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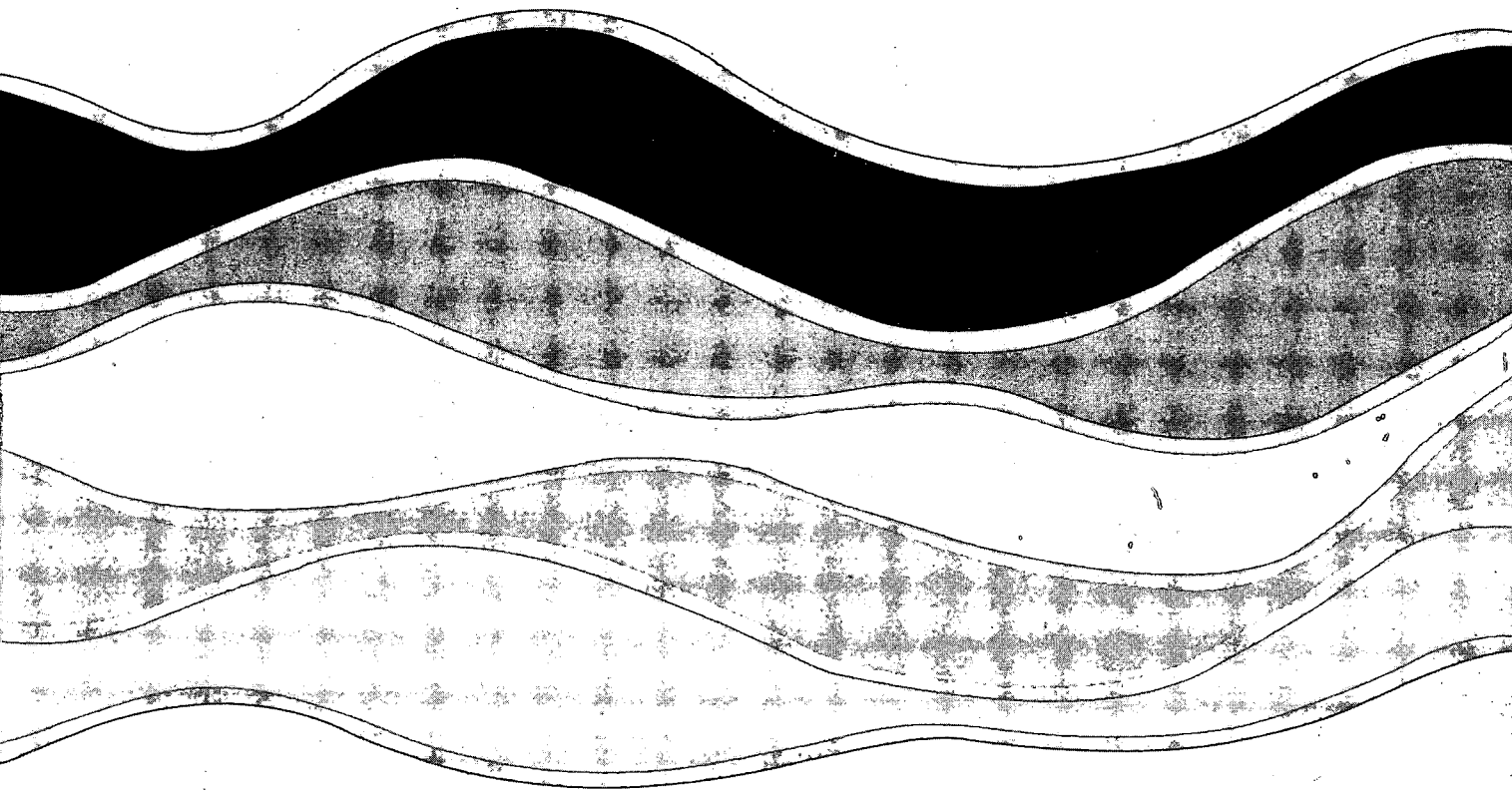

EFFECTS OF EIGHT PESTICIDES ON THE INCORPORATION OF THYMIDINE AND LEUCINE IN AN AQUATIC ENVIRONMENT [A PRELIMINARY REPORT]

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NWRI Contribution No. 90-18

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MANAGEMENT PERSPECTIVE

Bacteria are the major decomposers of organic matter in the aquatic environment. The rates which the bacteria transport organic substrates across their cell membranes are affected by physical (i.e., temperature) and chemical (i.e., pH, oxygen, ionic strength, and chemical inhibitors) conditions. Over the past 20 years, methods have been developed to measure the maximum uptake rates at which bacteria take up radioactively labelled organic substrates. The incorporation of the amino acid, leucine, into protein has been shown to be a good estimate of bacterial protein synthesis and the incorporation of the nucleoside, thymidine, into DNA has been shown to give a good estimation of bacterial DNA synthesis. In the current context, DNA synthesis is also called bacterial productivity since there is a direct relationship between thymidine uptake into DNA and increase in bacterial biomass.

This report illustrates the effects of selected pesticides on bacterial metabolism as monitored by protein and DNA synthesis. The pesticides are currently used in Canada, but are only selected as examples to test the effects of pesticides on the metabolism of bacteria in the aquatic environment. The chlorophenols at a concentration of 5 ppm were the most inhibitory substances tested, but deltamethrin showed a sustained 80% inhibitory effect even at ppb concentration.

PERSPECTIVE DE GESTION

Les bactéries sont les principaux agents de décomposition des matières organiques en milieu aquatique. Les vitesse auxquelles les bactéries transportent les substrats organiques à travers leurs membranes cellulaires dépendent de facteurs physiques (la température, par exemple) et chimiques (le pH, l'oxygène, la force ionique et les inhibiteurs chimiques, par exemple). Au cours des 20 dernières années, des méthodes ont été mises au point pour mesurer les vitesses maximales auxquelles les bactéries absorbent les substrats organiques agissant comme traceurs radioactifs. L'incorporation à la protéine de la leucine (aminoacide) semble être un bon moyen d'évaluer la synthèse bactérienne des protéines; par ailleurs, l'intégration à l'ADN de la thymidine (nucléoside) paraît être une bonne façon d'évaluer la synthèse bactérienne de l'ADN. Dans la situation actuelle, la synthèse de l'ADN est également appelée productivité bactérienne, car il existe un rapport direct entre l'absorption de la thymidine dans l'ADN et l'augmentation de la biomasse bactérienne. Ce rapport illustre les effets de certains pesticides sur le métabolisme des bactéries tel qu'on peut l'observer par la synthèse des protéines et de l'ADN. Les pesticides sont actuellement employés au Canada, mais ils sont seulement sélectionnés comme exemples pour tester les

effets des pesticides sur le métabolisme des bactéries en milieu aquatique. Les chlorophénols, à une concentration de 5 ppm, ont été les substances testées les plus inhibitrices, mais la deltaméthrine a affiché un effet inhibiteur soutenu de 80 p. 10, même à des concentrations de l'ordre de parties par milliard.

RÉSUMÉ

On a réalisé des expériences pour étudier l'effet de huit agents de contamination sélectionnés sur l'absorption de la ^{3}H-thymidine (productivité bactérienne) et de la ^{3}H-leucine (synthèse bactérienne des protéines). Les agents de contamination ont été ajoutés dans l'eau ou l'éthanol suivant leur solubilité. Diverses concentrations jusqu'à 10 ppm ont été ajoutées à des échantillons d'eau de surface du port de Hamilton. Les échantillons ont été incubés pendant 1, 2, 4 et 24 heures. L'aptitude des microorganismes à absorber la leucine ou la thymidine agissant comme traceurs radioactifs a été testée à ces intervalles. Le pentachlorophénol et le tétrachlorophénol ont été les substances les plus inhibitrices, alors que le méthoxychlore a eu peu d'effet sur l'absorption des substrats faisant office de traceurs. Les autres substances (trifluraline, TFM, atrazine et dinosèbe) ont montré tout d'abord une certaine inhibition, mais, après 24 heures, l'absorption était généralement semblable à celle des substances de contrôle et même souvent supérieure (stimulation). Le dinosèbe a eu plus d'effet sur la productivité bactérienne (synthèse de l'ADN) que sur la synthèse des protéines. La deltaméthrine a affiché un effet inhibiteur soutenu de

80 p. 100, même à des concentrations de l'ordre de parties par milliard.

