REMEDIATION OF MUTAGENICITY EFFECT OF HERBICIDES: ATRAZINE, CHLORPYRIFOS, MONOSODIUM METHANE ARSONATE, AND METHYL MERCURY IN ARTIFICIAL WETLANDS

***Wen-Hsun Yang, *Jen-Rong Yang, and **Ken Overstreet**
***Bioremediation Education, Science and Technology Center, and Biology Department**
School of Science and Technology
Jackson State University
****Department of Biology, University of Mississippi**

INTRODUCTION

Atrazine (ATR) (2-Chloro-4-ethylamino-6-isopropylamino-s-triazine) was the first herbicide synthesized for control of weed in agriculture fields since 1959. About 6 years later, chlorpyrifos (CPF) (O.O-Dimethyl-O-[3.5.6-trichloro-2-pyridyl]phosphothioate) was introduced into the market to substitute for the more toxic insecticide, DDT [(trichloro-bis (p-chlorophenyl)ethane]. More selective contact grassweed herbicide, monosodium methane arsonate, (MA), was also produced thereafter. The popular use of ATR and MA with DDT or CPF to have combined effects of herbicide and insecticide became a routine practice for increase of agricultural production. However, the massive use of agrichemicals worried many investigators into the possible environmental disaster. It was estimated in 1982 that 95% of all the corn growing region in Iowa was treated with nearly 52 million pounds of herbicide, and 52% of the region was treated with nearly 7.9 million pounds of insecticide (Kelly 1986). It is with great concern that these agrichemicals were discovered and are increasing in our groundwater, as well as in surface waters (Kelly 1986; Cohen et al. 1984; Kross et al. 1990). It is also our great concern that these 3 agrichemicals may interact with increasing background of methyl mercury (HG) in this area (about 0.2 mg/Kg in wet weight in southeastern US) for unpredictable toxic effect. The soluble concentrations of mercury in Mississippi River (5.626 ug/L) and Pearl River (5.029 ug/L) were known to be very high in this area (Yang et al. 1994). As a single chemical, ATR was known to exert its inhibitory effect mainly on photosynthesis by blocking the electron transport chain of phtosystem II. This resulted in the destruction of chlorophyll and cell death in plants (Solomon et al. 1996). MA interfered with the normal metabolism and growth of plants by mimicking phosphate in absorption, translocation, and metabolic pathway. MA is also considered to be toxic to animal for neurologic, reproductive, and gene function (Menzer 1991; Tomlin 1994). CPF is mainly toxic to animals for its irreversible inhibitory function to acetylcholinestrase (Menzer 1991; Sorrano et al. 1995). HG accumulated in organisms by formation of analog close to methionine for entry into amino acids and proteins. Upon binding to thiol-containing enzymes, the activity of the protein will be greatly reduced (Clarkson 1994). Conversion of methyl mercury into inorganic mercury resulted in increased hydrogen peroxide production and disrupted electron transfer in electron transport chains also (Lindqvist 1991; Clarkson 1994). Current reviews of atrazine (Brusick 1994), mercury compounds (Flora et al. 1994), inorganic arsenic compounds (de la Rosa et al. 1994), and organophosphosphate (Gollapudi et al. 1995), about the genotoxicological effects of those chemicals were inconclusive. Most of the laboratory tests showed no mutagenecity of those chemicals in the Salmonella mutagenicity test. The current experiment intends to use a more sensitive strain of Salmonella, TA102 (Levin et al. 1982), for more sensitive testing of those chemicals in solitary application or in mixed application with various combinations to those mesocosms.

MATERIALS AND METHODS

Salmonella Mutagenetic Test Strain

The Salmonella mutagenicity test strain TA102 (Levin et al. 1982) used in the current experiment was obtained from Dr. Ames at the University of California at Berkeley. Upon its arrival, the test strain was kept in the master plate containing histidine and 10 ug/ml tetracyclin in minimal glucose plates. Following characterization of genetic background, a well tested colony of the strain was cultured in the Nutrient Broth No. 2 to late exponential phase in the shaker incubator (250 rpm, 30°C). The culture was mixed with autoclaved glycerol to 15% in total concentration for permanent storage in the deep freezer at -85°C.

Chemicals

Atrazine (2-Chloro-4-ethylamino-6-isopropylamino-s-triazine), MSMA (Monosodium methane arsonate), and Chloropyrifos (O.O-Dimethyl-O-[3.5.6-trichloro-2-pyridyl]phosphothioate), with the high purity of EPA standards were purchased from the Chem Service Company. Methyl mercury (Dimethyl mercury) was purchased from the Aldrich Chemical Company. Daunomycin was purchased from the Sigma Company. Most of the other

laboratory chemicals were obtained from the Fisher Scientific Company.

