Studies of the impact of glyphosate on fish and fishpond water under mesocosm conditions

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by

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Abstract

The purpose of this study was to examine the effects of glyphosate on fish survival and aquatic environment parameters in mesocosm experiments. Fishes used in the experiments where Tilapia (*Oreochromis niloticus*) and Lambari (*Astyanax bimaculatus*). In a "high concentration" test of glyphosate during a short period of time (6, 12 and 24 hours), survival and growth of young fishes was recorded. In a second experiment, "environmental effects" of glyphosate application for aquatic weed control were evaluated during 9 weeks.

There were no obvious differences in growth and mortality in the "high concentration" experiment, regardless of the different concentrations and times of exposure of glyphosate used.

In the "environmental effects" test, some Tilapia from the higher concentrations had developed exoftalmia (visible swelling in the ocular globe) and some Tilapia in the highest concentration had effects on the liver. Among Lambari, no individuals were effected.

In the "environmental effects" test the initial increase of chlorophyll content seemed to be lower in the control mesocosms than in the glyphosate treatments. Possibly, glyphosate degraded relatively fast, maybe accompanied by an elevated release of nutrients.

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1. Aim

The aim of this study was to measure possible ecotoxicological impacts of glyphosate on the aquatic environment under mesocosm conditions. Two different experiments were conducted, one short period and "high concentration" test for fish survival, and one long period test at similar glyphosate concentration when measuring aquatic environment parameters as well as fish survival.

2. Introduction

2.1 World wide use of glyphosate

Glyphosate (N-phosphonomethylglycine) is a broad-spectrum and non-selective herbicide, used for the control of emerged aquatic grasses, broadleaf weeds and brush. The isopropylamine salt of glyphosate is also used for aquatic weed control and registered for use in all kinds of aquatic systems (Westerdahl and Getsinger, 1988), although management of aquatic weeds with glyphosate is not allowed in Sweden. In many situations it is the additives or adjuvants in the product, that makes it more toxic than the technical-grade glyphosate. The LD_{50} value of aspirin is 1240 mg/kg for rats, while the value of glyphosate (5600 mg/kg) is about four times as high (McNabb, 1996). The toxicity is, thus, lower for glyphosate than for aspirin. As a reference, the LD_{50} value is 200 mg/kg for caffeine (McNabb, 1996).

In Atkinson and Grossbard (1985) it was stated that "glyphosate has no remarkable subacute or chronic toxicity, nor does it have mutagenic or teratogenic effects at doses well above those expected to be received by ingestion of crops treated with glyphosate".

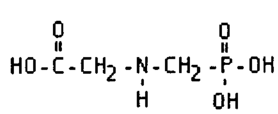
For more than 20 years glyphosate has been extensively used and its use is expected to increase even more as more glyphosate-resistant crops become available. This post-emergence herbicide is registered for use in more than 50 crops. Although primarily employed to control unwanted vegetation within an agricultural setting, herbicides containing glyphosate are used in many other areas, such as industrial, aquatic weed control, ornamental garden, and residential weed management.

Glyphosate is often used for perimeter and drainage canal weed control and for eliminating weeds from old polyethylene-mulched vegetable fields in preparation for reuse of the mulch for a second vegetable crop. With increased usage of glyphosate there is increased likelihood of damage from drift to nearby non-target crops, the extent of which would be dependent of the dosage to which the crop was exposed and the number of exposures. The existence of drift related injury to tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum annum*) has been documented, but the potential for damage to adjacent vegetable crops due to spray drift of glyphosate has been the subject to little research (Gilreath *et al.*, 2000). An obvious fact is that the intentional application of glyphosate formulations to emergent aquatic vegetation can result in higher concentrations in aquatic systems than can be expected from terrestrial uses (Giesy *et al.*, 2000).

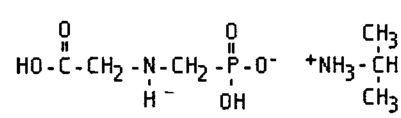
In agriculture, the use of glyphosate-based products is increasing, especially in applications involving genetically modified plant varieties engineered to tolerate glyphosate treatment. Other signs to expect an increase in the use of glyphosate is that farmers utilise glyphosate as a routine step in field preparations. In Sweden, the use of glyphosate has increased dramatically over the last years (KemI, 2001). Contributing factors to an increased sale of glyphosate are many, such as increased fuel price and decreased price of glyphosate. The phasing-out of the Swedish Conversion of Arable land scheme, the set-aside requirement in the EU Compensatory Payment scheme and the recently introduced requirements on autumn- and wintergrown land are some other possible explanations. Glyphosate use instead of mechanical methods in agriculture has become financially more beneficial (SJV, 2001).

The use of glyphosate, and its possible environmental effects, is of global concern. In Sweden, 30% of the total amount pesticides used contain glyphosate (Swedish Board of Agriculture, 1999). Compared to other pesticides, little research has been carried out about environmental effects of glyphosate, despite its use for nearly 30 years. A possible cause has been difficulties in measuring glyphosate and its residues. Further, action and symptoms of glyphosate are not necessarily herbicidal. As an example, glyphosate and its analogue glyphosine have for many years been used to enhance sucrose concentrations in sugar cane at harvest (Boehm, 1997).

In this review, the term glyphosate refers to the free acid form of N-phosphonomethyl-glycine (figure 1), even though much of the literature on glyphosate considers the isopropylamine salt of the herbicide. Isopropylamine is only one of the glyphosate salts. Other salts are sesquisodium and trimesium salts, also for pesticide use. Glyphosate is the active ingredient (a.i.) of herbicides such as Roundup and Rodeo, which both are based on the isopropylamine salt. The active ingredient (a.i) is the chemical in a formulated product that is principally responsible for the herbicidal effects and is shown as active ingredient on herbicide labels.



glyphosate
N-phosphonomethylglycine



isopropylamine salt of glyphosate

Figure 1. Structure of glyphosate and its isopropylamine salt.

2.2 Aquatic application

The numbers of effective compounds for controlling aquatic plants are limited. The desired toxicological effects in the aquatic environment have to be met, and at the same time satisfy the environmental effects criteria, as demanded for the registration of a herbicide. This is not simple. There are many tests of environmental impact, residue analysis and environmental fate, which has to be made over a long period of time, before an approval of a new pesticide can be made. The reason that there are only a few aquatic herbicides compared to crop production herbicides is mainly because of the uniqueness of the aquatic environment. Aquatic herbicides must have the capacity to be taken up by plants quickly and in sufficient amounts from water to be toxic to target plants yet have sufficiently low toxicity to man and to other organisms in the aquatic environment. Systemic herbicides are absorbed into the living portion of the plant and move within the plant. These herbicides, such as glyphosate, are only active when applied to and absorbed by foliage. They act more slowly compared to contact herbicides, which are generally lethal to all plant cells that they contact. Although they have to move to the part of the plant where their site of action is, systematic herbicides are generally more effective than contact herbicides, especially for controlling perennial and woody plants (Langeland and Thayer, 1991).

Herbicides for macrophyte control are commonly introduced directly into the water in shallow areas. An older application method, where the total water column was treated, was the use of part per million system which entailed covering the entire surface of the water body with the pesticide formulation. More recently, a number of application methods include a variety of controlled-release formulations that can provide the required concentration of herbicide for a long time and allow the most efficient placement of the herbicide (Murphy and Barrett, 1990). With systemic herbicides, such as glyphosate, the chemical must be sprayed directly onto emergent or floating vegetation for effective use.

In Giesy *et al.*, (2000) a summary of acute and chronic toxicity reference values (TRV) for aquatic and terrestrial wildlife for Roundup and glyphosate (tested as acid) is given, were the chronic toxicity reference value for fish is 1 mg a.i. /l.

2.3 Mode of action

Glyphosate is a phosphonic acid herbicide and an aromatic amino acid synthesis inhibitor in the protein biosynthesis (Tomlin, 1997). The mode of action of glyphosate is inhibition of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), resulting in the depletion of essential aromatic amino acids needed for plant survival (Ahrens, 1994). EPSPS is present in all plants, bacteria and fungi, but not in animals (European Commission, 1998). The synthesis of the chlorophyll precursor, 5-aminolevulinic acid (ALA), is also inhibited by glyphosate (Kitchen *et al.*, 1981). Its effects on chlorophyll content of green tissues are much less apparent, but effects in chloroplast development are fairly pronounced, where the effects are likely to be caused by photobleaching as a secondary effect of altered chloroplast function (Kearney and Kaufman, 1988). Lee (1981) found glyphosate to accelerate photodegradation of chlorophyll, especially in young leaves.

The amphoteric nature of glyphosate accounts for its relatively great K_d-value for binding to soil particles. Glyphosate has several pK_a values and is an amphotheric compound. It is practically insoluble in organic solvents due to its polarity. Therefore, glyphosate herbicides have to be applied to plant surface to be effective (Giesy *et al.*, 2000).

The amount of vacant phosphate sorption sites is correlated to glyphosate adsorption, and may occur through binding of the phosphonic acid moiety (Ahrens, 1994). Although glyphosate is not intentionally soil applied, a significant concentration of material may reach the soil or water surface. In a study of the effects of the isopropylamine salt on soil microbial activity and biomass, Haney *et al.* (2000) showed that it significally stimulated the soil microbial activity as measured by C and N mineralisation but did not effect soil microbial biomass. Further, according to The Pesticide Manual (Tomlin 1997), glyphosate and all its salts are non-volatile, do not photochemically degrade and are stable in air. Although there are no pronounced effects of glyphosate on photosynthesis, glyphosate does have relatively strong effects on chlorophyll synthesis (Munoz-Rueda, *et al.*, 1986).

2.4 Degradation in the environment

Field and laboratory studies have reported microbial degradation of glyphosate to AMPA (aminomethylphosphonic acid), the principal microbial metabolite, and CO_2 (Brightwell and Malik, 1978), with rapid dissipation from both flowing and standing surface waters (Brønstad and Friestad, 1985). Dissipation of glyphosate from flowing waters, is mainly due to factors such as tributary dilution, dispersion, and loss through adsorption to suspended particulate matter or sediments, and microbial degradation (Feng *et al.*, 1990). Dissipation from non-flowing waters such as ponds, is more depending on local conditions and therefore considered specific (Giesy *et al.*, 2000).

The herbicide is strongly adsorbed by sediment colloids, silt and suspended solids within the water column. When sorbed to sediments, glyphosate is inactivated. Because glyphosate is an acid under most environmental conditions, ionic rather than hydrophobic interactions are expected to account for the strong adsorption potential. Glyphosate has a water solubility of 11.6 g/l (25 °C), and a negligible vapour pressure (Tomlin, 1997). Based on the water solubility, the herbicide is not expected to bioaccumulate in aquatic biota. Using glyphosate concentration 3 to 4 times the recommended level, laboratory controlled studies showed bioconcentration factor (BCF) values in fish tissues after 10 to 14 days of exposure in the range of 0.2 – 0.3 (Brandt 1984). A chemical is "bioaccumulative" if its BCF is 100 or larger (KemI, 2001). However, glyphosate residues have been found in fish, crustaceans and molluscs after exposure to water containing the herbicide. The residues though, declined to about 50-90% of the accumulated levels when these aquatic organisms where subsequently exposed to water free from glyphosate for 14-28 days (FAO/WHO, 1986). Transfer of glyphosate from water to the atmosphere is negligible due to a negligible vapour pressure, which confirms the non-volatile nature of glyphosate (WSSA, 1983; Brandt 1984).

Biodegradation is considered to be the major process affecting glyphosate persistence and its strong adsorption to sediment in aquatic environments. It biodegrades both aerobically and anaerobically by micro-organisms present in soil, water and sediment. Degradation occurs more rapidly under aerobic than under anaerobic conditions (CCME, 1989). In aquatic environments, a minimum half-life of two weeks has been observed, but in clean waters with low content of organic matter and low microbial activity, glyphosate can persist for years (Atkinson and Grossbard, 1985).

From what researchers know today, glyphosate resistance in soil is very variable. Initial degradation is faster than the subsequent degradation of what remains and in some cases the half-life may be as little as three to five days, as e.g. the label of Roundup says. However, long persistence has been measured in following studies: 239 days on Finnish agricultural soils; between 295 days and 296 days on eight Finnish forestry sites; 335 days on an Ontario forestry site; 360 days on 3 British Columbia forestry sites; and 1 to 3 years on eleven Swedish forestry sites (Cox, 1995). As Cox (1995) stated: it is not one of the most earth spoiling chemicals that are being used – but it does have consequences. Since an enormous amount of people around the world use glyphosate, the risks begin to multiply. Accordingly, many aspects such as inert additives of glyphosate herbicide preparations are not being completely explored.

Glyphosate has been shown to damage liver and kidneys in laboratory animals such as rats and mice exposed to high levels over their lifetimes. EPA (United States Environment Protection Agency) has set the drinking water standard for glyphosate at 700 µg/l to protect against the risk of adverse health effects (Shelton, 2001). In EU, a level of 0.1 µg/l for individual pesticides is recommended not to be exceeded, which has been adopted by the Swedish National Food Administration (1993).

2.5 Additives

Surfactants are commonly used herbicide additives providing an enhancement of penetration of the spray solution through the leaf tissues. Surfactants may serve a number of functions, including acting as a wetting agent, or spreader, to reduce the surface tension between the leaf surface and the spray droplet. Surfactants have also been shown to influence the effects of glyphosate. Some surfactants increase the phytotoxicity of glyphosate but a variability of the effects on glyphosate efficiency has been reported (O'Sullivan *et al.*, 1981). The report suggests that cationic (polyethoxylated tertiary fatty amine) surfactants such as the POEA (polyethoxylated tallowamine) surfactant in Roundup, are more effective than non-ionic ones in enhancing glyphosate activity. The POEA-surfactant has also been shown to be about 20-70 times more toxic to fish than glyphosate itself (Folmar *et al.*, 1979). In Rodeo, no surfactant is included as sold, but must be added to effectively control weeds. The surfactant recommended for use with the Rodeo herbicide is ortho X-77, which also have been shown to be more toxic than glyphosate (Henry and Higgins, 1992).

The presence of hard-water cations, such as Ca^{2+} and Mg^{2+}, in the spray solution can greatly reduce the efficiency of glyphosate. The cations potentially compete with the isopropylamine in the formulation for association with the glyphosate anion. By using NMR (Nuclear Magnetic Resonance), an association of calcium was indicated with both the carboxyl and phosphonate groups of the glyphosate molecule. However, the presence of ammonium sulphate was observed to reverse the antagonism of calcium (Thelen *et al.*, 1995). In an effort to overcome the antagonism Buhler and Burnside (1983) demonstrated an increased activity by adding H_2SO_4. It was proposed that SO_4^{2-} compete for Ca^{2+} forming $CaSO_4$.

Determination of glyphosate residues is difficult and was discussed by Bardalaye *et al.* (1985) to be complicated by the three polar functional groups of glyphosate, phosphonic acid, carboxylic acid and secondary amine. The high degree of polarity necessitates derivatisation for many separatory methods, which is not always easy. Normally, analysis of the product is made by high pressure liquid chromatography (AOAC Methods, 1984) or gas chromatography methods (Mogadati *et al.*, 1996).

2.6 Objective and relevance of study

In Brazil there are many glyphosate factories and the majority of transportation is made by ship at sea and in large rivers, or by trucks. In an attempt to simulate a technical disaster, the first study was to evaluate the effects of high exposure of glyphosate during a short period of time (6, 12 and 24 hours) on survival and growth of young fishes. Tilapia (*Oreochromis niloticus*) was chosen as test organism because it is a tropical fish and important to Brazil as well as many other countries. Tilapia origins in the Nile River and has been farmed around the world for decades. It is a tropical species that can suppress growth of phytoplankton and benthic algae, and occurs in a wide variety of freshwater habitats like rivers, lakes, sewage canals and irrigation channels.

In a second experiment, under mesocosm conditions, environmental effects of glyphosate application for aquatic weed control were evaluated during 9 weeks. The fact that glyphosate dissipates quickly from free water is quite well known, but side effects or consequenses in the aquatic environment are not well documented and were to be investigated. Tilapia was used in conjunction with Lambari (*Astyanax bimaculatus*), which feeds on zooplankton, detritus and higher plants. It originates from the Amazon and may inhabit large free flowing, clear rivers, small streams, drainage ditches and artificial ponds and impoundments.

Fish occupy a number of trophic levels. They are susceptible to contaminant exposure through their diet, but direct uptake of waterborne chemicals via the gill is often the primary route of exposure.

3. Materials and methods

The experiments were carried out at Dept. Biologia Aplicada, UNESP (Universidade Estadual Paulista)-University of Sao Paulo, Campus Jaboticabal (Brazil), as showed in figure 2.

Figure 2. Experimental placement at UNESP-Jaboticabal, Brazil. Twenty-four mesocosms used in completely randomised design. The experiment included four treatments and six replications.

3.1 Herbicide used for study

The herbicide used in the two experiments was Rodeo (glyphosate (480 g/l), containing the isopropylamine salt of glyphosate (648 g/l) and inert ingredients (as the label of Rodeo says) (558 g/l)). The herbicide Rodeo is at the moment approved for use in crops like rice, citrus and sugarcane but has in the past also been approved for use in aquatic weeds. An application for a new licence is being considered by the Brazilian authorities (Professor Robinson Antonio Pitelli, personal communication). In the U.S. Rodeo is a formulation of glyphosate registered for aquatic use (Giesy *et al.*, 2000). As mentioned earlier, a surfactant must be added to glyphosate before terrestrial application, to effectively control weeds. The two factors that most often guide surfactant selection are efficacy and toxicity to nontarget aquatic organisms. As an example, the POEA surfactant used in Roundup, is more toxic to aquatic animals than is the active herbicidal ingredient glyphosate (Giesy *et al.*, 2000). Since this study was undertaken to examine ecotoxicological effects of glyphosate as its isopropylamine salt, the Rodeo herbicide, which contains no surfactant was chosen. Calculations in this report are based on glyphosate moiety only.

