MITOTIC INDEX AND STAGE ANALYSES ASSOCIATED WITH OCHRATOXIN A AND PENICILLIC ACID

THESIS

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INTRODUCTION

Until the discovery of the aflatoxins in the early 1960's, mycotoxins (mold metabolites) were relatively obscure in the scientific literature. Since that time, numerous mycotoxins have been identified and the research on their roles in human and animal diseases has intensified greatly.

Members of the genus Aspergillus represent some of the most prevalent mycotoxin-producing fungi associated with food and feed materials. Two mycotoxins produced on improperly stored grains by Aspergillus ochraceus chosen for this study were ochratoxin A and penicillic acid.

Ochratoxin A and penicillic acid were used to determine the in vitro effects on root tip cells from Pisum sativum var. Alaska (a variety of pea). Exposure times included periods of 6 and 12 hours using four different toxin concentrations (0.1, 1.0, 10, 100 ug/ml).

The major objective of this study was to obtain information about cellular sensitivity to the toxins, individually and in combination. The parameters examined were the mitotic indices and the mitotic phase analysis distributions.

The investigation was designed to yield answers to the following questions:

1. Is there any interaction between the toxins, their treatments, and the time exposures that can affect the actively dividing cells? And, if so, is there an effect on the mitotic phase distribution (prophase, prometaphase, metaphase, anaphase, telophase) by this interaction?

2. At which concentration, if any, for each toxin will the effect on the actively dividing cells be the greatest? Will a longer time exposure necessarily inflict more damage?

3. Will ochratoxin A and penicillic acid in combination elicit any type of a synergistic effect?

4. Is there a characteristic effect elicited at some phase during active mitosis?

REVIEW OF LITERATURE

Mycotoxins (mold metabolites) were relatively obscure in the scientific literature until the discovery of the aflatoxins in the early 1960's. This discovery led to an increased awareness of the potential role of fungal toxins. These compounds have been shown to be probable causative agents in disease in humans which are induced by long term and relatively low-level ingestion of the toxins. Aflatoxins are found in a number of foods and many are of high enough quality to be consumed directly by humans.

The aflatoxins were discovered as a result of attempts to discover the agent responsible for a recognized disease; whereas, the ochratoxins were discovered in a screening program for toxigenic fungi (Butler, 1974; Newberne, 1976).

Extensive reviews of the studies done with the aflatoxins and mycotoxins in general have been published by Wogan (1965), Walbeek (1968), Glodblatt (1969), Wilson (1970), Jarvis (1971), Purchase (1974), Schlessinger (1975), Newberne (1976), Stoloff (1976), Hollaender and Serres (1978), Rodricks (1978), Moreau (1979), and Ciegler and Bennett (1980).

In 1961, mycologists of the South African Council for Scientific and Industrial Research undertook investigations

into the microflora of local legume and cereal products. Aspergillus ochraceus was frequently encountered in the survey. Both Raper and Fennell and van der Merwe (1965) reported that this storage mold occurs widely in nature and is often found on soil and on decaying vegetation. As a result of a general screening of molds isolated from grains, ochratoxin was discovered by van der Merwe et al. (1965) as a mycotoxin produced by Aspergillus ochraceus. The toxicity of the fungus was attributed to ochratoxin A, the main toxic component in culture extracts. Recently, ochratoxins have been isolated from other Aspergilli including Penicillium viridicatum.

The ochratoxins (Figure 1) comprise a group of closely related compounds which contain a 3,4-dihydro-3-methy-isocourmarin moiety which is linked to an L-β-phenylalanine through a carboxy group position 7 (van der Merwe, 1965; Searcy, 1969). Ochratoxin A is a colorless, crystalline compound with a molecular formula $C_{20}H_{18}O_6NCl$ and a molecular weight of 403.8.

During the isolation and chemical characterization of ochratoxin A, van der Merwe (1965) and others isolated the methyl and ethyl derivatives of ochratoxin A; the less toxic dechloroderivative, ochratoxin B; and the relatively, non-toxic (Nesheim, 1969; Newberne, 1976) ethyl ester

derivative of ochratoxin A, ochratoxin C. A single recent report indicates that the methyl ester of ochratoxin A and ochratoxin C may be as toxic as ochratoxin A (Moreau, 1979). The toxicity has been attributed to the dependence upon the combined presence of the chlorine atom and the free carboxyl group. Although ochratoxins A, B, and C have been isolated from laboratory cultures, only ochratoxin A has been detected in most cases of natural occurrence (Rodricks, 1978).

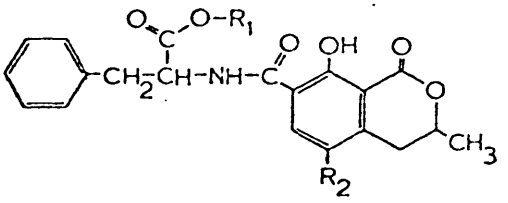


Figure 1.

ochratoxin A	R_1=H	R_2=Cl
ochratoxin B	R_1=H	R_2=H
ochratoxin C	R_1=CH_2CH_3	R_2=Cl

Aspergillus ochraceus has also been reported to concomitantly produce penicillic acid (Steyn, 1967; Natori, 1970; Ciegler, 1971; and Rodricks, 1978), a carcinogenic mycotoxin (Dickens, 1961), and is also synthesized by a

number of species of Penicillia and Aspergilli (Shibata, 1964; Ciegler, 1971). The quantity of ochratoxin A and penicillic acid produced both in culture and in nature is influenced by temperature and moisture; low temperatures (10°C and 20°C) favor penicillic acid synthesis and higher temperatures (28°C) favor ochratoxin A production. Generally, penicillic acid is produced in yields about one to three magnitudes greater than ochratoxin A (Ciegler, 1972).

Penicillic acid (Figure 2) is comprised of a 3-methoxy-5-methyl-4-oxy-2,5-hexadienoic acid. This compound has a molecular formula of $C_8O_4H_{10}$ ($CH_2{:}C(CH_3)COC(OCH_3){:}CHCO_2H$) and a molecular weight of 170.16 (Newberne, 1976). Ciegler et al. (1971) has reviewed the literature on penicillic acid and other lactone mycotoxins.

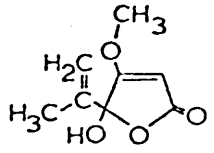


Figure 2. Penicillic acid

Most of the work done concerning the biological activity of mycotoxins in cell culture concerns the aflatoxins (Goldblatt, 1969). Legator and Withrow (1964) found that crude aflatoxin mixtures as well as crystallized aflatoxin

suppressed mitotic division in heteroploid and diploid human embryonic cells. This inhibition occurred four hours after exposure and reached a maximum in 8-12 hours. In addition, aflatoxin markedly inhibited the synthesis of DNA and affected normal cell morphology.

Lilly (1965) was first to investigate the action of aflatoxin on chromosomes. Using root seedlings of Vicia faba (broad bean), he found that most of the abnormalities consisted of chromosome fragments with occassional anaphase bridges. During an investigation into the induction of chromosome breaks in human blood in culture, results indicated that aflatoxin breaks human chromosomes.

Legator et al. (1964;1965) used cultured heteroploid human embryonic lung cells. The earliest effect of the aflatoxin was suppression of DNA synthesis and mitosis; this effect is detectable within the first few hours after exposure. The most notable result was the arrest of mitosis in the metaphase stage.

In 1969, Engelbrecht and Purchase exposed monkey kidney epithelial cell cultures to aflatoxin and ochratoxin to determine whether any specific morphological effects are produced which indicated the mode of action of the toxins. After 24 and 48 hours of exposure, aflatoxin produced a decrease in mitosis and fragmentation of the nucleolus, as

well as non-specific changes such as cytoplasmic vacuolation and pycnosis and karyorhexis. Ochratoxin produced enlarged nucleoli and a decrease in normal mitosis with an increase in abnormal forms. Prophase and metaphase blocks were observed along with non-specific degenerative changes.

Reiss (1971) reported on the action of aflatoxin on Allium cepa (onion) root tips. He observed on prepared squashes, clumping of individual chromosomes during anaphase and a reduction of the mitotic frequency under aflatoxin influence in human lung cells, human white blood corpuscles, and Vicia faba root cells.

In 1975, Reiss reported that patulin and two other mycotoxins caused a reduction of the mitotic index proportional to the toxin concentration in the root tips of Allium cepa. The damage resulted in strong inhibition of the development of anaphases and in vacuolization of the cytoplasm.

In 1979, Linnainmaa et al. studied the cytogenic effects of purified grain mycotoxins (T-2 and Saratoxin H) in the growing root meristem of Allium cepa. Mitotic activity of the cells was seen to decrease gradually when treatment time was increased and after 24 hour treatment the mitotic index was only 1/10 of the respective control. Typical C-mitotic action (Hyypio et al., 1955) was obtained

by both toxins and was comparable to the efficiency of colchicine. With T-2, an increase of time treatment led to a decrease in the frequency of anaphases. According to their observation, these toxins do not induce chromosome breaks.

Korte and Ruckert (1980) found that aflatoxins and patulin induce chromosomal damage in Chinese hamster bone marrow cells. Other studies concerning the use of aflatoxins and other mycotoxins on cell cultures include those by Legator (1966), Sporn (1966), Zuckerman (1966), Umeda (1971), Umeda (1972), Rodricks (1976), Umeda (1977), Tashiro (1979), Lorkowski (1980), Moreau (1979), and Moule (1980).

Although the presence of a toxin represents a hazard, there is a growing concern over the simultaneous occurrence of one or more toxins. Of particular interest is the cooperative effect of two or more substances that can elicit a total effect greater than the sum of the activities of individual agents - toxic synergism.

Reports of the natural contamination of grains by ochratoxin A and/or penicillic acid have stimulated work on the toxic interaction of these mycotoxins (Ciegler, 1972; Thorpe, 1974; Lillehoj and Ciegler, 1975; Sansing, 1976). In a preliminary study of the interaction effects

of the acute toxicities of ochratoxin A, penicillic acid, and citrinin in mice, combinations of the mycotoxins elicited a synergistic lethal response (Lindenfelser et al., 1973; Lillehoj and Ciegler, 1975; Sansing et al., 1976. The response was then expanded to examine effects of toxin pairs on nucleic acid metabolism in the liver and kidneys of mice. Generally, in this case, toxin combinations initiated effects similar to the independent functions of each mycotoxin. Also reported was that penicillic acid alone stimulated ribonucleic acid synthesis in liver, combinations with ochratoxin A or citrinin inhibited accumulation of the nucleic acid.

Umeda et al. (1972) reported on the effect of patulin and penicillic acid on HeLa cell chromosomes. This mycotoxin was found to induce accumulation of metaphase cells with elongation of the whole cell cycle, but was not found to demonstrate chromosome aberrations.

A study done by Reddy et al. (1979) postulated that penicillic acid and patulin, a mycotoxin produced by members of the genus Penicillium and Aspergillus, produced a synergistic effect. Enhancement of patulin toxicity by penicillic acid was indicated by the occurrence of deaths in dogs exposed simultaneously to sublethal doses of both mycotoxins and by other criteria.

Creppy et al. (1980) suggested a cooperative effect between ochratoxin A and citrinin, a mycotoxin produced by Penicillium viridicatum. Both mycotoxins are cytotoxic to hepatoma tissue culture cells. When both mycotoxins are added simultaneously to these culture cells, the inhibition of RNA and protein synthesis occurs immediately, that of DNA synthesis after a short lag time. They also found that while penicillic acid stimulates accumulation of RNA in mouse liver, a combination of ochratoxin A or citrinin (with penicillic acid) inhibits the accumulation of RNA.

Other literature concerning work with ochratoxin A or penicillic acid can be found published by Umeda (1971), Chu (1974), Creppy (1979), Galtier (1979), Lillehoj (1979), Chan (1980a), Chan (1980b), Chan (1980c), Galtier (1980), Hult (1980), and Stormer (1980).

The majority of the studies at the cellular level have concerned the aflatoxins. Within the past ten years, ochratoxin A has been recognized as being as pathogenic as the aflatoxins (Ciegler et al., 1971). Cytological studies such as mitotic disruptions and chromosomal abnormalities are logical points of reference for all cell morphogenesis and subsequent analyses of interaction at the molecular level. Penicillic acid has been included in the study because of its natural occurrence in connection with ochratoxin A.

MATERIALS AND METHODS

Experimental Scheme

Studies to determine selected cytological *in vitro* effects of ochratoxin A and penicillic acid on root tip cells from *Pisum sativum* var. Alaska (variety of pea) were conducted. Each series of experiments involved controls and four concentrations of ochratoxin A or penicillic acid (0.1, 1.0, 10, 100 µg/ml). In addition, to study possible synergism, experiments were carried out using various concentrations of ochratoxin A and penicillic acid together in a 1:3 ratio (0.1, 1.0, 10, 100 µg/ml).

Earlier investigations showed that the minimum mitotic cycle of *Pisum sativum* is about 10 to 12 hours (Van't Hof et al., 1960; Van't Hof et al., 1963; Van't Hof, 1963). The root tips were treated with toxins for periods of 6 and 12 hours. Run times included 6 a.m., noon, and 6 p.m. Preliminary runs with controls indicated active division at these times.

The criteria used in evaluating the cellular sensitivity to the toxins were: (1) the mitotic index and (2) the mitotic phase analysis.

Growth of Plant Materials

The experimental material for this study was Pisum sativum var. Alaska (Harpool Seed, Inc., Denton, Texas; Lot No. 6-86), a variety of pea. The procedure used for the preparation of root tips was a modification of the method of Van't Hof (1968). The pea seeds were soaked in a beaker of distilled water for 24 hours at room temperature. Following this, the seeds were removed and placed on wet paper toweling contained within a shallow pan. The pan was then covered to prevent dehydration of the peas. After 48 hours, non-germinated seeds were discarded and the remaining seeds were arranged so that the roots would grow "straight". After 60 to 72 hours, the pea roots were approximately 2-1/2 to 3 cm long and were ready for toxin treatment.

Toxin Treatment

Ochratoxin A (7-carboxy-5-chloro-8-hydroxy-3,4-dihyro-3-methyl isocoumarin amide of L-β-phenylalanine) and penicillic acid (3-methoxy-5-methyl-4-oxy-2,5-hexadienoic acid) were purchased from Sigma Chemical Company, St. Louis, Missouri (Ochratoxin A, Lot No. 47C-0139; Penicillic acid, Lot No. 126C-0063). The toxins were dissolved in 0.1M

sodium bicarbonate for a final concentration of 100 µg/ml. Length of exposure time for each concentration was 6 and 12 hours. Duplicate runs were carried out for each concentration and exposure time simultaneously. Each run and exposure time were at the same time of the day for each mycotoxin.

Five peas with roots 2-1/2 to 3 cm long were exposed per concentration per exposure time - one root tip per squash slide.

A receptacle was set with 20 wells to hold 20 individual vials so that the top of the receptacle and the vials was level. Each vial held 20 ml of the toxin concentration.

The vials were arranged so that there were five rows, one for each concentration, and two columns for each exposure time to allow for duplicate runs.

The germinated pea seedlings were suspended on a 1/4 inch wire mesh placed directly on top of the receptacle so that the roots extended down into the vial for exposure. The pea itself rested atop the wire mesh. This mesh allowed for more rapid and efficient ease in exposing the individual roots and for the removal of the individual roots.

Preparation of Root Tips

After toxin exposure, the terminal 1 cm of the root was removed by pinching off with forceps, and placed in a vial with fixative (6 parts methanol, 3 parts chloroform, 2 parts acetic acid). The tips were then evacuated at 15 mm Hg for 10 minutes or until no more air bubbles were visible. They were placed in a 60°C hydrolysis oven for 18 minutes. The fixative was then replaced with enough warm (60°C) 1N hydrochloric acid to cover the root tips. This was then placed once again in a 60°C oven for 18 minutes - the time being very critical. The acid was replaced with Schiff's reagent (See Appendix for details), the vial was stoppered and allowed to stain for 30-45 minutes.

Squash Technique

The root tips were removed from the staining solution and drained on paper toweling. The tips were placed on clean glass slides and the excess material just behind the highly stained (dark purple) region was cut off. The tip was then moved to the center of the slide. A 1/2 drop (Pasteur pipette) of fast green (pinch of fast green stain dissolved in 45% acetic acid) was added to make the cells more visible under the microscope. The tip was squashed

with a glass plunger using short, rapid, firm strokes confined to the immediate area. The slide was passed through an alcohol flame three short times. The slide was placed in a Coplin jar filled with 50% TBA (tertiary butyl alcohol) and left for 1-2 hours. The slides were removed from the TBA gently, the excess TBA was drained off by standing the slide on end and allowing a paper towel to absorb the excess alcohol. One drop of Permount mounting media (Fisher Scientific Company, Fair Lawn, New Jersey) was placed over the area of the cells, a cover slip was added and the slide was allowed to dry overnight on a flat surface.

Cell Counts - Mitotic Index and Stage Analysis

After the cells were mounted permanently on glass slides, mitotic indices were recorded. Using an American Optical Spencer light microscope with 450X magnification, between 2000 and 2500 cells were counted for each set of controls and concentrations per run of 6 and 12 hours. (Preliminary control runs were also analyzed at zero, 4, 6, 8, and 12 hours.) Using hand tally counters (Scientific Products), counts were recorded as the number of dividing cells per total number of cells, with a maximum total of 500 cells per slide.

Analysis of the mitotic stages was done under oil immersion on a Zeiss Photomicroscope I, using a neutral green filter to complement the staining technique. (Total magnification was 1562.5X : occular Kpl-W, 12.5; optovar, 1.25; objective, 100 Planapochromat.) For each control and concentration per run of 6 and 12 hours, between 400 and 500 dividing cells were chosen for stage analysis, with a maximum of 100 dividing cells per slide. Cells were scored into stages of prophase, prometaphase, metaphase, anaphase, and telophase. Cells not in one of these stages were considered to be non-dividing.

Areas to be scored both in the mitotic index and the stage analysis were chosen by mannerly, random, horizontal and vertical "sweeps" across the coverslip area. Any changes in cell appearance were noted during the mitotic stage analysis.

EXPERIMENTAL RESULTS

The investigation involved a comparison of selected *in vitro* effects of ochratoxin A and penicillic acid on pea root tips. Studies were made for each toxin treatment (0.1, 1.0, 10, 100 µg/ml) using individual toxins and both toxins in combination. Cellular sensitivity was evaluated by analysis of the mitotic index and mitotic stages.

Literature investigations involving cell cultures and mycotoxins reported studies at 24 and 48 hours with cell necrosis occurring around 48 hours exposure. This investigation explored involvement at 6 and 12 hours of toxin exposure. A preliminary experiment to determine a suitable time frame period utilized 10 control root tips (500 cells examined per root tip) per time. Table 1 shows the time range from 6 a.m. to 6 p.m. and the percentage of cells found dividing at the different time intervals. From these preliminary results, 6 a.m. to 6 p.m. showed the cells to be actively dividing during this time interval, thus 6 a.m. to 6 p.m. was deemed suitable for this investigation's time frame. Roots were excised at noon (6 hours) and 6 p.m. (12 hours). The preliminary study also analyzed these same root tips as to their mitotic stage distribution among prophase, prometaphase, metaphase, anaphase, and

TABLE 1

Mitotic Index for Preliminary Control Study

Time	No. Dividing Cells*	% of Total Dividing
6 a.m.	520	10.4
10 a.m.	246	4.9
12 p.m.	360	7.2
2 p.m.	395	7.9
6 p.m.	423	8.5

*Total of 5000 pea root tip cells examined per time.

telophase. Table 2 shows the percentage of cells at the various times as distributed among the five stages (100 dividing cells per root tip were scored). At both 12 p.m. and 6 p.m., there was a suitable distribution of dividing cells among the five phases so as to accommodate a comparison for the treatment distributions.

Tables 3 and 4 list the mitotic indices for ochratoxin A, penicillic acid, and ochratoxin A + penicillic acid treatments at 6 and 12 hours. All data was coded before being statistically analyzed with the use of SPSS (Statistical Package for the Social Sciences) and BMDP (Biomedical Computer Programs) on the DEC-20 computer. (See Appendix for programs used, printouts of computational results, and coded data - Note: a high coded score represents a low mitotic index mean count.) A visual interpretation of these mitotic indices for toxin treatments at 6 and 12 hours showed a decrease in actively dividing cells, with 12 hours showing a lower mean count.

A one-way analysis of variance was performed on the raw data scores to determine if there was a significant variation between the means for the treatments. The data proved to be highly significant by the F-ratios at the level of P=.0001 (see Appendix subprogram ONEWAY).

TABLE 2

Mitotic Analysis for Preliminary Control Study

Stage	Percentage* of Cells Dividing Time				
	6 am	10 am	12 pm	2 pm	6 pm
Prophase	44.4	83.8	85.2	88.8	81.4
Prometaphase	18.2	12.6	23.2	6.8	10.4
Metaphase	13.8	12.6	1.4	0.6	1.6
Anaphase	6.2	0.0	1.2	0.0	1.8
Telophase	17.6	0.0	1.2	2.6	3.0

*Percentage of dividing cells in each division stage from a total of 500 dividing cells per time.

TABLE 3

Mitotic Index* for Toxin Treatments at 6 Hours

Toxin**	Toxin Treatment (µg/ml) 0.0	0.1	1.0	10	100
OA	44.6	12.2	10.4	11.1	16.4
PA	31.3	7.6	1.9	4.9	8.0
OA+PA	36.4	20.6	14.0	6.4	13.7

*Values represent the mean of 2 replicate runs.

**OA= ochratoxin A

PA= penicillic acid

TABLE 4

Mitotic Index* for Toxin Treatments at 12 Hours

Toxin**	Toxin Treatment (µg/ml) 0.0	0.1	1.0	10	100
OA	39.8	8.2	7.1	7.9	11.8
PA	25.9	7.2	1.9	1.5	8.6
OA+PA	47.0	9.1	5.9	3.5	1.6

*Values represent the mean of 2 replicate runs.
**OA= ochratoxin A
PA= penicillic acid

An SPSS program was run (see Appendix subprogram ANOVA) to determine any significant interactions between toxins, treatments, and times. (This and subsequent analyses were done with coded data.) Table 5 shows that F-ratios for the main effects and all but one of the interactions was significant at the P=.0001 level. The interaction between treatment and time, ignoring toxin interaction, was not significant even at the P=.01 level. From this analysis it could also be noted that (1) penicillic acid is most effective on retarding cell division while ochratoxin A has the least effect, (2) 10 > 1.0 > 100 > 0.1 μg/ml, and (3) 12 hours is more effective than 6 hours of exposure.

