Bull. Environ. Contam. Toxicol. (1992) 48:772–780


Environmental Contamination and Toxicology

# Response of Rainbow Trout to a Two Month Exposure to Vision®, a Glyphosate Herbicide

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In Canada, Vision[R] is a major forest management herbicide, representing 81% of all herbicides sprayed on the forests (Trotter et al. 1990). It contains 356 g/L of glyphosate (N-(phosphonomethyl)glycine) as the active ingredient, and is believed to have its effect by inhibiting the shikimic acid pathway of plants. Since this is a biochemical pathway common to all plants, the herbicide is broad spectrum and acts during the active growth phase (Cole 1985). Vision is usually applied to forests aerially and because of this can enter aquatic systems by direct over spraying and/or drift. Studies following forest applications of Vision have found that the herbicide does, on occasion, enter ponds and streams and levels of glyphosate ranging from 0.75 µg/L to 270 µg/L have been found. Once in aquatic systems, the half-life of the herbicide varies from several days to ten weeks, depending on the pH of the water (Trotter et al. 1990).

Lethal levels of glyphosate for fishes are generally higher than the concentrations that have been found in waterways following spraying. The $96hLC_{50}$ for glyphosate ranges from 97 mg/L to 140 mg/L (Folmar et al. 1979). Operational formulations of the herbicide are more toxic than the technical grade chemical with the $96hLC_{50}$ being in the range of 2.0 to 50 mg/L (Folmar et al. 1979; Hildebrand et al. 1982). The increased toxicity of operational formulations comes from the presence of a surfactant (Folmar et al. 1979).

There have been few studies of the sublethal effects of glyphosate herbicides. Folmar et al. (1979) found that

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exposure of rainbow trout (Oncorhyncus mykiss) to up to 2.0 mg/L of glyphosate or Roundup® for 12 h had no effect on fecundity or gonadosomatic index. Mitchell et al. (1987) found that exposure to 2.78 mg/L Roundup for ten days did not affect seawater adaptation or growth in coho salmon (Oncorhynchus kisutch). There is a lack of longer-term exposure studies and this has hindered the establishment of guidelines for levels of glyphosate for freshwater organisms (Trotter et al. 1990). This study examines the effects of a two month exposure to glyphosate, as Vision, on the growth, behaviour, and gill and liver histopathology of rainbow trout.

## MATERIALS AND METHODS

All $LC_{50}$ tests were conducted in aerated water, under static conditions. Fish were tested in groups of five, in 20 L polycarbonate containers holding 10 L of aerated water, which was changed daily. Fish were 9.5 $\pm$ 0.1 cm ($\bar{x} \pm$ S.E.) in total length. Fish in both the $LC_{50}$ tests and the long term exposures were triploid females obtained from a hatchery. Water temperature was maintained at 12.3 $\pm$ 0.2°C which is typical of streams and ponds in Newfoundland, Canada during the time of spraying (Kendaris 1981). The pH of the water was 6.01, alkalinity 3.0 mg/L $CaCO_3$, hardness 9.6 mg/L $CaCO_3$, and the partial pressure of oxygen was greater than 150 torr throughout.

All long term exposures were done in duplicate using a proportional diluter to which the toxicant was delivered using a piston pump. Eight fish were housed in each polycarbonate container in 20 L of aerated water. There were 2 containers at each concentration for a total of 16 fish at each concentration. The diluter was calibrated to give nominal concentrations of 0, 6.25, 25 and 100 µg/L of glyphosate in Vision and delivered 0.5 L of water to each container every 15 min. The fish in each bucket were fed rations of two percent of their body weight, five times a week. Debris was siphoned from the buckets every second day.

Foraging and agonistic behaviour of the fish was measured following one and two months exposure to 0, 6.25 and 100 µg/L of glyphosate in Vision. Each fish was observed in a 25x30x70 cm stream tank with a 4.5 cm/s current. Details of the stream tank and foraging observations are given in Morgan and Kiceniuk (1990). Each fish was presented with ten, live, adult brine shrimp, one at a time. The reaction distance of fish to the brine shrimp, the number of ingestions and the ratio of attacks/approach and captures/attack were

recorded.