For single applications in the terrestrial environment, rates between 1-3.5 kg a.i./ ha are most common, but maximum single application rates up to 5.5 kg a.i./ha occur. The annual maximum total is 6,73 kg a.i./ha in any cropping situation, regardless whether it is a conventional crop or a glyphosate tolerant crop (Giesy *et al.*, 2000). The normal glyphosate rate used as an aquatic herbicide is about 2 kg/ha (Tomlin, 1997).

3.2 High concentration test - experimental procedure

Ten young Tilapias were put in a floating cage, totally 60 cages, keeping them in ponds for at least two days before exposure of glyphosate. The cages were perforated and made of plastic as shown in figure 3. The diameter was 0,3 m and the height was 0,4 m. One day before the experiment the total weight of the ten fishes inhabiting each cage was noted. In order to start, 60 litres of glyphosate solution was prepared. Four tanks (60 l) with four different concentrations of glyphosate, together with a control tank were prepared as shown in table 1. All treatments were replicated four times.

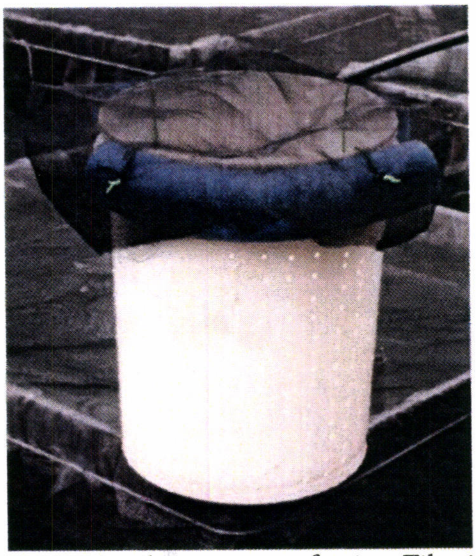


Figure 3. Floating cage for ten Tilapias (*Oreochromis niloticus*) during 22 days.

The concentration of glyphosate was estimated to be approximately equal throughout the experiment since there was negligible amount of organic matter in the water, and the inner side of each tank was covered with plastic. If adsorption to the walls of the cage and the covering net occured, this would have been of the same rate in each one of the cages and should therefore not have been the cause for any difference in glyphosate concentration among treatments. Normally an application rate of 75 ppm is regarded as very high.

The experimental situation for concentration and exposure time of glyphosate in the "high concentration" test is shown in table 1.

Table 1. Concentration and time of exposure to fishes of glyphosate (*Oreochromis niloticus*) in each treatment (1 ppm equals 1mg/l), replicated four times.

Treatment number	Concentration of glyphosate	Time of exposure
1	100 ppm	6 hours
2	100 ppm	12 hours
3	100 ppm	24 hours
4	50 ppm	6 hours
5	50 ppm	12 hours
6	50 ppm	24 hours
7	25 ppm	6 hours
8	25 ppm	12 hours
9	25 ppm	24 hours
10	12.5ppm	6 hours
11	12.5ppm	12 hours
12	12.5ppm	24 hours
13	Control	6 hours
14	Control	12 hours
15	Control	24 hours

3.2.1 Glyphosate concentration: High concentration test.

The five different treatments were 12.5ppm, 25ppm, 50ppm, 100ppm and control. Concentrations were based on weight per volume of water. To prepare the solution of glyphosate, calculations were made as shown in equations 1-2.

$$(X * 480 \text{ g/l}) / 60 \text{ l} = 100\text{ppm} \quad (1)$$

$$X = ((100 \text{ mg/l}) / 480000 \text{ mg/l}) * 60 \text{ l} = 12.5 \text{ ml} \quad (2)$$

where X is the amount of Rodeo to use in 60 l solution.

After exposure to glyphosate, all the fishes were transferred to clean water at the same time. The time for returning the fishes to clean water is therefore referred to as time 0. The sequence of introducing the fishes in glyphosate solution is also shown in table 1. For example, number 3, 6, 9, 12 and 15 were exposed to different concentrations 24 hours before time 0. When the fishes were transferred into fresh water at time 0 they were subjected to the same conditions of recovery. They were fed twice a day with a mixture of sojabean meal, wheat meal, salt, calcium carbonate, proteins and fish flour, supplied as fish food by a local dealer. Every day they were given food corresponding to 10% of their biomass, (appendix A, table A). At 22 days after the treatment the weight of the fishes was noted again. The fish mortality was evaluated continuously. The cages were kept away from rain and direct sunlight during time of exposure to glyphosate and then subjected to normal environmental factors. All measured data is shown in table A, B and C in appendix A.

3.3 Environmental effects test - experimental procedure

Twenty-four mesocosms (500 l; figure 4) were used in a completely randomised experimental design. The material of the mesocosms was eternite, which had the surface covered with a plastic film to reduce supposed adsorption of glyphosate. The experiment was based on three different treatments and control, with six replications. The three different treatments of glyphosate were 2,0 kg/ha, 4,0 kg/ha, and 12,0 kg/ha of active ingredient (a.i). The herbicide was applied (sprayed) directly on the water surface. High concentrations of glyphosate like this should not normally occur in the field (directly in the water) since glyphosate is used against unwanted growth and applied to the emerged parts of the plant. In fact, those concentrations are higher than in the "high concentration" test of glyphosate in section 3.2.

The water intake and output in the mesocosms were located at the surface. The water level was held at 400 l in the mesocosms, and 100 l of fresh water was put in each cage every day in order to keep the water renovation time at 4 days. In case of rainfall, small containers were used to collect the outflowing water and recycle it, in order not to exceed the input of 100 l of fresh water per day, and to keep control of possible glyphosate loss.

In order to minimise the predation factor from birds and to decrease the rate of evaporation, all the mesocosms were covered with sunshade (40% reduction of insolation), as shown in figure 4. The sunshade covered the mesocosms at all times except when measuring data. One week before starting the second experiment (measuring daily parameters), half a kilo of sediment from a nearby located lake (inhabiting Tilapias and Lambaris among fishes) was put into each cage to induce growth of algae.

Figure 4. All mesocosms covered with sunshade (40 % reduction of insolation).

The water quality parameters chlorophyll a, and total nitrogen and phosphorus concentration, were evaluated weekly for 8 weeks from the day of applying glyphosate. Additional measurements were taken five days before spraying, making it nine weekly measurements in total. Daily measurements were taken of dissolved and saturated oxygen concentration, temperature, pH, salinity, electrolytic conductivity, and the survival of young Tilapia (*Oreochromis niloticus*) and Lambari (*Astyanax bimaculatus*). Measurements of glyphosate residues were taken at four different occasions, one day, one week, one month and two months after applying glyphosate. For glyphosate and chlorophyll measurements one litre samples were taken while 250 ml samples were sufficient when measuring nutrients. The fishes were fed at a rate corresponding to 10% of their body weight per day.

For measuring the daily data, portable equipment was used. The oxygen-meter (YSI F-1085) can measure dissolved oxygen (mg/l), oxygen saturation (%), temperature (°C), conductivity (µS/cm) and salinity (‰). The value of conductivity was measured in two ways, at the temperature (not adjusted) and adjusted for a temperature of 25 °C. pH was measured using a CORNING® CHEKMITE pH-10.

3.3.1 Glyphosate concentration: Environmental effects test.

Three different concentrations (a.i) of glyphosate were to be applied, 2.0 kg/ha, 4.0 kg/ha and 12.0 kg/ha. Concentrations used will be referred to as LOW (low dose, 2 kg/ha), MEDIUM (medium dose, 4 kg/ha), HIGH (high dose, 12 kg/ha) and CONTROL (no glyphosate). The dose was area based and not calculated by volume.

As mentioned earlier the active ingredient (a.i) of Rodeo is 480 g/l, with the isopropylamine salt as 648 g/l. For application of glyphosate (spraying), a hand-equipment was used. The device normally sprays 200 l/ha, and for the experiment 2 litres of each concentration was enough. To know what quantity of Rodeo to use for a 2 l solution, the doses in l/ha were divided by 100, see equations 2.1-2.3. To prepare the spray solution a simple calculation was made.

$$2 \text{ kg/ha} / 0.48 \text{ kg/l (a.i)} = 4.17 \text{ l/ha} / 100 \Rightarrow 41.7 \text{ ml} / 2 \text{ l (LOW)} \quad (2.1)$$

$$4 \text{ kg/ha} / 0.48 \text{ kg/l (a.i)} = 8.33 \text{ l/ha} / 100 \Rightarrow 83.3 \text{ ml} / 2 \text{ l (MEDIUM)} \quad (2.2)$$

$$12 \text{ kg/ha} / 0.48 \text{ kg/l (a.i)} = 25 \text{ l/ha} / 100 \Rightarrow 250 \text{ ml} / 2 \text{ l (HIGH)} \quad (2.3)$$

The herbicide was applied directly on the water surface. The treatments were completely randomised (figure 5). Surrounding tanks were covered to avoid effects from spray drift. Control tank E 5 was only covered by 80 % when spraying tank D 4, see spraying scheme figure 5. Minor effects of spray drift may have taken place. The weekly measurements (total N, total P and chlorophyll content) and samples for glyphosate determination were taken 24 hours after spraying. All daily measured data are shown in table D, E, F, and G in appendix B and all weekly measured data are shown in table H, I and J in appendix C.

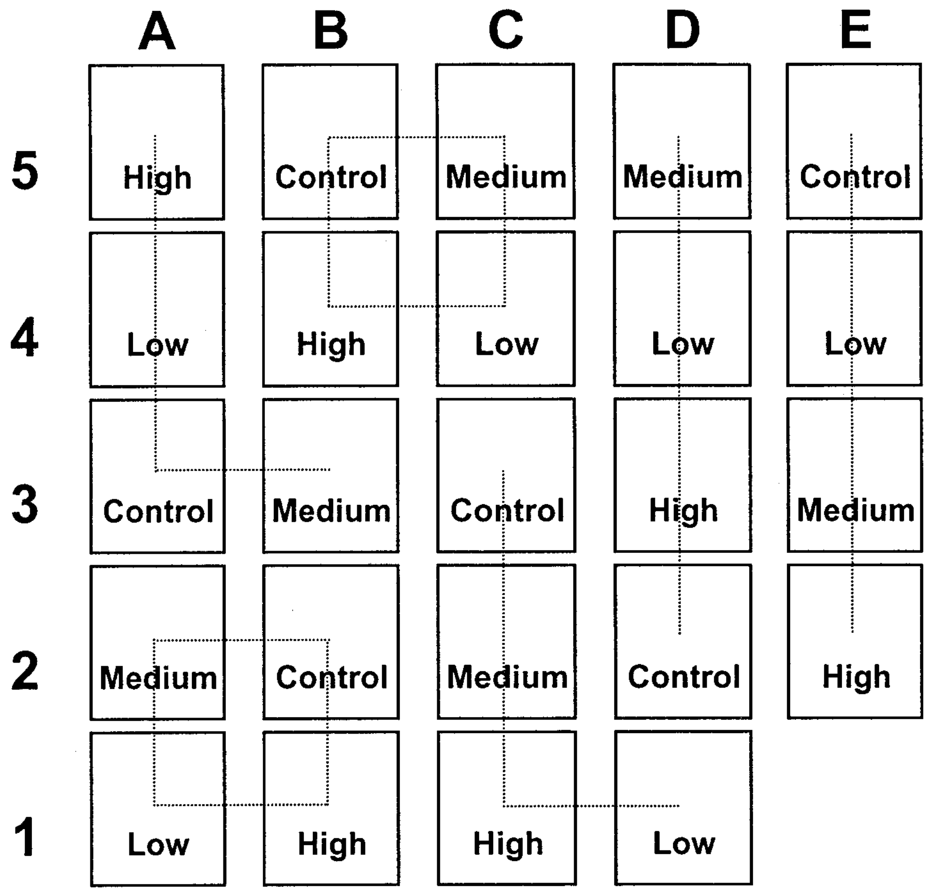


Figure 5. Spraying scheme of glyphosate rates in mesocosms. A1-E5 is the designation of the experimental mesocosms and the concentrations (a.i) are referred to as control (0 kg/ha), low (2 kg/ha), medium (4 kg/ha) and high (12 kg/ha). Dotted lines group mesocosms into blocks.

The experiment mesocosms were divided into six different blocks with all the different treatments in each block (table 2); high, medium, low dose and control. At the time of application (11.00 hrs local time), the temperature and humidity was 28 °C and 66% respectively (Renan Gravena, Dept. Biologia Aplicada, UNESP-Jaboticabal Brazil, personal communication).

Table 2. Grouping of mesocosms for the "environmental effects" test into blocks.

Block 1: A1, A2, B1 and B2	Block 4: C1, C2, C3 and D1
Block 2: A3, A4, A5 and B3	Block 5: D2, D3, D4 and D5
Block 3: B4, B5, C4 and C5	Block 6: E2, E3, E4 and E5

3.3.2 Mixing water samples for glyphosate determination.

For economical reasons the number of samples for glyphosate determination was minimised. The experiment had six replications and mixing of water samples was made by mixing different blocks from water samples taken at the same time, i.e. block 1 was mixed with block 2, block 3 mixed with block 4 and block 5 mixed with block 6. As an example, the control sample A3 (block 1) taken at 15 Mar-2001, was mixed with B2 (block 2) also taken at 15 Mar-2001. In table 3 the schedule for mixing of glyphosate samples is shown.

Table 3. Schedule of mixing glyphosate samples before analysis.

Location	Water sample Control	Low dose	Medium dose	High dose
Block 1+2	A3+B2	A1+A4	A2+B3	B1+A5
Block 3+4	B5+C3	C4+D1	C5+C2	B4+C1
Block 5+6	D2+E5	D4+E4	D5+E3	D3+E2

3.4 Experiment surroundings

Weather data for 2001-03-10 until 2001-05-10 is presented in figure 6a, 6b and 6c. Fig 6a shows the air temperature with minimum, mean and maximum values per day. Figure 6b displays the rainfall in mm and sunlight in hours/day, while figure 6c shows the relative humidity in % and atmospheric pressure in millibar.

According to figure 6a and 6b the amount of rainfall was during the 2001-03-10 until 2001-05-10 smaller than usual, even though the temperature was normal. The measurements of atmospheric pressure in figure 6c are all about 950 mbar, which is close to the "normal" pressure at sea level (1,013 bar). The relative humidity is quite low (30-50 %) due to small amounts of rain and high temperature.

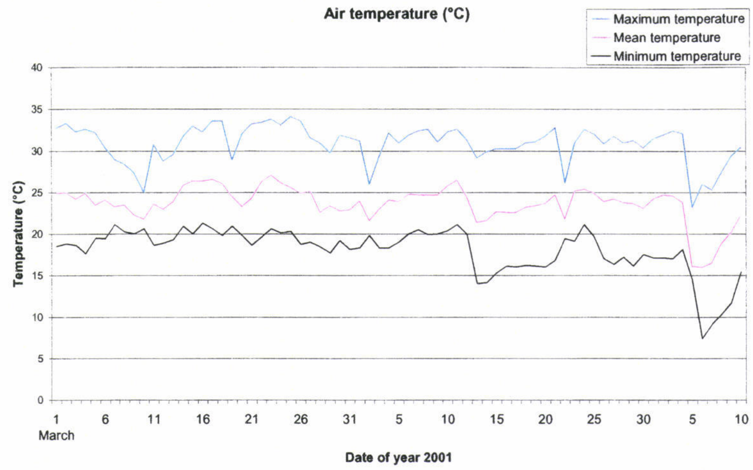


Figure 6a. Air temperature (°C) at Jaboticabal (Brazil), during 2001-03-10 until 2001-05-10.

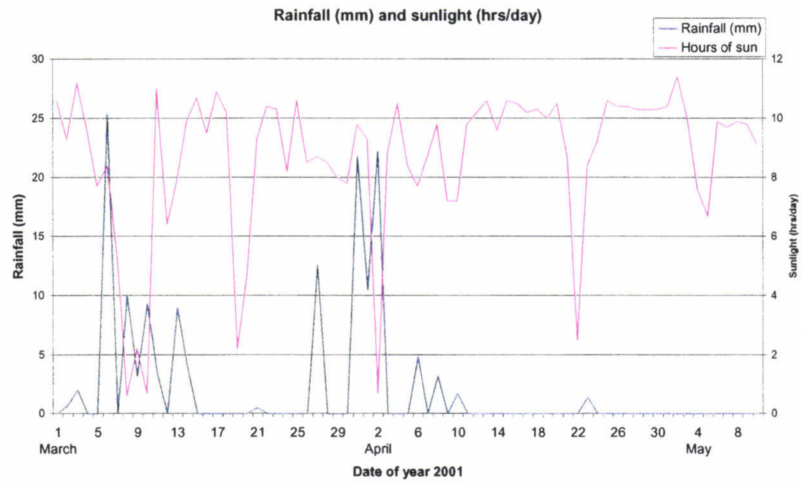


Figure 6b. Rainfall (mm) and sunlight (hrs/day) at Jaboticabal (Brazil), during 2001-03-10 until 2001-05-10.

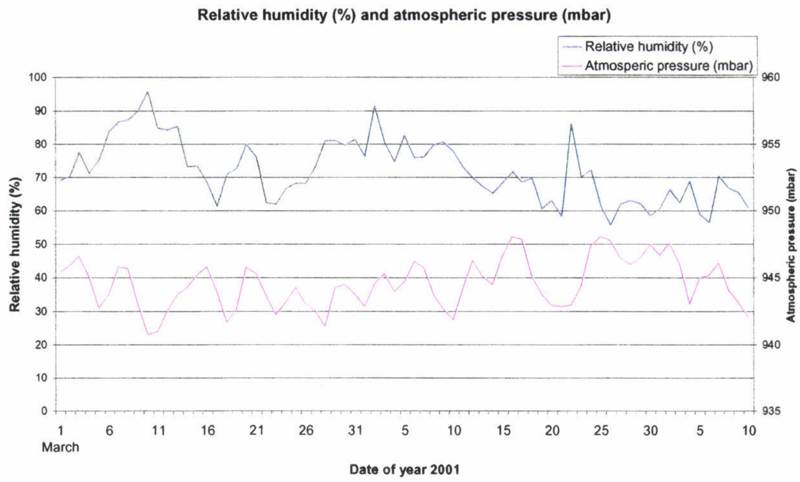


Figure 6c. Relative humidity (%) and atmospheric pressure (mbar) at Jaboticabal (Brazil), during 2001-03-10 until 2001-05-10.