Another SPSS program (see Appendix subprogram BREAKDOWN) provided a technique for further examination of the means differences for the mitotic indices. The mean differences for the breakdown analysis are shown in Figures 1 to 3 (two-way interaction) and in Figures 4 to 8 (three-way interaction). Again there seems to be no interaction when the means are broken down by just treatment and time (Figures 2(b) and 3(b)). The toxins, the various treatments, and the length of exposure time all interacted to affect the mitotic indices.

TABLE 5

(F) Values for Comparison of Analysis of Variance

Using Program SPSS-ANOVA

Source of Variation	(F)-Ratio	Significance of (F)
Main Effects		
Toxin	28.004	0.0001
Treatment	74.354	0.0001
Time	176.917	0.0001
2-Way Interactions		
Toxin Treatment	28.872	0.0001
Toxin Time	103.348	0.0001
Treatment Time	1.749	0.1840
3-Way Interactions		
Toxin Treatment Time	11.736	0.0001

Figure 1. Means Differences* For Mitotic Indices:

(a) Means broken down by toxin by treatment

(b) Means broken down by toxin by time

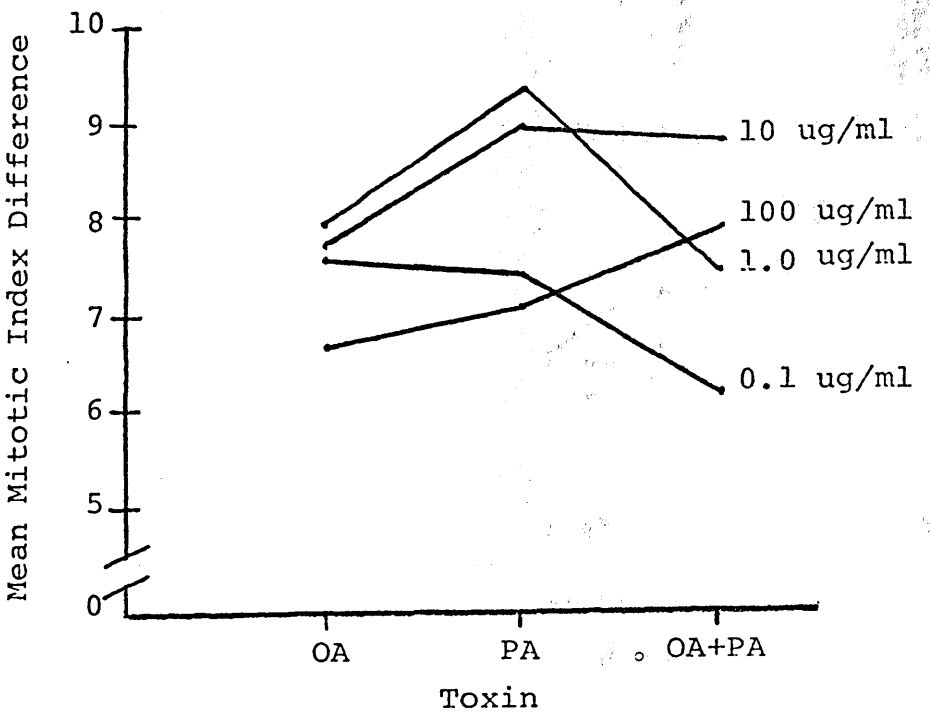
OA = ochratoxin A

PA = penicillic acid

OA+PA = ochratoxin A + penicillic acid

*Data reference: for all figures see Appendix SPSS subprogram Breakdown

(a)


Mean Mitotic Index Difference
10
9
8
7
6
5
0
10 ug/ml
100 ug/ml
1.0 ug/ml
0.1 ug/ml
OA
PA
OA+PA
Toxin

(b)

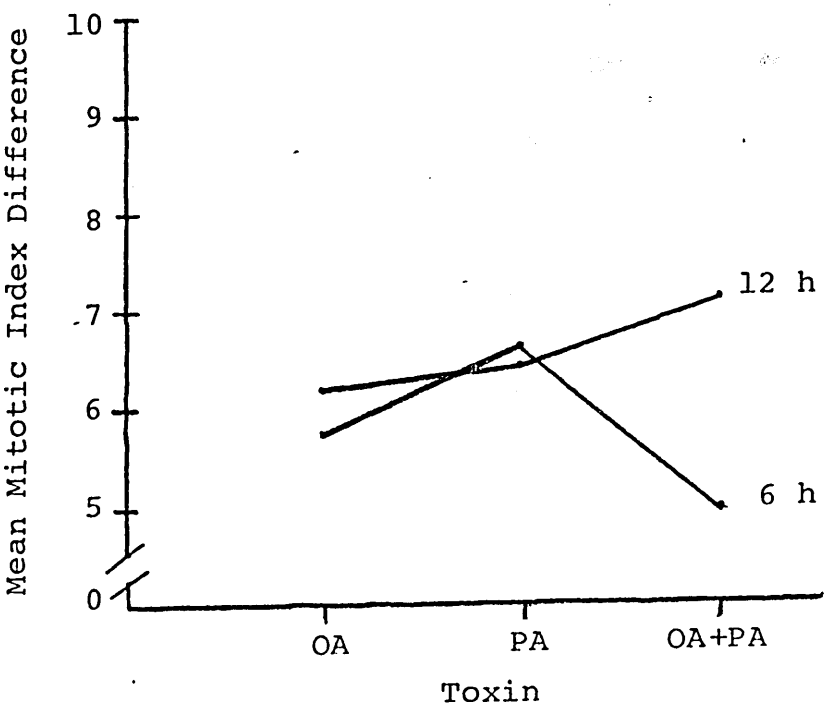

Mean Mitotic Index Difference
10
9
8
7
6
5
0
12 h
6 h
OA
PA
OA+PA
Toxin

Figure 2. Means Differences For Mitotic Indices:

(a) Means broken down by time by toxin

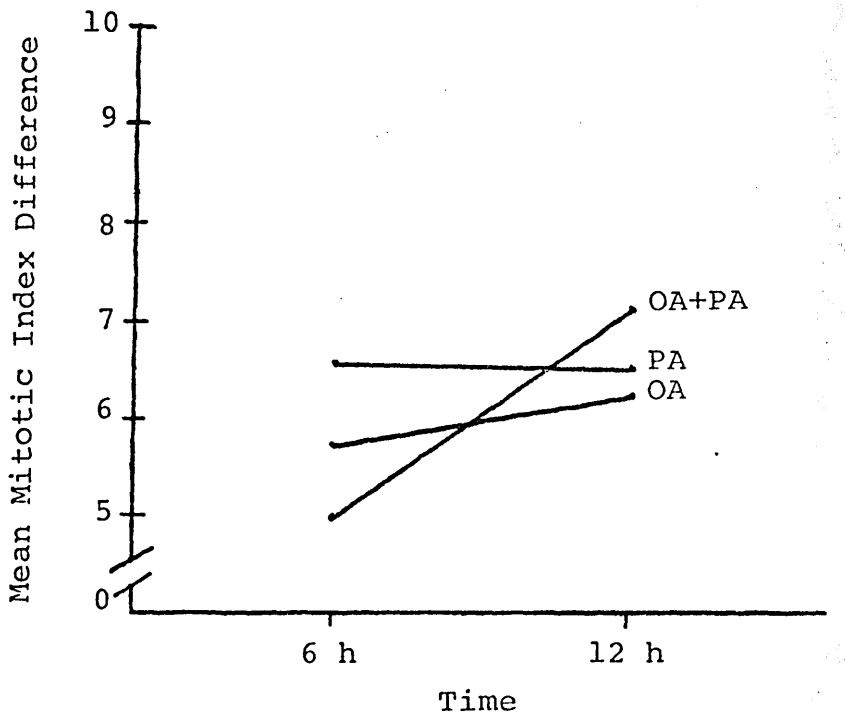
(b) Means broken down by time by treatment

OA = ochratoxin A

PA = penicillic acid

OA+PA = ochratoxin A + penicillic acid

(a)


Mean Mitotic Index Difference
10
9
8
7
6
5
0
OA+PA
PA
OA
6 h
12 h
Time

(b)

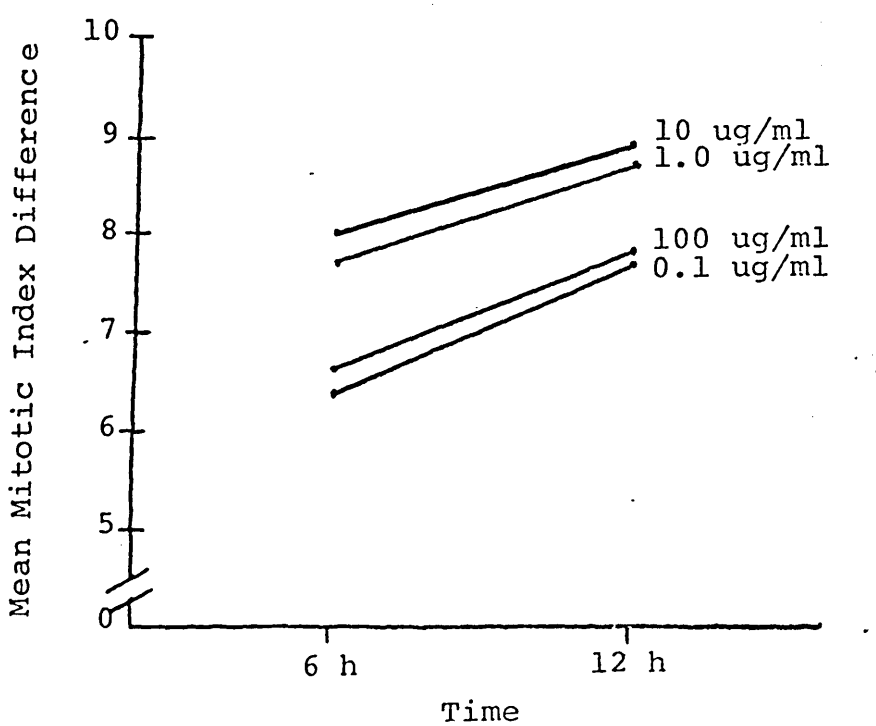

Mean Mitotic Index Difference
10
9
8
7
6
5
0
10 ug/ml
1.0 ug/ml
100 ug/ml
0.1 ug/ml
6 h
12 h
Time

Figure 3. Means Differences For Mitotic Indices:

(a) Means broken down by treatment by toxin

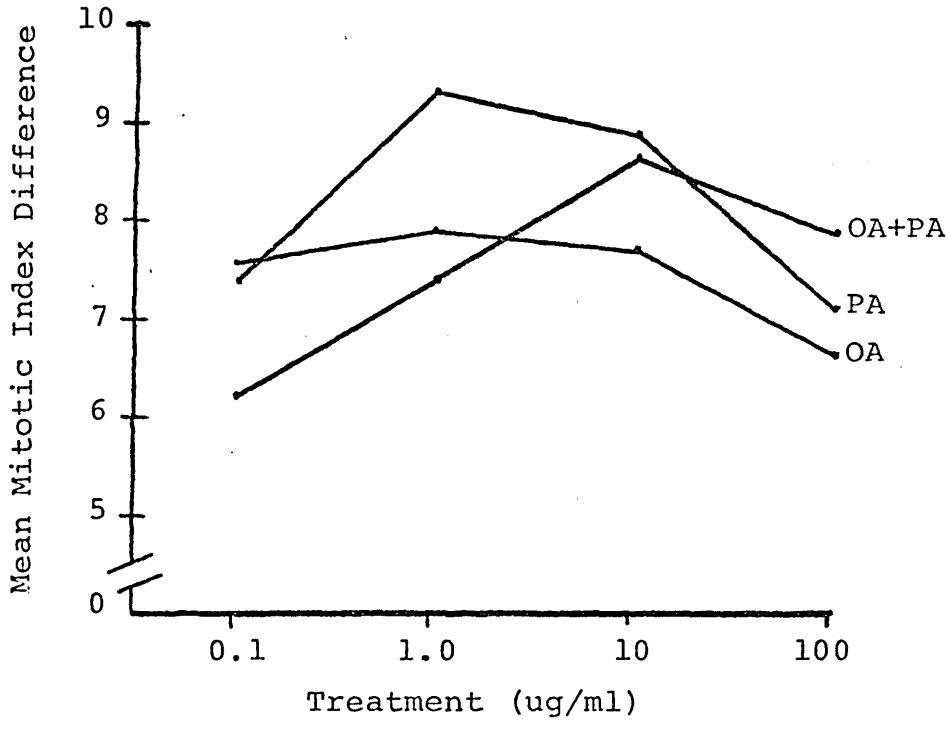
(b) Means broken down by treatment by time

OA = ochratoxin A

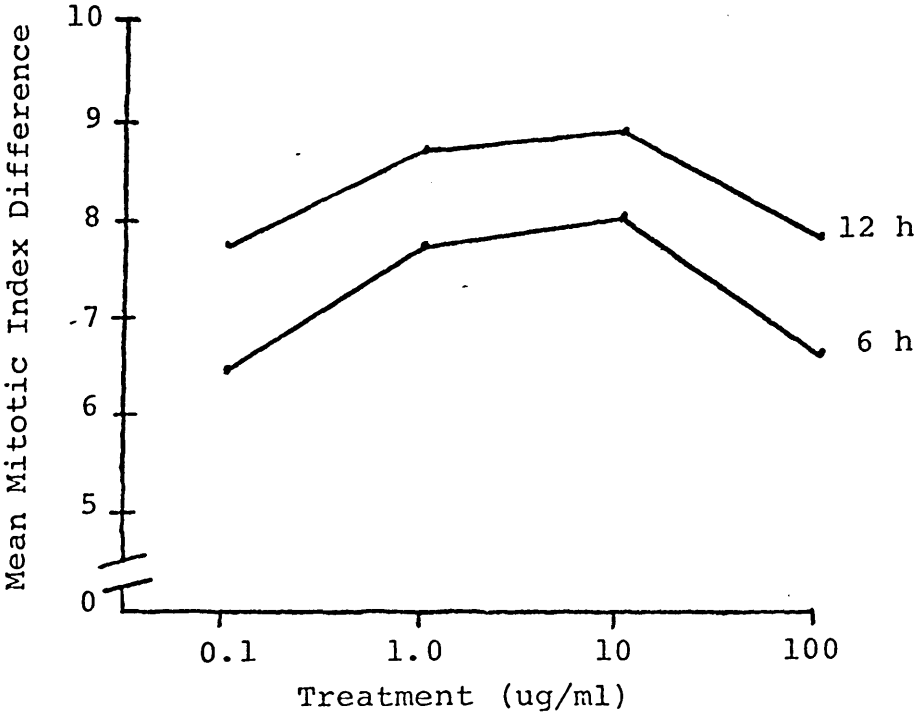
PA = penicillic acid

OA+PA = ochratoxin A + penicillic acid

(a)


Mean Mitotic Index Difference
10
9
8
7
6
5
0
0.1
1.0
10
100
Treatment (ug/ml)
OA+PA
PA
OA

(b)


Mean Mitotic Index Difference
10
9
8
7
6
5
0
0.1
1.0
10
100
Treatment (ug/ml)
12 h
6 h

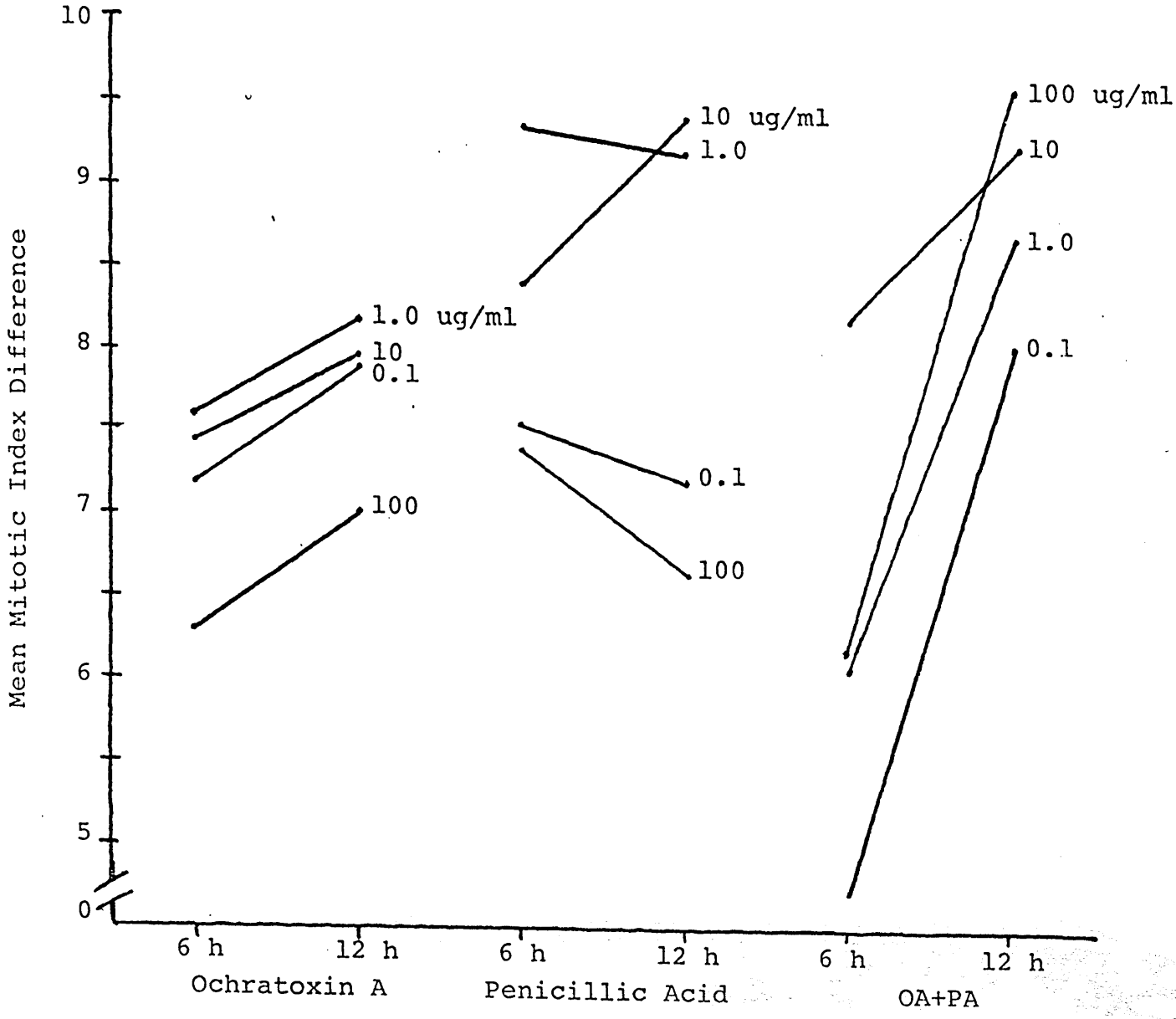
Figures 1(a) and 3(a) show the means broken down by toxin and treatment. For each toxin, the general effects on the mitotic indices are shown by the following sequence: ochratoxin A, 1 > 10 > 0.1 > 100; penicillic acid, 1 > 10 > 0.1 > 100; and ochratoxin A + penicillic acid, 10 > 100 > 1 > 0.1. This is the general effect without breakdown with respect to time exposure.

Figures 1(b) and 2(a) are the breakdown of the means by time and toxin. There was a more dramatic decrease in actively dividing cells for ochratoxin A + penicillic acid from 6 to 12 hours. Ochratoxin A showed its greatest effect at 12 hours, while penicillic acid showed its at 6 hours. This breakdown analysis segment deals with the treatments as a whole.

Figures 2(b) and 3(b) present the means broken down by time and treatment. For both 6 and 12 hours of exposure, the concentrations at 1 and 10 µg/ml showed the greatest effect on lowering the mitotic indices, with 12 hours showing the greater effect. For the toxins in general, the overall effect from 6 to 12 hours is: 10 > 1 > 100 > 0.1 µg/ml.

The three-way means breakdown analysis tested to see if, above and beyond any main effects or two-way interaction effects, there were any effects due to particular

Figure 4. Means Differences For Mitotic Indices: Means broken down by toxin by time by treatment


Mean Mitotic Index Difference
10
9
8
7
6
5
0
1.0 ug/ml
10
0.1
100
10 ug/ml
1.0
0.1
100
100 ug/ml
10
1.0
0.1
6 h
12 h
6 h
12 h
6 h
12 h
Ochratoxin A
Penicillic Acid
OA+PA

three-way combinations of the factors. Figure 4 shows the mitotic index breakdown by toxin by time by treatment. For ochratoxin A, each of the treatments showed the greater effect at 12 hours with an overall effect of 1 > 10 > 0.1 > 100. Penicillic acid showed the greater effect at 6 hours of exposure except at 10 µg/ml where there was approximately a 10% decrease from 6 to 12 hours. This was a larger decrease than for the other penicillic acid treatments. At 6 hours, penicillic acid showed an effect of 1 > 10 > 0.1 > 100; the effects of 1 and 10 µg/ml were reversed for 12 hours although there was < 1% difference for these two at this time. Concentrations of 0.1 and 100 µg/ml behaved similarly for penicillic acid. Ochratoxin A + penicillic acid showed the more dramatic mean count differences from 6 to 10 hours. At 6 hours, 1 and 100 µg/ml acted in a similar manner, 0.1 µg/ml showed the least effect. At 12 hours, ochratoxin A + penicillic acid showed the effect of 100 > 10 > 1 > 0.1.

