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Physiological Perturbations in Several Generations of *Daphnia magna* Straus Exposed to Diazinon

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Daphnia magna **was exposed to sublethal diazinon concentrations (0.05, 0.1, 0.5, 0.75, and 1.0 ng/L) for 21 days. The algae** ***Nannochloris oculata*** **(5×10^5 cells/mL) was used to feed the daphnids. Chronic toxicity tests were carried out using neonates of F_1 (first brood) and F_1 (third brood) offspring generations from parentals (F_0) preexposed to the organophosphate. The effect of diazinon on survival, reproduction, and growth was monitored for the selected daphnid generations. The parameters used to evaluate pesticide effect on reproduction were mean total young per female, mean brood size, time to first reproduction, mean number broods per female, and intrinsic rate of natural increase (*r*). Survival and growth (body length) were also determined after 21 days of exposure to the pesticide. Reproduction as well as survival was significantly reduced when diazinon concentration increased in the medium. This effect was greater in F_1 (first) and F_1 (third) offspring compared to the parental generation (F_0) daphnids. The intrinsic rate of natural increase (*r*) decreased with increasing concentrations of diazinon, especially in those animals from the first and third brood. However, the growth of the exposed organisms decreased in the same order of magnitude in all generations tested. The maximum acceptable toxicant concentration (MATC) was calculated for F_0, F_1 (first), and F_1 (third) generations of *D. magna* exposed to the pesticide using as parameter of evaluation the intrinsic rate of natural increase (*r*). The interpolation of these results gave MATC values of 0.62 and 0.07 ng/L pesticide for F_0–F_1 (first) generations and F_1 (third) generation, respectively. Therefore, F_1 (third) generation seems to be more sensitive to diazinon than generations F_0 and F_1 (first). The EC_{50} values have been derived for some selected parameters on the generations of *D. magna* exposed to diazinon. EC_{50} values decreased in F_1 (first) and F_1 (third) generations compared to the parental generation F_0. Expanding the reproduction tests to several generations revealed important information on chronic toxicity that could add to an increased cost-effectiveness in the protection of aquatic environments.**

Key Words: **multigeneration; toxicity; chronic; diazinon;** ***Daphnia magna*****.**

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INTRODUCTION

Adaptation of organisms to contaminated environments is an area of current concern in ecotoxicology. The occurrence of resistance in aquatic organisms at certain trophic levels could have fat-reaching implications for the equilibrium in the ecosystem (Bodar *et al*., 1990). There are several reasons why organisms exhibit resistance to pollutants such as pesticides. They may have acquired resistance by physiological acclimatization during exposure to sublethal concentration of that toxicant, or populations may have evolved a genetically based resistance (Bodar *et al*., 1990).

The evaluation of the effects of toxicants on aquatic organisms usually includes chronic toxicity tests as well as acute and sublethal studied (Day and Kaushik, 1987). Daphnids, especially *Daphnia magna*, have been used for many years in standard tests of toxicity (OECD, 1984) because of its high sensitivity, easy handling, and high reproduction rate (Münzinger and Monicelli, 1992). Generally, the criteria for assessment of toxicity with chronic studies are survival and total number of progeny produced.

Acute and chronic toxicity tests determine the toxicity of single chemicals and multiplication of the resulting data by an application factor to derive a safe concentration (Baillieul *et al*., 1993). Chronic toxicity tests give an estimate of the chemical concentrations that cause sublethal effects; however, these experiments start with neonates from previously unexposed mothers, thereby ignoring exposure during oogenesis and embryogenesis, and toxicant transfer from mothers to neonates. Therefore these tests do not really reflect the chronic effects of the toxicants. A more realistic figure of chronic toxicity could result from assays that also test the reproduction of a second generation (Van Leeuwen *et al*., 1985). Extending chronic toxicity tests to a second generation could therefore increase the cost-effectiveness of the assays.

Recently, several researchers have developed studies to evaluate the toxicity of metal compounds and effluents in several generations of an organism (Bodar *et al*., 1990;

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Münzinger, 1990; Münzinger and Monicelli, 1992; Bervoets *et al*., 1996) but no data are available that reflect the influence of pesticides during exposure over several generations of the investigated animals. In order to this, a cladocern (*D. magna*) was exposed to diazinon for several generations.

Diazinon is an organophosphate insecticide used extensively to control files, lice, nematodes, and soil insects in lawns and crop plants. It is used primarily for its broad effectiveness, short persistence, and relatively low mammalian toxicity.