4. Results

4.1 High concentration test

The amount of food (10% of biomass) seemed to be insufficient in the first experiment, because some of the fishes were cannibalised. In some cases it was not discovered that one fish was missing until days after or after the final day of experiment when the fishes were weighted again. This may have resulted in unequal amounts of food for periods of time and the gain of weight can therefore have been disturbed and not proportional as intended. In figure 7 it is accounted for dead together with missing fishes, and in figure 8 only for dead fishes. In figure 8 it is also visualised how many fishes that died (%) of total number. Days of recovery from glyphosate were 22. There was no obvious difference in growth of Tilapia (*Oreochromis niloticus*) between different treatments (figure 7 and 8).

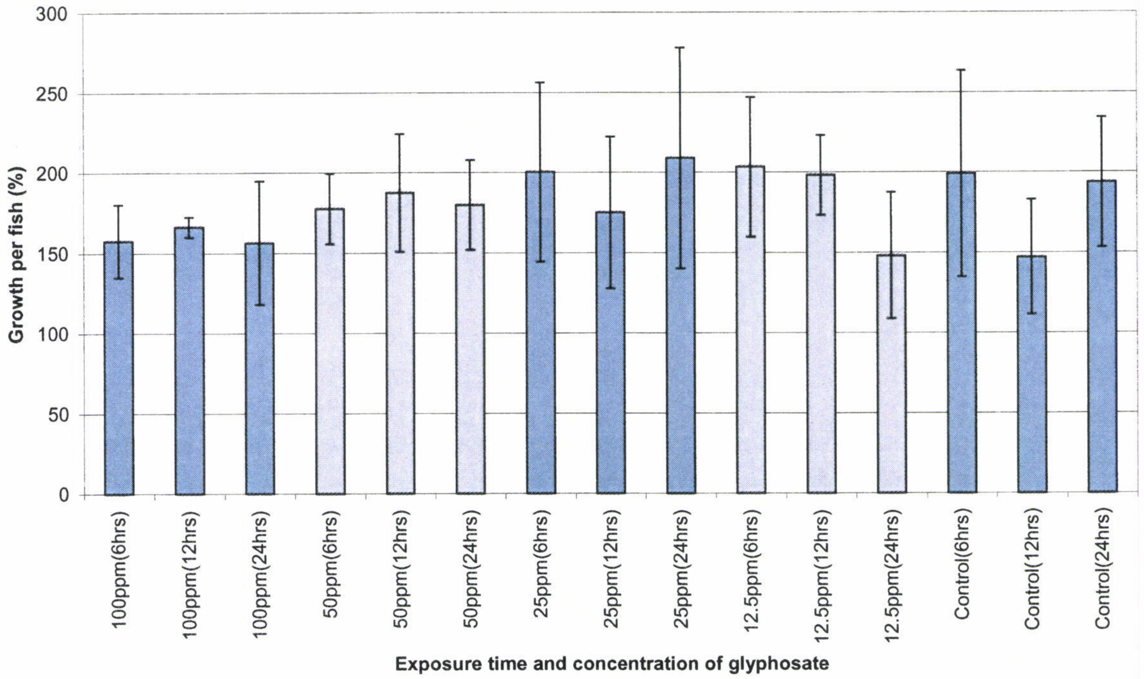


Figure 7. The growth (%) per fish (*Oreochromis niloticus*), after being exposed to glyphosate for 6, 12 and 24 hours at concentrations of 12.5, 25, 50 and 100 ppm and then fresh water for 22 days. Mean values of replications ± standard deviation, n=4. Each replication included ten fishes at start.

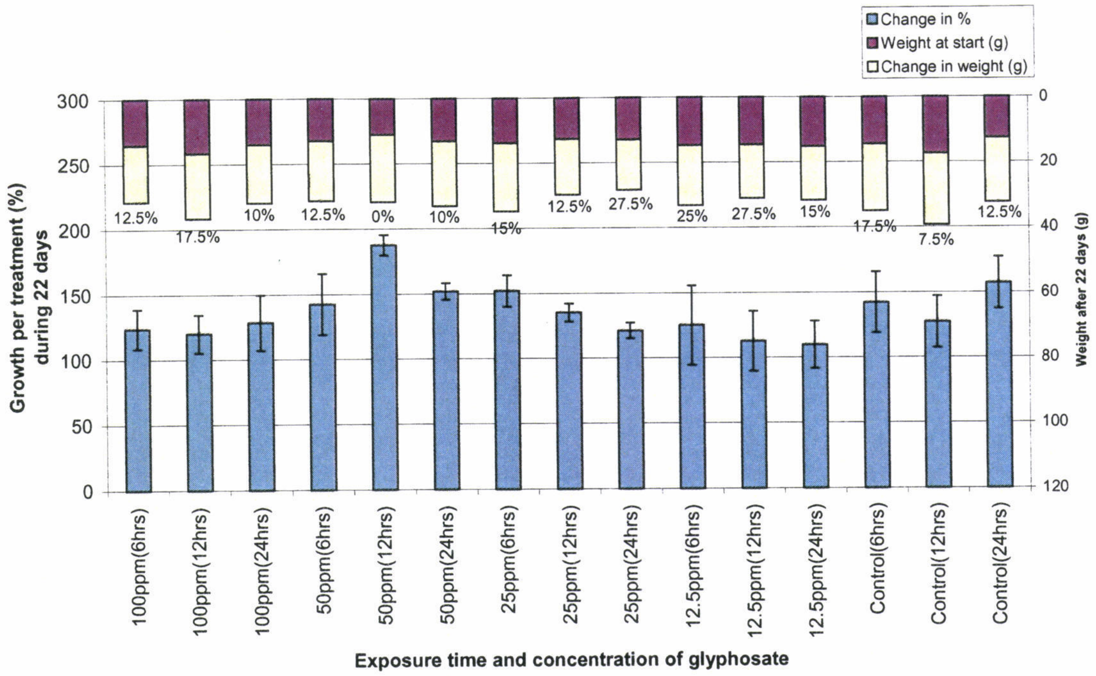
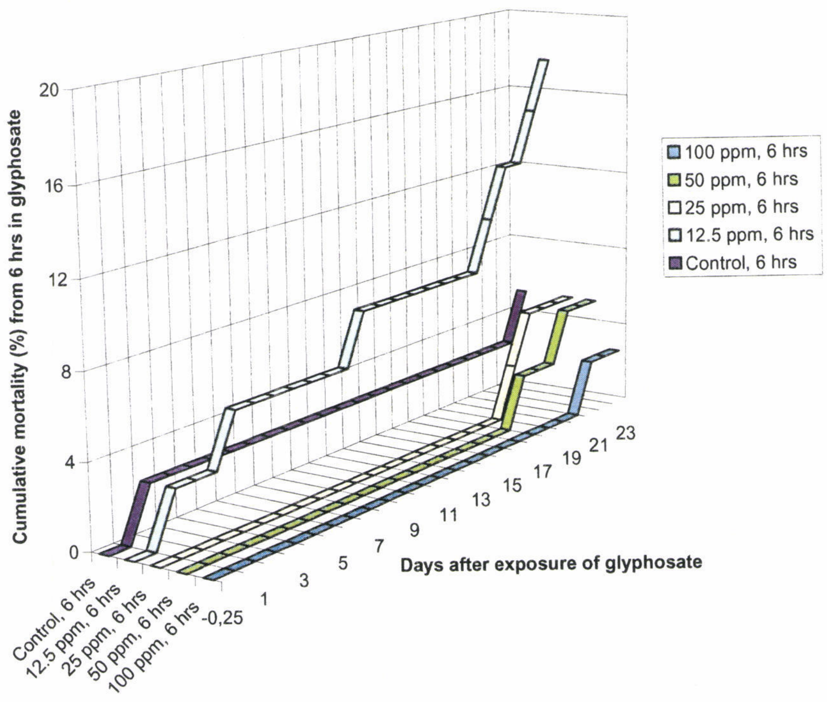


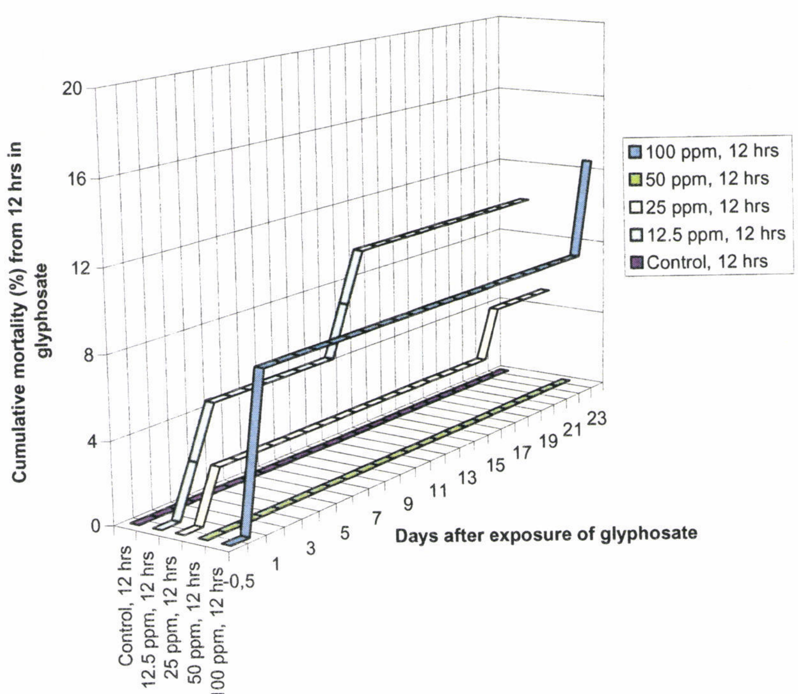
Figure 8. The growth (%) of fishes (*Oreochromis niloticus*) per treatment, after being exposed to glyphosate for 6, 12 and 24 hours at concentrations of 12.5, 25, 50 and 100 ppm, and change in weight (g). Dead fishes (%) of total under each stacked column. Mean values of replications ± standard deviation, n=4. Each replication included ten fishes at start and date of exposure was 18 Feb-01.

The mortality data were inconsistent (figure 9a-9c), did not seem to be related to glyphosate exposure, and the mortality in the 12.5 ppm for 6 hours treatment (figure 9a) can only be explained by the stress factor. The highest death-rate of fishes in the treatment of 100 ppm for 12 hours (figure 9b), occured during the first day in fresh water. Again, stress must have been an important factor. The mortality in the 12.5 ppm treatment for both 12 and 6 hours of exposure (figure 9a and 9b) was very high, although exposure for 24 hours (figure 9c) shows lower values of mortality. In the 24 hours treatment of 100 and 50 ppm (figure 9c) the values were also large but not conspicuously different from control.

a:


Cumulative mortality (%) from 6 hrs in glyphosate
20
16
12
8
4
0
Control, 6 hrs
12.5 ppm, 6 hrs
25 ppm, 6 hrs
50 ppm, 6 hrs
100 ppm, 6 hrs
-0,25
1
3
5
7
9
11
13
15
17
19
21
23
Days after exposure of glyphosate
100 ppm, 6 hrs
50 ppm, 6 hrs
25 ppm, 6 hrs
12.5 ppm, 6 hrs
Control, 6 hrs

b:


Cumulative mortality (%) from 12 hrs in glyphosate
20
16
12
8
4
0
Control, 12 hrs
12.5 ppm, 12 hrs
25 ppm, 12 hrs
50 ppm, 12 hrs
100 ppm, 12 hrs
-0,5
1
3
5
7
9
11
13
15
17
19
21
23
Days after exposure of glyphosate
100 ppm, 12 hrs
50 ppm, 12 hrs
25 ppm, 12 hrs
12.5 ppm, 12 hrs
Control, 12 hrs

c:

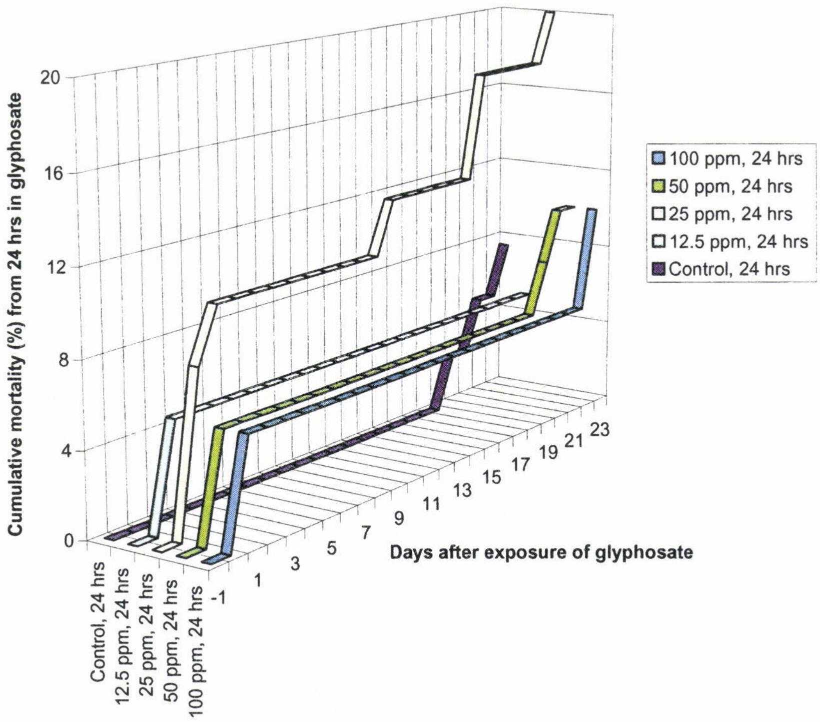


Figure 9. Cumulative mortality (%) of Tilapia (*Oreochromis niloticus*) from (a) 6, (b) 12 and (c) 24 hours in glyphosate, 100 ppm, 50 ppm, 25 ppm and 12.5 ppm. Mean value from replications with ten fishes in each, n=4. Date of exposure was 18 Feb-01, fishes were then transferred to fresh water, experiencing recovery during 22 days.

4.2 Environmental effects test

The oxygen content and conductivity of the water (figure 10 and 11) decreased during time of study (2001-03-10 until 2001-05-10). The mean LOW value of oxygen (figure 10) after application of glyphosate was always slightly higher, compared to other treatments. There was a large difference in conductivity between the two higher concentrations of glyphosate on one hand, and the low and control treatment on the other (figure 11) at the start of spraying.

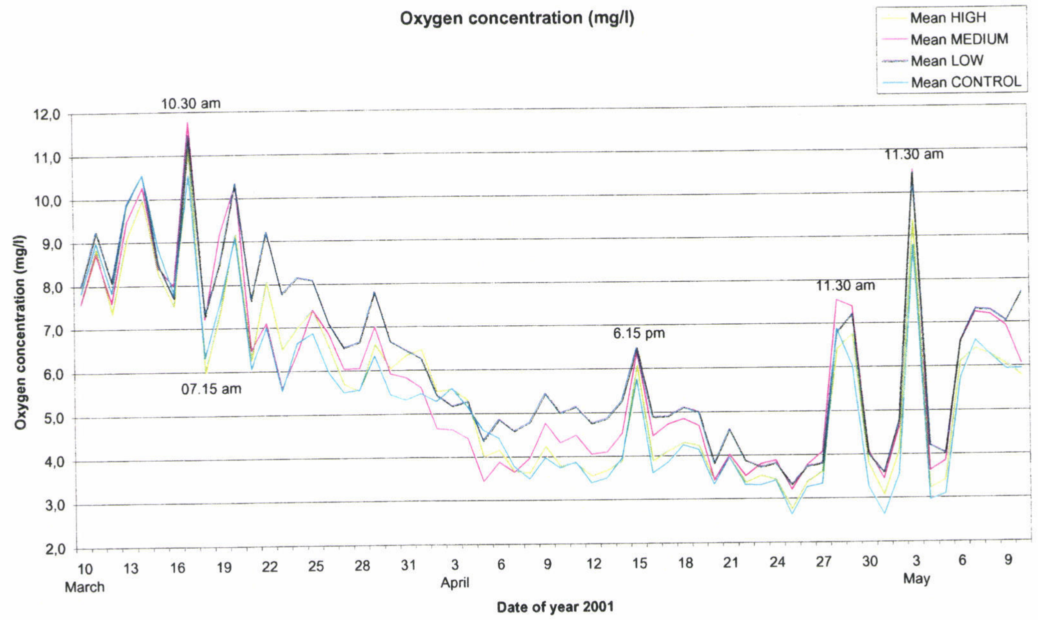


Figure 10. Oxygen concentration (mg/l) in the water for each treatment of glyphosate. Mean value of six replications. Concentrations (a.i) were HIGH(12 kg/ha), MEDIUM(4 kg/ha), LOW(2 kg/ha) and CONTROL(0 kg/ha). Date of applying glyphosate was 14 mar-2001. The normal time of measurement was 08.00-09.00 am and deviations are indicated.

