Toxicity determination of three sturgeon species exposed to glyphosate

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Abstract

Glyphosate, N–(phosphonomethyl) glycine, has been widely used to control agricultural weeds in the north of Iran. However, it is also supposed to have adverse effects on natural sturgeon population. The present study was undertaken to evaluate the acute toxicity of glyphosate to three different sturgeon species (*Huso huso*, *Acipenser stellatus*, and *A. persicus*) under laboratory conditions. Fish were exposed to one of ten glyphosate concentrations (10 to 100 mg l^{-1} with 10 mg l^{-1} intervals), along with a control group. The values of the median lethal concentration (LC50) for each experimental species were estimated using a standard probit regression analyses after each 6, 12, 24, 48, 96, and 168 hours as exposure times. Results showed that increase in glyphosate exposure times up to 168 hours was simultaneous to decrease of the lethal concentration (LC50). 96–h LC50 of glyphosate for *H. huso, A. stellatus* and *A. persicus* were 26.4, 23.2 and 27.5 mg l^{-1}, respectively. Glyphosate exhibited a slight to moderate toxicity in sturgeon species. However, it may negatively affect the natural population of sturgeons through decreasing of fry mass, smaller size of yolk sac and the initiation of unsafe behaviors.

Keywords: Glyphosate, Sturgeon, Toxicity, Caspian Sea, Iran

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Introduction

The sturgeon population of the Caspian Sea is the largest in the world (Levin, 1997) and Iran is the second major country which produces this resource (Josupeit, 1994; Pourkazemi et al., 2000). There are, however, evidences that their native population declined over 90% in the past three decades. According to the International Union for Conservation of Nature (IUCN) red list status, all sturgeon species in the Caspian Sea are currently considered as critically endangered in red list (IUCN, 2010). Some known causes of population disappearance are illegal catch, habitat loss, overfishing, degradation, and herbicide contamination (Nourouzi et al., 2009; Sadeghirad et al., 2009).

Coastal habitats are progressively being exposed to herbicide pollutions specially glyphosate from large farms and paddy fields close to this regions (Kottelat et al., 2009). Because of high water solubility and widespread application, the exposure of sturgeon species to this agrochemical leads to many problems including reduced reproductive success and extinction of the remaining natural wild population (Elandalloussi et al., 2008).

The agricultural activities are continuing to grow around the world and management strategies are shifting to intensive systems with higher efficiency lower herbicide applications. Contamination of aquatic ecosystems by application of agrochemicals which include a variety of herbicides is a crisis of worldwide importance (Oruς and Üner, 1999). Glyphosate, the isopropyl amine salt of N–(phosphonomethyl) glycine, is a non–selective post–emergence herbicide for weed control in agricultural, forestry and aquatic systems (Çavas and Könen, 2007). This herbicide is widely used in the world due to its high efficacy and cost effectiveness. (Vereecken, 2005; Glusczak et al., 2006). Many commercial herbicides have been formulated using glyphosate as active ingredient such as Rodeo®, Roundup® and Aquamaster® (Mozdzer et al., 2008; Papchenkova et al., 2009).

The major pathway for glyphosate uptake in plants is through the foliage; however, some root uptake may occur. It controls weeds by inhibiting the synthesis of aromatic amino acids necessary for protein formation in susceptible plants (Anthelme and Marigo, 1998). The glyphosate degradation is mostly determined by microbial metabolism with amino–methylphosphonic acid (AMPA) as the main degradation product (Rueppel et al., 1977; Kolpin et al., 2006; Cribner et al., 2007). The half–life ranges from a few days to several weeks and even years, but the averageis two months (Giesy et al., 2000; Glusczak et al., 2007) and its water solubility is around 157 µg l^{-1} (Rodrigues and Almeida, 1998).

Glyphosate has been broadly tested for acute toxicity on invertebrates, birds and mammals (Mann & Bidwell, 1999; Perkins et al., 2000; Smith, 2001; Edginton et al., 2004; Thompson et al., 2004). However, there are limited researches on the toxic characteristics of glyphosate and its effects on fish. Several results indicated this herbicide has relatively low acute toxicity against aquatic organisms and doesn't bioaccumulate in tissues of aquatic organisms (WHO, 1994; Giesy et al., 2000).

The present research was conducted to determinate the LC50 of three sturgeon fries (*Huso huso*, *Acipenser stellatus*, and *A. persicus*) to different concentrations of glyphosate (41% acid equivalent a.e. l^{-1}) ranging from 5 to 100 mg l^{-1} for 6 to 168 hours under laboratory conditions.