The breakdown means for treatment by toxin by time and for treatment by time by toxin are shown in Figures 5 and 6, respectively. At 0.1 µg/ml, ochratoxin A + penicillic acid showed little effect at 6 hours (only affecting some 20% of the dividing cells). The two individual toxins behaved similarly with a reversal of

Figure 5. Means Differences For Mitotic Indices: Means broken down by treatment by toxin by time

OA = ochratoxin A

PA = penicillic acid

OA+PA = ochratoxin A + penicillic acid

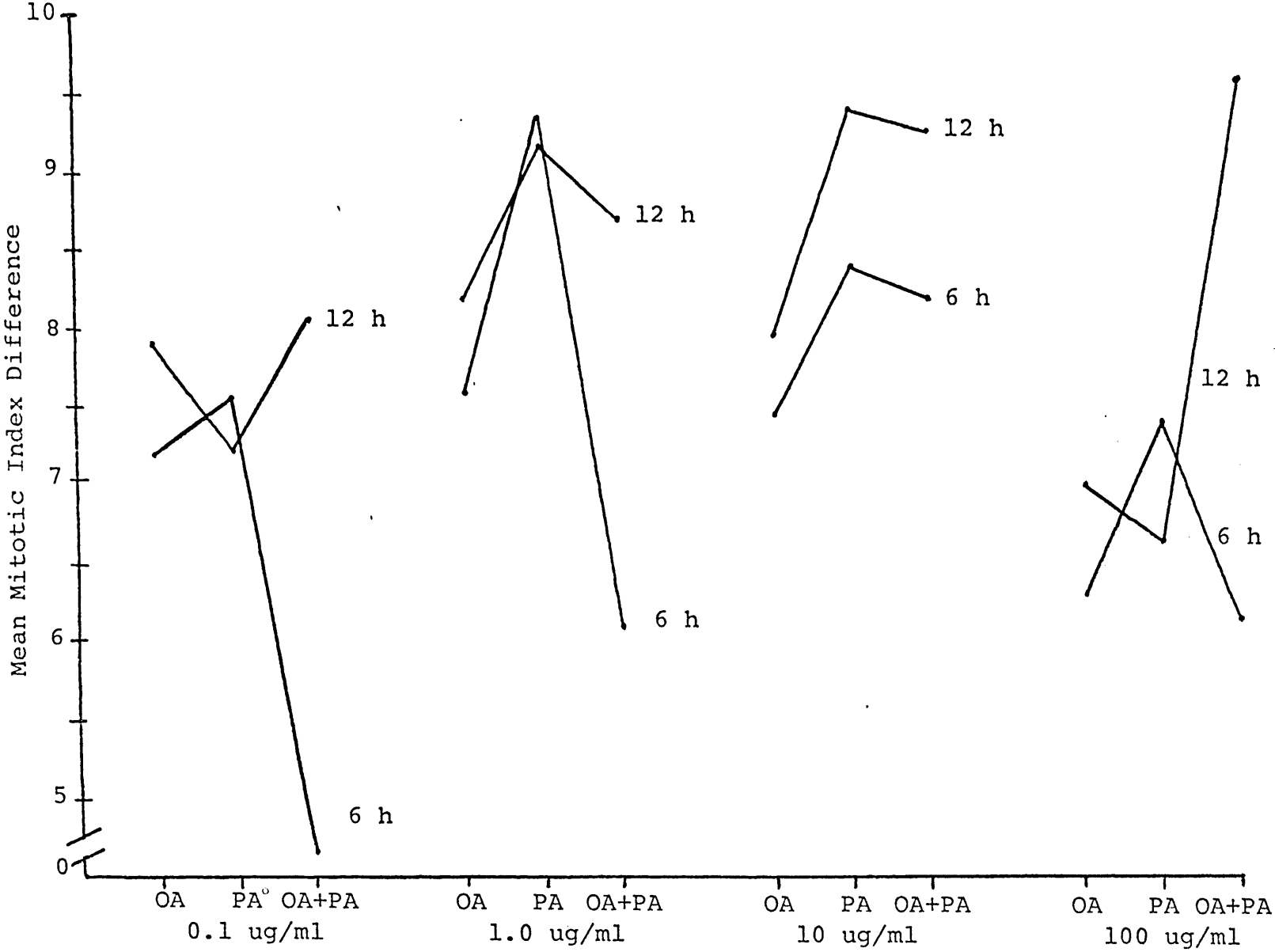
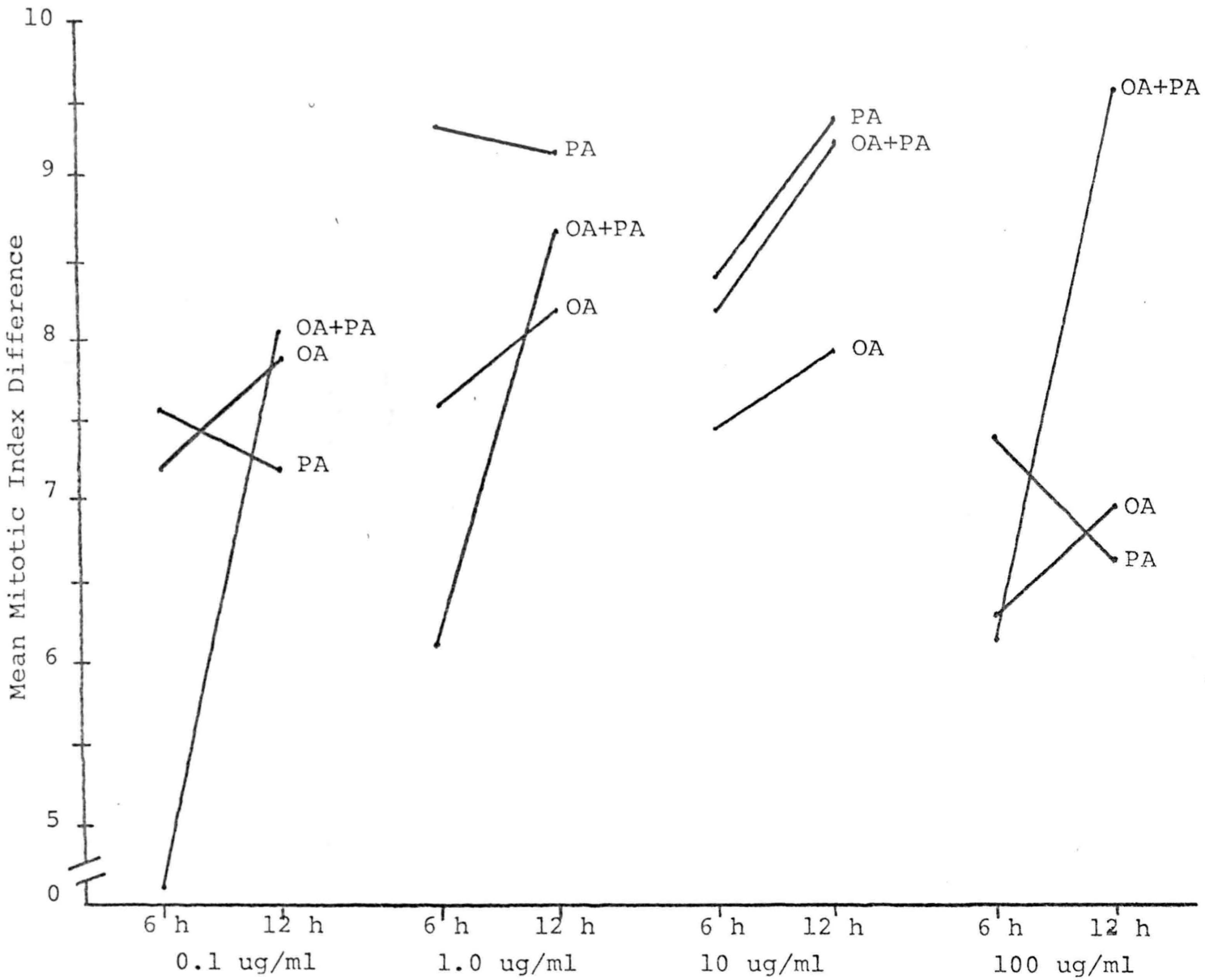

Mean Mitotic Index Difference
10
9
8
7
6
5
0
12 h
6 h
12 h
6 h
12 h
6 h
12 h
6 h
OA
PA
OA+PA
0.1 ug/ml
1.0 ug/ml
10 ug/ml
100 ug/ml

Figure 6. Means Differences For Mitotic Indices: Means broken down by treatment by time by toxin

OA = ochratoxin A

PA = pencillic acid

OA+PA = ochratoxin A + penicillic acid


Mean Mitotic Index Difference
10
9
8
7
6
5
0
OA+PA
OA
PA
PA
OA+PA
OA
PA
OA+PA
OA
OA+PA
OA
PA
6 h
12 h
0.1 ug/ml
6 h
12 h
1.0 ug/ml
6 h
12 h
10 ug/ml
6 h
12 h
100 ug/ml

their effects from 6 to 12 hours. At 12 hours, the toxins in combination elicited the greatest effect, now affecting approximately 80% of the dividing cells. At 1 µg/ml, penicillic acid showed the greater effect of the toxins at both 6 and 12 hours (approximately 92% of the cells undividing). At 6 hours, ochratoxin A showed an effect less than penicillic acid yet still greater than the combination effect; at 12 hours, ochratoxin A had the least effect at this concentration (76-82% of the cells were not dividing). With the toxins in combination, the decrease in the number of dividing cells was more dramatic affecting from 61 then 81% of the dividing cells. At 10 µg/ml, with all the toxins, 12 hours had the greatest effect with penicillic acid > ochratoxin A + penicillic acid > ochratoxin A. At 100 µg/ml ochratoxin A affected the dividing cells much less than it had at the previous three concentrations, although still stopping some 65-70% of the cells from dividing. Penicillic acid showed the opposite effect from that of ochratoxin A, showing the greatest effect at 12 hours for this concentration. Ochratoxin A + penicillic acid affected some 62% of the dividing cells at 6 hours and jumped to 96% at 12 hours.

Figures 7 and 8 are the three-way interactions for time by toxin by treatment and time by treatment by toxin.

Figure 7. Means Differences For Mitotic Indices: Means broken down by time by toxin by treatment

OA = ochratoxin A

PA = penicillic acid

OA+PA = ochratoxin A + penicillic acid

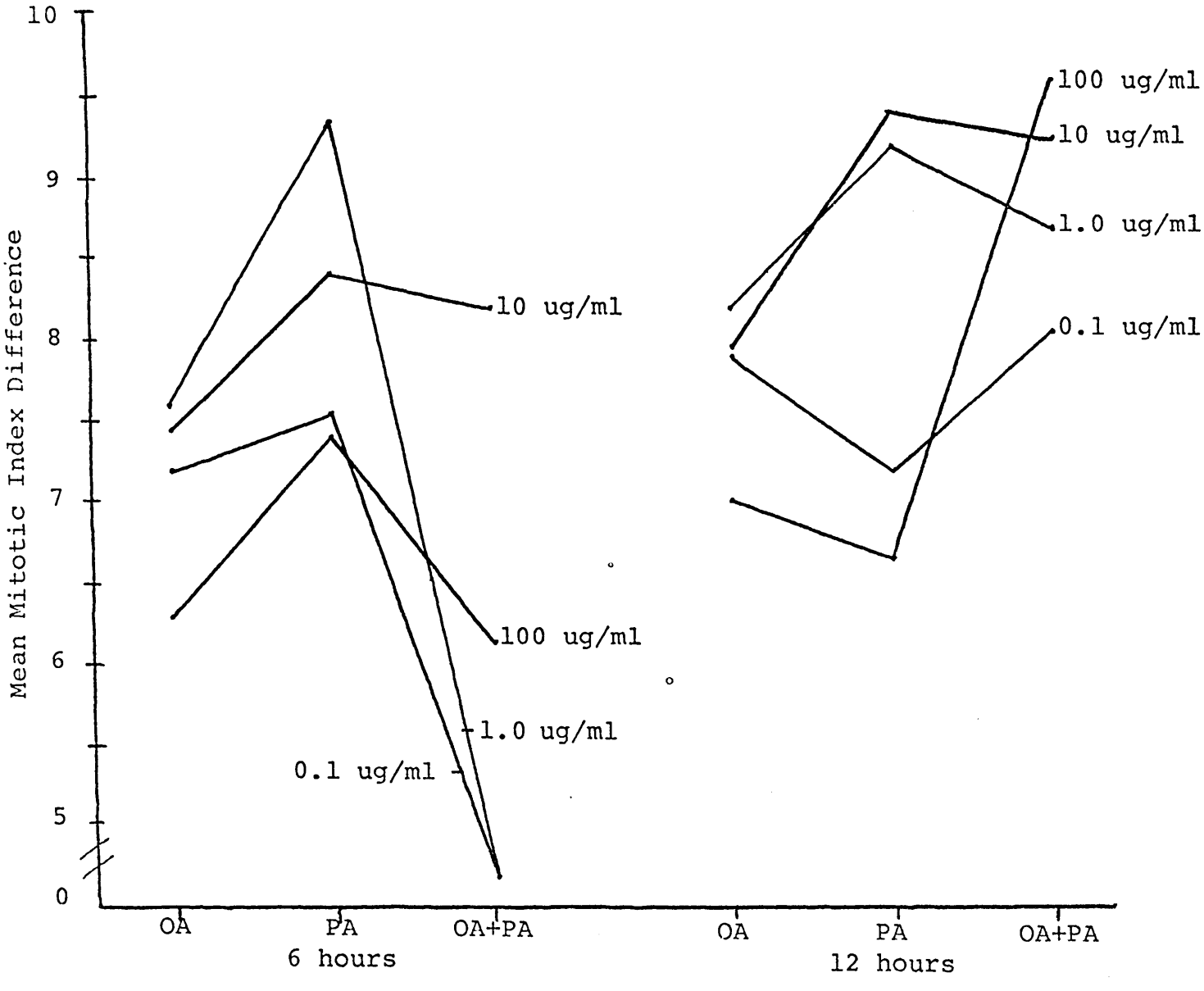
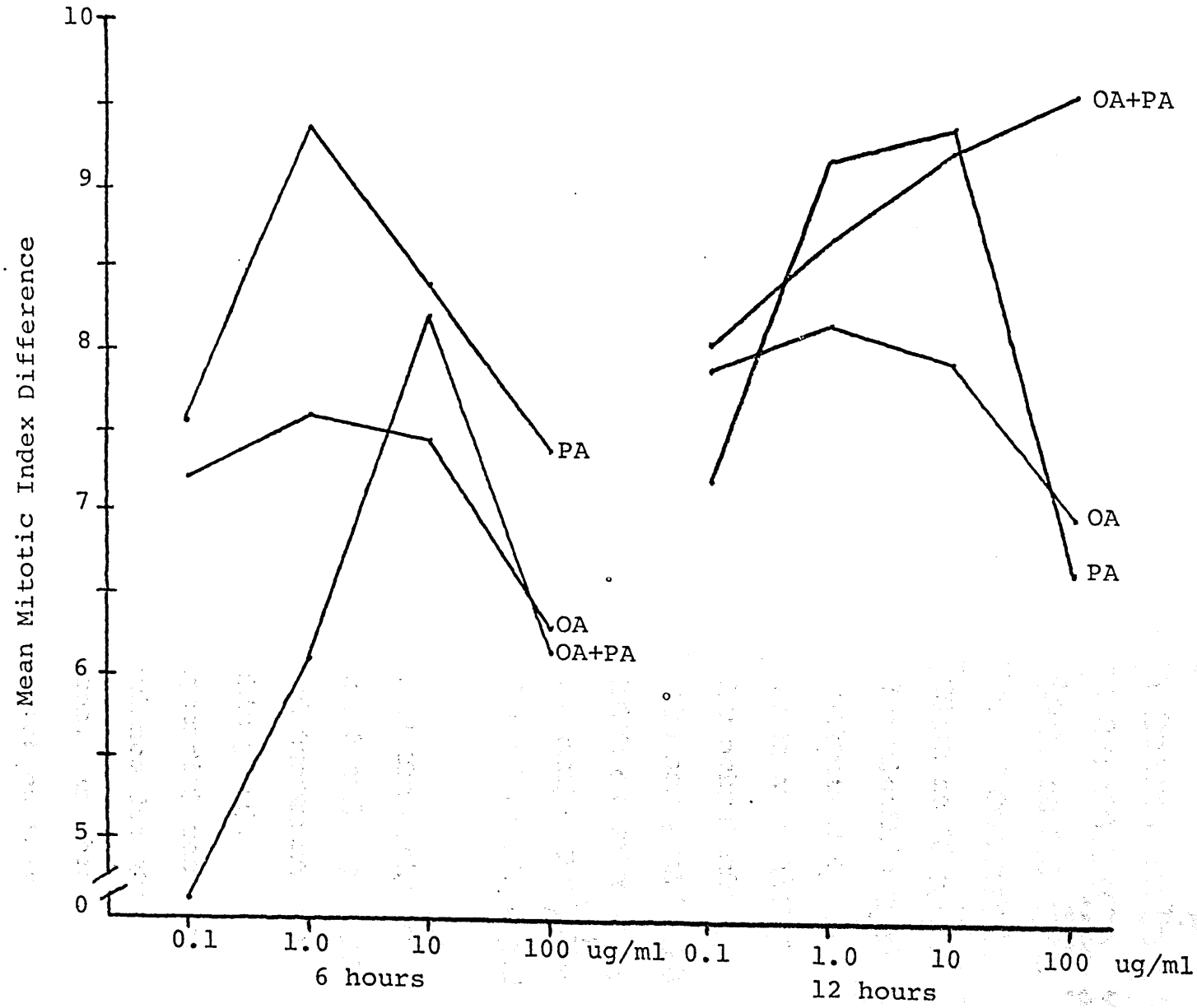

Mean Mitotic Index Difference
10
9
8
7
6
5
0
10 ug/ml
100 ug/ml
1.0 ug/ml
0.1 ug/ml
100 ug/ml
10 ug/ml
1.0 ug/ml
0.1 ug/ml
OA
PA
OA+PA
6 hours
OA
PA
OA+PA
12 hours

Figure 8. Means Differences For Mitotic Indices: Means broken down by time by treatment by toxin

OA = ochratoxin A

PA = penicillic acid

OA+PA = ochratoxin A + penicillic acid


Mean Mitotic Index Difference
10
9
8
7
6
5
0
PA
OA
OA+PA
OA+PA
OA
PA
0.1
1.0
10
100 ug/ml
6 hours
0.1
1.0
10
100 ug/ml
12 hours

At 6 hours, penicillic acid showed the greatest effect (1 > 10 > 0.1 > 100). For ochratoxin A the decrease in dividing cells rose from 0.1 to 10 µg/ml (at which time it affected some 82% of the dividing cells); at 100 µg/ml its effect was similar to that at 1 µg/ml. Ochratoxin A + penicillic acid showed the greatest effect at 10 µg/ml for 6 hours, behaving similar to that of penicillic acid at this concentration. At 12 hours, ochratoxin A + penicillic acid showed a continuous decrease in actively dividing cells from 0.1 to 100 µg/ml. For ochratoxin A the effects of 1 and 10 µg/ml were much the same, with 0.1 and 100 µg/ml showing the least effect (with ochratoxin A, the same trend was seen at both 6 and 12 hours). Penicillic acid showed the greatest effects at both 1 and 10 µg/ml for 6 and 12 hours.

The second half of the study involved the analysis of the mitotic phase distribution. Tables 6 and 7 present the distributions found for the toxins and treatments at 6 and 12 hours (data was not coded for this part).

First, the BMDP program P2V (see Appendix) was run. This program performed an analysis of variance for the fixed effects and for the repeated measures. In this program, a distinction is made between variables that classify cases into groups (toxins, treatments, times)

TABLE 6

Mitotic Phase Analysis* at 6 Hours

Treatment (µg/ml)	Phases** A	B	C	D	E
Ochratoxin A					
0.0	88.5*	10.8	0.7	0.1	0.0
0.1	89.5	6.5	0.0	0.0	0.2
1.0	85.5	1.5	0.0	0.0	0.0
10	94.1	1.1	0.0	0.0	0.0
100	73.9	0.0	0.0	0.0	0.0
Penicillic Acid					
0.0	81.0	11.3	3.2	0.4	4.4
0.1	92.9	5.2	0.0	0.0	2.1
1.0	91.5	5.6	1.8	0.4	0.2
10	89.7	7.9	1.4	0.1	1.6
100	85.6	8.7	0.4	0.2	0.6
OA + PA					
0.0	78.0	13.7	3.7	0.6	4.8
0.1	91.3	5.1	1.3	0.0	2.3
1.0	94.7	3.5	1.2	0.1	1.9
10	59.8	3.5	0.2	0.0	1.1
100	60.2	6.2	0.0	0.5	1.6

*Percentage of dividing cells in each division phase from a total of 500 cells per run. Values represent the mean of 2 replicate runs.

**Phases: A= prophase, B= prometaphase, C= metaphase, D= anaphase, E= telophase.

TABLE 7

Mitotic Phase Analysis* at 12 Hours

Treatment (µg/ml)	Phases** A	B	C	D	E
Ochratoxin A					
0.0	92.0*	5.8	1.2	0.5	0.6
0.1	24.0	3.2	0.0	0.0	0.0
1.0	25.8	3.0	0.0	0.0	0.0
10	21.1	3.1	0.2	0.0	0.0
100	27.1	4.1	0.0	0.0	0.0
Penicillic Acid					
0.0	84.6	7.4	1.7	0.7	1.6
0.1	83.1	5.3	0.6	0.4	1.2
1.0	66.9	3.7	0.0	0.4	1.0
10	73.0	2.9	0.8	0.2	0.8
100	67.6	8.5	0.6	0.8	2.7
OA + PA					
0.0	85.5	9.3	1.3	1.0	3.0
0.1	76.9	5.2	0.0	0.0	1.1
1.0	68.4	3.6	0.0	0.0	0.9
10	77.2	2.0	0.0	0.0	0.4
100	67.8	4.7	0.0	0.0	0.0

*Percentage of dividing cells in each division phase from a total of 500 cells per run. Value represent the mean of 2 replicate runs.

**Phases: A= prophase, B= prometaphase, C= metaphase, D= anaphase, E= telophase.

TABLE 8

Mitotic Phase Analysis of Variance for Repeated Measures

Source	(F)-Ratio	Tail Probability
Toxin	8.05	0.0121*
Treatment	3.06	0.0834
Time	21.24	0.0017*
Toxin x Time	9.72	0.0072*
Treatment x Toxin	1.45	0.3055
Treatment x Time	2.40	0.1361
Phases** x Toxin	5.69	0.0002*
Phases x Treatment	2.31	0.0213*
Phases x Time	19.78	0.0001*
Phases x Toxin x Time	8.95	0.0001*
Phases x Treatment x Toxin	0.99	0.5072
Phases x Treatment x Time	2.27	0.0234*

*Significant for P > = 0.05
**Dependent variables for repeated measures (Phases: prophase, prometaphase metaphase, anaphase, and telophase)

and repeated measures (mitotic phases). A summary of the F-ratios and their significance is presented in Table 8. The analysis showed a significant effect on the mitotic phases by the toxins, treatments, and time exposures.

A breakdown analysis of the means from the repeated measures analysis was run (see Appendix program BREAKDOWNS) in SPSS. This was to determine the interactions on the mitotic phase distribution by the toxins, treatments, and times. The mean differences for this breakdown analysis are shown in Figures 9 to 17. The mitotic phases (prophase, prometaphase, metaphase, anaphase, telophase) are shown broken down by toxin and treatment, by toxin and time, and by time and treatment.

The means breakdowns for the main effects from the repeated measures analysis are summarized in Table 9. The table shows the distribution trend for the phases in regard to their main effects. These trends showed that (1) the distribution was affected differently for each treatment and each toxin, and (2) 12 hours had a lower mean count in each division than did 6 hours.