Experimental Design and Sampling

For the entire complex experiment, sixty-six 500-liter plastic mesocosms were assembled at the University of Mississippi's Biological Field Station in northern Mississippi. They were filled with 15 cm of sand and a 5 cm thick top layer of sediment from a nearby pond-3. Six baskets of Juncus effusus were planted in each mesocosm. Thereafter, the mesocosms were filled with water from the near-by Pond-3. A center-point enhanced 2{+4} factorial design was used for treatment of methyl mercury (HG), monosodium acid methane arsonate (MSMA), atrazine (ATZ), and chlorpyrifos (CPF). Immediately before the experiment, methyl mercury was added into soil sediment with the concentration of 0 ug/kg (-1 in treatment code), 0.2 ug/Kg (0 in treatment code), and 0.4 ug/kg (+1 in treatment code), respectively. Three agrichemicals, atrazine, chlorpyrifos, and MSMA were applied to 66 mesocosms at 0% (-1 in treatment code number), 50% (0 in treatment code number), and 100% (+1 in treatment code number) of the expected environment concentration (EEC) model (192 ppb, 219 ppb and 51 ppb), respectively on June 10, 1996. The EEC model were expected concentration values of agrichemicals for wetlands downstream of agricultural fields receiving runoff two days after an agricultural application. The current experiment involved one third of the total mesocosms (66 tanks) treated on June 10, with about half of them (10) treated with redoses on Day 62 according to the code indicated on Table 1. Surface water of 100 ml were collected and filtrated aseptic filter with pore size of 0.2 um on Day 1 (June 10), Day 8 (June 18), and Day 64 (August 13) and thereafter frozen and stored in a deep freezer at -85°C. The entire experiment has been carried out with double blindness for prevention of bias in any experimental and statistical data until March 5, 1997. Filtered water from the Pond-3 was frozen to be used as standard (blank) of this experiment on June 10. Filtrated distilled water was also used as the negative control. In addition, Daunomycin at different concentrations, 10 ug/100 ul, 5 ug /100 ul, and 0.5 ug/100 ug was kept in a refrigerator as a positive control.

Salmonella Mutagenecity Test

For preparation of bacteria culture for test, one ml of frozen TA102 was mixed with 35 ml of Nutrient broth No. 2 for shaking culture (30^0 C, 250 rpm) to about mid logarithmic phase. For 6 different assays, respectively 0.1, 0.1, 0.1, 0.05, 0.1 and 0.2 ml of samples were preincubated with 0.1 ml of TA102 culture (at mid-logarthmic phase) for 30 minutes at 37°C. Thereafter, the primary were mixed with media A, B, C, D, E, and F. Three test media A, B, C, which contained 0.02 ml of uninduced liver enzyme fraction (S-9), 0.02 ml arclor 1254-induced liver enzyme fraction (S-9), 0.04 ml arclor 1254-induced S-9 with other minerals were used for assessment of enzyme effect on activation of test samples. Three other test media D, E, F, without S-9, were used to mix with the exponentially increased amount of sample waters 0.05, 0.1, and 0.2 ml which have been premixed with 0.1 ml of bacteria, for evaluation of concentration-related effect of mutagen in water samples (Table 2). Finally, the secondary mixtures for A, B, C, D, E, F assays were mixed with 0.2 ml of 0.5 mM Histidine/biotin and 1.8 ml of top agar (0, 5% agar in 1% NaCl solution, kept at 55°C) for vigorous vortex shaking followed by uniform coating at the top layer of MG plate (30 ml). The MG plate with coated top agar was incubated for revertants to grow at 48 hours of incubation at 37°C.

Statistical Analysis

The numbers of revertants formed by testing of one sample were compared with the numbers of those formed by the blank control water in 6 assays for analysis of the significance of differences in the sign test. Part of 6 assays, A, B, C, will be used as a subgroup for analysis of liver enzyme activation capability of the agrichemicals. Another part of 6 assays, D, E, F, will be used to examine the correlation between the amount of material and the number of revertants obtained.

