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TOXICITY AND GENOTOXICITY TESTING OF ROUNDUP

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Glyphosate, in the commercial formulation named Roundup, is a broad spectrum herbicide that is one of the most frequently applied pesticides in the world. However, there has been little evidence of Roundup toxicity or genotoxicity. Genotoxicity of glyphosate was carried out using the Escherichia coli *SOS chromotest. The glyphosate-induced dose response in the SOS chromotest suggests that glyphosate possesses genotoxic properties. Glyphosate at a 0.2 g/l concentration in toxicity bioassay caused 50% mortality of Daphnia magna (LD50 after 24 h — 0.22 g/l; after 48 h — 0.19 g/l), but 0.25 — 0.5 g/l — 100% death of organisms (LD100 after 24 h — 0.5 g/l; after 48 h — 0.25 g/l). Our results (*E. coli *SOS chromotest and daphnia test system) together with recent animal studies and epidemiological reports suggest that glyphosate, especially, Roundup possesses both toxic and genotoxic properties.*

Key words: *glyphosate (Roundup), toxicity, genotoxicity,* Escherichia coli *SOS chromotest, toxicity bioassay.*

INTRODUCTION

The US Environmental Protection Agency's estimated that 891 pesticide active ingredients were registered in 1997. Each active ingredient has a specific mode of action for controlling a pest, and each active ingredient has its own possible side effects on the wild-life and human exposed to it. Harmful effects of pesticides in the environment have been documented in many investigations — on soil microorganisms (Клинцаре, 1983; Ahitiainen *et al*., 2003), and aquatic flora and fauna (Blaustein and Johanson, 2003). Occupational exposure to pesticides may increase risk for adverse reproductive outcomes (Greenlee *et al*., 2003), brain and nervous system disturbances (Colborn, 2006), may cause immunodepression and lead to cancer in later life and can also induce heritable changes (Ames, 1992; Bull *et al*., 2006). Three million cases of pesticide poisoning, nearly 220,000 fatal, occur world-wide every year (Bolognesi, 2003).

Glyphosate [N-(phosphomethyl)glycine], commonly sold in the commercial formulation named Roundup (Monsanto, Belgium), has been a frequently used herbicide on both cropland and noncropland areas of the world since its introduction in the 1970s (Williams *et al*., 2000). Glyphosate is extensively used as a non-selective herbicide by both professional operators and growers and its use is likely to increase further as it is one of the first herbicides against which crops have been modified to increase their tolerance. Since 1996, Monsanto and other seed companies have introduced glyphosate-resistant canola, cotton, corn, sugar been, and alfalfa. Today, glyphosate-resistant soybeans make up more than 90% of the soybean in the United States, and corn more than 60% (Service, 2007). In 1995, US farmers used 4.5 million kilograms of glyphosate, now they use ten times that amount (Service, 2007).

Roundup is a combination of the active ingredient glyphosate and other chemicals: a surfactant polyoxyethylenamine (POEA) that enhances the spreading of spray droplets when they contact foliage, and a various minor components, including anti-foaming and colour agents, biocides and inorganic ions to provide pH adjustment. Commercial glyphosate-based formulations contain from 41% or more glyphosate to 1% glyphosate formulations marketed for home-garden use.

Glyphosate is a broad-spectrum herbicide, of which the primary mechanism is inhibition of the enzyme 5-enolpyruvoshikimate 3-phosphate synthase, which is essential for the formation of aromatic amino acids in plants. Since this specific biological pathway operates only in plants and microorganisms, the mechanism is not considered to be a risk for other organisms and humans. Although there has been little consistent evidence of genotoxicity or carcinogenicity from *in vitro* and animal studies, a few epidemiological reports have indicated potential health effects of glyphosate. Occupational exposure to herbicide may increase parental risk of infertility, and adverse pregnancy outcomes such as spontaneous abortion (De Roos *et al*., 2005).

Between 1 January to 30 September 1980, 93 cases of exposure to herbicides containing glyphosate and surfactant (Roundup) were treated at Changhua Christian Hospital. The average amount of the 41% solution of glyphosate herbicide ingested by non-survivors (seven patients) was 184 ± 70 ml (range 85–200 ml), but much larger amounts (500 ml) were reported to have been ingested by some patients and only resulted in mild to moderate symptomatology. Intentional ingestion (80 cases) resulted in erosion of the gastrointestinal tract (66%), seen as sore throat (43%), dysphagia (31%), and gastrointestinal haemorrhage (8%). Other organs were affected less frequently (non-specific leucocytosis 65%, lung 23%, liver 19%, cardiovascular 18%, kidney 14%, and CNS 12%) (Cox, 1995). Human poisoning with Roundup is not due to the active ingredient glyphosate alone, but with the complex and variable mixtures.