The mean differences for the mitotic phase analysis broken down for prophase, metaphase, prometaphase, and telophase by toxin and time are shown in Figures 9, 10 and 11. There were a larger number of cells in prophase and

TABLE 9

Means Breakdown from Repeated Measures Analysis

Phases x (source)	Phase* A	B	C	D	E
Treatment					
0.0 µg/ml	84.67	0.92	5.50	0.60	0.33
0.1 µg/ml	75.67	0.48	2.50	0.15	0.00
1.0 µg/ml	71.33	0.30	4.83	0.17	0.00
10 µg/ml	68.83	0.30	4.17	0.27	0.00
100 µg/ml	63.17	0.48	3.83	0.17	0.00
Toxin					
Ochratoxin A	61.80	0.36	3.10	0.18	0.00
Penicillic acid	81.10	0.61	5.50	0.45	0.10
OA + PA	75.30	0.52	3.90	0.18	0.10
Time					
6 hours	83.13	0.56	4.40	0.27	0.00
12 hours	62.33	0.43	3.93	0.27	0.13

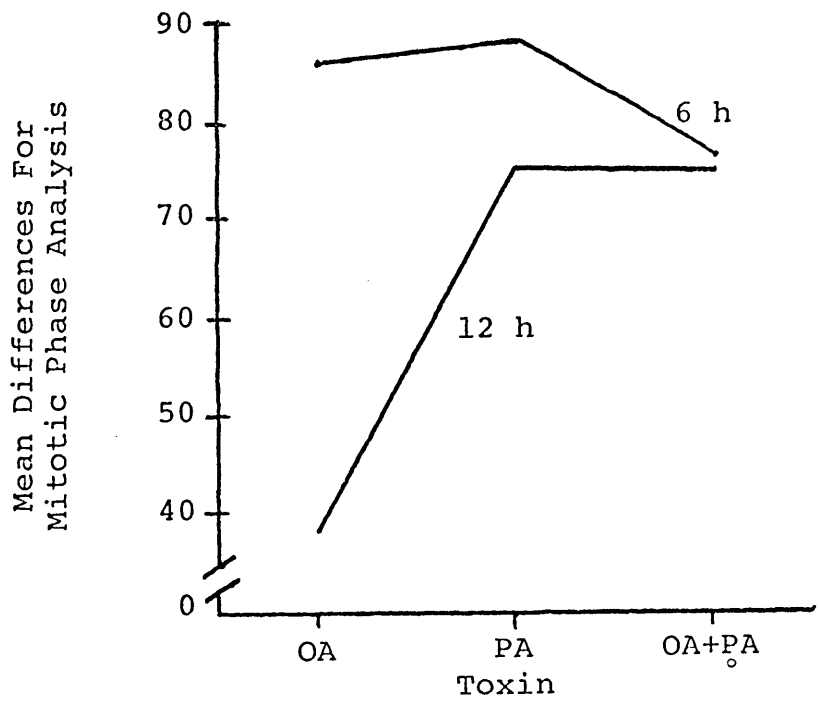
*Phases: A= prophase, B= prometaphase, C= metaphase, D= anaphase, E= telophase.

Figure 9. Means Differences For Mitotic Phase Analysis: Means broken down for prophase and prometaphase by toxin and by time

OA = ochratoxin A

PA = penicillic acid

OA+PA = ochratoxin A + penicillic acid


Prophase
Mean Differences For Mitotic Phase Analysis
90
80
70
60
50
40
0
6 h
12 h
OA
PA
OA+PA
Toxin

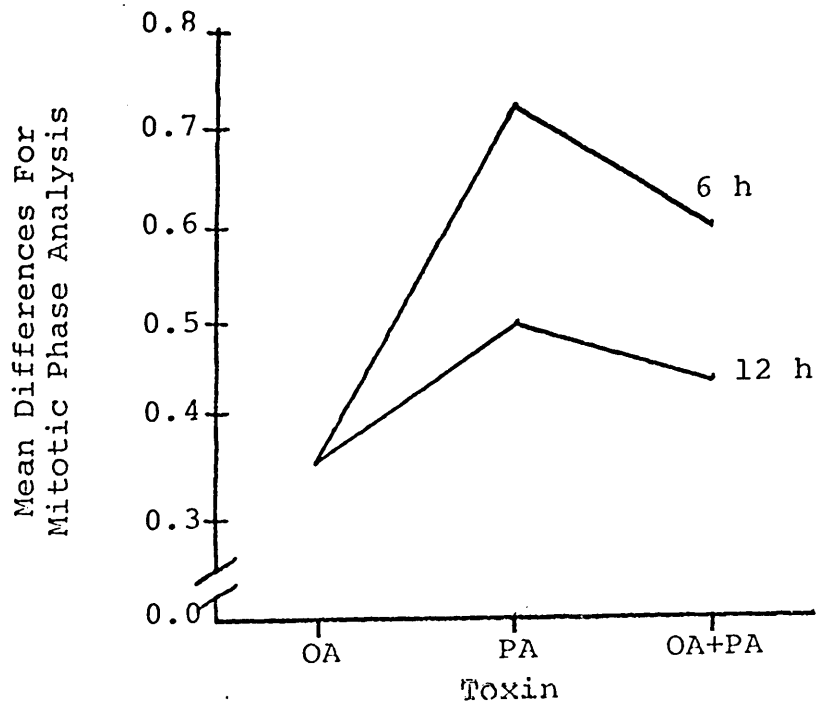
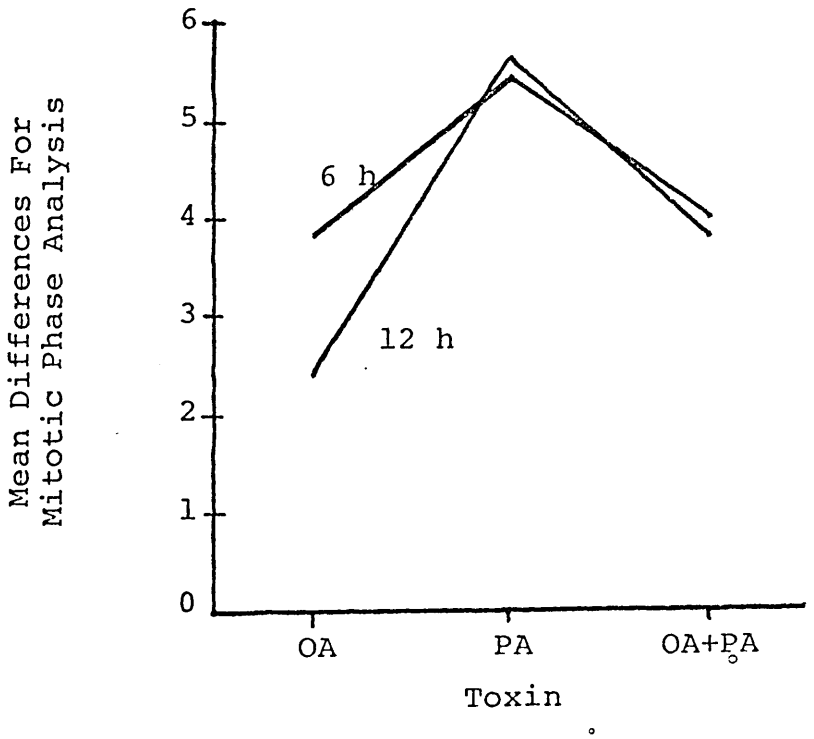

Prometaphase
Mean Differences For Mitotic Phase Analysis
0.8
0.7
0.6
0.5
0.4
0.3
0.0
6 h
12 h
OA
PA
OA+PA
Toxin

Figure 10. Means Differences For Mitotic Phase Analysis: Means broken down for metaphase and anaphase by toxin and by time

OA = ochratoxin A

PA = penicillic acid

OA+PA = ochratoxin A + penicillic acid


Metaphase
Mean Differences For
Mitotic Phase Analysis
6
5
4
3
2
1
0
6 h
12 h
OA
PA
OA+PA
Toxin

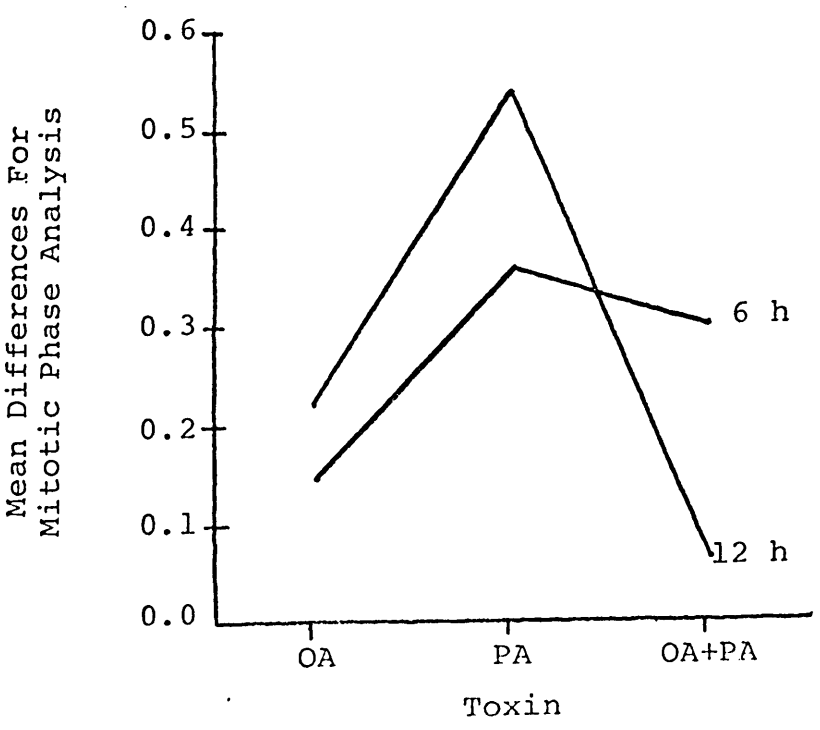
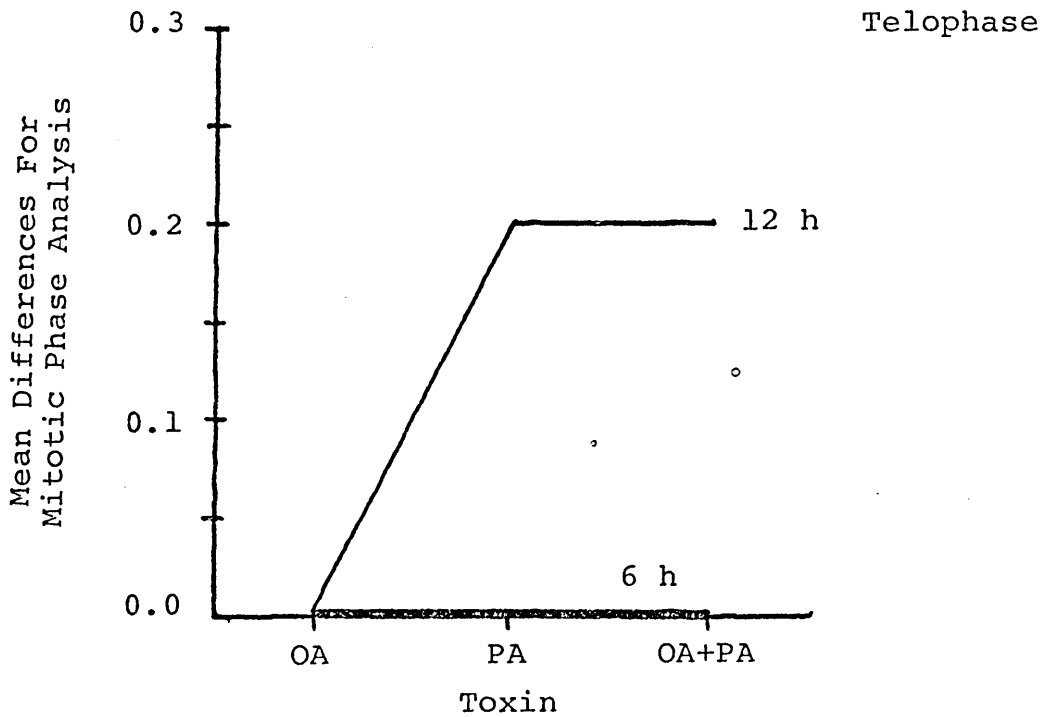

Anaphase
Mean Differences For
Mitotic Phase Analysis
0.6
0.5
0.4
0.3
0.2
0.1
0.0
6 h
12 h
OA
PA
OA+PA
Toxin

Figure 11. Means Differences For Mitotic Phase Analysis: Means broken down for telophase by toxin and by time

OA = ochratoxin A

PA = penicillic acid

OA+PA = ochratoxin A + penicillic acid


Telophase
0.3
0.2
0.1
0.0
Mean Differences For
Mitotic Phase Analysis
12 h
6 h
OA
PA
OA+PA
Toxin

prometaphase for all the toxins for 6 hours. Ochratoxin A + penicillic acid had approximately the same number of dividing cells in prophase at 6 and 12 hours; the difference was more dramatic for ochratoxin A. At 6 and 12 hours, ochratoxin A had approximately the same number of dividing cells in prometaphase. As a whole, penicillic acid contained greater numbers of dividing cells in prophase and prometaphase than did the other toxins. In metaphase, the effect was greater at 12 hours although for penicillic acid, the effect was approximately the same at either time exposure. At 6 hours, ochratoxin A and penicillic acid had fewer dividing cells scored in anaphase at 6 than at 12 hours; for the two toxins in combination, few cells scored were in anaphase at 12 hours (25% less than found at 6 hours). Only at 12 hours, were cells scored in telophase.

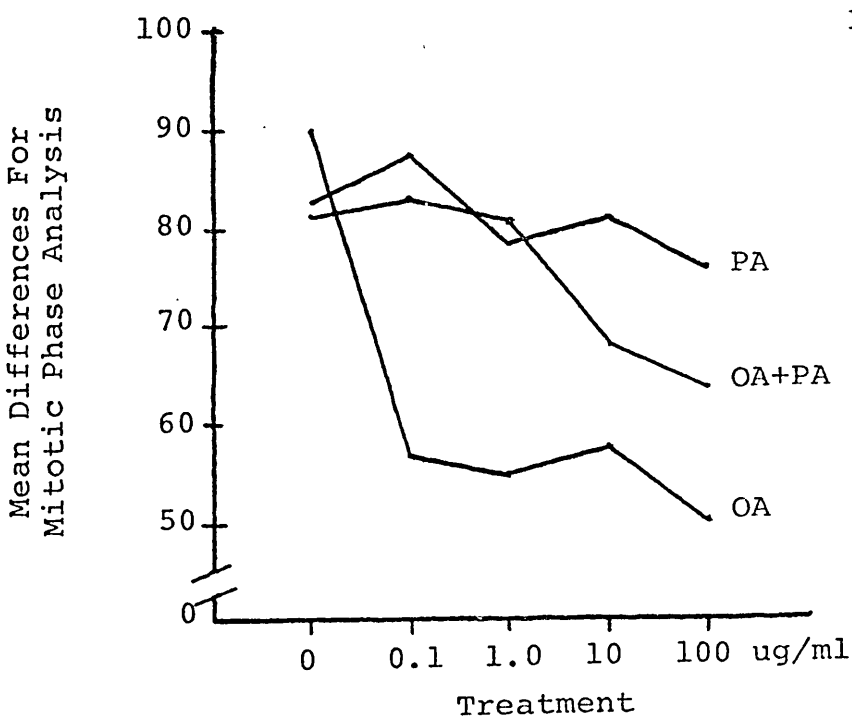
Figure 12, 13, and 14 show the mitotic phase analysis means differences broken down for prophase, prometaphase, metaphase, anaphase, and telophase by treatment and toxin. Penicillic acid seemed to contain more cells in prophase than did ochratoxin A + penicillic acid and ochratoxin A, respectively. For the treatments, penicillic acid showed its greatest number in prophase at 0.1 µg/ml, ochratoxin A at 10 µg/ml, and for the two in combination at 0.1 µg/ml.

Figure 12. Means Differences For Mitotic Phase Analysis: Means broken down for prophase and prometaphase by treatment and by toxin

OA = ochratoxin A

PA = penicillic acid

OA+PA = ochratoxin A + penicillic acid


Prophase
Mean Differences For Mitotic Phase Analysis
100
90
80
70
60
50
0
PA
OA+PA
OA
0
0.1
1.0
10
100 ug/ml
Treatment

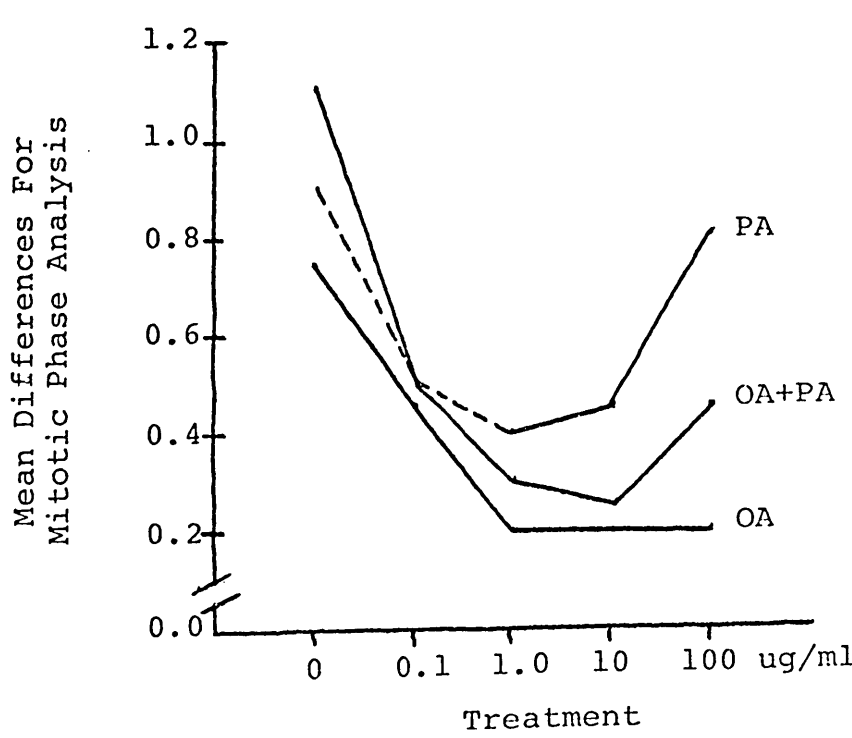

Prometaphase
Mean Differences For Mitotic Phase Analysis
1.2
1.0
0.8
0.6
0.4
0.2
0.0
PA
OA+PA
OA
0
0.1
1.0
10
100 ug/ml
Treatment

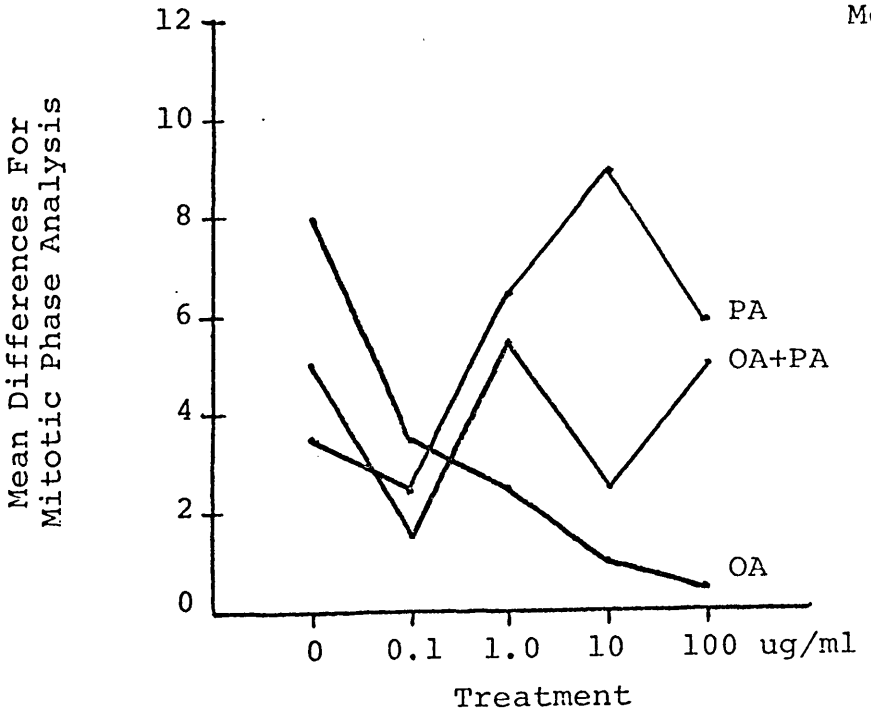
Figure 13. Means Differences For Mitotic Phase Analysis: Means broken down for metaphase and anaphase by treatment and by toxin