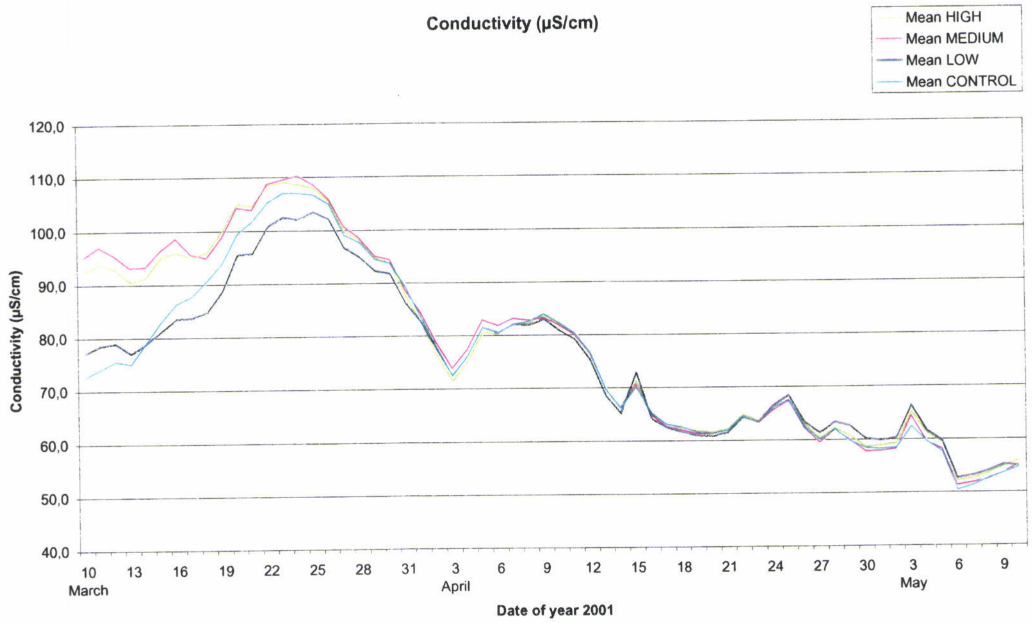


Figure 11. Conductivity (µS/cm) in the water for each treatment of glyphosate. Mean value of six replications. Concentrations (a.i) were HIGH(12 kg/ha), MEDIUM(4 kg/ha), LOW(2 kg/ha) and CONTROL(0 kg/ha). Date of applying glyphosate was 14 mar-2001.

Both pH and watertemperature tended to decrease (figure 12 and 13). Measurements of pH started 16 Mar-2001 due to technical problems. The higher values were all measured late in the day.

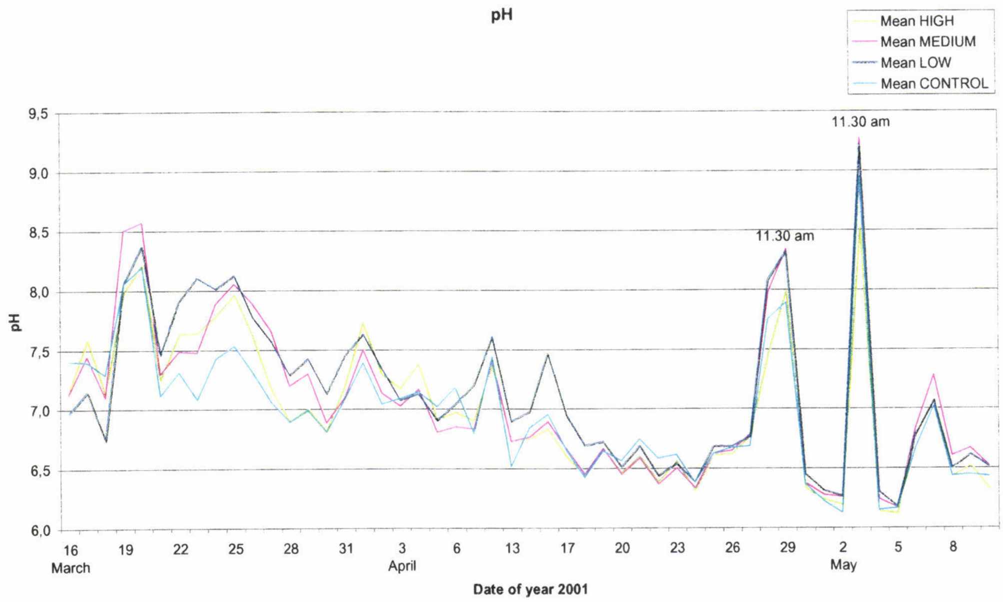


Figure 12. pH in water for each treatment of glyphosate. Mean value of six replications. Concentrations (a.i) were HIGH(12 kg/ha), MEDIUM(4 kg/ha), LOW(2 kg/ha) and CONTROL (0 kg/ha). Date of applying glyphosate was 14 mar-2001.

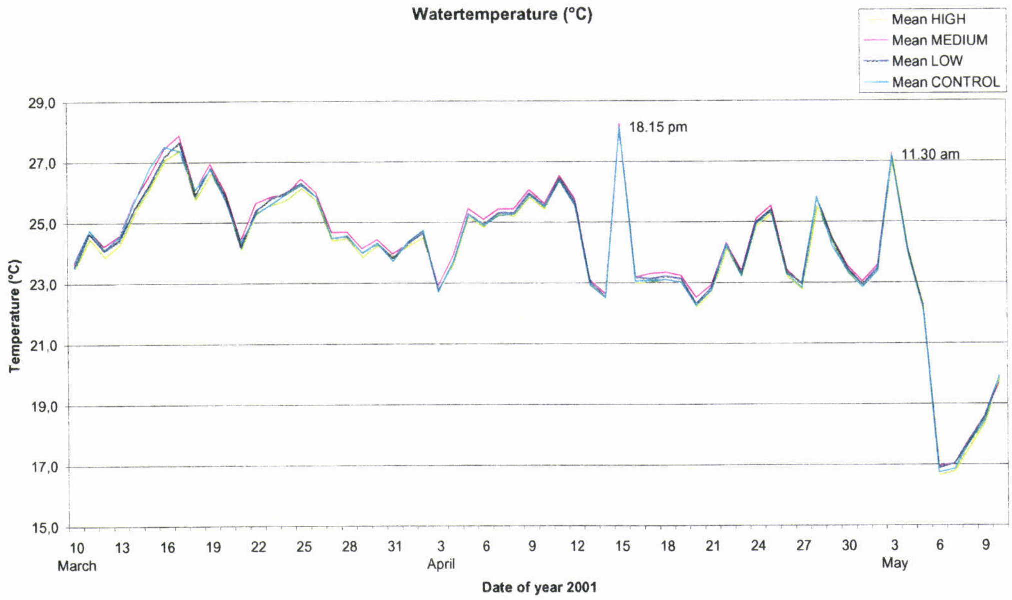


Figure 13. Water temperature (°C) for each treatment of glyphosate. Mean value of six replications. Concentrations (a.i) were HIGH(12 kg/ha), MEDIUM(4 kg/ha), LOW(2 kg/ha) and CONTROL (0 kg/ha). Date of applying glyphosate was 14 mar-2001.

The chlorophyll concentration tended to increase (figure 14), and in the medium and low treatment, concentrations seemed to be higher than in the control.

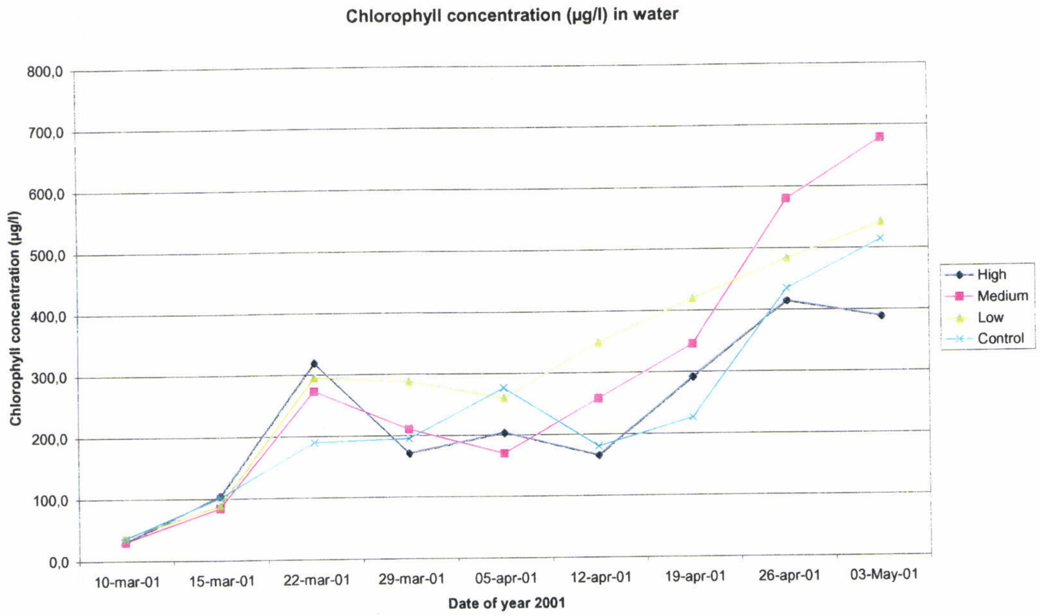


Figure 14. Chlorophyll concentration (µg/l) in water from each treatment of glyphosate. Mean value of replications, n=6. Concentrations (a.i) were 12 kg/ha (High), 4 kg/ha (Medium), 2 kg/ha (Low) and 0 kg/ha (Control). Application date of glyphosate was 14 mar-2001.

Nitrogen and phosphorus concentrations (figure 15a and 15b) did not seem to show any trends related to the different treatments.

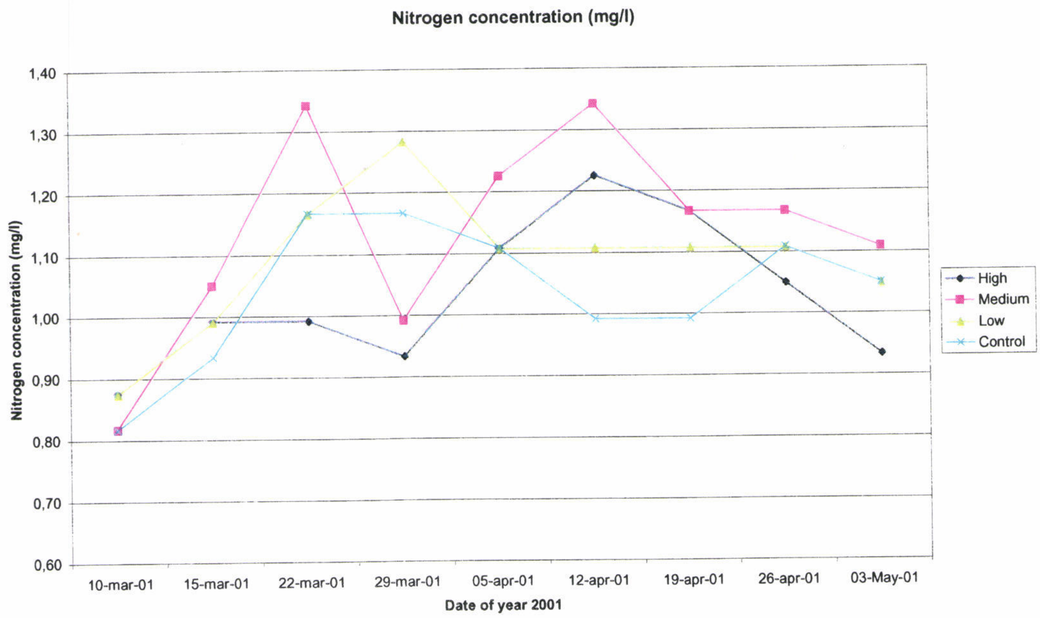


Figure 15a. Nitrogen concentration (%) of the water from each treatment of glyphosate. Mean value of replications, n=6. Concentrations (a.i) were 12 kg/ha (High), 4 kg/ha (Medium), 2 kg/ha (Low) and 0 kg/ha (Control). Application date of glyphosate was 14 mar-2001.

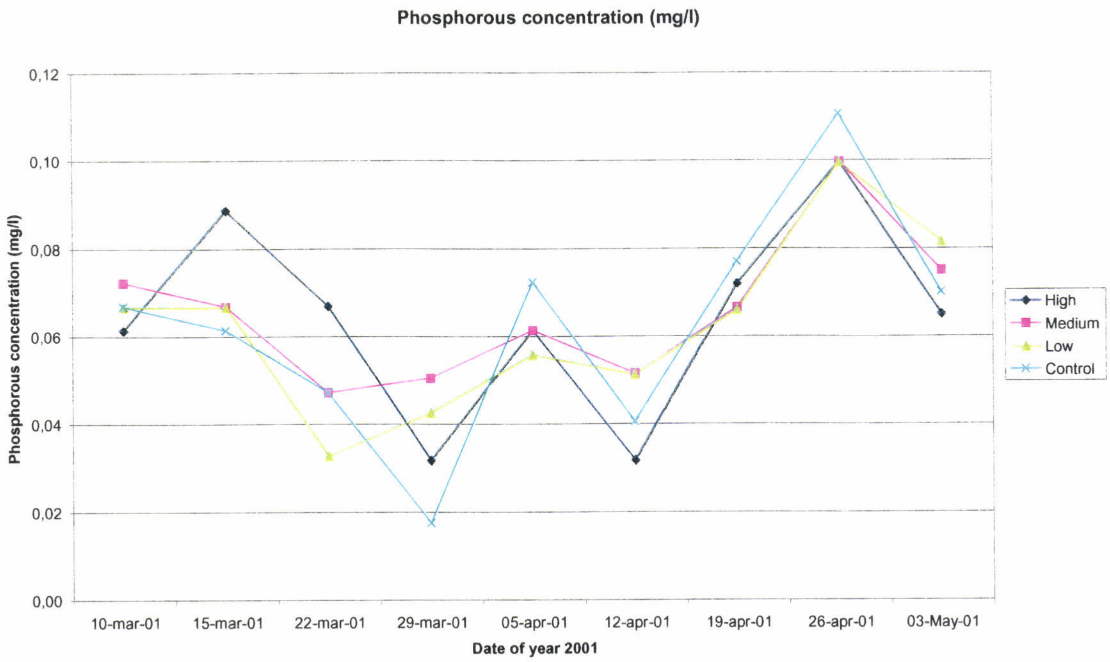


Figure 15b. Phosphorus concentration (%) of the water from each treatment of glyphosate. Mean value of replications, n=6. Concentrations (a.i) were High(12 kg/ha), Medium(4 kg/ha), Low(2 kg/ha) and Control(0 kg/ha). Application date of glyphosate was 14 mar-2001.

In the "environmental effects" test there seemed to be some effects on the fishes. Some Tilapia (*Oreochromis niloticus*) from the higher concentrations had developed exoftalmia (visible swelling in the ocular globe of the eye), and some in the highest concentration had effects on the liver. Among Lambari (*Astyanax bimaculatus*), no individuals were observed to be effected.

Glyphosate concentration values can not be presented, because the results of the glyphosate determination was not of hand at the time of printing of this report.

5. Discussion

The amount of food in the "high concentration" test was not sufficient, which resulted in cannibalism (*Oreochromis niloticus*). In the "high concentration" test study, mortality and weight change did not differ among different treatments. Probably more replications than four were needed. In future work, the stress factor has to be minimised, in order not to overrule possible treatment effects.

Control operations made with herbicides can have an indirect effect on the aquatic environment. If large amounts of aquatic vegetation are being killed, there may be a secondary effect on phytoplankton, such as nutrient release of nitrogen and phosphorus causing phytoplankton growth, blooms. Decaying vegetation and lack of oxygen production may cause dissolved oxygen to become so low that fish can not survive in the water. The indirect effect of lowered dissolved oxygen in the water, is the most common reason for fish-kills due to aquatic herbicide application (Langeland, 2000). In the "environmental effects" test this should not be the fact, since there were no emerged plants or macrophytes involved. Reduced light or darkness makes plants and algae consume oxygen. Therefore, the low concentration of oxygen usually occurs during early morning hours, especially in productive lakes as well as in the experimental mesocosms. Other aquatic organisms, in particular those associated with decaying organic matter, also consume a great deal of oxygen. Normally, the danger of fish-kills is less in cool water because it can hold more oxygen than warm water. Because it requires light, photosynthesis occurs only during daylight hours while respiration and decomposition occur 24 hours a day.

The reason for the possible initial difference in conductivity between the two higher concentrations, and the low and control treatments, respectively, is unclear in the "environmental effects" test. Larger values after application of glyphosate may indicate an addition of ions. However, the difference in conductivity is also the case before spraying and can not be connected to added glyphosate. The slow decrease over time in both oxygen content and conductivity is probably due to a decrease in oxygen and conductivity of inflowing water, which showed lower values over time. The sudden peaks of oxygen content, are explained by late or early measurement times. The falling temperature of water is due to decreasing air temperature, which was especially obvious 3-4 of May 2001.

Glyphosate contains both phosphorus (18%) and nitrogen (8%) and can hypothetically be one of many contributing factors to eutrophication, although probably not a major one. If 2 kg glyphosate / ha is applied, accordingly, by looking at the molecular weights, 16.6 mg/l (H_2O) of nitrogen and 36.6 mg/l (H_2O) of phosphorus will applied, calculated on 1 m depth. Added glyphosate may therefore influence the content of nutrients in the water. Since the amount of food was the same in each mesocosm, the food supply can not have been the cause for any difference in nutrient concentration among treatments. Another explanation for the tendency for initial increase of chlorophyll content, even though glyphosate is degraded fast, is that glyphosate initially kill phytoplankton. Those phytoplankton will be degraded, thereby releasing sources of nutrients for photosynthesising organisms.

The pH values were higher when measured a few hours later (11.30 am) than early in the morning (9.00 am) as intended. This is possibly explained by CO_2 removal through photosynthesis producing oxygen, which reduces the acidity of the water and increases the pH. During dark hours, respiration and decomposition processes occur alone, lowering pH.

The analysis of glyphosate would be most interesting to have. The analysis could not be made in time because of economical shortcomings. Whether glyphosate residues occurred in the water days, weeks or a month after application, would have been important to know, and could have cast explanatory light on some of the results recorded.

According to the European Commission (1998), considering aquatic uses of glyphosate as the isopropylamine salt, values of TER (toxicity exposure ratio) were calculated to be lower than the relevant trigger indicating an unacceptable risk for aquatic organisms. The loss of glyphosate to the atmosphere via vaporisation is negligible, since the compound has no significant vapour pressure (Franz *et al.*, 1997). However, movement of glyphosate away from the area of treatment is possible through spray drift.

Sublethal effects in fish have been reported (Monroe, 1990; Holtby and Baillie 1989). Effects include erratic swimming, laboured breathing, behavioural aberrations, and altered migration and reproduction. The effects on liver (Tilapia) in this study was expected since microscopic liver and kidney changes in fish have been reported, in contrast no observable differences in function or toxic effects have been seen in comparison to controls after lifetime administration of glyphosate to test animals (EPA, 1992).

