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Detection of Mutagenic Activities of Various Pesticides in *Neurospora crassa*

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Pesticides are chemicals intentionally introduced to the environment and have become a necessity in agriculture as well as in pest control. Among the potential hazardous effects of pesticides. mutagenesis and carcinogenesis are of special concern. For this reason the mutagenic activities of various pesticides have been the object of extensive research. The studies indicate the potential of pesticides tocause cancer (Ames et al.. 1975; Waters et al.. 1980; Rosenkraz et al.. 1984; Gichner et al.. 1990; Canna-Michaelidou and Nicolaou. 1996; Kornuta et al.. 1996; Lewalter and Leng. 1999).

The mutagenicity of several pesticides known to be carcinogenic in the Salmonella microsome test system (Ames. 1979) were tested for the induction of recessive lethal mutations in the adenine-3 (ad-3) region in *Neurospora crassa*. The ad-3 forward mutation system of *N. crassa* developed by de Serres and coworkers (Dee Serres and Malling. 1971) is one of the comprehensive microbial assay systems for chemical mutagenesis studies.

However the disadvantages of this system are as follows; the time required for recovery of induced mutants. a requirement for processing large volumes of incubation medium and lastly the requirement for specialized facilities . For this reason the Neurospora reverse-mutation assays developed by Westergaard and Mitchell was one of the first assays used to test the mutagenic activity of environmental chemicals . During the past years. attempts have been made to develop a tester set and to develop spot. plate and suspension tests in *N. crassa* to provide a highly sensitive. rapid and inexpensive assay system in an eukaryotic organism (De Serres and Brockman. 1995; De Serres et al. 1997; Ong, 1978).

Cypermethrin and its dervatives are synthetic pyrethroid insecticides and propoxur is a widely used carbamate insecticides that are used for pest control in agriculture (in cotton. vegetables and other crops) and are also used in veterinary products.

In this study the mutagenic activities of six pesticides (cypermethrin and four derivatives and propoxur) were studied in Neurospora-reverse mutation assay and N23, N24 strains of *Neurospora crassa* have been used as testers for the suspension tests.

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MATERIALS AND METHODS

N. crassa N23 and *N. crassa* N24 were obtained from The Fungal Genetics Stock Center Department of Microbiology. University of Kansas Medical School. Kansas City. K. S. 66160 7420 Fed ID 48-60 299925.

The products were obtained commercially from the sources indicated: cypermethrin and cypermethrin derivatives (α, β, cis and trans cyp.) (Hektas Chemical Company). propoxur (Bayer Company). $MgCl_2.6H_2O$, sorbose, glucose, NaCl, KCl, $CaCl_2.2H_2O$, $NaH_2PO_4.2H_2O$, $Na_2HPO_4.2H_2O$, $N_2H_4O_3$, glycerol and silica gel 60 (Merck), ammonium tartarate (piedel de Haen), adenine sulphate. Pesticides were of 94-99 % purity.

Vegetative cultures and stocks were prepared according to Ong (1978). A silica gel stock was prepared for each tester and was kept at 4^0C. Vegetative cultures of the testers were started by transferring a few silica gel crystals from stocks. Fries' minimal medium supplemented with 0.75% fructose, 0.75% glucose, 10μg/ml calcium pentothenate, 100 μg/ml adenine sulfate and 1.2% Difco agar was used for slants (Horowitz and Beadle. 1943). After 7 of days incubation at 25 ^{0}C to permit vegetative growth and conidiation. cultures were ready to be used. These cultures can be stored at 4^0C for three months.

The vegetative cultures of two strains were used to prepare conidial suspensions, one day before the experiments according to Ong (1978). The final concentration of conidia was adjusted to $2x10^7$ conidia/ml. The conidial suspensions were stored at 4^0C until used in the experiment.

The method for the suspension test was performed basically the same as according to Malling and de Serres (1968). In briefly, a total of $1x10^8$ conidia (N23 or N24) were treated with 0.1ml pesticide solution in 4ml of 0.06 M phosphate buffer (pH 7) at 30^0C in a test-tube rotator. In the control 0.1ml of solvent was added instead of the pesticide solution. After 2h treatment, the conidia were washed twice with pH 8 Fries' basal medium and were suspended into 5 ml of the same medium.