SIGN TEST OF DIFFERNCE IN THE NUMBERS OF REVERTANTS

Significance of the result was analyzed with the nonparametric sign of either positive or negative number shown in the difference of revertants formed between the test sample and the control in the 6 assays (A, B, C, D, E, F). In practice, the number of revertants formed by testing of the sample was substructed by the number of revertants formed by testing of the blank control water (Pond-3 spring water). The result of substraction was recorded as either positive or negative with regard to its numerical values for nonparametric test. The accumulation of 6 assay results were listed in Tables 2 and 3 as XpYn, with X indicating the number of positive and Y indicating the number of negative in 6 assays (X+Y=6). The significant level of the comparison between the sample and the control can be evaluated from the probability distributions of 6 outcomes. The probability to have at least 6 positive signs in the current test is calculated to be 0.015625 (or $1.5625\%_{,6}C_0(0.5)^6$), whereas the probability to have at least 5 positive sign is calculated to be 0.109375 ($10.5625\%_{,6}C_0(0.5)^6$ $_6C_1(0.5)^5(0.5)^1$) according to the outcome of binomial distribution in the sign test.

MUTAGENETIC INDEX

Intensity of mutagentic effect was also evaluated by the mutagenetic index to be calculated by the following formula.

> M.I. =[(No of revertants obtained by testing sample) - (No. of revertants obtained by testing blank)] / (No. of revertants obtained by testing blank).

C/B RATIO

For evaluation of liver microsomal enzyme effect on mutagens, the number of revertants obtained for the assay C was divided by the number of revertants formed for the B assay to calculate C/B ratio in each sample or standards. The value of C/B ratio indicated the degree of enhancement of mutagenicity effect by the Aroclor 1254-induced liver enzyme in S-9 fraction.

CORRELATION COEFFICIENT BETWEEN SAMPLE CONCENTRATIONS AND NUMBERS OF REVERTANTS

The correlation coefficient between sample concentrations and numbers of revertants in D, E, F assays was calculated by the ratio of the covariance of X (concentrations of sample) and Y (numbers of revertants) to the square root of the product of Variance X with Variance Y in the following formula.

$$\gamma = \text{Covariance } XY / (\text{Variance } X)^{1/2}(\text{Variance } Y)^{1/2}$$

RESULTS AND DISCUSSION

The ambient environment created in the mesocosms (#10 mesocosm, Table 1) as the control for the examination of agrichemical effect on various organisms including microorganisms, algae, macrophytes, invertebrates (hydra, tadpole), and vertebrates (catfish), is mutagenetic. As indicated in 6 positive differences (6p0n) in 6 assays and higher mutagenetic index (5.32), it is significantly mutagenetic at the level of 1.56% in probability distribution (Table 3). There is a significant correlation between the amounts of water and the number of revertants formation also (γ=1.00). Other two negative controls in the same experiment using distilled waters also supported similar findings. Since Pond-3 has been used to supply all water and sediment required for assembly of all mesocosms, it was considered that the additional components of the mesocosm such as amacrophyte, invertebrates, and vertebrates be responsible for increase of such mutagenecity in #10 mesocosms. As indicated in the moderately high C/B ratio (1.45), those mutagens appeared to be biodegradable and degraded into non-detectable level of mutagenecity following several weeks of incubation with biorganisms and microorganisms in the mesocosm (#10). Not a few number of plant contents were already known to be mutagenetic following disruption of plant with or without isolation procedure. Both quercetin and kaempferol are well-known flavonoids of plant and fruit which frequently cause mutagenecity in grape wines or juices (Bjeldanes and Chang 1977; Harfdigree and Epler 1978; Sugimura et al. 1977). Chlorophyll was also known to have mutagenecity effect (Sarkar et al. 1996). It is possible that early phase of mutagenecity effect in the ambient control (#10) might have resulted from interaction between macrophytes and invertebrates, or between macrophytes and vertebrates. The eating process in that interaction might result in the mechanical disruption of macrophyges, algae, and other planktons in the mesocosms for leakage of flavonoids and chlorophylls in the surface water. This early phase of mutagenecity activity was probably followed by biodegradation of those mutagens at late phase by microorganisms in biodegradation process, or complete enzymatic digestion of those mutagens in the digestive tract by invertebrates or vertebrates in digestive process. Subsequently the mutagenicity of the mesocosm was reduced to no mutagenecity as indicated in the sign test of (5p1n), or reduced mutagencity index to 0.24, or 0.18 on Day 8 or Day 64, respectively.