The aim of the present study was to investigate whether the toxicity of diazinon toward *D. magna* increases during subsequent generations when generations are exposed to sublethal concentrations of this pesticide or whether the population achieves greater tolerance. Individual parameters, such as body length, survival, and brood size and population parameters such as the intrinsic rate of population growth (*r*) were monitored to detect chronic cumulative effects. The maximum acceptable toxicant concentration (MATC) for the three daphnid broods exposed to the organophosphate was also determined.

MATERIALS AND METHODS

Test Organisms

D. magna were obtained from continuous culture maintained in this laboratory in 6-L aquaria at $22 \pm 1°C$, in dechlorinated tap water (total hardness, 181.8 ± 18.8 mg/L as $CaCO_3$; pH 7.9 ± 0.2; alkalinity, 4.1 mmol/L), with a 12:12-h light:dark photoperiod and a density below 50 animals per liter.

The medium was renewed two times each week and the daphnids were fed daily with the algae *Nannochloris oculata*. This algae was also continuously cultivated in the laboratory using a nutrient medium (Bischoff and Bold, 1983).

Offspring were separated at regular intervals from cultures that were 3–5 weeks old and the test animals used were juveniles ≤ 24 h old.

Test Chemical

The diazinon used in the experiments was 96.1% pure as assayed by CEQUISA Company (Spain). Stock solutions were prepared by dissolving the toxicant in acetone immediately prior to each experiment.

Ferrando *et al*. (1992) determined that diazinon concentration in experimental conditions is almost 90% of the original concentration after 24 h. Based on that, test solutions were renewed every day, so the animals were transferred to clean beakers containing fresh medium and food every 24 h. Diazinon concentration in the experimental media was determined following the method of Zweigh and Sherma (1972). Pesticide was extracted with hexane. After the solvent was dried with sodium sulfate, it was evaporated to dryness on a rotary evaporator with the water bath at 45°C. The residue was dissolved in 5 ml of hexane and injected into the gas chromatograph. The gas chromatograph used was a Varian 6000.

Multigeneration Toxicity Tests

Preliminary acute toxicity tests were conducted in order to calculate diazinon EC_{50} data (Fernández *et al*., 1995). Based on these results, daphnids were exposed for 21 days to the following sublethal diazinon concentrations: 0 (control), 0.05, 0.1, 0.5, 0.75 and 1.0 ng/L, plus the acetone control (10^{-4} μl/L). *N. oculata* at a density of 5×10^5 cells/mL (Ferrando *et al*., 1995) was used as food. The experimental design is provided in Fig. 1.

In the parental generation (F_0) reproduction tests, 15 neonates (≤ 24 h old) per diazinon concentration were individually transferred from the culture to 60-mL glass beakers containing 50 mL of the test medium.

From the first brood of each treatment, 15 neonates (≤ 24 h old) were selected and individually transferred to 60-mL glass beakers to start the first generation (F_1—first brood) tests, in which they were exposed to the same sublethal diazinon concentrations as their parentals (F_0), plus the controls (acetone and blank controls).

To start another experiment, 15 neonates (≤ 24 h old) from the third brood of the parental generation (F_0) were collected from each exposure pesticide concentration and individually transferred to 60-mL beakers containing 50 mL of the same medium, plus the controls. Subsequently, these newborn daphnids (F_1—third brood) were exposed to the same pesticide concentrations as generations F_0 and F_1 (first).

The size (body length), fecundity, and survival (longevity) of the two *D. magna* generations (F_0 and F_1) studied were monitored for 21 days. Longevity (survival of the daphnids during the test), time to the first reproduction, total number of neonates per female, number of broods, and brood size were the criteria used. Neonates from the studied generations were counted daily and discarded.

Growth of the survival adults of each treatment was determined after 21 days of exposure. The length of each survival animal (cm) was measured from the apex of the helmet to the base of the tail spine (Zagatto, 1989).

The intrinsic rate of natural increase (*r*) was calculated using successive approximations of the formula of Lotka (1913): $\sum l_x m_x e^{-rx} = 1$, where l_x is the proportion of individuals surviving to age x, m_x is the age-specific fecundity (number of neonates produced per surviving female at age x), and x is expressed in days. As r calculated in *D. magna* organisms after 21 days is indistinguishable from r estimated for the entire life span, due to the great importance of early reproduction (Van Leeuwen *et al*., 1985), all calculations were based on 21-day experiments.