For the survival test 0.1 ml of the conidial suspension was diluted to $2x10^3$ conidia per ml and 0.5 ml of the diluted conidial suspension was added to 100 ml of Westergaards' medium supplemented with 1% sorbose, 0.05% glucose, 0.05% fructose, 5μg/ml calcium pantothenate, 0.1% vitamin solution, 0.02% bactocasamino acids, 25 μg/ml adenine sulphate and 1.5% Bacto agar (Ong. 1978). The medium was poured into 5 petri dishes and the colonies were counted after incubation at 30^0C for 2 days. For the reversion test, the remaining (4.9 ml) conidial suspension was added to 100 ml of a medium similar to that used for the survival test except that the concentration of adenine sulphate was reduced to 0.1μg/ml. The medium was then poured into 5 petri dishes. Reversion colonies were counted after 3-5 days of incubation at 30^0C. Reversion frequencies were determined from the number of revertants and the viable number of conidia.
Sprague-Dawley male rats were used for the preparation of the liver S-9 fraction. 3-methylcholanthrene and phenobarbital were used for induction of rat liver enzymes.

Consequently two types of cytochrome (cyt p-450 and cyt p-448) were activated by using these two chemicals (Singer and Grunberger, 1983) 3-methylcholanthrene was diluted in corn oil (125 mg/kg body weight) and injected intraperitoneally to each rat five days before sacrifice. The rats were given drinking water ad libitum for five days. Phenobarbital was added to drinking water (0.1% w/v) for five days before rat were sacrificed. Preparation of the liver S-9 fraction was based on the procedure of Garner et al, (1972). The protein concentration of the S-9 fraction was determined by the procedure of Lowry et al, (1951). The total protein content of the S-9 fraction was 9 mg/ml.

Aflatoxin B_1 (causes frame-shift mutation) and 4NQO (causes frame-shift mutation and base-pair substitution) were used as positive mutagens in the presence and absence of S-9 fraction in both strains, respectively (Ong. 1977; Purchase et al., 1981).

RESULTS AND DISCUSSION

In this study, the mutagenic activities of several pesticides were investigated by using the Neurospora test system. The results are shown in Tables 1-6. The data were analyzed using theMann-Whitney statistical method (Sokal and Rholf, 1995).

α-cyp was weakly mutagenic ($p<0.1$) in the presence of the S-9 fraction in *N. crassa* N24 strain at concentrations 5 mg/ml and 25 mg/ml. and highly mutagenic ($p<0.05$) at concentrations 10 mg/ml and 50 mg/ml. α-cyp, in the absence or presence of the S-9 fraction in *N. crassa* N23 have not been found mutagenic at all concentrations that have been tested. α-cyp significantly decreased the percentage of survivals ($p<0.05$) in the presence of S-9 fraction in the N24 strain at all concentrations,whereas no effect was observed toxic for N23 strain with or without S-9 fraction (Table 1).
β-cyp was weakly mutagenic ($p< 0.1$) in the presence of S-9 fraction in *N. crassa* N23 strain at concentrations 15 mg/ml and 30 mg/ml and. mutagenic ($p<0.05$) in the presence of S-9 fraction in *N. crassa* N24 strain at concentration 30 mg/ml. β-cyp significantly decreased the percentage of survivals ($p<0.05$) in the presence of S-9 fraction in N23 strain at concentration 7.5 mg/ml (Table 2).
cis-cyp was mutagenic ($p<0.05$) in the presence of S-9 fraction in the N24 strain at concentrations 10 mg/ml and 50 mg/ml. cis-cyp decreased approximately 50% of survivals degree in the absence of S-9 fraction in *N. crassa* N24 at concentration 25 mg/ml and it has been found cytotoxic at concentration 50 mg/ml (Table 3).
trans-cyp was weakly mutagenic ($p<0.1$) in the absence of S-9 fraction in *N. crassa* N23 strain at concentration 40 mg/ml and significantly mutagenic ($p<0.05$) in the presence of S-9 fraction in *N. crassa* N23 strain at concentrations 5 mg/ml and 20 mg/ml. Trans-cyp was significantly mutagenic ($p<0.05$) in the presence of S-9 fraction in *N. crassa* N24 strain at concentration 10 mg/ml. Generally to be considered mutagenic a chemical is expected to show a dose response. In addition, a chemical is considered mutagenic if it causes at least a doubling of the spontaneous mutation frequency. Occasionally, non-linear dose-response curves are obtained in Salmonella /microsome test system and in *Neurospora crassa* (Maron and Ames, 1983; Brockman et al., 1984).

