–10 times more sensitive than *S. capricornutum* to glyphosate either in acid form or in its salt form. The LC₅₀ value at 96-h exposure is found to be 2.27 mg/L (glyphosate) and 5.89 mg/L (isopropyl salt of glyphosate) for *S. costatum* species, whereas LC₅₀ values for *S. capricornutum* for glyphosate and its salt form are 24.7 and 41.0 mg/L, respectively. This variation in the LC₅₀ values for these herbicides in both

algal species may be due to phylogenetic structural differences between them (Tsui and Chu 2003).

(b) Invertebrates Glyphosate showed its toxic effects on many invertebrates found on both land and water. No relevant data were found on the toxicity of glyphosate on lower invertebrates like protozoa, porifera, coelenterates and platyhelminthes.

(i) Nematelminthes Nematelminthes are an important class of lower invertebrates. They are mostly parasitic, but their abundance, diversity and correlation with soil indicate the state of the ecosystem. While some of the nematodes cause diseases, but they are also helpful in maintaining the earth’s nutrient cycle and augment the assortment of natural ecosystem (Achiorno et al. 2008). Use of glyphosate in soil systems have also affected the nematodes and caused many toxic effects in them.

Achiorno et al. (2008) appraised the effect of various concentrations of glyphosate (both technical grade and formulation) on *Chordodes nobili*. Embryo, larvae and adults were used for performing the experiment. Test organisms were bared to all the concentrations ranging from 0.1 to 8 mg/L of glyphosate. Larvae hatched from the eggs treated with glyphosate (≥ 0.1 mg/L) do not show any deformity in their development; however, their infective capacity was appreciably decreased. Comparable results were also achieved when larvae were directly exposed to the herbicide. Both the technical grade and formation of glyphosate presented similar kind of results. However, the adult worms exposed to 1.76 mg/L of glyphosate (for 96 h) showed 50% mortality (Achiorno et al. 2008).

(ii) Annelids: Earthworms Earthworms are the essential component of soil biota. They are helpful in maintaining the quality and ecosystem of soil (Datta et al. 2016). Various acute and chronic toxicity tests were conducted by different researchers to evaluate the toxicological effects of glyphosate on these wigglers.

Correia et al. conducted laboratory tests on *Eisenia fetida* to investigate the toxicological effects produced by glyphosate on it. Five different concentrations of glyphosate (1, 10, 100, 500 and 1000 mg) were used as test concentrations. The experiment was carried out for 56 days. No mortality was observed in the soils treated with glyphosate at any of these concentrations. However, steady and considerable decrease in the mean body weight was found at all the test concentrations. Glyphosate revealed severe toxic effects on the reproduction and development of earthworms in the range of test concentrations. No cocoons or juveniles were found in the soil treated with the herbicide. Apart from this, significant anatomical changes were also observed after 30 days of the experiment. Morphological abnormalities like the elevation

of body, coiling and curling were observed in all the specimens exposed to the highest test concentration of soil treated with glyphosate (Correia and Moreira 2010).

Another research group checked the acute and chronic toxicological effects of aminomethylphosphonic acid, the main metabolite of glyphosate on *Eisenia andrei* at field-relevant concentrations. No significant mortality was observed in both acute and chronic assays. In acute toxicity test, momentous loss in the biomass of earthworms was recorded in case of control as compared to the earthworms treated with aminomethylphosphonic acid. However, in chronic test, larger loss in the biomass of earthworms was recorded at the highest concentration of aminomethylphosphonic acid. Also there was an increase in the number of juveniles and cocoons at the highest concentration of the herbicide. But the mean body weight of these juveniles was found to be decreased. These results confirmed that juveniles are more sensitive to aminomethylphosphonic acid than the adults (Domínguez et al. 2016).

Ecotoxicological effects of glyphosate were evaluated on *Eisenia fetida* and *Eisenia andrei*. In the bioassays, earthworms were rendered to the soil samples collected from soya farms (treated with glyphosate), from Argentina. Both behavioral and biological changes were noticed in the test organisms of both species. It was observed that glyphosate decreased the cocoon viability, thereby decreasing the number of juveniles produced. Apart from this, they also avoided the soils treated with glyphosate and show reduction in their feeding activity (Casabe et al. 2007).

Similar kind of studies was conducted by Yasmin and D'Souza on *Eisenia fetida* to check the toxicological effects of glyphosate and other pesticides on it. A regular diminution in the body weight of the test organisms was found, when they were exposed to glyphosate and mixture of glyphosate, carbendazim and dimethoate (Yasmin and D'Souza 2007).

Hazardous effects of commonly used herbicide glyphosate on two annelid species *Eisenia fetida* and *Octolasion tyrtaeum* were studied by García-Torre et al. (2014). Both these test organisms were exposed to five different concentrations of glyphosate. Results reveal that earthworm species *Octolasion tyrtaeum* was more prone to the highest concentration of glyphosate (50,000 mg kg⁻¹). 100% mortality was observed at this concentration after seventh day of treatment. However, in the case of *Eisenia fetida* no mortality was recorded, but a noticeable loss (40%) in the body weight was found. Adverse effects of the herbicide were also found on the adult fecundity and cocoon viability. The number of juveniles produced from the cocoons was also decreased (García-Torre et al. 2014).

Berghausen et al. also assessed the impact of glyphosate-based herbicides on two species of earthworms (*Lumbricus terrestris* and *Aporrectodea caliginosa*). The surface casting

activity of *Lumbricus terrestris* was decreased after three weeks of herbicide application. However, no change in this activity was recorded for other earthworm species (*Aporrectodea caliginosa*). Apart from this, reproduction rate in earthworms of both species was also reduced within 3 months after herbicide application (Gaupp-Berghausen et al. 2015).

Toxicity evaluation of two glyphosate-based herbicides was carried out by comparing their adverse effects on earthworm (*Eisenia andrei*). Glyphosate's commercial formulations, Roundup FG and Mon 8750, were used. Lethal concentration (LC-50) values reveal that Roundup FG was 4.5 times more toxic than Mon 8750. However, at sublethal concentrations noticeable weight loss was observed. Glyphosate acts as uncoupler of oxidative phosphorylation in the mitochondria of earthworms. Roundup FG showed venomous effects on the DNA of test organisms and caused lysosomal damage in them (Piola et al. 2013).

Sublethal effects on the population dynamics of earthworm species *Eisenia fetida* were carried out by Santadino et al. Two different concentrations of the herbicide were used. Glyphosate showed long-term effects on the test organisms with the decrease in the fertility of cocoons. This led to the local extinction of population of the earthworms in the soil (Santadino et al. 2014).

Assessment of the effect of the pesticide to the nontarget organisms present in the soil was done by Santos et al. Three commercial formulations containing insecticides (Chlorpyrifos, Endosulfan) and the herbicide (Glyphosate) were used. Treated soil was collected to verify the avoidance test and reproduction behavior of *Eisenia andrei*. These worms avoided the soil contaminated with Chlorpyrifos and Endosulfan. However, in the case of glyphosate, an equal number of worms were found on both sides indicating that glyphosate does not cause any harm to earthworms if used in recommended dose. Also it does not affect the reproduction activity of the worms (Santos et al. 2012).

Glyphosate molecule has many binding sites due to the presence of different functional groups present in it. It can easily combine with metal ions and form metal complexes. Fan Zhou et al. found that Cu ions present in the soil form complex with glyphosate and reduce the acute toxicity on earthworm caused by Cu ions. This complexation declined the mortality rate in earthworms. Along with this superoxide dismutase (SOD), glutathione (GSH) content and acetylcholinesterase activity were also reduced to the levels of control. These outcomes revealed that the complexation of glyphosate with metal ions present in soil could reduce the toxicity and accessibility of heavy metal ions present in the soil (Zhou et al. 2012).

Another research group used glyphosate-based herbicide Groundclear (containing 5% of isopropylamine salt of glyphosate), to examine its acute toxicity on *Eisenia fetida*. Earthworms were exposed to five different concentrations

of the herbicide; however, the worms exposed to the recommended dose for 24–48 h show very little mortality. But they show avoidance behavior against the herbicide. The presence of herbicide in the soil also affects the locomotor activity of the worms. Thus, the use of herbicide may not directly cause any harm to them, but it can cause severe long-term effects (Verrell and Van Buskirk 2004).

