Environmental Biology of Fishes **36**: 319–324, 1993.

Non lethal concentrations of pesticide impair ovarian function in the freshwater perch, *Anabas testudineus*

Chirashree Choudhury, Arun Kumar Ray[1], Samir Bhattacharya & Shelley Bhattacharya
Department of Zoology, Visva-Bharati University, Santiniketan-731 235, India
[1] *Author for correspondence*

Received 7.8.1991 Accepted 15.5.1992

Key words: Reproduction, Air-breathing teleost, Gonadosomatic index, Metacid-50, Carbaryl, Fish

Synopsis

Anabas testudineus were treated with non lethal levels of metacid-50 (0.106 ppb) and carbaryl (1.66 ppm) for 90 days covering the pre-spawning and spawning phases of the annual reproductive cycle. The main purpose of the present work was to identify the effects of metacid-50 and carbaryl on the gonado somatic index (GSI) and ovarian and plasma estrogen level. There was no alteration in GSI until 15 days, initiating the inhibition on day 20 which further intensified from 20 to 90 days of exposure. Plasma and ovarian estrogen level significantly increased up to 15 days of exposure followed by a decline till the end of the experiment. It is noteworthy that the effect of pesticides on GSI is reflected in the ovarian estrogen level. This highlights the fact that at short-term exposures the nonlethal levels of pesticides have no inhibitory effect while at long-term exposure, the pesticides have potent inhibitory effect on the reproduction of fish.

Introduction

Pesticides as a group of environmental contaminants cause severe toxicity, both acute and chronic in non-target species. Literature abounds with reports on the impact of pesticides on various fish tissues but studies on reproductive toxicity in fish are not as many. Studies on pesticidal effects on female reproduction are abortion in *Gambusia affinis* (Boyd 1964), reduction in reproductive efficiency in brown trout, *Salmo trutta,* and brook charr, *Salvelinus fontinalis* (Burdick et al. 1972), prevention of reproduction by Carbaryl (Carlson 1972), reduced hatching of embryos by PCB in brook charr (Freeman & Idler 1975), retardation of ovarian development by Fenitrothion and Carbaryl (Saxena & Garg 1978) and Phenthoate (Dey & Bhattacharya 1989) in *Channa punctatus* and by Malathion and Endrin in *Heteropneustes fossilis* (Singh & Singh 1980). Recently we reported the reproductive toxicity of Metacid-50 and Carbaryl with respect to serum GtH titre, pituitary GtH content and hypothalamic GnRH activity in *C. punctatus* (Ghosh et al. 1990). The present work is a follow up of our earlier observation to evaluate the effects of non lethal concentrations of metacid-50 (Methyl-parathion 50%) and carbaryl on the ovarian weights (GSI) and levels of estrogen in the ovary and plasma of the Indian climbing perch, *Anabas testudineus* (Bloch) during an exposure regimen of 90 days (metacid-50, 29 April through 29 July 1988 and carbaryl, 20 April through 20 July 1989) extending over the pre-spawning and spawning phases of the annual reproductive cycle.

Materials and methods

Fish

Adult, healthy, female *Anabas testudineus* (average weight 20 g ± 2.6 and average length 13 cm ± 0.9) were collected from local freshwaters. The fish were at first acclimatized under laboratory conditions in glass aquaria for 10 days and kept in batches of 10 in glass aquaria containing 30 litre of tap water. A 90 day exposure test with 0.106 ppb of metacid-50 (Bayer, India Ltd.) and 1.66 ppm of carbaryl (Paushak Pvt. Ltd. India) was set up covering prespawning and spawning phases of the annual reproductive cycle. The water characteristics were: dissolved oxygen 3–6 ppm; hardness (as $CaCO_3$) 215–245 ppm and pH 7.27–7.60; temperature 28–30° C.

Pesticides

Metacid-50 is a commercial organophosphate containing 50% methyl-parathion as the active ingredient. Carbaryl is also a commercial formulation containing 50% n-methyl naphthyl-1-carbamate as the active ingredient.