trans-cyp, decreased the percentage of survivals in N24 in the absence of S-9 fraction. Cypermethrin has not been found mutagenic in both of the two strains with or without S-9 fraction at all concentrations but it decreased the percentage of survivals ($p< 0.1$) in *N. crassa* N23 strain with or without S-9 fraction (Table 5). Propoxur was weakly mutagenic ($p< 0.1$) in the presence of S-9 fraction in *N. crassa* N23 strain at concentration 200 mg/ml. It was also weakly mutagenic ($p< 0.1$) in *N. crassa* N24 strain with S-9 mix at concentration 40 mg/ml. and it was mutagenic ($p< 0.05$) at concentrations 100 mg/ml and 200 mg/ml. Propoxur was weakly mutagenic ($p< 0.1$) in the presence of S-9 fraction in *N. crassa* N23 strain at concentration 200 mg/ml. It was also weakly mutagenic ($p< 0.1$) in *N. crassa* N24 strain with S-9 mix at concentration 40 mg/ml and it was mutagenic ($p< 0.05$) at concentrations 100 mg/ml and 200 mg/ml. Propoxur decreased the percentage of survivals in the absence or presence of S-9 fraction in *N. crassa* N23 strain at concentration 200 mg/ml. It significantly decreased ($p<0.05$) the percentage of survivals in *N. crassa* N24 strain with S-9 fraction at all concentrations tested. It has slightly decreased ($p< 0.1$) the percentage of survivals in *N. crassa* N24 strain without S-9 fraction at concentrations 20-40 mg/ml and significantly decreased ($p< 0.05$) at concentration 200 mg/ml (Table 6).

In this study, the mutagenic activity of six pesticides was tested in N23 and N24 using a suspension test. N23 and N24 strains selected from hundreds of ad-3 mutants (Ong, 1978) are highly sensitive to mutagens and are revertible by a spesific group of chemicals. N23 can be reverted from adenine dependence to adenine independence by agents which cause base-pair substitutions whereas N24 can be reverted by frameshift mutagens. Cypermethrin and its derivatives α, β, cis and trans-cyp were found to be nonmutagenic in the absence of S-9 fraction in both strains. Also cypermethrin has been found to be non-mutagenic by Pluijman et al (1984). In the presence of S-9 fraction, β and trans-cyp were mutagenic in both strains; α-cyp and cis-cyp were found to be mutagenic only in N24 strain in the presence of S-9 fraction (Table 1,2,3,4). β-cyp and trans-cyp seem to cause base-pair substitution as well as frameshift mutation under the same conditions. However, α-cyp and cis-cyp only revert N24 strain which carries frameshift mutations. These results are in agreement with previous studies on the mutagenicity of cypermethrin that have been performed in *Salmonella typhimirium* TA1538, TA98, TA100 by Brooks(1979) (Bhunya and Pati, 1988). Propoxur (carbamate insecticide), only in the presence of the S-9 fraction in N24 strain was observed to be mutagenic and seems to cause frameshift mutation (Table 6). The mutagenic action of this pesticide has been investigated by Sümer et al (1990) with *Salmonella typhimurium* TA98 and TA100 and was found mutagenic only in TA98 and causes frameshift mutation.

Except cypermethrin, the other pesticides tested in this study were found to be mutagenic, after S-9 activation. The results from *Neurospora crassa* test system are in agreement with Salmonella test system. The methods of suspension tests described here for Neurospora are very similar to those used for bacteria. These test systems are rapid, sensitive and valuable in proving the mutagenic activity. Since the correlation between carcinogenicity and mutagenicity is high the pesticides that give positive result in short-term mutagenicity test systems have carcinogenic potential (Mc Cann and Ames, 1976; Ramel and Rannung, 1980).