Following the foraging session a sheet of cardboard was removed from the outside of the tank to reveal a mirror along the rear, longitudinal wall of the tank. Ten minutes later the frequency and time spent in agonistic activities of the fish toward the mirror were recorded for 10 min. Three agonistic activities were recorded (Taylor and Larkin 1986). Erection of the dorsal and anal fins while motionless was classified as lateral display. Swim against mirror (SAM) was any swimming motion by a fish with its snout pressed against the mirror. Wigwag was swimming parallel to the mirror usually with the head up or down and the body stiffened.

All fish in the long term exposures were anesthetized with Tricaine Methanesulfonate and fin clipped for individual identification. The fish were weighed to the nearest 0.1 g and total length was measured to the nearest 0.1 cm. Measurements were taken prior to exposure and after one and two months exposure to nominal concentrations of 0, 6.25, 25 and 100 µg/L. Fish used in the growth and behavioural studies were 9.1 $\pm$ 0.1 cm in total length and had a mean weight of 7.2 $\pm$ 0.2 g at the beginning of the experiment.

Fish 11.2 $\pm$ 0.1 cm in total length and 14.2 $\pm$ 0.6 g in weight were exposed for two months to 0, 6.25, 25 and 100 µg/L under the same conditions as those used in the growth and behavioural studies. At the end of two months the fish were killed by severing the spinal column and the liver and one gill arch removed. These organs were chosen because the detergent in the formulation could cause gill irritation and because the liver is the main organ of detoxification. Samples were preserved in bouins for 24 h and then placed in alcohol until processing. They were embedded in paraffin, sectioned, stained with Mayers Haematoxylin and Eosin, and examined for abnormalities under a light microscope at 100 X. 5 sections of liver from random locations within the organ from each fish were examined for tumors and melanomacrophage. All of the secondary lamellae on 5 primary lamellae were examined from each fish for lesions. Distal hyperplasia, epitheal lifting, clubbing, basal hyperplasia and fusion were identified. A description of these lesion types can be found in Khan and Kiceniuk (1984). Histopathological examinations were performed as a blind test.

Water samples were taken on two occassions and sent to Fenwick Labs, Halifax, NS, Canada for analysis to confirm the presence of glyphosate. An aliquot of the water sample was filtered and concentrated by rotary

evaporation. It was then derivatized with 9-fluorenylmethylchloroformate. Analysis was by reversed phase HPLC with fluorescence detection (J. C. Marr, pers. comm.)

If data met the assumptions of parametric tests or could be transformed to do so they were analysed using ANOVA, otherwise there were anlayzed with chi-square or Kruskal-Wallis. All frequencies were analyzed with chi-square analyses. The mean reaction distance for each fish was calculated and the means subjected to ANOVA. The percent change in growth in length and weight, time spent in each aggressive activity, and percentage of gill lesions, were compared across concentration using Kruskal-Wallis tests. Foraging attack sequence ratios were transformed using a modified arcsine transformation and analyzed using ANOVA (Zar 1984). The significance level was set at 0.05.

## RESULTS AND DISCUSSION

The $96hLC_{50}$ at 12 C was 10.42 mg/L glyphosate (95% fiducial limits 9.37 - 11.67 mg/L) in the Vision formulation. This amount of glyphosate is contained in 0.029 ml/L of Vision. This is consistent with the results of other studies. For rainbow trout similar in size to those used here, the $96hLC_{50}$ for Roundup (the same formulation as Vision) has been found to range from 8.3 to 54.8 mg/L (Folmar et al. 1979; Hildebrand et al. 1982). Increased temperature and pH both result in an increase in the toxicity of glyphosate and the acute toxicity also varies with species and life history stage, with fry being the most vulnerable (Folmar et al. 1979).

HPLC analyses confirmed the presence of glyphosate in the water, although the measured levels were lower than the nomimal concentrations. The means ($\pm$ SE) of the measured concentrations were 4.25 $\pm$ 0.8, 8.0 $\pm$ 0.7, and 45.75 $\pm$ 6.3 µg/L for nominal concentrations of 6.25, 25 and 100 µg/L. This indicates that there was adsorption of the glyphosate onto the bucket walls and debris and/or uptake by the fish.