The U.S. Environmental Protection Agency (U.S. EPA 1993) and the World Health Organization (WHO 1994) reviews on the toxicology of glyphosate considered that it is not mutagenic or carcinogenic. The U.S. EPA classified glyphosate as category E, indicating "evidence of non-carcinogenicity for humans" (U.S. EPA 1993). Despite this conclusion, recent case-control studies suggested an association between reported glyphosate use and the risk of non-Hodgkin lymphoma (NHL) and multiple myeloma. Myeloma has been associated with agents that cause either DNA damage or immune supression (De Ross *et al*., 2005). Some studies observed that glyphosate treatment of human lymphocytes *in vitro* resulted in increased sister chromatide exchanges, chromosomal aberrations, and indicators of oxidative stress (Bolognesi *et al*., 1997).

The aim of our study was to analyse toxic and genotoxic activity of herbicide roundup by two methods: standartised daphnia test for analysing toxicity, and *Escherichia coli* SOS chromotest, a very sensitive and short-term method for detecting genotoxicity. We did not find in the literature investigations of the hazardous effects of Roundup using these tests.

MATERIALS AND METHODS

We used for our experiments Roundup BIO from Monsanto Brusells Belgia.

We determined Roundup toxicity following the standard ISO 6341:1996 test (Anonymous, 1996) (*Daphnia magna* strains) and genotoxicity using the *Escherichia coli* SOS chromotest. Toxicity bioassays can be used predict the herbicide impact in environment, either in aquatic ecosystems (Stratton and Giles, 1990).

The test principle is determination of the initial concentration, which within 24 h, immobilizes 50% of exposed *Daphnia magna*, under the conditions defined in the ISO 6341:1996 standard test. LD_{50} is the level at which 50% of a population dies when exposed to the chemical. As the test organism *D. magna* less than 24 h old was used. At the end of the test period (after 24 h or 48 h) the mobile *D. magna* in containers were counted and the percentage immobilization for each concentration (in relation to the total number of used *D. magna*) was calculated by using Probit analysis.

The test was carried out in two stages:

- preliminary test, which gives an approximate value of the 24 h LD_{50} or 48 h LD_{50} and determines the range of concentrations to be tested in the final toxicity test;

- definitive test, to determine the final percentages of *D. magna* immobilised by different herbicide concentrations and the 24 h LD_{50} or 48 h LD_{50}.

Time of exposure was 24 and 48 hours. Test conditions — 16h/8h-light/dark photoperiod, temperature 20 ^{o}C.

Ten individuals (24 h old) were treated in each container with 100 ml test solution (concentration of glyphosate — 0.1 g/l–60 g/l). The control was tap water at the same experimental conditions without addition of glyphosate.

Genotoxicity assay of Roundup (glyphosate) was carried out using the SOS chromotest. This test is based on using the genetically modified *Escherichia coli* PQ37 strain, in which the *lac*Z is under the control of the *sfi*A gene (Quillardat *et al*., 1985). In short, genotoxicity was detected measuring the activation of SOS response of the test organism by evaluating β-galactosidase (*de novo* synthesis) induction and alkaline phosphatase (constitutive) expression. The β-galactosidase and alkaline phosphatase activities were calculated according to Quillardet and Hofnung (1993) units = A420 × 10^3/t (A420 = optical density units = A420 × 10^3/t at 420 nm; t – substrate conversion time in min.).

The induction factor (IF) was calculated as the ratio Rx/Ro (Rx = β-galactosidase activity/alkaline phosphatase activity determined for the test compound at concentration x; Ro — β-galactosidase activity/ alkaline phosphatase activity at test compound concentration zero). Roundup was dissolved in saline solution just before each experiment. The control was saline solution alone.

RESULTS

Roundup toxicity. Based on the results of the acute toxicity test, mortality of *D. magnus* was observed already at a very low concentration of herbicide. Inhibition was observed during the first day. Probit analysis shows that both LD50 after 24 h (Fig. 1) and 48 h (Fig. 1) exposure are very similar (Fig. 2). According to the Roundup work instructions, recommended concentrations for use outdoors are 7.2 g/l and 72 g/l. However, according to our experiments, already 0.2 g/l concentrations can initiate 50% mortality of test organisms (LD50 after 24 h: 0.22 g/l; after 48 h: 0.19 g/l), and 0.25–0.5 can cause 100% death of organisms (LD50 after 24 h: 0.5 g/l; after 40 h: 0.25 g/l) (Fig. 2) indicating that Roundup is a really toxic herbicide.