Materials and methods

Sturgeon fries (weight, 10.2±1.0 g; length, 8.0±0.4 cm) were obtained from International Sturgeon Research Institute (ISRI), Rasht, Iran. Prior to the bioassays, fish were adapted to laboratory conditions for approximately 4 weeks in 400–L tanks with constantly aerated water. They were kept in a static system with constant aeration and natural photoperiod (12:12 h L:D photoperiod). Water quality parameters were daily evaluated throughout the experiment and were as follow: temperature 22±1.5 °C, pH 7.4±0.2 units, dissolved oxygen 6.8±1.3 mg l^{-1}, ammonia–N 0.009±0.004 mg l^{-1}, nitrite–N 0.02 ± 0.01, and nitrate–N 0.63 mg l^{-1}. All water parameters were measured according to APHA (1992). Fish were fed once a day during the acclimation period using commercial pellet food. Feces and pellet remains were removed by suction every day.Acute toxicity tests were carried out in a static way according to the procedure described by Antón et al. (1994). The commercial formulation Roundup® (41% a.e. Monsanto Company, St Louis MO, USA) was used in this study. Experiments were done in 100–L aquariums each containing eight fish, constant aerated water and the same characteristics explained for the adaptation period.

The experiment was composed of ten groups for each species exposed to one of ten glyphosate concentrations (10 to 100 mg l^{-1} with 10 mg l^{-1} intervals) and a control exposed merely to water (without herbicide addition). All tests were performed in triplicates and no feeding was done during the experiment. The herbicide was added to the aquaria at the beginning of the test. The number of dead fish was recorded for six interval times (6, 12, 24, 48, 96 and 168 h).The value of the mean lethal concentration (LC50) for each experimental species was estimated using Finney's standard probit regression analyses and 95% probability for respective confidence limits (Finney, 1971).

Behavioral changes of experimental fish including swimming speed, anomalous swimming, orientation, and breathing frequency were daily observed and recorded during the toxicity experiment. The findings were examined for conformity to normality with Kolmogorov–Smirnov's test. The SAS statistical program (2008) was used to assess mortality among experiments. The effects of glyphosate concentration for each exposure time were compared to the sturgeon species survival using one–way analyses of variance (ANOVA) with subsequent use of Tukey's HSD test to separate means. The p–value <0.05 was considered statistically significant.

Results

Glyphosate concentration and exposure times have been recognized as important factors in the survival of sturgeon species. A rise in these factors determines an increase in metabolic rate with negative effects on the survival rate. Many injury appearances were observed in Sturgeon fries in whole concentrations during the study. In case of the control group, no mortality occurred throughout the experiment. Exposure to more than 50 mg l^{-1} glyphosate resulted in more than 80% mortality.

The statistical analysis indicated that glyphosate concentrations and exposure times had significant effects on sturgeon mortality. The LC50 value obtained for each exposure time of every species was never lower than those obtained for succeeding exposure times. The 6–h LC50 and 168–h LC50 of glyphosate was the highest and lowest values for each species, respectively (Table 1). The quantitative comparison of LC50 exhibited no significant differences between three sturgeon species by increasing exposure duration to 48 h (ANOVA, p<0.05). However, acute toxicity after 48 h exposure time differed significantly between the species (ANOVA, p<0.05). The 168–LC50 for experimental species was in a decreasing order of *H. huso* > A. *persicus* > *A. Stellatus* (Table 1).

Results of Finney's probit regression analyses indicated glyphosate has lower acute toxicity to *H. huso* compared to *A. stellatus* and *A. persicus*. However, all sturgeon species exposed to 100 mg l^{-1} died within 24 hours from the beginning of exposure. Sturgeon fries exposed to concentrations of 60 to 100 mg l^{-1} showed a high increase in mortality.

Normal behavior with no fish mortality was observed in control aquaria during the experiment. After exposure of experimental species to sub lethal concentration of glyphosate (less than 25 mg l^{-1}), the fish revealed abnormal behavioral changes such as sluggish swimming, respiratory alterations and excessive mucus secretion. However, fish tended to show more outward signs of toxicity by rising glyphosate concentration. The acutely intoxicated fish exhibited convulsive disorders, loss of equilibrium, color darkening of body surface and finally death compared to the control fish after 196 h.

