World Journal of Fish and Marine Sciences 4 (5): 512-516, 2012
ISSN 2078-4589

DOI: 10.5829/idosi.wjfms.2012.04.05.64100

Mortality Response of *Xiphophorus maculatus* (Cyprinodontiformes: Poeciliidae) to Some Agricultural Pesticides

Reza Tarkhani and Mohammad Reza Imanpoor

Department of Fisheries, Faculty of Fisheries and Environment,
Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Abstract: This research was performed to determine and compare acute toxicity of diazinon and Deltamethrin as potential dangerous organic pesticides to assess mortality effects of these chemicals to the freshwater Platy (*Xiphophorus maculatus*). LC_{50} of 24 h, 48 h, 72 h and 96 h was attained by probit analysis by Finney's and using the maximum-likelihood procedure (SPSS). The 24-96h LC_{50} for the Diazinon were 35.89 ± 0.01, 24.99 ± 0.01, 16.27 ± 0.02 and 10.49 ± 0.46 ppm respectively. The 24-96h LC_{50} for the Deltamethrin were 0.26 ± 0.01, 0.22 ± 0.01, 0.15 ± 0.01 and 0.15 ± 0.01 ppm respectively. In the present study, LC_{50} values indicated that deltamethrin is more toxic than diazinon to this species. LC_{50} obtained in the present study compare with corresponding values that have been published in the literature for other species of fishes, show different LC_{50}.

Key words: LC_{50} % Diazinon % Deltamethrin % Toxicity % Platy

INTRODUCTION

During the last decades, significant amounts of pesticides belonging to the classes of organophosphates have been released into the environment. The organophosphorus pesticides were developed by chemical manipulation of nerve gases and further modifications have resulted in chemicals with greater species selectivity [1]. Chemical pesticides with persistent molecules (long half-life periods) pose a threat to fish and also to the human population consuming the affected fish. Organophosphorus pesticides (OPs) are largely used in agriculture and the aquatic environment near to fields is under influence of OPs such as diazinon [O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimiinyl) phosphorothioate [2] and deltamethrin [3]. In this context, more and more researchers assess the environmental risk of these and other pollutants with the help of indices of toxicity [4-13]. In the search for effective pesticides risk management tools, many risk assessment methods have been adopted over time. Organophosphate compounds are useful as pesticides due to their ability to inhibit acetylcholinesterase, an enzyme responsible for inactivating the neurotransmitter acetylcholine [14-15].

Diazinon is a contact organophosphate pesticide and extensively used, both in agriculture and households to control insects in soil, plants, fruit and vegetable crops. Agricultural spray contains 85-90% diazinon. After its application on crops and plants, diazinon is easily washed into surface waters and enters the ground water. Eventually, it enters the aquatic environment in large quantities [16]. Diazinon degrades rapidly, but under conditions of low temperature, low moisture, high alkalinity and lack of suitable microbiological degraders, it may remain biologically active in soils for six months or longer, Because of its aquatic distribution, diazinon affects a wide range of non-target organisms, like invertebrates, mammals, birds and fishes, especially those inhabiting aquatic environment [17-18]. The pyrethroids including deltamethrin are widely used as pediculicides and are among the most potent insecticides known [19]. Pyrethroids have been proved to be extremely toxic to fish and some aquatic arthropods, for example shrimp [20]. The toxicity of Pyrethroids on mammals, birds and amphibians have been reviewed by Bradbury and Coats [21].

In fishes, exposure to diazinon in sublethal doses is known to affect the nervous system by inhibition of acetylcholinesterase activity [22]. Sublethal doses may lead to reduced growth and reproduction in aquatic invertebrates [17]. Acute toxicity tests of adult fish using diazinon have shown that 96 h sublethal values vary by several orders of magnitude between species [23-24].

Corresponding Author: Reza Tarkhani, Department of Fisheries, Faculty of Fisheries and Environment, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

The present study was performed to determine acute toxicity of diazinon on the platy (*Xiphophorus maculatus*). Platies are from common freshwater fishes which are capable of tolerating a wide range of fluctuations in water quality and are good model fish for ecotoxicological studies. The *X. maculatus* was selected for the bioassay experiments because of it is widespread and presently cultured all over Asia, in most parts of Europe and on a small scale in some countries of Africa and Latin America.