ABSTRACT

Experiments were carried out to study the effect of eight selected contaminants on the uptake of ^{3}H-thymidine (bacterial productivity) and ^{3}H-leucine (bacterial protein synthesis). The contaminants were added in water or ethanol depending on their solubilities. Varying concentrations up to 10 ppm were added to samples of Hamilton Harbour surface water. The samples were incubated for 1, 2, 4, and 24 hours. The ability of the microorganisms to take up the labelled thymidine or leucine was tested at these times. Pentachlorophenol and tetrachlorophenol were the most inhibitory substances, while methoxychlor had little effect on the uptake of the labelled substrates. The remaining substances (trifluralin, TFM, atrazine, and dinoseb) showed initial inhibition but by 24 hours the uptake was usually similar to the controls and often even higher (stimulation). Dinoseb had a greater effect on bacterial productivity (DNA synthesis) than it did on protein synthesis. Deltamethrin showed a sustained 80% inhibitory effect even at ppb levels.

INTRODUCTION

Bacteria are the major decomposers of organic matter in the aquatic environment. The methodology to determine the rates at which bacteria take up organic solutes has been a subject of intensive study for at least 25 years. Model compounds, (i.e., simple sugars, amino acids and organic acids) labelled with ^{14}C or ^{3}H have been used to measure the potential for organic flux. Obviously, various environmental conditions (temperature, pH, ionic strength, etc.) affect the rate at which the bacterial population take up the organic substrates. Recent improvements in methodologies have shown that the use of radioactively labelled leucine (3) will give a good indication of bacterial protein synthesis and the incorporation of radioactively labelled thymidine into DNA is a good indicator of bacterial productivity (4).

In recent years many pesticides have been added to freshwater, either by design (pesticide added to control nuisance growth of certain aquatic plants or animals) or by agricultural run-off. The effect these pesticides have on aquatic bacteria has received little attention. The effects of some aromatic pollutants (anthracene, p,p'-dichlorodiphenyltrichloroethane, naphthalene, and pentachlorophenol [PCP]) have been studied on sediment bacteria using the metabolism of ^{14}C-glucose and incorporation of [^{3}H]thymidine (2). Sediment communities were also studied by monitoring ^{14}C-acetate incorporation and glucosidase activity in the presence of various heavy metals and the organic toxicants sodium deoxycholate, sodium dodecyl sulphate, and PCP (1).

MATERIALS AND METHODS

Protein synthesis. The procedure as outlined by Chin-Leo and Kirchman (3) was used. A final concentration of leucine was 11 nM (1 nM 4,5-[^{3}H]-leucine [ICN Biomedicals, specific activity, 54 Ci·mmole^{-1}] and 10 nM unlabelled leucine). After the incubation time, trichloroacetic acid (TCA) was added to a final concentration of 5%. The sample was heated to 85-90°C for 30 min, cooled on ice, and filtered through Sartorius filters (pore size, 0.45 μm) which had previously been soaked in a solution of 10 mM unlabelled leucine. The filters were rinsed with 5% TCA and dissolved in scintillation fluor (ACS II, Amersham). The samples were then counted by liquid scintillation (Canberra Packard model 4300) using the external standard procedure. To ensure the recommended leucine concentration and incubation time were adequate for Hamilton Harbour water, control experiments were run varying the incubation time (5 to 60 min.) and concentration of leucine (5 to 100 nM).

Bacterial productivity (DNA synthesis). The procedure as outlined by Fuhrman and Azam (4) was followed using 11 nM [methyl-^{3}H]thymidine and incubating the samples for 20 minutes. Cold TCA was added to a final concentration of 5%, held on ice for 30 min. and filtered through Sartorius filters (pore size, 0.45 μm) which had previously been soaked in a solution of 10 mM unlabelled thymidine. The filters were then dissolved and counted as above.

Pesticide effect studies. Eight pesticides were tested using the natural aquatic bacteria found in Hamilton Harbour. The pesticides were trifluralin, TFM (3-trifluoromethyl-4-nitrophenol), atrazine, deltamethrin, methoxychlor, dinoseb, 2,3,4,5-tetrachlorophenol, and pentachlorophenol. Milli-Q water was used to dissolve the trifluralin and TFM, and ethanol was used to dissolve the remaining

pesticides. The final pesticide concentrations in the samples were less than 10 ppm. The water solubility of deltamethrin and methoxychlor only allowed the highest concentration to be 5 ppb. Dilutions of the various pesticides were prepared so that 100 µl of each dilution was added to 100 ml of Hamilton Harbour surface water (prescreened through 30 µm Nitex). The control was the addition of 100 µl of Milli-Q water or ethanol depending on the pesticide solvent. After 1, 2, 4, and 24 hours, 10 ml of sample was removed and assayed for bacterial productivity and protein synthesis (as above). In this preliminary study, only single determinations at each concentration and time were made.

RESULTS AND DISCUSSION

Protein synthesis conditions. The conditions as outlined in Chin-Leo and Kirchman (3) were tested for use in Hamilton Harbour. Figure 1C illustrates that the recommended 30 min. incubation period was sufficient, but we decided on a 20 min. incubation to complement the thymidine incubation time. Calculating the V_{max} by the Wright-Hobbie procedure (5) (Figure 1A) gave a value of 1.89 $nM \cdot L^{-1} \cdot h^{-1}$. Figure 1B shows that at the recommended concentration (11 nM leucine) V_{max} was not attained. However to reach this velocity we would either have to added an additional 39 nM unlabelled leucine, which would have reduced the radioactivity by 4.5-fold, or add the same ratio of labelled and unlabelled leucine to attain 50 nM. The difference in the uptake velocity between 11 and 50 nM (1.5 and 1.8 $nM \cdot L^{-1} \cdot h^{-1}$, respectively) was considered small so neither option was felt to be beneficial.

Atrazine. Figure 2A shows how thymidine incorporation into DNA is effected by the addition of the herbicide, atrazine. The lowest

concentration was 0.05 ppm and it had the greatest sustained effect during the first four hours of incubation. The 0.1 ppm initially stimulated DNA synthesis and then only slight inhibition was noted at 95% of the control. The two higher concentrations had little effect on the DNA synthesis as compared to the control and all concentrations, except 0.1 ppm, stimulated DNA synthesis at 24 hours. Figure 2B shows the effect of atrazine on protein synthesis. Initially all concentrations inhibited leucine incorporation into protein. By four hours the bacteria were recovering and even stimulated.

Trifluralin. DNA synthesis was not run on the herbicide, trifluralin, but Figure 3 shows the effect of 0.5 to 10 ppm on protein synthesis. The lowest concentration tested showed a slight stimulation in leucine uptake into protein, but by 24 hours it had inhibited synthesis by 77% as compared to the control. We obtained variable results in the higher concentrations of trifluralin. The 1 and 10 ppm had an equal initial effect (80% inhibition at 2 hrs), but the 2 and 5 ppm didn't inhibit protein synthesis. By 24 hours the two higher concentrations were stimulatory while the 1 and 2 ppm concentrations only gave slight inhibition at 95% of the control.

TFM. The lampricide, TFM, initially inhibited DNA synthesis at all concentrations tested (0.5 to 10 ppm)(Figure 4A). The two lower concentrations (0.5 and 1 ppm) had returned to the control value by 24 hours, but the three highest concentrations continued to inhibit DNA synthesis by 20% of the control. Figure 4B shows slight initial inhibition of protein synthesis, but by 24 hours there was no effect.

Deltamethrin. Even at very low ppb levels this insecticide gave decreasing DNA synthesis with time (Figure 5A). The lowest concentration (0.5 ppb) gave the most inhibition. The inhibition of all concentrations did not recover by 24 hours. Figure 5B shows a similar trend in the bacterial protein synthesis.

Methoxychlor. DNA synthesis was not effected by the presence of o.05 to 5 ppb of methoxychlor (Figure 6A). Although there was initially some slight inhibition of protein synthesis at the 0.5 to 5 ppb concentrations (Figure 6B), by 24 hours all treated samples were above the control values. The lack of bacterial effects from this pesticide is not surprising since methoxychlor is an insecticide which acts at the nerve cell membrane level.

