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Cytogenotoxic Effects of Carbaryl (Sevin) in Channa Punctatus in Vivo

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Abstract: ***Cytogenotoxic effect of the carbamate insecticide carbaryl (Seven®) to live fish Channa punctatus was studied. We analysed the induced sister chromatid exchanges (SCE) frequencies, chromosomal aberrations (CA), cellular proliferation rate in mitotically dividing cells of kidney and micronucle (MN) i in peripheral erythrocytes of Channa punctatus following in vivo exposure to three different concentrations of carbamate insecticide (Carbaryl). Our result revealed that the insecticide induced significantly high incidences of micronuclei in peripheral erythrocyte, chromosomal aberrations as well as sister chromatid exchanges, inhibited mitotic index and caused considerable delay in the generation time of kidney cells in treated organisms. The effects were found to be depend upon dose as well as period of exposure to the chemical. The experimental data showed carbaryl as cytogenotoxic to fish per se. These results have implications in the use of pesticides in the agricultural field.***

Keywords: Genotoxic, Pesticide toxicity; carbaryl; freshwater fish, Micronucleus, Sisterchromatid Exchanges, Chromosomal aberration, Channa punctatus

1. Introduction

Carbamates are large group of synthetic pesticides extensively applied in modern agriculture [1] Carbaryl (L - naphthy N - methyl carbamate), is a carbamate pesticide that is being used widely to control a variety of pests of different crops like cotton, tobacco, paddy, vegetable crops. It is considered to be environmentally safe because of its shorter persistence and lower mammalian toxicity [2] and hence is more acceptable than the 'environmentally hazardous' organochlorine and organophosphate pesticides.1 - Naphthol, the main degradation product of carbaryl, especially in the aquatic environment [3] is supposed to be nontoxic to the non target organisms. However, the toxicity of 1 - naphthol to the aquatic organisms does not seem to have been adequately tested and in the few studies on the toxicity to aquatic organisms of carbaryl and its degradation product the latter has been reported to be more toxic [4, 5, 6, 7]

Fish are excellent subjects for the study of the mutagenic and/or carcinogenic potential of contaminants present in water samples since they can metabolize, concentrate and store waterborne pollutants [8]Since fish often respond to toxicants in a similar way to higher vertebrates, they can be used to screen for chemicals that are potentially teratogenic and carcinogenic in humans. The main application for model systems using fish is to determine the distribution and effects of chemical contaminants in the aquatic environment [9, 10]

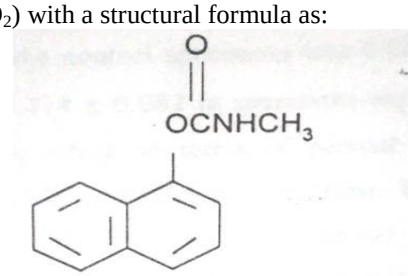
The present investigation was undertaken to study the Mutagenic, carcinogenic and clastogenic effect of carbaryl to the Indian fresh water fish Channa punctatus in vivo as Cytogenetic test model applying Sister Chromatid Exchanges (SCE) frequencies, Chromosomal Aberrations (CA), cellular proliferation rate in mitotically dividing cells of kidney and micronucle (MN) in peripheral erythrocytes [11]

2. Materials and Methods

Test animal: Specimens of Channa punctatus measuring about 10 - 12 cm collected from the local ponds and maintained in laboratory aquaria were used for seven days before treatment.

Test Chemical: Carbaryl is abroad spectrum carbamate insecticide. It was first introduced in market in 1956 under the trade name of Sevin in India by M/S Rallis India Ltd., Mumbai. To control insect pests of fruits, vegetables, forage, cotton and other crops as well as poultry pests.

Chemically, Carbaryl is L - Naphthyl N - Methyl Carbamate ($C_{12}H_{11}NO_2$) with a structural formula as:



Structure of Carbaryl

In pure form it is a white crystalline solid with melting point of 142 - 143^0. It has a vapour pressure of less than 0.005 mm Hg at $260^{0.}$ It is stable to heat, light and hydrolysis except under alkaline conditions. It is highly soluble in most solvent but is relatively less soluble in water (about 40 ppm). The compound is a reversible inhibitor of cholinesterase and is rapidly metabolized in mammals. It also has a very short residua life and therefore, can be use in agriculture right up to harvest period

Doses and route of exposure: From among the specimens acclimatized for at least a fortnight in the laboratory aquaria, only strong and active fishes were released into different aquaria containing 125, 67.5 and 25 microgram of Carbaryl