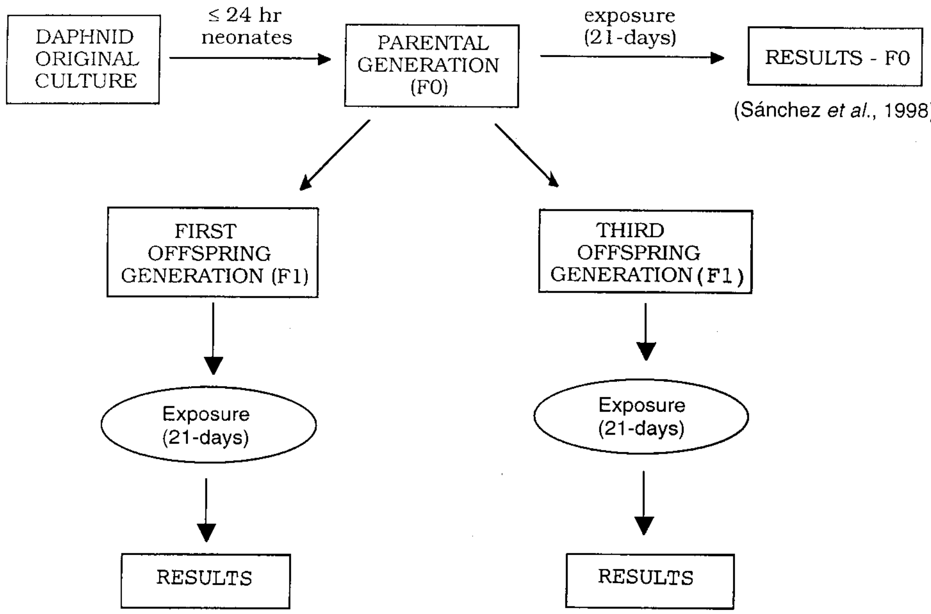

FIG. 1. Experimental design of the multigeneration toxicity test.

Also estimated was the maximum acceptable toxicant concentration (MATC) for *r*. The MATC is defined (Stephan *et al*., 1985) as the estimated toxic threshold concentration falling between the highest concentration revealing no effect (NOEC) and the next highest concentration indicating a toxic effect (LOEC) when compared to the controls.

A useful, quantitative parameter, analogous to the LC_{50} derived in acute toxicity tests, is the EC_{50}, the median effective concentration of the toxicant at which the value of a given parameter is reduced to 50% of that in controls. The EC_{50} values were derived for the parameters studied (longevity, number of young per female, number of broods per female, brood size, and intrinsic rate of natural increase) on *D. magna* after exposure to the pesticide tested using regression equations.

Statistical Analysis

Data from the three generations of *D. magna* studied were analyzed using analysis of variance (ANOVA) to detect significant differences between treated groups and control values, followed by Duncan test ($P \leq 0.05$) with an SPSS computer program (Nie and Hull, 1981).

RESULTS

The diazinon 24-h EC_{50} value for *D. magna* in experimental conditions was 0.86 μg/L (Fernández *et al*., 1995). The effect of sublethal diazinon concentrations on the survival and reproduction of the *D. magna* generations studied is presented in Tables 1–3. As can be seen in those tables, all parameters studied in the chronic experiments were affected by the sublethal levels of the pesticide to which the offspring generations were exposed.

Survival (longevity) of *D. magna* parental generation (F_0) decreased significantly ($P < 0.05$) with increasing concentration of diazinon, particularly above 0.50 ng/L (Table 1). At 1.0 ng/L pesticide, all daphnids died before the potential age of reproduction had been reached (Days 7–8). Exposure to diazinon concentrations as low as 0.75 ng/L was lethal to daphnids from F_1 (first brood) generation, which survived only 2.5 days (Table 2). Daphnids from F_1 (third brood) generation survived only 6.5 days of exposure to 0.5 ng/L diazinon (Table 3). Mortality in the acetone and blank controls of generations F_0, F_1 (first), and F_1 (third) never exceeded 10% at the end of the experiment. These results suggest that first and third brood daphnids were more sensitive to the effects of diazinon exposure than was the parental generation.

