CYCLOHEXIMIDE

Task 1: Review and Evaluation of Individual Studies

Contract No. 68-01-5830

Final Report

October 9, 1981

SUBMITTED TO:

Environmental Protection Agency
Arlington, Virginia 22202

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CYCLOHEXIMIDE

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STUDY 1

CHEMICAL: CYCLOHEXIMIDE , ACTI-DIONE

FORMULATION: 00 - Active Ingredient

FICHE/MASTER ID: 05013827

CITATION: Kornfeld, E.C. 1949. The structure and chemistry of Actidione, an antibiotic from Streptomyces griseus. J. Am. Chem. Soc. 71(1):150-159.

DIRECT RVW TIME = 16 (MH) START-DATE END DATE

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SIGNATURE C. Christian , Richard L Hebert DATE: July 20, 1981

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CONCLUSIONS:

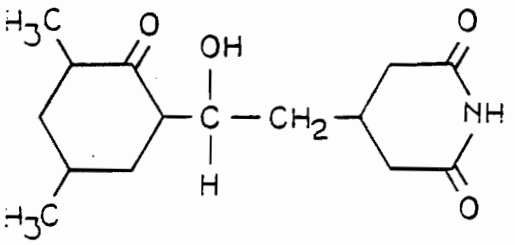
Degradation - Hydrolysis

1. This study is scientifically valid.

2. Cycloheximide undergoes hydrolysis in an alkaline solution to form d-2,4-dimethylcyclohexanone, propionaldehyde-3,3-diacetic acid, and ammonia. Rates of hydrolysis and accumulation and decline patterns were not studied.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-(2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-)glutarimide

Cycloheximide (Actidione, Upjohn Co.; purified by repeated recrystallization) was dissolved in 5 N NaOH (unspecified temperature and length of time) and distilled. The distillate was ether extracted after saturation with NaCl. The combined ether extracts were dried over Na_2SO_4, ether was removed, and the residue was distilled. The fragrant volatile product was analyzed by UV and IR spectroscopy, elemental analysis, and optical rotation analysis. Oxime, semicarbazone, and 2,4-dinitrophenylhydrazone derivatives were obtained in "the usual manner," presumably according to Ford and Leach (1948, J. Am. Chem. Soc. 70:1223). The p-nitrophenylhydrazone derivative was prepared in ethanol with acetic acid as a catalyst and separated as golden needles from dilute ethanol. An l-menthydrazone derivative was prepared by the method of Woodward et al. (1941, J. Am. Chem. Soc. 63:120) and recrystallized from methanol.

In another experiment, the residue remaining after distillation of the 5 N NaOH hydrolysis mixture was cooled and neutralized with H_2SO_4, and $KMNO_4$ was added. The mixture was shaken until permanganate color was observed. After warming on a steam bath for a few minutes, it was made alkaline with NaOH, and MnO_2 was filtered off. The filtrate was extracted with ether after saturation with Na_2SO_4 and acidification with H_2SO_4. A crude syrupy product was obtained after ether evaporation. On long standing, the ether solution yielded a crystalline product. The melting points of the product and a bromophenylacyl ester derivative were analyzed. The derivative was prepared by neutralizing the product with $NaHCO_3$, adding bromophenacyl bromide in ethanol, refluxing, and removing the ethanol. The derivative was recrystallized from aqueous acetone.

Cycloheximide was also refluxed in 12 N NaOH for 30 minutes. The evolved gas was trapped in ice water. Phenyl isothiocyanate was added and crystals were obtained and analyzed.

REPORTED RESULTS:

The fragrant volatile product obtained from the distillate of the NaOH solution had the formula $C_8H_{14}O$, was dextrarotatory, and had an absorption peak at 284 nm, characteristic of a carbonyl group. The presence of a carbonyl group was confirmed by the preparation of oxime, semicarbazone, p-nitrophenylhydrazone, and 2,4-dinitrophenylhydrazone derivatives. Aldehyde tests were negative, and therefore it was a cyclic ketone. Its boiling and

melting points, IR spectrum, and refractive index corresponded to those of synthetic d-2,4-dimethylcyclohexanone (Figure 1). To confirm the identity, the l-menthydrazone derivative of the product was subjected to mixed melting point analyses with synthetic l-menthydrazone derivatives of d- and l-2,4-dimethylcyclohexanone. The melting point remained undepressed upon mixing with the d-2,4-dimethylcyclohexanone derivative. Further confirmation was obtained by X-ray diffraction analysis of the menthydrazone derivative. Its identity was further confirmed by oxidizing cycloheximide with chromic acid, which yielded a 1,3-diketone (dehydroactidione, Figure 1). This compound was characterized by its UV absorption and conversion to a copper complex. The 1,3-diketone, on alkaline degradation, also gave 2,4-dimethylcyclohexanone.

The residue remaining after distillation of the hydrolysis mixture consisted of an impure seven-carbon acidic fragment. On oxidation, it gave methanetriacetic acid (Figure 1), which was confirmed by mixed melting point analysis with an authentic sample and with bromophenacyl ester derivatives of the product and synthetic methanetriacetic acid. Thus, the hydrolytic cleavage of cycloheximide can be looked upon as a reverse aldol reaction accompanied by opening of the glutarimide ring. This was confirmed by reacting benzylamine with cycloheximide, which yielded ring opening and condensation of the resulting aldehyde with benzylamine (I, Figure 1).

When cycloheximide was refluxed with 12 N NaOH, ammonia was evolved, as determined by trapping the gas in water and adding phenyl isothiocyanate. The product was identified as phenylthiourea, on the basis of mixed melting point analysis with authentic phenylthiourea.

DISCUSSION:

1. The experiments were conducted in 5 N and 12 N NaOH solutions. However, cycloheximide reportedly was extremely labile even under very dilute alkaline (0.01 N NaOH) conditions.

2. Propionaldehyde-2,2-diacetic acid cannot be derived from cycloheximide, nor can it yield methanetriacetic acid, as was described. The nomenclature in the report must be in error, and the correct term for the compound in question must be propionaldehyde-3,3-diacetic acid (this is the term used in Figure 1).

3. In Studies 2 and 3 (00011594 and 00011595), it was found that cycloheximide was dehydrated when treated either with a dilute acidic solution or with a NaOH solution, resulting in the formation of anhydrocycloheximide. In the present study, anhydrocycloheximide was not found, but when it was treated with NaOH it also yielded 2,4-dimethylcyclohexanone.

4. The experiments were not performed in sterile solutions in the dark. However, microorganisms would not survive in the highly basic solutions used here. Also, imide hydrolysis is a common reaction and does not require light. Thus hydrolysis of the glutarimide ring to yield ammonia can be expected to readily occur in the dark. However, light could have affected the hydrolysis reaction that occurred at the α-carbon site.

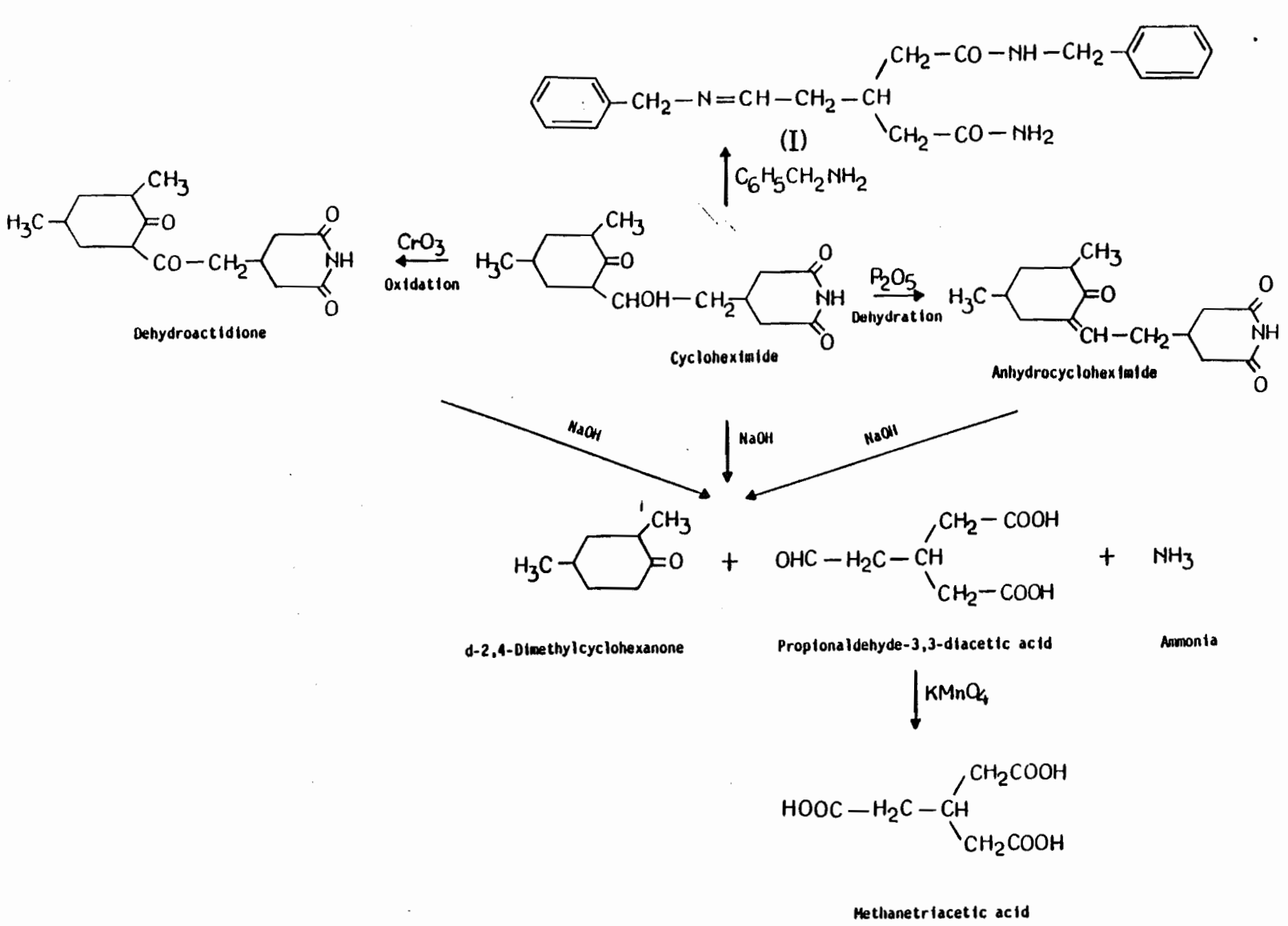


Figure 1. Cycloheximide degradation mechanisms. Propionaldehyde-3,3-diacetic acid was reported as a 2,2-diacetic acid in this study.

CHEMICAL: CYCLOHEXIMIDE, ACTI-DIONE

FORMULATION: 00 - Active Ingredient

FICHE/MASTER ID: 00011594

CITATION: Garrett, E.R., and R.E. Notari. 1966. Cycloheximide transformations. I. Kinetics and mechanisms in aqueous acid. J. Org. Chem. 31:425-434.

DIRECT RVW TIME = 17 (MH) START-DATE END DATE

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CONCLUSIONS:

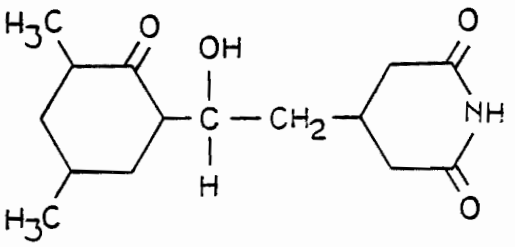
Degradation - Hydrolysis

1. This study is scientifically valid.

2. Cycloheximide is dehydrated in dilute acidic solutions (0.01-0.9 M HCl) at 40-80 C, producing anhydrocycloheximide and unidentified isomers of cycloheximide that are most likely α-epicycloheximide and α-epi-Naramycin B. Simultaneously, hydrolysis of the glutarimide ring occurs to yield first the monocarboxylic acid amide and then the dicarboxylic acid and ammonia.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

Cycloheximide was purified by recrystallization (method unspecified). The anhydrocycloheximide used in this study was prepared by dissolving 10 g cycloheximide in 150 ml of 6 N HCl and warming the mixture slowly until a flocculant precipitate formed at 40-50 C. The mixture was cooled immediately and the precipitate was allowed to crystallize. The precipitate was then recrystallized twice from aqueous methanol. The dehydration and hydrolysis of cycloheximide and anhydrocycloheximide were studied in solutions containing HCl at various concentrations and at temperatures ranging from 40 to 75 C. Cycloheximide disappearance and anhydrocycloheximide formation were followed spectrophotometrically at 245 nm. Quantitative thin-layer chromatography (TLC) of cycloheximide was performed by constructing a calibration curve of mean spot area versus known concentration. TLC plates were developed with ethyl acetate. The data were used to obtain rate constants and were fitted to predictions from computer models based on equations derived for the specific reactions.

Hydrolysis was also studied with solutions of 0.01 M cycloheximide in 0.1 M HCl at 80 C. Aliquots were removed immediately and at different time intervals (up to 500 hours) and diluted with ethanol. After the excess HCl was partially neutralized, the mixture was titrated potentiometrically with NaOH. The acidic hydrolysis of anhydrocycloheximide was studied similarly.

REPORTED RESULTS:

Cycloheximide at 0.01 M in 0.1 M HCl was dehydrated, resulting in the formation of anhydrocycloheximide and an unknown isomer of cycloheximide (Figure 1). The rate of dehydration increased with time and temperature and reached a maximum at about 6 hours at 80 C. The catalytic rate constants were dependent on hydrogen ion concentrations. The average yields of anhydrocycloheximide at 6 hours were 68% at 40 C, 70% at 50 C, 71% at 61.8 C, 72% at 70 C, and 73% at 74.6 C. The different yields at different temperatures were statistically significant, but not the yields at different HCl concentrations (approximate range, 0.01-0.9 M).

The decrease in absorbance at 245 nm was followed in solutions of anhydrocycloheximide. The curve reached an asymptotic value at about 20 hours, when the yields of cycloheximide were calculated as 34, 39, 48, and 52% at 40, 50, 61.8, and 70 C, respectively.