(Sevin) which correspond to MC, MC/2 and MC/5 doses respectively. MC represent the maximum tolerable concentration of the test compound at which no death of animal beyond 5% was observed during the period of treatment and was determined from preliminary experiments on groups of 20 specimens in aquaria containing 100 litter of water. The test lasted for 25 days with change of water, chemical and food every alternate day. The lowest concentration leading to 50 % death after the treatment was considered as LC_{50} and half of this corresponds of MC. MC/2 and MC/5 represent 1/2 and 1/5 of MC. The treated specimens received in intramuscular injection of 0.02% colchicine solution at the rate of 1ml per 100 mg body weight 2 h prior to their sacrifice on completion of 5, 10, 15, 20 and 25 days of exposure to the test chemical. Control received injection of an equal amount to distilled water Specimens used for analyses of SCEs and proliferation kinetics received, in addition to intra muscular injection of an aqueous solution of Bromo deoxy Uridine (BrdU) at the rate of 50 mg per 100 g body weight at least 24h prior to their sacrifice.

Micronucleus Test: The smear of peripheral blood drawn from the caudal vein with a heparinized syringe, was prepared and well - dried slides were stained in 10% Giemsa solution (Stock solution diluted with Sorensen's buffer at pH 6.8) for 30 min following the method of [12]. Four thousand cells per animals (1000 cells per slide) were scored for micro - nuclei and nuclear anomalies.

Chromosome aberration (CA) test: Mitotic metaphase chromosome spread from kidney cells were obtained following colchicines - citrate - flame drying Giemsa technique of [13]. Chromosome aberrations of various kinds were scored from 100 metaphase cells. Sister - chromatid exchange (SCEs) and proliferative kinetics: Air - dired preparations of mitotic spreads from kidney of BrdU treated specimens were stained for induction of differential staining of sister - chromatids ([14]. and metaphase cells were classified as M1, M2 and M3 according to staining pattern. At each point of time 600 cells were examined, Proliferation Rate Index (PRI) was determined from the count of M1, M2 and M3 cells by using the formula of [15]. Where PRI= (M1+2M2+3M3) /100. SCEs were scored from M2 cells only.

Mitotic indices: Mitotically dividing cells were scored from slides prepared and stained for metaphase spreads from kidney of both treated and control specimens. Cells at interphase were omitted from the analysis to avoid any possible error in the result.

Statistical analyses: For comparison of mean, Student's t - test was applied. Two - way analysis of multiple variance (ANOVA) was done for dose and time response study.

3. Results

Chromosome Aberration Test: Carbaryl (Sevin) was found to be the most potent inducer of chromosome aberrations in the kidney cells at each concentration and the period of exposure. As can be judged from the average data on chromosome aberrations presented in table 1, all the treated groups of specimens, irrespective of concentration or the period of exposure, had highly elevated frequency of chromosome aberrations as compare to the control. While the frequency of chromosome aberrations in the control specimens was 0.084 ± 0.031, it was0.489 ± 0.064, 0.592± 0.056 and 0.714± 0.082 in specimens exposed for 5 days to MC/5, MC/2 and maximum tolerable concentration (MC) respectively which in terms of percent increase. in Chromosome aberration was highest, of all pesticides described earlier. Furthermore, the frequency increased with the increase in the concentration or the period of exposure up to 20 days. The frequency increased progressively with the increase in the period of exposure and reached maximum to 0.86± 0.067, 0.982 ± 0.074, 1.176± 0.109 in specimens exposed for 20 days to MC/5, MC/2 and MC respectively. The specimens exposed for25days to any of the three concentrations, on the other hand, had slightly low frequency thereby indicating a reversal in the, although the same was still very high as compared to controls Two way analysis of multiple variance (ANOVA test) testified that the pesticide induced Chromosomal Aberrations in dose as well as period dependent manner. In fact, the calculated values of 'F' for concentration (F=101.18; d. f14, 2, p<0.001) and period of exposure (F=98.18., d. f.14.4p<0.001) were much higher than their respective tabulated values.