The effects of diazinon on the fecundity of the parental generation (F_0) of daphnids after 21 days of exposure are summarized in Table 1. Reproduction (number of young per female, brood size, and number of broods per female) was significantly reduced at pesticide concentrations higher than 0.05 ng/L; however, the onset of reproduction was not clearly affected. Number of neonates born declined from 132 young (control) to 7.0 young at 0.75 ng/L. The results of the reproduction tests with the first generation of daphnids (F_1—first) are presented in Table 2. Reproductive

TABLE 1
Size, Survival, and Fecundity of Parental Generation (F_0) of *D. magna* Exposed to Several Concentrations of Diazinon in a 21-Day Life Study[a]

Diazinon (ng L^{-1})	Length (cm)	Longevity (days)	Days to first brood	No. young per female	Brood size	No. broods per female
Blank control	0.49 ± 0.01	21.0 ± 0	7.8 ± 0.1	131.7 ± 15.1	25.9 ± 2.5	5.1 ± 0.1
Acetone control	0.51 ± 0.01	18.7 ± 2.1	8.2 ± 0.3	136.8 ± 21.0	30.2 ± 1.7	4.4 ± 0.5
0.05	0.47 ± 0.008*	16.8 ± 3.6*	8.2 ± 0.2	104.4 ± 22.5*	22.3 ± 2.0	4.6 ± 0.8
0.1	—	15.6 ± 0.6*	8.0 ± 0.5	66.6 ± 14.2*	19.3 ± 3.0*	3.4 ± 0.2*
0.5	0.45 ± 0.02*	13.3 ± 2.4*	8.8 ± 0.7*	50.5 ± 14.1*	15.2 ± 3.9*	2.4 ± 0.4*
0.75	—	8.9 ± 2.5*	8.2 ± 0.2	7.0 ± 2.3*	5.8 ± 3.0*	0.8 ± 0.1*
1.0	—	7.1 ± 0.7*	—	0*	0*	0*

Note. Values are means ± SD.
[a] Sánchez *et al*. (1998).
* $P < 0.05$.

parameters such as number of young per female, brood size, and number of broods per female decreased as the concentrations of diazinon increased in the medium. Significant differences ($P \leq 0.05$) between treated and control groups were found at diazinon concentrations of 0.05 ng/L and higher. Number of neonates per female decreased significantly from 134 young (control) to 86 young (0.05 ng/L), and the number of broods per female declined from 5 broods (control) to 3.9 broods (0.05 ng/L). No reproduction was detected when animals were exposed to 0.75 ng/L. In the third brood (F_1—third) (Table 3) significantly lower reproductive output compared to the control was observed, especially when animals were exposed to the highest concentrations tested (0.5 and 0.75 ng/L). The number of neonates per female was reduced to 10 young at 0.5 ng/L and the number of broods to 0.9 brood at the same diazinon concentration. In addition, comparison of the F_1 (first) and F_1 (third) broods with the parental generation (F_0) revealed a decrease in reproduction for all diazinon concentrations used. No data were available for F_1 (first) and F_1 (third) broods exposed to 1.0 ng/L diazinon, because their parents (F_0) exposed to that concentration did not reproduce.

In the present study, the intrinsic rate of natural increase (r) is found to be a sensitive parameters of toxicity due to the effect of diazinon on reproduction and survival. Diazinon concentrations > 0.75 ng/L significantly reduced r values of F_0 and F_1 (first) generations from 0.32 and 0.31 (F_0 and F_1—first controls, respectively) to 0.23 and 0 after exposure to 0.75 ng/L, respectively (Figs. 2 and 3). However, pesticide concentrations > 0.1 ng/L significantly ($P < 0.05$) reduced r values in F_1 (third brood) from 0.31 (controls) to 0.20 (0.1 ng/L) (Fig. 4).

A significant reduction ($P \leq 0.05$) in mean carapace length of 21-day-old daphnids from F_0, F_1 (first), and F_1 (third) generations was detected as diazinon concentration

TABLE 2
Size, Survival, and Fecundity of F_1 (First Brood) Generation of *D. magna* Exposed to Several Concentrations of Diazinon in a 21-Day Life Study