Zaller et al. analyzed the effects of glyphosate-based herbicide on the correlation between earthworms (*Lumbricus terrestris*) and symbiotic mycorrhizal fungi. Herbicide application on the soil decreased the earthworm activity in the mesocosms containing arbuscular mycorrhizal fungi. It further declined the soil mycorrhizal fungi spore biomass, vesicles and reduced the root mycorrhization. This resulted in the poor interactions between the worms and the mycorrhizal fungi which pose a serious threat to the natural systems (Zaller et al. 2014).

(iii) Arthropods Arthropods constitute 90% of the animal kingdom and are one of the biggest groups of invertebrates. They play an important role in maintaining ecological balance, provide livelihood and nutrition to human communities (Whiles and Charlton 2006). Extreme use of herbicides has many direct and indirect effects on them. Different research groups evaluated the impacts of glyphosate on arthropods as follows.

(iii(a)) Crustaceans Crustaceans are the very large group of arthropods found in freshwater and sea water. They form an important part of human diet. Apart from this, they are beneficial to the aquatic ecosystem as they help in the destruction and decaying of microscopic plants present in water. They themselves are eaten up by larger sea animals and maintain the balance of aquatic food chains. Leaching of glyphosate from soil to the water system has affected many aquatic animals and poses severe toxic impacts over them (Pérez et al. 2011). Toxicological impacts of glyphosate on sea animals were evaluated on *Daphnia* (as a test organism) by various researchers.

Daphnia Toxicity level of glyphosate (in its common formulation RON-DO) in water was checked on two planktonic crustacean species of *daphnia*. *Daphnia magna* and *D. spinulata* were exposed to glyphosate at different concentration (18, 32, 54, 90, 150 and 250 mg glyphosate a.i/L) for 24 and 48 h. It has been found that after 24-h exposure to the herbicide all the organisms of both the species become immobilized only at the highest concentration of 250 mg/L. And after 48-h exposure, immobility of organisms was found only at the concentration of 150 mg/L. These results showed that glyphosate is moderately toxic to *daphnia* (Alberdi et al. 1996).

Negative effects of glyphosate and its common formulation Roundup, on *Daphnia magna*, were also analyzed by another research group. They found that both glyphosate and Roundup are toxic to these aquatic invertebrates. Roundup proved somewhat lesser acute toxicity than pure glyphosate. The EC₅₀ values of Roundup are 3.7–10.6 mg/L, while for glyphosate these values vary from 1.4 to 7.2 mg/L. However, Roundup was found to show more chronic toxicity in the tests spanning the complete life cycle of *Daphnia*. It has been found that at minimal test concentration of 0.05 mg/L, there occurs reduction of juvenile size. At 0.45 mg/L of Roundup, the growth, fertility and abortion rate were also affected. Significant negative effects were observed for both these herbicides at concentrations of 1.35 and 4.05 mg/L. Roundup at concentration of 1.35 mg/L showed 100% abortion of eggs. Also at this concentration, the embryonic stages are unfavorably affected (Cuhra et al. 2013).

The effect of common inorganic suspended sediment (Bentonite clay) on the acute toxicity of glyphosate on the aquatic organisms was checked by Hartman and Martin. *Daphnia pulex* was used to evaluate the toxicity level of the herbicide in aquatic systems. It has been established that the presence of suspended sediment increased the short-term toxicity of glyphosate at all the test concentrations. There was a significant decrease in the number of total population of *D. pulex* on exposure to Roundup at different concentrations (with both and without the suspended sediment). However, this reduction in the number of organisms was less in the case of glyphosate solutions without the suspended sediment. Glyphosate showed its selective toxicity to immature individuals, and this reduction in immature population was more when suspended sediments were used (Hartman and Martin 1984).

Toxicity of glyphosate and its commercial formulation Faena on fresh water invertebrate *Daphnia magna* was investigated by Dominguez-Cortinas et al. They found that both glyphosate and Faena are toxic to aquatic nontarget organisms. Faena is found to be 1.7-fold more toxic to *Daphnia Magna* than pure glyphosate. Glyphosate is an organophosphate pesticide and targets the esterases system of animals. It inhibits the activity of this enzyme and affects various metabolic functions in animals. EC₅₀ values for glyphosate are 0.6 μM, 0.1 mg L⁻¹ (Dominguez-Cortinas et al. 2008).

In another toxicity evaluation of glyphosate and its commercial formulation Roundup by Szekacs et al., two different populations of *Daphnia magna* were used. One was obtained from standard laboratory (originated from LAB Research Kft., Veszprem, Hungary) and other was wild species (collected in Pest County, Hungary). Roundup showed acute 48-hour LD₅₀ values in 560–1700 μg/mL range on the laboratory species. However, the wild species was more sensitive toward Roundup and it was found to be twice times sensitive to this herbicide formulation. Toxicity tests on standard

laboratory species of *D. magna* showed that Roundup was 35 times more toxic than glyphosate (Szekacs et al. 2014).

Hyalella castroi Dutra et al. observed the effects of glyphosate's commercial formulation Roundup on the biochemical composition, Na^+/K^+ ATPase activity, levels of lipoperoxidation and reproductive traits in the freshwater amphipoda, *Hyalella castroi*. Test organisms were exposed to four different concentrations of the herbicide (0.36, 0.52, 1.08 and 2.16 mg/L). It was found that glyphosate exposure affected the reproductive activity of the animal. No mating pairs, ovigerous females or eggs in the marsupium of the matured females were observed at any of the tested concentration. Also the survival rate of the organism was lowered at all the concentrations (0.36 mg/L-73.92%, 0.52 mg/L- 61.12%, 1.08 mg/L-55.32% and 2.16 mg/L-47.72%). Glycogen level was also found to decrease in the tested organisms, and this decrease was dose dependent. But the decrease was more intense in males. Protein level was also decreased, and this decrease was again dose dependent. It was found that in the case of male *Hyalella castroi* the total protein content increased with the increase in the glyphosate concentration. Also the lipid level, triglycerides level, cholesterol content, lipoperoxidation and Na^+/K^+ ATPase activity were significantly reduced at all the test concentrations of glyphosate (Dutra et al. 2011).

Crayfish Effects of glyphosate and the common surfactant polyoxyethyleneamine (POEA) were verified on freshwater crayfish (*Cherax quadricarinatus*) by Frontera et al. Sublethal effects of 50-day exposure to glyphosate (22.5 mg/L), polyoxyethyleneamine (7.5 mg/L) and the mixture of both in the ratio of 3:1 were checked on the growth of the juvenile crayfish. Exposure of crayfish to the mixture of glyphosate and polyoxyethyleneamine resulted in lower somatic cell growth and decreased muscle protein level in the fish. It also reduced the muscle glycogen stores and lipid reserves of the fish. Also, an appreciable amount of weight loss was noticed in the fish when exposed to either of these glyphosate or the surfactant polyoxyethyleneamine (Frontera et al. 2011).

Glyphosate toxicity was checked on early juveniles of crayfish, by exposing them for 60 days to two test concentrations (10 and 40 mg/L). 33% mortality was perceived in the crayfish juveniles, exposed to highest concentration of the glyphosate. No significant divergence was noted in the molting process of the animal at any of the test concentrations. Decrease in the weight gain was recorded at both the glyphosate concentrations. However, a significant decrease was observed in the weight gain after first month of exposure at 40 mg/L of glyphosate. Apart from this a considerable decrease was found in the lipid level in muscles and protein level in both hepatopancreas and muscles of the crayfish (Avigliano et al. 2014).

(iii(b)) Insects Insecta is the largest group of invertebrates in phylum arthropoda. In terrestrial ecosystems, insects play a vital role as pollinators, herbivores, seeds dispersers, predators and detritivores. They work as ecosystem engineers.

Honey bee Excessive use of glyphosate has also affected the population of bees all over the world. It kills the potential food source for honey bees by destroying the non-crop plants. Apart from this, it exterminates the beneficial bacteria found in the gut of honey bees (Burlew 2010) and caused many toxicological effects on them. Toxicological impact of glyphosate on honey bees was evaluated by different researchers all over the world.