Table 1: Frequency of chromosome aberrations (per 100 chromosomes) in kidney cells of Channa punctatus following their in vivo exposure to three different concentrations of Carbaryl for 5 - 25 days and in control

Periods of Exposure (in days)	Concentrations		
	MC/5	MC/2	MC
0	0.084 ± 0.031	0.084±0.031	0.084±0.031
5	0.489±0.064	0.592±0.056	0.714±0.082
10	0.613±0.076	0.772±0.081	0.844±0.074
15	0.755±0.056	0.866±0.061	0.965±0.039
20	0.861±0.094	0.981±0.074	1.176±0.109
25	0.765±0.078	0.915±0.055	1.011±0.072

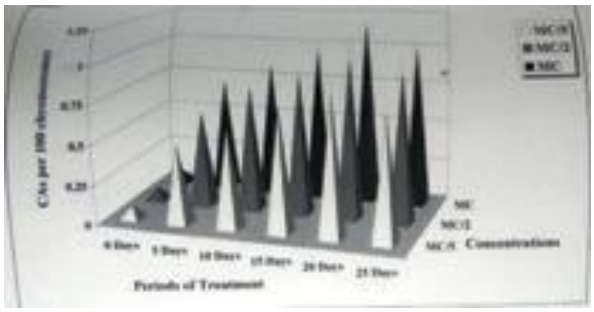


Figure 1: Average frequency of chromosomal aberrations (CAs) per100 chromosomes of Channa punctatus following their in vivo exposure to three different concentrations of carbarylfor5 - 25daysand in controls

Micronucleus Test:
The frequency of micronuclei and nuclear anomalies of various kinds in the peripheral erythrocytes in Channa punctatus exposed to different concentrations of Carbaryl for varying periods of time and in control is summarized in table 02. Evidently the frequency in all the treated group of specimens had higher of erythrocytes with MN and nuclear anomalies as compared to controls. While the frequency in control specimens is 0.063±0.011, it ranged from 0.198 ± 0.014, 0.543±0.047 and 0.290±0.026 to 0.642 ±0.071 and

0.370±0.082 to0.678±0.066 specimens exposed MC/5, MC/2 and MC respectively. Furthermore, the frequency increased progressively with the increase in the concentration and or the period of exposure till 20 days but thereafter decline, as was the case with chromosome aberrations. Statistical analyses of the data applying Student's 't' test however, revealed that the increase in the frequency in all specimens was statistically significant as compared to the controls and that the level of significance increased with increase in the concentration or period of exposure. Two - way analyses multiple variance, too, testified that Carbaryl, induced MN and nuclear anomalies in concentration as well as period of exposure dependent. In fact, the calculated values of 'F' for concentration (F=85.106; d. f.14.2, p<0.001) and period of exposure (F=200.824, df 14.4, p<0.001) were significantly higher than their respective tabulated values.

Table 2: Frequency of peripheral erythrocyte with micronuclei and nuclear anomalies of various kidney in Channa punctatus following their in vivo exposure to three different concentrations of Carbaryl for 5 - 25 days and in control.

Periods of Exposure (in days)	Concentrations		
	MC/5	MC/2	MC
0	0.063 ± 0.011	0.063 ± 0.011	0.063 ± 0.011
5	0.198±0.014	0.290±0.026	0.370±0.082
10	0.326±0.026	0.412±0.041	0.476±0.044
15	0.450±0.041	0.539±0.039	0.581±0.036
20	0.543±0.047	0.642±0.071	0.678±0.066
25	0.523±0.049	0.580±0.051	0.611±0.062

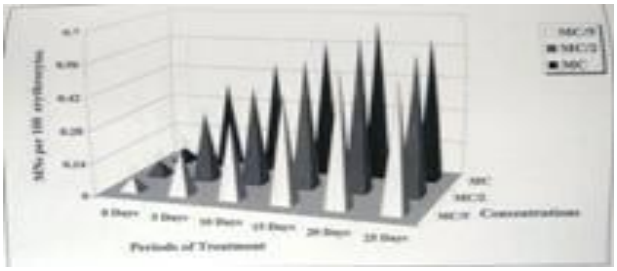


Figure 2: Average frequency of micronuclei (MNs) and nuclear anomalies per100 peripheral erythrocytes of Channa punctatus following their in vivo exposure to three different concentrations of carbarylfor5 - 25daysand in controls

Sister - chromatide Exchanges

The frequency of sister chromatid exchanges in the kidney cells of specimens of Channa punctatus exposed to different concentrations (MC/5, MC/2 and MC) of Carbaryl for varying period of time and controls is enumerated in table no.3. Evidently, the kidney cells in all the treated groups of specimens had a significant higher frequency SCE as compare to those of controls While the frequency 0.197±0.011 in control it was respectively to 0.473±0.048, 0.557±0.066 and 0.557±0.066 and 0.667±0.081, in specimens exposed for 20 days and to MC/5, MC/2 and MC concentrations. The specimens those exposed for 25 days, however, had a relatively low frequency of SCEs as compared to those exposed for 20 days, which indicated a reversal in increasing trend of the frequency as ws the case with chromosome aberration and micronuclei tests. This notwithstanding, statistical analyses of the data by way of Student's 't' test testified that elevated rate of sister - chromatid exchanges inin all the group of specimens exposed to each concentration and/or each period of exposure was significant, as compared to the controls. Also, the level of significance increase with the increase in the concentration and /or the period of exposure. Two - way analyses of the multiple variance, too reinforced that induction of sister - chromatid exchanges by Carbaryl was dependent significantly both on the concentration as well as the period of exposure. The calculated values of 'F' for concentration (F=119.309; d. f.14, 2, p<0.001) and period of exposure (F - 115.811; d. f.14, 4. p<0.001) were unexpectedly higher than their respective tabulated values.

