ORIGINAL RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF BIOASSAYS
ISSN: 2278-778X
CODEN: IJBNHY
OPEN ACCESS

Bioassay Indication of Nemagon Toxicity on *Daphnia magna* (Straus 1820)

Moshe Gophen
MIGAL-Scientific Research Institute, POB 831, Kiryat Shmone-11016, Israel.

Received for publication: December 12, 2015; **Accepted**: December 27, 2015

Abstract: A Bioassay test of the toxic effect of Nemagon on the survival rate of *Daphnia magna* (Straus 1820) was carried out. Seven levels of dilution (10^{-2} - 10^{-9}) were tested in a 100 ml volume of filtered tap water, and the Daphnia conditions were observed and documented. Two trials were carried out: (1): Nemagon dilutions which were not incubated and (2): 24 hours of dilutions incubation prior to Daphnid's placing. Nemagon was less effective after incubation. A percentage of 50% and 100% mortalities were recorded. The high level of toxicity of Nemagon was confirmed.

Key words: Nemagon, Daphnia magna, Toxicity, Bioassay.

Introduction

Nemagon (D.B.C.P) was commonly used in Israeli agricultural crops, especially for Bananas, which is used for the removal of soil Nematodes. It was also used as soil fumigant. Nevertheless, it was found that in mammals, it results in male sterility at high levels of exposure. D.B.C.P causes a dramatic decrease in male fertility, ranging from Oligospermia (low sperm count) to Azoospermia (lack of sperm). In the late 1970`s after the awareness discovery of its deleterious health effects on humans, it was banned from use in Israel, although, not completely. In several countries in South America, Nemagon was commonly used by Banana growers and after severe scandals and court cases, it was banned, but not completely ((Singhal & Rai, 1982; Campos 2012; Silva 2007). The potential presence of the material in underground water or in the bottom sediments in lakes and reservoirs, might be a problem for years after stoppage of use. The active ingredient of the Nematicid (or Fumigant) known as Nemagon (Fumazone) is 1,2-Dibromo-3-chloropropane, (Dibromochloropropane) (D.B.C.P). In the U. S. A, until the year 1977, D.B.C.P was used as a soil fumigant and Nematocide on over 40 different crops. From the year 1977 to 1979, US-Environmental Protection Agency (E. P. A) suspended the registration of all D.B.C.P containing products, except for pineapples in Hawaii. In the year 1985, the use of existing stocks of D.B.C.P was prohibited by the E.P.A. Human exposure to D.B.C.P could result from the ingestion of contaminated drinking water and food products. Human exposure could also result from inhalation and/ or skin contact with the product.

In the past, the release of D.B.C.P to the environment occurred primarily from its fumigant and from the use of Nematocide. Due to the cancellation of all D.B.C.P uses, environmental exposure is expected to decline significantly with time. D.B.C.P residues have persisted in contaminated soil and groundwater, long after applications have ceased. For example in agricultural areas, around Turlock, in the Central Valley of California, D.B.C.P was applied to crops in the 1970s. As late as the year 1989, D.B.C.P persistence was reported in groundwater which was previously used for beneficial purposes, and numerous nearby wells had to be shut down at that time. Nemagon is also widely applied in the control of plant and sheep (*Haemonchus contoruts* Rudolphi, 1803; Cobb, 1898), as well as parasitic nematodes. *Haemonchus contortus*, also known as the barber's pole worm, is a very common parasite and it is one of the most pathogenic nematodes of ruminants (Singhal 1982). In Costa Rica, Law Act NO. 8130, "Social and Economic Benefits Compensation for the affected population "D.B.C.P" was signed in San Jose, 27.8. 2011 and it was formally enforced on 6.9.2011. Many years after the stoppage of Nemagon use in Nicaragua, the population of Chinandega still suffers misery (Agritoxicos Nicaragua). Traces of poisoned drinking waters were found in 15 wells. In the early 1970`s, Nemagon was applied as soil Nematocide by Banana growers in close vicinity, to the shore line of Lake Kinneret. Therefore, a bioassay test of Nemagon toxicity was carried out.