Exposure of rainbow trout to glyphosate in the form of Vision at sublethal concentrations had little effect on the fish. There was no significant effect of exposure for either one or two months on any of the foraging variables that were measured (Table 1). There was no significant effect of exposure to Vision for either one or two months on the time spent performing any agonistic activity (Table 2). There was no significant effect on growth in length or weight of exposure to

Table 1. Mean (± SE) of foraging activities for rainbow trout exposed to Vision. Results of statistical tests are also shown.

| | | | | | |
|---|---|---|---|---|---|
| One month | 0 µg/L | 4.25 µg/L | 45.75 µg/L | | |
| Reaction distance | 17.0 ± 1.2 | 15.1 ± 1.0 | 17.1 ± 1.2 | $F = 0.9$ | df = 2,30 NS[a] |
| Attacks/approach | 0.88 ± 0.02 | 0.88 ± 0.03 | 0.82 ± 0.05 | $F = 0.9$ | df = 2,30 NS |
| Captures/attack | 0.97 ± 0.002 | 0.96 ± 0.01 | 0.95 ± 0.02 | $F_2 = 1.2$ | df = 2,30 NS[b] |
| Ingestions | 9.8 ± 0.1 | 9.6 ± 0.2 | 9.4 ± 0.3 | $x = 0.1$ | df = 2 NS[b] |
| Two months | | | | | |
| Reaction distance | 20.4 ± 2.3 | 21.5 ± 1.3 | 20.7 ± 1.2 | $F = 0.1$ | df = 2,25 NS[a] |
| Attacks/approach | 0.85 ± 0.04 | 0.82 ± 0.05 | 0.89 ± 0.14 | $F = 0.8$ | df = 2,25 NS |
| Captures/attack | 0.96 ± 0.009 | 1.0 ± 0 | 0.95 ± 0.01 | $F_2 = 1.7$ | df = 2,25 NS[b] |
| Ingestions | 9.8 ± 0.2 | 10.0 ± 0 | 9.7 ± 0.2 | $x = 0.2$ | df = 2 NS[b] |

[a] ANOVA
[b] chi-square

Table 2. Mean (± SE) frequency of and time spent in agonistic activities for rainbow trout exposed to Vision. Results of statistical tests are also given. For activities showing significant differences, concentrations with the same letter are not significantly different. For all tests df = 2.

| | | | | |
|---|---|---|---|---|
| | | Time(s) | | |
| One month | 0 µg/L | 4.25 µg/L | 45.75 µg/L | |
| Lateral display | 23.7 ± 7.8 | 53.0 ± 18.2 | 38.8 ± 13.2 | $x^2_2$ = 0.4 NS[a] |
| SAM | 52.8 ± 16.2 | 82.6 ± 21.1 | 66.4 ± 5.1 | $x^2_2$ = 0.4 NS |
| Wigwag | 56.6 ± 19.9 | 54.6 ± 13.6 | 84.5 ± 20.0 | $x^2$ = 0.2 NS |
| Two months | | | | |
| Lateral display | 21.4 ± 2.0 | 7.8 ± 2.9 | 17.1 ± 8.2 | $x^2_2$ = 0.8 NS |
| SAM | 31.6 ± 9.6 | 22.9 ± 7.6 | 46.7 ± 13.9 | $x^2_2$ = 0.5 NS |
| Wigwag | 62.0 ± 26.0 | 44.0 ± 24.9 | 78.0 ± 30.0 | $x^2$ = 0.5 NS |
| | | Frequency | | |
| One month | | | | |
| Lateral display | 6.8 ± 2.3 | 8.8 ± 2.5 | 8.4 ± 2.0 | $x^2_2$ = 3.2 NS[b] |
| SAM | 17.7 ± 2.9 | 21.0 ± 3.5 | 20.9 ± 3.9 | $x^2_2$ = 4.0 NS |
| Wigwag | 12.0 ± 2.7 A | 12.4 ± 2.1 A | 18.2 ± 4.1 B | $x^2$ = 18.9 p<0.001 |
| Two months | | | | |
| Lateral display | 5.0 ± 2.0 | 3.9 ± 1.5 | 5.8 ± 1.6 | $x^2_2$ = 2.9 NS |
| SAM | 12.8 ± 2.3 AB | 9.6 ± 2.3 B | 15.7 ± 4.1 A | $x^2_2$ = 11.4 p<0.005 |
| Wigwag | 14.5 ± 4.4 A | 8.3 ± 2.4 B | 15.4 ± 4.5 A | $x^2$ = 24.8 p<0.001 |