Treatment

Before deciding the exposure dose the median tolerance limits (Tlm) were determined following the method of Doudoroff et al. (1951). The Tlm for Metacid-50 was 5.33 ppb and that for Carbaryl was 15.83 ppm. The exposure doses were selected on the basis of no mortality and absence of any sign of physiological distress over a period of 90 days. A concurrent control was maintained throughout the experiment. Since the duration of treatment was prolonged, feeding of fish was continued once daily. As per Anon. (1960) regulations the water was changed daily and 0.106 ppb of Metacid-50 and 1.66 ppm of Carbaryl were at first dissolved in 20 ml of the aquarium water and then added freshly to negate the effect of metabolites on the toxicity of the poison. Fish were sampled for determination of GSI and hormonal profiles in the ovary and plasma on day 3, 7, 15, 20, 30, 60 and 90 both from the control and experimental set.

Gonadosomatic Index (GSI)

The gonads were dissected out and weighed on a single pan balance. The GSI was calculated on a 100 g body weight basis following the formula described by Abidin (1986).

Collection of blood and ovary

Blood was collected from the caudal peduncle region and the plasma was separated by centrifugation at 2500 g at 4° C for 10 min. The entire ovary was carefully dissected out and immediately homogenized in 0.6% saline; after centrifugation the supernatant was decanted and stored at 4° C for future use.

Radioimmunoassay of 17β-Estradiol

Estradiol-17β (E_2) was determined by RIA as described by Sower & Schreck (1982). Extraction efficiency was more than 85%. Anti-Estradiol-17β was obtained as gift from J.Y.L. Yu, Academia Sinica, Taipei, Taiwan. Anti-Estradiol-17β was diluted 1 : 25000 in phosphate buffered saline-gelatin (PBSG). 100 µl of antiserum were added to the standards which ranged from 10 to 1000 pg. After the samples were incubated for 1 h, 100 µl of ^3H-E_2 (10000 dpm in PBSG) were added to each tube. Samples were then incubated at 4° C overnight and then placed in an ice bath for 15 min. 500 µl of dextran coated charcoal suspension (0.625% charcoal and 0.4% dextran in PBSG buffer) were added. Samples were then centrifuged, decanted into scintillation fluid and the radioactivity counted in a liquid scintillation system (Model No. LSS-20, Electronic Corporation of India). The results were expressed as ng per ml plasma and pg per mg of ovarian protein. Samples were prepared and stored at − 24° C until the end of the sampling period. Inter-assay variations were not more than 8% while intra-assay variations were less than 5%.

Protein estimation

Ovarian protein was assayed by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