Material and Methods

The content of the commercial substance from the marketed containers was diluted by filtered (0.45 Milipore filtered paper) tap water as follows: 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} and 10^{-2}. Test organisms were 10 adults of *Daphnia magna* (Straus 1820) from batch culture [fed by Chlorella sp. (Beyerinck)] in each jar, and the trials were carried out in 100 ml jars for each dilution. Two trials were carried out: (1) 24 hours' exposure duration and Daphnids were placed immediately after being collected from the batch culture; (2) 57 hours' exposure duration, Daphnids were placed in filtered waters for 24 hours, prior to the toxic exposure. For each trial, a control jar with Daphnids in filtered water, without toxic substance was maintained. Time intervals in the 1st trial are as follow: 0 h, 0.5h, 2h, 3h,4 h, 22 h, and 29 hours; In the 2nd trial, time interval are as follow: 1h, 2h, 6h, 9h ,12h, 25h, 47h and 57 hours; no observations were made during night time. All trials were carried out under constant room temperature (22^0C -24^0C) with 24 hours diffused neon light. The physiological conditions of the Daphnids were classified as follows: A- Normal: free swimming in the entire water column; B- Floating: close or adhered to surface, fast legs beating; C- Moriband: Settled at the bottom and flutter; D- Settled Dead not moving at the bottom.

Results

A summary of observations is given in Table 1- First Trial and Table 2-Second Trial.

***Corresponding Author:**
Prof. Moshe Gophen
MIGAL-Scientific Research Institute,
POB 831, Kiryat Shmone-11016, Israel.

Table 1: A, B, C, D, Daphnid physiological conditions (see text); 1-10 number of Daphnids Per Respective Jar; 0-29 Time in hours; 10^{-2}-10^{-9}-Dilutions.

Time	Control	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
0	A10	A10	A10	A10	A10	A10	C6;D4	D10	D10
0.5	A10	A10	A10	A10	A10	A5;B5	C10	D10	D10
2	A8;C2	A9;D1	A7;B1;C2	A9;C1	A8;B2	A4;B1;C4	A1;C9	D10	D10
3	A9;D1	A6;B2;C2	A3;B1;C6	A4;B2;C4	A5;B5	A4;B4;C2	C5;D5	D10	D10
4	A9;D1	A7;B1;C2	A1;D9	A3;D7	A2;B7;D1	A1;B4;D5	D10	D10	D10
22	A9;D1	A1;D9	D10	D10	C2;D8	C2;D8	D10	D10	D10
29	A9;D1	D10	D10	D10	D10	C1;D9	D10	D10	D10

Table 2: A, B, C, D, Daphnid physiological conditions (see text); 1-10 number of Daphnids Per Respective Jar ;1-57 Time in hours; 10^{-4}-10^{-9}-Dilutions.

Time	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	Control
1	C10	AQ10	A8;B1;C1	A10	A10	A10	A10
2	C10	A3;B5;C2	A7;B3	A10	A10	A10	A10
6	C7;D3	A4;B2;C4	A6;B4	A10	A10	A10	A10
9	C4;D6	A1;B2;C7	A7;B1;C2	A8;B2	A10	A8;C2	A10
12	C4;D6	A4;B2;C4	A7;B3	A10	A10	A9;B1	A10
25	C5;D5	B3;C2;D5	A3;B5;C2	A10	A8;C2	A8;C2	A10
47	D10	A3;D7	A7;B2;D1	A5;C2;D3	A5;C4;D1	A10	A8;C2
57	D10	D10	A3;B1;C2;D4	A5;C3;D2	A2;C3;D5	A9;C1	A7;C3

Table 3: Time (hr) of 50% and 90%-100% mortality in the 1st trial

Dilution	Time (hr) of 50% mortality	Time (hr)of 90-100% mortality
Control	no mortality	no mortality
10^{-9}	4-22	22-29
10^{-8}	3-4	4-22
10^{-7}	3-4	4-22
10^{-6}	4-22	22-29
10^{-5}	3-4	22-29
10^{-4}	2-3	3-4
10^{-3}	no 50% mortality	no 100% mortality
10^{-2}	no 50% mortality	no 100% mortality

Table 4: Time (hr) of 50% and 90%-100% mortality in the 2nd trial.