Dinoseb. Dinoseb (2-sec-butyl-4,6-dinitrophenol) had a greater effect on bacterial DNA synthesis than protein synthesis (Figure 7). The midrange concentrations of dinoseb gave sustained inhibition of thymidine incorporation around 70-80% of the control (Figure 7A). The highest concentration (6.2 ppm) was intially very inhibitory (40%) and had only recovered to 65% of the control by 24 hours. Protein synthesis (Figure 7B) showed a continual loss of protein synthesis at the 0.12 ppm level, but the higher concentrations were recovered by 24 hours. Dinoseb, however, is highly toxic to fish (6), all goldfish which were being tested died within 24 hours after being exposed to dinoseb at 0.4 ppm. All fish tested survived 0.1 ppm. Dinoseb is not intended for use in aquatic areas (6).

Tetrachlorophenol. DNA synthesis was immediately effected by the presence of tetrachlorophenol (Figure 8A). The bacteria seemed to adapt to the TCP at 0.1 and 0.5 ppm concentrations in time, but the 2 and 5 ppm levels reduced the synthesis to 50% and 10% of control

values, respectively. The same pattern was noted in the protein synthesis (Figure 8B) although at the 5 ppm level, the data was giving negative percentages of the control and have not be included in the figure.

Pentachlorophenol. PCP, as did TCP, had a pronounced effect on DNA synthesis at 2 and 5 ppm, while the lower concentrations had little effect (Figure 9A). The bacteria slowly recovered from the 2 ppm, but by 24 hours, the 5 ppm treated sample was only 6% of the control value. Protein synthesis appeared to be more sensitive to the effects of PCP (Figure 9B). Again, the lowest concentration (0.1 ppm) had no effect, but 0.5 ppm decreased the synthesis to 40% of the control value at 4 hours. All concentrations had recovered full protein synthesis by 24 hours except the 5 ppm PCP sample which was recovering but only to the 60% of the control value.

CONCLUSIONS

Bacterial metabolism is negatively affected by most of the pesticides tested. The trend of the native aquatic bacteria in Hamilton Harbour was toward complete recovery of initial activity by 24 hours. The rate of this recovery was one of the distinguishing characteristics of the different pesticides. The chlorophenols, PCP and TCP, decreased DNA and protein syntheses to the greastest degree and when present at concentrations exceeding 2 ppm needed more than 24 hours to reach the activities of the control. Deltamethrin had a sustained effect on bacterial metabolism and the bacteria did not recover to control values from the exposure to ppb concentrations. In future studies only one metabolic activity (with replicates) need be tested since DNA synthesis and protein synthesis showed similar trends. We

recommend the use of tritiated leucine since the radioisotope does not have the radiodecay problems of thymidine which leads to a loss of activity and formation of labelled thymine at a rate of 3% per month.

LITERATURE CITED

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2. **Bauer, J. E. and D.G. Capone.** 1985. Effects of four aromatic organic pollutants on microbial glucose metabolism and thymidine incorporation in marine sediments. Appl. Environ. Microbiol. **49:** 828-835.

3. **Chin-Leo, G. and D.L. Kirchman.** 1988. Estimating bacterial production in marine waters from the simultaneous incorporation of thymidine and leucine. Appl. Environ. Microbiol. **54:**1934-1939.

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5. **Wright, R.T. and J.E. Hobbie.** 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology **47:**447-464.

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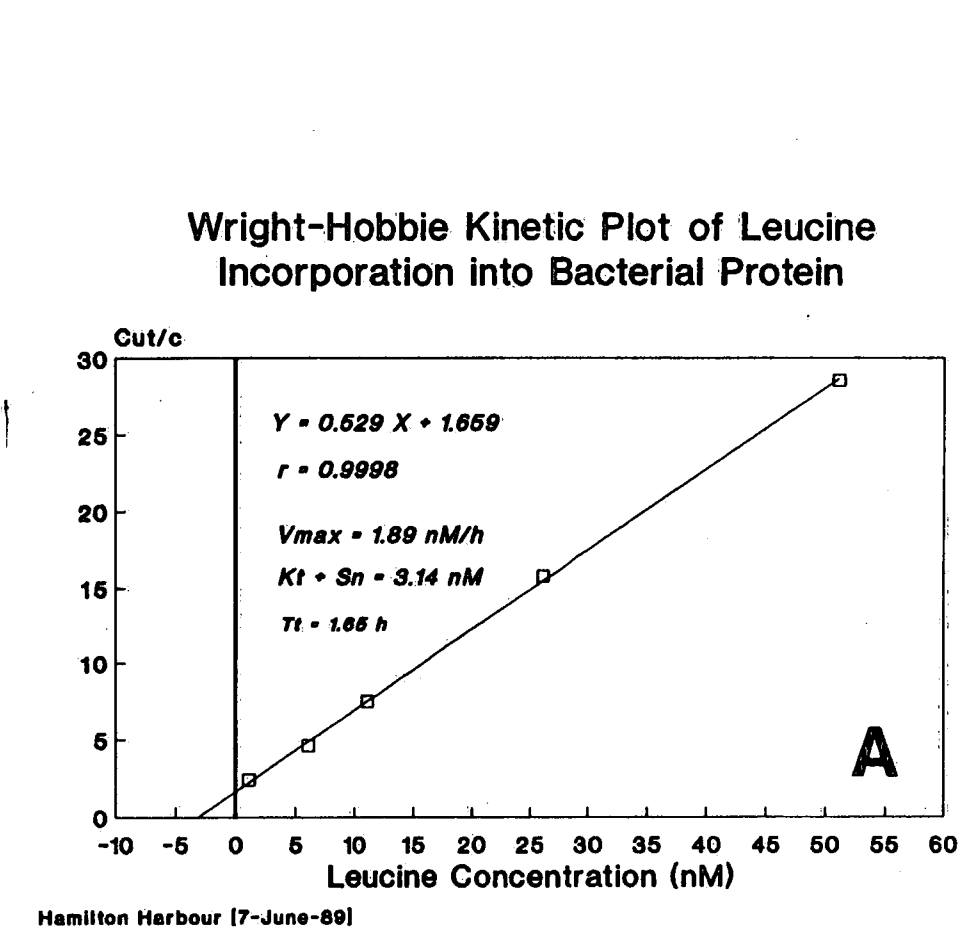
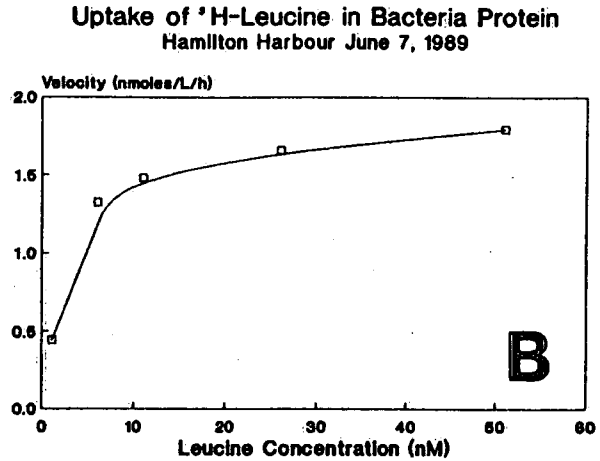

Wright-Hobbie Kinetic Plot of Leucine Incorporation into Bacterial Protein
Cut/c
Y = 0.529 X + 1.659
r = 0.9998
Vmax = 1.89 nM/h
Kt = Sn = 3.14 nM
Tt = 1.66 h
A
Leucine Concentration (nM)
Hamilton Harbour [7-June-89]


Uptake of ³H-Leucine in Bacteria Protein
Hamilton Harbour June 7, 1989
Velocity (nmoles/L/h)
B
Leucine Concentration (nM)