TLC indicated that both the solutions of cycloheximide and anhydrocycloheximide in dilute HCl at 80 C produced three common spots, which were developed by ethyl acetate, and a fourth spot that remained at the origin. The anhydrocycloheximide spot (R_f 0.61) was visible both by UV light and reaction with 2,4-dinitrophenylhydrazine (DNPH). The cycloheximide spot (R_f 0.47) was visible on reaction with DNPH. The third spot (R_f 0.53) was detected by charring with H_2SO_4 and heat.

The latter spot (P) could not be conclusively characterized, but it was identical to cycloheximide in elemental analysis, molecular weight determination, and UV and IR spectroscopy. Based on TLC, NMR and UV spectroscopy, optical rotary dispersion data, and an extensive analysis of theoretical and known mechanisms of isomerization and epimerization, it was determined that P was either α-epi-Naramycin B or α-epicycloheximide. The cycloheximide spot lost biological activity with time. Based on possible or known isomerization and epimerization schemes as well as NMR data for isolates of the spot, the major component(s) of the cycloheximide spot was identified as isocycloheximide and/or α-epi-Naramycin B. The reactions in Figure 2 summarize the conclusions regarding reaction schemes.

Along with dehydration, hydrolysis also took place (Figure 3). When solutions of 0.01 M cycloheximide or anhydrocycloheximide in 0.1 M HCl at 80 C were titrated as a function of time, curves with three end-points were obtained. The first end-point represented the volume of NaOH consumed by the excess HCl. The second end-point was attributed to the sum of carboxylic acid obtained. The third end-point was attributed to the ammonium ion formed through hydrolysis of the mono-carboxylic acid amide. Although the absorbance of anhydrocycloheximide remained constant after 20 hours, the formation of ammonium ion from imide hydrolysis continued for 500 hours. The apparent contradiction in the absorbancy data was due to the similar absorption pattern of the dicarboxylic acid. The hydrolysis was about 20, 55, and 90% complete by 25, 100, and 500 hours, respectively. The percent yield of mono-carboxylic amide never exceeded 20% at any time and was generally at 10-15%.

When the spectral data obtained were fed into the computer, the curves generated for the degradation of cycloheximide in 0.15 M HCl and also for the production of anhydrocycloheximide as a function of time were in agreement with those obtained by TLC. The data were fitted into the computer model to generate curves of the mole fraction of each product versus time for 0.01 M cycloheximide in 0.1 M HCl at 80 C.

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The results show that ∿70% of the initial cycloheximide is transformed immediately, and ∿10% remains after 6 hours. At this time, ∿70% is in the form of anhydrocycloheximide and ∿15% is present as P. Anhydrocycloheximide concentrations decline to 25-30% by 100 hours. P reaches levels of ∿30% after 25-30 hours and declines to ∿20% by 100 hours. The levels of dicarboxylic acids of anhydrocycloheximide and P increase at a relatively constant rate, and reach ∿22 and ∿30%, respectively, by 100 hours. The concentration of the dicarboxylic acid of cycloheximide remains negligible and the amount of cycloheximide isomers other than P remain <10%.

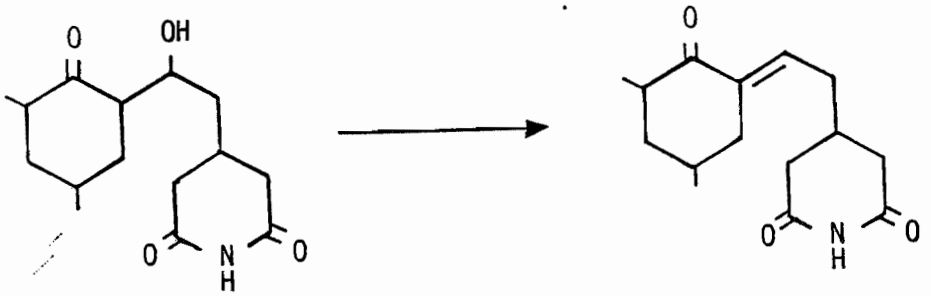
DISCUSSION:

1. In this pharmacokinetic study of cycloheximide, two chemical reactions took place simultaneously. Dehydration in HCl produced anhydrocycloheximide, whereas hydrolysis produced monocarboxylic acid amide, dicarboxylic acid, and ammonia.

2. Hydrolysis took place in ≥0.01 M HCl at 40-80 C; these conditions would not normally exist in the environment. Therefore, the reaction rates determined here are not applicable to environmental situations.

3. The experiments were not performed in sterile solutions in the dark. However, microorganisms would not survive or proliferate in the acidic solutions and at the high temperatures used. Also, imide hydrolysis is a common reaction that does not require light. Therefore it can be expected to occur in the dark. However, light could have affected the dehydration reaction.

4. The hydrolysis products were not identified here, but were presumed based on the nature of the titration curves and their identification in Study 1 (05013827). This is a valid presumption because the third end-point was high (pK_3, 8.8) and therefore indicative of ammonium ion formation from the one amine in cycloheximide. A spot at the origin of TLC plates was observed to enlarge with time. This spot was not identified but was attributed to acids. This latter conclusion is unwarranted as no data were obtained for the spot.



Cycloheximide

Anhydrocycloheximide

Figure 1. Dehydration of cycloheximide.

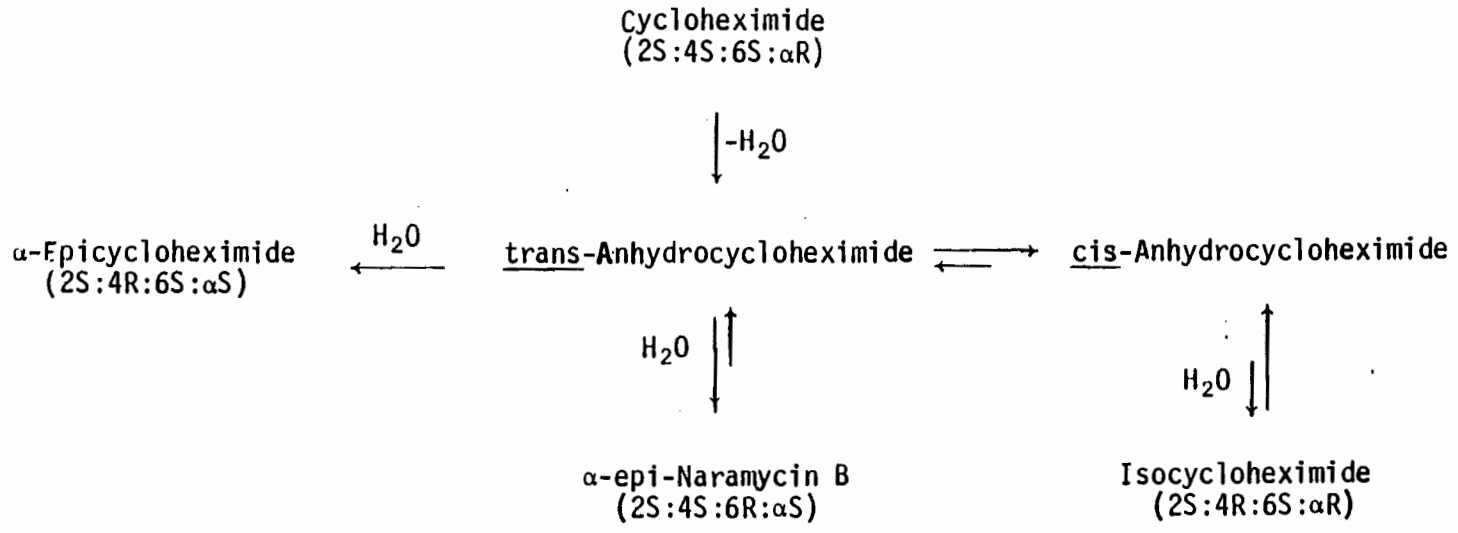


Figure 2. Postulated transformations of cycloheximide in acidic solutions.

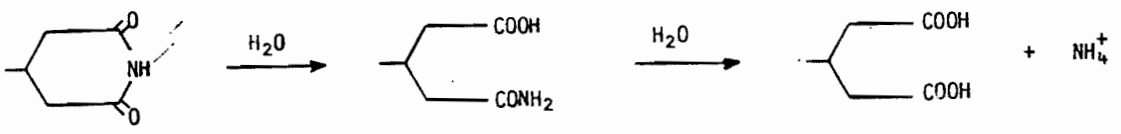


Figure 3. Hydrolysis of glutarimide ring of cycloheximide, its isomers, and anhydrocycloheximide.

STUDY 3

CHEMICAL: CYCLOHEXIMIDE, ACTI-DIONE

FORMULATION: 00 - Active Ingredient

FICHE/MASTER ID: 00011595

CITATION: Garrett, E.R., and R.E. Notari. 1965. Cycloheximide transformations. II. Kinetics and stability in a pharmaceutically useful pH range. J. Pharm. Sci. 54(2):209-215.

DIRECT RVW TIME = 16 (MH) START-DATE END DATE

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CONCLUSIONS:

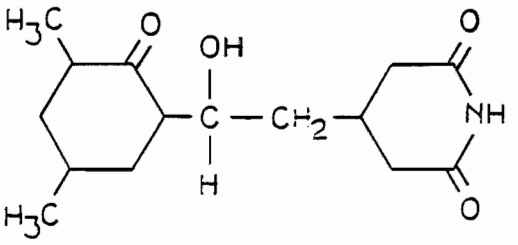
Degradation - Hydrolysis

1. This study is scientifically valid.

2. Cycloheximide is dehydrated to anhydrocycloheximide at 50 and 80 C in acetate buffer at pH 3-5.5, and at 25-43 C in NaOH at pH 7-10. In alkaline solutions, the amount of anhydrocycloheximide that can be formed is formed instantly. In addition, the glutarimide ring is hydrolyzed in alkali, forming monocarboxylic amide, dicarboxylic acid and ammonia. The times required for 10% of the hydrolysis to occur at pH 7 and 9 are 11 days and 2.6 hours at 20 C and 6.8 days and 1.6 hours at 30 C, respectively.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

Cycloheximide (Upjohn Co.) was purified by recrystallization (method unspecified). The anhydrocycloheximide used in this study was prepared by dissolving 10 g cycloheximide in 150 ml of 6 N HCl, and warming the mixture slowly until a flocculant precipitate formed at 40-50 C. The mixture was cooled immediately and the precipitate was allowed to crystallize. The compound then was recrystallized twice from aqueous methanol.

To study dehydration, standardized buffers of pH 3-5.5 were made by mixing standard NaOH solutions with standard solutions of acetic acid and KCl. Acetic acid and acetate concentrations were determined by titration with NaOH and HCl, respectively. Cycloheximide was added at 1.42×10^{-4} M and the solutions were incubated at 50 and 80 C. Samples were removed at various times (up to 200 hours) and cooled immediately. The absorbance was read on a spectrophotometer at 245 nm. Complete spectra of the samples were also obtained on a recording spectrophotometer. The reactions of cycloheximide in the buffers were monitored by thin-layer chromatography (TLC) using ethyl acetate developer.

Transformations in alkaline solutions were examined in a thermostated cell chamber of a recording spectrophotometer. Thermally equilibrated aqueous solution of 6.4×10^{-4} M cycloheximide and NaOH (at various concentrations) were mixed. The samples were immediately transferred into the sample cell and the spectra were recorded as a function of time.

The constant pH hydrolysis of cycloheximide was studied with a potentiometer standardized with pH 7.0 and pH 10.0 buffers after thermal equilibration of the thermostated titration vessel. The pH-stat mechanism was adjusted to maintain the observed pH. The titration vessel held NaOH solutions at various concentrations and temperatures. An aliquot of a thermally equilibrated aqueous cycloheximide solution was added into the vessel at 5.21×10^{-3} M and constantly agitated. The volume of NaOH solution required to maintain a constant pH was recorded as a function of time.

To study the alkaline hydrolysis of cycloheximide, 1-ml aliquots of a solution of cycloheximide in 0.166 M NaOH at 25 C were removed at different time intervals and diluted with 20 ml of 25% ethanol. The samples were then adjusted to pH 3 with HCl and titrated potentiometrically with 0.166 M NaOH. The first end-point indicated the amount of remaining HCl. The concentration of the generated carboxyl groups could be calculated from the total standard alkaline titer for the first and second end-points. The concentration of the generated ammonium group could be calculated from the total standard alkali titer between the second and third end-points. The percent hydrolysis of cycloheximide and the acid amide could also be determined as a function of time.

REPORTED RESULTS:

Cycloheximide, when treated with acetate buffer solutions at 50 and 80 C, was dehydrated, forming only anhydrocycloheximide as determined spectrophotometrically and by TLC. The rate constants and maximum yields of anhydrocycloheximide increased with the concentration of acetate in buffers of identical pH (values between 3.16 and 5.63). Hydroxyl ion concentrations were calculated, and rate constants were also found to be dependent on their concentrations.

Cycloheximide (6.4×10^{-4} M) was also dehydrated rapidly in NaOH buffers (unspecified pH) at 28-37 C. When the hydroxyl ion concentration was $1\text{-}2 \times 10^{-3}$ M, disappearance of NaOH was too rapid to allow the measurement of rate constants. When the hydroxyl ion concentration was maintained constant by using 10-300 times as much NaOH as cycloheximide, the conversion was complete within a few seconds. Maximum yields of anhydrocycloheximide were 10% and were obtained at time zero.

On plotting readings of NaOH obtained in the constant pH alkaline hydrolysis of cycloheximide, the initial portions of the curves were linear and constant for different temperatures (30.2-42.7 C) and hydroxyl ion concentrations. Further, the first-order rate constants obtained were consistent with the premise that hydrolysis was catalyzed by hydroxyl ions. Cycloheximide consumed an equimolar amount of hydroxyl ions within a few minutes at 25 C in 0.166 M NaOH. Subsequent consumption, up to two equivalents, was not complete after 24 hours. The rate constants for imide solvolysis were calculated for 20 and 30 C. The $t_{0.9}$ values for complete hydrolysis were then calculated for 20 and 30 C. At 20 C, these values were 1.7 minutes, 2.6 hours, and 11 days at pH 11, 9, and 7, respectively. At 30 C, the values were 1 minute, 1.6 hours, and 6.8 days at pH 11, 9, and 7, respectively.