Table 1. Mutagenic activity of α-Cyp in the presence or absence of S9 fraction in *N. crassa* N23 and N24 strains.•

Dose mg/ml	N23				N24			
	$S9^-$ mean±SD		$S9^+$ mean±SD		$S9^-$ mean±SD		$S9^+$ mean±SD	
α-Cyp	Survivals (%)	Revertants	Survivals (%)	Revertants	Survivals (%)	Revertants	Survivals (%)	Revertants
0	100 ±0.000	0.40 ±0.00	100 ±0.00	2.40 ±2.07	100 ±0.00	0.50 ±0.10	100 ±0.00	1.20 ±0.44
5	99.01 ±10.42	0.00 ±0.00	101.17 ±8.49	0.60 ±0.55	96.55 ±15.55	0.30 ±0.95	70.51 ±4.50	0.40 ±0.55
10	97.53 ±11.38	0.50 ±0.85	99.81 ±4.96	1.60 ±0.55	100.10 ±16.62	0.00 ±0.84	65.04 ±4.08	0.20 ±0.44
25	98.98 ±6.14	0.30 ±0.94	96.63 ±6.40	1.20 ±0.44	94.22 ±10.43	0.40 ±0.70	67.59 ±2.65	2.00 ±0.70
50	98.98 ±7.50	1.00 ±1.41	101.10 ±1.44	2.20 ±2.95	101.33 ±8.66	0.50 ±1.08	72.77 ±3.41	34.80 ±6.22
4NQO 20	83.94 ±9.02	10.20 ±3.70	-	-	88.14 ±8.71	2.00 ±2.33	-	-
40	62.09 ±7.95	22.10 ±4.72	-	-	82.60 ±6.69	5.60 ±1.89	-	-
▼Afl.B_1 20	-	-	96.80 ±3.76	0.40 ±0.50	-	-	97.30 ±8.43	2.00 ±2.32
P_5	1.000	0.143	0.841	0.151	0.739	0.353	0.048^*	0.095^{**}
P_{10}	0.684	0.971	1.000	0.690	0.579	0.631	0.008^*	0.032^*
P_{25}	0.853	0.353	0.841	0.421	0.280	0.739	0.008^*	0.095^{**}
P_{50}	0.912	0.684	0.832	0.690	0.875	0.631	0.024^*	0.008^*
$P_{20(4NQO)}$	0.053^{**}	0.000^*	-	-	0.059^{**}	0.196	-	-
$P_{40(4NQO)}$	0.000^*	0.000^*	-	-	0.053^{**}	0.052^{**}	-	-
$P_{20(Afl.B1))}$	-	-	0.782	0.286	-	-	0.751	0.062^{**}

*: P< 0.05 (significantly different) **: P< 0.1 (weakly different) ▼: Aflatoxin B_1

•: Each value is the mean of three seperate experiments with five plates each.

Table 2. Mutagenic activity of β-Cyp in the presence or absence of S9 fraction in *N. crassa* N23 and N24 strains. •

Dose mg/ml	N23				N24			
	$S9^-$ mean±SD		$S9^+$ mean±SD		$S9^-$ mean±SD		$S9^+$ mean±SD	
β-Cyp	Survivals (%)	Revertants	Survivals (%)	Revertants	Survivals (%)	Revertants	Survivals (%)	Revertants
0	100 ±0.00	0.20 ±0.42	100 ±0.00	0.80 ±1.09	100 ±0.00	0.30 ±0.67	100 ±0.00	1.40 ±1.51
7.5	103.67 ±6.81	0.40 ±0.70	92.04 ±3.83	1.00 ±1.22	102.62 ±10.88	0.50 ±0.70	97.03 ±7.12	1.40 ±1.14
15	105.50 ±11.00	0.60 ±0.96	94.82 ±4.14	3.00 ±2.23	101.65 ±12.52	0.50 ±0.97	104.61 ±6.45	0.00 ±0.00
30	94.71 ±7.53	0.60 ±0.96	94.03 ±4.20	3.40 ±2.07	92.57 ±11.77	0.10 ±0.31	99.04 ±7.03	13.40 ±3.97
60	94.46 ±7.53	0.10 ±0.31	101.30 ±3.94	2.20 ±1.92	86.58 ±15.24	0.60 ±1.07	104.29 ±5.39	0.20 ±0.44
4NQO 20	83.94 ±9.02	10.20 ±3.70	-	-	88.15 ±8.71	2.00 ±2.33	-	-
40	62.09 ±7.95	22.10 ±4.72	-	-	82.60 ±6.69	5.60 ±1.89	-	-
▼Afl.B_1 20	-	-	96.80 ±3.76	0.40 ±0.50	-	-	97.30 ±8.43	2.00 ±2.32
$P_{7.5}$	0.247	0.684	0.216	0.841	0.802	0.529	0.708	1.000
P_{15}	0.218	0.436	0.256	0.095**	0.971	0.739	0.625	0.151
P_{30}	0.280	0.436	0.256	0.056**	0.280	0.684	0.728	0.008*
P_{60}	0.280	0.739	0.841	0.222	0.072**	0.684	0.624	0.222
$P_{20(4NQO)}$	0.060**	0.000*	-	-	0.069**	0.196	-	-
$P_{40(4NQO)}$	0.005*	0.000*	-	-	0.058**	0.052**	-	-
$P_{20(Afl.B1)}$	-	-	0.782	0.286	-	-	0.751	0.062**