To analyze the toxicity level of insecticides and herbicides on insects, Boily et al. evaluate acetylcholinesterase activity in honey bees (*Apis mellifera*) by exposing them to maize treated with three different pesticides (neonicotinoids, glyphosate and acephate). Honey bees were revealed to sublethal concentrations of insecticide (neonicotinoids) and herbicides (glyphosate and acephate) under controlled conditions. Abnormal increase in the acetylcholinesterase level was found in the bees treated with neonicotinoids, while slight decrease was recorded in the case of glyphosate. (Boily et al. 2013).

Another research group checked the toxicological effects of glyphosate on honey bee (*Apis mellifera*) by analyzing their appetite behavior. Honey bees were exposed chronically and acutely to the recommended doses of glyphosate. Diminished sensitivity to nectar and poor learning performance were observed in the case of young adult bees treated chronically to the herbicide. However, in acute toxicity test performed at the recommended doses, decrease in the elemental learning and diminished short-term memory retention was examined. In spite of this, non-elemental associative learning was also damaged, though rummage for nectar was not affected by this herbicide. But a serious problem may arise when bees might carry nectar with glyphosate traces in it; this could distress the other nest mates and has all long negative consequences (Herbert et al. 2014).

Effects of sublethal concentrations of glyphosate on honeybee navigation were carefully investigated. Three different sublethal concentrations (2.5, 5.0 and 10 mg L⁻¹) of glyphosate were mixed with sucrose solutions, and bees were nourished with them. Steering path followed by the bees from the source of food to their hives was detected using harmonic radar system. It was observed that honey bees fed with sucrose solution containing highest dose of glyphosate took more time to come to their hives than the control bees. They also show more indirect flights to their nests. These results revealed that glyphosate impaired the intellectual capability of bees, thereby making it difficult for them to return to their nests (Balbuena et al. 2015).

To study the effect of different pesticides on honey bees (*Apis mellifera*), larvae were treated with different concentrations. All the pesticides triggered the apoptosis in the treated larvae. The cell death was detected by DNA fragmentation labeling from midgut, salivary glands and ovaries of the treated larvae. 69% cell death was observed in the midgut of larvae treated with glyphosate (Gregorc and Ellis 2011).

Wasps Bueno et al. evaluated the pernicious nature of pesticides used in soybean crops on the *Trichogramma pretiosum*. Collection of different insecticides and herbicides was appraised, and varying results were obtained from different pesticides. Glyphosate-based herbicide (Roundup Ready) was found to be harmful for the eggs of the parasite but harmless for other parasitoid stages. However, glyphosate's other formulation, Glyphosate 960 (Roundup original) was slightly harmful for eggs, but it remained harmless for pupae of the parasitoid (Bueno et al. 2008).

(iv) Molluscs Molluscs are considered to be ecological indicators, and their condition reflects the vigor of the entire ecosystem. They are the important food source for many aquatic animals and act as recyclers of plants and animal waste in aquatic systems. Seepage of glyphosate from agricultural lands has caused havoc to these tiny eco-friendly critters.

Snails Toxicity effects of glyphosate were also studied on different species of snails both aquatic and terrestrial by different groups of ecologists.

Tate et al. studied the long-term effects of glyphosate on the development and survival of aquatic snails (*Pseudosuccinea columella*) which act as intermediate host for liver fluke (*Fasciola hepatica*). Three successive generations of the snails were assessed to three different concentrations of glyphosate (0.1, 1.0, 10 mg/L) for 12 days. Glyphosate showed little effect on the first and second generations of snails. However, at concentration of 1.0 mg/L, the embryos of third generation developed much faster than other two concentrations. Hatching of eggs was repressed at the highest concentration of 10 mg/L and slightly inhibited at 0.1 mg/L. Snails exposed to the glyphosate concentrations of 0.1 and 10 mg/L showed an abnormal increase in egg-laying capacity and polyembryony in their eggs. This means that glyphosate affects the reproduction and development of snails (Tate et al. 1997).

Effect of sublethal concentration of glyphosate on the protein content and aminotransferase activity in *Pseudosuccinea columella* was checked by Christian et al. Three different concentrations of glyphosate were used (0.1, 1.0 and 10 mg/L). Results showed a significant increase in the

aminotransferase/glutamic oxaloacetic transaminase activity in all the snails reared at all the test concentrations of glyphosate. However, alanine aminotransferase/glutamic pyruvic transaminase activity was decreased. Also there was an unequal enhancement in the aminotransferase activity in the body fluids of the snails exposed to glyphosate. Apart from this, slight decrease was observed in the aminotransferase activity/glutamic oxaloacetic transaminase/glutamic pyruvic transaminase in the snails cultured at glyphosate concentrations of 0.1 and 10 mg/L. But the most significant results were shown at the lowest test concentration (0.1 mg/L), which specified that the lower concentrations of glyphosate were more easily absorbed and metabolized by the test organism. Also it was found that over an extended period of time, there comes a saturation of the toxification in an organism at about 1 mg/L for glyphosate. This proved the lack of consistent dose-dependent effects with in glyphosate concentration in the snails (Christian et al. 1993).

Assessment of the long-term effects of glyphosate on the terrestrial snail species *Helix aspersa* was done by Druart et al. Newly hatched snails were exposure to the soil and food contaminated with the herbicide for 168 days. Recommended field dose and tenfold the recommended dose of glyphosate were used for the experiment. No effects on the survival and growth of the snail were observed. But the presence of glyphosate was detected in the tissues of the snails which were continuously fed on the food contaminated with the herbicide. This concluded that there will be risk of transfer of glyphosate to food chain (Druart et al. 2011).

(v) Echinoderms Echinoderms are the important invertebrates that play numerous roles in ecological balance. They burrow deep in the sand and provide more oxygen at greater depths of sea floor. Some of the echinoderms like starfish prevent the growth of algae on coral reefs. Glyphosate has proved risky effects on these sea creatures and has caused devastation to them.

Sea Urchins To comprehend the injurious effects of the commonly used pesticide, sea urchins were used as the model animals by Marc et al. The pervasive use of glyphosate herbicides has influenced the early development in sea urchins and hampered their hatching process. Along with this, the active surfactant polyoxyethylene amine was also found to be highly noxious to the embryos. This illustrated that these herbicides have impinged the transcription process in sea urchins and affected their health (Marc et al. 2005).

Alterations caused by glyphosate-based herbicides in the cell division of sea urchins were studied by Marc et al. Different glyphosate formulations with different concentrations were used, and it was established that glyphosate at the highest concentration of 2 mM detained the cell cycle in these

organisms and the embryos developed into unhealthy adults (Marc et al. 2004).

Table 2 shows the toxic effects of glyphosate on various species of invertebrates.

(c) Vertebrates Toxic effects of glyphosate were also studied on different classes of vertebrates.

(i) Fish Fish is one of the most important classes of chordates in aquatic ecosystems. Fish are an integral part of natural ecosystem and provide immense economic, ecological and cultural values through food fishing. Fish forms an important part of food chain and maintains natural balance of food webs. Different researchers from all round the world evaluated the toxic impacts of glyphosate on different types of fish.

Acute and subacute toxic effects of sublethal glyphosate in water were studied by Neskovic et al. on fresh water fish carp (*Cyprinus carpio* L.). Toxicity test were performed on three different concentrations of glyphosate (2.5, 5 and 10 mg/L). LC₅₀ values of glyphosate for the fish after 48- and 96-h exposure were 645 and 620 mg/L, respectively. This shows that glyphosate is slightly toxic to carp. Biochemical analysis on the liver, heart, kidneys and serum of the fish was done to check the noxious effects of glyphosate. An increase in the alkaline phosphatase activity was observed in the liver of the fish at all the test concentrations. At 10 mg/L of glyphosate, an increase in the alkaline phosphatase activity was recorded in the heart of the fish. Apart from this, at concentrations of 2.5 and 5 mg/L there was an increase in the glutamic oxaloacetic transaminase activity in the liver and kidneys. Also, an increase in glutamic pyruvic transaminase activity in the serum of the fish was recorded at 5 and 10 mg/L of glyphosate. Histopathological studies of glyphosate on carp showed that on exposure to 5 mg/L, the gills of fish developed epithelial hyperplasia and subepithelial edema. Similar changes were observed at concentration of 10 mg/L, but the results were more pronounced. Leukocyte infiltration, slight hypertrophy of chloride cell, lifting and rupture of the respiratory epithelium were also observed on some secondary lamellae. Apart from this, at glyphosate concentration of 10 mg/L congestion of sinusoids and early signs of fibrosis were also recorded (Neskovic et al. 1996).