The rate constants obtained here and in Study 2 (00011594) for dehydration of cycloheximide in acidic solutions were plotted against pH values. The curves show an increase with temperature and a minimum at pH 4.4 for all temperatures. The heat of activation (ΔH) was calculated from rate constants obtained at pH 4 and 4.7 at 50 and 80 C. The ΔH was used to estimate rate constants at pH 4.4 at 20 and 30 C. These constants showed that, if buffer systems are neglected, the time to reach 10% of the

anhydrocycloheximide that will form ($t_{0.9}$) is 105 and 36 days at 20 and 30 C, respectively.

DISCUSSION:

1. In the alkaline hydrolysis study, the formation of the acid amide was faster than the subsequent hydrolysis of the amide to yield ammonia. This is in contrast to the acidic hydrolysis of cycloheximide (Study 2, 00011594), in which the rate-determining step was solvolysis of the imide function.

2. It was theorized that only 10% of anhydrocycloheximide was obtained in alkali because the alkaline catalyzed retraldolization, yielding dimethylcyclohexanone and glutarimide β-acetaldehyde. Although these compounds were not identified here, their formation was documented in Study 1 (05013827); therefore this postulate is reasonable.

3. The experiments were not performed in the dark in sterile solutions. However, microorganisms would not be expected to survive or proliferate at the high temperatures and highly basic and acidic solutions used in most portions of this study. The hydrolysis of the imide function is a common reaction that does not require light. However, light may affect the dehydration reaction.

4. The hydrolysis products were not identified here, but were presumed present based on the nature of the titration curves and their identification in Study 1 (05013827). This is reasonable because the third endpoint was high and therefore indicative of ammonium ion formation from the only amine group in cycloheximide.

CASE GS0038 CYCLOHEXIMIDE STUDY 4 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 30 TOPIC 050525 GUIDELINE 40 CFR 163.62-9b/c/d

FORMULATION 00 = ACTIVE INGREDIENT

FICHE/MASTER ID 00011196 CONTENT CAT 01

Petzold, E.N.; Chapman, D.D. (1970) The Stability of Cycloheximide in a Controlled Soil Environment: Report No. 120-9760-30. (Unpublished study received Jul 28, 1970 under 1023-EX-27; submitted by Upjohn Co., Kalamazoo, Mich.; CDL:210033-D)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
SEC: EFB -30-050516

DIRECT RVW TIME = 7 (MH) START-DATE END DATE

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CONCLUSIONS:

Metabolism - Aerobic Soil

1. This study is scientifically valid.

2. Cycloheximide at 0.2 ppm had a biphasic decline pattern in a mixture of three Florida soils incubated aerobically at 35 C. The initial half-life was ~2.8 days, and the second and third half-lives were 4.5-5 days, as determined with a cylinder-plate bioassay analytical technique. Ninety percent dissipated by the 13th day.

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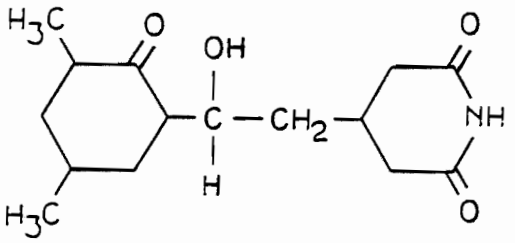
MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

Soils from three localities in Florida were air dried and sifted through an 8-mesh screen (2.38 mm). The soils were designated as Delray Beach, Wintergarden, and Lake Alfred, and their characteristics were not specified. The respective soils were blended together in the approximate proportions of 5:3:2. An aqueous solution of cycloheximide (purity not specified, Upjohn Co.) was added to yield a cycloheximide concentration of 0.2 ppm, a soil moisture content of 7.7%, and a pH of 6.8. Aliquots (100 g) were placed in stoppered brown glass 4 oz. bottles fitted with glass tubes. Twelve bottles were analyzed immediately, and the remainder were incubated in a water bath at 35 C. Each sample was purged daily with four volumes of air. Bottles were taken daily for analysis until ~90% of the applied cycloheximide dissipated (13 days). One sample was taken on day 1, two on day 2, and three to six samples were taken daily thereafter. The samples were extracted with chloroform and analyzed by a cylinder plate bioassay that is accurate at levels of 0.015-0.020 ppm, has a range in random error of ±0.01 ppm, and has a range in recovery levels of 99-106.5% for samples fortified at 0.02-1 ppm. This assay is described in Study 38 (00012869, 00011224, 00011225, and 00011195).

REPORTED RESULTS:

The data were subjected to a computer analysis, and the best fit for a curve was observed when the results were analyzed by two regressions, the first on the data from the 0-3 day interval, and the second on the 3-13 day interval. It was postulated that at least two independent mechanisms for degradation exist. The initial half-life was ~2.8 days, and the second and third half-lives were ~4.5 and ~4.8 days, respectively. Ninety percent of the applied cycloheximide had dissipated by the 13th day.

DISCUSSION:

The incubation temperature was relatively high. It is possible that dissipation would be slower at lower temperatures that would better reflect usual environmental temperatures. Metabolites were not isolated and no attempt was made to define degradation mechanisms.

CASE GS0038 CYCLOHEXIMIDE STUDY 5 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 30 TOPIC 050520

FORMULATION 00 = ACTIVE INGREDIENT

FICHE/MASTER ID 00012845 CONTENT CAT 01

Petzold, E.N.; Chapman, D.D. (1971) Fate of Cycloheximide When Incorporated into Sterile vs. Non-Sterile Soil: Report No. 120-9760-51. (Unpublished study received Mar 22, 1972 under 2F1252; submitted by Upjohn Co., Kalamazoo, Mich.; CDL:095124-F)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
SEC: EFB -30-05052010

DIRECT RVW TIME = 7 (MH) START-DATE END DATE

REVIEWED BY: R. Hebert
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ORG:
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SIGNATURE: DATE:

CONCLUSIONS:

Metabolism - Aerobic Soil

1. This portion of the study is scientifically valid.

2. Cycloheximide had a half-life of ~1.5 days in a soil mixture (three Florida soils) treated with randomly labeled [^{14}C]cycloheximide at 0.2 ppm and incubated aerobically at 35 C. Extractable ^{14}C had a half-life of 2.25 days. More than 80% of the applied ^{14}C dissipated in the form of volatile compounds after 9 days of incubation, and <15% was unextractable.

29

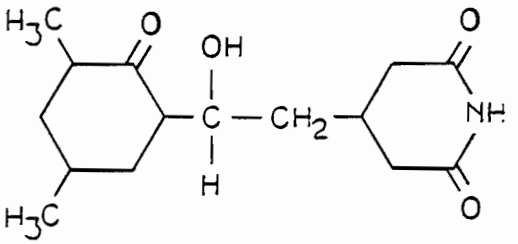
Microbiological - Effects of Microbes on Pesticides

1. This portion of the study is scientifically valid.

2. Microorganisms contributed to cycloheximide degradation in soil, as demonstrated by much faster degradation in nonsterile soil than in presterilized soil. Quantitative estimates of the microbial impact could not be derived because samples became contaminated.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

The general protocols of this experiment were identical to those of Study 4 (MRID 00011196), but with the following exceptions. The degradation of cycloheximide (Upjohn Co., unspecified purity) in presterilized soil samples (autoclaved overnight at 15 psi) was also examined. Randomly labeled [^{14}C]cycloheximide was added to the soil at 0.2 ppm. Samples were subjected to a preliminary water extraction and the extract was analyzed for ^{14}C by liquid scintillation counting (LSC). The water was then extracted with chloroform and the residue was partitioned between hexane and water (25:5). The water was assayed for cycloheximide by bioassay as described and discussed in review of Study 4 and for ^{14}C by LSC. All data were subjected to computerized linear regression analyses.

REPORTED RESULTS:

The half-life values for ^{14}C and cycloheximide are presented in Table 1. Less degradation of cycloheximide occurred in presterilized samples than in nonsterile samples, although the presterilized samples became contaminated during the experiment. The amounts of extractable and nonextractable ^{14}C are shown in Table 2.

DISCUSSION:

1. The differences in rates of degradation between nonsterile and presterilized samples demonstrate that microorganisms play a major role in the degradation of cycloheximide in soil. However, the samples were contaminated so that quantitation of the microbial impact is impossible.

2. The difference in the results obtained by LSC and a bioassay of the second water extract demonstrate that the bioassay detected only biologically active compounds (probably only cycloheximide), whereas the LSC assayed all compounds containing ^{14}C.

3. The data in Table 2 show that unextractable residues do not accumulate. Therefore, the ^{14}C that dissipated must have been in the form of volatile compounds, which by day 9 represented >80% of the nominal concentrations of cycloheximide.

4. The incubation temperature was 35 C. It is possible that dissipation would be slower at lower temperatures, which would better reflect usual environmental temperatures.

Table 1. Half-life of extractable ^{14}C and cycloheximide in a mixture of three soils treated with [^{14}C]cycloheximide at 0.2 ppm.[a]

Test type	Half-life (days)	
	Presterilized soil	Nonsterile soil
^{14}C in preliminary water extract	16.8	2.25
^{14}C in water used for bioassay[b]	14.0	2.12
Bioassay for cycloheximide	9.0	1.49

[a]Soils and incubation conditions are cescribed in Study 4 (00011196).

[b]Water after hexane partitioning of a chloroform extract.

Table 2. Distribution of ^{14}C between extractable and nonextractable fractions of a soil mixture treated with [^{14}C]cycloheximide at 200 ppb.[a]

Incubation time (days)	^{14}C expressed as ppb cycloheximide			
	1st water extract	2nd water extract	Nonextractable	Total
1	111	34	28	173
2	84	22	31	134
3	62	13	16	91
4	57	15	45	117
5	24	7	49	80
6	27	8	37	72
7	17	4	12	33
8	6	15	27	48
8	4	13	27	44
9	6	5	28	39
10	7	3	28	38

[a]Soils and incubation conditions described in Study 4 (00011196).

CASE GS0038 CYCLOHEXIMIDE STUDY 6 PM 12/08/80

CHEM. 043401 Cycloheximide

BRANCH EFB DISC 20 TOPIC 1015 GUIDELINE 40 CFR 163.62-8f3

FORMULATION 00 = ACTIVE INGREDIENT

FICHE/MASTER ID 05014316 CONTENT CAT 01

Di Menna, M.E. (1962) The antibiotic relationships of some yeasts from soil and leaves. Journal of General Microbiology 27:249-257.

SUBST. CLASS = S.

DIRECT RVW TIME = 5 (MH) START-DATE END DATE

REVIEWED BY: R. Hebert
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SIGNATURE: Richard L Hebert DATE: Apr. 23, 1981

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SIGNATURE: DATE:

CONCLUSIONS:

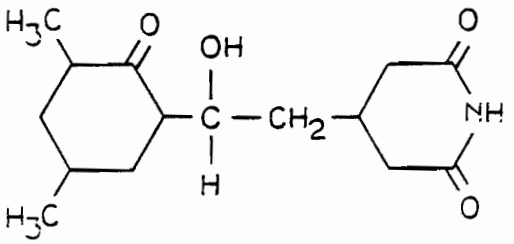
Microbiological - Effects of Pesticides on Microbes

1. This study is scientifically valid.

2. The growth of 11 yeast species from soil, 7 from leaves, and 7 of uncertain habitat was inhibited by cycloheximide at 1,000 mg/disc on agar plates. Quantitative data were not available from this study.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

The activity of cycloheximide was tested in 25 species of yeasts (Table 1) collected from soil and leaves in New Zealand. The yeasts were grown for 2-7 days on soybean agar at 20 C to obtain inocula for the tests. Sterile filter paper discs were soaked in an aqueous solution of purified cycloheximide (Actidione, Upjohn Co., purity unspecified) that was diluted to 1:1,000 and filter sterilized. The discs were placed on seeded test plates of soybean agar. After 3 days of incubation at 20 C, the plates were examined for zones of inhibition.

REPORTED RESULTS:

Results were reported simply as inhibition, weak inhibition, or no inhibition. All yeasts except Candida humicola were inhibited, and C. curvata was weakly inhibited.

DISCUSSION:

The primary purpose of this study was to test several bacterial and fungal isolates for production of antibiotics antagonistic to the yeasts. Some purified antibiotics, including cycloheximide, were also tested. Cycloheximide was in solution at 1,000 ppm. However, no attempt was made either to quantify the amount of antibiotic placed on the discs, or to determine minimum inhibitory concentration.

Table 1. Yeasts used in this study to test for susceptibility to cycloheximide.

Habitat	Organism
Soil	*Trichosporon cutaneum*
	T. pullulans
	Candida muscorum
	C. curvata
	C. humicola
	Cryptococcus albidus
	Cr. terreus
	Cr. diffluens
	Hansenula saturnus
	H. mrakii
	H. californica
Leaves	*Cryptococcus laurentii*
	Sporobolomyces roseus
	Torulopsis ingeniosa
	Rhodotorula glutinis
	R. mucilaginosa
	R. marina
	R. graminis
Uncertain	*Saccharomyces delbrueckii*
	Debaryomyces kloeckeri
	Schizoblastosporion starkeyi-henricii
	Candida tropicalis
	C. guilliermondii
	C. parapsilosis
	Cryptococcus luteolus

CASE GS0038 CYCLOHEXIMIDE STUDY 7 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 20 TOPIC 1015 GUIDELINE 40 CFR 163.62-8f3

FORMULATION 00 = ACTIVE INGREDIENT

FICHE/MASTER ID 05013615 CONTENT CAT 01

Hacskaylo, E. (1961) Influence of cycloheximide on growth of mycorrhizal fungi and on mycorrhizae of pine. Forest Science 7(4):376-379.

SUBST. CLASS = S.