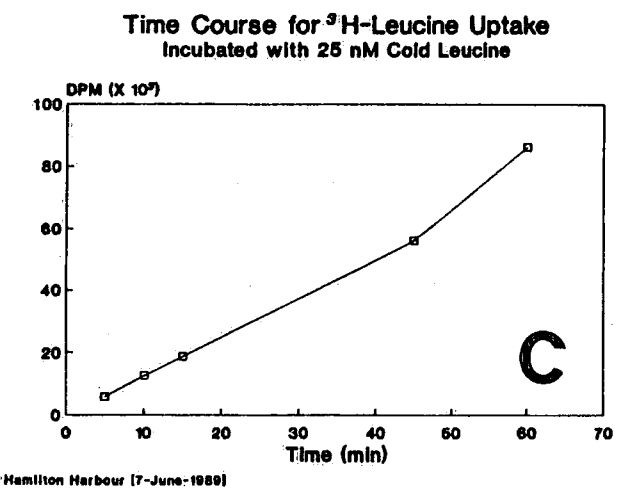
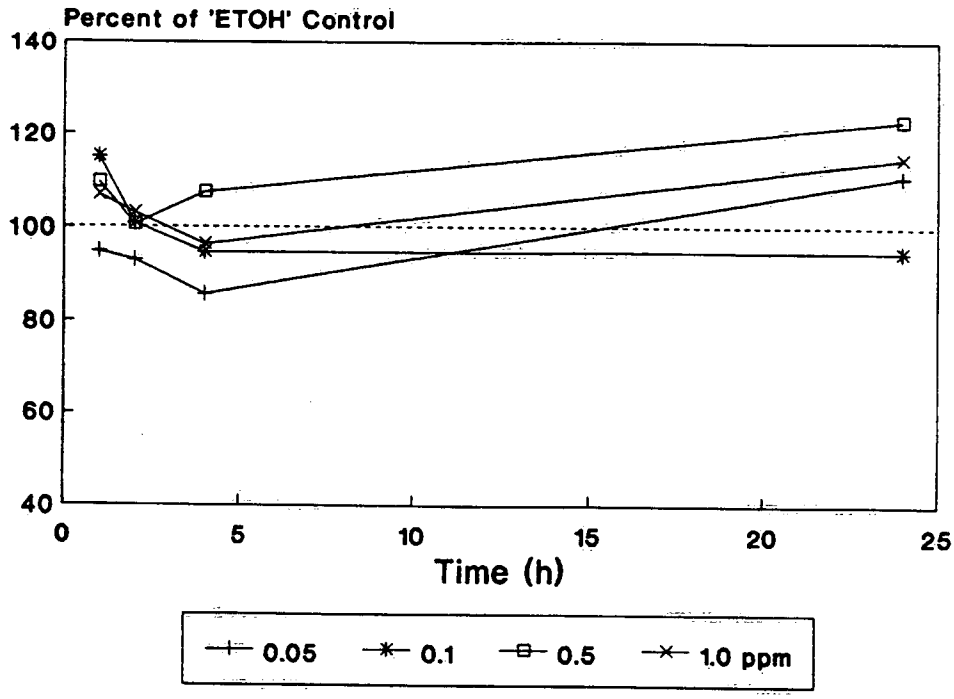

Time Course for ³H-Leucine Uptake
Incubated with 25 nM Cold Leucine
DPM (X 10²)
C
Time (min)
Hamilton Harbour [7-June-1989]

Figure 1

EFFECT OF ATRAZINE ON DNA SYNTHESIS



A

EFFECT OF ATRAZINE ON BPS[1]