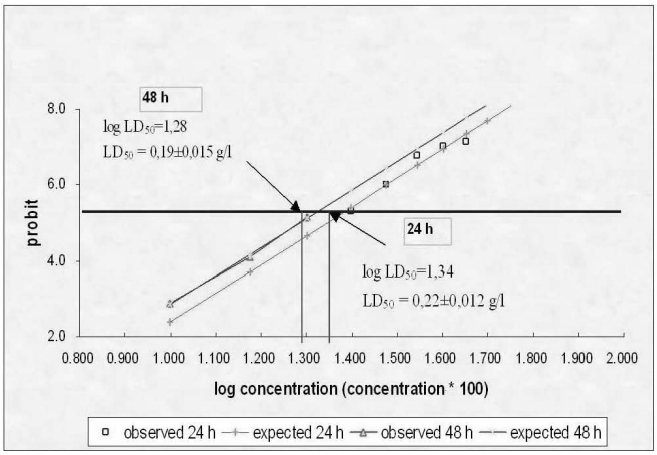

Fig. 1. D. magna LD_{50} values after 24 h and 48 h mobility inhibition test.

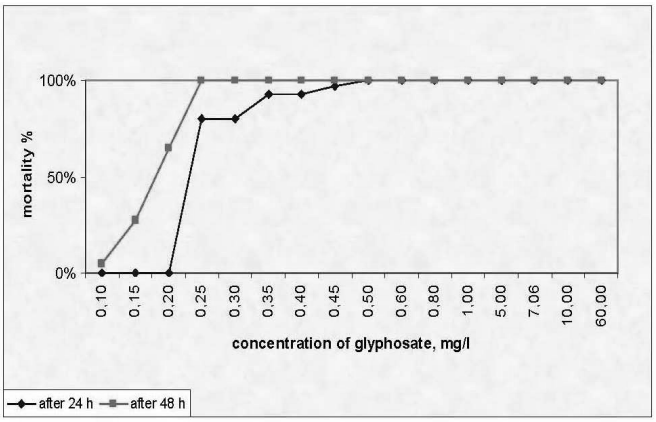

Fig. 2. Mortality of *D. magna* after 24 h and 48 h.

The standard ISO 6341:1996 test (Stratton and Giles, 1990) is widely used for determination of acute toxicity *Daphnia magna* Straus (*Cladocera, Crustacea*) by: 1) chemical substances, which are soluble under the conditions of the test, 2) industrial effluents, (3) sewage effluents, and (4) surface or ground waters.

D. magna are excellent organisms to use in bioassays because they are sensitive to changes of water chemistry. Roundup and its ingredients cause harmful effects on aquatic microbial biota in environment. It is difficult to separate the toxicity of glyphosate alone and commercial formulations alone.

Roundup genotoxicity. The genotoxic properties of glyphosate were studied using the *E. coli* SOS chromotest. The results show (Fig. 3) that glyphosate induced the SOS-repair system. The maximum value of IF was 4.8 at dose 0.25 μm glyphosate per sample. According to Mesch-Sundermann *et al.* (1992), a compound can be classified as genotoxic if the Ifmax exceeds 2.0 and increases with increasing compound concentration. Since glyphosate induced more then 2.0 IF dose the response was dose dependent the results suggest that glyphosate possesses genotoxic properties.

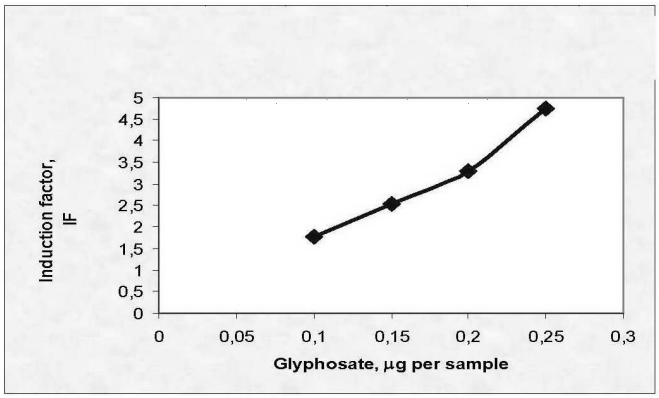

Fig. 3. Dose response in the *E. coli* SOS chromotest for glyphosate.