[a]Kruskal-Wallis
[b]chi-square

Vision for either one or two months (Table 3). No livers, either from control or exposed fish, showed any evidence of tumors or melanomacrophages. All gill lesions were observed in small numbers, so the total number of lesions was used in the analyses. There was no significant difference between the number of lesions on gills of fish exposed to Vision and the control fish (mean number of lesions per fish ± S.E.: 0 µg/L 28.1 ± 6.0; 4.25 µg/L 22.6 ± 3.7; 8.0 µg/L 22.1 ± 3.8; 45.75 µg/L 24.8 ± 2.9; $\chi^2$ = 0.92, NS).

Table 3. Mean (± SE) percentage change in length and weight for rainbow trout exposed to Vision. Results of Kruskal-Wallis tests are also shown.

| | 0 µg/L | 4.25 µg/L | 8.0 µg/L | 45.75 µg/L | |
|---|---|---|---|---|---|
| One month | | | | | |
| length | 7.9±3.6 | 8.2±0.6 | 6.2±1.3 | 7.5±0.5 | $\chi^2$=3.0 NS |
| weight | 19.9±0.6 | 14.3±2.7 | 19.7±4.7 | 11.0±2.4 | $\chi^2$=4.3 NS |
| Two months | | | | | |
| length | 7.4±0.8 | 7.1±0.8 | 5.5±1.5 | 6.6±0.8 | $\chi^2$=1.8 NS |
| weight | 27.2±2.9 | 30.5±3.5 | 33.4±8.5 | 33.2±8.7 | $\chi^2$=0.6 NS |

Shorter term studies have found no significant effect of glyphosate herbicides on fecundity or gonadosomatic index in rainbow trout (Folmar et al. 1979) or on sea water adaptation and growth in coho salmon (Mitchell et al. 1987). Acute toxicity studies have shown that much of the toxicity of herbicide formulations containing glyphosate comes from the surfactant (Folmar et al. 1979). It is not surprising that glyphosate itself is relatively nontoxic to animals given that its primary mode of action is thought to be the inhibition of a biochemical pathway not found in animals (Cole 1985). Although the surfactant results in a lower $LC_{50}$ for commercial formulations of the herbicide than for the technical grade compound, this study indicates that sublethal concentrations of a formulation containing this surfactant are also relatively nontoxic.

Even though there was little overall effect of exposure to Vision on rainbow trout, there were significant differences between control and exposed fish in one aspect of agonistic behaviour (Table 2). After one month of exposure fish in the highest concentration (45.75 µg/L) had a significantly higher frequency of wigwags. After two months, fish exposed to the lowest concentration of Vision performed significantly fewer wigwags. The ability of salmonids to maintain preferred feeding stations, through the agonistic activities measured in this study, results in increased growth (Grant 1990). It is not known what the

implications of a change in one agonistic activity in the repetoire of aggressive behaviour would be in terms of a fish's ability to hold a feeding territory. Also, the dose response was inconsistent over time, making interpretation of the results more difficult. It should be noted however, that this effect occurred at a concentration well below the level that has been measured following some spray operations (Trotter et al. 1990).

Acknowledgments. We thank G. Sheppard and J. Ryder for technical assistance. S. Winsor conducted the histopathological examinations. P. Pepin and D. Shaw gave helpful comments on an earlier version of the manuscript.

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Received September 30, 1991; accepted December 15, 1991.