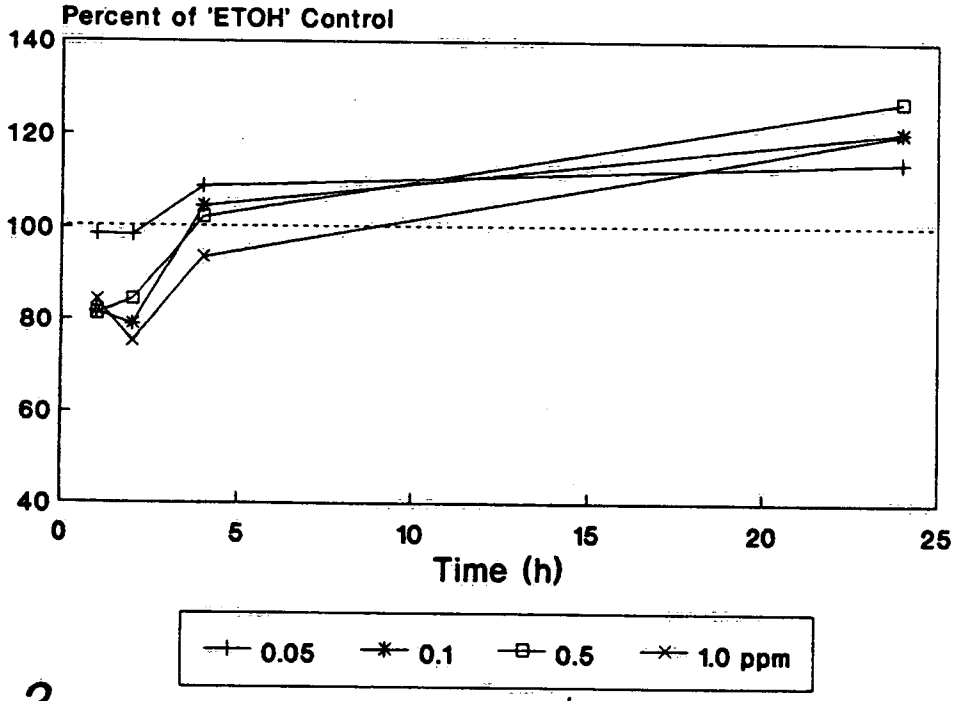


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Figure 2

[1] *BPS = bacterial protein synthesis*

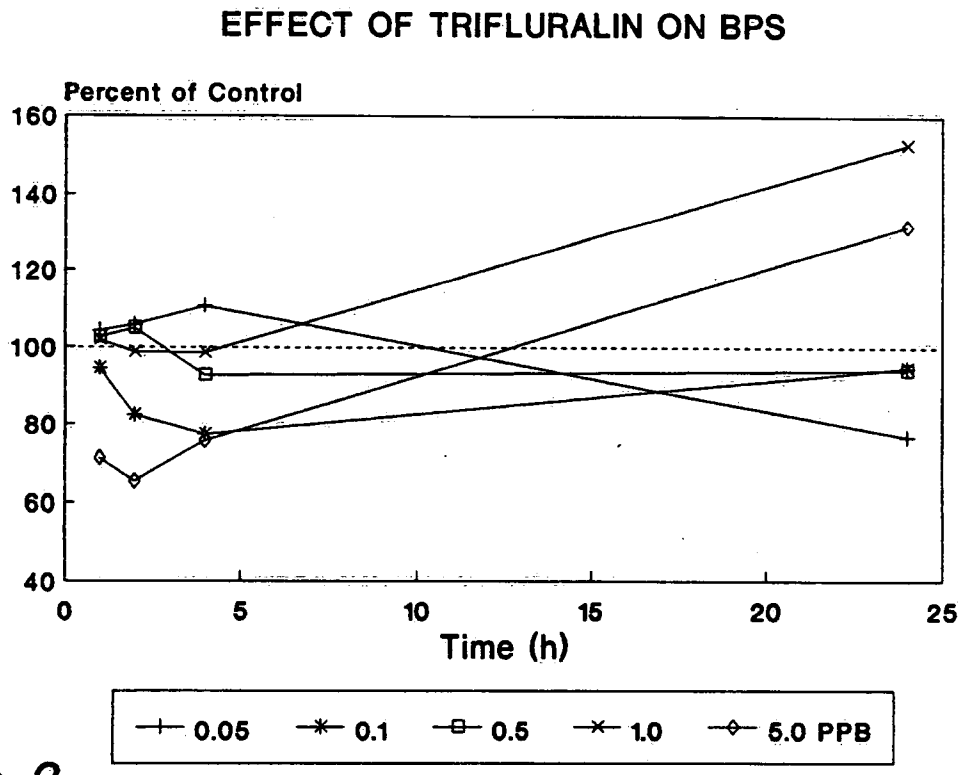
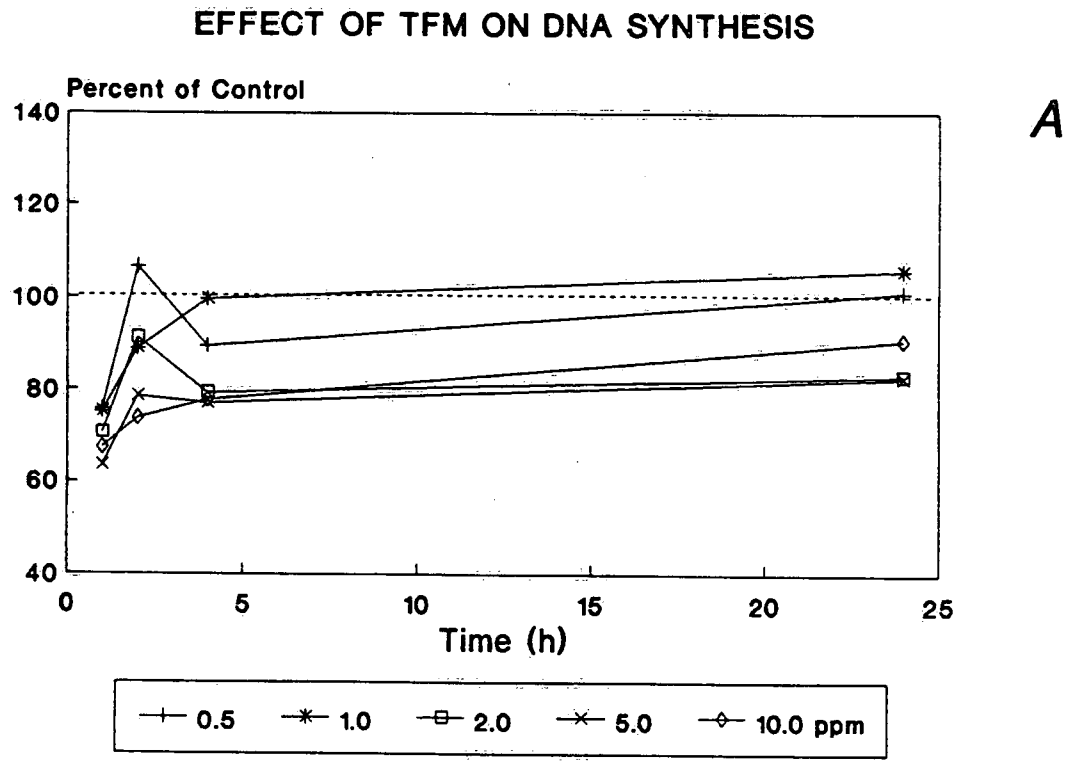
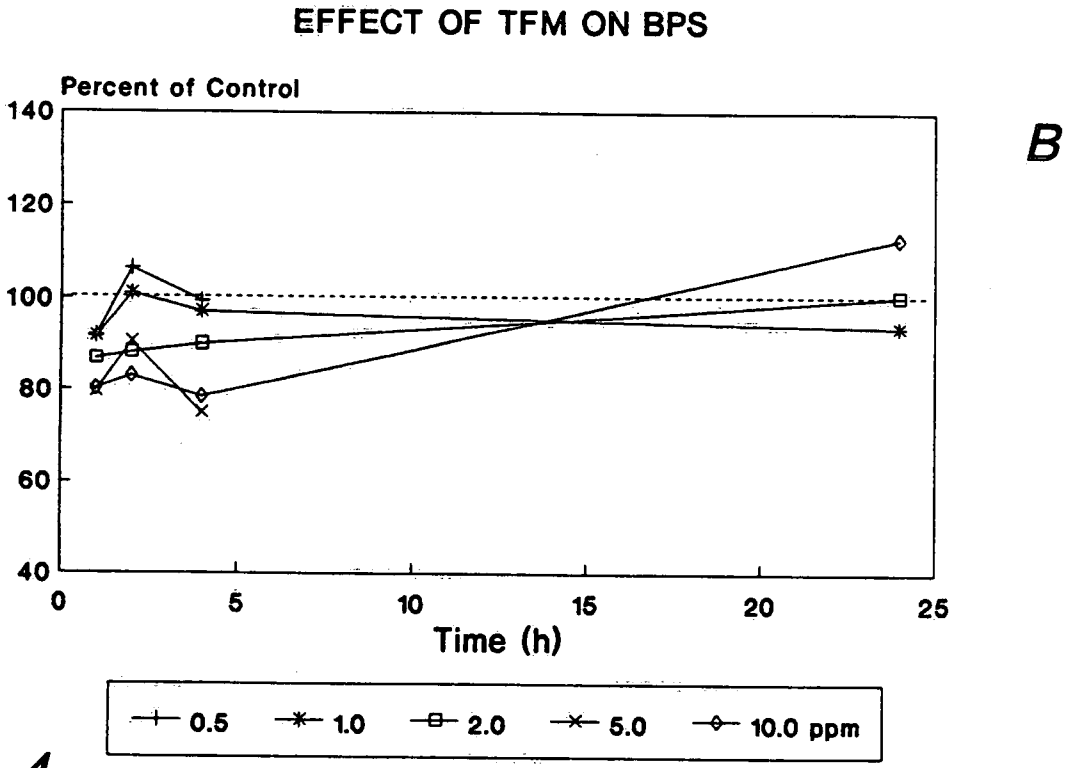

EFFECT OF TRIFLURALIN ON BPS
Percent of Control
160
140
120
100
80
60
40
0
5
10
15
20
25
Time (h)
0.05
0.1
0.5
1.0
5.0 PPB

Figure 3

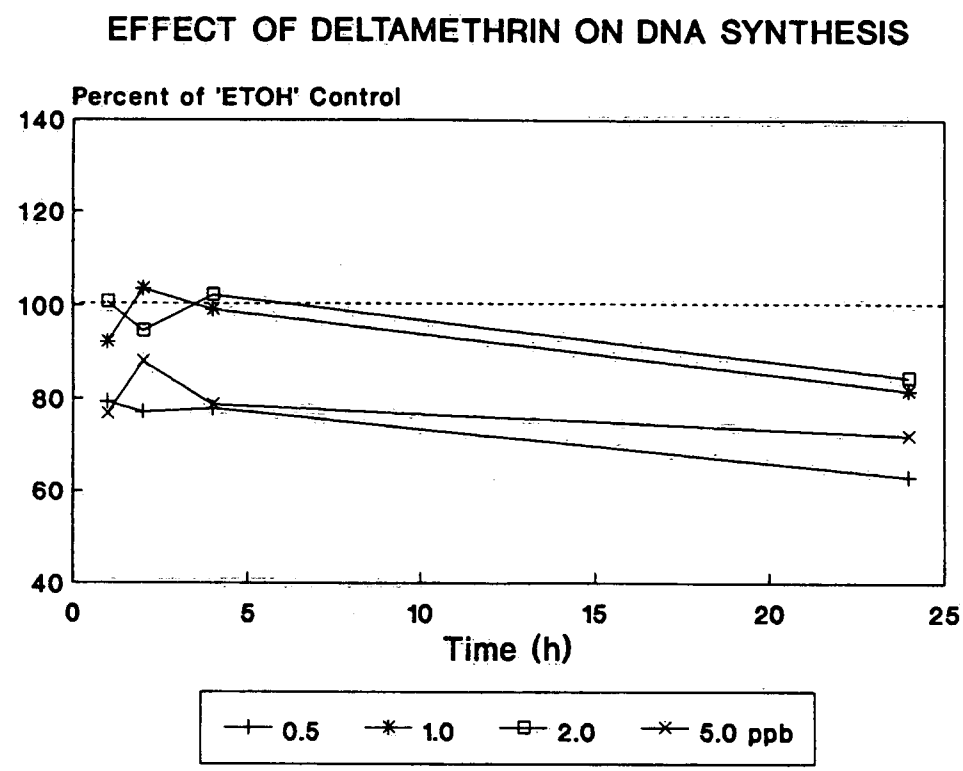

EFFECT OF TFM ON DNA SYNTHESIS
Percent of Control
140
120
100
80
60
40
0
5
10
15
20
25
Time (h)
0.5
1.0
2.0
5.0
10.0 ppm

