Genotoxic potential of Carbaryl in the peripheral blood erythrocytes of *Anabas testudineus*

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ABSTRACT

Aquatic ecosystems in agricultural areas are inadvertently dosed through the use of large number of agrochemicals including pesticides, which has direct or indirect effect on human health. So there is utter need to evaluate the genotoxic potential, if any, of such environmental pollutants. In the present communication we report the genotoxic properties of carbaryl (1-naphthyl-N-methyl-carbamate) or sevin*, an insecticide, employing micronucleus test (MNT) in the peripheral blood erythrocytes of *Anabas testudineus*. Different doses (50, 100 and 150 mg/kg) of carbaryl were injected intraperitoneally and specimens were sacrificed after 24, 48 and 72 hours. Peripheral blood (from caudal region) smear slides were prepared and stained with Giemsa (pH 7.0). Besides micronuclei the chemical also induced other nuclear and cytoplasmic anomalies. The present results revealed the genotoxic potential of carbaryl.

Introduction

Now a days-large number of pesticides are in use to control the pests and to increase the animal and plant productivity. Pesticides in water cause massive fish kills and contamination of aquatic animals (Fuller and Weissner, 1976) which would ultimately be consumed and enter into human body. Carbaryl (sevin*) is a widely used broad spectrum insecticide (Wagner, 1983). Pesticides enter into the aquatic ecosystem through its application, accidental release, through runoff water and rain. The primary insecticidal action of carbamate is to inhibit the acetyl cholinesterase in the nervous systems of insects and other animals (Matsumura, 1985; Cramner, 1986). Besides all these the genotoxic properties of carbaryl are also on record. It gave positive results in Ames *Salmonella* test and for chromosome aberration both in vivo and in vitro mammalian test systems (Ishidate et al., 1988). Carbaryl (85% purity) caused an increased number of recessive lethal in *Drosophila melanogaster* (Epstein et al., 1972) and has also been reported to be toxic in aquatic bivalves *Corbicula* in vivo system (Jadhav et al., 1996). From the point of human exposure and risk assessment, the evaluation of genotoxic effect in fish
are more relevant since fish constitute one of the important components of human food chain. Besides there are some advantages of using fish as a test model. Fish can be exposed to the toxic chemicals in the laboratory similar to higher vertebrate, be handled easily and relatively less expensive.

In genotoxicity testing, micronucleus test (MNT) is preferred over chromosomal aberration (CA) and sister chromatid exchange (SCE). It is more so in fish because of its high diploid number and smaller size of chromosomes without marked variation. Furthermore, karyotype of all fish are not ideal; fluctuation of mitotic frequency at different times and from tissue to tissue, which make chromosome analysis difficult and time consuming (Rahman and Khuda-Bukhsh, 1992).

Occurrence of micronuclei (MN) and other nuclear anomalies (NA) in fish exposed to various chemical, physical or living mutagens has been studied earlier in fish to assign the degree of genotoxicity of these agents. Chemicals like ethyl methane sulphonate induced micronuclei in Umbra pygmaea (Hooftman an de Raat, 1982); mitomycin C and paper mill effluents induced micronuclei in Heteropeucestes fossilis (Das and Nanda, 1986). Chlorpyriphos has been reported to be genotoxic in Channa punctatus (Porichha et al., 1998) and pyrethroid lamda-cyhalothrin induced micronuclei in Cheirodon interruptus interruptus (Campana et al., 1999). Recently genotoxic potential of sevin® in Heteropeucestes fossilis has been reported (Sahoo and Bhunya, 2001). So it was thought pertinent to study genotoxicity of carbaryl (sevin®) in Anabas testudineus employing micronucleus test in the peripheral blood erythrocytes as the cytogenetic end point.

Materials and methods

Commercial grade carbaryl (1-naphthyl-N-methyl-carbamate) or sevin® (CAS no. 63-25-2 purity 50%, Rhone-pouline Agrochem (India) Ltd.) procured from the authorised pesticides dealer served as the test chemical. Live Anabas testudineus (15 to 20 grams) were collected from non-contaminated ponds of domestic use. Prior to chemical treatment fish were kept in the laboratory aquarium for 2 to 3 days for acclimatization.

Micronucleus test assay (MNT)

Following different dosing of the chemical (50, 100, 150 mg/kg) blood smear slides were prepared after 24, 48 and 72 hours by caudal incision in accordance with Al-Sabti (1986) and Das and Nanda (1986) with some modifications. Then the slides were just dried in air and fixed in absolute methanol for 10-15 minutes and stained in 15-20% Giemsa (pH 7.0) for 1 to 1 ½ hours. From each individual 4000 erythrocytes (1000 per slide) were examined.

Statistical analysis

The frequencies of abnormalities were determined for each animal and then the mean ± standard error for each group was calculated. For comparison of experimental means of the treated with control animals, student’s t-test was employed. Data sets that were either not normally distributed or possessed unequal variances were square root transformed and then retested using two-way analysis of variance (ANOVA) for comparison between more than two treatment means. Linear regression equation was derived between doses of carbaryl and frequency of micronuclei.
Results

Physiological toxicity

After the administration of the chemical, the frequency of surfacing and gulping air gradually increased with dose. The frequency of these activities was 14-15 per hour in the control, while 17-18 per hour with lowest dose (50mg/kg), 20-22 per hour (100mg/kg) and 26-28 per hour (150mg/kg). Mucous secretion was also observed in the treated individuals only. Mucous secretion was more with higher doses. These irregular activities continued for 30-60 minutes following almost motionless for a while for 2-3 hours after which the fish gradually returned to normalcy.