DIRECT RVW TIME = 4 (MH) START-DATE END DATE

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CONCLUSIONS:

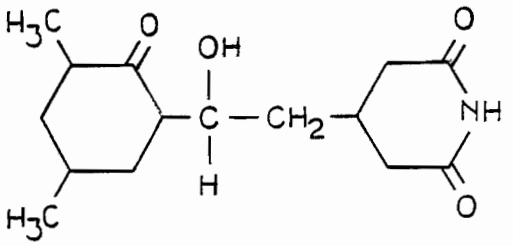
Microbiological - Effects of Pesticides on Microbes

1. This study is scientifically valid.

2. The growth of 9 of 10 strains of mycorrhizal fungi was significantly inhibited on agar containing cycloheximide at 1 ppm. The growth of all but one species was completely inhibited at 100 ppm. Cycloheximide at 0.1 ppm had relatively little effect on growth. The treatment of the bark of white pines with cycloheximide at ≤25 µg per plant had no effect on mycorrhizal development. The data indicate that beneficial mycorrhizal fungi may be eliminated if cycloheximide persists and accumulates in soil.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

Ten strains of mycorrhizal fungi (Table 1) were grown on malt extract-glucose agar plates for 4 weeks at 21 C. The colonies were aseptically cut into 5-mm square pieces that were transferred to fresh plates. After 48 hours, these pieces were transferred to nutrient agar plates containing filter-sterilized cycloheximide (crystalline Actidione, Upjohn Co., purity unspecified) at 0, 0.1, 1, 10, 100, and 1,000 ppm. Three replicates of each species were inoculated onto agar containing cycloheximide at each concentration. The diameters of the colonies were measured after 4 weeks of incubation at 21 C.

In a separate experiment, 2-year-old white pine trees were planted in 7-inch pots containing sterilized soil that subsequently was inoculated with leaf mold from a pine woods. The trees were grown in a greenhouse using 18-hour photoperiods. After 6 months, the trees were well established and ectotrophic mycorrhizae were well developed on actively growing roots. Fuel oil (No. 1) solutions of cycloheximide were applied to the basal portion of the stems between the lowest needles and the soil. Cycloheximide was applied to the stems at 25, 12.5, or 6.25 µg per plant. Five trees received the compound at each dose, five received fuel oil alone, and five were untreated. The solutions were not permitted to touch the needles or the soil. After 90 days, the trees were examined for mycorrhizal development.

REPORTED RESULTS:

The average colony diameters of the fungi grown on media with and without cycloheximide are shown in Table 1. All of the fungi, except Cenococcum graniforme, were completely inhibited by cycloheximide at 10 or 100 ppm. C. graniforme was completely inhibited at 1,000 ppm. Very little inhibition occurred at 0.1 ppm, but most species were markedly inhibited at 1 ppm. The growth of Amanita caesaria and isolate 2 of Amanita rubescens was significantly stimulated by cycloheximide at 0.1 ppm.

No differences were noted on any part of the trees, and mycorrhizae were well developed on all trees.

DISCUSSION:

1. The species tested were all basidiomycetes except C. graniforme, which cannot yet be classified because sporulating structures have not been observed in this fungus. This may be a reason why C. graniforme was more resistant to cycloheximide than the other species tested.

2. The results of the white pine experiments indicate that cycloheximide is not translocated in quantities sufficient to inhibit mycorrhizal development.

Table 1. Average colony diameters of mycorrhizal fungi grown on nutrient agar with and without cycloheximide.

Species	Colony diameter (mm) on agar containing cycloheximide at various concentrations (ppm)					
	0	0.1	1	10	100	1,000
Cenococcum graniforme	29	27[a]	27[a]	26[a]	11[a]	5[a]
Boletus felleus	68	69	69	45[a]	5[a]	5[a]
B. rubellus	37	40	23[a]	5[a]	5[a]	5[a]
Russula emetica	38	34[a]	21[a]	5[a]	5[a]	5[a]
Amanita muscaria	34	32	27[a]	5[a]	5[a]	5[a]
A. rubescens Fr. 2	51	60[a]	45[a]	5[a]	5[a]	5[a]
A. rubescens Fr. 1	39	34[a]	31[a]	23[a]	5[a]	5[a]
A. caesaria	54	57[a]	34[a]	17[a]	5[a]	5[a]
A. flavorubescens	17	18	13[a]	10[a]	5[a]	5[a]
A. verna	56	56	37[a]	15[a]	5[a]	5[a]

[a]For comparisons within species; significant differences from the control at the 1% level (initial diameter was 5 mm).

CASE GS0038 CYCLOHEXIMIDE STUDY 8 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 29 TOPIC 1099 GUIDELINE 40 CFR 163.62-8f3

FORMULATION 00 = ACTIVE INGREDIENT

FICHE/MASTER ID 05016063 CONTENT CAT 01

Partridge, A.D. (1966) Some effects of cycloheximide on selected forest fungi. Plant Disease Reporter 50(7):497-499.

SUBST. CLASS = S.

DIRECT RVW TIME = 6 (MH) START-DATE END DATE

REVIEWED BY: R. Hebert
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CONCLUSIONS:

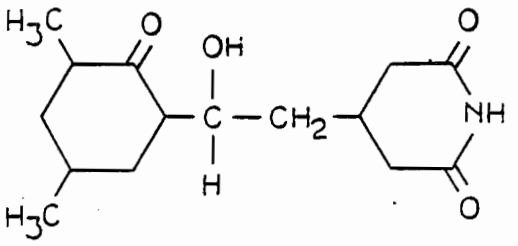
Microbiological - Effects of Pesticides on Microbes

1. This study is scientifically valid except for the wood experiments, which could not be properly evaluated due to the omission of the methods used to quantitate fungal growth.

2. Cycloheximide, at 50 ppm, completely inhibited the growth of 11 forest fungi in culture. Ten of the species were completely, or nearly completely, inhibited by cycloheximide at 10 ppm. Moderate to severe inhibition of all species occurred at 1 ppm, and half were markedly inhibited at 0.1 ppm.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-(2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-)glutarimide

Eleven forest fungi (Table 1) were tested for their susceptibility to cycloheximide (Acti-dione crystalline, 85-100% purity, Upjohn Co.). The fungi were grown on Hagem's or malt agar (for boletes and nonboletes, respectively) at room temperature. Five test methods were examined. First, mycelia and spores on slants were washed with solutions of cycloheximide at 0.1-1,000 ppm and the suspensions were streaked onto fresh slants. Second, the first procedure was performed but the spores and mycelia were washed with sterile distilled water prior to inoculation of the slants. Third, the first and second procedures were repeated except that a 1-hour exposure period was used. Fourth, the fungi were transferred to agar media with cycloheximide at 0.1-1,000 ppm. Growth measurements were made after 6 weeks. After 12 weeks, fungi that grew at a particular concentration were transferred to media with a higher concentration. Media were prepared by adding filter sterilized cycloheximide to molten agar cooled to 50 C. Fifth, all nonboletes were cultured on malt agar for 6 weeks, and oven-dried blocks of grand-fir wood saturated with cycloheximide solutions of varying concentrations were placed on sterile woven glass mats on top of the colonies. Growth measurements were made after 6-12 weeks, depending on the growth rates observed.

Each test method was repeated three times with 10 cultures of each fungus. Controls were included as well.

REPORTED RESULTS:

Little or no growth of any of the fungi resulted when mycelia and/or spores were suspended momentarily or for 1 hour in cycloheximide solutions and then streaked onto agar. This was also true for suspensions washed with distilled water after cycloheximide exposure. The growth of the fungi on media containing cycloheximide is shown in Table 1. Most of the fungi were markedly inhibited by cycloheximide at 1 ppm. All, except Armillaria mellea, were completely or nearly completely inhibited on media containing cycloheximide at 10 ppm. None grew at 50 ppm or more. The table shows that six fungi that barely grew at a particular concentration grew much better when, after 12 weeks, they were transferred

to media with an increased cycloheximide concentration. These six fungi all grew on wood soaked in a solution containing cycloheximide at 10 ppm. Severe inhibition occurred at 100 ppm. Generally, there was better growth after prolonged exposure (1 year), indicating that cycloheximide was degraded with time.

DISCUSSION:

The methods used for measuring fungal growth on wood were not specified. Therefore, the wood experiments are considered invalid.

Table 1. Relative growth of forest fungi incubated for 6 weeks on agar with cycloheximide.

Fungus	Relative growth at various cycloheximide concentrations (ppm)[a]							
	0.1	1	2	5	10	20	30	50
Armillaria mellea	95	81	69	--[b]	46	(39)[c]	(86)	0
Boletinus ochraceoroseus	85	75	42	0	0	0	0	0
Boletus elegans	33	10	0	0	0	0	0	0
B. granulatus	70	18	0	0	0	0	0	0
B. subluteus	58	35	18	10	5	(8)	0	0
Echinodontium tinctorium	59	22	22	4	(29)	0	0	0
Fomes annosus	100	63	--[b]	--[b]	1	(65)	(72)	0
F. pini	66	16	33	9	(9)	(46)	0	0
Polyporus schweinitzii	100	43	25	10	(84)	(43)	0	0
Poria weirii	100	78	--[b]	--[b]	1	(100)	(78)	0
Suillus tomentosus	1	0	0	0	0	0	0	0

[a]Growth is expressed as percent of growth on cycloheximide-free agar.

[b]No data were reported.

[c]Results in parentheses represent growth after previous growth for 12 weeks at the next lowest concentration.

CASE GS0038 CYCLOHEXIMIDE STUDY 9 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 20 TOPIC 1015 GUIDELINE 40 CFR 163.62-8f3

FORMULATION 00 = ACTIVE INGREDIENT

FICHE/MASTER ID 05013346 CONTENT CAT 01

Vaartaja, O.; Agnihotri, V.P. (1969) Interaction of nutrients and four antifungal antibiotics in their effects on "Pythium" species in vitro and in soil. Plant and Soil XXX(1):49-61.

SUBST. CLASS = S.

DIRECT RVW TIME = 6 (MH) START-DATE END DATE

REVIEWED BY: R. Hebert
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CONCLUSIONS:

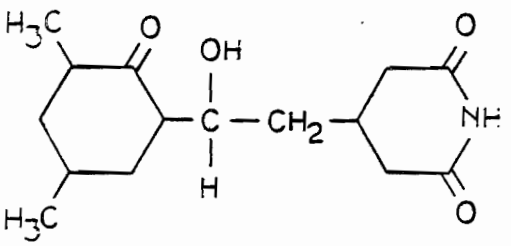
Microbiological - Effects of Pesticides on Microbes

1. This study is scientifically valid.

2. Cycloheximide inhibits the growth, sporulation, and sporangial germination of Pythium species. The inhibition of germination is more pronounced in soil than in culture. The inhibition of growth of four Pythium strains at 20 C ranged from 10 to 70% and from 40 to 80% on water agar supplemented with cycloheximide at 1 and 3 ppm, respectively.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

The four fungi used were two strains of Pythium ultimum, P. irregulare, and P. rostratum. All grow saprophytically, but the first two species are also virulent plant pathogens. The fungi were first grown in water agar, and then transferred to water agar with and without cycloheximide (unspecified source and purity) at 1 and 3 ppm. The cycloheximide was added aseptically to molten agar cooled to 65 C. The plates were incubated at 20 C for 72 hours, at which time colony diameters were measured. The presence of sporangia was recorded after 10 days. The effects of nutrients on the activity of cycloheximide were examined by using water agar containing cycloheximide at 1, 3, or 5 ppm and yeast extract, sucrose, or asparagine at 500 ppm.

The effects of cycloheximide on sporangial germination were examined with P. irregulare, which produces abundant large sporangia that are easily detected. The fungus was first grown on cornmeal agar to induce abundant sporangia. The surface mycelia were stripped off the agar, blended, and filtered (5 μm). The residue, which consisted mostly of sporangia and empty hyphal fragments, was washed and suspended in sterile water. The suspension contained ∿3.6 x 10^4 sporangia/ml, and was combined with a cycloheximide-nutrient solution (cycloheximide at 1, 3, or 5 ppm and yeast extract, sucrose, or asparigine at 500 ppm). A control solution containing only yeast extract, sucrose, or asparagine was also used. The solution was incubated at 20 C for 16 hours, at which time germination was observed microscopically on stained smears. Three replicate counts were made (70 sporangia/count). Germination was examined in similar suspensions added to soil that had been repeatedly watered and aerated for 4 months.

REPORTED RESULTS:

Colony diameters of P. rostratum were reduced approximately 50 and 75% by cycloheximide at 1 and 3 ppm, respectively. Growth of P. ultimum strain I was reduced approximately 70 and 80% at the respective concentrations. P. irregulare and P. ultimum strain II were inhibited by about 10-20% and 40-50% at the respective concentrations. Sporulation was inhibited by cycloheximide at 1-5 ppm. The addition of nutrients to the media did not prevent the inhibitory action of cycloheximide, except that sporulation was not inhibited by cycloheximide at 1 ppm when yeast extract was added.

Cycloheximide inhibited the germination of sporangia in soil to a much greater extent than in solutions (Table 1). The addition of nutrients to the antibiotic solutions greatly reduced the effects of cycloheximide, but the nutrients had little if any beneficial effects in the cycloheximide-spiked soil. Cycloheximide induced multiple abnormalities in the morphology of germinating sporangia.

DISCUSSION:

The data demonstrate that the toxic effects of cycloheximide on spore germination of Pythium species are greatly enhanced in soil versus in culture. This could be due to a combination of factors, including the presence of additional toxic antibiotics in soil as well as a deficiency in factors required for germination.

Table 1. Effect of cycloheximide and cycloheximide-nutrient solutions on germination of sporangia of Pythium irregulare in vitro and in soil.[a]

Cycloheximide concentration (ppm)	Germination in solution and in soil (%)[b]			
	Without nutrient	With yeast extract	With sucrose	With asparagine
In solution				
1	62 a	95 a	91 a	92 a
3	39 b	86 a	91 a	86 a
5	16 c	91 a	84 a	79 a
In soil				
1	0	27	15	0
3	0	0	0	0
5	0	0	0	0

[a]Data represent an average of three counts (70 sporangia/count).

[b]Values within each column followed by different letters were significantly different at the 5% level (Duncan's multiple range test).

42

CASE GS0038 CYCLOHEXIMIDE STUDY 10 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 20 TOPIC 1099 GUIDELINE 40 CFR 163.62-8f3

FORMULATION 01 = TECHNICAL CHEMICAL

FICHE/MASTER ID 05012807 CONTENT CAT 01

Martin, J.F.; Nicolas, G. (1970) Physiology of spore germination in "Penicillium notatum" and "Trichoderma lignorum". Transactions of the British Mycological Society 55(1):141-148.