A

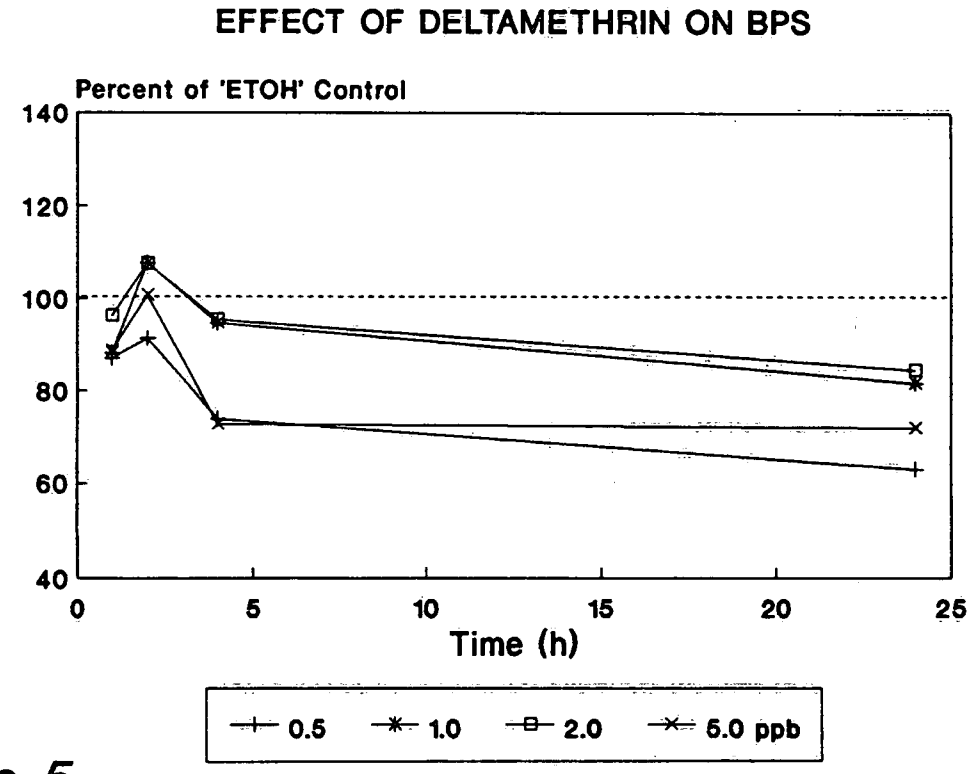

EFFECT OF TFM ON BPS
Percent of Control
140
120
100
80
60
40
0
5
10
15
20
25
Time (h)
0.5
1.0
2.0
5.0
10.0 ppm

B

Figure 4


EFFECT OF DELTAMETHRIN ON DNA SYNTHESIS
Percent of 'ETOH' Control
140
120
100
80
60
40
0
5
10
15
20
25
Time (h)
0.5
1.0
2.0
5.0 ppb

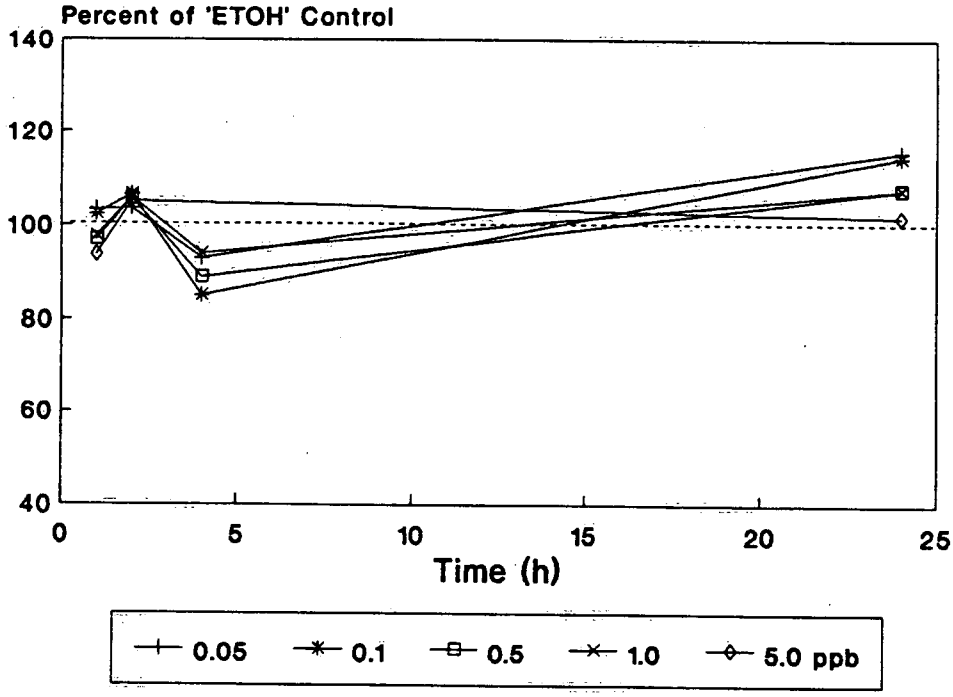
A


EFFECT OF DELTAMETHRIN ON BPS
Percent of 'ETOH' Control
140
120
100
80
60
40
0
5
10
15
20
25
Time (h)
0.5
1.0
2.0
5.0 ppb

B

Figure 5

EFFECT OF METHOXYCHLOR ON DNA SYNTHESIS



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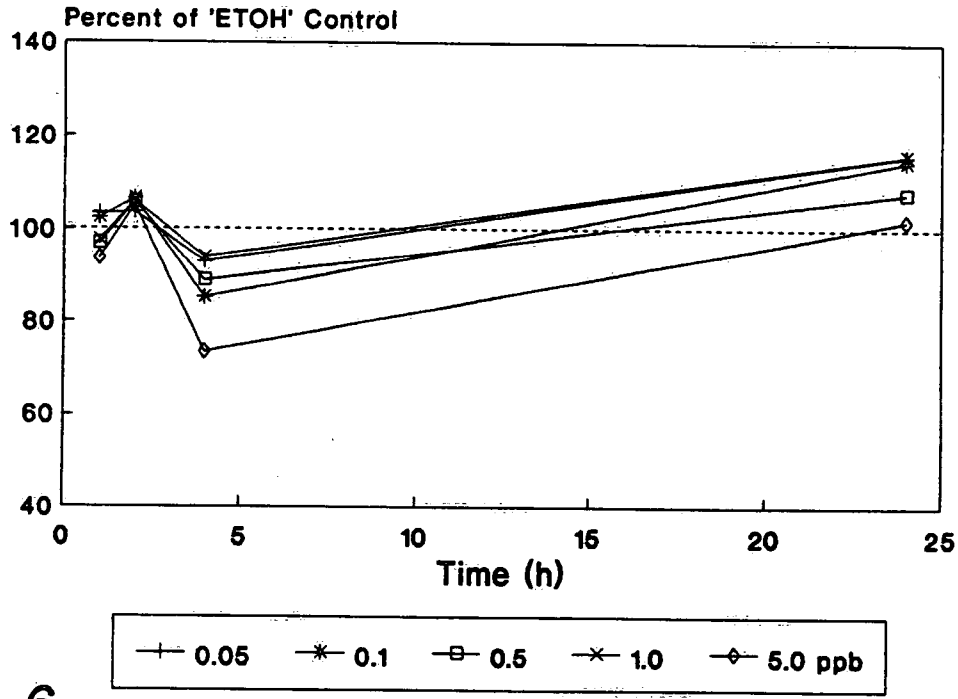
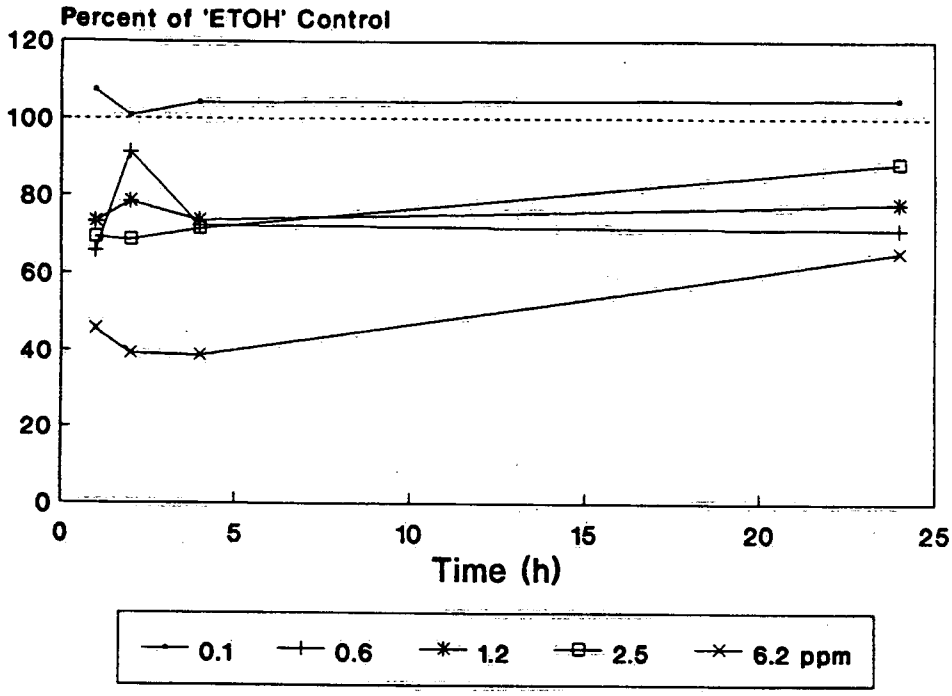
EFFECT OF METHOXYCHLOR ON BPS