Table 1: Significant differences between experimental species exposed to glyphosate. Different subscripts within a same row indicate significant differences at p<0.05.

	Huso Huso	*Acipenser stellatus*	*Acipenser persicus*	p–value
6 hours	$74.42\pm6.69^{n.s.}$	$69.72\pm3.48^{n.s.}$	$77.21\pm8.49^{n.s.}$	0.2873
12 hours	$67.19\pm5.84^{n.s.}$	$61.15\pm11.30^{n.s.}$	$61.34\pm14.12^{n.s.}$	0.3324
24 hours	$41.30\pm12.39^{n.s.}$	$46.17\pm3.69^{n.s.}$	$44.82\pm8.51^{n.s.}$	0.2246
48 hours	26.92 ± 3.16^{c}	39.70 ± 4.52^{a}	34.18 ± 6.25^{b}	0.0346
96 hours	19.83 ± 5.35^{b}	24.72 ± 6.73^{a}	26.05 ± 3.20^{a}	0.0117
168 hours	8.31 ± 2.40^{c}	12.98 ± 5.19^{a}	10.59 ± 1.82^{b}	0.0078

n.s. = not significant (p<0.05).

Discussion

This study demonstrated that glyphosate herbicide can be toxic to *H. Huso*, *Acipenser stellatus* and *A. persicus* in concentrations varied from 8.31 to 77.21 mg l^{-1}. It means that glyphosate has moderate to low toxicity against these species. The results of our study exhibited that longer exposure times caused greater toxic effects of the bioconcentrated chemical and lower glyphosate was needed to reach lethality. It is implied that sensitivity of non-target organisms to toxicants for long exposure times contribute to reduced life expectancy (Yu et al., 1999).

Each sturgeon species exhibited significantly different sensitivity to glyphosate with increasing exposure duration (48, 96 and 168 h) in the order of *H. huso*, *A. persicus*, and *A. stellatus* ($p<0.05$). Therefore, more sensitivity of *H. huso* may be related to its higher longevity, food behaviors and lifestyles in comparison with *A. stellatus* and *A. persicus*.

Besides, the lethal toxicity of commercial toxicant formulation such as Roundup® is more than the glyphosate technical grade substance. The surfactants such as polyethoxylated tallowamine (POEA) used in the Roundup formulation are the principal toxic compound of the glyphosate–based herbicide to aquatic organisms (26.8 mg l^{-1} for *Salmo gairdneri*, 27.7 mg l^{-1} for *Oncorhynchus nerka*, 4.9 mg l^{-1} for *Ictalurus punctatus* and 4.4 mg l^{-1} for *Lepomis microchirus*; Servizi et al., 1987; Williams et al., 2000; Tsui and Chu, 2003; Langiano and Martinez, 2008). Consequently, lifelong exposure to glyphosate could have more adverse effects on the natural population of sturgeon species in the Caspian Sea.

The median toxicity tests of glyphosate have been performed for various aquatic species (Gardner and Grue, 1996; Simenstad et al., 1996; Williams et al., 2000; Relyea, 2005; Glusczak et al., 2006; Langiano & Martinez, 2008). The LC50 of glyphosate obtained in the present study for sturgeon species were close to those obtained for *Oncorhynchus mykiss* by Servizi et al. (1987). However, *Prochilodus lineatus* and *Oreochromis niloticus* showed much greater sensitivity to the glyphosate herbicide (Jiraungkoorskul et al., 2002; Langiano and Martinez, 2008). It could be emphasized that harmless concentrations of glyphosate for temperate climate species, like sturgeon species, could be lethal for tropical species.

Ecological conditions could also be implicated in the lethal toxicity of aquatic animals (Neskovic et al., 1996). For instance, glyphosate toxicity of salmonids (*Onchorhychus kisutch, O. tshawytsha, O. keta, O. gorbuscha* and *O. mykiss*) in hard water is around two–folds lower than soft waters (Wan et al., 1989). It is known that temperature has considerable effects on metabolic rate with adverse effects on physiological processes (Wilson & Parker, 1996; Bat et al., 2000). Folmar et al., (1979) showed that value of LC50 for rainbow trout (*Salmo gairdneri*) and bluegills (*Lepomis macrochirus*) rised by increasing of water temperature. Technical grade glyphosate was less toxic as pH increased from 6.5 to 9.5 and Roundup® became more toxic as the pH increased from 6.5 to 7.5 (Folmar et al., 1979).