*: P< 0.05 (significantly different) **: P< 0.1 (weakly different) ▼Afl. B_1: Aflatoxin B_1

•: Each value is the mean of three seperate experiments with five plates each.

Table 3. Mutagenic activity of cis-Cyp in the presence or absence of S9 fraction in *N. crassa* N23 and N24 strains. •

Dose mg/ml	N23				N24			
	$S9^-$ mean±SD		$S9^+$ mean±SD		$S9^-$ mean±SD		$S9^+$ mean±SD	
Cis-Cyp	Survivals (%)	Revertants	Survivals (%)	Revertants	Survivals (%)	Revertants	Survivals (%)	Revertants
0	100 ±0.00	0.40 ±0.51	100 ±0.00	2.40 ±2.07	100 ±0.00	0.50 ±0.70	100 ±0.00	1.20 ±0.44
5	82.97 ±6.81	0.20 ±0.42	93.20 ±2.15	2.20 ±1.303	94.32 ±12.84	0.30 ±0.48	91.47 ±6.57	1.60 ±1.14
10	82.30 ±4.67	0.20 ±0.42	75.20 ±3.28	1.40 ±1.67	101.27 ±7.40	0.10 ±0.31	100 ±3.43	0.20 ±0.44
25	83.47 ±9.77	0.30 ±0.67	94.00 ±4.65	0.60 ±0.89	51.85 ±27.94	0.00 ±0.00	99.74 ±3.90	0.60 ±0.89
50	84.87 ±7.95	0.40 ±0.96	94.70 ±3.44	1.20 ±2.16	0.00 ±0.00	0.10 ±0.31	101.97 ±3.67	0.00 ±0.00
4NQO 20	92.45 ±6.53	3.20 ±2.92	-	-	86.27 ±7.45	1.30 ±2.05	-	-
40	58.77 ±8.01	10.70 ±4.88	-	-	88.62 ±6.33	37.20 ±12.07	-	-
▼Afl.B_1 20	-	-	106.12 ±4.76	2.00 ±0.70	-	-	104.80 ±10.25	4.80 ±2.49
P_5								
P_{10}	0.055**	0.481	0.216	1.000	0.481	0.631	0.786	0.548
P_{25}	0.055**	0.481	0.048*	0.421	0.165	0.247	1.000	0.032*
P_{50}	0.062**	0.579	0.215	0.151	0.000*	0.143	0.841	0.222
$P_{20(4NQO)}$	0.058**	0.579	0.215	0.310	0.000*	0.247	0.690	0.008*
$P_{40(4NQO)}$	0.280	0.008*	-	-	0.076**	0.247	-	-
$P_{20(Afl.B1)}$	0.000*	0.000*	-	-	0.063**	0.000*	-	-
	-	-	0.795	0.151	-	-	0.656	0.032*

*: P< 0.05 (significantly different) **: P< 0.1 (weakly different) ▼Afl.B_1: Aflatoxin B_1

•: Each value is the mean of three seperate experiments with five plates each.