When a single chemical of either HG (#24 mesocosm), AS (#48 mesocosm), or ATR (#47 mesocosm) was applied on Day 0 with redose on Day 62, neither HG, AS, nor ATR appeared to be mutagenetic. The positive mutagenetic effect shown on Day 1 might be the comutagenetic effect with the internal factors. The only single chemical to show mutagenenicity in current experiment was CPF (#28 mesocosm) which demonstrate a concrete positive result on Day 64 with considerably high mutagenecity index 7.59 and very low C/B ration (0.42). This suggests a possible mutagenic effect caused by irreversible and non-degradable binding of phosphate to enzyme for inhibition of enzyme related with mutation. Similar action was claimed for irreversible inbibition of acetylcholine estrase by CPF for the insecticide activity (Menzer 1991; Tomlin 1994). When two chemicals were simultaneously given on Day 0 for evaluation of mutagenecity on Day 1 and Day 64, HG was comutagenetic with AS (#42) to demonstrate strong mutagenetic effect not only on Day 1 and Day 64, but also on Day 8. This effect is accompanied with strong mutagenetic index (6.30) and CB ratio (2.23) also. Nevertheless, HG was moderately counter-mutagenetic to CPF (#18) so that mutagenecity of CPF was suppressed on Day 64. By contrast, the simultaneous application of HG and ATR (#49) appeared to be very counter-mutagenetic, so that mutagenecity effect of the mesocosms (#49) was not seen Day 1 to Day 64. Although a single chemical effect of AS (#48) or ASR (#47) was not mutagenetic, the combination of AS with ATR in mesocosm (#61) appeared to have co-

mutagenetic effect to increase mutagenetic effect of both to detectable level with increased mutagenetic index (3.17) and high C/B ratio(3.29). Simultaneous application of AS and CPF was mutagenetic also (#51 mesocosm). When three chemicals were given in different combination to the mesocosms, only the combination of HG with AS and CPF appeared to be mutagenetic. Any other combinations including HG+ATR with AS or with CPF appeared to be not mutagenetic as the extention of antimutagenetic effect of HG+ATR. Nevertheless, when either 50% (#3, #11, #53, #4, #16, #40) or 100 % doses (#35) of all 4 agrichemicals, HG, MSMA, ATR, and CPF were given together to the mesocosms, mutagenetic effect was seen in nearly all of 7 mesocosms with considerably increased mutagenetic index (1,80-20.71), suggesting comutagenetic effect of 4 agrichemicals. The mixtures of all 4 chemicals in the mesocosms in full dose (#35) was very mutagenetic. With 50% half dose, 66% of treated mesocosms (#3, #4) were mutagenetic on Day 64, whereas one third of the treated mesocosms (#11) appeared to have significant reduction of mutagenecity to undetectable level possible by the process of bioremediation. With redose, all mesocosms ((#4, #16, #46) treated with half doses of all chemicals were mutagenetic on Day 64 .

SUMMARY AND CONCLUSION

Salmonella test strain TA102 was used to test mutagenicity effect of methyl mercury (HG) and other three agrichemicals, monosodium acid methane arsonate (MA), atrazine (ATR), chlorpyrifos (CPF), and remediation effect of artificial wetlands on the chemicals. Sixty-six 500-litter plastic mesocosms were assembled with sand, soil, and water from the nearby pond-3. HG, MA, ATR, CPF were included at ambient levels or added according to a center-point enhanced-2^4 factorial design; the elevated levels were 0.4 mg HG/Kg wet weight sediment, and 219, 192, and 51 ppb of MA, ATR, and CPF in water, respectively. Surface water was sampled from each mesocosm 1, 8, and 64 days after chemicals were added. On Day 62, half of the mesocosms were redosed. For 6 different assays, respectively 0.1, 0.1, 0.1, 0.05, 0.1 and 0.2 ml of samples were preincubated with 0.1 ml of TA102 culture (at mid-logarthmic phase) for 30 minutes at 37°C before mixing with media A, B, C, D, E, F, to be included in top agar to cover the basal minimal glucose agar for 48 hours incubation. Three test media A, B, C, which contained 0.02 ml of uninduced liver enzyme fraction (S-9), 0.02 ml arclor 1254-induced liver enzyme fraction (S-9), 0.04 ml arclor 1254-induced S-9 with other minerals were used for assessment of enzyme effect on activation of test samples. Three other test media D, E, F, without S-9, were used to mix with the exponentially increased amount of sample waters 0.05, 0.1, and 0.2 ml which have been mixed with 0.1 ml of bacteria, for evaluation of concentration-related effect of mutagen in water samples. Colony numbers calculated as reversed mutations were used for statistical analysis. Differences of colony numbers between the sample and the control in 6 assays were particularly useful for non-parametric sign test for testing of significant difference at the probability level of 0.015625. The result indicated a significantly increased internal mutagenecity following the creation of mesocosms by mixing macrophytes, invertebrated, and vertebrates in considerably increased density. This internal mutagenecity appeared to be remediated to non-detectable range in the mesocosm on Day 8 or Day 64 of the experiment. The only single chemical to show mutagenecity without ambiguity is CPF which showed 6 positive signs in the sign test on Day 64 following redose of CPF on Day 62 in the absence of interanal mutagenicity effect. Nevertheless, the interaction of HG and MA appears to be comutagenetic to obtain the mutagenecity by cooperation of 2 originally non-mutagentic chemical into very strong mutagenetic activity. By contrast, the interaction of HG with ATR appears to be countermutagenetic even in the presence of internal mutagen on Day 1. The interaction of HG with CPF was inhibitory, but the interaction of ATR with CPF was not inhibitory to the mutagenicity of CPF. When 3 of 4 chemicals were mixed together in 4 different ways, the only combination to have mutagenecity is the mixture of HG, MA, and CPF. The mixtures of all 4 chemicals in the mesocosms in full dose were very mutagenetic. Nevertheless, with half doses one third of mesocosms appear to have remediated to non-mutagenetic level on Day 64. It appears to be very clear from this experiment, that 3 agrichemicals and methyll mercury interact with each other in different ways for enhancement or inhibition of mutagenecity. Therefore, adequate combination of those chemicals is important to obtain insecticide and herbicide effect without mutagenecity effect.