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
SEC: EFB -20-1299

DIRECT RVW TIME = 4 (MH) START-DATE END DATE

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TITLE:
ORG:
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SIGNATURE: DATE:

CONCLUSIONS:

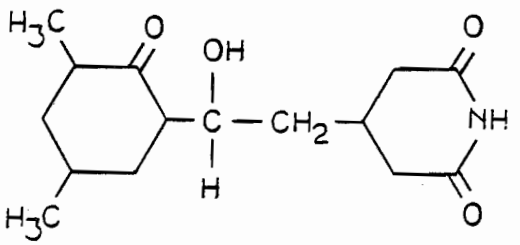
Microbiological - Effects of Pesticides on Microbes

1. This study is scientifically valid.

2. Spore germination in the common soil fungi Penicillium notatum and Trichoderma lignorum was inhibited by 90-100% during 3 days of incubation at 26 C in the presence of cycloheximide at 50 ppm.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

Trichoderma lignorum and Penicillium notatum were obtained from an institutional collection in Spain, and grown on potato-dextrose agar in flasks at 26 C. After 3 weeks, conidia were gently brushed off the surface and suspended in sterile distilled water. They then were filtered through sterile gauze and washed three times, which yielded spores free of mycelia and agar. The spores were added at 7×10^5 conidia/ml to 30 ml of Czapek-Dox broth (pH adjusted to 5.4) containing Tween 80 at 0.01%. Another set of flasks also contained cycloheximide (source and purity unspecified) at 50 ppm. The flasks were incubated at 26 C on a reciprocal shaker. After 12, 14, 16, 20, and 36 hours, a few drops were removed and examined microscopically for spore swelling and subsequent germination. A spore was considered swollen if its diameter was at least 1.5 times its original diameter, and as germinated when the germ tube was at least as long as its diameter after swelling. At least 300 spores were counted in each observation and results were expressed as percentage of swollen or germinated spores. The results represent the averages of at least three experiments.

REPORTED RESULTS:

Cycloheximide had a very noticeable inhibitory effect on spore germination (Table 1). Only 8% of the P. notatum spores had germinated after 36 hours in the presence of cycloheximide, whereas 95% of the control groups had germinated by this time. Similar results occurred with T. lignorum. Cycloheximide had little effect on swelling of P. notatum spores but inhibited swelling of T. lignorum spores by ~70%.

DISCUSSION:

The toxic effects of cycloheximide on eucaryotic cells have been studied in great detail, and cycloheximide is known to inhibit protein synthesis. When fungal spores swell prior to germ tube emergence, many of the macromolecules required for the process already have been synthesized and are waiting for the proper trigger to begin the sequence of normal events. Therefore it is not surprising that spore swelling was not markedly inhibited in P. notatum. Germ tube formation and elongation are known to require the synthesis of new proteins and therefore they are expected to be inhibited by cycloheximide.

Table 1. Effects of cycloheximide at 50 ppm on swelling and germ tube formation in spores of *Trichoderma lignorum* and *Penicillium notatum*.

Fungus	Condition	Swelling (S) and germ tube formation (G) during incubation period (%)									
		12 hours		14 hours		16 hours		20 hours		36 hours	
		S	G	S	G	S	G	S	G	S	G
P. notatum	Control	40	0	50	10	65	20	75	40	100	95
	Cycloheximide	30	0	50	0	60	0	70	0	80	8
T. lignorum	Control	70	0	75	0	80	10	85	20	90	70
	Cycloheximide	0	0	5	0	5	0	10	0	20	0

CASE GS0038 CYCLOHEXIMIDE STUDY 11 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 20 TOPIC 1099 GUIDELINE 40 CFR 163.62-8f3

FORMULATION 00 - ACTIVE INGREDIENT

FICHE/MASTER ID 05021327 CONTENT CAT 01

Ryder, N.S.; Peberdy, J.F. (1979) Chitin synthetase activity and chitin formation in conidia of "Aspergillus nidulans" during germination and the effect of cycloheximide and 5 -fluorouracil, Experimental Mycology 3(3):259-269.

SUBST. CLASS = S.

DIRECT RVW TIME = 6 (MH) START-DATE END DATE

REVIEWED BY: R. Hebert
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CONCLUSIONS:

Microbiological - Effects of Pesticides on Microbes

1. This study is scientifically valid.

2. Cycloheximide in broth at 10-100 ppm temporarily inhibits germination of conidia of Aspergillus nidulans. After 24 hours the inhibitory effect virtually disappears.

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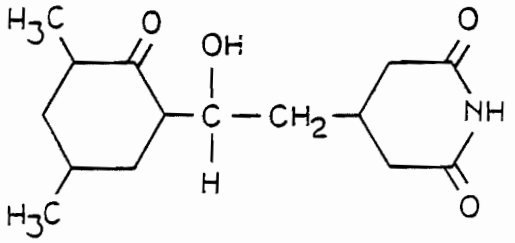
MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

Aspergillus nidulans Eidan, strain BWB 50 (requires biotin), was grown on malt extract agar for 7 days at 37 C. Conidia were harvested and inoculated into a fermenter containing 3 liters of Vogel's medium with glucose and biotin. The fermenter was maintained at 37 C with stirring at 250 rpm and sparged aeration at 200 ml/min. The effect of cycloheximide on germination was examined by adding filter-sterilized cycloheximide (Sigma, unspecified purity) at 10 and 100 mg/l. Samples (500 ml) were removed aseptically at various times and conidia were collected on membrane filters for microscopic examination to assess germination. Spores were considered to have germinated when the length of the germ tube was at least half the spore diameter. Measurements were made with samples containing at least 300 spores. Samples were also collected on glass fiber disks for dry weight determinations.

REPORTED RESULTS:

Germ tubes started to appear after 3 hours in control cultures, with germination frequencies of 19, 67, and 98-100% occuring after 4.5, 6, and 7.5 hours. The dry weight of the conidia increased 10-fold during a 9-hour period. In the presence of cycloheximide at 10 and 100 ppm, germination frequencies after 9 hours were 20 and 0%, respectively. Conidial dry weight increased twofold after 9 hours with cycloheximide at 10 ppm, and there was no increase at 100 ppm. After 24 hours, there was 90% germination at each treatment level.

DISCUSSION:

1. Most of the study was concerned with the effects of cycloheximide on biochemical processes associated with germination; specifically, glucose uptake and the synthesis of protein and cell wall polysaccharides. These aspects were not reviewed because they are not related to the environmental fate of cycloheximide.

2. The temporary nature of the effects of cycloheximide is probably due to the rapid degradation of the compound in solution, as observed elsewhere (Study 26, 00012843; Study 37, 00012880 and 00012864). Cycloheximide also is degraded rapidly in soil (Study 4, 00011196; Study 5, 00012845; Study 25, no MRID; Study 26, 00012843). Therefore, the effect probably would be temporary in the environment.

CASE GS0038 CYCLOHEXIMIDE STUDY 12 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 20 TOPIC 0525 GUIDELINE 40 CFR 163.62-8f2

FORMULATION 00 = ACTIVE INGREDIENT

FICHE/MASTER ID 05013498 CONTENT CAT 01

Tani, M.; Ishida, N.; Furusawa, I. (1977) Effects of temperature and antibiotics on appressorium formation in spores of "Colletotrichum lagenarium*". Canadian Journal of Microbiology 23(5):626-629.

SUBST. CLASS = S.

DIRECT RVW TIME = 4½ (MH) START-DATE END DATE

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CONCLUSIONS:

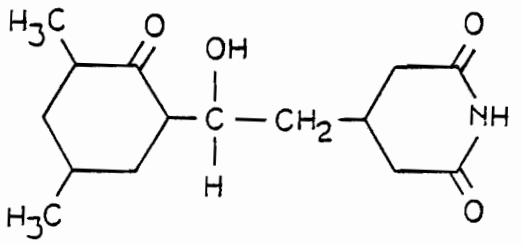
Microbiological - Effects of Pesticides on Microbes

1. This study is scientifically valid.

2. Cycloheximide in solution at 1 ppm inhibits spore germination of the plant pathogen Colletotrichum lagenarium. The data were not sufficient to determine long-term effects. Appressorium formation by pregerminated spores incubated at 32 C, but not at 24 C, was markedly enhanced by cycloheximide at 0.7-1.5 ppm, suggesting that cycloheximide may enhance infectivity at 32 C.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

Colletotrichum lagenarium (Pass.) Ellis and Halsted was grown on potato-sucrose agar at 24 C for 7 days. Conidia were collected aseptically and were washed three times by centrifugation in ice-cold distilled water to increase the germination rate. About 1 x 10^5 spores/ml were suspended in sterile deionized water. Drops were placed on glass slides in humidified Petri dishes and incubated at 24 or 32 C for up to 23 hours. Spores were similarly placed in drops containing cycloheximide (unspecified source and purity) at 0.7-1.5 ppm. For microscopic observation, spores were stained with cotton blue in lactophenol. The number of germinated spores and the percentage with appressoria were recorded. Four hundred spores were examined in each test. Germination was determined by measuring germ tubes with a micrometer.

REPORTED RESULTS:

Complete germination of control spores occurred at either 24 or 32 C, but appressorium formation was inhibited at 32 C. The temperature sensitive stage was found to occur between 4 and 8 hours of incubation, when spores initiated swelling.

Spore germination at 24 and 32 C was inhibited 98 and 100%, respectively, by cycloheximide at 1 ppm. Cycloheximide treatment after 1 hour of incubation at 24 C permitted all spores to germinate, but none germinated at 32 C. Treatment after 4, 6, and 8 hours at 32 C permitted germination of 42, 85, and 100% of the spores, respectively. These values represented the percentage of spores already germinated before addition of cycloheximide.

The formation of appressoria by spores germinated at 24 C was not inhibited by cycloheximide added at 1 ppm at 0 or 1 hour. Cycloheximide helped to overcome the temperature effect observed when spores were incubated for 4 hours at 24 C and shifted to 32 C. About 30% of the germinated spores formed appressoria when cycloheximide was added at 0.7-1.5 ppm at the time of the shift, compared with 4.5% without cycloheximide. It was suggested that suppression of protein synthesis at 32 C shifted the metabolic pathway from that for germ tube growth to that for appressorium formation.

DISCUSSION:

Colletotrichum species and related fungi are well-known plant pathogens. The appressorium is a flattened, hyphal pressing organ from which an infection peg grows and enters the epidermal cell of the host. The results of this study suggest that cycloheximide, a fungicide, may enhance the infectivity of *C. lagenarium* at high temperatures (32 C), although it would be inhibitory at low temperatures (24 C).

CASE GS0038 CYCLOHEXIMIDE STUDY 13 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 20 TOPIC 1099 GUIDELINE 40 CFR 163.62-8f3

FORMULATION 00 = ACTIVE INGREDIENT

FICHE/MASTER ID 05016140 CONTENT CAT 01

Naumova, T.I.; Naumov, G.I. (1974) Indutsirovannaya eliminatsiya tsitogenov ubiystva (k¤1¬) i (k¤2¬) i tsitogena neytral'nosti (n) drozhzhey~Saccharomyces~. ¢Induced elimination of killer cytogenes (k¤1¬) and (k¤2¬) and the neutral cytogene (n) of the~Saccharomyces~yeasts.! Biologicheskie Nauki (Moscow), ¢Biological Sciences.! 17(2):108-110.

SUBST. CLASS = S.

DIRECT RVW TIME = 8½ (MH) START-DATE END DATE

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CONCLUSIONS:

Microbiological - Effects of Pesticides on Microbes

1. This study is scientifically valid.

2. Cycloheximide induces mutations in extrachromosomal (nonnuclear) DNA in Saccharomyces cerevisiae. At 0.66 ppm, cycloheximide induced the elimination of gene(s) responsible for the killer phenotype in S. cerevisiae.

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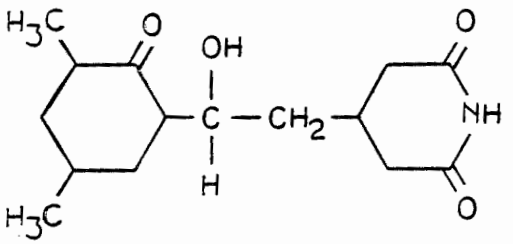
MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

The following strains of yeast were used to examine the capacity of cycloheximide to induce mutations: Saccharomyces cerevisiae M-437 (killer [k_2]), K7 (killer [k_1]), N2 (neutral [n]), and S. globosus SBY-257C (sensitive [o]). The killer strains produce a toxic compound that kills sensitive strains. Based on prior research, the characteristic was presumed to be determined by a cytoplasmic gene rather than a nuclear gene. Killer strains with cytogenes k_1 and k_2 kill each other. Strains with the neutral cytogene (n) are resistant to killer strain k_1, but not to k_2. This study sought to examine if cycloheximide, as well as several mutagens, would induce the elimination of these genes, and thus provide further proof of the cytoplasmic location of the gene.

The cells of killer and neutral strains were grown on a complete medium (unspecified composition) in the presence of cycloheximide (unspecified source and purity) at 0.66 ppm. After an unspecified amount of time cells from the colonies were transferred to dishes containing a layer of either a killer or sensitive strain. Cell death was studied with a medium containing methylene blue, which specifically stains dead cells. Cells were also streaked out to see if they would turn blue, thus indicating the presence of mixed sensitive and killer cells. Respiratory-deficient (ρ^-) mutations were detected by streaking cells on a complete medium in which glucose was replaced by ethyl alcohol at 2%.

REPORTED RESULTS:

Strains K7 and N2 were very sensitive to cycloheximide. Therefore, cycloheximide-induced elimination of cytogenes k_1 and n in these strains could not be studied. Cytogene k_2 was eliminated in 179 of 258 colonies of strain M-437 grown in the presence of cycloheximide. The remaining 74 contained mixed sensitive and killer cells. Four of 74 became ρ^-.

DISCUSSION:

1. It is well established that the toxic effects of cycloheximide on eucaryotic cells are due to inhibition of protein synthesis by cytosol ribosomes (i.e., nonmitochondrial). The mutagens studied here are known to alter mitochondrial DNA. Respiratory deficient mutants induced by acridine dyes arise from the deletion of mitochondrial DNA. Cycloheximide behaved similarly, indicating that mitochondrial DNA replication may have been indirectly affected by cycloheximide.

2. Reportedly, 258 colonies of strain M-437 were tested but results were reported for 253 colonies (179 sensitive and 74 mixed).