6. Conclusion

In the described experiments glyphosate showed no obvious effects on the environmental parameters tested. Tilapia (*Oreochromis niloticus*) were possibly effected when exposed to higher concentrations (12 kg a.i / ha).

Additional studies would be of considerable interest. Investigated concentrations could not be regarded as either safe or harmful.

7. Acknowledgements

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Appendix A. Data on food and growth for high concentration test

Table A. Amount of food (g) per replication in high concentration test, 10 % food per kg fish (*Oreochromis niloticus*).

Number of treatment	Concentration and exposure time	Repl 1	Repl 2	Repl 3	Repl 4
1,00	100 ppm, 6 hrs	1.50	1.20	1.35	1.35
2,00	100 ppm, 12 hrs	1.50	1.50	1.35	1.80
3,00	100 ppm, 24 hrs	1.20	1.20	1.20	1.50
4,00	50 ppm, 6 hrs	1.50	1.20	1.20	1.20
5,00	50 ppm, 12 hrs	1.05	1.03	1.35	0.90
6,00	50 ppm, 24 hrs	1.05	1.20	1.35	1.20
7,00	25 ppm, 6 hrs	1.35	1.20	1.50	1.35
8,00	25 ppm, 12 hrs	1.50	1.35	1.05	1.20
9,00	25 ppm, 24 hrs	1.05	0.90	1.20	1.35
10,00	12.5 ppm, 6 hrs	1.35	1.50	1.50	1.35
11,00	12.5 ppm, 12 hrs	1.35	1.65	1.50	1.05
12,00	12.5 ppm, 24 hrs	1.20	1.80	1.35	1.35
13,00	Control, 6 hrs	1.50	1.35	1.35	1.35
14,00	Control, 12 hrs	2.10	1.65	1.95	1.20
15,00	Control, 24 hrs	1.50	1.35	1.05	1.20

Table B. Weight (g) of ten fishes (*Oreochromis niloticus*) at day 0 in high concentration (glyphosate) test.

Number of treatment	Concentration and exposure time	Repl 1	Repl 2	Repl 3	Repl 4	Mean
1,00	100 ppm, 6 hrs	16.18	12.7	1.4	13.6	14.1
2,00	100 ppm, 12 hrs	18.0	16.1	15.1	17.5	16.7
3,00	100 ppm, 24 hrs	15.0	13.2	13.0	14.7	14.0
4,00	50 ppm, 6 hrs	14.5	13.1	11.2	12.9	13.0
5,00	50 ppm, 12 hrs	10.4	10.8	14.1	9.00	11.0
6,00	50 ppm, 24 hrs	11.3	14.4	13.9	13.0	13.1
7,00	25 ppm, 6 hrs	14.0	12.1	14.8	14.3	13.8
8,00	25 ppm, 12 hrs	14.8	13.4	11.2	11.3	12.7
9,00	25 ppm, 24 hrs	13.6	10.5	13.2	14.1	12.9
10,00	12.5 ppm, 6 hrs	14.3	15.1	15.9	13.7	14.8
11,00	12.5 ppm, 12 hrs	13.9	18.1	15.0	11.5	14.6
12,00	12.5 ppm, 24 hrs	11.6	20.6	13.9	14.6	15.2
13,00	Control, 6 hrs	15.7	13.7	15.1	13.5	14.5
14,00	Control, 12 hrs	20.5	1.7	19.4	12.6	17.4
15,00	Control, 24 hrs	15.8	13.1	10.3	11.3	12.6

Appendix A (continued)

Table C. Weight (g) of ten fishes (*Oreochromis niloticus*) at day 22 in high concentration (glyphosate) test.

Number of treatment	Concentration and exposure time	Repl 1	Repl 2	Repl 3	Repl 4	Mean
1,00	100 ppm, 6 hrs	37.4	28.8	31.3	28.7	31.5
2,00	100 ppm, 12 hrs	38.0	35.6	31.8	41.2	36.7
3,00	100 ppm, 24 hrs	31.6	34.5	26.1	35.4	29.4
4,00	50 ppm, 6 hrs	37.5	34.5	26.2	27.3	31.4
5,00	50 ppm, 12 hrs	33.3	29.8	34.9	29.2	31.8
6,00	50 ppm, 24 hrs	32.5	34.6	32.0	33.2	33.1
7,00	25 ppm, 6 hrs	32.1	35.1	34.4	37.5	34.8
8,00	25 ppm, 12 hrs	31.5	32.1	27.0	28.6	29.8
9,00	25 ppm, 24 hrs	30.4	25.4	28.1	29.9	28.4
10,00	12.5 ppm, 6 hrs	28.8	40.9	29.5	33.7	33.2
11,00	12.5 ppm, 12 hrs	26.1	36.6	29.4	32,0	31.0
12,00	12.5 ppm, 24 hrs	29.6	32.7	32.2	32.7	31.8
13,00	Control, 6 hrs	41.4	37.4	31.9	29.6	35.1
14,00	Control, 12 hrs	37.9	44.6	42.4	32.6	39.4
15,00	Control, 24 hrs	31.3	37.8	28.4	32.3	32.4

Appendix B. Data on oxygen, conductivity, pH and water temperature for environmental effects test.

Table D. Oxygen concentration (µg/l) in mesocosms measured daily from one week before glyphosate application in environmental effects test.

	Mesocosm														
Date	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	C1	C2	C3	C4	C5
10-mar	7.14	7.34	8.9	8.04	7.68	7.73	8.09	8.49	7.7	7.71	7.63	6.82	7.02	8.01	6.88
11	9.22	8.49	10.2	8.9	8.91	8.47	9.45	9.41	9.21	8.9	10.22	8.3	8.12	9.1	8.02
12	7.89	6.8	9	7.66	7.12	6.91	8.08	8.2	7.57	7.39	9.2	7.9	7.48	8.28	7.05
13	10.29	8.29	11.58	9.71	9.13	8.74	10.33	10.55	9.54	9.1	11.25	9.81	9.26	9.86	8.77
14	10.46	8.86	13.13	11.52	10.43	9.98	10.86	11.98	10.73	9.09	11.5	10.86	10.16	10.21	8.89
15	8.41	7.71	11.11	9.5	7.66	8.99	8.69	9.84	9.26	6.41	9.3	8.19	8.74	8.26	7.28
16	8.21	8.6	9.62	9.61	7.87	8.37	7.3	8.44	8.69	6.24	7.38	7.28	7.37	6.95	7.18
17	11.55	12.82	13.61	14.62	13.24	12.37	7.88	13.13	13.99	9.76	7.58	7.51	9.81	10.6	11.62
18	7.26	7.85	9.43	9.73	9.68	7.47	3.5	8.96	7.93	5.41	1.71	5.27	7.26	7.61	6.1
19	9.94	10.68	10.22	9.71	11.63	10.17	5.16	10.06	9.75	7.36	2.32	9.43	9.21	11.21	6.58
20	12	12.34	10.59	10.6	12.63	10.82	6.96	11.66	11.01	9.22	6.04	9.2	10.66	13.24	8.12
21	9.21	8.73	7.83	8.31	9.85	6.92	5.23	7.79	6.17	6.79	4.48	3.17	7.92	9.43	5.28
22	10.4	9.15	7.45	10.36	11.18	8.36	7.41	8.35	7.84	7.98	8.3	3.54	8.43	10.14	6.26
23	8.11	7.24	5.48	9.75	8.65	6.43	5.27	4.44	6.44	6.02	6.27	5.21	6.56	8.08	4.69
24	9.26	8.44	5.76	10.13	8.08	7.18	6.42	1.98	7.22	6.69	5.9	9.02	8	7.43	4.99
25	9.96	9.41	6.02	7.52	9.02	7.48	5.79	3.16	8.33	7.38	6.46	11.32	9.56	7.79	5.76
26	10.45	8.5	5.8	5.68	7.74	6.91	4.37	3.53	7.56	6.77	6.27	9.84	9.12	7.07	5.65
27	9.66	7.53	5.51	5.84	6.31	6.15	4.88	4.49	6.43	5.84	5.24	6.85	8.26	6.57	5.36
28	8.9	6.83	6.05	5.81	6.17	6.08	5.19	6.23	6.68	5.97	3.66	5.66	7.58	6.4	5.4
29	9.48	7.52	7.02	7.01	6.87	7.56	6.77	8.24	7.72	6.68	4.35	6.11	7.99	7.59	5.96
30	7.24	5.65	5.96	6.11	5.53	7.33	6.41	7.31	6.17	6.07	5.01	5.31	6.39	6.42	5.62
31	7.09	5.97	5.84	6.44	5.9	7.14	6.48	6.35	6.28	5.91	6.1	6.29	5.59	5.87	5.34
01-apr	6.94	5.55	5.83	6.63	5.72	7.17	6.95	6.08	6.06	5.89	6.39	6.79	6.5	5.72	5.56
2	5.87	4.84	5.19	6.02	5.59	5.92	5.9	5.01	4.44	5.12	4.93	5.55	6.45	4.58	4.58
3	5.65	5.11	5.87	6.26	5.96	6.12	6.36	5.01	5.09	5.81	5.68	6.2	6.39	4.6	5.2
4	5.52	4.61	5.22	6.06	6.34	6.09	5.75	4.65	4.26	5.6	5.12	6.31	5.94	4.83	4.77
5	4.41	3.56	4.2	5.17	5.43	4.76	7.46	3.38	3.1	4.49	2.98	5.11	5.07	3.86	3.25
6	4.86	4.01	4.64	5.41	5.63	5.03	4.68	3.93	3.27	4.4	3	5.13	5.01	4.07	3.37
7	3.63	3.44	4.31	5.39	4.98	4.5	4.33	3.64	3	2.97	2.55	4.95	4.43	3.64	3
8	3.5	3.65	4.7	5.52	5.06	4.34	4.33	4.52	2.85	0.77	3.05	5.11	4.46	3.6	3.13
9	3.9	3.99	5.11	5.75	5.39	5.11	4.92	5.69	3.7	1.12	3.85	5.78	5.23	4.19	4.05
10	3.2	3.67	4.74	5.44	5.08	4.3	4.27	5.03	3.23	1.77	3.32	5.23	4.69	3.84	3.35
11	3.1	4.01	4.66	5.19	5.38	4.05	4.25	5.26	3.3	2.53	3.63	5.16	4.35	4.08	3.44
12	3.18	3.63	4.29	4.82	4.8	3.69	4.11	4.72	3.42	2.68	3.71	4.38	2.77	3.81	3.17
13	3.45	3.62	4.23	5.02	4.91	3.78	4.17	4.47	3.27	3.7	4.15	4.54	2.12	3.87	3.21
14	3.85	4.14	4.43	5.21	5.36	3.67	4.46	4.95	3.49	4.12	4.34	4.65	2.82	4.36	3.99
15	4.95	5.63	6.43	6.34	7.01	6.77	5.42	6.15	6.29	5.53	6.19	6.62	4.35	5.81	6.51
16	3.71	4.23	4.11	4.63	5.29	4.03	4.24	4.24	3.29	4.26	4.21	4.55	1.29	3.4	4.26
17	3.71	4.77	4.36	4.74	5.57	4.23	4.27	4.37	3.16	4.55	4.88	4.6	2.04	3.22	4.89
18	3.95	5.12	4.48	5.09	5.84	4.23	4.67	4.38	3.35	5.13	5.26	4.53	2.83	3.18	5.01
19	4.08	4.94	4.04	4.79	5.64	4.3	4.15	3.94	2.88	4.7	5.69	4.4	3.84	2.85	5.15
20	3.45	4.26	3.05	4.12	4.55	3.4	3.4	2.72	2.4	3.95	4.77	2.74	3.28	1.29	4.08
21	4.15	5.16	3.62	4.74	5.3	4.12	3.91	3.38	2.64	4.75	5.28	3	4.12	1.86	5.14
22	3.52	4.16	3.06	3.87	4.41	3.71	3.18	2.99	2.16	4.44	4.84	2.68	3.53	1.23	4.71
23	3.5	4.28	3.35	4.09	4.45	4.09	3.23	3.19	2.43	4.46	5.08	3.38	3.36	1.18	5.02
24	3.42	4.18	3.25	4.01	4.36	3.85	3.29	2.65	1.93	4.53	5.1	3.6	3.45	0.94	4.96
25	2.51	3.07	2.55	2.98	3.58	2.69	2.36	1.33	1.57	3.98	4.59	3.09	2.22	0.69	4.9
26	2.89	3.62	3.31	3.4	4.23	3.42	2.93	1.77	2.39	4.64	5.59	3.89	2.42	0.84	5.39
27	3.25	3.93	3.47	3.61	4.39	3.86	3.31	1.6	2.5	4.86	5.67	4.03	1.92	0.73	5.72
28	5.42	5.74	6.18	5.82	6.71	5.96	6.98	5.6	6.88	7.86	8.42	8.22	4.65	3	9.08
29	6.65	7.2	6	7	7.54	6.35	5.72	5.54	7.46	7.26	8.91	8.24	3.73	2.8	8.75
30	3.43	4.21	2.87	3.61	4.31	2.93	3.3	2.19	3.81	4.82	5.94	4.81	1.36	0.91	5.6
May 1	3.17	3.65	1.98	3.89	3.42	2.34	2.67	1.38	3.15	4.02	4.75	4.05	0.92	0.81	5.07
2	4.42	4.48	2.98	5.31	4.56	3.31	3.74	2.75	4.15	5.13	6.28	5.42	1.53	1.37	6.49
3	10.33	9.81	7.98	11.22	9.98	8.32	8.07	10.53	11.31	9.06	11.94	11.86	8.38	6.68	12.07
4	3.72	3.47	2	5.31	3.42	2.18	3.12	3.29	3.72	4.23	5.12	4.25	2.57	1.02	4.58
5	3.69	3.76	2.01	5.31	3.51	2.25	3.36	3.33	4.34	4.1	5.2	4.34	2.73	0.91	5.2
6	6.47	6.18	5	7.96	6.67	4.32	5.89	6.24	7.44	6.34	7.96	7.45	5.91	2.87	7.7
7	6.6	6.67	6.09	8.49	7.38	4.44	6.95	6.9	7.3	7.3	8.53	8.24	6.7	3.81	8.28
8	6.7	6.44	5.38	8.22	7.21	4.68	6.92	6.78	6.7	6.98	8.34	8.35	6.65	3.75	8.11
9	6.36	6.59	4.98	7.86	6.85	4.99	6.82	6.67	6.67	6.8	7.68	7.48	6.36	3.67	7.7
10	6.52	6.71	4.92	7.49	5.94	5.42	6.57	6.43	5.78	6.72	7.58	8.11	6.37	7.8	3.34

Appendix B (continued)

Table D (continued). Oxygen concentration (µg/l) in mesocosms measured daily from one week before glyphosate application in environmental effects test.

	Mesocosm								
Date	D1	D2	D3	D4	D5	E2	E3	E4	E5
10-mar	8.26	7.54	7.62	8.23	7.81	7.24	8.1	8.22	7.62
11	9.87	8.81	8.67	9.12	8.86	7.64	9.3	9.25	8.45
12	8.65	7.51	7.3	7.95	7.81	6.08	7.8	8.01	7.45
13	10.98	9.58	8.77	9.39	9.59	7.14	9.86	9.07	9.16
14	12.2	9.93	9.22	9.86	10.33	8.04	10.62	9.03	10.09
15	10.64	9.77	7.69	7.98	9.15	6.81	8.33	6.16	8.45
16	9.15	7.37	7.75	7.98	9.2	5.11	7.17	4.29	9
17	12.23	8.96	10.47	12.68	13.64	9.39	11.88	6.95	13.19
18	7.55	4.23	5.11	6.96	8.07	4.05	7.02	4.57	8.08
19	9.11	5.69	6.38	8.24	10.13	4.05	8.23	3.03	7.97
20	11.41	7.97	9.92	8.9	10.78	4.66	9.73	5.85	9.11
21	8.38	3.22	6.12	4.86	6.97	4.12	6.96	5.61	5.37
22	11.29	4.2	7.65	5.66	8.56	5.05	6.76	7.37	6.46
23	9.02	4.76	5.75	5.25	7.18	5.51	4.89	6.43	5.2
24	8.68	5.89	6.63	6.29	9.05	7	5.01	7.11	7.05
25	9.82	5.01	6.48	5.78	9.41	6.66	5.3	7.63	7.38
26	8.66	3.53	3.85	4.91	8.35	7.04	5.26	5.76	6.21
27	7.53	3.51	3.2	5.16	7.16	6.89	4.88	4.41	5.06
28	7.87	3.99	3.79	5.66	7.63	6.84	4.56	5.29	4.55
29	8.72	4.94	5.72	7.25	8.6	7.43	5.54	6.76	4.6
30	7.64	4.85	5.78	7.11	7.16	6.55	4.48	5.42	3.01
31	6.69	4.99	6.19	6.53	5.97	6.48	5.04	6.13	3.12
01-apr	6.01	5.24	7.07	5.95	4.23	6.47	5.34	6.37	2.36
2	5.31	5.19	6.48	5.23	3.41	5.8	4.57	5.56	3.87
3	4.95	5.56	6.07	4.92	2.79	4.49	3.46	4.67	3.48
4	5.19	5.01	6.16	5.48	3.43	3.94	2.8	4.55	3.42
5	4.44	3.83	5.23	5.15	3.53	2.63	1.83	3.25	2.7
6	5.39	4.47	5.67	5.88	4.19	2.41	2.62	3.53	3.4
7	5.51	3.9	5.34	6.24	4.08	1.71	2.77	3.14	2.76
8	6.17	4.01	5.16	6.63	4.09	1.42	3.32	3.3	2.69
9	7.19	4.5	5.99	7.26	4.89	1.46	4.15	4.38	2.98
10	6.55	4.13	5.59	6.57	4.68	1.18	3.9	4.35	2.82
11	6.793	4.17	5.6	6.78	4.98	1.13	4.02	4.89	3.28
12	5.95	3.55	4.77	6.26	4.48	0.93	3.87	4.49	2.96
13	5.98	3.65	4.62	6.16	4.82	1.36	3.89	4.65	3.13
14	6.45	4.17	4.74	6.55	5	1.79	4.38	5.16	3.75
15	6.81	6.33	6.06	7.41	7	4.2	6.29	7.53	6.47
16	5.98	4.1	4.58	6.43	4.95	1.95	4.47	5.14	3.67
17	6.01	4.19	4.73	6.49	5.07	2.11	4.6	5.22	3.72
18	6.21	4.33	4.91	6.63	5.18	2.23	4.8	5.56	4.04
19	6.46	4.15	4.71	6.5	5.23	2.2	4.36	5.26	3.96
20	4.97	3.6	3.59	4.98	3.69	1.86	3.05	4.11	2.74
21	5.98	4.12	4.41	5.68	3.81	2.01	3.49	5.13	3.16
22	5.46	3.52	3.33	4.81	3.43	1.83	3.08	4.31	2.21
23	5.38	3.44	3.43	4.56	4.03	1.72	2.82	3.62	2.05
24	5.4	3.45	3.23	4.93	4.14	2.2	3.69	4.17	2.51
25	5.15	2.68	2.55	4.73	4.09	1.61	2.69	3.82	2.01
26	5.49	3.44	2.66	5.18	4.61	2.02	3.27	4.58	2.84
27	4.79	3.69	2.83	5.64	5.88	2.48	3.22	4.76	2.78
28	8.88	8.54	6.35	9.02	8.4	4.23	8.13	8.5	7.04
29	9.45	7.29	6.29	9.08	7.98	3.98	6.57	8.26	6.16
30	5.61	4.05	3.24	5.66	4.58	2.64	3.04	4.83	3.16
May 1	4.73	3.3	2.65	5.18	4.18	2.14	2.24	3.71	2.84
2	5.93	4.23	3.62	6.55	5.33	2.71	3.18	5	3.67
3	12.14	9.67	10.02	12.62	10.89	4.65	7.9	9.76	9.57
4	5.36	3.1	3.25	5.67	4.55	1.69	1.54	4.14	2.75
5	4.84	3.22	3.13	5.16	4.54	2.09	1.86	4.2	3.11
6	7.56	5.58	6.6	8.12	7.36	3.78	4.29	6.49	5.72
7	8.29	6.18	6.1	8.84	7.83	4.73	5.43	7.82	6.33
8	8.44	5.76	6.11	8.99	8.03	4.63	5.36	7.56	5.82
9	8.2	5.51	5.87	8.77	7.95	4.27	5.1	7.32	5.15
10	8.19	5.73	5.83	8.88	6.75	4.21	5	7.21	5.35

Appendix B (continued)

Table E. Conductivity (µS/cm) in mesocosms measured daily from one week before glyphosate application in environmental effects test.