OA = ochratoxin A

PA = penicillic acid

OA+PA = ochratoxin A + penicillic acid

Metaphase



Anaphase

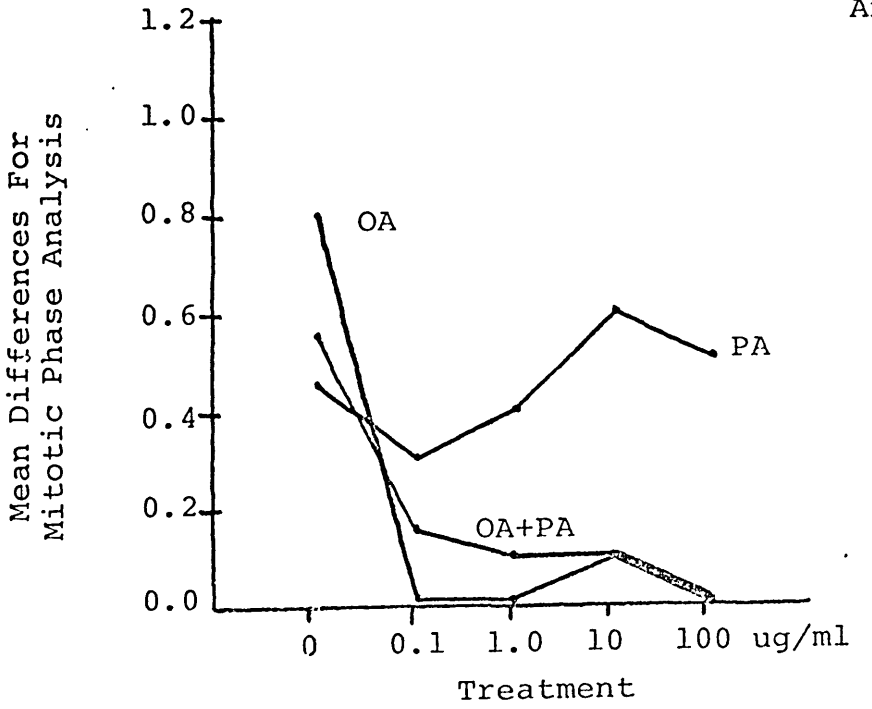
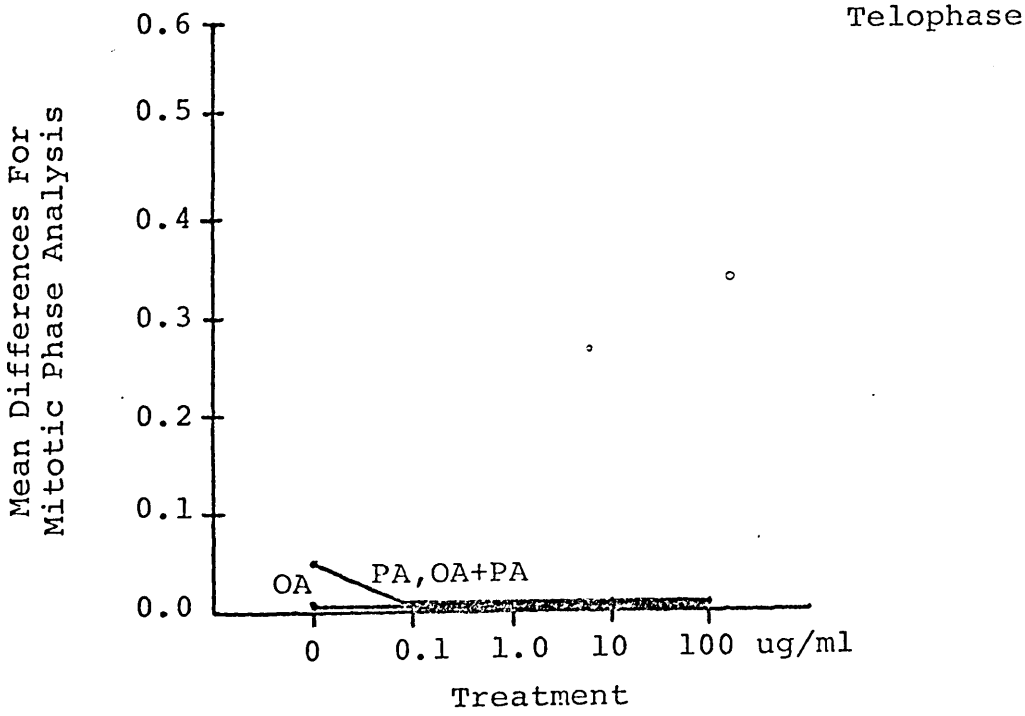


Figure 14. Means Differences For Mitotic Phase Analysis: Means broken down for telophase by treatment and by toxin

OA = ochratoxin A

PA = penicillic acid

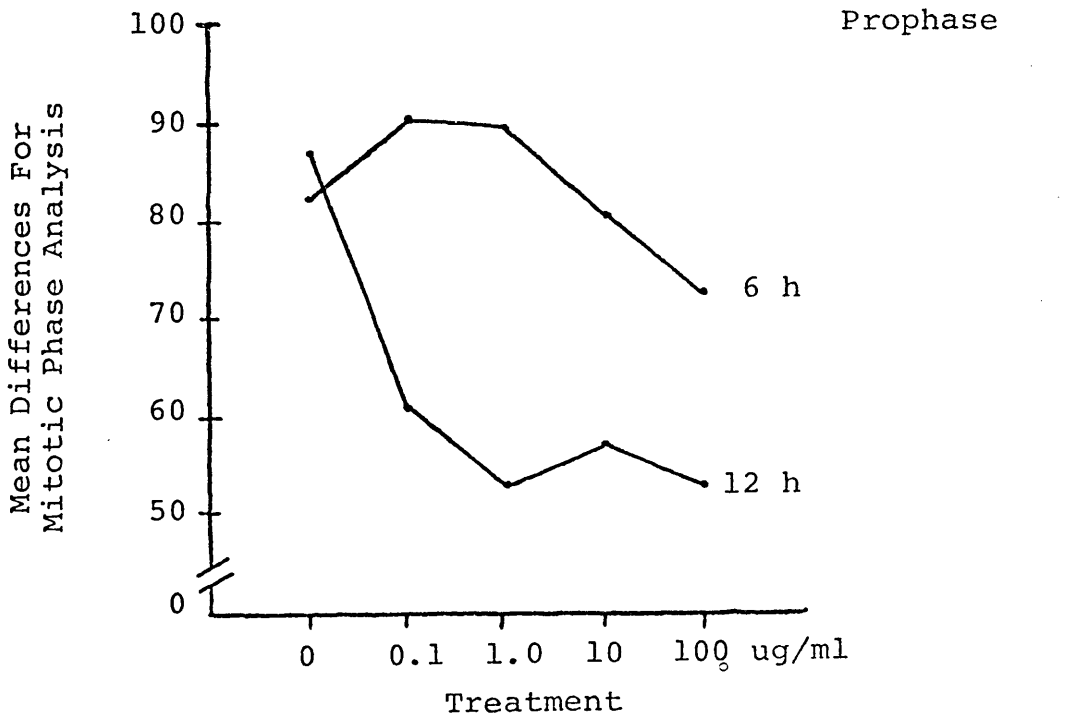
OA+PA = ochratoxin A + penicillic acid


Telophase
Mean Differences For
Mitotic Phase Analysis
0.6
0.5
0.4
0.3
0.2
0.1
0.0
OA
PA, OA+PA
0
0.1
1.0
10
100 ug/ml
Treatment

Ochratoxin A showed the sharper decrease from the controls. The greater number of cells scored in prometaphase show penicillic acid > ochratoxin A + penicillic acid > ochratoxin A. For all the toxins, there was a drop in this phase from the controls, with 0.1 and 1 µg/ml. From these last two concentrations, there was a slight rise at 10 and 100 µg/ml although the number never reached that of the controls. Again, there was a drop from the control mean counts in scoring for metaphase. Penicillic acid showed the greatest number of dividing cells in metaphase occurring at 10 µg/ml. At 1 and 100 µg/ml ochratoxin A + penicillic acid showed similar numbers in this phase. Ochratoxin A showed a steady decline from the control mean counts from 0.1 to 100 µg/ml. For metaphase mean counts, penicillic acid > ochratoxin A + penicillic acid > ochratoxin A. Relatively few, if any, cells scored for ochratoxin A + penicillic acid and ochratoxin A were found in anaphase or telophase. Penicillic acid had some cells in anaphase although still < 1% of the total.

The mean breakdowns for the phases broken down by treatment and toxin are shown in Figure 15, 16 and 17. For all the treatments, fewer cells were shown in prophase at 12 hours than at 6, as was the case for prometaphase. At 6 hours, more cells are found in metaphase except at

Figure 15. Means Differences For Mitotic Phase Analysis: Means broken down for prophase and prometaphase by treatment and by time


Prophase
Mean Differences For
Mitotic Phase Analysis
100
90
80
70
60
50
0
6 h
12 h
0
0.1
1.0
10
100 ug/ml
Treatment

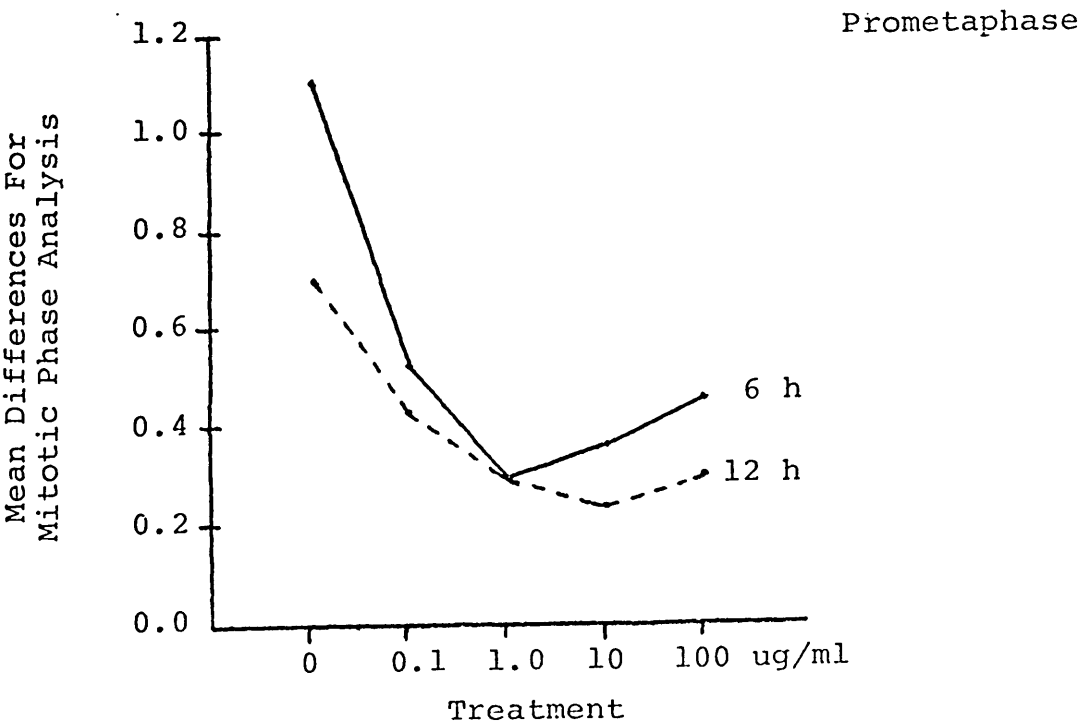
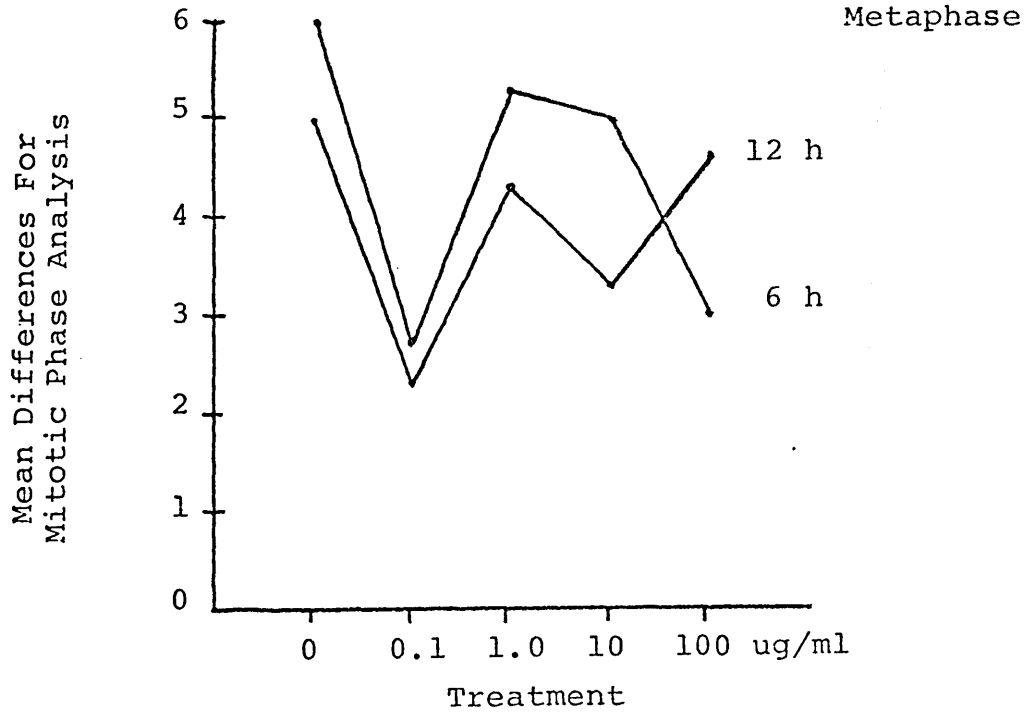

Prometaphase
Mean Differences For
Mitotic Phase Analysis
1.2
1.0
0.8
0.6
0.4
0.2
0.0
6 h
12 h
0
0.1
1.0
10
100 ug/ml
Treatment

Figure 16. Means Differences For Mitotic Phase Analysis: Means broken down for metaphase and anaphase by treatment and by time


Metaphase
Mean Differences For
Mitotic Phase Analysis
6
5
4
3
2
1
0
12 h
6 h
0
0.1
1.0
10
100 ug/ml
Treatment

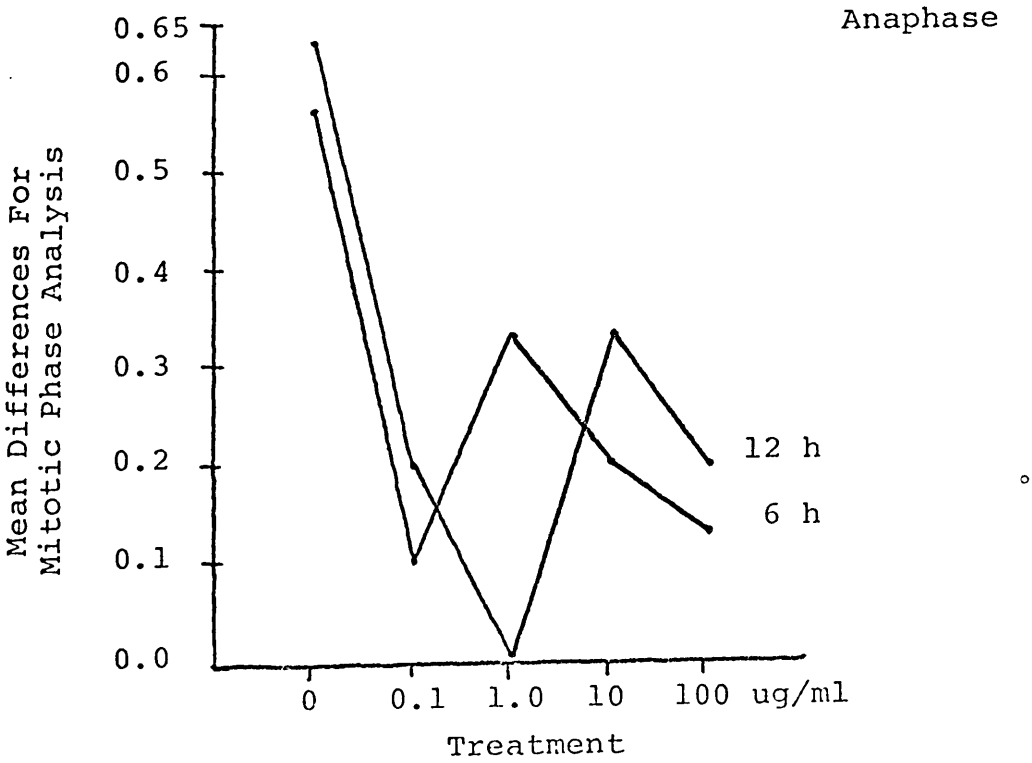
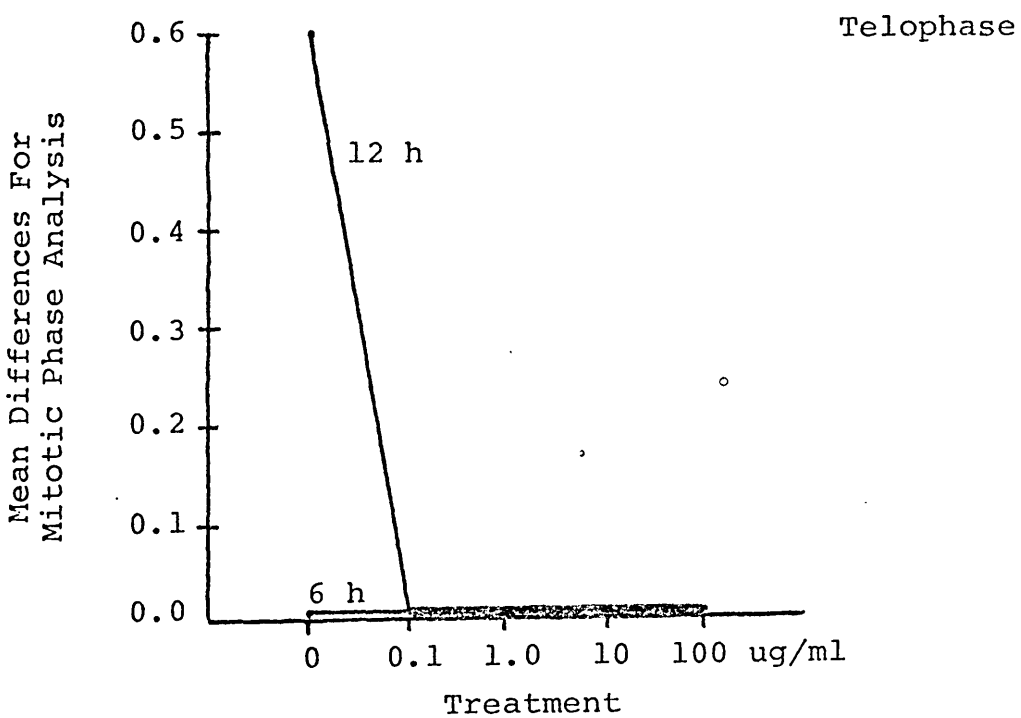

Anaphase
Mean Differences For
Mitotic Phase Analysis
0.65
0.6
0.5
0.4
0.3
0.2
0.1
0.0
12 h
6 h
0
0.1
1.0
10
100 ug/ml
Treatment

Figure 17. Means Differences For Mitotic Phase Analysis: Means broken down for telophase by treatment and by time


Telophase
Mean Differences For Mitotic Phase Analysis
0.6
0.5
0.4
0.3
0.2
0.1
0.0
12 h
6 h
0
0.1
1.0
10
100 ug/ml
Treatment

100 µg/ml where the results are reversed. For 0 to 10 µg/ml, the results were similar for 6 to 12 hours. Relatively no cells were found at 6 or 12 hours for anaphase and telophase. For each phase, the cells behaved relatively alike for each treatment at 6 and 12 hours.

DISCUSSION

In the past and recent years, root tips have been regarded as the ideal plant tissue in which to study the effect of chemical substances on chromosomes. Root tips are relatively easy to handle and the root meristem contains a large number of dividing cells that are readily obtainable. These plant materials are inexpensive and easily available all year round, and have large chromosomes and a low chromosome number. Pisum sativum (pea) was chosen for these reasons; and because extensive work has been done with Pisum sativum in connection with cytological studies (Wilson, 1963).

Initial counts for the mitotic indices and mitotic phase analyses done on preliminary control groups are summarized in Tables 1 and 2 (Experimental Results). At the beginning time of 6 a.m. the cells were actively dividing and approximately 38% of the cells were in either metaphase, anaphase, or telophase. At both 12 p.m. and 6 p.m., again there was a good distribution among the mitotic phases. This preliminary study showed the cells to be actively dividing before any application of treatment at the start time of 6 a.m. and at the times chosen for excision of the root tips.

The mitotic indices for toxin treatments at 6 and 12 hours (Tables 3 and 4) visually showed a decrease in actively dividing cells, with 12 hours showing a lower mean count than at 6 hours. A one-way analysis of variance on the mitotic indices data showed the F-ratios to be highly significant at the level of P=.0001. An analysis of variance on the main effects and two- and three-way interactions provided F-ratios highly significant at the P=.0001 level. Hence, each of the main effects - toxins, treatments, times - could not possibly be representative of the same population. The two-way interaction between just treatment and time showed significance at the P=.025 level. These results (Table 5). were considered strong support that ochratoxin A, penicillic acid, and the two in combination along with the various treatments (0.1, 1.0, 10, 100 µg/ml), and the time exposures (6 and 12 hours) all interacted in some way to suppress the number of cells actively dividing. This analysis also presented indications that penicillic acid was most effective on lowering the mitotic index while ochratoxin A, although effective, was the least effective of the three. Overall, 10 µg/ml appeared to be more effective than 1 µg/ml and 100 µg/ml with 0.1 µg/ml having the least

effect; and, as was assumed, 12 hours of exposure did more damage than 6 hours of exposure.

A further examination of the mitotic indices was done by breaking down the differences among the means. The two-way interactions (Figures 1-3) showed that each of the mycotoxins suppressed the mitotic acitvity although not in the same way for each of the treatments. For ochratoxin A and ochratoxin A + penicillic acid the greater effect was shown at 12 hours of exposure; penicillic acid showed a greater effect at 6 hours of exposure although the means difference between 6 and 12 hours was only 0.05. A breakdown then for time with respect to the other conditions supported this conclusion. The greatest mean difference from 6 hours to 12 hours of exposure resulted from treatment with ochratoxin A + penicillic acid; again, there was relatively little mean difference from 6 to 12 hours for penicillic acid. Ochratoxin A + penicillic acid in combination increased its effect steadily from 0.1 to 10 µg/ml and dropped off at 100 µg/ml, although still greater than at 1 µg/ml.

The three-way breakdown interactions (Figures 4-8) provided a better view of the overall effect. With ochratoxin A the increase was similar from 6 to 12 hours for each of the treatments, with an overall effect of

1.0 > 10 > 0.1 > 100 µg/ml. Penicillic acid showed 6 hours of exposure to have a greater effect than at 12 hours except at 10 µg/ml; at this treatment there was a sharp decrease in the number of actively dividing cells for 12 hours. This decrease was larger (10%) than for any of the other treatments at 6 or 12 hours. Concentrations of 0.1 and 100 µg/ml behaved in a similar manner for penicillic acid. With regard to the actions of ochratoxin A + penicillic acid, the changes in mean counts were more dramatic than for the individual mycotoxins from 6 to 12 hours (Figure 4). At 6 hours, 1.0 and 100 µg/ml affected the mitotic indices in much the same way; the most dramatic differences being between 0.1 and 10 µg/ml. At 12 hours of exposure, the sequence of effect was : 100 > 10 > 1.0 > 0.1. Thus far, the breakdown interactions indicated that (1) the longer the dividing cells were exposed to the toxins, the lower the mean counts for the mitotic indices, (2) penicillic acid behaved in much the same manner at either time exposure, and (3) each of the toxins exhibited different effects for each of the treatments.

The next assessment from the three-way interactions was with interest to the various treatments and toxins,

and their effect on the mitotic indices from 6 to 12 hours. At 0.1 g/ml, ochratoxin A and penicillic acid behaved similarly at 6 hours leaving only approximately 25-30% of the cells actively dividing. Ochratoxin A + penicillic acid had little effect at 6 hours (80% of cells still dividing). But at 12 hours, ochratoxin A + penicillic acid had an effect similar to ochratoxin A alone, affecting some 80% of the dividing cells; penicillic acid acted alike for 6 and 12 hours, affecting 70-75% of the dividing cells. At this concentration, ochratoxin A + penicillic acid elicited a greater effect on the mitotic indices when exposed for 12 hours; penicillic acid elicited the least effect. The effects of ochratoxin A and penicillic acid separately are reversed for this treatment over the two exposure times.