B

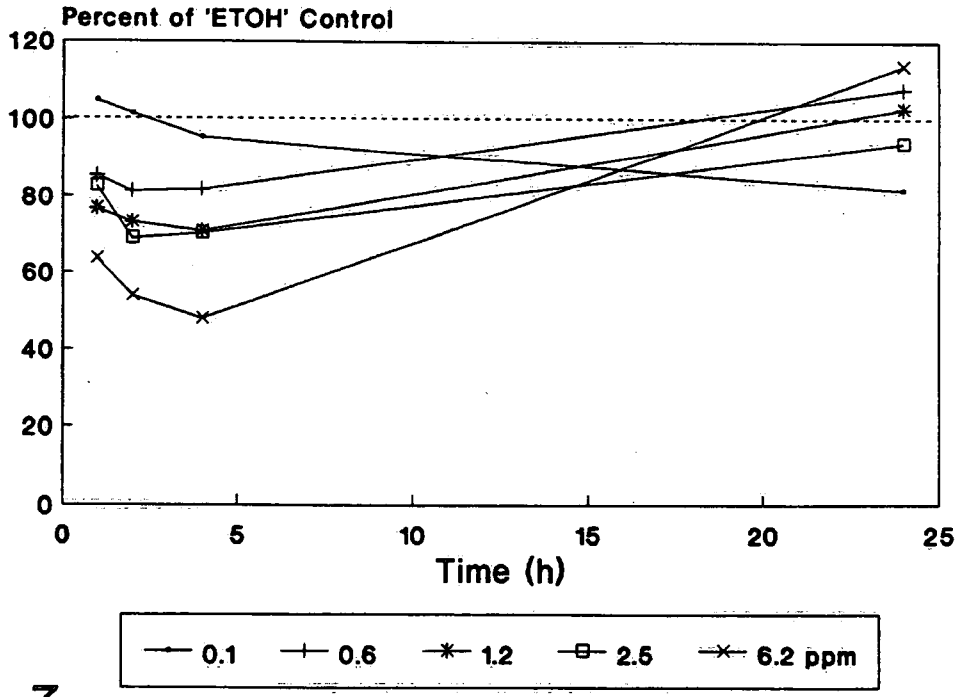
Figure 6

EFFECT OF DINOSEB ON DNA SYNTHESIS



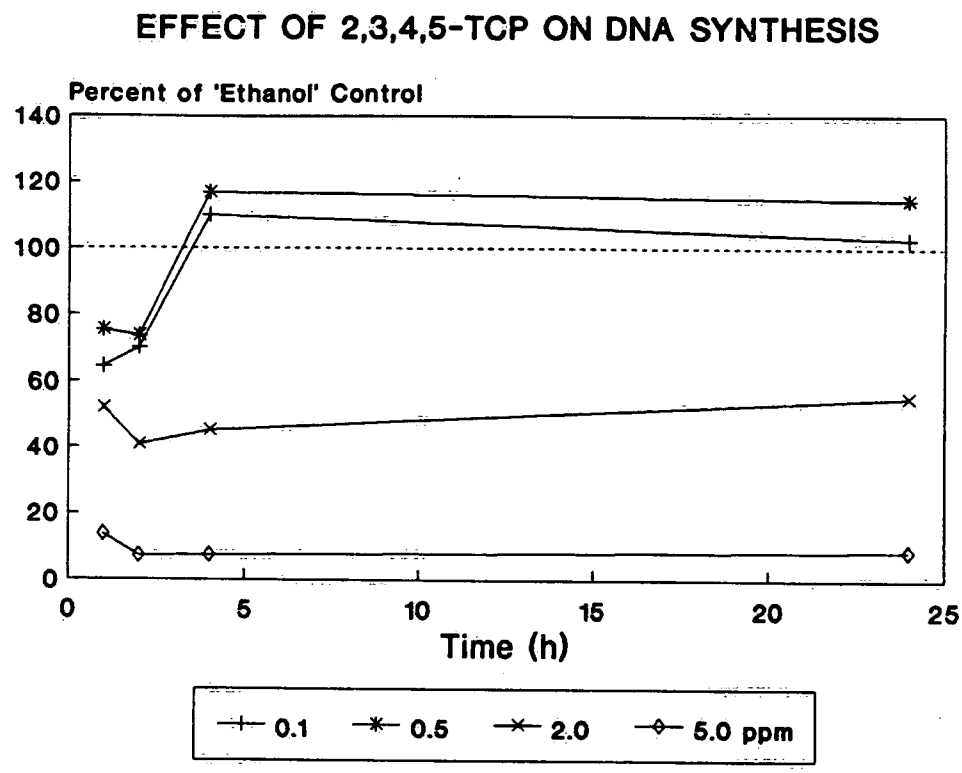
A

EFFECT OF DINOSEB ON BPS

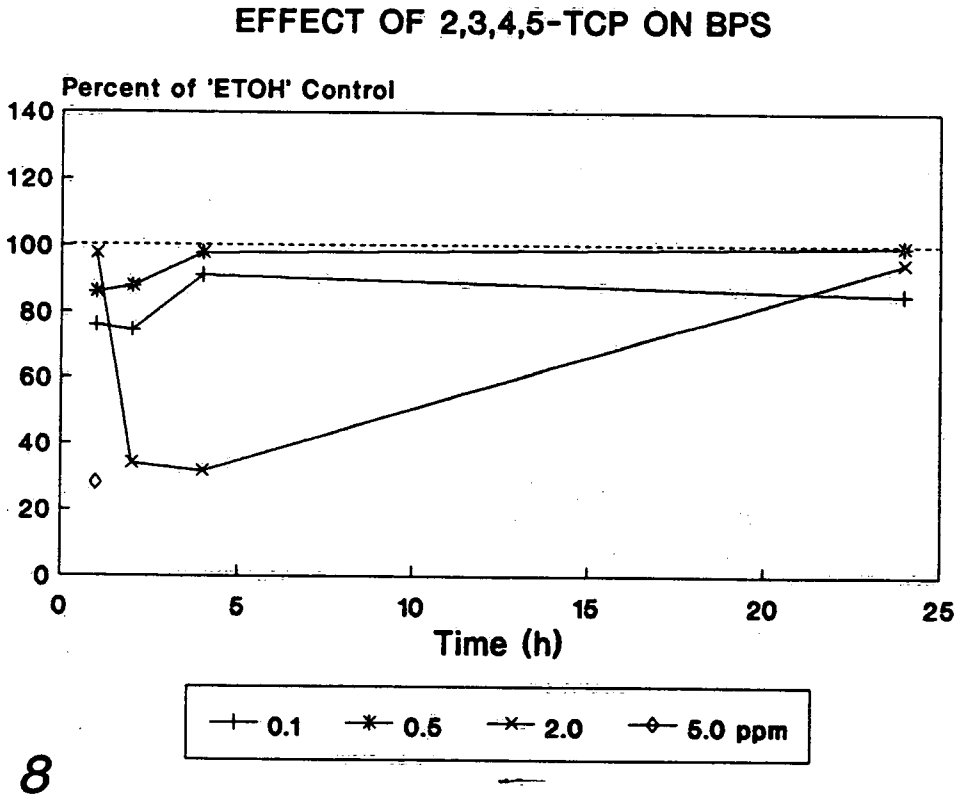


B

Figure 7


EFFECT OF 2,3,4,5-TCP ON DNA SYNTHESIS
Percent of 'Ethanol' Control
140
120
100
80
60
40
20
0
0
5
10
15
20
25
Time (h)
0.1
0.5
2.0
5.0 ppm