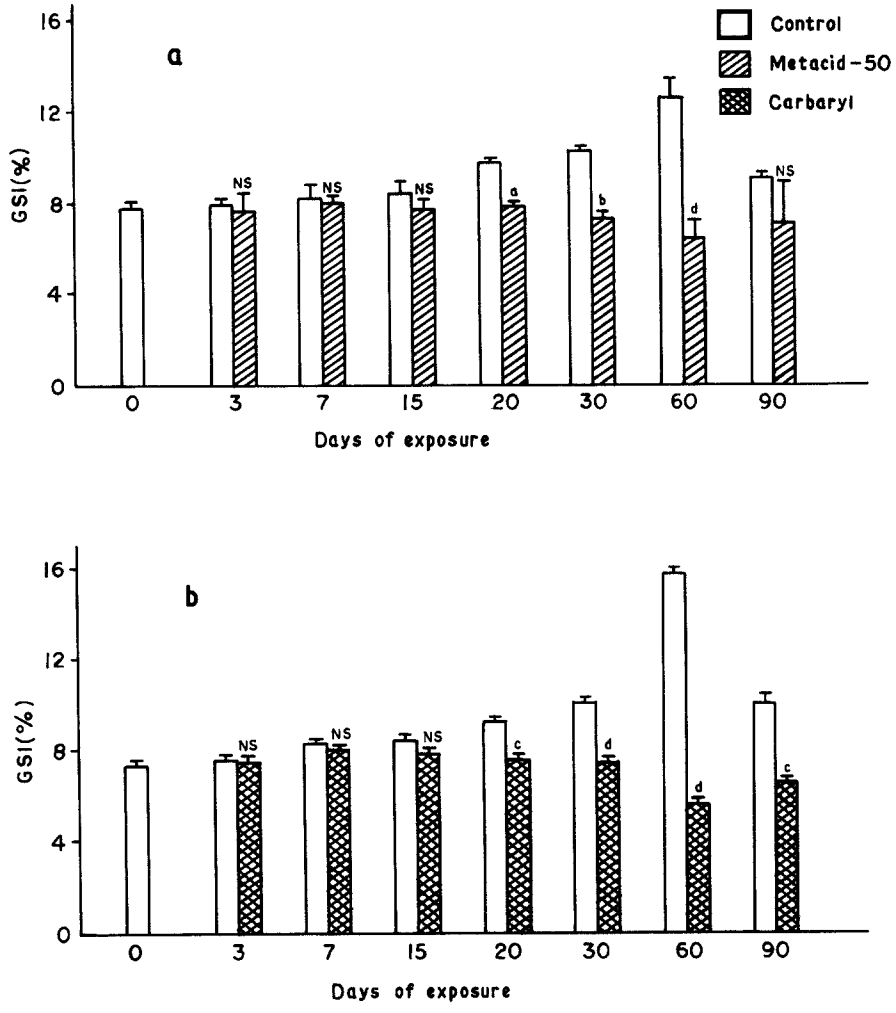

Fig. 1. Gonadosomatic index (mean ± S.E.) of *Anabas testudineus* exposed to metacid-50 (a) and carbaryl (b) for 90 days. a – $p < 0.05$, b – $p < 0.01$, c – $p < 0.005$, d – $p < 0.001$, NS – not significant; number of fish per sample was five.

Statistical analysis

All data were subjected to Student's 't' test (Snedecor & Cochran 1971).

Results

Figure 1a demonstrates no significant change in the GSI value until day 15 of the organophosphate treatment. The decrease in the GSI was observed only on day 20 which continued until the end of the exposure period. The decline in the GSI was highest on the 60th day of exposure which coincided with the prespawning phase of the fish.

With the carbaryl treatment (Fig. 1b) the GSI did not deviate from the control value significantly until after 15 days of exposure. However, from 20 days onwards untill the termination of the experiment GSI level varied significantly from the respective control value.

The profiles of the plasma and ovarian estradiol-17β (E_2) of the control fish showed a gradual increase until 60 days of observation (Fig. 2, 3). A 90 days of sampling the ovarian E_2 level demonstrated a slight decrease from those of the 60 day levels.

In the case of plasmal and ovarian E_2 levels (Fig. 2a, b) in the organophosphate treated fish a gradual increase was observed from day 3 to day 15 of