MATERIALS AND METHODS

The selected fish species for the present study was platy fish (*Xiphophorus maculatus*). Test chambers were glass aquaria of 120 liter. Lethal experiments were conducted using 125 young platy fish. The mean values for test water qualities were as follows: temperature 27±1°C, pH 7.1±0.2, dissolved oxygen 7.4±0.1 mgL^{-1} and total hardness 125.1±2.2 mgL^{-1}. Fish were feed twice daily with formulated feed and dead fish were immediately removed to avoid possible water quality deterioration [25].

Nominal concentrations of active ingredient tested were 0, 5, 15, 30 and 50 for diazinon and 0, 0.03, 0.04, 0.06, 0.10, 0.20, 0.30 and 0.40 ppm for Deltamethrin, following preparation from a stock solution. The stock solutions were renewed every 12 h. The control group received acetone at the maximum acetone volume used in the dilution of the dosing concentrations. Groups of 21 Angel fish were exposed for 96h in aerated glass aquaria with 120 liter of test medium. No food was provided to the specimens during the assay and test media was renewed daily [25]. Mortality rates were recorded at time 0, 24, 48, 72 and 96 h. Acute toxicity tests was carried out according to Hotos and Vlahos [26].

The nominal concentration of diazinon and Deltamethrin estimated to result in 50% mortality of Angle fish within 24 h (24-h LC_{50}), 48 h, 72 h and 96 h was attained by probit analysis by Finney's [27] method and using the maximum-likelihood procedure (SPSS 18, SPSS Inc. Chicago, Illinois, USA). The LC_{50} value is obtained by fitting a regression equation arithmically and also by graphical interpolation by taking logarithms of the diazinon and deltamethrin concentrations versus probit value of percentage mortality. The 95% confidence limits for LC_{50} are estimated by using the formula LC_{50} (95% CL) = LC_{50} ± 1.96 [SE (LC_{50})]. The SE of LC_{50} is calculated from the formula: $SE(LC50) = 1/b\sqrt{pnw}$ Where: b = the slope of the chemical / probit response (regression) line; p = the number of chemical used, n = the number of animals in each group, w = the average weight of the observations (Hotos and Vlahos, 1998). After the acute toxicity test, the LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) were determined for each measured endpoint.

RESULTS

No fish died during the acclimation period before exposure and no control fish died during acute toxicity tests. The mortality of *X. maculatus* were 5, 10, 20, 30 and 50 ppm for Diazinon and, 0.03, 0.04, 0.06, 0.10, 0.20, 0.30 and 0.40 ppm for Deltamethrin were examined during the exposure times at 24, 48, 72 and 96 h (Table 1and2).

Median lethal concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% test presented in Table 3 and 4. Because mortality (or survival) data are collected for each exposure concentration in a toxicity test at various exposure durations (24, 48, 72, or 96 hours), data can be plotted in other ways; the straight line of best fit is then drawn through the points. These are time–mortality lines. The LT_{50} (median lethal survival time) can be estimated for each concentration.

Toxicity Testing Statistical Endpoints are in tow part: 1- Hypothesis Testing: is there a statistically significant difference between the mean response in the treatments and mean response in control or reference sample? LOEC: Lowest Observed Effect Concentration; NOEC: No Observed Effect Concentration. 2- Point Estimates: what toxicant concentration will cause a specific effect on the test population? LC_{50}: the median Lethal Concentration. Our result for Toxicity Testing Statistical Endpoints is in Figs. 1 and 2.

Table 1: Cumulative mortality of Platy Fish (21 fish for each concentration) exposed to acute commercial diazinon

Concentration (ppm)	No. of mortality 24h	48h	72h	96h
0.00	0	0	0	0
5	0	0	0	0
10	0	2	6	8
20	5	8	15	21
30	7	13	20	21
50	17	21	21	21

Significantly increased mortality of *X. maculatus* was observed with increasing concentrations from 10 ppm to higher concentrations.