Adverse effects and global mechanism of toxicity of glyphosate and its common formulation Roundup on brown trout (*Salmo trutta*) were analyzed by using RNA-sequencing by Webster and Santos. Juvenile female brown trout was exposed to different concentrations (0, 0.01, 0.5 and 10 mg/L) of glyphosate and Roundup for 14 days. Transcriptional profiling showed that 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 (Webster and Santos 2015).

Another research group Murussi et al. investigate the effect of three different formulations of glyphosate 48% (Orium[®], Original[®] and Biocarp[®]) on silver catfish (*Rhamdia quelen*) at different concentrations (0.0, 2.5 and 5.0 mg/L) for 96 h. Enzymological studies were conducted on the liver and plasma of the fish, and certain alterations were observed in the enzymatic activity. 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 (Murussi et al. 2016).

Toxicity evaluation of glyphosate on acetylcholinesterase activity in the fish species *Cnesterodon decemmaculatus* was done by Helman et al. Three sublethal concentrations of glyphosate (1, 17.5 and 35 mg/L) were used. It was found that the fish remains alive even at the highest concentration of 35 mg/L. Inhibitory effects on the activity of acetylcholinesterase were observed in the anterior body section of the fish. The inhibition ranged between 23 and 36%. Decrease in the acetylcholinesterase activity was recorded in the anterior and middle body sections, but no change in the activity of this enzyme in the posterior part of the fish was recorded. This showed that acetylcholinesterase presented different sensitivity to glyphosate depending upon the enzyme location in the body (Menéndez-Helman et al. 2012).

Another research group Salbego et al. determined the toxic effects of glyphosate formulation Roundup on piava fish (*Leporinus obtusidens*). The fish was exposed to three different concentrations of Roundup (0, 1 and 5 mg/L) for 90 days. It was found that the acetylcholinesterase activity in the brain of the fish was decreased when the fish was exposed to Roundup concentration of 5 mg/L. Liver

Table 2 Effect of glyphosate on invertebrates

Phylum	Species	Exposure time	Response	References
Nemathelminthes	<i>Chordodes nobili</i>	96 h	Decrease in the infective capacity of larvae derived from exposed eggs, 50% Mortality at concentrations > 1.76 mg/L	Achiomo et al. (2008)
Annelids	<i>Eisenia fetida</i>	56 days	Reduction in mean bodyweight, no cocoons and juveniles, morphological abnormalities like the elevation of body, coiling and curling	Correia and Moreira (2010)
	<i>Eisenia fetida</i> and <i>Eisenia andrei</i>	28 days	Decreased cocoon viability and earthworms avoided the soil containing glyphosate	Casabe et al. (2007)
	<i>Eisenia fetida</i>	28 days	Regular diminution in the body weight	Yasmin and D'Souza (2007)
	<i>Eisenia fetida</i> and <i>Octolasion tyraeum</i>	28 days	100% mortality (<i>Octolasion tyraeum</i> at 50,000 mg/kg) weight loss, decreased cocoon viability and reduced fecundity	García-Torre et al. (2014)
	<i>Lumbricus terrestris</i> and <i>Aporrectodea caliginosa</i>	32 days	Decrease in surface casting activity and reduced rate of reproduction	Gaupp-Berghausen et al. (2015)
	<i>Eisenia andrei</i>	72 h	Weight loss, DNA damage and lysosomal damage	Piola et al. (2013)
	<i>Eisenia fetida</i>	40 days	Decrease in the fertility of cocoons	Santadino et al. (2014)
	<i>Eisenia fetida</i>	124 h	Affect locomotor activity and worms show avoidance behavior	Verrell and Van Buskirk (2004)
	<i>Lumbricus terrestris</i>	14 days	Declined soil mycorrhizal fungi spores biomass, poor interactions between worms and mycorrhizal fungi	Zaller et al. (2014)
Arthropods (Crustaceans)	<i>Daphnia magna</i> and <i>D. spinulata</i>	48 h	Organisms of both the species become immobilized	Alberdi et al. (1996)
	<i>Daphnia magna</i>	55 days	Reduction in juvenile size, growth rate decreased, fertility rate was affected and abortion rate was increased	Cuhra et al. (2013)
	<i>Daphnia pulex</i>	48 h	Decrease in the number of total population	Hartman and Martin (1984)
	<i>Daphnia magna</i>	48 h	It targets the esterases system of animals and inhibits the activity of this enzyme and affects various metabolic functions in animals	Dominguez-Cortinas et al. (2008)
	<i>Hyalella castroi</i>	7 days	No mating pairs, no ovigerous females and no eggs. Survival rate was decreased, and glycogen, protein, lipid, cholesterol and triglycerides level were also decreased. Lipo peroxidation and Na ⁺ /K ⁺ ATPase activity decrease	Dutra et al. (2011)
	<i>Cherax quadricarinatus</i>	50 days	Decreased somatic cell growth, decreased muscle protein, reduced muscle glycogen, reduced level of lipids and loss in weight	Frontera et al. (2011)
	<i>Cherax quadricarinatus</i>	60 days	33% mortality of juveniles, decrease in weight gain, decrease in lipid level in muscles and decrease in protein level in heptopancreas and muscles	Avigliano et al. (2014)

Table 2 (continued)

Phylum	Species	Exposure time	Response	References
Arthropods (Insecta)	<i>Apis mellifera</i>	14 days	Abnormal decrease in acetylcholinesterase level	Boily et al. (2013)
	<i>Apis mellifera</i>	Prolonged exposure	Reduced sensitivity to nectar, poor learning performance, diminished short-term memory retention, damaged non-elemental associative learning	Herbert et al. (2014)
	<i>Apis mellifera</i>	1 h	Impaired intellectual capability of bees; they become more wayward in their flights back home to their hives	Balbuena et al. (2015)
	<i>Apis mellifera</i>	4 days	69% cell death in the midgut of treated larvae	Gregorc and Ellis (2011)
	<i>Trichogramma pretiosum</i>	72 h (egg), 144 h (larvae), 192 h (pupae)	Harmful for eggs	Bueno et al. (2008)
Mollusca	<i>Pseudosuccinea columella</i>	12 days	Delayed hatchings of eggs, abnormal increase in egg-laying capacity and polyembryony in cells	Tate et al. (1997)
	<i>Pseudosuccinea columella</i>	4 weeks	Increase in aminotransferase/glutamic oxaloacetic transaminase activity, decrease in alanine aminotransferase/glutamic pyruvic transaminase activity, unequal enhancement in the aminotransferase activity in the body fluids	Christian et al. (1993)
	<i>Helix aspersa</i>	168 days	Glyphosate residues were present in the tissues of snails	Druart et al. (2011)
Echinoderms	<i>Sphaerechinus granularis</i>	–	Early development in the organism, hampered hatching process, invade the transcription process	Marc et al. (2005)
	<i>Sphaerechinus granularis</i>	–	Detained cell cycle, embryos develop into unhealthy adults	Marc et al. (2004)

glycogen content was decreased at both the round up concentrations. Also the hepatic glucose content was reduced when the fish was exposed to 5 mg/L of Roundup. Lactate levels in both liver and muscles of the fish were increased at both the Roundup concentrations. However, hepatic protein content remained constant at 1 mg/L but increased at 5 mg/L concentration of Roundup. In muscles, protein content has decreased with the increase in concentration of Roundup (Salbego et al. 2010).

Toxicity effects of Roundup on another fish *Jenynsia multidentata* were studied by Hued et al. (2012). Fish were exposed to 5, 10, 20, 35, 60 and 100 mg/L of Roundup concentrations. The LC₅₀ was found to be 19.02 mg/L at 96-h exposure for both male and female. All fish exposed to higher concentrations (60 and 100 mg/L) died during the exposure period. Sexual activity of male fish was also reduced when exposed to 0.5 mg/L Roundup for 7 and 28 days. Fish exposed to 5 mg/L concentration presented lifting of secondary lamellar epithelium, edema formation and hypertrophy of epithelial cells. Also, at this concentration there occurred hydropic degeneration in the liver of the fish. At concentration of 10 mg/L, lifting of secondary lamellar epithelium and hypertrophy of chloride cells were observed in the fish while hydropic degeneration accompanied by blood sinusoid dilation and foci of leukocyte infiltration occurred in the liver. At 20 mg/L of Roundup, hypertrophy of chloride cells and slight thickening of secondary lamellae were observed in the gills of the fish and in the liver focal necrosis and infiltration of leukocytes along with blood sinusoid dilation and vascular congestion were observed. At concentration of 35 mg/L, there occur more pronounced mucous cell proliferation and severe hyperplasia of epithelium cells in the gills of the fish. As the concentration of the herbicide increases, more severe effects were observed in the fish (Hued et al. 2012).