Diazinon (ng L^{-1})	Length (cm)	Longevity (days)	Days to first brood	No. young per female	Brood size	No. broods per female
Blank control	0.48 ± 0.004	21.0 ± 0	8.4 ± 0.4	134.5 ± 8.4	26.9 ± 1.7	5.0 ± 0
Acetone control	0.49 ± 0.006	20.1 ± 1.7	7.6 ± 0.2	161.9 ± 27.2	32.4 ± 5.4	4.7 ± 0.7
0.05	0.46 ± 0.01*	17.8 ± 1.8*	8.5 ± 0.3	85.9 ± 33.8*	20.7 ± 4.8	3.9 ± 0.6*
0.1	0.41 ± 0.01*	13.5 ± 1.7*	8.7 ± 0.4	33.4 ± 12.5*	10.3 ± 4.7*	2.2 ± 0.6*
0.5	—	10.7 ± 2.4*	8.1 ± 0.8	28.5 ± 19.4*	10.2 ± 7.2*	1.6 ± 0.8*
0.75	—	2.5 ± 0.8*	—	0*	0*	0*
1.0	—	—	—	—	—	—

Note. Values are means ± SD.
* $P < 0.05$.

TABLE 3
Size, Survival, and Fecundity of F_1 (Third Brood) Generation of *D. magna* Exposed to Several Concentrations of Diazinon in a 21-Day Life Study

Diazinon ($ng\,L^{-1}$)	Length (cm)	Longevity (days)	Days to first brood	No. young per female	Brood size	No. broods per female
Blank control	0.48 ± 0.007	20.7 ± 0.5	8.1 ± 0.1	114.7 ± 4.8	23.2 ± 0.5	4.9 ± 0.1
Acetone control	0.49 ± 0.008*	19.7 ± 2.5	7.6 ± 0.5	157.3 ± 18.6*	31.4 ± 3.7*	4.7 ± 0.7
0.05	0.46 ± 0.003*	19.5 ± 1.4	8.3 ± 0.3	115.3 ± 15.8	24.8 ± 1.5	4.5 ± 0.4
0.1	0.40 ± 0.005*	16.1 ± 2.1*	11.2 ± 3.2*	31.6 ± 18.0*	9.8 ± 4.2*	2.1 ± 0.9*
0.5	—	6.5 ± 3.1*	8.2 ± 0.4*	10.2 ± 6.8*	4.5 ± 2.5*	0.9 ± 0.6*
0.75	—	—	—	—	—	—
1.0	—	—	—	—	—	—

Note. Values are means ± SD.
* $P < 0.05$.

increased from 0.05 ng/L diazinon to 0.5 ng/L (Tables 1–3). Daphnids exposed to 0.75 and 1.0 ng/L diazinon (F_0 generation) and to 0.5, 0.75, and 1.0 ng/L (F_1—first and F_1—third broods) did not survive 21 days, so the lengths of those animals could not be measured.

Acetone controls did not differ significantly ($P > 0.05$) in any of the studied parameters of *D. magna* F_0 and F_1 (first) generations (Table 1 and 2, Figs. 2 and 3). However, those animals from F_1 (third brood) exposed to the solvent (acetone controls) indicated an increase in the number of young per female and in the brood size (Table 3), which means better reproduction in those conditions because of the presence of acetone in the medium.

Data used to estimate the chronic value (MATC) for diazinon were based on the r values (Figs. 2–4). Interpolation of the data indicates that MATC lies between 0.5 (NOEC) and 0.75 (LOEC) ng/L diazinon for F_0 and F_1 (first) generations, and between 0.05 (NOEC) and 0.1 (LOEC) ng/L for F_1 (third) generation. The interpolation of these results gave MATC values of 0.62 and 0.07 ng/L pesticide for F_0–F_1 (first) generations and F_1 (third) generation, respectively. Therefore, F_1 (third) generation daphnids seems to be more sensitive to diazinon than generations F_0 and F_1 (first).

Another useful parameter is the EC_{50}. The EC_{50} values were derived for some selected parameters on the generations of *D. magna* exposed to diazinon using the regression equations given in Tables 4–6. As can be seen in those tables, EC_{50} values decreased in F_1 (first and third broods) generation compared to the parental generation F_0. These results mean that less toxicant would be necessary to reduce the selected parameters (survival and reproduction) to 50% in the first and third broods of *D. magna*. Furthermore, it can also be assumed that the sensitivity was very similar for F_1 first and third broods because EC_{50} values were similar. The results also indicated that reproductive parameters (number of young per female, brood size, and number of broods per female) were more affected by the toxicant than