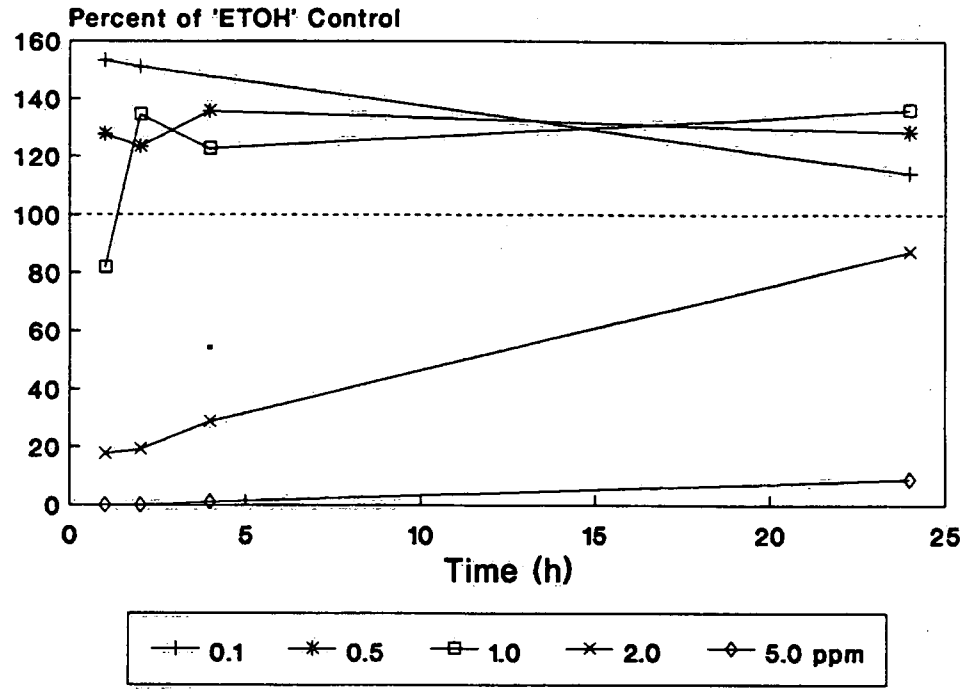
A


EFFECT OF 2,3,4,5-TCP ON BPS
Percent of 'ETOH' Control
140
120
100
80
60
40
20
0
0
5
10
15
20
25
Time (h)
0.1
0.5
2.0
5.0 ppm

B

Figure 8

EFFECT OF PCP ON DNA SYNTHESIS



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EFFECT OF PENTACHLOROPHENOL ON BPS

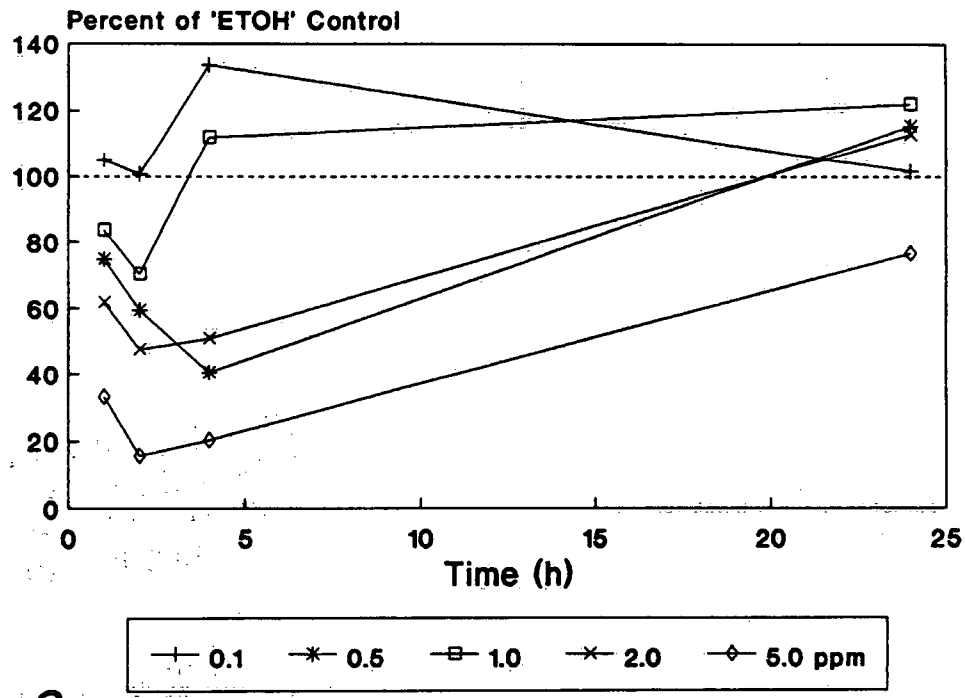

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Figure 9

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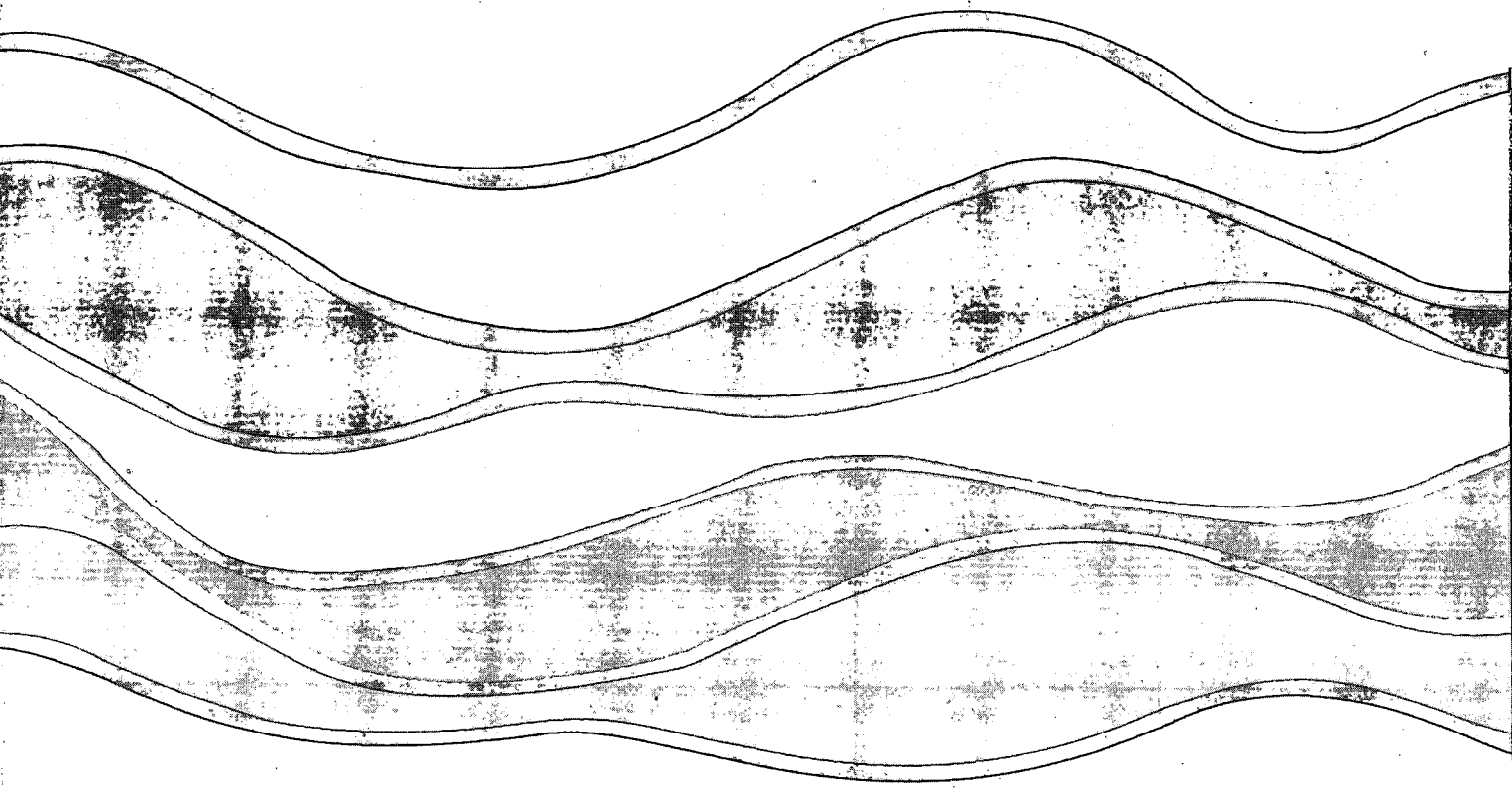

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