Another research group also studied the toxic effects of glyphosate (Roundup) on the fresh water fish surubim (*Pseudoplatystoma*). Different concentrations of Roundup (0, 2.25, 4.5, 7.5 and 15 mg/L) were used to test the metabolic and behavioral changes in the fish after the exposure of 96 h. 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 (Sinhorin et al. 2014). Mutagenic and genotoxic effects of glyphosate's formulation Roundup were studied by exposing poecilia reticulata for micronucleus test, comets assay and nuclear abnormalities. The fish were exposed to 0, 1.41, 2.83, 4.24 and 5.65 µL/L of the herbicide for 24 h. On analysis it was found that 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. Also the concentration of the herbicide used and the damage caused are positively correlated with each other (De Souza Filho et al. 2013).

Toxicity of the technical grade glyphosate, isopropylamine salt of glyphosate, formulated herbicide Roundup and Roundup surfactant was checked on four different fresh water fish, rainbow trout (*Salmo gairdneri*), fathead minnows (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*) and bluegills (*Lepomis macrochirus*) by Folmar et al. Acute toxicity test showed that the LC50 value at 96 h for Roundup varied from one species of fish to other species. It was 2.3 mg/L for fathead minnow and 140 mg/L for the rainbow trout. Toxicity of surfactants was found to be similar to that of Roundup formulations. However, Roundup was found to be more toxic at higher temperature and at pH of 7.5 for rainbow trout and bluegills. But toxicity does not changes with the increase in pH value. Toxicity effects of the herbicide depend upon the life cycle of the fish. Eyed eggs were in the least sensitive stage, while sac fry and early swim-up stages are prone to glyphosate. Reproduction and gonadosomatic index in adult rainbow trout were not affected by this herbicide (Folmar et al. 1979).

Cattaneo et al. analyzed the effects of commercial glyphosate's formulation Roundup, on the activity of acetylcholinesterase enzyme and oxidative stress in *Cyprinus carpio*. Five different concentrations of the herbicide were used (0, 0.5, 2.5, 5 and 10 mg/L), and the fish was exposed to these concentrations for 96 h. It was found that after exposure, the acetylcholinesterase activity was repressed in the brain and muscle of the fish. Oxidative stress produced by the Roundup was measured by the amount of thiobarbituric acid-reactive species present in the fish. After exposure, an increase in thiobarbituric acid-reactive species was observed at all the test concentrations of the herbicide. These results confirmed the lipid peroxidation and anti-acetylcholinesterase action, stimulated by the Roundup on the fish (Cattaneo et al. 2011).

(ii) Amphibians Amphibians play an essential role in ecosystem as secondary consumers in both aquatic and terrestrial habitats and maintain balance in many food chains. Excessive use of pesticides has adverse effects on them. Decline

in amphibian population will have large-scale everlasting effects on ecosystem; it will boost up algal population and will affect primary production in the ecosystem (Whiles et al. 2006). Toxic nature of glyphosate and its adverse effects on frogs and toads were studied by different research groups.

Frogs Appraisal of venomous nature of glyphosate and its commercial formulation Roundup on the early developmental stages of frog (*Leptodactylus latrans*) was deliberated by Bach et al.

A broad range of concentrations varying from (0.0007–9.62 mg of acid equivalent per liter) of Roundup and (3–300 mg/L) of glyphosate were used. Larvae were fed with blended lettuce regularly till they reached the developmental stages Gosner stage 25 and 36. In the case of larvae fed with glyphosate, no mortal effects were monitored during any of these stages. However, Roundup affected the swimming and other morphological activities of the tadpoles. Oral abnormalities and edema were the common symptoms of herbicidal poisoning. Commercial formulation Roundup was found to be more toxic to frogs (Bach et al. 2016).

Noxious effects of glyphosate on the hepatic tissues and erythrocytes of frog species *Leptodactylus latinasus* were evaluated. Anurans were exposed to three different concentrations of glyphosate (100, 1000, 10,000 $\mu\text{g g}^{-1}$). Blood samples were collected from the frogs and were investigated for any malfunctioning caused by the herbicide in them. 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 (Pérez-Iglesias et al. 2016).

Acute toxicity of a mixture of two herbicides dicamba and glyphosate was verified on the late developmental stage of *Rhinella arenarum* larvae. Both these herbicides have capability to interact with the DNA present in the blood stream of the frog. Genotoxicity of these herbicides was verified by the breaking of DNA strands which cause lesions in the peripheral blood cell of the frog when exposed for 96 h. Mixture of the herbicides induces cell damage to much higher frequency than when applied alone. Glyphosate synergically increases the toxicity of dicamba when used together (Soloneski et al. 2016).

A very authentic and reliable method was used by Lajmanovich et al. to evaluate the injurious effects caused by dermal uptake, of organophosphate pesticides (2,4-D, chlorpyrifos and glyphosate) on the common toad *Rhinella arenarum*. 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 (Lajmanovich et al. 2015).

Pernicious effects of glyphosate and its other commercial formulations were evaluated on adult frogs and tadpoles of four different species (*Crinia insignifera*, *Heleioporus eyrei*, *Limnodynastes dorsalis*, and *Litoria moorei*) found locally in southwestern Australia. The toxicity tests were conducted for 48 h. Glyphosate isopropylamine was found to be non-toxic to the tadpoles, and no mortality was recorded at concentrations between 503 and 684 mg/L. However, technical grade of glyphosate was found to be toxic. The toxicity was due to acid intolerance of tadpoles toward glyphosate. However, the tadpoles of species *Litoria moorei* were showing greater sensitivity toward the herbicides. It was also established that the adults and newly evolved metamorphs were less sensitive than tadpoles toward the toxicity of the herbicides (Mann and Bidwell 1999).

Dornelles et al. investigated the pernicious effects of the common herbicides (atrazine, glyphosate and quinclorac) on the endurance and biochemical changes in the blood and body of bull frog tadpoles (*Lithobates catesbeianus*). Exposure to glyphosate resulted in a significant reduction in the glycogen and triglyceride level in all the organs of tadpoles. Along with this, an increase in lipid peroxidation and cholesterol level in the gills of the tadpoles was observed. However, muscles and total protein content in the gills were decreased. These alterations in the biochemical parameters of tadpoles showed the toxicity produced by the herbicides on the tadpoles (Dornelles and Oliveira 2016).

To evaluate the pestilential effects of glyphosate on frogs, another group of researchers exposed tadpoles of species *Rhinella arenarum* to four different commercial formulations of glyphosate (Round Ultra- Max, Infosato, Glifoglex and C-K YUYOS FAV). Tadpoles were treated with eight different concentrations of all the herbicides for 48 h. Results exhibited the consequential decrease in the activity of the main enzymes (acetylcholinesterase, butyrylcholinesterase, carboxylesterase and glutathione S-transferase) used in the catalyses of neurotransmitters (Lajmanovich et al. 2011).

Acute and chronic toxicological studies of glyphosate-based herbicides containing surfactant polyethoxylated tallowamine (POEA) were carried out by Howe et al. Four North American amphibian species (*Rana clamitans*, *R. pipiens*, *R. sylvatica* and *Bufo americanus*) were used for toxicity analysis. Evaluation between the amphibian species demonstrated that the toxic level of the tested herbicides varied from species to species. However, *Rana pipiens* when exposed to the recommended dose of POEA or any glyphosate-based herbicide containing POEA showed adverse effects with the decreased snout-vent length at metamorphosis, tail damage with gonadal abnormalities. It also resulted in delayed metamorphosis. These results concluded that the surfactant POEA along with glyphosate is posing greater threat to the nontarget organisms (Howe et al. 2004).