The present study confirms earlier researches that glyphosate (technical grade) is slightly to moderately toxic to fish (WHO, 1994; Neskovic et al., 1996; Giesy et al., 2000). Comparing the toxicity of glyphosate with diazinon, another agrochemical abundantly utilized in the north of Iran, it can be declared firmly that glyphosate has much lower toxicity regarding the 96 h–LC50 of diazinon for *A. persicus* and *A. Stellatus* as 4.34 mg l^{-1} and 2.54 mg l^{-1}, respectively (Pajand et al., 2003).

Visual observations demonstrated a series of abnormal behavioral in sturgeon fries, as expected, by increasing exposure time and glyphosate concentration. Nonetheless, normal behaviors of the sturgeon were detected after glyphosate removal, suggesting a lack of herbicide aggregation to the fish tissue. The present findings support previous studies that glyphosate bioaccumulation in aquatic animals is not expected to be high due to its high water solubility and ionic characteristics (Giesy et al., 2000; van der Oost et al., 2003; Contardo–Jara et al., 2009).

Our result demonstrated that glyphosate has low toxicity to experimental sturgeons. In spite of the fact that glyphosate may have no direct effect, it may negatively affect natural population of sturgeons through decreasing of fry mass, smaller size of yolk sac and the initiation of unsafe behaviors. Global fisheries statistics demonstrate that there has been a drastic decrease of sturgeon catch from 1992 to 2007 (FAO, 2009). Further studies are needed to more clarify the direct and indirect effect of glyphosate sewage on the dynamic of natural sturgeon populations.

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References

Anthelme, F. and Marigo, G., 1998. Glyphosate uptake in *Catharanthus roseus* cells: involvement of a plasma membrane redox system? *Pesticide Biochemistry and Physiology*, 62, 73–86.

Antón, F. A., Laborda, E. and Ariz, M., 1994. Acute toxicity of the herbicide glyphosate to fish. *Chemosphere*. 28,745–753.

APHA, 1992. Standard Methods for the Examination of Water and Wastewater, 18th ed. American Public Health Association, Washington, DC.

Bat, L., Akbulut, M., Çulha, M., Gündoğdu, A. and Satilmiş, H.,

2000. Effect of temperature on the toxicity of Zinc, Copper and Lead to the freshwater amphipod *Gammarus pulex pulex*. *Turkish Journal of Zooplanktons*, 24**,** 409–415.

Çavas, T, and Könen, S., 2007. Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (*Carassius auratus*) exposed to a glyphosate formulation using the micronucleus test and the comet assay. *Mutagenesis*, 22, 263–268.

Cribner, E. A., Battaglin, W. A., Gilliom, R. J. and Meyer, M. T., 2007. Concentrations of Glyphosate, Its Degradation Product, Aminomethylphosphonic Acid, and Glufosinate in Ground– and Surface–Water, Rainfall, and Soil Samples Collected in the United States, 2001–06, Scientific Investigations Report 2007–5122, <http://pubs.usgs.gov/sir/2007/5122.> U.S. Geological Survey, U.S. Department of the Interior.

Contardo–Jara, V., Klingelmann, E. and Wiegand, C., 2009. Bioaccumulation of glyphosate and its formulation Roundup Ultra in *Lumbriculus variegatus* and its effects on biotransformation and antioxidant enzymes. *Environmental Pollution*, 157**,** 57–63.

Edginton, A. N., Sheridan, P. M., Stephenson, G. R., Thompson, D. G. and Boermans, H. J., 2004. Comparative effects of pH and Vision® herbicide on two life stages of four anuran amphibian species. *Environmental toxicology and chemistry*, 23, 815–822.

Elandalloussi, L. M., Leite, R. B., Rodrigues, P. M., Afonso, R. and Cancela, M. L., 2008. Effect of the Herbicide Roundup ((R)) on *Perkinsus olseni* in vitro Proliferation and in vivo Survival when Infecting a Permissive Host, the Clam *Ruditapes decussatus*. *Bulltine of Environmental Contamination and Toxicology,* 80(**6**), 512–515.

Food and Agriculture Organization, 2007. FAO year book. Fishery and Aquaculture Statistics. Rome. [www.fao.org/fishery/statistics/en]. Access: 24 June 2010.

Finney, D.J., 1971. Probit Analysis :New York. Cambridge University Press.

Folmar, L.C., Sanders, H.O., and Julin, A.M., 1979. Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. *Archives of Environmental Contamination and Toxicology*, 8, 269–278.