	Mesocosm														
Date	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	C1	C2	C3	C4	C5
10-mar	70	67	63	99	108	105	69	100	62	65	142	140	124	74	149
11	71	68	65	101	111	107	70	101	62	66	144	143	127	75	152
12	72	69	66	99	108	105	70	99	66	68	136	136	126	75	144
13	70	69	67	96	103	100	70	95	66	68	133	133	125	74	140
14	72	69	81	98	105	102	73	97	67	69	129	130	125	76	137
15	73	72	91	101	109	106	74	100	70	73	133	135	130	78	141
16	77	85	96	100	109	106	77	100	75	76	130	131	131	81	137
17	77	76	105	98	108	102	78	98	76	76	125	126	126	81	130
18	78	75	110	98	105	101	81	98	79	79	126	125	127	82	128
19	84	85	120	104	112	106	82	102	82	80	130	127	130	85	131
20	92	92	121	108	115	109	89	107	92	88	129	128	130	94	131
21	92	90	118	107	112	106	92	106	96	96	123	126	126	92	127
22	97	95	120	111	116	110	96	111	102	97	125	129	128	98	130
23	98	96	120	111	115	110	98	112	104	100	123	126	127	100	129
24	100	98	119	111	114	110	98	115	105	101	121	120	123	101	126
25	100	99	118	111	113	110	99	114	104	102	118	117	120	101	124
26	100	97	114	109	111	108	100	111	101	100	115	113	116	100	119
27	95	93	107	103	104	102	95	104	95	95	107	106	108	95	115
28	92	91	104	101	103	100	94	100	94	95	105	104	106	94	111
29	90	89	100	97	99	95	89	95	91	92	101	100	103	91	106
30	89	89	98	96	98	94	89	94	91	92	99	99	101	91	103
31	84	85	91	90	92	88	93	89	84	86	91	92	94	86	96
01-apr	82	80	85	86	87	84	79	84	78	82	85	87	89	82	91
2	77	76	80	82	82	79	74	78	69	77	79	81	83	77	85
3	72	71	74	76	76	74	69	73	65	72	73	76	77	73	79
4	76	75	77	79	79	77	72	77	72	76	75	79	80	76	81
5	81	81	83	84	84	82	77	83	81	81	78	83	85	82	86
6	81	80	81	83	83	81	77	82	82	80	77	82	83	81	84
7	83	82	82	84	84	83	79	84	83	82	79	84	84	83	85
8	82	82	83	83	84	82	79	83	83	83	80	83	84	83	84
9	84	83	83	84	85	84	81	83	84	87	84	84	84	84	85
10	82	82	81	83	83	82	79	81	82	86	83	83	83	82	84
11	82	80	79	81	82	80	78	79	79	83	80	80	81	80	83
12	78	77	75	77	77	77	74	76	75	79	77	77	80	76	80
13	71	69	67	70	70	70	68	69	68	71	69	69	73	69	73
14	68	66	64	67	67	67	65	66	65	67	66	67	69	65	69
15	74	70	70	73	73	72	60	70	70	73	70	71	77	71	73
16	67	65	63	66	65	66	63	65	65	66	65	66	71	65	67
17	65	62	62	64	63	63	61	62	63	63	63	64	68	65	64
18	65	61	61	64	62	63	61	62	63	63	62	63	68	63	63
19	64	60	60	63	61	62	61	62	64	62	60	63	66	62	62
20	63	60	60	62	61	62	61	62	64	61	60	63	64	62	61
21	63	60	61	63	61	62	62	63	65	61	60	64	64	63	61
22	65	63	63	65	64	64	64	65	68	63	62	66	66	66	62
23	64	62	62	64	63	63	64	64	67	62	62	65	65	66	62
24	67	65	65	66	66	66	67	67	70	65	64	67	68	69	63
25	68	67	67	68	68	67	69	70	73	65	64	69	70	72	64
26	64	62	62	63	63	63	64	66	66	60	58	63	66	67	58
27	62	60	58	61	61	60	61	64	62	57	56	60	64	65	55
28	63	62	61	63	63	62	62	65	62	59	59	61	68	68	61
29	62	61	60	61	62	61	60	65	60	58	59	59	66	67	59
30	61	59	59	60	60	59	59	63	59	55	53	56	64	65	54
May 1	61	59	59	59	60	59	58	63	60	56	55	56	64	64	55
2	62	60	60	59	61	60	58	63	60	56	55	55	65	64	55
3	66	65	65	65	65	64	61	67	67	62	67	63	67	69	68
4	62	62	61	60	63	62	58	63	62	58	57	55	66	67	57
5	61	60	60	58	61	59	55	61	59	56	56	54	63	64	56
6	53	53	52	51	54	52	48	54	52	50	49	47	56	56	50
7	54	53	53	51	54	53	49	55	53	50	50	48	56	56	50
8	55	55	55	53	55	54	50	56	56	52	51	48	58	56	51
9	57	56	57	54	56	54	52	56	58	53	52	50	59	57	52
10	58	57	58	55	58	54	55	57	59	54	54	53	59	51	57

Appendix B (continued)

Table E (continued). Conductivity (µS/cm) in mesocosms measured daily from one week before glyphosate application in environmental effects test.

	Mesocosm								
Date	D1	D2	D3	D4	D5	E2	E3	E4	E5
10-mar	88	61	62	65	62	76	53	68	54
11	90	61	63	65	64	77	55	69	55
12	88	64	65	68	66	80	58	71	59
13	86	63	63	67	65	78	58	70	58
14	86	65	66	70	67	80	60	72	61
15	88	67	70	72	69	83	62	76	63
16	90	71	73	75	72	85	67	79	67
17	90	72	74	77	75	85	69	80	69
18	90	76	77	78	75	88	70	82	72
19	92	77	80	81	78	91	71	86	75
20	99	86	87	89	87	98	81	92	83
21	99	91	90	92	89	101	86	93	88
22	104	97	95	98	95	104	92	98	94
23	105	100	98	102	98	105	96	100	98
24	99	101	98	102	105	105	99	101	99
25	105	102	99	104	100	104	99	101	100
26	103	102	99	103	99	101	98	100	98
27	97	97	95	97	94	96	94	95	93
28	95	96	94	96	93	95	93	93	92
29	93	93	91	93	91	92	91	91	91
30	92	92	91	93	91	92	91	91	91
31	86	86	85	87	85	86	85	85	86
01-apr	83	84	80	83	83	82	81	81	82
2	77	78	74	77	78	76	75	75	76
3	72	72	69	72	74	71	70	70	71
4	76	76	73	76	78	75	74	74	76
5	82	82	77	81	83	81	82	79	83
6	81	80	78	80	81	81	81	79	82
7	82	82	80	81	83	84	83	81	84
8	82	82	80	80	83	84	82	81	85
9	82	83	81	82	83	85	82	82	86
10	80	82	80	79	81	84	81	80	84
11	77	80	78	78	79	82	78	77	81
12	73	76	75	75	76	78	74	73	77
13	67	70	68	69	69	73	68	66	70
14	63	66	65	66	66	68	65	62	66
15	70	70	68	74	70	74	70	76	71
16	62	64	63	64	64	67	63	60	64
17	60	62	61	62	62	65	61	58	62
18	60	62	61	62	62	64	61	57	62
19	59	61	60	62	61	64	60	57	61
20	59	61	61	61	62	63	60	57	61
21	60	61	61	63	63	64	61	58	62
22	63	64	64	65	66	66	64	61	66
23	63	63	63	64	65	65	62	60	65
24	66	65	66	67	67	67	64	64	68
25	68	66	69	69	69	70	66	65	69
26	62	61	64	64	64	65	61	60	64
27	61	58	61	61	59	62	58	58	61
28	62	60	62	64	63	65	59	60	62
29	61	56	60	63	60	63	57	60	58
30	58	56	60	60	59	62	55	57	57
May 1	58	56	59	59	59	61	56	57	57
2	59	56	60	60	59	61	56	58	57
3	66	60	64	69	64	65	59	63	60
4	61	55	61	61	61	64	59	60	60
5	59	56	60	59	59	62	59	58	57
6	52	48	53	52	53	54	51	52	50
7	53	48	53	52	53	54	52	53	51
8	53	48	53	54	54	55	52	53	52
9	54	48	54	55	55	56	53	55	54
10	56	48	55	56	54	56	52	54	55

Appendix B (continued)

Table F. pH in mesocosms measured daily from one week before glyphosate application in environmental effects test.

	Mesocosm														
Date	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	C1	C2	C3	C4	C5
10-mar	N.m (not measured)														
11	N.m														
12	N.m														
13	N.m														
14	N.m														
15	N.m														
16	6.81	7	8.8	7.26	7.47	7.49	7.43	7.41	7	7	7.12	7.21	7.2	6.91	7.28
17	7.78	8.47	8.68	8.26	8.47	8.22	7	8.16	8.52	6.77	6.76	6.75	7	6.72	7.34
18	6.68	8	8.86	7.78	8.31	7.62	6.47	7.48	7.42	6.51	6.36	6.49	7	6.49	7
19	8.84	9.25	9.2	8.63	9	9	7.24	8.63	8.74	7.36	7	8.11	8.37	8.8	7.91
20	9	9.34	9	8.68	9.1	9	7.37	8.8	8.74	8.12	7.12	8	8.53	9	8
21	8.5	8.77	8.26	7.78	8.56	7.78	6.7	7.36	7	6.73	6.72	6.64	7.54	8	7
22	8.8	8.86	7.59	8.26	8.63	8.38	7.24	7.72	7.71	7.42	7.33	6.7	7.84	8.44	7.18
23	9.08	9.08	7	8.76	8.68	8.3	7	7.15	7.27	7.12	7.3	7.12	7.37	8.23	7.21
24	9.39	9.34	7.33	8.84	8.63	8.56	7.35	7.08	7.46	7.53	7.27	8.11	7.6	7.66	7.28
25	9.47	9.47	7.35	8	8.56	8.68	7.39	7.21	8.08	7.72	7.8	9.34	8	7.78	7.33
26	9.6	9.35	7.21	7.35	7.9	8.24	7.14	7	7.64	7.4	7.46	9.08	7.85	7.59	7.3
27	9.53	9.17	7.1	7.41	7.27	7.46	7	7	7.17	7	7	8.35	7.44	7.22	7.33
28	9.21	8.63	7	7.14	7	7	6.84	6.82	7	7	6.76	7	7.13	6.82	7
29	9.31	8.63	7	7.24	7	7.12	7	7.12	7.18	7	6.82	7	7.24	7	7
30	8.56	7.3	6.86	6.78	6.71	7	6.83	6.88	6.82	6.83	6.69	6.73	7	6.7	6.82
31	8.69	8	7.28	7.46	7.15	7.46	7.33	7.23	7.24	7.3	7.36	7.21	7.21	7.7	7
01-apr	9	8	7.12	7.56	7.23	8.61	7.65	7.37	8	7.66	8.11	8.24	7.61	7.53	7.4
2	8.56	7.553	6.63	7.36	7.16	8	7.14	7	7.16	7.14	7.16	7.24	7.33	7	7
3	7.75	7.22	7	7.19	7.09	8	7.26	7.3	7	7.23	7.15	7.21	7.27	6.82	7
4	7.33	7	7	7.27	7.4	8.29	7.46	7.4	7.15	7.36	7.27	7.69	7.28	7.15	7.14
5	7	6.76	7	7	7	7.38	7.21	6.83	6.71	7.21	6.71	7.12	7.16	6.84	6.79
6	7.29	6.84	7.21	7	7.26	7.57	7.46	6.89	6.57	7.33	6.57	7.18	7.29	6.82	6.79
7	N.m														
8	N.m														
9	6.68	6.68	7.08	7	7	7.21	7	7	6.78	6.51	6.68	7.25	6.9	6.78	6.64
10	N.m														
11	N.m														
12	7.32	7.39	7.59	7.49	7.54	7.37	7.6	7.48	7.36	7.32	7.32	7.51	7.38	7.34	7.3
13	6.64	6.69	6.55	6.86	6.91	6.76	6.62	6.82	6.69	6.51	6.71	6.85	6.45	6.72	6.66
14	6.7	6.76	6.93	6.82	6.94	6.71	6.96	6.83	6.71	6.83	6.73	6.84	6.73	6.69	6.68
15	N.m														
16	6.78	6.86	7.01	6.88	7.08	6.8	7.16	6.83	6.74	7.14	6.76	6.88	6.67	6.71	6.78
17	6.54	6.7	6.74	6.65	6.86	6.59	6.75	6.58	6.48	6.83	6.63	6.65	6.46	6.48	6.67
18	6.35	6.52	6.46	6.46	6.63	6.4	6.51	6.41	6.37	6.58	6.52	6.47	6.3	6.33	6.45
19	6.52	6.61	6.85	6.53	6.81	6.73	6.72	6.72	6.64	6.71	6.82	6.72	6.54	6.58	6.8
20	6.5	6.61	6.61	6.53	6.61	6.44	6.63	6.43	6.39	6.63	6.58	6.36	6.52	6.3	6.52
21	6.65	6.84	6.83	6.68	6.75	6.55	6.84	6.53	6.51	6.87	6.78	6.46	6.71	6.4	6.67
22	6.44	6.58	6.67	6.46	6.52	6.38	6.65	6.32	6.3	6.75	6.56	6.23	6.53	6.19	6.48
23	6.45	6.5	6.73	6.61	6.77	6.51	6.67	6.53	6.55	6.79	6.73	6.48	6.54	6.46	6.67
24	6.35	6.43	6.51	6.31	6.38	6.24	6.37	6.19	6.23	6.57	6.47	6.23	6.29	6.24	6.52
25	6.45	6.53	6.69	6.45	6.56	6.48	6.65	6.46	6.58	6.83	6.87	6.52	6.55	6.59	7.02
26	6.49	6.53	6.77	6.43	6.62	6.44	6.57	6.41	6.49	6.96	7	6.52	6.6	6.53	7.11
27	6.61	6.62	6.65	6.5	6.74	6.65	6.46	6.52	6.61	7.02	7.23	6.66	6.64	6.63	7.32
28	6.69	7.02	7.92	6.92	7.27	6.81	6.9	6.6	7.45	9.52	10.13	8.46	6.5	6.3	9.97
29	6.93	8.11	8.72	7.57	8.44	6.87	7.22	6.77	8.83	9.39	10.15	9.17	6.58	6.56	9.98
30	6.2	6.33	6.37	6.43	6.35	6.09	6.31	6.1	6.28	6.69	6.87	6.37	6.27	6.19	6.93
May 1	6.23	6.32	6.22	6.27	6.3	6.1	6.18	6.13	6.27	6.38	6.46	6.28	6.17	6.19	6.58
2	6.18	6.24	6.16	6.23	6.24	6.05	6.02	6.05	6.19	6.32	6.57	6.31	6.05	6.09	6.72
3	9.35	9.19	9.45	9.65	8.83	6.96	8.91	9.41	9.87	9.53	10.83	10.37	7.67	6.79	10.35
4	6.17	6.23	6.19	6.31	6.15	5.87	6.13	6.04	6.25	6.37	6.48	6.35	6.12	6.12	6.57
5	6.17	6.17	6.18	6.19	6.08	5.94	6.12	6.09	6.28	6.29	6.35	6.21	6.16	6	6.38
6	6.69	6.71	6.7	6.95	6.84	6.36	6.65	6.65	7.16	6.83	7.28	7.17	6.68	6.43	7.2
7	6.97	6.95	6.98	7.25	7.08	6.51	6.93	6.83	7.12	7.19	8.57	8.32	7.12	6.51	8.15
8	6.43	6.4	6.41	6.56	6.52	6.12	6.52	6.26	6.51	6.61	7.03	7.18	6.55	6.08	7.03
9	6.49	6.57	6.39	6.65	6.63	6.18	6.72	6.35	6.58	6.65	7.07	7.14	6.49	6.25	7.02
10	6.46	6.52	6.32	6.54	6.4	6.18	6.66	6.29	6.34	6.7	6.67	6.89	6.45	6.08	6.67

Appendix B (continued)

Table F (continued). pH in mesocosms measured daily from one week before glyphosate application in environmental effects test.