At the next concentration, 1.0 µg/ml, the results were similar for each of the toxins. This was characterized with ochratoxin A, where 76-82% of the cells were not dividing from 6 to 12 hours after exposure. Again, penicillic acid behaved relatively alike at 6 and 12 hours after exposure with a loss of 92% of the cells not dividing. Ochratoxin A + penicillic acid showed a more dramatic decrease from 6 to 12 hours affecting 61 then 81% of the dividing cells. When treated at 1 µg/ml, penicillic acid

elicits a much greater effect at either time exposure. Ochratoxin A + penicillic acid together had a greater effect than ochratoxin A if exposed for 12 hours.

Next, at 10 µg/ml, all the mycotoxins behaved in a similar manner from 6 to 12 hours after exposure: ochratoxin A had from 25 then 20% of the cells dividing, with penicillic acid it was 16 then 6%, and for ochratoxin A + penicillic acid it was 18 then 7% (Figure 5). At this concentration the longer exposure showed the greatest number of cells not actively dividing. As before, penicillic acid > ochratoxin A + penicillic acid > ochratoxin A.

At the last concentration of 100 µg/ml, ochratoxin A halted some 65-70% of the division for either time and penicillic acid stopped 65-75%. As noted earlier, ochratoxin A + penicillic acid showed a sharper decrease in division after 12 hours (from 62% at 6 hours to 96% of the total cells not dividing).

At 0.1 and 100 µg/ml, after 12 hours of exposure, ochratoxin A + penicillic acid elicited an effect greater than each of the mycotoxins alone suggesting some type of cooperative effect. (Toxic synergism is defined in the present literature as "the cooperative effect of two or more substances when they elicit a total effect greater

than the sum of the activities of the individual substances". The effects in this study, although exhibiting a cooperative effect, were not synergistic by literature definition (Schlessinger, 1975)). At 1 and 10 µg/ml, penicillic acid had a greater effect. In general, after 6 hours: penicillic acid > ochratoxin A > ochratoxin A + penicillic acid.

After 6 hours of exposure, penicillic acid was most effective at 1 µg/ml, ochratoxin A and 1 and 10 µg/ml, and ochratoxin A + penicillic acid at 10 µg/ml. After 12 hours of exposure, penicillic acid showed the greatest effect at 1 and 10 µg/ml, ochratoxin A was effective over 0.1 to 10 µg/ml, and the two in combination showed a continuous decrease in actively dividing cells from 0.1 to 100 µg/ml. In most cases, after 12 hours of exposure the least amount of cells were found dividing except in the case of penicillic acid, where the most damage appeared to be after 6 hours exposure. Hence, in general, the concentrations of 1 and 10 µg/ml seemed to elicit the greatest effects at both 6 and 12 hours.

After it was determined from the analysis of the mitotic indices that ochratoxin A, penicillic acid, and the two in combination affected actively dividing cells, the next part was to determine where the effects were

showing up in regard to active mitosis. The mitotic phases examined were prophase, prometaphase, metaphase, anaphase, and telophase. These phases were analyzed for their distribution after exposures to the toxins, treatments, and times (Tables 6 and 7).

An analysis of variance for the fixed effects and the repeated measures is summarized with F-ratios and their significance in Table 8 (Experimental Results). Interactions for the phases with toxins and times and with toxins and treatments are highly significant at the P=.0001 level. From this, a breakdown analysis of the means for the phases was done (Table 11). This was to determine what interaction the toxins, treatments, and exposure times had on the phase distribution.

With the toxin treatments in general, there were more dividing cells in prophase at 6 hours than at 12 hours. As a basis for comparison, with controls (0.0 µg/ml) approximately 83-89% of the cells scored were in prophase. Overall the greatest count in this phase, sequences as : penicillic acid > ochratoxin A + penicillic acid > ochratoxin A. At 6 hours for ochratoxin A, treatment counts differed by the most from $\pm$ 5% from the controls; at 12 hours, the contrast was sharper. Penicillic acid had a larger prophase count at 6 hours than the controls (+5-12%);

after 12 hours, the counts for prophase declined 18 to 22%. Ochratoxin A + penicillic acid behaved in a different manner. At 6 hours the counts increased for 0.1 and 1 µg/ml but decreased for 10 and 100 from the controls by 10 to 17%.

Approximately 10 to 13% of the control cells scored were in prometaphase. Again, more cells for the treatments were found in this stage of division at 6 hours than at 12. At 0.1 µg/ml for all the toxins, the counts in prometaphase were reduced by approximately 50% both at 6 and 12 hours. One and 10 µg/ml generally showed a lower prometaphase count. From the treatments of 0.1 and 100 µg/ml the mycotoxins all behaved in much the same manner (Figure 12). The means analysis indicated a buildup in this stage at the higher concentrations, although the mean counts were lower than the controls.

In the controls, 1-3% were in the metaphase division. Ochratoxin A showed less than 1% of the counts in this division and virtually no cells in any of the next mitotic divisions. The mycotoxins showed a general trend of more cells in this division at 6 hours than at 12. At 6 hours penicillic acid and ochratoxin A + penicillic acid showed approximately 1% of the control count. At 12 hours, ochratoxin A and ochratoxin A + penicillic acid showed

relatively few cells in this mitotic stage (1%). Penicillic acid showed approximately 50% less than the control count.

Only about 1% of the control cells scored were in anaphase. Basically, only penicillic acid had cells scored in anaphase. (Ochratoxin A and the two in combination had < 1%). The mean counts for penicillic acid differed from the controls at 10 and 100 µg/ml.

Approximately 4% of the cells from the controls scored were in telophase. For penicillic acid or ochratoxin A + penicillic acid, only 1% or less of the cells were scored in this phase. At 12 hours, as previously noted, no cells were in this phase except for the controls.

The means analyses showed that overall for the toxins, a greater percentage of the total number of cells scored were in prophase, prometaphase, and metaphase at 12 hours for treatments 0.1 and 100 µg/ml.

The conclusions to be drawn from the statistical analyses of the experimental data follow. The toxins elicit a greater reduction of the mitotic index at 12 hours, with penicillic acid having the greater effect. Only at 12 hours of exposure and at 10 µg/ml and 100 µg/ml were there any indications of some type of cooperative effect between ochratoxin A and penicillic acid. For the

most part, ochratoxin A + penicillic acid elicited an effect somewhere between that of the individual toxins (penicillic acid > ochratoxin A + penicillic acid > ochratoxin A).

Ochratoxin A and penicillic acid have been shown to induce accummulation of metaphase cells in other systems, as for example, C3H mouse mammary carcinoma cell cultures (Umeda et al., 1977), and cultured hepatoma cells (Creppy et al., 1980). For this study, at 12 hours of exposure, relatively few cells can be found in anaphase or telophase; and even at 6 hours, the counts were low. At 6 hours generally more cells were found in prophase for the treatments than for the controls, although only 50% of the control counts for prometaphase were found for the treatments. The greatest percentage of the treated cells were found in prophase and prometaphase indicative of a possible metaphase block.

From this study, there were not any substantial indications of a synergistic effect (the effect being greater than the sum of the two individual toxins) between ochratoxin A and penicillic acid. This was in contrast to the observations of Lindendelser (1973) who found a synergistic lethal response in acute toxidity tests in mice. Another type of synergistic effect was

observed by Creppy et al. (1980) who noticed a synergistic effect on the accumulation of RNA in cultured mammalian cells. There was some indication rather of a "cooperative effect" since the effect of the two mycotoxins in combination yielded an effect between that of the individual toxins.

Ochratoxin A has been found to preferentially inhibit protein synthesis (Creppy et al., 1979) with no indication of binding to RNA or DNA (proteins include arginase and catalase). Penicillic acid has been shown to inhibit protein, RNA, and DNA synthesis. (Penicillic acid can react with arginine, histidine, and lysine.) Both mycotoxins are capable of interacting with the sulfhydryl groups of enzymes (Rodrick, 1978).

The general tendency of the cells to remain in the prometaphase stage of division is indicative of a possible "C-mitotic effect" (Hyypio et al., 1955). The reason possibly being the lactone ring reactivity with the microfillament proteins, perhaps preventing polymerization of these for spindle or depolymerizing those already existing. (Epoxytrichothecene mycotoxins have been shown by Linnainmaa (1979) to arrest cells in metaphase stage by possible epoxide ring reactivity with the microfillar

proteins and their SH-groups.) (References for lactone reactivity can be found by Dickens (1965) and by Van Duuren (1969).)

Further investigation to characterize any chromosome aberrations or abnormalities due to toxin treatment and characterization of the effect of these toxin combinations on cellular protein, RNA, and DNA synthesis would provide information of particular relevance in pinpointing any cooperative effect and for any comparisons with other toxin combinations of this nature in the literature. The results in this study are a good start for a further investigation since interactions of these toxins, treatments, and times on the mitotic indices and on the mitotic phase distributions were highly significant at the P=.0001 level.

SUMMARY

The investigation focused on a comparison of selected *in vitro* effects of ochratoxin A and penicillic acid on *Pisum sativum* (pea variety) root tips. Each mycotoxin was tested individually and in combination at concentrations of 0.1, 1.0, 10, and 100 ug/ml. Time exposures were for 6 and 12 hours. Cellular sensitivity was evaluated by analysis of the mitotic index and mitotic phase distribution.

1. Analysis of the mitotic indices was highly significant (P=.0001) for all interactions of the toxins, treatments, and time exposures.
2. Twelve hours of exposure inflicted more damage on the mitotic indices than did six hours.
3. Penicillic acid elicited a greater effect on lowering the mitotic indices with ochratoxin A eliciting the least effect.
4. For penicillic acid, 1.0 and 10 ug/ml were more effective as was it with ochratoxin A; for the two mycotoxins in combination, 10 and 100 ug/ml elicited the greatest effect on the mitotic indices, perhaps indicative of a possible cooperative effect.

5. The analysis of the mitotic phases showed a highly significant effect (P=.0001) on the distribution by the interaction with the toxins, treatments, and time exposures.

6. There were indications of a prophase build-up and a general tendency for the cells to remain in prometaphase as the time and concentrations were increased suggesting a "C-mitotic" effect.

APPENDIX

Schiff's Reagent

To each 100 ml of 0.15N HCl, add 1 gram of basic fuchsin and 1.9 grams of sodium or potassium metabisulfite. Heat, with continuous stirring, until boiling. Allow the mixture to stand for 24 hours, then decolorize with activated charcoal (a heaping reaspoon). Filter the mixture with a Buchner funnel (moisten the filter paper with a few drops of 1N HCl). The final product should be almost colorless.

Coded Mitotic Index for Table 3*

Toxin**	Toxin Treatment (µg/ml) 0.1	1.0	10	100
OA	72.7	76.7	75.1	63.3
PA	75.8	93.9	84.4	74.5
OA+PA	43.5	61.6	82.4	62.4

*Calculated as: Code= ((mean of control-mean of treatment) /mean of control) x 100
A high coded score represents a low mitotic index.

**OA= ochratoxin A
PA= penicillic acid

Coded Mitotic Index for Table 5*

Toxin**	Toxin Treatment (µg/ml) 0.1	1.0	10	100
OA	79.4	82.3	80.2	70.4
PA	72.4	92.7	94.3	66.8
OA+PA	80.8	87.5	92.7	96.6

*Calculated as: Code=((mean of control-mean of treatment) /mean of control) x 100
A high coded score represents a low mitotic index.

**OA= ochratoxin A
PA= penicillic acid

```
                1 RUN NAME          MITOTIC INDEX:OA AND PA (MYCOTOXINS)
                2 VARIABLE LIST     TREAT,TOXIN,RUN,TIME,SCORE,ROW
                3 INPUT FORMAT      FIXED(4F1.0,1X,1F3.1,1F2.0)

According to your INPUT FORMAT, variables are to be read as follows:

Variable  Record     Columns  Print Format

TREAT          1     1 -   1  (0)
TOXIN          1     2 -   2  (0)
RUN            1     3 -   3  (0)
TIME           1     4 -   4  (0)
SCORE          1     6 -   8  (1)
ROW            1     9 -  10  (0)

The INPUT FORMAT provides for   6 variables and  1 record(s) per case.

                4 N OF CASES        60
                5 INPUT MEDIUM      OP.DAT
                6 VALUE LABELS      TREAT (0)NO TREATMENT (1)0.1 UG'ML
                7                   (2)1.0 UG'ML (3)10. UG'ML
                8                   (4)100 UG'ML/
                9                   TOXIN (1)OCHRATOXIN A
               10                   (2) PENICILLIC ACID
               11                   (3) BOTH/
               12                   RUN (1)FIRST (2)DUPLICATE/
               13                   TIME (1)6 HOURS (2)12 HOURS/
               14 ONEWAY            SCORE BY TREAT(0,4)/
               15                   POLYNOMIAL=4/
               16 STATISTICS        ALL

 ***** ONEWAY problem requires       102 words WORKSPACE *****

               17 READ INPUT DATA

- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

MITOTIC INDEX:OA AND PA (MYCOTOXINS)
File    NONAME    (Creation date =  3-Aug-81)
- - - - - - - - - - - - - - - - - - - - - - - - - - O N E W A Y - - - - -
```

ONEWAY

Source	D.f.	Sum of squares	Mean squares	F-ratio	F-prob.
Between groups	4	8464.2667	2116.0667	65.906	0.0000
LINEAR term	1	4416.5333	4416.5333	137.554	0.0000
Deviation from LINEAR	3	4047.7334	1349.2445	42.023	0.0000
QUAD. term	1	3584.3810	3584.3810	111.637	0.0000
Deviation from QUAD.	2	463.3524	231.6762	7.216	0.0016
CUBIC term	1	381.6333	381.6333	11.886	0.0011
Deviation from CUBIC	1	81.7191	81.7191	2.545	0.1164
Within groups	55	1765.9167	32.1076		
Total	59	10230.1830			

Group	Count	Mean	Standard deviation	Standard error	Minimum	Maximum
GRP00	12	37.2500	8.7918	2.5380	25.0000	54.0000
GRP01	12	10.4167	4.9260	1.4220	6.0000	21.0000
GRP02	12	6.4167	4.5619	1.3169	1.0000	14.0000
GRP03	12	5.4167	3.2879	0.9491	1.0000	11.0000
GRP04	12	9.4167	5.2303	1.5099	0.0000	17.0000
Total	60	13.7833	13.1679	1.7000	0.0000	54.0000
	Fixed effects model		5.6664	0.7315		
	Random effects model			5.9387		

ONEWAY

Random effects model - estimate of between component variance 173.6633

Tests for homogeneity of variances

Cochran's C = Max. variance/Sum(variances) = 0.4815, P = 0.004 (approx.)
Bartlett-Box F = 2.881, P = 0.022
Maximum variance / Minimum variance = 7.150

```
  1 RUN NAME        MITOTIC INDEX:OA AND PA (MYCOTOXINS)
  2 VARIABLE LIST   TREAT,TOXIN,RUN,TIME,SCORE,ROW,PERSCORE
  3 INPUT FORMAT    FIXED (4F1.0,1X,1F3.1,1F2.0,1X,1F3.1)
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According to your INPUT FORMAT, variables are to be read as follows:

Variable	Record	Columns	Print Format
TREAT	1	1 - 1	(0)
TOXIN	1	2 - 2	(0)
RUN	1	3 - 3	(0)
TIME	1	4 - 4	(0)
SCORE	1	6 - 8	(1)
ROW	1	9 - 10	(0)
PERSCORE	1	12 - 14	(1)

The INPUT FORMAT provides for 7 variables and 1 record(s) per case.

```
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  5 INPUT MEDIUM    OP.DAT
  6 VALUE LABELS    TREAT (0)NO TREATMENT (1)0.1 UG'ML
  7                 (2)1.0 UG'ML (3)10. UG'ML
  8                 (4)100 UG'ML/
  9                 TOXIN (1)OCHRATOXIN A
 10                 (2) PENICILLIC ACID
 11                 (3) BOTH/
 12                 RUN (1)FIRST (2)DUPLICATE/
 13                 TIME (1)6 HOURS (2)12 HOURS/
 14 ANOVA           PERSCORE BY TOXIN(1,3) TREAT(1,4) TIME(1,2)
 15 STATISTICS      ALL
```

ANOVA problem requires 1188 words of SPACE.

```
 16 READ INPUT DATA
```

MITOTIC INDEX:OA AND PA (MYCOTOXINS)

File NONAME (Creation date = 3-Aug-81)

* * * * * * * * * A N A L Y S I S O F V A R I A N C E

PERSCORE
by TOXIN
TREAT
TIME

* *

Source of variation	Sum of Squares	df	Mean Square	F	Signif of F
Main effects	36.865	6	6.144	76.011	0.000
TOXIN	4.534	2	2.267	28.044	0.000
TREAT	18.031	3	6.010	74.354	0.000
TIME	14.301	1	14.301	176.917	0.000
2-way interactions	31.135	11	2.830	35.016	0.000
TOXIN TREAT	14.003	6	2.334	28.872	0.000
TOXIN TIME	16.708	2	8.354	103.348	0.000
TREAT TIME	0.424	3	0.141	1.749	0.184
3-way interactions	5.692	6	0.949	11.736	0.000
TOXIN TREAT TIME	5.692	6	0.949	11.736	0.000
Explained	73.692	23	3.204	39.637	0.000
Residual	1.940	24	0.081		
Total	75.633	47	1.609		

MITOTIC INDEX:OA AND PA (MYCOTOXINS)

File NONAME (Creation date = 3-Aug-81)

* * * M U L T I P L E C L A S S I F I C A T I O N A N A L Y S I S

PERSCORE
by TOXIN
TREAT
TIME

* *

Grand mean = 7.71

Variable + category	N	Unadjusted Dev'n	Unadjusted Eta	Adjusted for independents Dev'n	Adjusted for independents Beta
TOXIN					
1 OCHRATOXIN A	16	-0.26		-0.26	
2 PENICILLIC ACID	16	0.43		0.43	
3 BOTH	16	-0.17		-0.17	
			0.24		0.24
TREAT					
1 0.1 UG'ML	12	-0.68		-0.68	
2 1.0 UG'ML	12	0.48		0.48	
3 10. UG'ML	12	0.73		0.73	
4 100 UG'ML	12	-0.53		-0.53	
			0.49		0.49
TIME					
1 6 HOURS	24	-0.55		-0.55	
2 12 HOURS	24	0.55		0.55	
			0.43		0.43
Multiple R squared					.487
Multiple R					.698

```
  1 RUN NAME          MITOTIC INDEX:OA AND PA (MYCOTOXINS)
  2 VARIABLE LIST     TREAT,TOXIN,RUN,TIME,SCORE,ROW,PERSCORE
  3 INPUT FORMAT      FIXED(4F1.0,1X,1F3.1,1F2.0,1X,1F3.1)
```

According to your INPUT FORMAT, variables are to be read as follows:

Variable	Record	Columns	Print Format
TREAT	1	1 - 1	(0)
TOXIN	1	2 - 2	(0)
RUN	1	3 - 3	(0)
TIME	1	4 - 4	(0)
SCORE	1	6 - 8	(1)
ROW	1	9 - 10	(0)
PERSCORE	1	12 - 14	(1)

The INPUT FORMAT provides for 7 variables and 1 record(s) per case.

```
  4 N OF CASES        60
  5 INPUT MEDIUM      OP.DAT
  6 VALUE LABELS      TREAT (0)NO TREATMENT (1)0.1 UG'ML
  7                   (2)1.0 UG'ML (3)10. UG'ML
  8                   (4)100 UG'ML/
  9                   TOXIN (1)OCHRATOXIN A
 10                   (2) PENICILLIC ACID
 11                   (3) BOTH/
 12                   RUN (1)FIRST (2)DUPLICATE/
 13                   TIME (1)6 HOURS (2)12 HOURS/
 14 BREAKDOWN         TABLES=PERSCORE BY TOXIN BY TREAT/
 15                   PERSCORE BY TOXIN BY TIME/
 16                   PERSCORE BY TREAT BY TOXIN/
 17                   PERSCORE BY TREAT BY TIME/
 18                   PERSCORE BY TIME BY TOXIN/
 19                   PERSCORE BY TIME BY TREAT/
```

***** Given workspace allows for 1990 cells and 2 dimensions for suppl