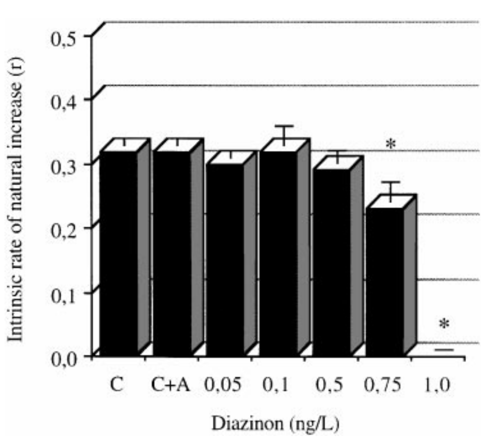

FIG. 2. The effect of diazinon on the intrinsic rate of natural increase (r) in F_0 generation of *D. magna*. *$P \leq 0.05$.

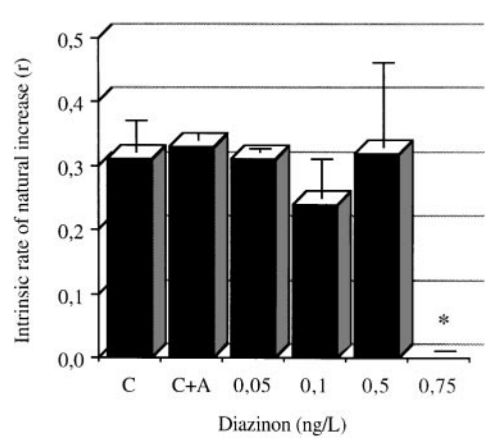

FIG. 3. The effect of diazinon on the intrinsic rate of natural increase (r) in F_1 (first brood) generation of *D. magna*. *$P \leq 0.05$.

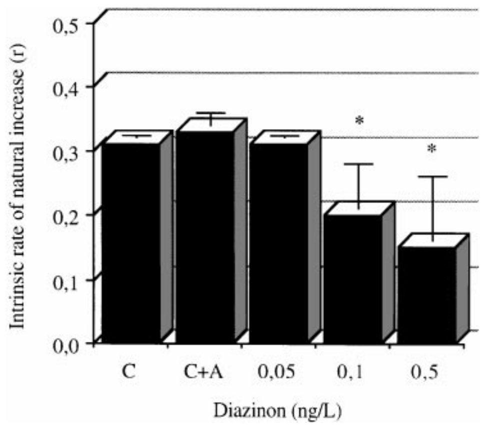

FIG. 4. The effect of diazinon on the intrinsic rate of natural increase (r) in F_1 (third brood) generation of *D. magna*. *$P \leq 0.05$.

TABLE 5
Regression Equations and Correlation Coefficients (r^2) Describing the Relation between Diazinon Concentration (ng/L) (x) and the Values of the Different Studied Parameters (y) of *D. magna* F_1 (First Brood) Generation (F_0)

Parameter	Regression equations	Correlation coefficients (r^2)	EC_{50} (ng L^{-1})
Longevity	$y = 18.84 - 20.49x$	0.95	0.41
No. young per female	$y = 93.42 - 132.0x$	0.81	0.20
Brood size	$y = 21.31 - 27.48x$	0.87	0.29
No. broods per female	$y = 4.05 - 5.41x$	0.91	0.29
Intrinsic rate of natural increase	$y = 0.31 - 0.35x$	0.90	0.44

Note. EC_{50} is the effective concentration of diazinon at which the parameters observed are reduced to 50% of that in controls.

longevity for the generations studied, and therefore their EC_{50} values were lower. EC_{50} values for the intrinsic rate of natural increase (r) were greater in all cases (F_0, F_1—first, F_1—third) because of the influence of survival in the value of that parameter.

DISCUSSION

Several researchers (Daniels and Allan, 1981; Ingersoll and Winner, 1982) have suggested that survival in chronic toxicity tests is the best index of toxicity because it is less variable than reproductive parameters. Day and Kaushik (1997), however, suggested that survival is only a good indicator when exposure to toxicants continues through the entire life cycle of the organism. Other investigators have reported that reproduction was a more sensitive index of chronic pesticide toxicity to *D. magna* than survival (Buhl *et al*., 1993).

Bervoets *et al.* (1993) observed that the second daphnid generation exposed to an industrial effluent reduced reproduction at a dilution of 0.5% of the effluent. This effect was greater in the second generation than in the parental daphnids. Additionally, Baldwin *et al.* (1995) also observed that the second generation *D. magna* exposed to 50 mg/L of the estrogen diethylstibestrol demonstrated in a reduction in fecundity, with cumulative offspring per female lower than in the first and the parental generation daphinds.