Table 4. Mutagenic activity of trans-Cyp in the presence or absence of S9 fraction in *N. crassa* N23 and N24 strains. •

Dose mg/ml	N23				N24			
	$S9^-$ mean±SD		$S9^+$ mean±SD		$S9^-$ mean±SD		$S9^+$ mean±SD	
Trans-Cyp	Survivals (%)	Revertants	Survivals (%)	Revertants	Survivals (%)	Revertants	Survivals (%)	Revertants
0	100 ±0.00	0.20 ±0.42	100 ±0.00	0.80 ±1.09	100 ±0.00	0.30 ±0.67	100 ±0.00	1.40 ±1.51
5	93.61 ±4.62	0.20 ±0.42	93.83 ±2.26	3.80 ±0.83	102.20 ±23.21	0.20 ±0.42	92.65 ±6.30	1.00 ±0.00
10	98.36 ±10.19	0.30 ±0.48	91.14 ±3.84	1.60 ±0.89	103.75 ±14.18	0.00 ±0.00	98.44 ±6.25	10.8 ±4.65
20	98.48 ±8.49	0.10 ±0.31	94.42 ±4.57	8.00 ±4.84	87.33 ±17.04	0.10 ±0.31	89.60 ±6.03	0.40 ±0.54
40	95.23 ±6.25	1.80 ±2.04	93.43 ±4.64	1.40 ±0.54	78.61 ±5.38	0.00 ±0.00	94.7 ±8.32	1.00 ±1.41
4NQO 20	83.94 ±9.02	10.20 ±3.70	-	-	88.14 ±8.71	2.00 ±2.33	-	-
40	62.09 ±7.95	22.10 ±4.72	-	-	82.60 ±6.69	5.60 ±1.89	-	-
▼Afl.B_1 20	-	-	96.80 ±3.76	0.40 ±0.50	-	-	97.30 ±8.43	2.00 ±2.32
P_5	0.143	1.000	0.148	0.008*	0.796	0.971	0.208	1.000
P_{10}	0.796	0.739	0.216	0.310	0.529	0.481	0.351	0.008*
P_{20}	0.853	0.739	0.151	0.008*	0.063**	0.684	0.078**	0.421
P_{40}	0.280	0.075**	0.195	0.421	0.059**	0.481	0.476	0.690
$P_{20(4NQO)}$	0.061**	0.000*	-	-	0.079**	0.196	-	-
$P_{40(4NQO)}$	0.000*	0.000*	-	-	0.058**	0.052**	-	-
$P_{20(AflB1)}$	-	-	0.476	0.286	-	-	0.751	0.062**

*: $P < 0.05$ (significantly different) **: $P < 0.1$ (weakly different) ▼Afl.B_1: Aflatoxin B_1

•: Each value is the mean of three seperate experiments with five plates each.

Table 5. Mutagenic activity of Cyp in the presence or absence of S9 fraction in *N. crassa* N23 and N24 strains. •

Dose mg/ml	N23				N24			
	$S9^-$ mean±SD		$S9^+$ mean±SD		$S9^-$ mean±SD		$S9^+$ mean±SD	
Cyp	Survivals (%)	Revertants	Survivals (%)	Revertants	Survivals (%)	Revertant	Survivals (%)	Revertants
0	100 ±0.00	0.20 ±0.42	100 ±0.00	0.80 ±1.09	100 ±0.00	0.30 ±0.67	100 ±0.00	1.40 ±1.51
20	86.28 ±10.78	0.00 ±0.000	81.39 ±6.02	0.20 ±0.44	104.01 ±3.96	0.10 ±0.31	93.12 ±2.95	0.60 ±0.89
40	95.89 ±9.43	0.20 ±0.42	81.94 ±3.81	0.80 ±0.83	101.32 ±5.74	0.30 ±0.67	96.01 ±10.80	0.60 ±0.54
100	89.72 ±9.24	0.20 ±0.42	92.06 ±1.83	1.60 ±1.14	103.39 ±5.99	0.00 ±0.00	100.30 ±7.65	2.80 ±1.64
200	90.87 ±10.08	0.10 ±0.31	86.61 ±5.72	0.40 ±0.54	101.82 ±7.33	0.30 ±0.67	99.80 ±7.34	0.00 ±0.00
4NQO 20	92.45 ±6.53	3.20 ±2.92	-	-	86.27 ±7.45	1.30 ±2.05	-	-
40	58.77 ±8.01	10.70 ±4.88	-	-	88.62 ±6.33	37.20 ±12.07	-	-
▼Afl.B_1 20	-	-	106.12 ±4.76	2.00 ±0.707	-	-	104.80 ±10.25	4.80 ±2.49
P_{20}	0.072**	0.481	0.058**	0.548	0.218	0.684	0.332	0.421
P_{40}	0.163	1.000	0.058**	1.000	0.219	1.000	0.310	0.548
P_{100}	0.061**	1.000	0.288	0.310	0.205	0.481	0.356	0.222
P_{200}	0.129	0.739	0.076**	0.690	0.263	1.000	0.318	0.151
$P_{20(4NQO)}$	0.280	0.008*	-	-	0.075**	0.247	-0.656	-
$P_{40(4NQO)}$	0.000*	0.000*	0.795	-	0.071**	0.000*	-	-
$P_{20(Afl.B1)}$	-	-	-	0.151	-	-		0.032*