Table 3: Incidence of sister - chromatid exchanges (per 100 chromosomes) in kidney cells of Channa punctatus following their in vivo exposure to three different concentrations of Carbaryl for 5 - 25 days and in control

Periods of Exposure (in days)	Concentrations		
	MC/5	MC/2	MC
0	0.197 ± 0.011	0.197 ± 0.011	0.197 ± 0.011
5	0.473±0.048	0.557±0.066	0.667±0.081
10	0.568±0.069	0.698±0.061	0.793±0.079
15	0.699±0.063	0.829±0.081	0.915±0.085
20	0.813±0.087	0.954±0.091	1.122±0.123
25	0.710±0.071	0.857±0.079	0.967±0.102

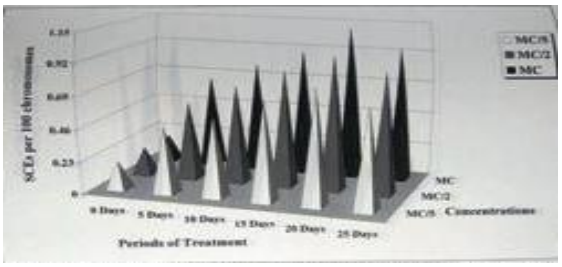


Figure 3: Average frequency of sister chromatid exchanges per100 chomosomes of kidney cells of Channa punctatus following their in vivo exposure to three different concentrations of carbaryl for 5 - 25daysand in controlsz

Mitotic index

Table 04 portrays the frequency of mitotically dividing cells in the kidney of Channa punctatus following their in vivo exposure to three sub lethal concentrations (MC/5, MC/2 and MC) of Carbaryl of the period varying from 5 - 25 days and in controls. A cursory survey of the data would make it evident that all the treated groups of specimens had highly depressed mitotic index as compared to the control, thereby indicating that Carbaryl is also mitostatic to larger cells. However, similar to the observation made in chromosome aberration test, MNT test an SCE probe the specimens exposed for 25 days had relatively higher frequency of MI than those exposed for 20 days - . Statistical analyses of the data employing Student's 't' test clearly indicated that the decrease in the rate of mitotically dividing cells in kidney of control specimens was significant as compared to the controls while application of ANOVA test (two - way analysis of multiple) testified that Carbaryl affected the mitotically dividing kidney cells in both concentration as well as period dependent manner. The calculated values of 'F' for concentration (F=22.206, d. f.14.2, p<0.001) and period of exposure (F=22.311, d. f.14, 4, p<0.001) were in fact signific antly higher than their respective tabulated values

Table 4: Frequency of mitotically dividing cells in kidney cells of Channa punctatus following their in vivo exposure to three different concentrations of f Carbaryl or 5 - 25 days and in control

Periods of Exposure (in days)	Concentrations		
	MC/5	MC/2	MC
0	2.431± 0.123	1.431± 0.172	2.431 ± 0.123
5	2.254±0.181	1.564±0.121	1.273±0.112
10	1.862±0.192	1.322±0.153	0.914±0.103
15	1.392±0.202	1.023±0.151	0.854±0.101
20	0.804±0.073	0.663±0.081	0.484±0.041
25	1.032±0.191	0.772±0.071	0.562±0.082

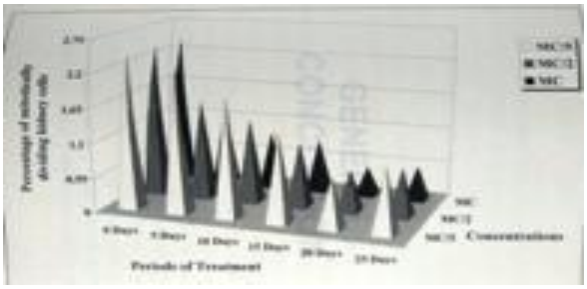


Figure 4: Average frequency of mitoticaly dividing cells in kidney of Channa punctatus following their in vivo exposure to three different concentrations of carbarylfor5 - 25daysand in controls