However, *D. magna* preexposed to sublethal cadmium concentrations become more resistant to the toxic effects of cadmium (Bodar *et al*., 1990). The degree to which preexposed daphnids developed resistance to cadmium remained similar for three sucessive generations.

TABLE 4
Regression Equations and Correlation Coefficients (r^2) Describing the Relation between Diazinon Concentration (ng/L) (x) and the Values of the Different Studied Parameters (y) of *D. magna* Parental Generation (F_0)

Parameter	Regression equations	Correlation coefficients (r^2)	EC_{50} (ng L^{-1})
Longevity	$y = 18.52 - 11.85x$	0.95	0.67
No. young per female	$y = 107 - 117.6x$	0.93	0.35
Brood size	$y = 24.18 - 23.59x$	0.98	0.47
No. broods per female	$y = 4.63 - 4.79x$	0.98	0.43
Intrinsic rate of natural increase	$y = 0.34 - 0.25x$	0.85	0.72

Note. EC_{50} is the effective concentration of diazinon at which the parameters observed are reduced to 50% of that in controls.

TABLE 6
Regression Equations and Correlation Coefficients (r^2) Describing the Relation between Diazinon Concentration (ng/L) (x) and the Values of the Different Studied Parameters (y) of *D. magna* F_1 (Third Brood) Generation

Parameter	Regression equations	Correlation coefficients (r^2)	EC_{50} (ng L^{-1})
Longevity	$y = 20.23 - 27.86x$	0.99	0.35
No. young per female	$y = 99.17 - 192.14x$	0.80	0.22
Brood size	$y = 21.43 - 36.04x$	0.82	0.27
No. broods per female	$y = 4.27 - 7.20x$	0.86	0.25
Intrinsic rate of natural increase	$y = 0.29 - 0.30x$	0.85	0.47

Note. EC_{50} is the effective concentration of diazinon at which the parameters observed are reduced to 50% of that in controls.

A reduction in the intrinsic rate of natural increase (*r*) resulted as a consequence of chronic toxicant stress of pesticides on *D. magna* (Fernandez *et al*., 1995; Ferrando *et al*., 1995). Only toxicants that cause a decrease in the number and/or size of the first few broods of daphnids will cause *r* to decrease significantly. However, no data are available in the literature that reflect the effect of a pesticide on the *r* values of several generations of *D. magna*. Münzinger (1990) studied the effect of nickel on seven generations of *D. magna* and he found an adaptation toward nickel as the intrinsic rate of population growth (*r*) increased in the exposed generations.

The current study found that there was a positive relationship between adult body size (21 days) and reproduction; both parameters were reduced in the three studied generations as diazinon concentration increased. Hanazato (1998) also detected a strong positive relationship between maternal body size and clutch size with *D. magna* was exposed to different concentrations of the insecticide carbaryl.

Typically, endpoints pertaining to individual attributes such as survival, number of young produced, and growth are used to determine the MATC of a toxicant, but several researchers have advocated the use of *r* to estimate chronic toxic effect at the population level (Day and Kaushik, 1987; Ferrando *et al*., 1993, 1995).

Van Leeuwen *et al*. (1985) demonstrated that the response of daphnids exposed to a toxicant from the earliest stages of development gives a better estimate of chronic toxicity than the response of the offspring of previously unexposed parents. Studies have found that the ability of neonatal organisms to metabolize xenobiotics can be elevated when organisms are exposed to chemicals prenatally (Baldwin *et al*., 1995). In the current study, effects on daphnid reproduction of F_1 (first) generation were found even at a diazinon concentration of 0.05 ng/L.

CONCLUSIONS

The results indicate that after one generation, the daphnids from the first and third broods seem not to be adapted to the pesticide diazinon and they demonstrated more mortality and less reproduction than the parentals. It could be possible that the parental generation (F_0) exposed to sublethal concentrations of diazinon accumulated the pesticide in their bodies and then the toxicant was transferred from the mothers to their progeny (first and third broods). Then pesticide effects would be greater in the first and third broods when diazinon was again present in the medium. It would be interesting to investigate the population dynamics of these affected progeny in a medium free of toxicant in order to test their survival and reproduction during a recovery period.

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