(iii) Reptiles Reptiles play an essential role both as predators and as prey species. They maintain the ecological balance of most of the food webs. Devastating effects of glyphosate on the reptiles was examined by a number of research groups.

Crocodiles Natural habitats of crocodiles are found in the vicinity of agricultural lands, so the application of various herbicides and pesticides in the fields has noxious effect on the growth and development of them.

Genotoxicity and effects on growth of glyphosate-based herbicide (Roundup) on crocodilian species (*Caiman latirostris*) were evaluated by Gonzalez et al. Twenty-day-old hatchlings of *Caiman latirostris* were exposed to two field recommended doses of Roundup for 2 months. At the end of the experiment, blood samples were collected from the test organisms and micronucleus test was applied in erythrocytes. Significant increase in the frequency of micronucleus was found in the samples. This indicated an increased DNA damage in the cells which may result in retarded growth in the organisms (Gonzalez et al. 2013). Another similar type of work was done by Poletta et al. to determine the genotoxic effects of Roundup in the erythrocytes of *Caiman latirostris*, *in ovo* treatment. Early Caiman embryos were exposed to different sublethal concentrations of Roundup. At the time of hatching, blood samples were collected from each organism and two short-term tests (the comet assay and micronucleus tests) were performed. Results showed a significant increase in DNA damage at higher concentrations of the herbicide (Poletta et al. 2009).

Distressing effects due to the overuse of glyphosate on nontarget organisms were appraised by Latorre et al. (2013) on crocodilian species *Caiman latirostris*. Twenty-day-old crocodiles were exposed to two different concentrations of glyphosate for 2 months. They were weighed before and after the test period. Blood samples from the exposed crocodiles were taken to substantiate the total protein content and total WBC count. Results confirmed the decrease in total WBC count with increase in the percentage of heterophils and total protein content. It also showed the negative impact on the growth of the organisms. Thus, *in vivo* exposure of the reptiles to this herbicide altered their immune system (Latorre et al. 2013).

Another research group also evaluated the hazardous effects of Roundup on the immune system of *Caiman latirostris*. Test organisms were exposed to two different concentrations of the herbicide (recommended doses for the field application). After the exposure time (2 months), WBC count and complement system activity were checked. The crocodiles were then injected with a solution of lipopolysaccharide from *E. Coli* to activate the immune response and to appraise the parameters associated with it. A momentous decrease in the activity of the complement system was

observed along with the decrease in heterophils and lymphocytes count (Siroski et al. 2016).

Lizards Lizards are an important part of ecosystem. They help in maintaining ecological balance in food chain by thwarting the overpopulation of lower organisms. They are less imperative to humans but are significant for the complete food web. The common habitats of lizards are affected by the use of agricultural practices. Overuse of herbicides and pesticides also has negative impacts on these organisms.

Genotoxicity of the most commonly used herbicide Roundup on tegu lizard (*Salvator merianae*) was established by Schaumburg et al. *In ovo* treatment with six different sublethal concentrations of the herbicide was performed on the lizards. Blood samples were collected for micronucleus test, nuclear abnormalities and comet assay. Any external structural abnormality was also evaluated. Outcomes of the study revealed that the most commonly used herbicide has genotoxicity effects on tegu lizard. DNA damage was observed on the erythrocytes of the reptile after exposure to sublethal concentrations which may impede the developmental process of the neonates (Schaumburg et al. 2016).

An ecotoxicological study for the negative impact of glyphosate-based herbicides on common skink (*Oligosoma polychroma*) was carried out by Carpenter et al. Test organisms were exposed to two different formulations (Agpro Glyphosate 360, Yates Roundup Weed killer) at precise dose (144 mg/L of water) for 4 weeks. The lizards were confined during the experiment period for preferred temperature and mass. Any kind of change in the performance and behavior of lizards was noticed. None of these glyphosate formulations had any considerable effect on the mass of the organisms. Conversely, the lizards exposed to Yates Roundup Weed killer develop higher temperature to increase their metabolism and to thwart the physiological stress caused by herbicidal exposure (Carpenter et al. 2016).

(iv) Birds Birds are an important part of ecosystem and maintain balance in various food chains. Several studies have shown that the use of glyphosate in proposed extent does not cause any toxicological effect to many birds. However, their presence is affected by the glyphosate-treated areas due to non-availability of food and shelter (Forest info.ca).

Effect of use of glyphosate on wetland's vegetation which affected the densities of territorial male Red-winged Blackbirds (*Agelaius phoeniceus*), Marsh Wrens (*Cistothorus palustris*) and Yellow-headed Blackbirds (*Xanthocephalus xanthocephalus*) was appraised by Linz et al. Wetland containing cattail was randomly selected with 0% glyphosate (as control) and treated wetland as 50, 70 and 90% areal spray coverage of glyphosate. After 2 years of treatment, the densities of the birds were evaluated. The densities of Red-winged Blackbirds were higher in the wetland where no

glyphosate was sprayed. Similarly the abundance of Yellow-headed Blackbirds and Wrens was also found to be more in untreated wetlands as compared to the treated wetlands. This illustrates that the use of glyphosate reduced the wetlands vegetation which further affected the bird population (Linz et al. 1996).

Santillo et al. examined the effect of glyphosate-treated and untreated clearcuts on the breeding bird population. Results showed that the clearcuts treated with glyphosate had decreased complexity as compared to the untreated clearcuts. Therefore, the availability of total bird population was less in case of glyphosate-treated clearcuts. Birds like common yellowthroats (*Geothlypis trichas*), Lincoln's sparrows (*Melospiza lincolni*) and alder flycatchers (*Empidonax alnorum*) were fewer in treated areas. These results exemplify that the use of glyphosate affected the versatility of the birds and reduced their natural habitats that affect their breeding population (Santillo et al. 1989).

Deleterious effects of glyphosate-based herbicide Roundup on the reproductive system of drake *Anas platyrhynchos* were analyzed by in vivo studies. Disclosure to the herbicide has affected androgen and estrogen synthesis which causes severe damage to the reproductive system of the male bird. It also resulted in the variation in the morphology of the testis and epididymal region, thereby affecting the male genital organs of the bird. All these effects were dose dependent and severely affect the reproduction of the species (Oliveira et al. 2007).

(v) Mammals Mammals are placed at the top most position in hierarchy of kingdom animalia. They play a crucial role for maintaining services and functions for the balance in ecosystem. Excessive use of glyphosate has also caused several toxic effects on lower and higher mammals. Different studies have been conducted to investigate the lethal effects of glyphosate on these organisms.

Rats Long-term toxic impacts of glyphosate and its salutary effects with zinc were determined by the histopathological changes that occur in the stomach, kidney, liver, brain, spleen and pancreas of the rats by Tizhe et al. The rats were firstly pretreated with zinc (with dose of 50 mg/kg body weight) and then were exposed to two different concentrations of glyphosate (14.4 and 375 mg/kg bodyweight) for 2 months. Some rats were exposed to the herbicide after their treatment with zinc. Severe histopathological changes were observed in the case of rodents exposed to higher concentration of glyphosate. Mucosal epithelial cells were deteriorated along with the degeneration of hepatic cells. Glomerular degeneration, mononuclear cells infiltration and tubular necrosis were noticed in the kidneys of the rats. Pancreas and spleen also got damaged, while no pathological changes were noticed in the rats supplemented with zinc

(Tizhe et al. 2014a). Tizhe et al. in another work assessed the subchronic toxic effects of glyphosate and its supplemented effect with zinc on the hepatic and renal functions of Wistar rats. Rats were orally fed for 8 weeks with different concentrations of glyphosate and zinc. At the end of the study, blood samples were collected and assayed for total protein content, albumin, alanine, aspartate aminotransferase, alkaline phosphates and other ions present in the blood. From the results, it was concluded that exposure of rats to glyphosate caused both renal and hepatic toxicity which was alleviated by the presence of zinc with it (Tizhe et al. 2014b).

Another research group tested the effects of glyphosate and the common surfactant polyoxyethyleneamine used in herbicide Roundup on the reproductive system of male and female offsprings of Wistar rats. Lactating parent and pregnant female rats were orally fed with different concentrations of glyphosate (50, 150, 450 mg/kg). Results showed that glyphosate does not generate any maternal toxicity to female rats; only a delay in the vaginal canal opening was noticed. However, it affects the male reproductive system by decreasing the total sperm content in the adult male rats. Moreover, an increase in the number of abnormal sperms and a decrease in serum testosterone level were also observed in male rats (Dallegrave et al. 2007).