For Diazinon there were 100% mortality at 20 and higher concentrations within the 96h after exposure and 100% mortality at 30 ppm within the 96 h whereas 100% mortality for 50 ppm was 48h.

Table 2: Cumulative mortality of Platy Fish (21 fish for each concentration) exposed to acute commercial Deltamethrin

Concentration (ppm)	No. of mortality 24h	48h	72h	96h
0.00	0	0	0	0
0.03	0	0	0	0
0.04	0	0	0	0
0.06	0	0	0	0
0.10	1	3	6	8
0.20	3	7	11	14
0.30	16	19	20	21
0.40	19	20	21	21

Significantly increased mortality of *X. maculatus* was observed with increasing concentrations from 0.10 ppm to higher concentrations.
For Deltamethrin there were 100% mortality at 0.10 and higher concentrations within the 96h after exposure and 100% mortality at 0.30 ppm within the 96 h whereas 100% mortality for 0.40 ppm was 72h.

Table 3: Lethal Concentrations (LC_{1-99}) of Diazinon (mean ± Standard Error) depending on time (24-96h) for Platy fish

Point	Concentration (ppm) (95 % of confidence limits) 24h	48h	72h	96h
LC_1	2.77 ± 0.01	1.62 ± 0.01	-	6.43 ± 0.46
LC_{10}	17.64 ± 0.01	12.12 ± 0.01	7.27 ± 0.02	8.25 ± 0.46
LC_{20}	23.91 ± 0.01	16.54 ± 0.01	10.36 ± 0.02	9.02 ± 0.46
LC_{30}	28.42 ± 0.01	19.72 ± 0.01	12.59 ± 0.02	9.57 ± 0.46
LC_{40}	32.28 ± 0.01	22.45 ± 0.01	14.49 ± 0.02	10.05 ± 0.46
LC_{50}	35.89 ± 0.01	24.99 ± 0.01	16.27 ± 0.02	10.49 ± 0.46
LC_{60}	39.50 ± 0.01	27.54 ± 0.01	18.05 ± 0.02	10.93 ± 0.46
LC_{70}	43.36 ± 0.01	30.26 ± 0.01	19.96 ± 0.02	11.40 ± 0.46
LC_{80}	47.87 ± 0.01	33.45 ± 0.01	22.19 ± 0.02	11.96 ± 0.46
LC_{90}	54.14 ± 0.01	37.87 ± 0.01	25.28 ± 0.02	12.73 ± 0.46
LC_{99}	69.01 ± 0.01	48.36 ± 0.01	32.62 ± 0.02	14.55 ± 0.46

Table 4: Lethal Concentrations (LC_{1-99}) of Deltamethrin (mean ± Standard Error) depending on time (24-96h) for Platy fish

Point	Concentration (ppm) (95 % of confidence limits) 24h	48h	72h	96h
LC_1	0.07 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
LC_{10}	0.16 ± 0.01	0.11 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
LC_{20}	0.19 ± 0.01	0.15 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
LC_{30}	0.22 ± 0.01	0.18 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
LC_{40}	0.24 ± 0.01	0.20 ± 0.01	0.14 ± 0.01	0.14 ± 0.01
LC_{50}	0.26 ± 0.01	0.22 ± 0.01	0.15 ± 0.01	0.15 ± 0.01
LC_{60}	0.28 ± 0.01	0.24 ± 0.01	0.17 ± 0.01	0.17 ± 0.01
LC_{70}	0.31 ± 0.01	0.26 ± 0.01	0.18 ± 0.01	0.18 ± 0.01
LC_{80}	0.33 ± 0.01	0.29 ± 0.01	0.20 ± 0.01	0.20 ± 0.01
LC_{90}	0.37 ± 0.01	0.32 ± 0.01	0.23 ± 0.01	0.23 ± 0.01
LC_{99}	0.46 ± 0.01	0.41 ± 0.01	0.29 ± 0.01	0.29 ± 0.01