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Table 1. Codes and doses of each chemicals in sediment (soil) or in water in the mesoscosms containing about 400-liter of water mixed with sediment.

Chemicals	Doses Codes		
	-1	0	+1
Methyl Mercury (HG)	0 ug/Kg sediment	0.2 ug/Kg sediment	0.4 ug/Kg sediment
Monosodium acid methane arsonate (MSMA)	0 ppb water	25.5 ppb water	51.0 ppb water
Atrazine (ATR)	0 ppb water	96.0 ppb water	192.0 ppb water
Chlorpyrifos (CPF)	0 ppb water	109.5 ppb water	219.0 ppb water

HG: Methyl mercury; MSMA: Monosodium acid methane arsonate; ATZ: Atrazine; CPF: Chlorpyrifos

Table 2. Preparation of mixtures for A, B, C, D, E, F assays.

Category of Assay	A	B	C	D	E	F
unit/assay (ul)	0.5	0.5	0.5	0.45	0.5	0.4
Total volume (ml)	20.0	20.0	20.0	18.0	20.0	16.0
Dist. water (ml) (add to total volume as indicated)	12.89	12.89	12.09	12.6	14.6	10.6
.4 M Phosph.B. (ml)	5.0	5.0	5.0	5.0	5.0	5.0
Mg-K salt sol. (ml)	0.4	0.4	0.4	0.4	0.4	0.4
Uninduced rat S-9 (ml)	0.8	-	-	-	-	-
Induced rat S-9 (ml)	-	0.8	1.6	-	-	-
.95 M Gluc. phos. (ml)	0.11	0.11	0.11	-	-	-
.1 M NADP (ml) 0.8	0.8	0.8	-	-	-	

0.4 M Phosphate buffer was prepared by mixture of 0.4 M NaH_2PO_4 with 0.4M M Na_2HPO_4 in the ratio of about 60 ml to 440 ml to obtain pH 7.4 for autoclave at 121°C for 20 minutes.

Mg-K salt solution was prepared from dilution of 61.5 g KCl and 40.7 g $MgCl_2.6H_2O$ into 500 ml distilled water for autoclave at 121°C for 20 minutes.

184 mg of NADP was disolved into 2.4 ml of distilled water and mixed with 0.33 ml of 0.95 M glucosed phosphate and sterilized by filtration before divided into 3 portions to be added to A, B, and C mixtures.

Table 3. Salmonella mutagenecity test of mesocosms containing different combinations of methyl mercury(HG), monosodium arsenate(AS), atrazine (ATR) and chlorpyrifos(CPF) with or without redoses on Day 62.