Similar kind of work was also done by Romano et al. to evaluate the perturbing effects caused by glyphosate on the reproductive system of the Wistar rats. Exposure of rats to the herbicide had significantly altered their progression of puberty. It had also reduced the testosterone production in male rats (Romano et al. 2010).

Swine Toxic effects of various components of glyphosate-surfactant herbicides on the cardiovascular system of higher mammals were evaluated by Lee et al. Five different groups of male piglets were infused with different concentrations of glyphosate along with the various components of glyphosate (used in commercial formulations). Results showed that the surfactants as well as the active component (isopropylamine salt of glyphosate) have caused detrimental effects on the cardiovascular system of the swine (Lee et al. 2009).

Humans Glyphosate is a water-soluble herbicide, so it gets accumulated in water and soil systems from where it enters the food chain. UK Food Standard Agency conducted residue testing of glyphosate in the samples of bread and found 0.2 mg/kg of glyphosate in 27 out of 109 samples. (Myers et al. 2016). US department of agriculture in 2011 divulges the presence of glyphosate residues in 90.3% of 300 soybean samples at the concentration of 1.9 ppm. Also, they detect the presence of residues of aminomethylphosphonic acid in 95.7% of soybean samples at concentration of 2.3 ppm (Osteen and Fernandez-Cornejo 2013). The presence of glyphosate and its metabolites in food is posing serious

threat to mankind and is disrupting the natural ecological balance. Various researchers have conducted toxicity tests to evaluate the harmful effects of the herbicide on humans.

Toxicity evaluation of glyphosate and its common formulation Roundup on the human placental JEG 3 cells was carried by Richard et al. Results confirmed that glyphosate-based herbicides have perturbed the activity of enzyme aromatase (responsible for the synthesis of estrogen in females) and mRNA level present in the placenta of humans. Glyphosate also binds with the active sites of the enzyme and inhibits its activity. The toxic effects of glyphosate are facilitated by Roundup formulation. Roundup acts as a potent endocrine disruptor (Richard et al. 2005).

Cytotoxic and genotoxic properties of glyphosate and Roundup in the buccal epithelial cell line (TR146) of humans were evaluated by Koller et al. Workers were exposed to the herbicide via inhalation. Results confirmed that the use of Roundup damaged the epithelial cell membrane and also caused an impairment in mitochondrial functions. Significant increase in the nucleoplasmic bridges nuclear aberrations and micronuclei indicates DNA damage. Moreover, an increased release of extracellular lactate dehydrogenase shows plasma damage in the cells. This study indicates that epithelial cells are more susceptible to the cytotoxic and DNA damaging by the use of these herbicides (Koller et al. 2012).

Mesnager et al. checked the toxic effects of glyphosate and its common adjuvant polyethoxylated tallowamine (POE-15) in nine glyphosate-based commercial formulations on the human cell line. Toxicity evaluation was performed on hepatic (Hep G2), embryonic (HEK 293) and placental (JEG 3) cell lines after 24-h exposure. Mitochondrial activities, membrane degradation and caspases 3/7 activities were taken as the criterion for toxicity evaluation. Results depicted that all the glyphosate formulations were more toxic than pure glyphosate. Adjuvant polyethoxylated tallowamine (POE-15) was found to be most toxic against human cells. The toxic effects of POE-15 were dose dependent and induce the necrosis of cells even after its first micellization process. However, glyphosate induced its endocrine disruption only after entering the cell. This study concluded that the addition of surfactants in the commercial formulations increases their toxicity (Mesnager et al. 2013).

Another research group evaluated the toxic effects of glyphosate-based herbicides, containing not only glyphosate but also different adjuvant on the 3T3-L1 cell line. Three different glyphosate formulations were taken to study their impact on 3T3-L1 fibroblast proliferation and differentiation. An abrupt increase in the cell number or cytosolic lipid accumulation was observed. The commercially available glyphosate formulations containing the adjuvant are more potent inhibitors of proliferation and cell differentiation of adipocytes of 3T3-L1 fibroblasts. This study demonstrates

that not only polyethoxylated adjuvant but non-polyethoxylated adjuvant also contributes to cell toxicity. Thus, it was finally concluded that glyphosate-based herbicides disturbed the cell physiology and induces many cellular alterations (Martini et al. 2016).

Glyphosate's toxicity was also affirmed by another research group. According to them, glyphosate's toxicity is negligible when its residues were found in the food stuff like sugar, corn, soy and wheat. They intended that glyphosate interfered with cytochrome P450 (CYP) enzymes and interrupted the biosynthesis of aromatic amino acids by gut bacteria. It also impaired the serum sulfate transport in the blood. Disruption of CYP enzymes boosted the harmful effects of other food-borne chemical residues and environmental toxins which resulted in the damage of cellular systems all over the body. Eventually it makes the human body prone to many serious diseases like gastrointestinal disorders, obesity, heart disease, depression, autism, diabetes, infertility and cancer (Samsel and Seneff 2013).

Thongprakaisang et al. studied the toxic effects of technical grade glyphosate on the endocrine system of humans. Adverse effects of pure glyphosate were checked on estrogen receptors-mediated transcriptional activity and their expressions. They proposed that glyphosate exerts significant effects only in human hormone-dependent breast cancer T47D cells but does not affect hormone-independent breast cancer MDA-MB231 cells at 10^{-12} to 10^{-6} M in estrogen withdrawal condition. The considerable concentrations of glyphosate that persuade the commencement of estrogen response element transcription activity were 5–13 times more than in control (in T47D-KBluc cells). Also this activation was inhibited by an estrogen antagonist ICI 18278 which indicates that estrogenic activity of glyphosate was arbitrated by estrogen receptors. These findings suggested that the low and environmentally recommended concentrations of glyphosate can disrupt the hormonal system of humans (Thongprakaisang et al. 2013).

Another study by Gasnier et al. investigates the cytotoxicity, genotoxicity, anti-estrogenic and anti-androgenic effects of glyphosate-based formulations on human liver HepG2 cells. The cells were exposed to different concentrations of four different glyphosate formulations (Roundup Express, Bioforce, Grands Travaux and Grands Travaux plus) and pure glyphosate. Results suggested that all the parameters were disrupted with all the herbicidal formulations of glyphosate even after 24-h exposure. Concentrations above 0.5 mg/L of Grands Travaux (the most active formulation of glyphosate) caused human cell endocrine disruption on androgen receptor in MDA-MB453-kb2 cells. Concentration level above 2 mg/L inhibited transcriptional activities on estrogen receptors and hepatic cells HepG2. The concentration level above 10 mg/L caused severe cytotoxic effects

Table 3 Effect of glyphosate on vertebrates

Phylum	Species	Exposure time	Response	References
Fish	<i>Cyprinus carpio</i>	96 h	Increase in the alkaline phosphatase activity in liver, heart, increase in glutamic oxaloacetic transaminase activity in liver and kidneys, increase in glutamic pyruvic transaminase activity in the serum, epithelial hyperplasia and subepithelial edema, lifting and rupture of the respiratory epithelium and early signs of fibrosis	Neskovic et al. (1996)
	<i>Salmo trutta</i>	14 days	Increase in cell proliferation and cellular turnover	Webster and Santos (2015)
	<i>Rhamdia quelen</i>	96 h	Alterations in enzymatic activity, vacuolization, leukocyte infiltration, degradation of cytoplasm and melanomacrophage	Murussi et al. (2016)
	<i>Cnesterodon decemmaculatus</i>	90 days	Acetylcholinesterase activity in brain was decreased, liver glycogen content was reduced, hepatic glucose content was decreased, lactate level in liver and muscles was increased, and protein content in muscle was decreased	Menéndez-Helman et al. (2012)
	<i>Jenynsia multidentata</i>	7 and 28 days	Lifting of secondary lamellar epithelium, edema formation, hypertrophy of epithelial cells, hydropic degeneration in the liver, thickening of secondary lamellae in gills, focal necrosis and infiltration of leukocytes in liver	Hued et al. (2012)
	<i>Pseudoplatystoma</i>	96 h	Decrease in glucose level in plasma and increase in the glucose level of liver, lactate level increase in plasma and liver, decrease in protein and glycogen level of the plasma and muscles, decrease in cholesterol level, altered the enzymatic activity, ventilator frequency was increased and change in swimming behavior of fish	Sinhorin et al. (2014)
	<i>Poecilia reticulata</i>	24 h	Increase in the number of micronucleus and comets (increase in the number of damaged cells)	De Souza Filho et al. (2013)
	<i>Pimephales promelas</i> , <i>Salmo gairdneri</i> , <i>Ictalurus punctatus</i> , <i>Lepomis macrochirus</i>	96 h	Toxicity effects depend upon the life cycle of the fish	Folmar et al. (1979)
	<i>Cyprinus carpio</i>	96 h	Repression of acetylcholinesterase activity, increase in thiobarbituric acid-reactive species and stimulation of lipid peroxidation	Cattaneo et al. (2011)