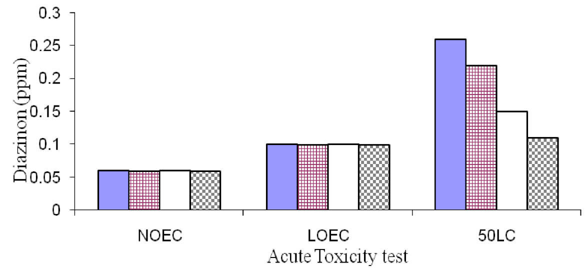

Fig. 1: Acute toxicity testing statistical endpoints in Platy fish exposed to crude Diazinon in different times (24h, 48h, 72 h and 96 h respectively)

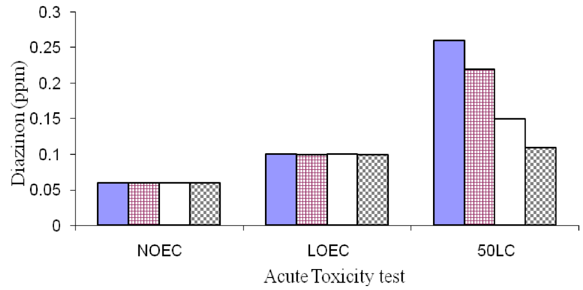

Fig. 2: Acute toxicity testing statistical endpoints in Platy fish exposed to crude Deltamethrin in different times (24h, 48h, 72 h and 96 h respectively)

DISCUSSION

The results of present study indicate that both chemicals diazinon and deltamethrin varied in their acute toxicity to Platy fish. The toxicity of deltamethrin and diazinon on Platy fish increased with increasing concentration and exposure time. The 96h LC_{50} was calculated to be 10.49 ± 0.46 ppm for diazinon and 0.15 ± 0.01 ppm for Deltamethrin and here we report deltamethrin to be highly toxic to fish. In addition we found that both Diazinon and Deltamethrin are lethal substrates to *X. maculatus*.

Contamination of aquatic environment with pesticides via rainfall runoff is very possible [28]. Serious concerns remains due to their potential to cause adverse effects on human and wildlife populations. Occurrence of pesticides in high concentrations in agricultural wastewaters and their toxicity to aquatic organisms especially fish species have been reported by many researchers [29-31].

Previous studies indicate the high toxicity of deltamethrin to fish species and our results are in good agreement with these reports. Value of diazinon 96h LC_{50} was 8 mg LG^1 for zebra fish (*Brachydanio rerio*) but for guppy (*Poecilia reticulata*) was 0.8 mgLG^1 [32-33].

The 96h LC_{50} values of diazinon on different fishes reported from tenths to several tens of mgL^{-1} [32, 34].

Boateng *et al*. [35] reported that young fish are more susceptible and different species respond unlike to concentrations of chemicals. LC_{50} value of deltamethrin in Tilapia, *Oreochromis niloticus* as 15.47 mg L^{-1} was reported by Boateng *et al*. [35]. Mittal *et al*. [36] estimated deltamethrin toxicity to *P. reticulate* to be LC_{50} = 0.016 ppm. Viran *et al*. [19] report LC_{50} value of deltamethrin in guppies as 5.13 mg L^{-1}. Mestres and Mestres [37] found 96-h fish LC_{50} values as follows: *Salmo gairdneri*, 0.39 mg L^{-1}; *Cyprinus carpio*, 1.84 mg L^{-1}; and *Sarotherodon mossambica*, 3.50 mgL^{-1}. Although deltamethrin is thought to be less toxic in field conditions due to its adsorption to sediments, these data are useful to assessment of potential ecosystem risks.

ACKNOWLEDGMENTS

The authors thank the Aquaculture Research Center and Fishery group for the supply of research material. This work was supported by the Gorgan University of Agricultural Sciences and Natural Resources.