CASE GS0038 CYCLOHEXIMIDE STUDY 14 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 20 TOPIC 1015 GUIDELINE 40 CFR 163.62-8f3

FORMULATION 00 = ACTIVE INGREDIENT

FICHE/MASTER ID 05013679 CONTENT CAT 01

Robinson, A.C. (1968) The effect of anti-fungal antibiotics on the nodulation of "Trifolium subterraneum" and the estimation of "Rhizobium trifolii" populations. Australian Journal of Experimental Agriculture and Animal Husbandry 8(32):327-331.

SUBST. CLASS = S.

DIRECT RVW TIME = 5 (MH) START-DATE END DATE

REVIEWED BY: J. Caplan and R. Hebert
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CONCLUSIONS:

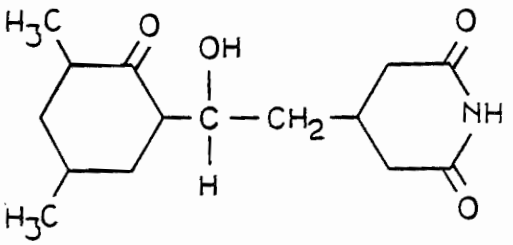
Microbiological - Effects of Pesticides on Microbes

1. This study is scientifically valid.

2. Cycloheximide inhibits and delays nodulation on roots of Trifolium subterraneum (clover) seedlings inoculated with Rhizobium trifolii. Nodules were not formed during 18 days after treatment with cycloheximide at 100 ppm, and only 25% of the plants formed nodules after treatment with cycloheximide at 20 ppm. This indicates that cycloheximide, at those rates, has a severe effect on the symbiotic relationship between clover and the nitrogen-fixing bacterium R. trifolii.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

The effects of cycloheximide (Upjohn Co., purity unspecified) on nodulation and growth of Trifolium subterraneum (subterranean clover) was studied. Cycloheximide at two concentrations (20 and 100 ppm) was aseptically introduced to T. subterraneum seedlings grown in test tubes (Gibson, 1963, Australian J. Biol. Sci. 16:28). Immediately following cycloheximide addition, each tube was inoculated with approximately 1×10^6 cells of Rhizobium trifolii TA1. Twelve replications of each concentration were used. Plants were observed daily for 18 days for nodule formation and were harvested for dry weight determinations after 32 days.

REPORTED RESULTS:

No plants nodulated when treated with cycloheximide at 100 ppm, and only 3 of 12 plants formed nodules 18 days after treatment with cycloheximide at 20 ppm. The mean time to nodulation in the plants treated at 20 ppm was 16.7 days as compared with 9.7 days in controls. The average dry weights of the cycloheximide-treated plants were 5.6 and 9.8 mg/plant for the 100-ppm and 20-ppm treatments, respectively, compared with an average dry weight of 22.8 mg/plant for controls.

DISCUSSION:

Cycloheximide inhibits protein synthesis in eucaryotic cells, but not in procaryotic cells. Therefore the inhibitory effects noted here were probably due to effects on the plants. At 20 ppm, the effect was probably temporary, and if nodulation had been examined for a longer duration the numbers may have approached those of controls. However, at 100 ppm, it is possible that irreversible inhibition occurred but the limited observation time does not allow for an unequivocal conclusion

CASE GS0038 CYCLOHEXIMIDE STUDY 15 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 20 TOPIC 1099 GUIDELINE 40 CFR 163.62-813

FORMULATION 00 = ACTIVE INGREDIENT

FICHE/MASTER ID 05019056 CONTENT CAT 01

Anilkumar, T.B.; Chakravarti, B.P. (1970) Effect of root feeding with antibiotics on rhizosphere microflora of maize seedlings. Biochemie und Physiologie der Pflanzen 161(5):442-446.

SUBST. CLASS = S.

DIRECT RVW TIME = 7 (MH) START-DATE END DATE

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CONCLUSIONS:

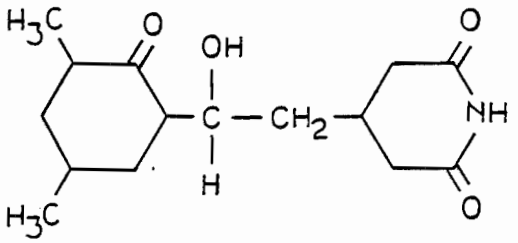
Microbiological - Effects of Pesticides on Microbes

1. This study is scientifically valid.

2. Maize seedlings treated with cycloheximide at 1 ppm had reduced numbers of rhizosphere fungi (50% of control levels), whereas bacterial and actinomycete populations remained comparable to those in controls.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

Hybrid maize seeds were surface sterilized with 1% NaOCl, washed with sterile distilled water, and sown in 6-inch pots containing quartz sand. Seedlings of uniform height were carefully removed from the pots, washed with distilled water, and placed in beakers containing solutions of cycloheximide at 1 or 5 ppm (Actidione, Fluka, Switzerland; purity unspecified). The beakers were kept in a glass chamber for 48 hours. The seedlings then were removed, washed, and transplanted to 6-inch pots filled with soil (characteristics unspecified) from a field where maize was grown in the previous season. Seven days after transplantation, the plants were dug up and the roots were gently tapped to remove loosely adhering soil. The roots then were placed in flasks containing 100 ml of sterile distilled water. The flasks were vigorously shaken and further dilutions were made by pipetting 1-ml aliquots of rhizosphere soil suspension in 9 ml of sterile distilled water. Aliquots (1 ml) of the diluted suspensions were pour plated with Martin's rose bengal agar (fungi), Thornton's standardized medium (bacteria), and water agar (actinomycetes). The plates were incubated at 27 C for 3-7 days.

REPORTED RESULTS:

The effects of cycloheximide on populations of rhizosphere microorganisms are shown in Table 1. Bacterial and actinomycete counts were comparable to those of controls, whereas fungal counts were about 50% those of controls. None of the plants treated with cycloheximide at 5 ppm survived after transplantation.

DISCUSSION:

1. The medium chosen for the isolation of actinomycetes (water agar) has no selective or enrichment agents in it for the propagation or isolation of actinomycetes. Therefore, the counts obtained from this medium would apply to a select group that can grow on this agar.

2. The plants treated at 5 ppm did not grow. This was probably because cycloheximide irreversibly inhibited protein synthesis in the fragile seedlings.

Table 1. Effect of cycloheximide at 1 ppm on rhizosphere microflora of maize seedlings.[a]

Treatment	Rhizosphere microflora[b]		
	Fungi ($x10^3$)	Bacteria ($x10^7$)	Actinomycetes ($x10^6$)
Cycloheximide	33.0	38.9	2.6
Distilled water control	75.3	39.5	2.1

[a]Samples were run in triplicate.

[b]Per gram of oven-dried soil.

(TOR03B) DATA EVALUATION RECORD PAGE 1 OF

CASE GS0038 CYCLOHEXIMIDE STUDY 16 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 20 TOPIC 1015 GUIDELINE 40 CFR 163.62-8f3

FORMULATION 00 = ACTIVE INGREDIENT

FICHE/MASTER ID 05019094 CONTENT CAT 01

Smiley, R.W.; Craven, M.M. (1979) Microflora of turfgrass treated with fungicides. Soil Biology and Biochemistry 11(4):349-353.

SUBST. CLASS = S.

DIRECT RVW TIME = 9½ (MH) START-DATE END DATE

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CONCLUSION:

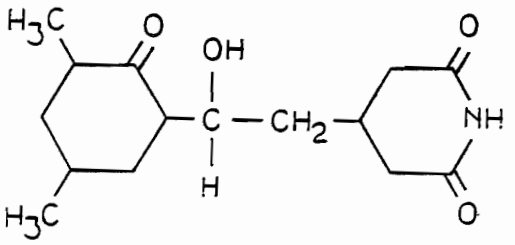
Microbiological - Effects of Pesticides on Microbes

This study is scientifically invalid because the application rate of cycloheximide was not provided, thus making it impossible to interpret the results.

60

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

Two-year-old Kentucky bluegrass (Poa pratensis L.) turf was installed as sod in 1975 at Cornell University. The height of the turf was maintained at 4.4 cm and clippings were removed. Cycloheximide (Actidione TGF, 2.1% ai WP, Upjohn Co.) was applied repeatedly at unspecified rates (nine times annually; every 21 days from April to September) during 1975, 1976, and 1977, to replicated 1- x 5-m plots. In April, July, and September, 1977, five cores (2.54-cm diameter, 3-cm length) from each plot were removed for microbial analysis. Each core sample (including thatch) was homogenized, and an aliquot was diluted in sterile distilled water. Serial dilutions of this suspension were made in 10 mM Tris-HCl buffer (pH 7.2) and aliquots (containing the appropriate dilution) were spread-plated on 0.3% tryptic soy agar (TSA), Pseudomonas isolation agar (PIA), actinomycete isolation agar (AIA), and rose bengal streptomycin agar (RBSA). The plates were incubated at 28 C. Total bacteria and Bacillus spp. spores (germinated by holding dilutions at 80 C for 10 minutes) were counted after 4 and 3 days, respectively, on TSA; actinomycetes, after 6 days on AIA; pseudomonads, after 6 days on PIA; and fungi, after 7 days on RBSA. All counts were converted to a proportion of the untreated control.

On October 9, 1977, prilled $(NH_4)_2SO_4$ was applied to the plots at 1 kg N/ha. Core samples were obtained 2 and 32 days after fertilization. The samples were homogenized and aliquots were suspended in 2 N KCl, shaken, and filtered. Ammonium- and nitrate-N were determined by steam distillation (Bremner, 1965, Agron. Monograph No. 9 Am. Soc. Agron., 1179-1237) by using MgO_2 and Devarda alloy. On June 11, 1978, $(NH_4)_2SO_4$ was applied at the same rate to the plots. Samples were collected 3 days before, and 2, 5, and 9 days after fertilization and analyzed for ammonium- and nitrate-N as previously described.

REPORTED RESULTS:

Cycloheximide had no observable effect on bacteria, actinomycetes, and fungi. The numbers in all three groups did not vary significantly

($p = 0.05$) from the control samples at any time. However, the numbers of Penicillium frequentans were reduced in the cycloheximide-treated plots (data were not shown in the report).

The concentrations of ammonium-N in the cycloheximide-treated turf plots are shown in Table 1. The ammonium-N levels in the cycloheximide-treated samples closely approximated those in the fertilized controls throughout the study. Nitrate-N levels never exceeded 10 µg/g in any of the samples (data were not shown).

DISCUSSION:

1. The cycloheximide application rate was not provided. Without application rate information it is not possible to correlate microbial toxicity with pesticide concentration. Additionally, cycloheximide residues should have been analyzed in the turf samples such that dissipation rates could be calculated as an aid in determining toxicity levels of cycloheximide.

2. No reason was given for providing a statistical comparison of the ammonium-N concentrations in the cycloheximide-treated samples with those in the unfertilized controls (Table 1). A statistical analysis of the ammonium-N levels in the treated samples compared with the fertilized controls would have had more meaning.

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Table 1. Ammonium-nitrogen concentrations in cycloheximide-treated Kentucky bluegrass thatch and soil prior to and following applications of $(NH_4)_2SO_4$ during the summer or autumn.

Treatment	NH_4^+-N concentration (µg/g)				
	Nov. 1977	June 1978			
	+30 days[a]	-3 days	+2 days	+5 days	+9 days
Cycloheximide	11	23[b]	64	15	5
Fertilized control	17	11	80[b]	22[b]	5
Unfertilized control	10	8	14	6	5

[a]Days before or after application (day 0) of $(NH_4)_2SO_4$. Ammonium-N concentrations at +2 days in Nov. 1977 were 1 µg/g in unfertilized plots and 126 µg/g in fertilized controls.

[b]Concentrations differed significantly ($p = 0.05$) from the unfertilized control.

CASE GS0038 CYCLOHEXIMIDE STUDY 17 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 20 TOPIC 1015 GUIDELINE 40 CFR 163,62-8f3

FORMULATION 00 = ACTIVE INGREDIENT

FICHE/MASTER ID 05016745 CONTENT CAT 01

Vancura, V.; Kunc, F. (1977) The effect of streptomycin and Actidione on respiration in the rhizosphere and non-rhizosphere soil. Zentralblatt fuer Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abteilung II 132(5/6):472-478.

SUBST. CLASS = S.

DIRECT RVW TIME = 12 (MH) START-DATE END DATE

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CONCLUSIONS:

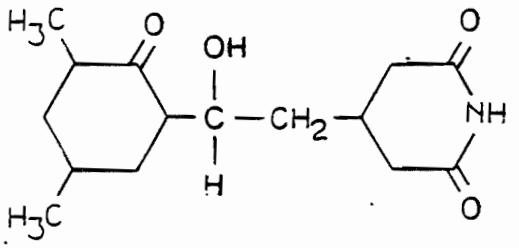
Microbiological - Effects of Pesticides on Microbes

1. This study is scientifically valid.

2. Treatment of rhizosphere (wheat, sugarbeet, or cucumber) or nonrhizosphere soils of two types with cycloheximide at 4,000 ppm resulted in decreased oxygen consumption, relative to that of untreated soils, during a 6-hour incubation period at 30 C. The observed decrease was greater in nonrhizosphere soil than in any rhizosphere soil treated. Unequivocal conclusions with respect to the environmental fate of cycloheximide cannot be made on the basis of these results.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

Wheat, sugarbeet, or cucumber seeds were planted in pots containing 1.5 kg of either a chernozem soil of pH 7.5, or a brown soil (of unspecified characteristics) of pH 6.6. Unseeded soil pots were prepared to serve as sources of nonrhizosphere soil. After adjustment of soil to 60% of moisture holding capacity, all pots were incubated under unspecified conditions for 21 days (wheat) or 35 days (beet and cucumber). At the end of the specified growing period, soil samples were collected from seeded and unseeded pots; rhizosphere soil was removed from roots by vigorous shaking followed by manual extraction of visible root segments.

Collected soils were suspended in distilled water and shaken for 10 minutes. For measurement of O_2 consumption, 2 ml of a soil suspension (containing between 0.4 and 0.5 g soil) was added to 1 ml of water containing 15 mg glucose and 12 mg cycloheximide (Actidione, Upjohn Co., purity not stated). Determinations were made at unspecified intervals during 6 hours of incubation at 30 C.