ID	AGRICHEMICALS USED				RE-	SIGN TEST			MUTAG. IND. AVE. [C/B RATIO]	CORR.COEF
No.	HG	AS	ATR	CPF	DOSE	Day 1	Day 8	Day 64	Day 1	Day64
#10	-1	-1	-1	-1	No	6p0n	5p1n	5p1n	5.32[1.45]1.00	0.18[1.37]0.99
#03	0	0	0	0	No	6p0n	3p3n	6p0n	9.66[0.91]0.99	0.40[1.32]0.92
#11	0	0	0	0	No	6p0n	5p1n	5p1n	5.66[0.91]0.99	0.30[0.99]0.88
#53	0	0	0	0	No	6p0n	5p1n	6p0n	5.82[1.58]0.98	0.30[1.14]0.42
#04	0	0	0	0	Yes	6p0n	5p1n	6p0n	7.53[1.05]0.98	0.39[0.85]0.89
#16	0	0	0	0	Yes	6p0n	4p2n	6p0n	9.34[1.24]0.98	0.29[0.96]0.59
#46	0	0	0	0	Yes	6p0n	4p2n	6p0n	9.67[4.50]1.00	0.23[0.91]0.76
#31	+1	+1	+1	+1	No	6p0n	5p1n	6p0n	5.08[1.05]0.73	0.26[1.01]0.95
#24	+1	-1	-1	-1	Yes	6p0n	5p1n	5p1n	5.08[1.08]0.98	0.20[2.41]0.83
#42	+1	+1	-1	-1	No	6p0n	6p0n	6p0n	6.30[2.23]0.79	0.47[0.65]0.99
#49	+1	-1	+1	-1	No	4p2n	5p1n	5p1n	0.40[0.75]0.76	0.20[0.66]0.85
#18	+1	-1	-1	+1	No	6p0n	4p2n	5p1n	5.28[0.79]0.93	0.20[1.51]0.50
#48	-1	+1	-1	-1	Yes	6p0n	6p0n	5p1n	4.51[0.83]0.99	0.15[1.11]0.12
#42	+1	+1	-1	-1	No	6p0n	6p0n	6p0n	6.30[2.23]0.79	0.47[0.65]0.99
#61	-1	+1	+1	-1	No	6p0n	5p1n	6p0n	3.17[3.29]0.93	0.38[1.19]0.19
#51	-1	+1	-1	+1	No	6p0n	5p1n	6p0n	6.15[1.54]0.99	0.35[1.11]0.84
#47	-1	-1	+1	-1	Yes	6p0n	4p2n	3p3n	11.55[1.72]0.99	0.21[1.11]0.12
#49	+1	-1	+1	-1	No	4p2n	5p1n	5p1n	0.40[0.75]0.76	0.20[1.70]0.85
#61	-1	+1	+1	-1	No	6p0n	5p1n	6p0n	3.17[3.29]0.96	0.20[1.19]0.19
#08	-1	-1	+1	+1	No	6p0n	5p1n	6p0n	5.96[0.77]0.74	0.25[0.88]0.99
#28	-1	-1	-1	+1	Yes	6p0n	5p1n	6p0n	7.59[0.42]0.32	0.22[0.88]0.33
#18	+1	-1	-1	+1	No	6p0n	4p2n	5p1n	5.28[0.79]0.93	0.20[1.51]0.50
#51	-1	+1	-1	+1	No	6p0n	5p1n	6p0n	6.15[1.54]0.99	0.35[0.93]0.94
#08	-1	-1	+1	+1	No	6p0n	5p1n	6p0n	5.96[0.77]0.74	0.25[0.88]0.99
#65	+1	+1	+1	-1	Yes	6p0n	5p1n	4p2n	6.56[2.05]0.93	0.07[1.58]0.95
#40	+1	+1	-1	+1	Yes	6p0n	5p1n	6p0n	0.40[0.64]0.98	0.30[0.95]0.83
#67	+1	-1	+1	+1	Yes	6p0n	4p2n	5p1n	1.94[0.35]0.80	0.10[0.93]0.19
#35	-1	+1	+1	+1	Yes	6p0n	6p0n	3p3n	6.37[2.25]0.92	0.15[1.70]0.14
H_2O	Negative Control					4p2n	4p2n	5p1n	0.12[1.50]0.99	0.16[1.02]0.56
Daunom.(5ug/100ml)	Pos. control					6p0n	6p0n	6p0n	3.42[0.75]0.80	0.42[0.77]0.96

Sign test was performed by the analysis of the number of positive or negative signs obtained by substraction of the number of revertants formed in the control from that formed in the sample in 6 differnt assays.
MUTAG. IND. AVE. is the mean value of mutagenetic index in 6 assays.
CORR. COEF. is the correlation coefficient between sample concentration (x variable) and the number of reversed mutation (y variable).