Table 3 (continued)

Phylum	Species	Exposure time	Response	References
Amphibians	<i>Leptodactylus latrans</i>	24 h	Affected swimming, oral abnormalities and edema	Bach et al.
	<i>Leptodactylus latrans</i>	48 h	Alterations in the hepatic tissue and erythrocyte nuclear abnormalities	Pérez-Iglesias et al. (2016)
	<i>Rhinella arenarum</i>	96 h	Breaking of DNA strands, lesions in peripheral blood cell	Soloneski et al. (2016)
	<i>Rhinella arenarum</i>	48 h	Neurotoxicity, oxidative stress and immunological depression	Lajmanovich et al. (2015)
	<i>Lithobates catesbeianus</i>	14 days	Significant reduction in glycogen and triglyceride level in all organs, increase in lipid peroxidation and cholesterol level in gills, decrease in total protein content in gills	Dornelles and Oliveira (2016)
	<i>Rhinella arenarum</i>	48 h	Decrease in the activity of important enzymes used in the catalyses of neurotransmitters	Lajmanovich et al. (2011)
Reptiles	<i>Rana clamitans</i> , <i>Rana sylvatica</i> , <i>Rana pipiens</i> , <i>Bufo americanus</i>	96 h	Decreased snout-vent length, tail damage, gonadal abnormalities and delayed metamorphosis	Howe et al. (2004)
	<i>Caiman latirostris</i>	2 months	Increase in the frequency of micronucleus, DNA damage with retarded growth	Gonzalez et al. (2013)
	<i>Caiman latirostris</i>	<i>In Ovo</i> exposure	DNA damage	Poletta et al. (2009)
	<i>Caiman latirostris</i>	2 months	Decrease in WBC count, increase in heterophils and total protein count	Latorre et al. (2013)
	<i>Caiman latirostris</i>	2 months	Decrease in heterophils and lymphocytes count	Siroski et al. (2016)
	<i>Salvator merianae</i>	<i>In Ovo</i> exposure	External structural abnormality, DNA damage	Schaumburg et al. (2016)
	<i>Oligosoma polychrome</i>	4 weeks	Increase in body metabolism and increase in body temperature	Carpenter et al. (2016)
	<i>Agelazus phoeniceus</i> , <i>Cistothorus palustris</i> , <i>Xanthocephalus xanthocephalus</i>	–	Reduced the wetlands vegetation and affected bird population	Linz et al. (1996)
	<i>Geothlypis trichas</i> , <i>Melospiza lincolni</i> , <i>Empidonax alhorum</i>	–	Affected versatility of birds and reduced their natural habitats and their breeding populations	Santillo et al. (1989)
	<i>Anas platyrhynchos</i>	15 days	Damage reproductive system of male bird and affected male genital organs	Oliveira et al. (2007)

Table 3 (continued)

Phylum	Species	Exposure time	Response	References
Mammals	Wistar Rats	2 months	Damaged mucosal epithelial cells, degeneration of hepatic cells, glomerular degeneration, tubular necrosis in kidneys, damaged spleen and pancreas	Tizhe et al. (2014a, b)
	Wistar Rats	21–23 days (during pregnancy), 21 days (during lactation)	Delay in vaginal canal opening in pregnant female rats, decrease in total sperm content in adult male rats, increase in the number of abnormal sperms and decrease in serum testosterone level	Dallegrave et al. (2007)
	Wistar Rats	–	Altered the progression of puberty in rats	Romano et al. (2010)
	Landrace piglets	24 and 48 h	Decreased cardiac index, increased pulmonary capillary wedge pressure, central venous pressure and mean pulmonary arterial pressure	
	<i>Homo sapiens</i>	18 h	Perturbed the activity of enzyme aromatase and mRNA level present in the placenta	Richard et al. (2005)
	<i>Homo sapiens</i>	20 min	Damaged epithelial cell membrane, impairment in mitochondrial functions, increase in nucleoplasmatic bridges, nuclear aberrations and micronuclei, increased release of extracellular lactate dehydrogenase	Koller et al. (2012)
	<i>Homo sapiens</i>	24 h	Negative dose-dependent effects on cellular respiration, necrosis of cells and endocrine disruption	Mesnager et al. (2013)
	<i>Homo sapiens</i>	24 h	Disturb cell physiology and induces cellular alterations	Martini et al. (2016)
	<i>Homo sapiens</i>	–	Interfered with cytochrome P450(CYP) enzymes, interrupted biosynthesis of aromatic amino acids and impaired the serum sulfate transport in the blood	Samsel and Seneff (2013)
	<i>Homo sapiens</i>	5 days	Disrupts hormonal system in humans	Thongprakashang et al. (2013)
	<i>Homo sapiens</i>	24 h	Disruption of endocrine system, inhibited transcriptional activities on estrogen receptors, DNA damage	Gasnier et al. (2009)
	<i>Homo sapiens</i>	48 h	DNA damage with chromosomal aberrations	Manas et al. (2009)

with DNA damage at concentration of 5 mg/L (Gasnier et al. 2009).

Genotoxicity of glyphosate on humans was evaluated by Manas et al. by performing cytogenetic tests on Hep-2 cells. Four different concentrations (3, 4.50, 6 and 7.5 mM) of glyphosate were used for comet assay and chromosome aberrations test. A significant increase in DNA damage was observed at glyphosate concentration ranging from 3–7.5 mM. These observations showed the genotoxic nature of glyphosate in comet assay in Hep-2 cells (Manas et al. 2009).

Table 3 shows the toxic effects of glyphosate on various species of vertebrates.

Conclusion

Glyphosate is one of the most commonly used herbicide worldwide. Earlier it was thought that glyphosate is environment-friendly and does not cause any harm to nontarget organisms present in the ecosystem. But due to its overuse it has leached into ground water and soil systems where it is posing serious threats to the organisms found in aquatic and terrestrial systems. This review presents a complete toxicity evaluation of glyphosate and its common formulations on kingdom animalia and other lower group of organisms. Its noxious effects are not only bounded to unicellular organisms but also creating many distresses in multicellular organisms. It shows its negative effects from lower invertebrates to higher chordates. Overuse of glyphosate had seriously affected the earthworms by decreasing their rate of reproduction, loss of biomass, DNA damage and reduced surface casting activity. In aquatic systems, many lower invertebrates are also directly affected by the lethal nature of glyphosate. Apart from this, overuse of glyphosate in soil and its leaching in aquatic systems had reduced the egg-laying capacity and have hampered the hatching process in snails and sea urchins. Not only lower mammals but humans are also severely exaggerated by this herbicide. Roundup is found to be potent endocrine disruptor in human beings. It is causing serious damage to placental cells with the decrease in the activity of enzyme aromatase. It caused DNA damage, plasma damage and epithelial cell damage in humans. Surfactant polyethoxylated tallowamine showed harmful effects on hepatic, embryonic and placental cell lines.

Thus, we can conclude that the extreme use of herbicide glyphosate has caused toxic effects on nontarget organisms found in soil and water. It has affected almost all organisms of animal kingdom. This is a serious concern as it had affected the whole food chain and produced many unwanted changes in it. Glyphosate has reduced the availability of weeds which may be an important food source for many

species. Thus, certain sustainable agricultural practices are needed to be adopted by farmers so as to maintain the interactions between biotic and abiotic components of ecosystems to get the ecological balance and to save the food webs.

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