	Mesocosm								
Date	D1	D2	D3	D4	D5	E2	E3	E4	E5
10-mar	N.m								
11	N.m								
12	N.m								
13	N.m								
14	N.m								
15	N.m								
16	7.29	7	7	6.89	7	6.85	6.85	6.63	7
17	6.82	6.61	6.77	6.89	7.24	6.82	6.7	6.4	8.32
18	6.76	6.47	6.42	6.45	6.91	6.64	6.72	6.28	8.44
19	8	7.18	7.2	7.39	8.77	7	8.35	6.7	9
20	8.54	7.42	8.15	8	8.72	7.18	8.56	7	8.74
21	7.24	6.58	6.76	6.67	7	6.7	7	6.59	6.88
22	8.31	6.78	7	6.78	7.45	6.78	7	6.88	7
23	8.56	7	7.2	7	7.31	7.14	7	7	7
24	7.59	7.3	7.31	7.21	8.37	7.51	7.14	7.4	7.45
25	8.56	7.3	7.33	7.22	7.76	7.37	7.21	7.71	7.45
26	7.84	7	7	7	7.46	7.46	7.15	7.27	7.27
27	7.26	6.82	6.83	7	7.21	7.26	6.9	7	7
28	7	6.69	6.63	6.76	7	7	6.71	6.77	6.7
29	7	6.84	6.76	7	7.14	7.14	6.89	7	6.84
30	7	6.76	6.88	7	6.88	6.81	6.69	6.73	6.56
31	7	7.08	7	7	6.33	7	6.82	6.87	6.32
01-apr	7.3	7.3	7.45	7.28	7	7	7	7.12	7
2	7	7.1	7.19	7	7	7.12	7	7.12	6.91
3	6.88	7.08	7	6.79	6.71	6.82	6.71	7	6.69
4	7	7	7.32	7	7	6.88	6.76	7	6.76
5	6.78	6.91	7.06	7	6.76	6.63	6.56	6.79	6.63
6	7	7.08	7.33	7.31	6.82	6.56	6.56	6.82	6.71
7	N.m								
8	N.m								
9	7.64	6.68	7.35	8.33	6.76	6.42	6.65	6.76	6.59
10	N.m								
11	N.m								
12	7.67	7.46	7.52	8.13	7.4	7.12	7.38	7.69	7.3
13	7.02	6.56	6.86	7.32	6.78	6.41	6.55	6.81	6.43
14	7.01	6.86	6.8	7.3	6.74	6.63	6.72	7.32	6.72
15	N.m								
16	7.25	7.02	7.05	8.38	6.85	6.57	7.14	8.79	6.71
17	6.87	6.63	6.69	7.41	6.64	6.37	6.71	7.71	6.49
18	6.66	6.4	6.53	7.05	6.5	6.2	6.35	7.3	6.3
19	6.81	6.56	6.68	6.95	6.69	6.3	6.45	6.93	6.49
20	6.52	6.55	6.39	6.52	6.36	6.38	6.42	6.7	6.44
21	6.68	6.7	6.53	6.78	6.47	6.49	6.53	6.92	6.52
22	6.48	6.54	6.29	6.47	6.27	6.28	6.32	6.59	6.36
23	6.47	6.54	6.49	6.63	6.52	6.32	6.3	6.62	6.4
24	6.41	6.31	6.36	6.54	6.36	6.22	6.28	6.47	6.25
25	6.88	6.56	6.79	6.92	6.76	6.4	6.46	6.8	6.46
26	6.81	6.61	6.66	6.9	6.75	6.53	6.55	6.92	6.51
27	6.82	6.74	6.74	7.01	6.96	6.63	6.62	6.99	6.58
28	9.28	8.96	6.87	9.36	8.38	6.28	7.39	9.91	6.7
29	9.62	8.59	7.23	9.49	9	6.41	6.98	9.71	6.83
30	6.51	6.43	6.28	6.58	6.4	6.15	6.11	6.79	6.14
May 1	6.35	6.27	6.2	6.45	6.29	6.1	6.05	6.38	6.12
2	6.29	6.19	6.13	6.47	6.26	6	5.97	6.34	6
3	9.92	9.48	8.45	10	9.32	6.2	6.95	9.57	8.64
4	6.31	6.09	6.18	6.51	6.34	5.91	5.9	6.4	6.01
5	6.08	6.17	6.1	6.31	6.21	5.99	5.95	6.31	6.07
6	6.77	6.61	6.74	7.04	6.89	6.32	6.35	6.79	6.52
7	7.26	7.2	6.71	7.35	7	6.47	6.42	7.05	6.7
8	6.56	6.32	6.38	6.83	6.63	6.06	6.11	6.54	6.21
9	6.76	6.23	6.47	6.97	6.72	6.2	6.18	6.56	6.2
10	6.56	6.25	6.31	6.82	6.61	6.09	6.12	6.6	6.2

Appendix B (continued)

Table G. Water temperature (°C) in mesocosms measured daily from one week before glyphosate application in environmental effects test.

Date	Mesocosm A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	C1	C2	C3	C4	C5
10-mar	23.5	23.7	23.7	23.6	24	23.5	23.6	23.6	23.4	23.9	23.4	23.5	23.7	23.6	24
11	24.9	24.8	24.5	24.6	25.1	24.7	24.6	24.1	23.9	25.1	24.5	24.7	24.8	24.8	25.2
12	24	23.9	23.7	23.8	24.1	23.9	23.8	23.6	23.5	24.3	23.9	23.9	23.8	23.8	24.6
13	24.2	24.3	24.2	24.3	24.7	24.3	24.3	24.2	24.1	24.8	24.3	24.3	24.2	24.3	25
14	25.2	25.4	25.3	25.2	25.7	25.4	25.6	25.2	25.1	26.2	25.2	25.4	25.6	25.6	26.5
15	26.2	26.3	26.6	26.1	26.5	26.2	26.7	26.3	26	27.1	26.1	26.3	26.8	26.4	27.1
16	27.2	27.3	27.1	27	27.4	27.4	27.6	27.2	26.9	27.7	26.8	27.1	27.5	27.3	28
17	27.5	27.7	27.1	27.2	27.8	27.6	27.2	27.3	26.8	27.9	27.2	27.4	27.3	27.8	28.5
18	25.7	25.9	25.8	25.7	25.8	25.8	25.8	25.7	25.6	26.1	25.5	25.6	26	25.8	26.5
19	26.6	26.7	26.6	26.6	26.7	26.7	26.5	26.7	26.5	26.9	26.5	26.7	26.7	26.9	27.4
20	25.6	25.8	25.5	25.5	25.7	25.9	25.4	25.8	25.9	25.8	25.7	26	26	26.2	26.6
21	24	24.2	24.2	24.1	24.3	24.1	24.1	24.1	24.1	24.5	23.9	24.6	24.3	24.3	24.6
22	25.1	25.3	25.1	25.1	25.4	25.5	25.1	25.6	25.2	25.3	25.2	25.6	25.5	25.6	26.1
23	25.5	25.6	25.4	25.5	25.7	25.5	25.4	25.6	25.6	25.7	25.3	25.6	25.6	26	26.4
24	25.7	25.8	25.8	25.6	25.8	25.7	25.7	25.8	25.6	26	25.5	25.7	25.9	26	26.4
25	26	26.1	26.2	26.1	26.4	26.1	25.9	26.2	25.9	26.3	25.9	26.1	26.1	26.3	26.9
26	25.6	25.7	25.8	25.6	25.9	25.8	25.5	25.8	25.4	25.9	25.4	25.6	25.7	25.8	26.3
27	24.3	24.5	24.4	24.3	24.5	24.4	24.2	24.4	24.2	24.5	24.2	24.3	24.3	24.4	25.2
28	24.3	24.5	24.5	24.3	24.6	24.5	24.4	24.5	24.3	24.6	24.4	24.3	24.4	24.7	25.2
29	23.6	23.8	23.8	23.6	23.8	23.8	23.7	23.9	23.6	23.9	23.7	23.9	23.9	24	24.5
30	24	24.2	24.2	24	24.2	24.2	24.2	24.3	24.1	24.3	24.1	24.2	24.1	24.2	24.7
31	23.5	23.7	23.7	23.5	23.8	23.7	23.5	23.8	23.7	23.3	23.7	23.7	23.7	23.7	24.4
01-apr	24.2	24.2	24.3	24.1	24.3	24.2	24	24.3	24.1	24.5	24.1	24.1	24.3	24.3	24
2	24.2	24.4	24.6	24.4	24.5	24.5	24.5	24.7	24.5	24.7	24.3	24.4	24.7	24.8	25.2
3	22.5	22.7	22.7	22.6	22.9	22.7	22.6	22.9	22.7	22.6	22.9	22.7	22.7	23	23.4
4	23.4	23.6	23.7	23.6	23.8	23.6	23.5	23.7	23.6	24	23.6	23.7	23.6	23.8	24.4
5	24.9	25.1	25.1	25.1	25.3	25.2	25.1	25.3	25.1	25.4	25.1	25.3	25.1	25.3	25.9
6	24.7	24.8	24.8	24.7	24.9	24.9	24.7	24.9	24.6	25	24.9	25	24.8	25	25.5
7	25.2	25.2	25.1	25	25.3	25.3	25	25.3	25.1	25.4	25.2	25.3	25.1	25.3	25.8
8	25.1	25.2	25.1	25	25.2	25.1	25	25.2	25.1	25.4	25.1	25.2	25.1	25.3	25.8
9	25.8	25.9	26	25.7	25.9	25.7	25.6	25.9	25.7	26	25.8	25.8	25.6	25.9	26.4
10	25.4	25.5	25.5	25.3	25.4	25.3	25.3	25.5	25.4	25.6	25.4	25.4	25.3	25.5	25.9
11	26.3	26.4	26.3	26.3	26.4	26.2	26.1	26.4	26.3	26.5	26.3	26.3	26.2	26.4	26.8
12	25.6	25.6	25.6	25.4	25.6	25.1	25.2	25.5	25.4	25.6	25.5	25.5	25.3	25.5	26.1
13	22.5	22.6	22.6	22.4	22.6	22.5	22.6	22.8	22.7	22.6	22.8	22.8	22.7	22.8	23.3
14	22	22	22.3	22.1	22.4	22.2	22.2	22.5	22.4	22.4	22.3	22.4	22.2	22.3	22.8
15	28.1	28.3	28.3	28.1	28.4	28.5	28	28	28.2	28	27.7	28	27.7	27.8	28.3
16	22.9	22.9	22.9	22.9	23	22.8	22.9	23	22.9	23.2	23	23.1	23	23.6	23.5
17	22.9	23	23	22.8	23.1	22.8	22.7	23	22.8	23.1	22.9	23.1	22.9	23	23.6
18	23	23.1	23	22.9	23.1	22.9	22.7	23.1	22.9	23.1	22.9	23.1	22.9	23	23.6
19	22.9	23	23	22.9	23.1	22.8	22.7	23	22.9	23.1	22.8	23.1	22.8	23	23.5
20	22.2	22.2	22.2	22	22.3	22	22	22.3	22.1	22.3	22	22.5	22.1	22.1	22.7
21	22.7	22.7	22.7	22.6	22.8	22.6	22.5	22.8	22.5	22.8	22.6	22.7	22.6	22.6	23.2
22	24.1	24.2	24.4	24	24.1	24	24	24.1	24	24.2	24	24.1	24	24	24.4
23	23.1	23.2	23.1	23.1	23.2	23.1	23	23.3	23.1	23.3	23.2	23.3	23.1	23.3	23.4
24	24.9	24.9	24.9	24.8	24.9	24.8	24.8	25	24.8	25	24.8	25	24.8	24.9	25.3
25	25.3	25.3	25.3	25.2	25.3	25.2	25.1	25.4	25.2	25.4	25.2	25.3	25.2	25.3	25.8
26	23	23.1	23.2	23	23.2	23	23	23.3	23.1	23.3	23	23.2	23.2	23.3	23.6
27	22.6	22.7	22.8	22.6	22.9	22.6	22.6	22.9	22.6	22.9	22.6	22.8	22.7	22.8	23.3
28	25.5	25.6	25.7	25.6	25.7	25	25.5	25.4	25.6	26.1	25.2	25.6	25.6	25.8	26.1
29	24.2	24.3	24.2	24.2	24.4	24.2	23.8	24.3	24.2	24.3	24.3	24.3	24	24.3	24.9
30	23.3	23.3	23.2	23	23.3	23.1	23.1	23.3	23.1	23.3	23.1	23.2	23.3	23.2	23.8
May 1	22.8	22.8	22.8	22.6	22.8	22.7	22.6	22.9	22.7	22.7	22.6	22.7	22.7	22.7	23.3
2	23.3	23.3	23.4	23.2	23.4	23.3	23.2	23.4	23.2	23.4	23.2	23.4	23.2	23.4	23.8
3	27.1	27.4	27.2	27.1	26.8	27.1	27	27.1	27.2	27.1	27	27.2	27	27.3	27.6
4	24	24	23.9	23.8	23.8	23.9	23.8	24	23.9	24.1	23.8	24	24	24.1	24.4
5	22	22	22.1	21.8	21.7	22	21.9	22.1	21.8	21.8	21.9	22.1	22.1	22	22.3
6	16.5	16.5	16.6	16.2	16.4	16.5	16.3	16.8	16.5	16.6	16.3	16.6	16.8	16.9	17.2
7	16.7	16.6	16.6	16.4	16.6	16.6	16.5	16.8	16.6	16.7	16.4	16.6	16.8	16.9	17.2
8	17.3	17.4	17.5	17.4	17.5	17.4	17.4	17.5	17.3	17.5	17.3	17.5	17.5	17.6	18
9	18.3	18.3	18.3	18.2	18.3	18.3	18.1	18.3	18.1	18.3	18.2	18.2	18.3	18.4	18.8
10	19.9	19.8	19.9	19.6	19.8	19.7	19.7	19.7	19.6	19.8	19.8	19.7	19.7	19.7	19.8

Appendix B (continued)

Table G (continued). Water temperature (°C) in mesocosms measured daily from one week before glyphosate application in environmental effects test.

	Mesocosm								
Date	D1	D2	D3	D4	D5	E2	E3	E4	E5
10-mar	23.6	23.8	23.7	23.7	23.8	23	23.3	23.3	23.5
11	24.8	25.1	24.8	24.7	25.5	23.9	24.3	24.1	24.5
12	24.3	24.4	23.9	24.3	24.8	24	24.5	24.3	24.7
13	24.7	24.8	24.2	24.7	25.1	24.2	24.5	24.4	24.9
14	25.6	25.7	25.5	25.9	26.5	25.1	25.5	25.3	25.8
15	26	26.8	26.3	26.6	26.9	25.9	26.4	26.3	26.9
16	27	27.5	27.2	27.5	27.8	26.8	27.2	27.1	27.7
17	27.7	27.4	27.8	28.2	28.6	27.1	27.7	27.5	27.3
18	25.7	26.2	25.9	26.3	26.3	26	26.4	26.2	26.6
19	26.7	26.9	26.9	27.2	27.2	26.5	26.9	26.8	27
20	26	25.8	26.2	26.4	26.3	25.1	25.5	25.5	25.7
21	24	24.4	24.2	24.5	24.5	24.1	24.4	24.3	24.6
22	25.3	25.3	25.5	26	25.8	25.2	25.4	25.3	25.4
23	25.7	25.8	25.8	26.4	26.1	25.6	25.8	25.6	25.8
24	26.2	26	25.9	26.3	25.7	26	26.2	26.1	26.2
25	26.3	26.3	26.3	26.7	26.8	26.2	26.5	26.3	26.5
26	25.8	26	25.9	26.2	26.3	26	26.2	26.1	26.2
27	24.6	24.8	24.5	24.8	24.9	24.7	24.7	24.5	24.7
28	24.5	24.7	24.5	24.9	24.9	24.6	24.7	24.5	24.8
29	24.1	24.3	24.1	24.4	24.3	24.2	24.4	24.3	24.3
30	24.3	24.4	24.5	24.9	24.6	24.4	24.6	24.5	24.6
31	23.9	24	23.9	24.3	24.1	24.5	24	24	24.1
01-apr	24.1	24.5	24.4	24.9	24.6	24.3	24.5	24.2	24.6
2	24.4	24.8	24.6	25.2	24.8	24.7	24.8	24.9	25.1
3	22.5	22.8	22.7	23.1	22.9	22.6	22.7	22.7	22.8
4	23.6	23.7	23.7	24.1	24.2	23.6	23.8	23.9	24
5	25.1	25.2	25.2	25.6	25.6	25.2	25.5	25.6	25.6
6	24.9	25	24.9	25.3	25.3	24.9	25	24.9	25.1
7	25.3	25.3	25.4	25.6	25.6	25.3	25.4	25.5	25.4
8	25.2	25.4	25.4	25.6	25.7	25.4	25.5	25.6	25.6
9	25.8	26	26	26.3	26.3	25.9	26.1	26.2	26.2
10	25.4	25.6	25.6	25.8	25.7	25.6	25.7	25.8	25.7
11	26.4	26.4	26.5	26.7	26.7	26.4	26.6	26.6	26.6
12	25.5	25.6	25.8	26	26	25.8	25.9	25.9	26
13	23	23.3	23.3	23.6	23.3	23.7	23.7	23.8	23.6
14	22.5	22.6	22.6	23	22.9	23	23.2	23.3	23.2
15	27.8	27.9	28	28.1	28.3	27.8	28.4	28.7	28.8
16	22.9	22.9	23	23.3	23.2	23.2	23.4	23.5	23.4
17	23	23.1	23.2	23.5	23.5	23.4	23.6	23.6	23.6
18	23.1	23.2	23.3	23.6	23.5	23.4	23.6	23.7	23.7
19	22.9	23	23.2	23.5	23.4	23.3	23.4	23.6	23.4
20	22.1	22.3	22.3	22.7	22.6	22.5	22.7	22.7	22.6
21	22.7	22.7	22.8	23.1	23.1	22.8	23	23.2	23
22	24.1	24.3	24.3	24.5	24.5	24.3	24.5	24.7	24.7
23	23.2	23.2	23.3	23.6	23.7	23.2	23.4	23.5	23.5
24	24.8	24.9	25	25.2	25.3	24.9	25.1	25.3	25.2
25	25.2	25.3	25.2	25.6	25.7	25.5	25.6	25.8	25.7
26	23.1	23.2	23.2	23.7	23.5	23.7	23.8	23.9	23.8
27	23.1	22.7	22.8	23.3	22.7	23.1	23.2	23.4	23.2
28	25.7	25.6	25.6	25.9	26.1	26	26	26.2	26.4
29	24.6	24.1	24.5	24.7	24.6	24.4	24.4	24.7	24.8
30	23.4	23.4	23.5	23.8	23.8	23.8	23.9	24	24
May 1	22.8	22.8	22.9	23.2	23.2	23.2	23.3	23.4	23.4
2	23.3	23.3	23.6	23.8	23.8	23.6	23.8	23.9	23.7
3	27.2	27.2	27.5	27.4	27.5	26.5	26.6	27	27.6
4	23.8	24	24	24.5	24.2	24	24.1	24.3	24.3
5	22.1	22.5	22.2	22.5	22.3	22.7	22.7	22.7	22.5
6	16.6	16.8	16.9	17.6	17.2	17.4	17.4	17.5	17.3
7	16.8	16.9	17	17.5	17.3	17.5	17.6	17.8	17.6
8	17.6	17.7	17.7	18.1	18.1	18.2	18.3	18.6	18.5
9	18.3	18.4	18.4	18.8	18.8	18.9	19	19.4	19.3
10	19.8	19.8	19.8	20.1	18.9	20.2	20.2	20.4	20.7

Appendix C. Data on chlorophyll, nitrogen and phosphorous content for environmental effects test.