DISCUSSION

The toxicity of the Roundup and its components on aquatic organisms (bacteria, microalgae, protozoa, and crustaceans) in artificial systems follow the order: polyoxyethylene amine > Roundup > glyphosate acid > isopropylamine salt of glyphosate. However, in studies on photosynthetic microalgae, the toxicity contribution of POEA accounted for more than 86% of Roundup effect, and the effect was species-dependent and pH-dependent (Tsui and Chu, 2003). These results suggest that POEA toxicity is greater then other Roundup ingredients. All POEA formulations were found to be extremely toxic to laboratory and field collected fairy shrimp *Thamnocephalus platyurus* (Brausch and Smith, 2007).

In a *Salmonella* assay only Roundup was tested (Rank *et al*., 1993), which showed a week mutagenic effect for concentrations of 360 μg/plate in TA98 (without S9) and 720 μg/plate in TA100 (with S9). These concentrations are close to the toxic level. The anaphase-telophase *Allium* test showed no effect for the glyphosate isopropilamine salt, but a significant increase in chromosome aberration appeared after treatment with Roundup at concentrations of 1.44 and 2.88 mg/l when calculated as glyphosate isopropilamine. The most frequent aberrations observed could be characterised as disturbance of the spindle (Rank *et al*., 1993). For chronic cytotoxicity a dose-dependent effect was observed in normal human cells (GM38) and human fibrosarcoma (HT1080) cells after treatment with 5.2–8.5 mM and 0.9–3.0 mM glyphosate, respectively. In an acute cytotoxicity study, GM38 cell exposed to 4.0–7.0 mM glyphosate, and HT1080 cells exposed to 4.5–5.8 mM glyphosate, cell viability counts were higher than 80%. Genotoxic effects were observed in GM38 cells at glyphosate concentrations of 4.0–6.5 mM and in HT 1080 cells at glyphosate concentrations of 4.0–6.5 mM (Monroy *et al*., 2005).

Our results (*E.coli* SOS chromotest and daphnia test system) together with recent animal studies and epidemiological reports (Richard *et al*., 2005) suggest that glyphosate, especially, Roundup, possesses both toxic and genotoxic properties.

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RAUNDAPA TOKSICITĀTE UN GENOTOKSICITĀTE

Kā vienu no videi un cilvēkam nekaitīgākajiem herbicīdiem jau kopš tā ieviešanas 1970. gadā reklamē glifosātu (N-fosfonometilglicīnu), kura komercnosaukums ir raundaps (Monsanto, Beļģija). Raundapu plaši izmanto gan graudaugu, gan negraudaugu lauku nezāļu iznīcināšanai. Raundaps ir maisījums, kurā glifosātam ir pievienots deterģents polioksietilenamīns. Lai gan tiek apgalvots, ka raundaps ir videi un cilvēkam nekaitīgs, tomēr ir arī ziņojumi par šī herbicīda izraisītajiem kaitīgajiem efektiem. Mēs raundapa toksicitāti analizējām ar *Daphnia magna* testkultūrām un genotoksicitāti ar *Escherichia coli* SOS hromotesta metodi. 0,2 g/l glifosāta toksicitātes testā izraisīja 50% *Daphnia magna* letalitāti (LD50 – 0,22 g/l pēc 24 studām, 0,19 g/l pēc 48 stundām), bet 0,25 – 0,5 g/l – 100% organismu nāvi (LD100 – 0,5 g/l pēc 24 stundām, 0,25 g/l pēc 24 stundām). Tātad raundaps koncentrācijās no 0,2 g/l – 0,5 g/l, kas ir ievērojami zemāka nekā tiek izmantota lauku apstrādei, izraisa testobjekta dafnijas *(Daphnia magna*) bojāeju, kas liecina par šī preparāta toksiskumu. Eksperimentos ar *E. coli* SOS hromotesta metodi, analizējot herbicīdu tādās pašās koncentrācijās kā toksicitātes testā, tika konstatēta ģenētisko bojājumu palielināšanās, pieaugot glifosāta koncentrācijai analizējamajā materiālā – tas liecina par glifosāta genotoksisko aktivitāti. Ar abām metodēm iegūtie rezultāti apstiprina, ka raundapam ir gan toksiskā iedarbība, gan genotoksiskie efekti.