REPORTED RESULTS:

Results of O_2 consumption studies are shown in Table 1. Oxygen consumption was reduced in all treated soils, compared with that of untreated soils. Cycloheximide inhibited O_2 consumption to a greater extent in nonrhizosphere soil than in rhizosphere soil. Similar experiments were performed with streptomycin, a bacterial inhibitor. In these cases, inhibition was greater in rhizosphere soil than in nonrhizosphere soil. Reductions observed in experiments using both antibiotics applied together were approximately equal to the sum of the reductions caused by individual antibiotics. It was concluded that bacteria play a greater role in O_2 consumption in rhizosphere soil than fungi, and that the opposite situation exists in nonrhizosphere soil.

DISCUSSION:

The procedures used in this study were proper, but the duration of the experiments was too short to allow extrapolation regarding effects of cycloheximide on soil respiration beyond 6 hours after application.

Table 1. Effects of cycloheximide on O_2 consumption by rhizosphere and nonrhizosphere soil suspensions during 6 hours of incubation at 30 C.[a]

Soil	Crop	Treatment	Nonrhizosphere soil[b]		Rhizosphere soil	
			O_2 consumption (µl/g/h)	Reduction (% of control)	O_2 consumption (µl/g/h)	Reduction (% of control)
Chernozem	Wheat	Control	24.2	--	31.3	--
		Cycloheximide	16.7	31.0	29.5	5.8
Brown	Wheat	Control	24.4	--	40.6	--
		Cycloheximide	16.8	31.2	33.3	18.0
Chernozem	Sugarbeet	Control	20.2	--	21.8	--
		Cycloheximide	19.5	23.2	19.5	10.6
Chernozem	Cucumber	Control	20.2	--	21.1	--
		Cycloheximide	19.5	23.2	18.2	13.7

[a]Means are presented from two replications.

[b]Unseeded soil.

CASE GS0038 CYCLOHEXIMIDE STUDY 18 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 20 TOPIC 1005 GUIDELINE 40 CFR 163.62-813

FORMULATION 00 = ACTIVE INGREDIENT

FICHE/MASTER ID 05018934 CONTENT CAT 01

Yopp, J.H.; Albright, G.; Miller, D.M. (1979) Effects of antibiotics and ultraviolet radiation on the halophilic blue-green alga,"Aphanothece halophytica‡". Botanica Marina XXII(5):267-272.

SUBST. CLASS = S.

DIRECT RVW TIME = 4 (MH) START-DATE END DATE

REVIEWED BY: R. Hebert
TITLE: Staff Scientist
ORG: Enviro Control, Inc., Rockville, MD
LOC/TEL: 468-2500

SIGNATURE: Richard L Hebert DATE: June 30, 1981

APPROVED BY:
TITLE:
ORG:
LOC/TEL:

SIGNATURE: DATE:

CONCLUSIONS:

Microbiological - Effects of Pesticides on Microbes

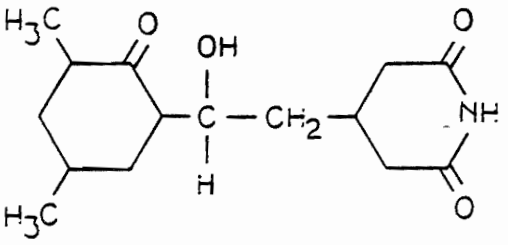
1. This study is scientifically valid.

2. The growth of the halophilic blue-green alga Aphanotheca halophytica was not inhibited by cycloheximide at 0.5-10 ppm. Approximately 10 and 25% growth inhibition was caused by cycloheximide at 50-100 and 500 ppm, respectively.

67

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

Aphanotheca halophytica, a halophilic blue-green alga, was grown in a basal salts medium supplemented with 2 M NaCl. The cultures were incubated on a shaker at 22 C under light. The effects of several antibiotics on growth were determined on log phase cells at an initial concentration of 1.9×10^7 cells/ml. Cycloheximide (unspecified source and purity) was added in a fresh filter-sterilized 2 M NaCl solution to yield concentrations of 0.5-500 ppm. The effects were expressed as a percentage of control levels. The incubation period and method of measuring algal growth were referenced (Yopp et al., 1978, Phycologia 17:172-178).

REPORTED RESULTS:

Cycloheximide at 0.5-10 ppm had no effect on growth of A. halophytica. Growth was inhibited by 11, 12, and 25% at cycloheximide concentrations of 50, 100, and 500 ppm, respectively.

DISCUSSION:

1. Based on the available information, it appears that growth was determined by measuring the optical density (750 nm) of cultures grown photoautotrophically (i.e., without an added carbon source).

2. Cycloheximide inhibits protein synthesis in eucaryotic cells, but not in procaryotes. A. halophytica is a procaryote; therefore, the results of this study are to be expected.

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CASE GS0038 CYCLOHEXIMIDE STUDY 19 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 20 TOPIC 1005 GUIDELINE 40 CFR 163.62-8f3

FORMULATION 00 = ACTIVE INGREDIENT

FICHE/MASTER ID 05021783 CONTENT CAT 01

Kapoor, K.; Sharma, V.K. (1979) Effects of cycloheximide and maleic hydrazide on a nitrogen-fixing cyanophyte. Biochemie und Physiologie der Pflanzen 174(5/6):509-512.

SUBST. CLASS = S.

DIRECT RVW TIME = 7 (MH) START-DATE END DATE

REVIEWED BY: J. Caplan
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SIGNATURE: J. Caplan DATE: May 19, 1981

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CONCLUSIONS:

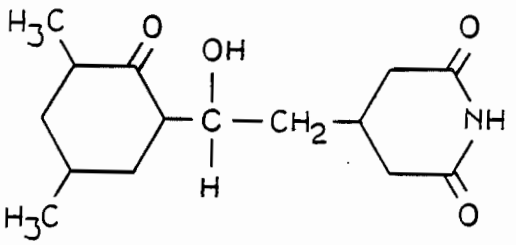
Microbiological - Effects of Pesticides on Microbes

1. This study is scientifically valid.

2. Cycloheximide at concentrations exceeding 350 ppm inhibits the growth of Anabaena doliolum in an elemental nitrogen medium. At 50-150 ppm, growth was stimulated. Nitrogen fixation was inhibited 30 and 70% by cycloheximide at 50 and 100 ppm, respectively.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

A culture of Anabaena doliolum was grown with cycloheximide (source and purity unspecified) at various concentrations on an elemental nitrogen medium (pH 7.5) under light for 15 days at 30 ± 2 C. Following incubation, growth of the culture was determined by measuring optical density, and cellular fixed nitrogen content was analyzed by a semimicro-Kjeldahl method.

REPORTED RESULTS:

Cycloheximide at 50-150 ppm stimulated A. doliolum growth, but A. doliolum growth was comparable with that of the control when cycloheximide was present at 200-350 ppm (Figure 1). Cell growth was inhibited by cycloheximide at 400 ppm. Nitrogen fixation was depressed at all concentrations, with inhibition increasing at higher levels of cycloheximide (Figure 1).

DISCUSSION:

1. Cells treated with cycloheximide at the higher concentrations reportedly stopped growing because they no longer could synthesize protein. But Figure 1 shows that only cycloheximide at 400 ppm reduced A. doliolum growth below control levels.

2. No duplicates were run.

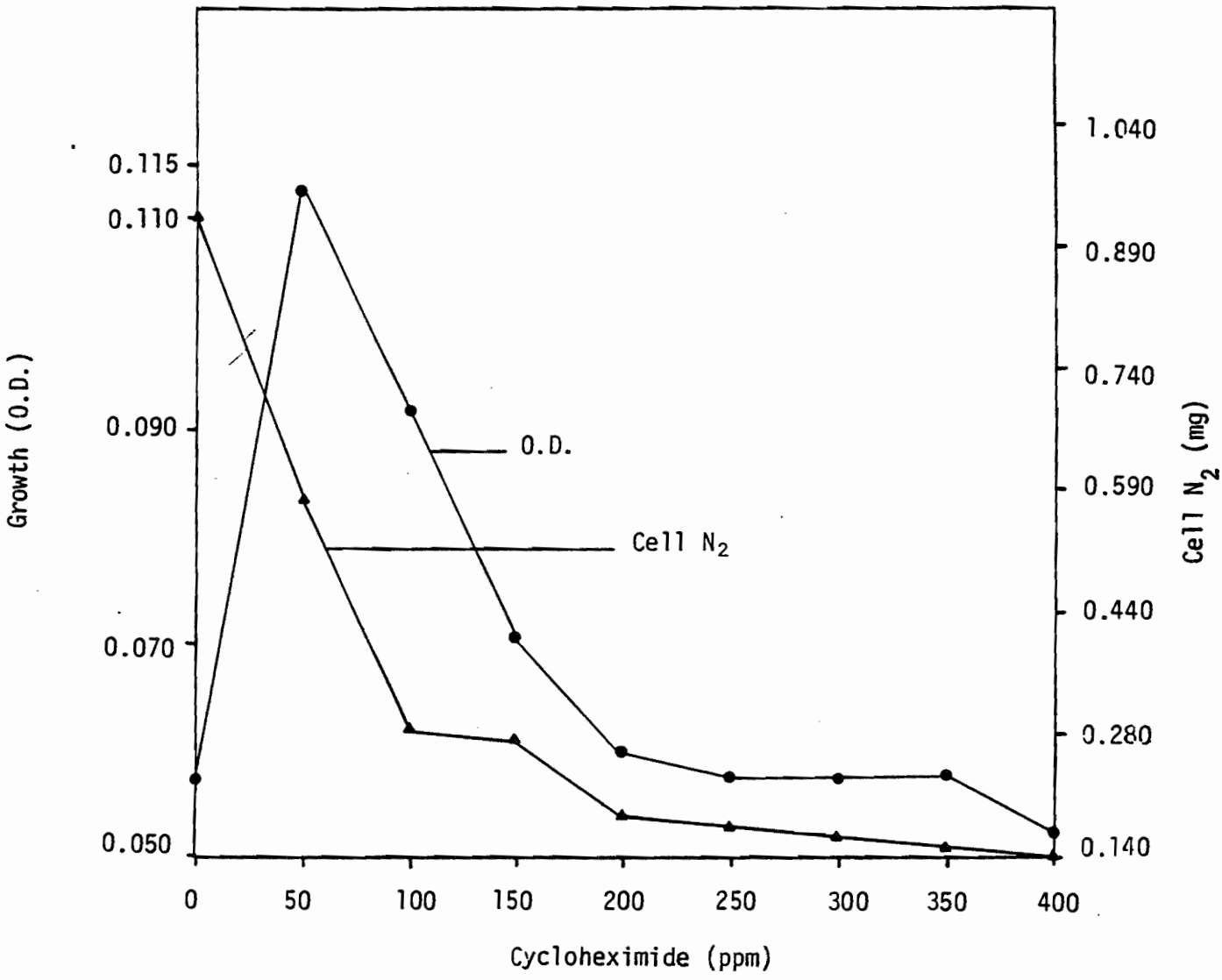


Figure 1. The effect of cycloheximide on growth and cell nitrogen content of Anabaena doliolum.

STUDY 20

CHEMICAL: CYCLOHEXIMIDE, ACTI-DIONE

FORMULATION: 00 - Active Ingredient

FICHE/MASTER ID: 05013934

CITATION: Coursen, B.W., and H.D. Sisler. 1960. Effect of the antibiotic, cycloheximide, on the metabolism and growth of Saccharomyces pastorianus. Am. J. Bot. 47(7):541-549.

DIRECT RVW TIME = 5 (MH) START-DATE END DATE

REVIEWED BY: J. Caplan
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SIGNATURE: J. Caplan DATE: May 28, 1981

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SIGNATURE: DATE:

CONCLUSIONS:

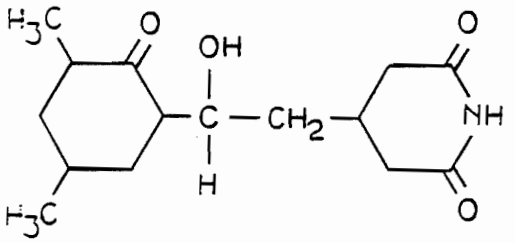
Microbiological - Effects of Pesticides on Microbes

1. This study is scientifically valid.

2. The concentration of cycloheximide required to inhibit the growth of Saccharomyces pastorianus in culture by 50% is 0.018 ppm.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

A pure culture of Saccharomyces pastorianus was grown in a nutrient medium at room temperature for 18-24 hours. The yeast cells were diluted with nutrient medium to an optical density of 1.0 at 450 nm and poured in culture tubes. Cycloheximide, its oxime, and its semicarbazone (Upjohn Co., purity unspecified) were added in a range of concentrations (not provided) to the yeast culture tubes, and the tubes were incubated on a shaker for 16-20 hours. Following incubation at room temperature, the concentrations of cycloheximide and its derivatives necessary to inhibit the growth of S. pastorianus by 50% (ED_{50}) were determined.

REPORTED RESULTS:

The ED_{50} values for cycloheximide, its semicarbazone derivative, and its oxime derivative were 0.018, 0.37, and 12.0 ppm, respectively.

DISCUSSION:

This study dealt primarily with the biochemical effects (i.e., amino acid synthesis) of cycloheximide on S. pastorianus. Only the portion related to the effects on growth were reviewed. This portion of the study was adequately performed.

CASE GS0038 CYCLOHEXIMIDE STUDY 21 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 20 TOPIC 1099 GUIDELINE 40 CFR 163.62-8f3

FORMULATION 00 - ACTIVE INGREDIENT

FICHE/MASTER ID 05017110 CONTENT CAT 01

Mefferd, R.B., Jr.; Loefer, J.B. (1954) Inhibition of respiration in "Tetrahymena pyriformis"S by Actidione. Physiological Zoology 27:115-118.