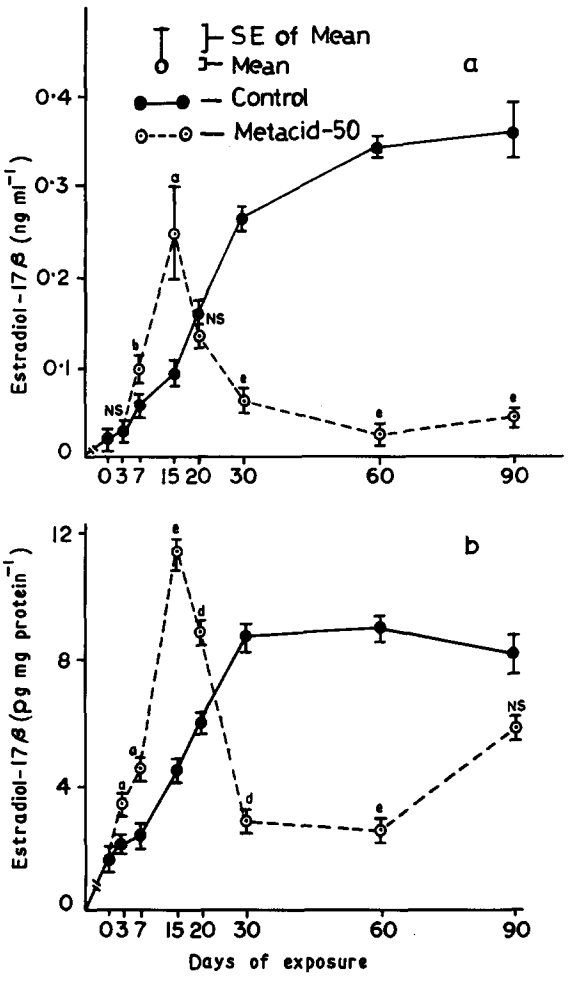

Fig. 2. Profiles of estradiol-17β in the plasma (a) and ovary (b) of *Anabas testudineus* exposed to metacid-50 for 90 days. a – $p < 0.05$, b – $p < 0.01$, d – $p < 0.001$, e – $p < 0.0001$, NS – not significant; number of fish per sample was five.

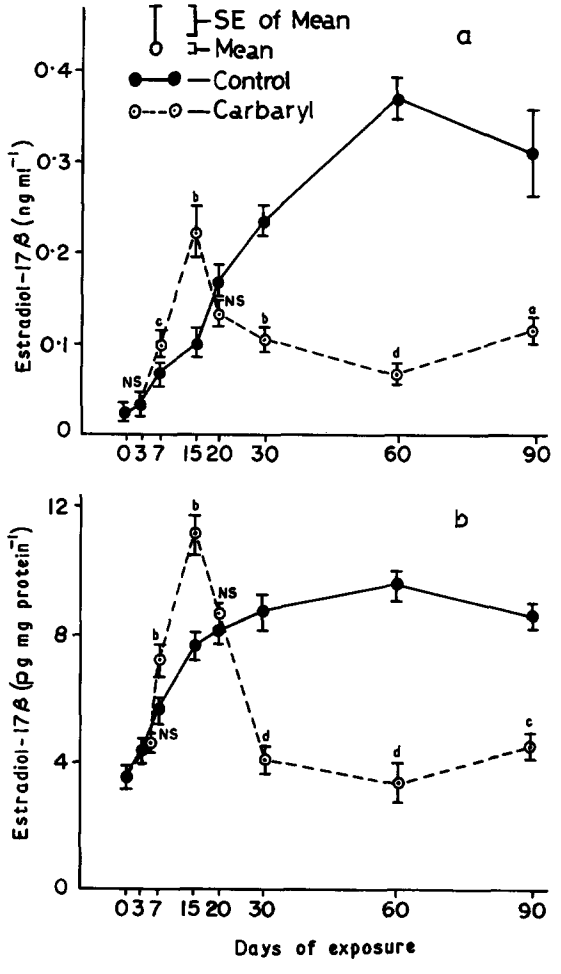

Fig. 3. Profiles of estradiol-17β in the plasma (a) and ovary (b) of *Anabas testudineus* exposed to carbaryl for 90 days. a – $p < 0.05$, b – $p < 0.01$, c – $p < 0.005$, d – $p < 0.001$, NS – not significant; number of fish per sample was five.

treatment, thereafter showing a sharp decline until day 60 of treatment. However, at 90 days of sampling both plasma and ovarian E_2 profiles improved a little showing 87% decline of plasma E_2 and 29% in the ovarian E_2 level over the control.

Figure 3a demonstrates that until 15 days of carbaryl treatment the plasma E_2 titre showed significant increase (18%–128%) while in the later period from 20–60 days of exposure the hormone level decreased (19%–83%) significantly. However, at 90 days, the degree of depletion was not as remarkable. Ovarian E_2 (Fig. 3b) on the other hand, showed a small decline on day 3 leading to 25–45% increment on day 7 and day 15, respectively. This increase subsided to only an 8% increase on day 20 which was followed by significant depletion from day 30 to 90.