Dilution	Time (hr) of 50% mortality	Time (hr) of 90 %-100% mortality
Control	no 50% mortality	no 100% mortality
10^{-9}	no 50% mortality	no 100% mortality
10^{-8}	47-57	no 100% mortality
10^{-7}	47	no 100% mortality
10^{-6}	47	no 100% mortality
10^{-5}	12-25	47
10^{-4}	6-9	47

Discussion

The human health deterioration by the Nematocid (or Fumigant) Nemagon was confirmed several years ago and therefore, it was deleted out of law. Nevertheless, in some cases, this toxic substance is still in use. The advantage and disadvantage of testing material toxicity by an indication of the survival of *Daphnia magn*a was highly discussed (Baird *et al.,* 1989; Xiu *et al.,* 1989; Coniglio and Baudo 1989; among others). The toxicological Bioassay test of Nemagon (D. C. B. P) on *Daphnia magna,* clearly indicate a high level of poisoning effect on animals. Presently, Daphnia magna is not commonly used for the Bioassays of toxicity due to the lower sensitivity of this zooplankter, in comparison with *Ceriodaphnia reticulata* (Jurine 1820) or Bacteria. I used *Daphnia magna* because of their availability and ease of maintenance and handling. I correlated the mortality of *Daphnia* and the toxicity of Nemagon as a significant related confirmation. Fifty percent mortality in less than 24 hours or 100% mortality at the dilution level of 10^{-9} indicates a high risk of zooplankton in freshwater bodies. The level of toxicity of Nemagon is reduced in water, as was confirmed in the 2nd trial. The Nemagon was "incubated" for 24 hours (diluted) in tap water and mortality level of the Daphnids declined.

Conclusions

Bioassay test of the toxic effect of Nemagon on Daphnia magna was carried out. The high level of toxicity was confirmed: 50-100% of the test organisms died within 6-47 hours with respect to dilution grade (10-2-10-9) of the commercial Nemagon staff in filtered tap water. Moreover, it was found that level of toxicity decline occurs when Nemagon was incubated 24 hours in filtered tap water. Beside known damage to mammals (including human being) these Bioassays tests clearly confirm and fully justification of the tight usage restriction on this Nematocide-Fumicide staff.

References

1. Baird, Donald. J. Barber, Ian. Bradley, Mairead Bradley, Peter. Calow and Amadeu M.V.M. Soars. The Daphnia Bioassay: a critique; in: Environmental Bioassay Techniques and their Application, Springer-Science and Busines Media B.V. (Munawar M., G. Niuxon, C.I. Mayfield, T. Reynoldson, & M.H. Sadar, eds), 1989, pp 403-406.

2. Campos, Marta Jimenes, The impact of Nemagon in the Working Population in Costa Rica, Xcaret 2 (Cancum Center) March 22, 2012.

3. Conglio, Lidia, and Renauto Baudo. Life–Tables of Daphnia obtusa (Kurz), Surviving Exposure to Toxic Concentration of Chromium. in: Environmental Bioassay Techniques and their Application, Springer-Science and Busines Media B.V. (Munawar M., G. Niuxon, C.I. Mayfield, T. Reynoldson, & M.H. Sadar, eds), 1989, pp 407-410.

4. Silva, Jose Adan. Nemagon is Still Alive and Kicking, Agrotoxicos Nicaragua, Nuevo Diario (Nicaragua) UITA-Secretaria Regional Latinoamericana-Montevideo-Uraguay. 2007; pp1-5.

5. Singhal, Krishan Chandra, and Pryamvada Ral; Role of Nemagon in the Control of Soil Transmitted Nematodes., Vol.14; (Vol.14; (3); 1982; pp 239-245.

6. Xiu Ruiquin, Yongxiang Xu, and Shirong Gao. Toxicity of the New Pyrethroid insecticide Deltamethrin to Daphnia mgna. in: Environmental Bioassay Techniques and their Application, Springer-Science and Busines Media B.V. (Munawar M., G. Dixon, C.I. Mayfield, T. Reynoldson, & M.H. Sadar, eds), 1989; pp 411-414.

Cite this article as:
Moshe Gophen. Bioassay Indication of Nemagon Toxicity on *Daphnia magna* (Straus 1820). *International Journal of Bioassays* 5.1 (2016): 4739-4741.

Source of support: Nil
Conflict of interest: None Declared