REFRENCES

1. Rahmi Aydın and Kenan Kööprucu, 2005. Acute toxicity of diazinon on the common carp (*Cyprinus carpio*) embryos and larvae. Pesticide Biochemistry and Physiology. 82: 220-225.
2. Tinoco-Ojanguren, R. and D.C. Halperin, 1998. Poverty, production and health: inhibition of erythrocyte cholinesterase via occupational exposure to organophosphate insecticides in Chiapas, Mexico. Arch. Environ. Health, 53: 29-35.
3. Smith, T.M. and G.W. Stratton, 1986. Effects of synthetic pyrethroid insecticides on nontarget organisms. Res. Rev., 97: 93-119.
4. Farre, M., M.J. Garcia, L. Tirapu, A. Ginebreda and D. Barcelo, 2001. Wastewater toxicity screening of non-ionic surfactants by Toxalert (R) and Microtox (R) bioluminescence inhibition assays. Anal. Chim. Acta, 427: 181-189.
5. Farre, M., E. Martinez and D. Barcelo, 2007. Validation of inter laboratory studies on toxicity in water samples. Tr. AC-Trends Anal. Chem., 26: 283-292.
6. Travisi, C.M., P. Nijkamp, M. Vighi and P. Giacomelli, 2004. Managing pesticide risks for non-target ecosystems with pesticide risk indicators: a Multicriteria Approach. Tinbergen Institute, Amsterdam, TI 2004-009/3.
7. Liess, M. and P.C. Von Der Ohe, 2005. Analyzing effects of pesticides on invertebrate communities in streams. Environ. Toxicol. Chem., 24: 954-965.
8. Tremolada, P., A. Finizio, S. Villa, C. Gaggi and M. Vighi, 2004. Quantitative interspecific chemical activity relationships of pesticides in the aquatic environment. Aquat. Toxicol., 67: 87-103.
9. Barata, C., J. Damasio, M.A. Lopez, M. Kuster, M.L. De Alda, D. Barcelo, M.C. Riva and D. Raldua, 2007. Combined use of biomarkers and in situ bioassays in *Daphnia magna* to monitor environmental hazards of pesticides in the field. Environ. Toxicol. Chem., 26: 370-379.
10. Schafer, R.B., T. Caquet, K. Siimes, R. Mueller, L. Lagadic and M. Liess, 2007. Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. Sci. Total. Environ., 382: 272-285.
11. Kock, M., M. Farre, E. Martinez, K. Gajda-Schrantz, A. Ginebreda, A. Navarro, M.L. De Alda and D. Barcelo, 2010. Integrated ecotoxicological and chemical approach for the assessment of pesticide pollution in the Ebro River delta (Spain). J. Hydrol., 383: 73-82.
12. Munoz , I., M.J. Gomez-Ramos, A. Aguera, J.F. Garcia-Reyes, A. Molina-Diaz and A.R. Fernandez-Alba, 2009. Chemical evaluation of contaminants in wastewater effluents and the environmental risk of reusing effluents in agriculture. Trac-Trends Anal. Chem., 28: 676-694.
13. Von Der Ohe, P.C., V. Dulio, J. Slobodnik, E. De Deckere, R. Kühne, R.U. Ebert, A. Ginebreda, W. De Cooman, G. Schüürmann and W. Brack, 2011. A new risk assessment approach for the prioritization of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants under the European Water Framework Directive. Sci. Total Environ., 409: 2064-2077.
14. Ecobichon, D.J. and R.M. Joy, 1994. Organophosphorus ester insecticides in pesticides and neurological diseases, second ed. CRC Press, Boca Raton, FL. pp: 171-249.