Discussion

The exposure doses of the two different classes of pesticides caused no mortality of the fish nor did it manifest any sign of physiological distress. However, they were both potent enough to cause significant reproductive impairment in terms of specific damage to ovarian tissue. Histological examination

revealed a preponderance of stage I and destruction of stage II and stage III oocytes in the fish treated with the pesticides (unpublished observation). As reported by Shukla (1982), GSI is greatly affected by DDT, BHC, endosulfan, chlordane and toxaphane. Dey & Bhattacharya (1989) observed the preponderance of stage I and destruction of stage II and stage III oocytes in association with decreased ovarian weight in phenthoate exposed *C. punctatus*.

Anabas is found to be a more tolerant species to these pesticides, compared to other species such as rainbow trout where the 24 h LD 50 for carbaryl is 1.41 ppm (Zinkl et al. 1987), and *Mystus vittatus* where the 98 h LD 50 for carbofuran is 310 ppb (Verma et al. 1980). Reports on *C. punctatus*, another Indian airbreathing teleost demonstrated a high degree of atresia at 2 ppm carbaryl exposure for 150 days (Saxena & Garg 1978) and a lower degree of ovarian recrudescence at 5 ppm of carbofuran exposure for 120 days. In contrast, the deleteriousness of carbaryl was effected in *Anabas* at a much lower level of exposure both in respect of acute and chronic toxicity. The present work thus substantiates further the potency of metacid-50 and carbaryl in terms of reproductive damage.

Previous workers have suggested an impairment of steroidogenic activity in the gonad of pesticide treated fish (Freeman & Idler 1975, Saxena et al. 1986, Kapur et al. 1978). In the present study a declining trend in the steroid hormone level of both plasma and the ovary was noted in the later periods of exposure, providing evidence for a delayed reproductive activity. Ghosh et al. (1990) have clearly established the mediation of the pituitary and hypothalamic hormones in affecting gonadal function of pesticide treated *C. punctatus*. The present study provides further data on the severity of pesticidal action on ovarian maturation.

It is not clear why the E_2 level in the ovary and plasma increased at the initial period of pesticide exposure (both metacid-50 and carbaryl). We presume it to be a stress effect which might be influencing a greater release of gonadotropin from the pituitary. We do not have any supportive data in favour of this presumption but this appears to be a plausible explanation for unexpected increase in E_2 level from day 2 to day 15 in response to pesticide, when the control level of E_2 in both the ovary and plasma remained far below the level of treated fish. To provide a better explanation, including the level of fish brain GnRH profile, further investigation is required.

Ghosh et al. (1990) recorded a decreased release of GtH in *Channa punctatus* exposed to sublethal doses of metacid-50 and carbaryl for 30 days. It may, therefore, be suggested that the decreased steroidogenic activity in *Anabas testudineus* during this period may be due to the decreased release of GtH during a long term exposure. It can be concluded that sublethal, long term exposure of an organophosphate or a carbamate pesticide impairs seriously the reproductive potential. A short term exposure upto 15 days may not be as harmful as far as the steroidogenic activity of the fish is concerned.

Acknowledgements

The authors gratefully acknowledge the Department of Science and Technology, New Delhi for financial support. The authors are also grateful to Amar Krishna Majumder for typing the manuscript.

References cited

Abidin, A.Z. 1986. The reproductive biology of a tropical cyprinid, *Hampala macrolepidota* (Vanhessett) from Zoo Negara Lake, Kualalumpur, Malaysia. J. Fish Biol. 29: 381–391.

Anon. 1960. Standard methods for the examination of water and wastewater including bottom sediments and sludges. American Public Health Association. 1960, New York. 626 pp.

Boyd, C.E. 1964. Insecticides cause mosquito fish to abort. Progr. Fish Cult. 26: 138.