```
 20 READ INPUT DATA
```

Criterion variable PERSCORE
broken down by TOXIN
by TREAT

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			370.2000	6.1700	3.3107
TOXIN	1.	OCHRATOXIN A	119.2000	5.9600	3.1110
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG/ML	30.2000	7.5500	0.5196
TREAT	2.	1.0 UG/ML	31.6000	7.9000	0.3916
TREAT	3.	10. UG/ML	30.8000	7.7000	0.4082
TREAT	4.	100 UG/ML	26.6000	6.6500	0.5323
TOXIN	2.	PENICILLIC ACID	130.3000	6.5150	3.4736
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG/ML	29.5000	7.3750	0.3862
TREAT	2.	1.0 UG/ML	37.1000	9.2750	0.1893
TREAT	3.	10. UG/ML	35.6000	8.9000	0.6055
TREAT	4.	100 UG/ML	28.1000	7.0250	0.4425
TOXIN	3.	BOTH	120.7000	6.0350	3.4785
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG/ML	24.7000	6.1750	2.1716
TREAT	2.	1.0 UG/ML	29.6000	7.4000	1.5188
TREAT	3.	10. UG/ML	34.9000	8.7250	0.6076
TREAT	4.	100 UG/ML	31.5000	7.8750	2.0023

Total cases = 60

Criterion variable PERSCORE
broken down by TIME
by TOXIN

VariableCode		Value label	Sum	Mean	Std dev
For entire population			370.2000	6.1700	3.3107
TIME	1.	6 HOURS	172.0000	5.7333	3.1374
TOXIN	1.	OCHRATOXIN A	57.1000	5.7100	3.0574
TOXIN	2.	PENICILLIC ACID	65.4000	6.5400	3.5258
TOXIN	3.	BOTH	49.5000	4.9500	2.9209
TIME	2.	12 HOURS	198.2000	6.6067	3.4729
TOXIN	1.	OCHRATOXIN A	62.1000	6.2100	3.3084
TOXIN	2.	PENICILLIC ACID	64.9000	6.4900	3.6109
TOXIN	3.	BOTH	71.2000	7.1200	3.7944

Total cases = 60

Criterion variable PERSCORE
broken down by TOXIN
by TIME

VariableCode		Value label	Sum	Mean	Std dev
For entire population			370.2000	6.1700	3.3107
TOXIN	1.	OCHRATOXIN A	119.2000	5.9600	3.1110
TIME	1.	6 HOURS	57.1000	5.7100	3.0574
TIME	2.	12 HOURS	62.1000	6.2100	3.3084
TOXIN	2.	PENICILLIC ACID	130.3000	6.5150	3.4736
TIME	1.	6 HOURS	65.4000	6.5400	3.5258
TIME	2.	12 HOURS	64.9000	6.4900	3.6109
TOXIN	3.	BOTH	120.7000	6.0350	3.4785
TIME	1.	6 HOURS	49.5000	4.9500	2.9209
TIME	2.	12 HOURS	71.2000	7.1200	3.7944

Total cases = 60

Criterion variable PERSCORE
broken down by TREAT
by TOXIN

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			370.2000	6.1700	3.3107
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TOXIN	1.	OCHRATOXIN A	0.0000	0.0000	0.0000
TOXIN	2.	PENICILLIC ACID	0.0000	0.0000	0.0000
TOXIN	3.	BOTH	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG'ML	84.4000	7.0333	1.3446
TOXIN	1.	OCHRATOXIN A	30.2000	7.5500	0.5196
TOXIN	2.	PENICILLIC ACID	29.5000	7.3750	0.3862
TOXIN	3.	BOTH	24.7000	6.1750	2.1716
TREAT	2.	1.0 UG'ML	98.3000	8.1917	1.1689
TOXIN	1.	OCHRATOXIN A	31.6000	7.9000	0.3916
TOXIN	2.	PENICILLIC ACID	37.1000	9.2750	0.1893
TOXIN	3.	BOTH	29.6000	7.4000	1.5188
TREAT	3.	10. UG'ML	101.3000	8.4417	0.7428
TOXIN	1.	OCHRATOXIN A	30.8000	7.7000	0.4082
TOXIN	2.	PENICILLIC ACID	35.6000	8.9000	0.6055
TOXIN	3.	BOTH	34.9000	8.7250	0.6076
TREAT	4.	100 UG'ML	86.2000	7.1833	1.2291
TOXIN	1.	OCHRATOXIN A	26.6000	6.6500	0.5323
TOXIN	2.	PENICILLIC ACID	28.1000	7.0250	0.4425
TOXIN	3.	BOTH	31.5000	7.8750	2.0023

Total cases = 60

Criterion variable PERSCORE
broken down by TREAT
by TIME

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			370.2000	6.1700	3.3107
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TIME	1.	6 HOURS	0.0000	0.0000	0.0000
TIME	2.	12 HOURS	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG'ML	84.4000	7.0333	1.3446
TIME	1.	6 HOURS	38.1000	6.3500	1.6208
TIME	2.	12 HOURS	46.3000	7.7167	0.4792
TREAT	2.	1.0 UG'ML	98.3000	8.1917	1.1689
TIME	1.	6 HOURS	46.1000	7.6833	1.4662
TIME	2.	12 HOURS	52.2000	8.7000	0.4858
TREAT	3.	10. UG'ML	101.3000	8.4417	0.7428
TIME	1.	6 HOURS	48.1000	8.0167	0.4665
TIME	2.	12 HOURS	53.2000	8.8667	0.7501
TREAT	4.	100 UG'ML	86.2000	7.1833	1.2291
TIME	1.	6 HOURS	39.7000	6.6167	0.6616
TIME	2.	12 HOURS	46.5000	7.7500	1.4543

Total cases = 60

Criterion variable PERSCORE
broken down by TIME
by TREAT

BREAKDOWN

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			370.2000	6.1700	3.3107
TIME	1.	6 HOURS	172.0000	5.7333	3.1374
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG/ML	38.1000	6.3500	1.6208
TREAT	2.	1.0 UG/ML	46.1000	7.6833	1.4662
TREAT	3.	10. UG/ML	48.1000	8.0167	0.4665
TREAT	4.	100 UG/ML	39.7000	6.6167	0.6616
TIME	2.	12 HOURS	198.2000	6.6067	3.4729
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG/ML	46.3000	7.7167	0.4792
TREAT	2.	1.0 UG/ML	52.2000	8.7000	0.4858
TREAT	3.	10. UG/ML	53.2000	8.8667	0.7501
TREAT	4.	100 UG/ML	46.5000	7.7500	1.4543

Total cases = 60

```
  1 RUN NAME          MITOTIC INDEX:OA AND PA (MYCOTOXINS)
  2 VARIABLE LIST     TREAT,TOXIN,RUN,TIME,SCORE,ROW,PERSCORE
  3 INPUT FORMAT      FIXED (4F1.0,1X,1F3.1,1F2.0,1X,1F3.1)
```

According to your INPUT FORMAT, variables are to be read as follows:

Variable	Record	Columns	Print Format
TREAT	1	1 - 1	(0)
TOXIN	1	2 - 2	(0)
RUN	1	3 - 3	(0)
TIME	1	4 - 4	(0)
SCORE	1	6 - 8	(1)
ROW	1	9 - 10	(0)
PERSCORE	1	12 - 14	(1)

The INPUT FORMAT provides for 7 variables and 1 record(s) per case.

```
  4 N OF CASES        60
  5 INPUT MEDIUM      OP.DAT
  6 VALUE LABELS      TREAT (0)NO TREATMENT (1)0.1 UG'ML
  7                   (2)1.0 UG'ML (3)10. UG'ML
  8                   (4)100 UG'ML/
  9                   TOXIN (1)OCHRATOXIN A
 10                   (2) PENICILLIC ACID
 11                   (3) BOTH/
 12                   RUN (1)FIRST (2)DUPLICATE/
 13                   TIME (1)6 HOURS (2)12 HOURS/
 14 BREAKDOWN         TABLES=PERSCORE BY TOXIN BY TIME BY TREAT/
 15                   PERSCORE BY TREAT BY TOXIN BY TIME/
 16                   PERSCORE BY TREAT BY TIME BY TOXIN/
 17                   PERSCORE BY TIME BY TOXIN BY TREAT/
 18                   PERSCORE BY TIME BY TREAT BY TOXIN/
```

***** Given workspace allows for 1791 cells and 3 dimensions for subprogr

```
 19 READ INPUT DATA
```

- -

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			370.2000	6.1700	3.3107
TOXIN	1.	OCHRATOXIN A	119.2000	5.9600	3.1110
TIME	1.	6 HOURS	57.1000	5.7100	3.0574
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG/ML	14.4000	7.2000	0.5657
TREAT	2.	1.0 UG/ML	15.2000	7.6000	0.2828
TREAT	3.	10. UG/ML	14.9000	7.4500	0.0707
TREAT	4.	100 UG/ML	12.6000	6.3000	0.4243
TIME	2.	12 HOURS	62.1000	6.2100	3.3084
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG/ML	15.8000	7.9000	0.0000
TREAT	2.	1.0 UG/ML	16.4000	8.2000	0.1414
TREAT	3.	10. UG/ML	15.9000	7.9500	0.4950
TREAT	4.	100 UG/ML	14.0000	7.0000	0.4243
TOXIN	2.	PENICILLIC ACID	130.3000	6.5150	3.4736
TIME	1.	6 HOURS	65.4000	6.5400	3.5258
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG/ML	15.1000	7.5500	0.0707
TREAT	2.	1.0 UG/ML	18.7000	9.3500	0.0707
TREAT	3.	10. UG/ML	16.8000	8.4000	0.2828
TREAT	4.	100 UG/ML	14.8000	7.4000	0.1414
TIME	2.	12 HOURS	64.9000	6.4900	3.6109
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG/ML	14.4000	7.2000	0.5657
TREAT	2.	1.0 UG/ML	18.4000	9.2000	0.2828
TREAT	3.	10. UG/ML	18.8000	9.4000	0.1414
TREAT	4.	100 UG/ML	13.3000	6.6500	0.0707
TOXIN	3.	BOTH	120.7000	6.0350	3.4785
TIME	1.	6 HOURS	49.5000	4.9500	2.9209
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG/ML	8.6000	4.3000	0.2828
TREAT	2.	1.0 UG/ML	12.2000	6.1000	0.2828
TREAT	3.	10. UG/ML	16.4000	8.2000	0.0000
TREAT	4.	100 UG/ML	12.3000	6.1500	0.3536
TIME	2.	12 HOURS	71.2000	7.1200	3.7944
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG/ML	16.1000	8.0500	0.0707
TREAT	2.	1.0 UG/ML	17.4000	8.7000	0.2828
TREAT	3.	10. UG/ML	18.5000	9.2500	0.0707
TREAT	4.	100 UG/ML	19.2000	9.6000	0.0000

BREAKDOWN

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			370.2000	6.1700	3.3107
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TOXIN	1.	OCHRATOXIN A	0.0000	0.0000	0.0000
TIME	1.	6 HOURS	0.0000	0.0000	0.0000
TIME	2.	12 HOURS	0.0000	0.0000	0.0000
TOXIN	2.	PENICILLIC ACID	0.0000	0.0000	0.0000
TIME	1.	6 HOURS	0.0000	0.0000	0.0000
TIME	2.	12 HOURS	0.0000	0.0000	0.0000
TOXIN	3.	BOTH	0.0000	0.0000	0.0000
TIME	1.	6 HOURS	0.0000	0.0000	0.0000
TIME	2.	12 HOURS	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG'ML	84.4000	7.0333	1.3446
TOXIN	1.	OCHRATOXIN A	30.2000	7.5500	0.5196
TIME	1.	6 HOURS	14.4000	7.2000	0.5657
TIME	2.	12 HOURS	15.8000	7.9000	0.0000
TOXIN	2.	PENICILLIC ACID	29.5000	7.3750	0.3862
TIME	1.	6 HOURS	15.1000	7.5500	0.0707
TIME	2.	12 HOURS	14.4000	7.2000	0.5657
TOXIN	3.	BOTH	24.7000	6.1750	2.1716
TIME	1.	6 HOURS	8.6000	4.3000	0.2828
TIME	2.	12 HOURS	16.1000	8.0500	0.0707
TREAT	2.	1.0 UG'ML	98.3000	8.1917	1.1689
TOXIN	1.	OCHRATOXIN A	31.6000	7.9000	0.3916
TIME	1.	6 HOURS	15.2000	7.6000	0.2828
TIME	2.	12 HOURS	16.4000	8.2000	0.1414
TOXIN	2.	PENICILLIC ACID	37.1000	9.2750	0.1893
TIME	1.	6 HOURS	18.7000	9.3500	0.0707
TIME	2.	12 HOURS	18.4000	9.2000	0.2828
TOXIN	3.	BOTH	29.6000	7.4000	1.5188
TIME	1.	6 HOURS	12.2000	6.1000	0.2828
TIME	2.	12 HOURS	17.4000	8.7000	0.2828

TREAT	3.	10. UG'ML	101.3000	8.4417	0.7428
TOXIN	1.	OCHRATOXIN A	30.8000	7.7000	0.4082
TIME	1.	6 HOURS	14.9000	7.4500	0.0707
TIME	2.	12 HOURS	15.9000	7.9500	0.4950
TOXIN	2.	PENICILLIC ACID	35.6000	8.9000	0.6055
TIME	1.	6 HOURS	16.8000	8.4000	0.2828
TIME	2.	12 HOURS	18.8000	9.4000	0.1414
TOXIN	3.	BOTH	34.9000	8.7250	0.6076
TIME	1.	6 HOURS	16.4000	8.2000	0.0000
TIME	2.	12 HOURS	18.5000	9.2500	0.0707
TREAT	4.	100 UG'ML	86.2000	7.1833	1.2291
TOXIN	1.	OCHRATOXIN A	26.6000	6.6500	0.5323
TIME	1.	6 HOURS	12.6000	6.3000	0.4243
TIME	2.	12 HOURS	14.0000	7.0000	0.4243
TOXIN	2.	PENICILLIC ACID	28.1000	7.0250	0.4425
TIME	1.	6 HOURS	14.8000	7.4000	0.1414
TIME	2.	12 HOURS	13.3000	6.6500	0.0707
TOXIN	3.	BOTH	31.5000	7.8750	2.0023
TIME	1.	6 HOURS	12.3000	6.1500	0.3536
TIME	2.	12 HOURS	19.2000	9.6000	0.0000

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			370.2000	6.1700	3.3107
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TIME	1.	6 HOURS	0.0000	0.0000	0.0000
TOXIN	1.	OCHRATOXIN A	0.0000	0.0000	0.0000
TOXIN	2.	PENICILLIC ACID	0.0000	0.0000	0.0000
TOXIN	3.	BOTH	0.0000	0.0000	0.0000
TIME	2.	12 HOURS	0.0000	0.0000	0.0000
TOXIN	1.	OCHRATOXIN A	0.0000	0.0000	0.0000
TOXIN	2.	PENICILLIC ACID	0.0000	0.0000	0.0000
TOXIN	3.	BOTH	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG'ML	84.4000	7.0333	1.3446
TIME	1.	6 HOURS	38.1000	6.3500	1.6208
TOXIN	1.	OCHRATOXIN A	14.4000	7.2000	0.5657
TOXIN	2.	PENICILLIC ACID	15.1000	7.5500	0.0707
TOXIN	3.	BOTH	8.6000	4.3000	0.2828

TIME	2.	12 HOURS	46.3000	7.7167	0.4792
TOXIN	1.	OCHRATOXIN A	15.8000	7.9000	0.0000
TOXIN	2.	PENICILLIC ACID	14.4000	7.2000	0.5657
TOXIN	3.	BOTH	16.1000	8.0500	0.0707
TREAT	2.	1.0 UG*ML	98.3000	8.1917	1.1689
TIME	1.	6 HOURS	46.1000	7.6833	1.4662
TOXIN	1.	OCHRATOXIN A	15.2000	7.6000	0.2828
TOXIN	2.	PENICILLIC ACID	18.7000	9.3500	0.0707
TOXIN	3.	BOTH	12.2000	6.1000	0.2828
TIME	2.	12 HOURS	52.2000	8.7000	0.4858
TOXIN	1.	OCHRATOXIN A	16.4000	8.2000	0.1414
TOXIN	2.	PENICILLIC ACID	18.4000	9.2000	0.2828
TOXIN	3.	BOTH	17.4000	8.7000	0.2828
TREAT	3.	10. UG*ML	101.3000	8.4417	0.7428
TIME	1.	6 HOURS	48.1000	8.0167	0.4665
TOXIN	1.	OCHRATOXIN A	14.9000	7.4500	0.0707
TOXIN	2.	PENICILLIC ACID	16.8000	8.4000	0.2828
TOXIN	3.	BOTH	16.4000	8.2000	0.0000
TIME	2.	12 HOURS	53.2000	8.8667	0.7501
TOXIN	1.	OCHRATOXIN A	15.9000	7.9500	0.4950
TOXIN	2.	PENICILLIC ACID	18.8000	9.4000	0.1414
TOXIN	3.	BOTH	18.5000	9.2500	0.0707
TREAT	4.	100 UG*ML	86.2000	7.1833	1.2291
TIME	1.	6 HOURS	39.7000	6.6167	0.6616
TOXIN	1.	OCHRATOXIN A	12.6000	6.3000	0.4243
TOXIN	2.	PENICILLIC ACID	14.8000	7.4000	0.1414
TOXIN	3.	BOTH	12.3000	6.1500	0.3536
TIME	2.	12 HOURS	46.5000	7.7500	1.4543
TOXIN	1.	OCHRATOXIN A	14.0000	7.0000	0.4243
TOXIN	2.	PENICILLIC ACID	13.3000	6.6500	0.0707
TOXIN	3.	BOTH	19.2000	9.6000	0.0000

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			370.2000	6.1700	3.3107
TIME	1.	6 HOURS	172.0000	5.7333	3.1374
TOXIN	1.	OCHRATOXIN A	57.1000	5.7100	3.0574
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG/ML	14.4000	7.2000	0.5657
TREAT	2.	1.0 UG/ML	15.2000	7.6000	0.2828
TREAT	3.	10. UG/ML	14.9000	7.4500	0.0707
TREAT	4.	100 UG/ML	12.6000	6.3000	0.4243
TOXIN	2.	PENICILLIC ACID	65.4000	6.5400	3.5258
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG/ML	15.1000	7.5500	0.0707
TREAT	2.	1.0 UG/ML	18.7000	9.3500	0.0707
TREAT	3.	10. UG/ML	16.8000	8.4000	0.2828
TREAT	4.	100 UG/ML	14.8000	7.4000	0.1414
TOXIN	3.	BOTH	49.5000	4.9500	2.9209
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG/ML	8.6000	4.3000	0.2828
TREAT	2.	1.0 UG/ML	12.2000	6.1000	0.2828
TREAT	3.	10. UG/ML	16.4000	8.2000	0.0000
TREAT	4.	100 UG/ML	12.3000	6.1500	0.3536
TIME	2.	12 HOURS	198.2000	6.6067	3.4729
TOXIN	1.	OCHRATOXIN A	62.1000	6.2100	3.3084
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG/ML	15.8000	7.9000	0.0000
TREAT	2.	1.0 UG/ML	16.4000	8.2000	0.1414
TREAT	3.	10. UG/ML	15.9000	7.9500	0.4950
TREAT	4.	100 UG/ML	14.0000	7.0000	0.4243
TOXIN	2.	PENICILLIC ACID	64.9000	6.4900	3.6109
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG/ML	14.4000	7.2000	0.5657
TREAT	2.	1.0 UG/ML	18.4000	9.2000	0.2828
TREAT	3.	10. UG/ML	18.8000	9.4000	0.1414
TREAT	4.	100 UG/ML	13.3000	6.6500	0.0707
TOXIN	3.	BOTH	71.2000	7.1200	3.7944
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG/ML	16.1000	8.0500	0.0707
TREAT	2.	1.0 UG/ML	17.4000	8.7000	0.2828
TREAT	3.	10. UG/ML	18.5000	9.2500	0.0707
TREAT	4.	100 UG/ML	19.2000	9.6000	0.0000

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			370.2000	6.1700	3.3107
TIME	1.	6 HOURS	172.0000	5.7333	3.1374
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TOXIN	1.	OCHRATOXIN A	0.0000	0.0000	0.0000
TOXIN	2.	PENICILLIC ACID	0.0000	0.0000	0.0000
TOXIN	3.	BOTH	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG'ML	38.1000	6.3500	1.6208
TOXIN	1.	OCHRATOXIN A	14.4000	7.2000	0.5657
TOXIN	2.	PENICILLIC ACID	15.1000	7.5500	0.0707
TOXIN	3.	BOTH	8.6000	4.3000	0.2828
TREAT	2.	1.0 UG'ML	46.1000	7.6833	1.4662
TOXIN	1.	OCHRATOXIN A	15.2000	7.6000	0.2828
TOXIN	2.	PENICILLIC ACID	18.7000	9.3500	0.0707
TOXIN	3.	BOTH	12.2000	6.1000	0.2828
TREAT	3.	10. UG'ML	48.1000	8.0167	0.4665
TOXIN	1.	OCHRATOXIN A	14.9000	7.4500	0.0707
TOXIN	2.	PENICILLIC ACID	16.8000	8.4000	0.2828
TOXIN	3.	BOTH	16.4000	8.2000	0.0000
TREAT	4.	100 UG'ML	39.7000	6.6167	0.6616
TOXIN	1.	OCHRATOXIN A	12.6000	6.3000	0.4243
TOXIN	2.	PENICILLIC ACID	14.8000	7.4000	0.1414
TOXIN	3.	BOTH	12.3000	6.1500	0.3536

BREAKDOWN

TIME	2.	12 HOURS	198.2000	6.6067	3.4729
TREAT	0.	NO TREATMENT	0.0000	0.0000	0.0000
TOXIN	1.	OCHRATOXIN A	0.0000	0.0000	0.0000
TOXIN	2.	PENICILLIC ACID	0.0000	0.0000	0.0000
TOXIN	3.	BOTH	0.0000	0.0000	0.0000
TREAT	1.	0.1 UG'ML	46.3000	7.7167	0.4792
TOXIN	1.	OCHRATOXIN A	15.8000	7.9000	0.0000
TOXIN	2.	PENICILLIC ACID	14.4000	7.2000	0.5657
TOXIN	3.	BOTH	16.1000	8.0500	0.0707
TREAT	2.	1.0 UG'ML	52.2000	8.7000	0.4858
TOXIN	1.	OCHRATOXIN A	16.4000	8.2000	0.1414
TOXIN	2.	PENICILLIC ACID	18.4000	9.2000	0.2828
TOXIN	3.	BOTH	17.4000	8.7000	0.2828
TREAT	3.	10. UG'ML	53.2000	8.8667	0.7501
TOXIN	1.	OCHRATOXIN A	15.9000	7.9500	0.4950
TOXIN	2.	PENICILLIC ACID	18.8000	9.4000	0.1414
TOXIN	3.	BOTH	18.5000	9.2500	0.0707
TREAT	4.	100 UG'ML	46.5000	7.7500	1.4543
TOXIN	1.	OCHRATOXIN A	14.0000	7.0000	0.4243
TOXIN	2.	PENICILLIC ACID	13.3000	6.6500	0.0707
TOXIN	3.	BOTH	19.2000	9.6000	0.0000

Program For BMDP2V

```
  PROGRAM CONTROL INFORMATION

/PROBLEM  TITLE IS 'REPEATED MEASURES'.
/INPUT    VARIABLES ARE 8.
                    FILE IS 'OPRESA.DAT'.
                    FORMAT IS '(1X,3F1.0,2(1X,F3.1),3(1X,F2.1))
/VARIABLE  NAMES ARE JA,QB,RC,V,W,X,Y,Z.
/DESIGN    DEPENDENT ARE 4 TO 8.
                    LEVEL IS 5.
                    NAME IS MITOSIS.
                    GROUPING ARE JA,QB,RC.  TREAT, TOXIN, TIME
/GROUP              CODES(1) ARE     0,    1,   2,   3,    4.
                    NAMES(1) ARE CONTR,AONET,KONE,TEN,HUNDR.
                    CODES(2) ARE     1,    2,   3.
                    NAMES(2) ARE OCHRA,PENIC,BOTH.
                    CODES(3) ARE   1,      2.
                    NAMES(3) ARE SIX,TWELVE.
/END

 PROBLEM TITLE . . . . . . . REPEATED MEASURES

NUMBER OF VARIABLES TO READ IN. . . . . :          8
NUMBER OF VARIABLES ADDED BY TRANSFORMATIONS. :    0
TOTAL NUMBER OF VARIABLES . . . . . :              8
NUMBER OF CASES TO READ IN. . . . . : 1000000
CASE LABELING VARIABLES . . . . . :
LIMITS AND MISSING VALUE CHECKED BEFORE TRANSFORMATIONS
BLANKS ARE. . . . . :                        ZEROS
INPUT UNIT NUMBER      3, FILE. . . . . :    OPRESA.DAT
REWIND INPUT UNIT PRIOR TO READING. : DATA. . :  YES
NUMBER OF WORDS OF DYNAMIC STORAGE. . . . . :  15363
INPUT FORMAT. . . . .
  VARIABLES TO BE USED
    1 JA          2 QB          3 RC    4 V          5 W
    6 X           7 Y           8 Z