Table H. Chlorophyll concentration (µg/l) in mesocosms measured weekly from one week before glyphosate application in environmental effects test.

Date	Number of treatment	Chlorophyll concentration (µg/l)	Date	Number of treatment	Chlorophyll concentration (µg/l)	Date	Number of treatment	Chlorophyll concentration (µg/l)
10-mar	A1.1	42	22-mar	A1.3	215	05-apr	A1.5	402
	A2.1	39		A2.3	472		A2.5	170
	A3.1	60		A3.3	163		A3.5	208
	A4.1	26		A4.3	405		A4.5	197
	A5.1	26		A5.3	371		A5.5	173
	B1.1	15		B1.3	315		B1.5	458
	B2.1	51		B2.3	176		B2.5	290
	B3.1	33		B3.3	310		B3.5	152
	B4.1	46		B4.3	519		B4.5	236
	B5.1	40		B5.3	290		B5.5	410
	C1.1	57		C1.3	371		C1.5	153
	C2.1	29		C2.3	159		C2.5	346
	C3.1	7		C3.3	149		C3.5	396
	C4.1	38		C4.3	296		C4.5	262
	C5.1	18		C5.3	162		C5.5	179
	D1.1	56		D1.3	385		D1.5	128
	D2.1	33		D2.3	153		D2.5	354
	D3.1	26		D3.3	312		D3.5	153
	D4.1	32		D4.3	227		D4.5	304
	D5.1	28		D5.3	248		D5.5	106
	E2.1	8		E2.3	22		E2.5	47
	E3.1	29		E3.3	285		E3.5	66
	E4.1	32		E4.3	248		E4.5	273
	E5.1	21		E5.3	204		E5.5	140
15-mar	A1.2	109	29-mar	A1.4	550	12-apr	A1.6	117
	A2.2	123		A2.4	261		A2.6	137
	A3.2	198		A3.4	38		A3.6	226
	A4.2	165		A4.4	181		A4.6	227
	A5.2	100		A5.4	117		A5.6	213
	B1.2	78		B1.4	349		B1.6	167
	B2.2	120		B2.4	198		B2.6	190
	B3.2	106		B3.4	234		B3.6	318
	B4.2	246		B4.4	271		B4.6	191
	B5.2	84		B5.4	312		B5.6	135
	C1.2	109		C1.4	124		C1.6	190
	C2.2	75		C2.4	114		C2.6	304
	C3.2	21		C3.4	329		C3.6	293
	C4.2	25		C4.4	186		C4.6	279
	C5.2	43		C5.4	173		C5.6	181
	D1.2	126		D1.4	246		D1.6	393
	D2.2	88		D2.4	33		D2.6	148
	D3.2	56		D3.4	121		D3.6	209
	D4.2	80		D4.4	201		D4.6	458
	D5.2	88		D5.4	184		D5.6	265
	E2.2	32		E2.4	42		E2.6	22
	E3.2	68		E3.4	293		E3.6	340
	E4.2	32		E4.4	368		E4.6	622
	E5.2	95		E5.4	259		E5.6	89

Appendix C (continued)

Table H (continued). Chlorophyll concentration (µg/l) in mesocosms measured weekly from one week before glyphosate application in environmental effects test.

Date	Number of treatment	Chlorophyll concentration (µg/l)	Date	Number of treatment	Chlorophyll concentration (µg/l)
19-apr	A1.7	195	May 3	A1.9	354
	A2.7	293		A2.9	296
	A3.7	181		A3.9	636
	A4.7	240		A4.9	561
	A5.7	335		A5.9	444
	B1.7	194		B1.9	315
	B2.7	209		B2.9	564
	B3.7	262		B3.9	611
	B4.7	243		B4.9	488
	B5.7	273		B5.9	396
	C1.7	636		C1.9	686
	C2.7	211		C2.9	1066
	C3.7	306		C3.9	396
	C4.7	427		C4.9	329
	C5.7	597		C5.9	1010
	D1.7	505		D1.9	795
	D2.7	247		D2.9	670
	D3.7	301		D3.9	365
	D4.7	352		D4.9	611
	D5.7	307		D5.9	600
	E2.7	39		E2.9	28
	E3.7	396		E3.9	483
	E4.7	801		E4.9	605
	E5.7	137		E5.9	419
26-apr	A1.8	292			
	A2.8	264			
	A3.8	502			
	A4.8	413			
	A5.8	466			
	B1.8	432			
	B2.8	269			
	B3.8	293			
	B4.8	222			
	B5.8	678			
	C1.8	1018			
	C2.8	617			
	C3.8	469			
	C4.8	469			
	C5.8	1138			
	D1.8	809			
	D2.8	530			
	D3.8	324			
	D4.8	425			
	D5.8	589			
	E2.8	20			
	E3.8	575			
	E4.8	497			
	E5.8	155			

Appendix C (continued)

Table I. Nitrogen concentration (mg/l) in mesocosms measured weekly from one week before glyphosate application in environmental effects test.

Date	Number of treatment	N (mg/l)	Date	Number of treatment	N (mg/l)	Date	Number of treatment	N (mg/l)
10-mar	A1.1	0.7	22-mar	A1.3	0.7	05-apr	A1.5	1.05
	A2.1	0.7		A2.3	1.4		A2.5	0.7
	A3.1	1.05		A3.3	1.4		A3.5	0.7
	A4.1	1.05		A4.3	0.7		A4.5	1.05
	A5.1	0.7		A5.3	1.4		A5.5	0.7
	B1.1	1.05		B1.3	1.05		B1.5	0.7
	B2.1	0.7		B2.3	1.4		B2.5	1.05
	B3.1	0.7		B3.3	2.1		B3.5	1.4
	B4.1	0.7		B4.3	1.05		B4.5	1.4
	B5.1	0.7		B5.3	1.4		B5.5	1.05
	C1.1	1.05		C1.3	0.7		C1.5	1.4
	C2.1	1.05		C2.3	1.4		C2.5	1.05
	C3.1	1.05		C3.3	1.05		C3.5	1.4
	C4.1	1.05		C4.3	1.4		C4.5	1.05
	C5.1	0.7		C5.3	0.7		C5.5	1.4
	D1.1	0.7		D1.3	1.4		D1.5	1.4
	D2.1	0.7		D2.3	1.05		D2.5	1.05
	D3.1	0.7		D3.3	1.05		D3.5	1.05
	D4.1	1.05		D4.3	1.4		D4.5	1.05
	D5.1	1.05		D5.3	1.4		D5.5	1.4
	E2.1	1.05		E2.3	0.7		E2.5	1.4
	E3.1	0.7		E3.3	1.05		E3.5	1.4
	E4.1	0.7		E4.3	1.4		E4.5	1.05
	E5.1	0.7		E5.3	0.7		E5.5	1.4
15-mar	A1.2	1.05	29-mar	A1.4	1.05	12-apr	A1.6	1.05
	A2.2	1.05		A2.4	1.05		A2.6	1.05
	A3.2	0.7		A3.4	1.05		A3.6	0.7
	A4.2	0.7		A4.4	1.05		A4.6	1.05
	A5.2	0.7		A5.4	1.05		A5.6	1.4
	B1.2	0.7		B1.4	0.7		B1.6	1.05
	B2.2	1.05		B2.4	1.05		B2.6	1.4
	B3.2	1.05		B3.4	1.05		B3.6	1.4
	B4.2	1.4		B4.4	1.05		B4.6	1.05
	B5.2	1.05		B5.4	1.05		B5.6	1.05
	C1.2	1.05		C1.4	0.7		C1.6	1.4
	C2.2	1.05		C2.4	1.05		C2.6	1.75
	C3.2	0.7		C3.4	1.05		C3.6	1.05
	C4.2	1.4		C4.4	1.4		C4.6	1.05
	C5.2	0.7		C5.4	0.7		C5.6	1.05
	D1.2	1.05		D1.4	1.4		D1.6	1.05
	D2.2	0.7		D2.4	1.4		D2.6	1.05
	D3.2	1.05		D3.4	1.05		D3.6	1.05
	D4.2	0.7		D4.4	1.4		D4.6	1.05
	D5.2	1.4		D5.4	0.7		D5.6	1.4
	E2.2	1.05		E2.4	1.05		E2.6	1.4
	E3.2	1.05		E3.4	1.4		E3.6	1.4
	E4.2	1.05		E4.4	1.4		E4.6	1.4
	E5.2	1.4		E5.4	1.4		E5.6	0.7

Appendix C (continued)

Table I (continued). Nitrogen concentration (mg/l) in mesocosms measured weekly from one week before glyphosate application in environmental effects test.

Date	Number of treatment	N (mg/l)	Date	Number of treatment	N (mg/l)
19-apr	A1.7	1.05	May 3	A1.9	0.7
	A2.7	1.05		A2.9	1.05
	A3.7	1.05		A3.9	1.05
	A4.7	1.05		A4.9	1.4
	A5.7	1.05		A5.9	1.05
	B1.7	1.4		B1.9	1.4
	B2.7	1.05		B2.9	1.05
	B3.7	1.4		B3.9	1.4
	B4.7	1.05		B4.9	1.05
	B5.7	1.05		B5.6	0.7
	C1.7	1.4		C1.9	0.7
	C2.7	1.05		C2.9	1.4
	C3.7	1.05		C3.9	1.4
	C4.7	0.7		C4.9	1.05
	C5.7	1.05		C5.9	0.7
	D1.7	1.4		D1.9	1.05
	D2.7	0.7		D2.9	1.05
	D3.7	1.05		D3.9	0.7
	D4.7	1.05		D4.9	1.05
	D5.7	1.05		D5.9	0.7
	E2.7	1.05		E2.9	0.7
	E3.7	1.4		E3.9	1.4
	E4.7	1.4		E4.9	1.05
	E5.7	1.05		E5.9	1.05
26-apr	A1.8	1.05			
	A2.8	1.05			
	A3.8	1.05			
	A4.8	1.05			
	A5.8	1.05			
	B1.8	0.7			
	B2.8	1.05			
	B3.8	1.05			
	B4.8	0.7			
	B5.8	1.05			
	C1.8	1.4			
	C2.8	1.4			
	C3.8	1.05			
	C4.8	1.4			
	C5.8	1.05			
	D1.8	1.05			
	D2.8	1.4			
	D3.8	1.4			
	D4.8	1.05			
	D5.8	1.4			
	E2.8	1.05			
	E3.8	1.05			
	E4.8	1.05			
	E5.8	1.05			

Appendix C (continued)

Table J. Phosphorous concentration (mg/l) in mesocosms measured weekly from one week before glyphosate application in environmental effects test.

Date	Number of measurement	P (mg/l)	Date	Number of measurement	P (mg/l)	Date	Number of measurement	P (mg/l)
10-mar	A1.1	0.04	22-mar	A1.3	0.07	05-apr	A1.5	0.04
	A2.1	0.07		A2.3	0.07		A2.5	0.07
	A3.1	0.07		A3.3	0.07		A3.5	0.07
	A4.1	0.07		A4.3	0.01		A4.5	0.07
	A5.1	0.07		A5.3	0.07		A5.5	0.07
	B1.1	0.04		B1.3	0.11		B1.5	0.11
	B2.1	0.07		B2.3	0.07		B2.5	0.07
	B3.1	0.07		B3.3	0.07		B3.5	0.07
	B4.1	0.04		B4.3	0.04		B4.5	0.07
	B5.1	0.04		B5.3	0.04		B5.5	0.07
	C1.1	0.04		C1.3	0.04		C1.5	0.04
	C2.1	0.07		C2.3	0.04		C2.5	0.04
	C3.1	0.07		C3.3	0.01		C3.5	0.07
	C4.1	0.07		C4.3	0.01		C4.5	0.04
	C5.1	0.07		C5.3	0.01		C5.5	0.07
	D1.1	0.07		D1.3	0.01		D1.5	0.07
	D2.1	0.07		D2.3	0.01		D2.5	0.07
	D3.1	0.11		D3.3	0.07		D3.5	0.04
	D4.1	0.11		D4.3	0.01		D4.5	0.04
	D5.1	0.11		D5.3	0.01		D5.5	0.04
	E2.1	0.07		E2.3	0.07		E2.5	0.04
	E3.1	0.04		E3.3	0.07		E3.5	0.07
	E4.1	0.04		E4.3	0.07		E4.5	0.07
	E5.1	0.07		E5.3	0.07		E5.5	0.07
15-mar	A1.2	0.07	29-mar	A1.4	0.07	12-apr	A1.6	0.04
	A2.2	0.07		A2.4	0.24		A2.6	0.04
	A3.2	0.07		A3.4	0.04		A3.6	0.04
	A4.2	0.07		A4.4	0.11		A4.6	0.01
	A5.2	0.11		A5.4	0.07		A5.6	0.01
	B1.2	0.07		B1.4	0.01		B1.6	0.04
	B2.2	0.07		B2.4	0.01		B2.6	0.04
	B3.2	0.07		B3.4	0.01		B3.6	0.01
	B4.2	0.11		B4.4	0.01		B4.6	0.01
	B5.2	0.04		B5.4	0.01		B5.6	0.01
	C1.2	0.11		C1.4	0.04		C1.6	0.01
	C2.2	0.07		C2.4	0.01		C2.6	0.04
	C3.2	0.07		C3.4	0.01		C3.6	0.07
	C4.2	0.07		C4.4	0.01		C4.6	0.07
	C5.2	0.07		C5.4	0.01		C5.6	0.07
	D1.2	0.07		D1.4	0.01		D1.6	0.04
	D2.2	0.07		D2.4	0.01		D2.6	0.04
	D3.2	0.11		D3.4	0.04		D3.6	0.04
	D4.2	0.04		D4.4	0.01		D4.6	0.07
	D5.2	0.07		D5.4	0.01		D5.6	0.07
	E2.2	0.04		E2.4	0.01		E2.6	0.07
	E3.2	0.04		E3.4	0.01		E3.6	0.07
	E4.2	0.07		E4.4	0.04		E4.6	0.07
	E5.2	0.04		E5.4	0.01		E5.6	0.04

Appendix C (continued)

Table J (continued). Phosphorous concentration (mg/l) in mesocosms measured weekly from one week before glyphosate application in environmental effects test.

Date	Number of measurement	P (mg/l)	Date	Number of measurement	P (mg/l)
19-apr	A1.7	0.07	May 3	A1.9	0.07
	A2.7	0.07		A2.9	0.10
	A3.7	0.07		A3.9	0.07
	A4.7	0.07		A4.9	0.10
	A5.7	0.07		A5.9	0.10
	B1.7	0.07		B1.9	0.07
	B2.7	0.07		B2.9	0.07
	B3.7	0.07		B3.9	0.07
	B4.7	0.07		B4.9	0.07
	B5.7	0.07		B5.6	0.07
	C1.7	0.11		C1.9	0.07
	C2.7	0.07		C2.9	0.07
	C3.7	0.11		C3.9	0.07
	C4.7	0.07		C4.9	0.07
	C5.7	0.07		C5.9	0.07
	D1.7	0.07		D1.9	0.14
	D2.7	0.07		D2.9	0.04
	D3.7	0.07		D3.9	0.04
	D4.7	0.04		D4.9	0.04
	D5.7	0.04		D5.9	0.07
	E2.7	0.04		E2.9	0.04
	E3.7	0.07		E3.9	0.07
	E4.7	0.07		E4.9	0.07
	E5.7	0.07		E5.9	0.10
26-apr	A1.8	0.07			
	A2.8	0.11			
	A3.8	0.11			
	A4.8	0.11			
	A5.8	0.11			
	B1.8	0.11			
	B2.8	0.11			
	B3.8	0.11			
	B4.8	0.07			
	B5.8	0.07			
	C1.8	0.07			
	C2.8	0.07			
	C3.8	0.14			
	C4.8	0.07			
	C5.8	0.11			
	D1.8	0.14			
	D2.8	0.14			
	D3.8	0.14			
	D4.8	0.11			
	D5.8	0.11			
	E2.8	0.11			
	E3.8	0.11			
	E4.8	0.11			
	E5.8	0.11			