Gardner, S.C., Grue, C.E., 1996. Effects of Rodeo and Garlon 3A on nontarget wetland species in central Washington. *Environmental Toxicology and Chemistry*, 15, 441–451.

Giesy, J.P., Dobson, S., and Solomon, K.R., 2000. Ecotoxicological Risk Assessment for Roundup Herbicide. *Reviews of Environmental Contamination and Toxicology*, 167, 35–120.

Glusczak, L., Miron, D. S., Crestani , da Fonseca, M. B., Pedron, F. A.,

Duarte, M. F., and Vieira, V. L. P., 2006. Effect of glyphosate herbicide on acetyl cholinesterase activity and metabolic and hematological parameters in piava (*Leporinus obtusidens*). *Ecotoxicology and Environmental Safety*, 65, 237–241.

Glusczak, L., Miron, D. D., Moraes, B. S., Simões, R. R., Schetinger, M. R. T., Morsch, V. M. and Loro, V. L., 2007. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*). *Comparative Biochemistry and Physiology*, 146, 519–524.

International Union for Conservation of Nature, 2010. IUCN Red List of Threatened Species. Version 2010.1. [www.iucnredlist.org]. Access: 24 June 2010.

Jiraungkoorskul, W., Upatham, E.S., Kruatrachue, M., Sahaphong, S., VichasriGrams, S. and Pokethitiyook, P., 2002. Histopathological effects of Roundup, a glyphosate herbicide, on Nile tilapia (*Oreochromis niloticus*). *Science of Asia*, 28,121–127.

Josupeit, H., 1994. World Trade of Caviar and Sturgeon. FAO, Rome. 100P.

Kolpin, D. W., Thurman, E. M., Lee, E. A., Meyer, M. T., Furlong, E. T. and Glassmeyer, S. T., 2006. Urban contributions of glyphosate and its degradate AMPA to streams in the United States. *The Science of the Total Environment*, 354, 191–197.

Kottelat, M., Gesner, J., Chebanov, M. and Freyhof, J., 2009. *Huso huso*. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.1. [www.iucnredlist.org]. Access: 24 June 2010.

Langiano, V. C. and Martinez, C. B. R., 2008. Toxicity and effects of a glyphosate–based herbicide on the Neotropical fish *Prochilodus lineatus*. *Comparative Biochemistry and Physiology, Part C*. 147, 222–231.

Levin, A. V., 1997. The distribution and migration of sturgeons in the Caspian Sea. Sturgeon stocks and caviar trade workshop. International Union for Conservation of Nature. Proceedings of a workshop on sturgeon stocks and caviar trade,1997 , Gland, Switzerland, and Cambridge, United Kingdom.

Mann, R. M. and Bidwell, J. R., 1999. The toxicity of glyphosate and several glyphosate formulations to four species of southwestern Australian frogs. *Archives of Environmental Contamination and Toxicology*. 26, 193–199.

Mozdzer, T. J., Hutto, C. J., Clarke, P. A. and Field, D. P., 2008. Efficacy of imazapyr and glyphosate in the control of non–native *Phragmites australis*. Restoration Ecology, 16(2), 221–224.

Neskovic, N. K., Poleksic, V., Elezovic, I., Karan, V. and Budimir, M., 1996. Biochemical and histopathological effects of glyphosate on carp (*Cyprinus carpio*). *Bulletin of Environmental Contamination and Toxicology*, 56, 295–302.

Nourouzi, M., Pourkazemi, M. and Fatemi, M., 2009. Application of microsatellite markers to study the genetic structure of stellate sturgeon population (*Acipenser stellatus* Pallas, 1771) in the south Caspian Sea. *Iranian Journal of Fisheries Sciences*, 8(1), 73–84.

Oruç, E.O., and Üner, N., 1999. Effects of 2,4–Diamin on some parameters of protein and carbohydrate metabolisms in the serum, muscle and liver of *Cyprinus carpio*. *Environmental Pollution*, 105, 267–272.

Pajand, Z. O., Esmaeli–Sari, A. and Piri–Zirkoohi, M., 2003. Effect of diazinon on the Persian sturgeon and stellate sturgeon fingerlings. *Pajouhesh & Sazandegi*, 58, 64–7.

Papchenkova, G. A., Golovanova, I. L. and Ushankova, N. V., 2009. The parameters of reproduction, sizes and activities of hydrolases in Daphnia magna Straus of successive generations affected by Roundup herbicide. *Inland Water Biology*. 2(**3**), 286–291.