Burdick, G.C., H.J. Dean, E.J. Harris, J. Skea, R. Karcher & G. Friska. 1972. Effect of rate and duration and feeding DDT on the reproduction of salmonid fishes reared and held under controlled condition. N.Y. Fish Game J. 19: 97–115.

Carlson, A.R. 1972. Effects of long term exposure to Carbaryl (Sevin) on survival, growth and reproduction of fathead minnow, *Pimephales promelas*. J. Fish. Res. Board Can. 29: 583–587.

Dey, S. & S. Bhattacharya. 1989. Ovarian damage to *Channa*

punctatus after chronic exposure to low concentrations of Elsan, mercury and ammonia. Ecotoxicol. Env. Safety 17: 247–257.

Doudoroff, P, B.G. Anderson, D.E. Burdick, P.S. Galtsoff, W.B. Hart, R. Patrick, E.R. Strong, E.W. Surber & W.M. Van Horn. 1951. Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish. Sew. Ind. Wastes 23: 1380–1397.

Freeman, H.C. & D.R. Idler. 1975. The effect of polychlorinated biphenyl (PCB) on steroidogenesis and reproduction in the brook trout, *Salvelinus fontinalis*. Can. J. Biochem. 53: 660–670.

Ghosh, P., S. Bhattacharya & S. Bhattacharya. 1990. Impairment of the regulation of gonadal function in *Channa punctatus* by Metacid-50 and Carbaryl under laboratory and field conditions. Biomed. Env. Sci. 3: 106–112.

Kapur, K., K. Kaur & H.S. Toor. 1978. The effect of fenitrothion on reproduction of a teleost fish, *Cyprinus carpio communis* (Linn.): a biochemical study. Bull. Env. Contam. Toxicol. 20: 438–442.

Lowry, O.H., N.J. Rosebrough, A.L. Farr & R.J. Randall. 1951. Protein measurement with the Folin-phenol reagent. J. Biol. Chem. 193: 265–275.

Mani, K. & P.K. Saxena. 1985. Effect of safe concentration of some pesticides on ovarian recrudescence in the freshwater murrel, *Channa punctatus* (Bloch): a quantitative study. Ecotoxicol. Env. Safety 9: 241–249.

Saxena, P.K. & M. Garg. 1978. Effect of insecticidal pollution on ovarian recrudescence in the freshwater teleost, *Channa punctatus* (Bloch). Ind. J. Exp. Biol. 16: 690–691.

Saxena, P.K., K. Mani & J.K. Kondal. 1986. Effect of safe application concentration (SAR) of some biocides on the gonads of freshwater murrel, *Channa punctatus*: a biochemical study. Ecotoxicol. Env. Safety 12: 1–24.

Shukla, L. 1982. Effects of insecticidal pollutants on the reproduction in fish. Ph. D. Thesis, Vikram University, Ujjain. 173 pp.

Singh, H. & T.P. Singh. 1980. Short-term effects of two pesticides on the survival, ovarian ^{32}P uptake and gonadotrophic potency in a freshwater catfish, *Heteropneustes fossilis* (Bloch). J. Endocrinol. 85: 193–199.

Sower, S.A. & C.B. Schreck. 1982. Steroid and thyroid hormones during sexual maturation of coho salmon (*Onchorhynchus kisutch*) in sea water or freshwater. Gen. Comp. Endocrinol. 47: 42–53.

Snedecor, G.W. & W.G. Cochran. 1971. Statistical methods. Iowa State University Press, Ames. 593 pp.

Verma, S.R., S. Rani, S.K. Bansal & R.C. Dalela. 1980. Effects of the pesticide thiotox, dichlorovos and carbofuran on the test fish *Mystus vittatus*. Water, Air, Soil Pollut. 13: 229–234.

Zinkl, J.G., P.J. Shea, R.J. Nakamoto & J. Callman. 1987. Brain cholinesterase activity of rainbow trout poisoned by Carbaryl. Bull. Env. Contam. Toxicol. 38: 29–35.