DESIGN SPECIFICATIONS

          GROUP =    1    2    3
          DEPEND =   4    5    6    7    8
          LEVEL =    5

 VARIABLE                CATEGORY    CATEGORY
 NO.  NAME               CODE        NAME

  1   JA                  0.0000     CONTR
      TREATMENT           1.00000    AONET
                          2.00000    KONE
                          3.00000    TEN
                          4.00000    HUNDR

  2   QB                  1.00000    OCHRA
      TOXIN               2.00000    PENIC
                          3.00000    BOTH

  3   RC  TIME            1.00000    SIX
                          2.00000    TWELVE
```

REPEATED MEASURES JA = TREAT QB = TOXIN RC = TIME

ANALYSIS OF VARIANCE FOR 1-ST DEPENDENT VARIABLE - V W X Y Z

	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F	TAIL PROBABILITY
	MEAN	36254.82667	1	36254.82667	1136.67	0.0000
	JA	390.47533	4	97.61883	3.06	0.0834
	QB	513.59573	2	256.79787	8.05	0.0121
	RC	677.55627	1	677.55627	21.24	0.0017
	JQ	370.16227	8	46.27028	1.45	0.3055
	JR	306.02707	4	76.50677	2.40	0.1360
	QR	620.17973	2	310.08987	9.72	0.0072
1	ERROR	255.16493	8	31.89562		
	MITO	122979.86067	4	30744.96517	946.79	0.0000
	MJ	1201.31733	16	75.08233	2.31	0.0213
	MQ	1478.41093	8	184.80137	5.69	0.0002
	MR	2569.13107	4	642.28277	19.78	0.0000
	MJQ	1032.49107	32	32.26535	0.99	0.5072
	MJR	1181.66560	16	73.85410	2.27	0.0234
	MQR	2326.25893	8	290.78237	8.95	0.0000
2	ERROR	1039.13640	32	32.47301		

```
     1 RUN NAME          BREAKDOWNS
     2 INPUT MEDIUM      OPRESA.DAT
     3 INPUT FORMAT      FIXED(1X,3F1.0,2(1X,F3.1),3(1X,F2.1))
     4 VARIABLE LIST     TREAT,TOXIN,TIME,P1,P2,P3,P4,P5
     5 N OF CASES        30
     6 COMPUTE           SUM=(P1+P2+P3+P4+P5)/5
     7 VALUE LABELS      TREAT (0) CONTR (1) ONET (2) ONE (3) TEN (4) HUNDR/
     8                   TOXIN (1) OCHRA (2) PENIC (3) BOTH/
     9                   TIME (1) SIX (2) TWELVE/
    10 BREAKDOWN         TABLES=SUM,P1 TO P5 BY TREAT,TOXIN,TIME/
    11                   SUM,P1 TO P5 BY TREAT BY TIME/
    12                   SUM,P1 TO P5 BY TREAT BY TOXIN/
    13                   SUM,P1 TO P5 BY TOXIN BY TIME/

workspace allows for  1990 cells and    2 dimensions for subprogram break
```

BREAKDOWNS

File NONAME (Creation date = 3-Aug-81)

D E S C R I P T I O N O F

Criterion variable SUM
broken down by TREAT

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			466.4000	15.5467	4.6484
TREAT	0.	CONTR	110.4200	18.4033	1.2508
TREAT	1.	ONET	94.5600	15.7600	5.2842
TREAT	2.	ONE	91.9600	15.3267	5.4995
TREAT	3.	TEN	88.2800	14.7133	5.5913
TREAT	4.	HUNDR	81.1800	13.5300	4.3258

Total cases = 30

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			466.4000	15.5467	4.6484
TREAT	0.	CONTR	110.4200	18.4033	1.2508
TIME	1.	SIX	54.0200	18.0067	1.3400
TIME	2.	TWELVE	56.4000	18.8000	1.2819
TREAT	1.	ONET	94.5600	15.7600	5.2842
TIME	1.	SIX	56.1800	18.7267	0.3177
TIME	2.	TWELVE	38.3800	12.7933	6.5805
TREAT	2.	ONE	91.9600	15.3267	5.4995
TIME	1.	SIX	57.3800	19.1267	0.9586
TIME	2.	TWELVE	34.5800	11.5267	5.6012
TREAT	3.	TEN	88.2800	14.7133	5.5913
TIME	1.	SIX	51.7400	17.2467	3.7855
TIME	2.	TWELVE	36.5400	12.1800	6.6763
TREAT	4.	HUNDR	81.1800	13.5300	4.3258
TIME	1.	SIX	45.7600	15.2533	3.1119
TIME	2.	TWELVE	35.4200	11.8067	5.3092
			2182.0000	72.7333	21.7461
TREAT	0.	CONTR	508.0000	84.6667	4.9666
TIME	1.	SIX	247.0000	82.3333	5.1316
TIME	2.	TWELVE	261.0000	87.0000	4.3589
TREAT	1.	ONET	454.0000	75.6667	25.9743
TIME	1.	SIX	271.0000	90.3333	1.5275
TIME	2.	TWELVE	183.0000	61.0000	32.2335
TREAT	2.	ONE	428.0000	71.3333	25.4139
TIME	1.	SIX	269.0000	89.6667	4.1633
TIME	2.	TWELVE	159.0000	53.0000	24.2693
TREAT	3.	TEN	413.0000	68.8333	26.4909
TIME	1.	SIX	242.0000	80.6667	18.9297
TIME	2.	TWELVE	171.0000	57.0000	31.2410
TREAT	4.	HUNDR	379.0000	63.1667	19.6002
TIME	1.	SIX	218.0000	72.6667	12.5033
TIME	2.	TWELVE	161.0000	53.6667	23.0940

Sum broken down by TREAT By TIME

For psychosis

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			14.9000	0.4967	0.3090
TREAT	0.	CONTR	5.5000	0.9167	0.2858
TIME	1.	SIX	3.4000	1.1333	0.1528
TIME	2.	TWELVE	2.1000	0.7000	0.2000
TREAT	1.	ONET	2.9000	0.4833	0.0983
TIME	1.	SIX	1.6000	0.5333	0.0577
TIME	2.	TWELVE	1.3000	0.4333	0.1155
TREAT	2.	ONE	1.8000	0.3000	0.1265
TIME	1.	SIX	0.9000	0.3000	0.2000
TIME	2.	TWELVE	0.9000	0.3000	0.0000
TREAT	3.	TEN	1.8000	0.3000	0.2098
TIME	1.	SIX	1.1000	0.3667	0.3055
TIME	2.	TWELVE	0.7000	0.2333	0.0577
TREAT	4.	HUNDR	2.9000	0.4833	0.3125
TIME	1.	SIX	1.4000	0.4667	0.4163
TIME	2.	TWELVE	1.5000	0.5000	0.2646
For entire population			125.0000	4.1667	2.8778
TREAT	0.	CONTR	33.0000	5.5000	2.4290
TIME	1.	SIX	18.0000	6.0000	2.6458
TIME	2.	TWELVE	15.0000	5.0000	2.6458
TREAT	1.	ONET	15.0000	2.5000	1.3784
TIME	1.	SIX	8.0000	2.6667	2.0817
TIME	2.	TWELVE	7.0000	2.3333	0.5774
TREAT	2.	ONE	29.0000	4.8333	2.4833
TIME	1.	SIX	16.0000	5.3333	0.5774
TIME	2.	TWELVE	13.0000	4.3333	3.7859
TREAT	3.	TEN	25.0000	4.1667	4.1191
TIME	1.	SIX	15.0000	5.0000	4.0000
TIME	2.	TWELVE	10.0000	3.3333	4.9329
TREAT	4.	HUNDR	23.0000	3.8333	3.3116
TIME	1.	SIX	9.0000	3.0000	3.6056
TIME	2.	TWELVE	14.0000	4.6667	3.5119

For prometaphase

For metaphase

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			8.1000	0.2700	0.3164
TREAT	0.	CONTR	3.6000	0.6000	0.2828
TIME	1.	SIX	1.7000	0.5667	0.3215
TIME	2.	TWELVE	1.9000	0.6333	0.3055
TREAT	1.	ONET	0.9000	0.1500	0.2510
TIME	1.	SIX	0.3000	0.1000	0.1732
TIME	2.	TWELVE	0.6000	0.2000	0.3464
TREAT	2.	ONE	1.0000	0.1667	0.3204
TIME	1.	SIX	1.0000	0.3333	0.4163
TIME	2.	TWELVE	0.0000	0.0000	0.0000
TREAT	3.	TEN	1.6000	0.2667	0.3011
TIME	1.	SIX	0.6000	0.2000	0.2000
TIME	2.	TWELVE	1.0000	0.3333	0.4163
TREAT	4.	HUNDR	1.0000	0.1667	0.2658
TIME	1.	SIX	0.4000	0.1333	0.2309
TIME	2.	TWELVE	0.6000	0.2000	0.3464
For entire population			2.0000	0.0667	0.2537
TREAT	0.	CONTR	2.0000	0.3333	0.5164
TIME	1.	SIX	0.0000	0.0000	0.0000
TIME	2.	TWELVE	2.0000	0.6667	0.5774
TREAT	1.	ONET	0.0000	0.0000	0.0000
TIME	1.	SIX	0.0000	0.0000	0.0000
TIME	2.	TWELVE	0.0000	0.0000	0.0000
TREAT	2.	ONE	0.0000	0.0000	0.0000
TIME	1.	SIX	0.0000	0.0000	0.0000
TIME	2.	TWELVE	0.0000	0.0000	0.0000
TREAT	3.	TEN	0.0000	0.0000	0.0000
TIME	1.	SIX	0.0000	0.0000	0.0000
TIME	2.	TWELVE	0.0000	0.0000	0.0000
TREAT	4.	HUNDR	0.0000	0.0000	0.0000
TIME	1.	SIX	0.0000	0.0000	0.0000
TIME	2.	TWELVE	0.0000	0.0000	0.0000

For anaphase

For telophase

BREAKDOWN

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			466.4000	15.5467	4.6484
TREAT	0.	CONTR	110.4200	18.4033	1.2508
TOXIN	1.	OCHRA	39.8200	19.9100	0.5233
TOXIN	2.	PENIC	35.1400	17.5700	0.7212
TOXIN	3.	BOTH	35.4600	17.7300	0.4384
TREAT	1.	ONET	94.5600	15.7600	5.2842
TOXIN	1.	OCHRA	24.1800	12.0900	9.6591
TOXIN	2.	PENIC	36.3200	18.1600	1.0465
TOXIN	3.	BOTH	34.0600	17.0300	1.8809
TREAT	2.	ONE	91.9600	15.3267	5.4995
TOXIN	1.	OCHRA	23.0800	11.5400	9.1641
TOXIN	2.	PENIC	34.3200	17.1600	3.5355
TOXIN	3.	BOTH	34.5600	17.2800	3.4224
TREAT	3.	TEN	88.2800	14.7133	5.5913
TOXIN	1.	OCHRA	23.5200	11.7600	10.2672
TOXIN	2.	PENIC	36.4200	18.2100	2.2769
TOXIN	3.	BOTH	28.3400	14.1700	1.7961
TREAT	4.	HUNDR	81.1800	13.5300	4.3258
TOXIN	1.	OCHRA	20.2800	10.1400	6.3074
TOXIN	2.	PENIC	33.3200	16.6600	2.8001
TOXIN	3.	BOTH	27.5800	13.7900	1.7961

Sum broken down by TREAT by TOXIN

Variable					
For entire population			2182.0000	72.7333	21.7461
TREAT	0.	CONTR	508.0000	84.6667	4.9666
TOXIN	1.	OCHRA	180.0000	90.0000	2.8284
TOXIN	2.	PENIC	165.0000	82.5000	2.1213
TOXIN	3.	BOTH	163.0000	81.5000	4.9497
TREAT	1.	ONET	454.0000	75.6667	25.9743
TOXIN	1.	OCHRA	113.0000	56.5000	45.9619
TOXIN	2.	PENIC	175.0000	87.5000	6.3640
TOXIN	3.	BOTH	166.0000	83.0000	9.8995
TREAT	2.	ONE	428.0000	71.3333	25.4139
TOXIN	1.	OCHRA	110.0000	55.0000	42.4264
TOXIN	2.	PENIC	157.0000	78.5000	17.6777
TOXIN	3.	BOTH	161.0000	80.5000	17.6777
TREAT	3.	TEN	413.0000	68.8333	26.4909
TOXIN	1.	OCHRA	115.0000	57.5000	51.6188
TOXIN	2.	PENIC	162.0000	81.0000	11.3137
TOXIN	3.	BOTH	136.0000	68.0000	12.7279
TREAT	4.	HUNDR	379.0000	63.1667	19.6002
TOXIN	1.	OCHRA	100.0000	50.0000	32.5269
TOXIN	2.	PENIC	152.0000	76.0000	12.7279
TOXIN	3.	BOTH	127.0000	63.5000	4.9497

For prophase

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			14.9000	0.4967	0.3090
TREAT	0.	CONTR	5.5000	0.9167	0.2858
TOXIN	1.	OCHRA	1.5000	0.7500	0.3536
TOXIN	2.	PENIC	1.8000	0.9000	0.2828
TOXIN	3.	BOTH	2.2000	1.1000	0.2828
TREAT	1.	ONET	2.9000	0.4833	0.0983
TOXIN	1.	OCHRA	0.9000	0.4500	0.2121
TOXIN	2.	PENIC	1.0000	0.5000	0.0000
TOXIN	3.	BOTH	1.0000	0.5000	0.0000
TREAT	2.	ONE	1.8000	0.3000	0.1265
TOXIN	1.	OCHRA	0.4000	0.2000	0.1414
TOXIN	2.	PENIC	0.8000	0.4000	0.1414
TOXIN	3.	BOTH	0.6000	0.3000	0.0000
TREAT	3.	TEN	1.8000	0.3000	0.2098
TOXIN	1.	OCHRA	0.4000	0.2000	0.1414
TOXIN	2.	PENIC	0.9000	0.4500	0.3536
TOXIN	3.	BOTH	0.5000	0.2500	0.0707
TREAT	4.	HUNDR	2.9000	0.4833	0.3125
TOXIN	1.	OCHRA	0.4000	0.2000	0.2828
TOXIN	2.	PENIC	1.6000	0.8000	0.0000
TOXIN	3.	BOTH	0.9000	0.4500	0.2121

for prometaphase

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			125.0000	4.1667	2.8778
TREAT	0.	CONTR	33.0000	5.5000	2.4290
TOXIN	1.	OCHRA	16.0000	8.0000	0.0000
TOXIN	2.	PENIC	7.0000	3.5000	0.7071
TOXIN	3.	BOTH	10.0000	5.0000	2.8284
TREAT	1.	ONET	15.0000	2.5000	1.3784
TOXIN	1.	OCHRA	7.0000	3.5000	2.1213
TOXIN	2.	PENIC	5.0000	2.5000	0.7071
TOXIN	3.	BOTH	3.0000	1.5000	0.7071
TREAT	2.	ONE	29.0000	4.8333	2.4833
TOXIN	1.	OCHRA	5.0000	2.5000	3.5355
TOXIN	2.	PENIC	13.0000	6.5000	0.7071
TOXIN	3.	BOTH	11.0000	5.5000	0.7071
TREAT	3.	TEN	25.0000	4.1667	4.1191
TOXIN	1.	OCHRA	2.0000	1.0000	0.0000
TOXIN	2.	PENIC	18.0000	9.0000	0.0000
TOXIN	3.	BOTH	5.0000	2.5000	3.5355
TREAT	4.	HUNDR	23.0000	3.8333	3.3116
TOXIN	1.	OCHRA	1.0000	0.5000	0.7071
TOXIN	2.	PENIC	12.0000	6.0000	1.4142
TOXIN	3.	BOTH	10.0000	5.0000	4.2426

for metaphase

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			8.1000	0.2700	0.3164
TREAT	0.	CONTR	3.6000	0.6000	0.2828
TOXIN	1.	OCHRA	1.6000	0.8000	0.1414
TOXIN	2.	PENIC	0.9000	0.4500	0.3536
TOXIN	3.	BOTH	1.1000	0.5500	0.3536
TREAT	1.	ONET	0.9000	0.1500	0.2510
TOXIN	1.	OCHRA	0.0000	0.0000	0.0000
TOXIN	2.	PENIC	0.6000	0.3000	0.4243
TOXIN	3.	BOTH	0.3000	0.1500	0.2121
TREAT	2.	ONE	1.0000	0.1667	0.3204
TOXIN	1.	OCHRA	0.0000	0.0000	0.0000
TOXIN	2.	PENIC	0.8000	0.4000	0.5657
TOXIN	3.	BOTH	0.2000	0.1000	0.1414
TREAT	3.	TEN	1.6000	0.2667	0.3011
TOXIN	1.	OCHRA	0.2000	0.1000	0.1414
TOXIN	2.	PENIC	1.2000	0.6000	0.2828
TOXIN	3.	BOTH	0.2000	0.1000	0.1414
TREAT	4.	HUNDR	1.0000	0.1667	0.2658
TOXIN	1.	OCHRA	0.0000	0.0000	0.0000
TOXIN	2.	PENIC	1.0000	0.5000	0.1414
TOXIN	3.	BOTH	0.0000	0.0000	0.0000

for anaphase

Variable			Sum	Mean	Std dev
For entire population			2.0000	0.0667	0.2537
TREAT	0.	CONTR	2.0000	0.3333	0.5164
TOXIN	1.	OCHRA	0.0000	0.0000	0.0000
TOXIN	2.	PENIC	1.0000	0.5000	0.7071
TOXIN	3.	BOTH	1.0000	0.5000	0.7071
TREAT	1.	ONET	0.0000	0.0000	0.0000
TOXIN	1.	OCHRA	0.0000	0.0000	0.0000
TOXIN	2.	PENIC	0.0000	0.0000	0.0000
TOXIN	3.	BOTH	0.0000	0.0000	0.0000
TREAT	2.	ONE	0.0000	0.0000	0.0000
TOXIN	1.	OCHRA	0.0000	0.0000	0.0000
TOXIN	2.	PENIC	0.0000	0.0000	0.0000
TOXIN	3.	BOTH	0.0000	0.0000	0.0000
TREAT	3.	TEN	0.0000	0.0000	0.0000
TOXIN	1.	OCHRA	0.0000	0.0000	0.0000
TOXIN	2.	PENIC	0.0000	0.0000	0.0000
TOXIN	3.	BOTH	0.0000	0.0000	0.0000
TREAT	4.	HUNDR	0.0000	0.0000	0.0000
TOXIN	1.	OCHRA	0.0000	0.0000	0.0000
TOXIN	2.	PENIC	0.0000	0.0000	0.0000
TOXIN	3.	BOTH	0.0000	0.0000	0.0000

for telophase

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			466.4000	15.5467	4.6484
TOXIN	1.	OCHRA	130.8800	13.0880	7.0200
TIME	1.	SIX	90.1000	18.0200	1.9885
TIME	2.	TWELVE	40.7800	8.1560	6.7908
TOXIN	2.	PENIC	175.5200	17.5520	1.8453
TIME	1.	SIX	94.0800	18.8160	1.1002
TIME	2.	TWELVE	81.4400	16.2880	1.5673
TOXIN	3.	BOTH	160.0000	16.0000	2.3510
TIME	1.	SIX	80.9000	16.1800	3.2724
TIME	2.	TWELVE	79.1000	15.8200	1.2832

Sum broken down by toxin by time

For entire population			2182.0000	72.7333	21.7461
TOXIN	1.	OCHRA	618.0000	61.8000	32.8255
TIME	1.	SIX	429.0000	85.8000	7.8549
TIME	2.	TWELVE	189.0000	37.8000	30.3760
TOXIN	2.	PENIC	811.0000	81.1000	9.4216
TIME	1.	SIX	438.0000	87.6000	4.5607
TIME	2.	TWELVE	373.0000	74.6000	8.5615
TOXIN	3.	BOTH	753.0000	75.3000	11.8138
TIME	1.	SIX	380.0000	76.0000	16.0779
TIME	2.	TWELVE	373.0000	74.6000	7.3689

for prophase

For entire population			14.9000	0.4967	0.3090
TOXIN	1.	OCHRA	3.6000	0.3600	0.2914
TIME	1.	SIX	1.8000	0.3600	0.4278
TIME	2.	TWELVE	1.8000	0.3600	0.0894
TOXIN	2.	PENIC	6.1000	0.6100	0.2644
TIME	1.	SIX	3.6000	0.7200	0.2490
TIME	2.	TWELVE	2.5000	0.5000	0.2550
TOXIN	3.	BOTH	5.2000	0.5200	0.3425
TIME	1.	SIX	3.0000	0.6000	0.4123
TIME	2.	TWELVE	2.2000	0.4400	0.2793

for prometaphase

Variable	Code	Value label	Sum	Mean	Std dev
For entire population			125.0000	4.1667	2.8778
TOXIN	1.	OCHRA	31.0000	3.1000	3.1429
TIME	1.	SIX	19.0000	3.8000	3.2711
TIME	2.	TWELVE	12.0000	2.4000	3.2094
TOXIN	2.	PENIC	55.0000	5.5000	2.5055
TIME	1.	SIX	27.0000	5.4000	2.8810
TIME	2.	TWELVE	28.0000	5.6000	2.4083
TOXIN	3.	BOTH	39.0000	3.9000	2.6854
TIME	1.	SIX	20.0000	4.0000	2.4495
TIME	2.	TWELVE	19.0000	3.8000	3.1937

for metaphase

Variable			Sum	Mean	Std dev
For entire population			8.1000	0.2700	0.3164
TOXIN	1.	OCHRA	1.8000	0.1800	0.3360
TIME	1.	SIX	0.7000	0.1400	0.3130
TIME	2.	TWELVE	1.1000	0.2200	0.3899
TOXIN	2.	PENIC	4.5000	0.4500	0.3028
TIME	1.	SIX	1.8000	0.3600	0.2966
TIME	2.	TWELVE	2.7000	0.5400	0.3130
TOXIN	3.	BOTH	1.8000	0.1800	0.2530
TIME	1.	SIX	1.5000	0.3000	0.3000
TIME	2.	TWELVE	0.3000	0.0600	0.1342

for anaphase

Variable			Sum	Mean	Std dev
For entire population			2.0000	0.0667	0.2537
TOXIN	1.	OCHRA	0.0000	0.0000	0.0000
TIME	1.	SIX	0.0000	0.0000	0.0000
TIME	2.	TWELVE	0.0000	0.0000	0.0000
TOXIN	2.	PENIC	1.0000	0.1000	0.3162
TIME	1.	SIX	0.0000	0.0000	0.0000
TIME	2.	TWELVE	1.0000	0.2000	0.4472
TOXIN	3.	BOTH	1.0000	0.1000	0.3162
TIME	1.	SIX	0.0000	0.0000	0.0000
TIME	2.	TWELVE	1.0000	0.2000	0.4472

for telophase

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