Perkins, P. J., Boermans, H. J., and Stephenson, G. R. 2000, Toxicity of glyphosate and triclopyr using the frog embryo teratogenesis assay–*Xenopus*. Environmental Toxicology and Chemistry. **19**:940–945.

Pourkazemi, M., Skibinski, D. O. F., and Beardmore, J. A., 2000. A preliminary Study on Phylogenetic Relationships between Five Sturgeon Species in the Iranian Coastline of the Caspian Sea. *Iranian Journal of Fisheries Sciences*, 2(1), 1–12.

Relyea, R. A., 2005. The Lethal Impacts of Roundup and Predatory Stress on Six Species of North American Tadpoles. Archive of Environmental Contamination and toxicology, **48:**351–357.

Rodrigues, B. N. and Almeida, F. S., 1998. Guia de Herbicidas. Londrina, Paraná–Brazil. 648P.

Rueppel, M. L., Brightwell, B. B., Schaefer, J. and Marvel, J. T. 1977. Metabolism and Degradation of Glyphosate in Soil and Water. *Journal of Agricultural and Food Chemistry*, 25, 517–528.

Sadeghirad, M., Amini Ranjbar, G. H. R. , Joushideh, H. and Arshad, A., 2009. Heavy metal concentration in the selected tissues of the Persian sturgeon, *Acipenser persicus*, from the southern cost of the Caspian Sea. *Iranian Journal of Fisheries Sciences*, 8(**2**), 175–184.

Servizi, J. A., Gordon, R. W. and Martens, D. W., 1987. Acute toxicity of Garlon 4 and Roundup herbicides to salmon and trout. *Bulletin of Environmental Contamination and Toxicology*, 39**,**15–22.

Simenstad, C. A., Cordell, J. R., Tear, L., Weitkamp L. A., Paveglio F. L., Kilbride, K. M., Fresh, K. L., et al., 1996. Use of Rodeo® and X–77® Spreader to control smooth cordgrass (*Spartina alterniflora*) in a southwestern Washington estuary: II. Effects on benthic microflora and invertebrates. *Environmental Toxicology and Chemistry*, 15, 969–978.

Smith, G. R., 2001. Effects of acute exposure to a commercial formulation of glyphosate on the tadpoles of two species of anurans. *Bulletin of environmental contamination and toxicology*, 67, 483–488.

Thompson, D. G., Wojtaszek, B. F., Staznik, B., Chartrand, D. T. and Stephenson, G. R., 2004. Chemical and biomonitoring to assess potential acute effects of Vision® herbicide on native amphibian larvae in forest wetlands. *Environmental toxicology and chemistry*, **23**, 843–849.

Tsui, M. T. K. and Chu, L. M., 2003. Aquatic toxicity of glyphosate–based formulations: comparison between different organisms and the effects of environmental factors. *Chemosphere*. 52,1189–1197.

van Der Oost, R., Beyer, J. and Vermeulen, N. P. E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental toxicology and pharmacology*, 13, 57–149.

Vereecken, H., 2005. Mobility and leaching of glyphosate: a review. *Pesticide Management Science,* 61,1139–1151.

Wan, M. T., Watts, R. G., and Moul, D. J., 1989. Effects of different dilution water types on the acute toxicity to juvenile Pacific salmonids and rainbow trout of glyphosate and its formulated products. *Bulletin of Environmental Contamination Toxicology*, 43, 378–385.

Word Health Organization, 1994. Glyphosate. Environmental Health Criteria No. 159, [http://www.inchem.org/documents/ehc/ehc/ehc159.htm.]. Access: 24 June 2010.

Williams, G. M., Kroes, R. and Munro, I.C., 2000. Safety Evaluation and Risk Assessment of the Herbicide Roundup and Its Active Ingredient, Glyphosate, for Humans. *Regulatory Toxicology and Pharmacology*, 31,117–165.

Wilson, W. H. Jr and Parker, K., 1996. The life history of the amphipod, *Corophium volutator*: the effects of temperature and shorebird predation. *Journal of Experimental Marine Biology and Ecology*, 196, 239–250.

Yu, Q., Chaisuksant, Y. and Connell, D. W., 1999. A model for non-specific toxicity with aquatic organisms over relatively long periods of exposure. *Chemosphere,* 38**,** 909–1918.