Genotoxicity

The incidence of micronuclei induced by different doses of carbaryl (50,100,150 mg/kg body weight) treated i.p. are summarised in the Table 1. Apart from the induction of micronuclei different types of nuclear anomalies like sickle shaped, notched and blabbed nucleus also noticed. Nuclei with 2 equal or unequal lobes were observed. Anomalies such as enucleated cell, cytoplasmic projection and constriction etc. were also produced by the chemical (Figs. 1-6).

Evidently, the mean values were significantly higher in all treated groups as compared to control (* p < 0.05, ** p < 0.01). Significant variations were observed (ANOVA) in the frequency of micronuclei according to the doses (F=24.57; d.f. = 36.3; p < 0.01) but no significant variations (ANOVA) were observed among the different time intervals (F=2.48; d.f. 36.2; p< 0.05). Insignificant difference is also noted between the dose and time interaction.

Table 1: Frequency of micronuclei (MN) and other nuclear anomalies (NA) induced by carbaryl

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Time of exposure in hrs.</th>
<th>Total No. of cells studied</th>
<th>Total No. of MN</th>
<th>% aberration ± S.E.</th>
<th>Total No. of NA</th>
<th>% aberration ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24</td>
<td>16000</td>
<td>02</td>
<td>0.12 ± 0.07</td>
<td>03</td>
<td>0.18 ± 0.06</td>
</tr>
<tr>
<td>50</td>
<td>48</td>
<td>&quot;</td>
<td>02</td>
<td>0.12 ± 0.07</td>
<td>03</td>
<td>0.18 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>&quot;</td>
<td>03</td>
<td>0.18 ± 0.06</td>
<td>02</td>
<td>0.12 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>&quot;</td>
<td>06</td>
<td>0.37 ± 0.07*</td>
<td>05</td>
<td>0.31 ± 0.11</td>
</tr>
<tr>
<td>100</td>
<td>48</td>
<td>&quot;</td>
<td>06</td>
<td>0.37 ± 0.07*</td>
<td>04</td>
<td>0.25 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>&quot;</td>
<td>07</td>
<td>0.43 ± 0.06*</td>
<td>04</td>
<td>0.25 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>&quot;</td>
<td>08</td>
<td>0.50 ± 0.10*</td>
<td>05</td>
<td>0.31 ± 0.11</td>
</tr>
<tr>
<td>150</td>
<td>48</td>
<td>&quot;</td>
<td>09</td>
<td>0.56 ± 0.06**</td>
<td>06</td>
<td>0.37 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>&quot;</td>
<td>11</td>
<td>0.68 ± 0.06**</td>
<td>06</td>
<td>0.37 ± 0.07</td>
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<td></td>
<td>24</td>
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<td>09</td>
<td>0.56 ± 0.11**</td>
<td>05</td>
<td>0.43 ± 0.11</td>
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</table>
| Results are mean %± SE of four fishes.
Result is significantly different from the control at *P < 0.05; **P< 0.01
(F=0.1442; F=36.6; p>0.05). Significant difference is found between the 50 and 100 mg/kg body weight, while no significant difference between the two higher doses i.e. 100 and 150 mg/kg of the body weight was noticed. Moreover, a linear increase in the frequency of micronuclei with the dose was observed (b=0.003; r=0.978; p<0.05) for peripheral blood erythrocytes as revealed by linear regression analysis (Fig. 7).

**Discussion**

As mentioned earlier carbaryl has been reported to produce positive results in Ames *Salmonella* test and chromosomal aberration in mammalian cell culture (Ishidate *et al.*, 1988).
Genotoxic effect of Carbaryl in A. testudineus

Carbaryl reacts with nitrous acid present in the stomach and leads to the formation of N-nitroso carbaryl (Elespuru and Lejinsky, 1973). N-nitroso carbaryl has been reported to be mutagenic in yeast (Elespuru et al., 1974).

In the present study all doses of carbaryl induced significantly higher number of micronuclei than that in controls. The frequency of micronuclei increased with dose indicating dose dependent effect of the chemicals. It is also evident that the highest dose used produced highest number of micronuclei at 72 hours (Table 1). Present results revealed the genotoxic potential of carbaryl.

Similar time related effects were observed in the peripheral blood erythrocytes of fish like Heteropneustes fossilis exposed to different concentrations of paper mill effluents (Das and Nanda, 1986). The present results are also in conformity with the previous findings regarding genotoxic effect of carbaryl in chick (Bhunuya and Jena, 1995), mouse in vivo systems (Pal and Bhunuya, 1998) and in fish in vivo systems (Sahoo and Bhunuya, 2001). Exposure to carbaryl causes some histopathological changes like degranulation and vacuolation in liver cell of fish and no distinction was observed in exocrine and endocrine parts due to distraction (Singh and Shrivastava, 1998).

Besides insecticidal properties, carbaryl has some adverse effects on the body weight of some fishes and developmental stages of some crustaceans (Robel et al., 1982). Carbamate pesticides like carbenazim and benomyl showed positive mutagenic effect with very high dose in certain systems. The benzimidazol moiety may act as base analogue for DNA and acts as spindle poison. These chemicals are also considered to be antimitotic agents and mitotic arresters, cause mitotic delay and low incidence of chromosome delay (WHO, 1986). So similar type of mechanism of action of carbaryl might be responsible for the induction of micronuclei in the presently tested fish system. Since large number of commercial pesticide formulations and mixtures manufactured by agrochemical industries are in our agroecological environment, it is difficult to evaluate all such chemicals. So it is suggested that the genotoxicity of commonly used pesticides should be evaluated with priority and comparatively safer pesticides should be recommended. It is also suggested that the use of carbaryl should be restricted.

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References


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