*: P< 0.05 (significantly different) **: P< 0.1 (weakly different) ▼Afl.B_1: Aflatoxin B_1

•: Each value is the mean of three seperate experiments with five plates each.

Table 6. Mutagenic activity of propoxur in the presence or absence of S9 fraction in *N. crassa* N23 and N24 strains.•

Dose mg/ml	N23				N24			
	$S9^-$ mean±SD		$S9^+$ mean±SD		$S9^-$ mean±SD		$S9^+$ mean±SD	
Propoxur	Survivals (%)	Revertants	Survivals (%)	Revertants	Survivals (%)	Revertants	Survivals (%)	Revertants
0	100 ±0.000	0.40 ±0.51	100 ±0.00	2.40 ±2.07	100 ±0.00	0.50 ±0.70	100 ±0.00	1.20 ±0.44
20	94.59 ±7.80	0.50 ±0.70	103.77 ±6.49	0.80 ±0.83	88.98 ±5.58	0.20 ±0.42	60.27 ±6.37	1.20 ±1.09
40	93.62 ±6.13	0.50 ±0.70	100.40 ±1.59	0.80 ±1.09	89.93 ±8.94	0.10 ±0.31	63.77 ±1.84	0.40 ±0.54
100	92.81 ±6.01	0.20 ±0.42	94.43 ±3.45	1.20 ±0.44	92.42 ±6.01	0.20 ±0.42	62.69 ±3.57	0.20 ±0.44
200	42.60 ±5.63	0.00 ±0.00	75.44 ±8.90	0.40 ±0.54	30.02 ±3.22	0.00 ±0.00	55.75 ±7.14	0.00 ±0.00
4NQO 20	92.45 ±6.53	3.20 ±2.92	-	-	86.27 ±7.45	1.30 ±2.05	-	-
40	58.77 ±8.01	10.70 ±4.88	-	-	88.62 ±6.33	37.20 ±12.07	-	-
▼Afl.B_1 20	-	-	106.12 ±4.76	2.00 ±0.70	-	-	104.80 ±10.25	4.80 ±2.49
P_{20}	0.105	0.912	0.421	0.222	0.071**	0.436	0.008*	0.841
P_{40}	0.119	0.912	0.548	0.222	0.063**	0.247	0.008*	0.095**
P_{100}	0.135	0.481	0.310	0.421	0.283	0.436	0.008*	0.032*
P_{200}	0.000*	0.143	0.058**	0.095**	0.000*	0.143	0.008*	0.008*
$P_{20(4NQO)}$	0.280	0.008*	-	-	0.076**	0.247	-	-
$P_{40(4NQO)}$	0.000*	0.000*	-	-	0.074**	0.000*	-	-
$P_{20(Afl.B1)}$	-	-	0.795	0.151	-	-	0.656	0.032*

*: P< 0.05 (significantly different) **: P< 0.1 (weakly different) ▼Afl.B_1: Aflatoxin B_1

•: Each value is the mean of three seperate experiments with five plates each.

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