15. Pesando, D., P. Huitorel, V. Dolcini, C. Angelini, P. Guidetti and C. Falugi, 2003. Biological targets of neurotoxic pesticides analyzed by alteration of developmental events in the Mediterranean sea urchin, *Paracentrotus lividus*, Marine Environ. Res., 55: 39-57.
16. Ansari, B.A. and K. Kumar, 1988. Diazinon toxicity on protein and nucleic acid metabolism in the liver of zebra fish, *Brachydanio rerio* (Cyprinidae), Sci. Total Environ., 76: 63-68.
17. Burkepile, D.E., M.T. Moore and M.M. Holland, 2000. The susceptibility of five nontarget organisms to aqueous diazinon exposure, Bull. Environ. Contam. Toxicol., 64: 114-121.
18. Hamm, J.T. and D.E. Hinton, 2000. The role of development and duration of exposure to the embryotoxicity of diazinon, Aquat. Toxicol., 48: 403-418.
19. Viran, R., F.U. Erkoc, H. Polat and O. Kocak, 2003. Investigation of acute toxicity of deltamethrin on guppies (*Poecilia reticulata*). Ecotoxicology and Environmental Safety. 55: 82-85.
20. Srivastav, A.K., S.K. Srivastava and S.K. Srivastav, 1997. Impact of deltamethrin on serum calcium and inorganic phosphate of freshwater catfish, *Heteropneustes fossilis*. Bull. Environ. Contam. Toxicol., 59: 841-846.
21. Bradbury, S.P. and J.R. Coats, 1989. Comparative toxicology of the pyrethroid insecticides. Rev. Environ. Contamin. Toxicol., 108: 133-177.
22. Dutta, H.M. and D. Arends, 2003. Effects of endosulfan on brain acetylcholinesterase activity in juvenile bluegill sunfish, Environ. Res., 91: 157-162.
23. Dutta, H.M. and H.J.M. Meijer, 2003. Sublethal effects of diazinon on the structure of the testis of bluegill, *Lepomis macrochirus*: a microscopic analysis, Environ. Pollut., 125: 355-360.
24. Alam, M.K. and O.E. Maughan, 1993. Acute toxicity of selected organophosphorus pesticides to *Cyprinus carpio* and *Barillus vagra*, J. Environ. Sci. Health, B 28: 81-89.
25. Ferrando, M.D. E. Sancho and E. Andreu-Moliner, 1991. Comparative acute toxicities of selected pesticides to *Anguilla anguilla*, J. Environ. Sci. Health, B 26: 491-498.
26. Gooley, G.J., F.M. Gavine, W. Dalton, S.S. De Silva, M. Bretherton and M. Samblebe, 2000. Feasibility of aquaculture in dairy manufacturing wastewater to enhance environmental performance and offset costs. Final Report DRDC Project No. MAF001. Marine and Freshwater Resources Institute, Snobs Creek, pp: 84.
27. Hotos, G.N. and N. Vlahos, 1998. Salinity tolerance of *Mugil cephalus* and *Chelon labrosus*, Pisces: Mugilidae/fry in experimental conditions. Aquaculture, 167: 329-338.
28. Finney, D.J., 1971. Probit Analysis. Univ. Press. Cambridge. pp: 333.
29. Willis, G.H. and L.L. McDowell, 1982. Review: Pesticides in agricultural runoff and their effects on downstream water quality. Environ. Toxicol. Chem., 1: 267-279.
30. Capel, P.D. S.J. Larson and T.A. Winterstein, 2001. The behavior of thirty-nine pesticides in surface waters as a function of scale. Hydrol. Process. 15: 1251-1269.
31. Larkin, D.J. and R.S. Tjeerdema, 2000. Fate and effects of diazinon. Rev. Environ. Contam. Toxicol., 166: 49-82.
32. Galloway, T. and R. Handy, 2003. Immunotoxicity of organophosphorous pesticides. Ecotoxicology, 12: 345-363.
33. Adedeji, O.B., A.O. Adedeji, O.K. Adeyemo and S.A. Agbede, 2008. Acute toxicity of diazinon to the African catfish (*Clarias gariepinus*). African Journal of Biotechnology. 7(5): 651-654.
34. Keizer, J.D., G. Gostino and L. Vittozzi, 1991. The importance of biotransformation in the toxicity of xenobiotics to fish.1.Toxicity and bioaccumulation of diazinon in guppy (*Poecilia reticulata*) and zebra fish (*Brachydanio rerio*). Aquat. Toxicol., 21: 239-254.
35. Tsuda, T., M. Kojima, H. Harada, A. Nakajimaand S. Aoki, 1997. Acute toxicity, accumulation and excretion of organophosphorus insecticides and their oxidation products in killifish. Chemosphere, 35: 939-949.
36. Boateng, J.O., F.K. Nunoo, E.H.R. Dankwa and M.H. Ocran, 2006. Acute toxic effects of deltamethrin on tilapia, *Oreochromis niloticus* (Linnaeus, 1758). West Africa Journal of Applied Ecology. 9: 1-5.
37. Mitta, P.K., T. Adak and V.P. Sharma, 1994. Comparative toxicity of certain mosquitocidal compounds to larvivorous fish *Poecilia reticulata*. Ind. J. Malariol., 31(2): 43-47.