4. Discussion

Cabaryl (Sevin) is another insecticide which has not been studied extensively in terms of genotoxicity. It has, however been reported to be non mutagenic in E. coli [[16]. **.** Bacillus subtilis [17] S. typhimurium strains [18, 19]. **.** and in stationary phase cells of yeast, S. cervisiae D 4 strain [20] though in the latter test system, it produced gene conversion as genetic end point. The compound has also been found not to yield revertants in significant frequency n the host mediated assay in mouse with S. typhimurium G 46 strain as indicator organism and micronuclei in bone marrow cells of swiss mice in vivo [21]and strand breakage in the human DNA [22]. Also, the sex linked recessive lethal test in Drossophila following larval feeding yielded negative result [23] But Carbaryl induced mosai spots both in the eye and wing through mitotic recombination and point mutation and mutagenicity test in Drossophila [24] it's larval stages by sex - linked recessive lethal (SLRL) test in Drossophilagerm cells [25].

In the earlier, it was reported that 1 - naphthol was more toxic than the parent compound to two size groups of the carp. Labeo rohita (Ham.) [6]. In a comparative study of sublethal and chronic effect of Carbaryl and Malathion found Crabaryl is, more toxic [26] In the present study We observed not only significantly high frequency of chromosome aberrations or SCEs in the kidney cells of all the treated groups of specimens but also micronuclei and nuclear anomalies of various kinds in the peripheral erythrocytes. We also observed a significant reduction in the frequency of mitotically dividing cells in kidney of all the treated groups of specimens, irrespective of the period of their exposure and the concentration of the pesticide. Furthermore the compound was found to be the most potent inducer of chromosome aberrations among the pesticides here in. The exact reason for such a contrast difference in the results between the earlier studies and the present study is difficult to explain. One possibility is perhaps the difference the mode of treatment. In the present study the specimens were kept exposed continuously to the pesticides which might have led to an accumulation of the pesticide in the kidney etc. in huge quantity mainly due to the slow rate of metabolic activity of per se. In fact, some recent studies have shown that sevin causes severe necrosis of hepatic cells as well as damage to the connective tissue of the liver of fishes following their prolonged exposure even to sub lethal concentration [27, 28] In a comparative study of acute toxicity of Carbaryl, Methicarb and Carbosulfan to the guppy (Poecilia reticulate) and rainbow trout (Onychorynchus mykiss) [29] said that guppy is more resistant to carbaryl and Methicarb but less sensitive to Carbofusan indicate carbaryl and Methicarb are less toxic than carbosulfan. Yet, another possibility to low toxicity of carbamate pesticides in general, to mammalian species. comparison of sublethal and chronic effect of carbaryl and malathion on Clarias batrachus**)** [26]found malathion is more toxic than carbaryl. (In rats they are slowly metabolizedto1 - napthal and other hydroxylation and conjugation products which are excreted in urine. Histo pathological study of carbaryl in the testis of albino rats [30] find out adverse effect of carbaryl affecting spermatogenesis in rats. A recent study by [3**1]** however, has clearly demonstrated carbaryl as genotoxic for chicks. In fact they observed a significantly high frequency of micronuclei in the bone marrow cells and peripheral erythrocytes of chick following two peritoneal injections at the rate of 100, 125 and 150mg per kg body wt. at interval of 24 hours and their sacrifice at 6^{th} hour after the2nd injection. Immunotoxicity of Carbaryl in chicken [32] studied immuno supessive effect is not significant, not onliy that They also used mitomycin C as positive control.

Thus our data as well as those of [31] clearly suggest carbaryl as highly genotoxic as well as mitostatic to higher animals. in a comperative study of genotoxic effect of the carbamate insecticide primore - 50 in Vicia faba root meristem in vivo and human lymphocyte in vitro [1]applying Sister Chromatid Exchanges (SCE) and Cell cycle progression and cell proliferation evaluated the difference in the role plant and animal metabolism on its genetic potential. Biochemical and Histopathological study of carbaryl in the hepatopancreas and gills of prawn deteriorates protein content and histological structure [33] warranted immediate prevention of indiscriminate use of carbaryl to make healthy dietary environment of man. A further study employing various in vivo short term test, is however, is necessary to confirm our contention.

5. Conclusion

Present study, reveals that the carbaryl induced Sister Chromatid Exchanges (SCE) and Cell cycle progression and cell proliferation, chromosomal aberrations, Mitoti index, and micronucleus were more significant when compared to control in Channa punctatus. Hence car baryl considered as mutagenic agent.

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Author Profile

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