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
SEC: TOX -45-50

DIRECT RVW TIME = 6 (MH) START-DATE END DATE

REVIEWED BY: J. Caplan
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CONCLUSIONS:

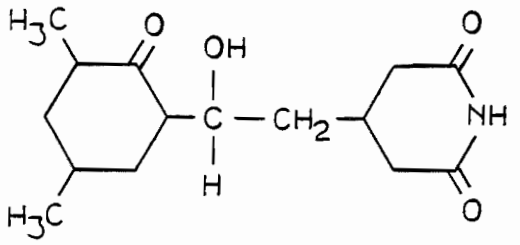
Microbiological - Effects of Pesticides on Microbes

1. This study is scientifically valid.

2. Cycloheximide at 33 ppm inhibits O_2 uptake by Tetrahymena pyriformis.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

Pure cultures of Tetrahymena pyriformis S. were grown at room temperature in 2-liter flasks containing 1 liter of a 2% casitone-salts medium. Ciliates from 48-hour cultures were concentrated by centrifugation and washed with sterile phosphate buffer (pH 6). The cells were adjusted to a constant number as determined by microscopic counts correlated with spectrophotometry. Yeast extract was added at a concentration of 0.05%. Aliquots (1 ml) of this suspension were placed in Warburg vessels and 1 ml of either phosphate buffer alone or phosphate buffer plus 100 µg cycloheximide (Upjohn Co., purity unspecified) were added. The vessels were immediately incubated (29.5 C), shaken, and allowed to equilibrate for 15 minutes before a number of substrates (Table 1) were added (1 ml) to the reaction chambers. Oxygen uptake was measured by standard manometric procedures.

REPORTED RESULTS:

The effect of cycloheximide on substrate utilization by T. pyriformis is shown in Table 1. In the presence of cycloheximide at 33 ppm, respiration of cell substrates was sharply inhibited. The inhibition ranged from 61% for tryptophane to 35% for pyruvate. Endogenous respiration was inhibited by 47%.

DISCUSSION:

Since live protozoa counts were not performed after respiration measurements, it cannot be determined whether substrate utilization was specifically inhibited (on a per cell basis) or cycloheximide was very toxic or lethal to the cells. The latter appears to be true because cycloheximide inhibited endogenous respiration.

Table 1. Influence of cycloheximide on the utilization of certain substrates by T. pyriformis.

Substrate	Percent inhibition of O_2 uptake relative to controls[a]
Tryptophane	61
Glucose	56
Malate	47
Acetate	45
Lactate	44
α-Ketoglutarate	41
Glutamate	41
Fructose	41
Galactose	40
Mannose	40
Maltose	40
Pyruvate	35
Endogenous[b]	47

[a]Results of triplicate determinations using a constant number of cells.

[b]Ribose, rhamnose, sucrose, citrate, succinate, and lysine were not utilized above the endogenous rate.

CASE GS0038 CYCLOHEXIMIDE STUDY 22 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 30 TOPIC 050525 GUIDELINE 40 CFR 163.62-9b/c/d

FORMULATION 00 = ACTIVE INGREDIENT

FICHE/MASTER ID 00011189 CONTENT CAT 01

Staten, F.W.; Wright, W.M.; Thornton, A.M. (1974) Soil Leaching Studies on Cycloheximide: Report No. 120-9760-93. (Unpublished study including letter dated Jan 25, 1974 from F.W. Staten, W.M. Wright and A.M. Thornton to A.W. Neff, received Sep 29, 1977 under 1023-59; submitted by UpJohn Co., Kalamazoo, Mich.; CDL: 097214-I)

SUBST. CLASS = S.

DIRECT RVW TIME = 8 (MH) START-DATE END DATE

REVIEWED BY: D. Harper
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SIGNATURE: David Harper DATE: May 13, 1981

APPROVED BY:
TITLE:
ORG:
LOC/TEL:

SIGNATURE: DATE:

CONCLUSIONS:

Mobility - Leaching

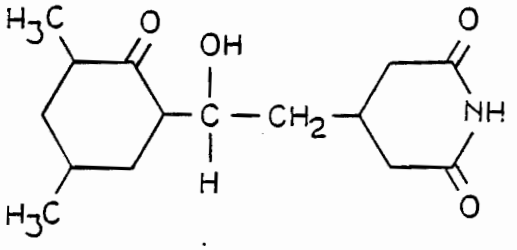
1. This study is scientifically valid.

2. [^{14}C]Cycloheximide is very mobile in sand soil containing 6.8% organic matter. Approximately 96% of the applied radioactivity leached through a 12-inch column of sand soil eluted with the equivalent of 20 acre-inches of water. About 64% of the ^{14}C was in the form of cycloheximide, as determined by a cylinder-plate bioassay analytical technique.

3. The data in this study satisfy part of the requirements in Section 163.163-1 of EPA's Guidelines for Registering Pesticides (1981) by demonstrating the rapid leaching of cycloheximide in a sand soil.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

Sand soil (95.2% sand, 1.4% silt, 3.4% clay, 6.8% organic matter, pH 7.2, and CEC 4.6 meq/100 g), obtained from a Florida orange grove was air dried and sieved to 1 mm. The soil was packed in a glass column (48-mm diameter) to a height of 12 inches and moistened. A 100-g sample of additional soil was placed in a beaker and treated with a methanol solution of [^{14}C]cycloheximide (purified by thin-layer chromatography, Upjohn Co.) to yield a cycloheximide concentration of 11.54 ppm. The treated soil was placed on top of the soil in the column and moistened. The column was eluted with the equivalent of 1 acre-inch of water per hour for 20 hours.

The leachate was mixed with scintillation solution and analyzed by liquid scintillation counting (LSC). The soil was removed from the column, cut into 2-inch segments, and air dried. Soil samples (150-250 mg) were combusted and analyzed by LSC. Subsamples were assayed for cycloheximide as described in Study 38 (00012869, 00011224, 00011225, and 00011195).

REPORTED RESULTS:

Almost all (95.8%) of the original radioactivity was found in the leachate. The cycloheximide assay indicated that 64.5% of the radioactivity leached was cycloheximide. The soil retained 2.7% of the original radioactivity (Table 1). The beaker used for soil treatment was washed with methanol and found to contain 3.23% of the original radioactivity.

DISCUSSION:

1. Acceptable standard procedures were employed, except that the soil was sieved to 1 mm, thus removing the very coarse sand. However, it is doubtful that the removal of the very coarse sand from the soil altered the soil texture.

2. Although it was not specifically stated, there was sufficient information to assume that the assay used to detect cycloheximide was the one used in other Upjohn Co. reports and described in Study 38 (00012869, 00011224, 0011225, and 00011195). The assay is accurate for levels as low as 0.02 ppm, has a range in random error of ±0.01 ppm, and has a range in recovery levels of 99-106.5% for samples fortified at 0.02-1 ppm.

Table 1. Cycloheximide and ^{14}C residues in a column of sand soil treated with [^{14}C]-cycloheximide and eluted with the equivalent of 20 acre-inches of water.

Sample depth (inches)	Cycloheximide (µg)	Radioactivity (% of applied)
0-2	5.9	0.6[a]
2-4	5.6	0.5[a]
4-6	4.8	0.4[a]
6-8	3.6	0.3[a]
8-10	2.7	0.2[a]
10-12	2.1	0.2[a]
12-15	5.7	0.5[a]
Total in soil	30.4	2.7
Eluate	1,070.9	95.8
Total recovered	1,101.3	98.5

[a]Calculated by reviewer.

CASE GS0038 CYCLOHEXIMIDE STUDY 23 DM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 30 TOPIC 45,520

FORMULATION 00 - ACTIVE INGREDIENT

FICHE/MASTER ID 00311119 CONTENT CAT 01

Staten, F.W.; Thornton, A.M.; Wright, W.M. (1974) Soil Leaching Studies on Florida Soil Fortified with 14C Cycloheximide and Aged 30 Days: Report no. 12C-9760-02. (Unpublished study including letter dated Jan 24, 1974 from F.W. Staten, A.M. Thornton and W.M. Wright to A.A. Neff, received Sep 29, 1977 under 1023-50; submitted by Upjohn Co., Kalamazoo, Mich.; CDL: 097214-J)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
SEC: EFB -30-45525

DIRECT RVW TIME = 8 (HR) START-DATE END DATE

REVIEWED BY: D. Harper
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SIGNATURE: Daniel Harper DATE: May 14, 1981

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TITLE:
ORG:
LOC/TEL:

SIGNATURE: DATE:

CONCLUSIONS:

Mobility - Leaching

1. This study is scientifically valid.

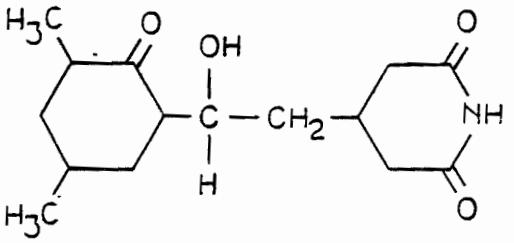
2. Residues of [^{14}C]cycloheximide aged in soil for 30 days are mobile in sand soil eluted with the equivalent of 0.5 acre-inches of water per day for 45 days. Approximately 27% of the radioactivity applied was lost during the aging process. The leachate contained 52% of the applied radioactivity and the soil column contained 15%. The leachate did not contain detectable (<0.015 ppm) cycloheximide as determined by a cylinder-plate bioassay analytical technique.

3. The data in this study satisfy part of the requirements in Section 163.163-1 of EPA's Guidelines for Registering Pesticides (1981) by providing information on the leaching of aged cycloheximide residues in sand soil.

81

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

A sand soil (95.2% sand, 1.4% silt, 3.4% clay, 6.8% organic matter, pH 7.2, and CEC 4.6 meq/100 g), obtained from a Florida orange grove was air dried and sieved to 1 mm. A 100-g subsample of the soil was moistened with methanol and treated with a methanol solution of [^{14}C]cycloheximide (Upjohn Co., purified by thin-layer chromatography) to yield a final concentration of 11.54 ppm. The soil was saturated with water and aged for 30 days. The remaining air-dried soil was packed into a glass column (48 mm in diameter) to a height of 12 inches and moistened. The aged and treated soil was placed on top of the soil column. The column was eluted with the equivalent of 0.5 acre-inches of water per day for 45 days. Leachate samples were collected daily, diluted with distilled water, and extracted three times with ethyl acetate. The ^{14}C in the water and ethyl acetate was determined by liquid scintillation counting (LSC). The leachate samples were also assayed as described in Study 38 (00012869, 00011224, 00011225, and 00011195). The soil was removed from the column with air pressure, cut into 2-inch segments, and air dried. The soil then was mixed with mannitol, combusted, and analyzed by LSC.

REPORTED RESULTS:

The aging of the treated soil resulted in the loss of 26.9% of the applied radioactivity. The leachate contained 70.7% of the radioactivity present in the soil after aging, and 20.8% remained in the soil column (Table 1). The leachate did not contain detectable (0.015 ppm) cycloheximide. About 0.1% of the radioactivity in the leachate was extractable in ethyl acetate.

DISCUSSION:

1. Acceptable standard procedures were employed, except that the soil was sieved to 1 mm, thus removing the very coarse sand. However, it is doubtful that the removal of the very coarse sand from the soil altered the texture.

82

2. Although it was not specifically stated, there was sufficient information to assume that the assay used to detect cycloheximide was the one used in other Upjohn Co. reports and described in Study 38. This assay is accurate for levels as low as 0.01-0.02 ppm, has a range in random error of ±0.01 ppm, and has a range in recovery levels of 99-106.5% for samples fortified at 0.02-1 ppm.

Table 1. Leaching of [^{14}C]cycloheximide aged in sand soil.[a]

Sample depth (inches)	Percent of ^{14}C soil residue levels after aging[b]	Percent of applied ^{14}C[c]
0-2	11.3[c]	8.3
2-4	3.2[c]	2.4
4-6	2.5[c]	1.8
6-8	1.6[c]	1.1
8-10	0.9[c]	0.6
10-12	0.7[c]	0.5
12-14	0.6[c]	0.4
Total in soil	20.8	15.1
Leachate	70.7	51.7
Total	91.5[b]	66.8

[a]Soil column aged for 30 days, and eluted with the equivalent of 0.5 acre-inches of water per day for 45 days.

[b]Soil contained 73.1% of applied ^{14}C after aging.

[c]Calculated by reviewer.

CHEMICAL: CYCLOHEXIMIDE, ACTI-DIONE

FORMULATION: 00 - Active Ingredient

FICHE/MASTER ID: 505001190

CITATION: Helling, C.S., D.G. Dennison, and D.D. Kaufman. 1974. Fungicide movement in soil. Phytopath. 64:1091-1100.

DIRECT RVW TIME = 5 (HH) START-DATE END DATE

REVIEWED BY: D. Harper
TITLE: Staff Scientist
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ORG:
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SIGNATURE: DATE:

CONCLUSIONS:

Mobility - Leaching

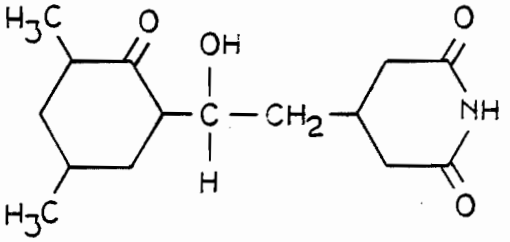
1. This study is scientifically valid.

2. Cycloheximide is mobile to very mobile in soil. The R_f value for cycloheximide in a silty clay loam soil sieved to <250 µm was 0.89, as was determined by examination of thin-layer chromatography plates. ←

3. The data from this study satisfy part of the requirements in Section 163.163-1 of EPA's Guidelines for Registering Pesticides (1981) by providing information on the mobility of cycloheximide in one soil type.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, NIZAROCIN



3-(2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-glutarimide

Samples of Hagerstown silty clay loam soil (39.5% clay, 2.5% organic matter, and pH 6.8) were dried, sieved to <250 µm, moistened, and applied to glass thin-layer chromatography (TLC) plates. The TLC plates were spotted with cycloheximide (Acti-Dione, Upjohn Co., 85-100% purity), developed with water for 2-3 hours, and air dried.

The TLC plates were sprayed with a molten (45 C) Czapek's agar suspension containing mycelia and/or spores of one of 10 soil fungi and an alga. The plates were incubated at 100% relative humidity and 28 C in the dark until zones of inhibition appeared. Data for all test organisms were used to establish the average R_f value for cycloheximide. Fungi used in the study for cycloheximide were Aspergillus fumigatus, Diplodia zeae, Fusarium roseum, Helminthosporium sativum, Pencillium chrysogenum, Rhizoctonia solani, Trichoderma viride, and two isolates of Fusarium moniliforme. The alga used was Chlorella sorokiniana.

REPORTED RESULTS:

The mean R_f value for cycloheximide was 0.89 (0.85-0.92).

DISCUSSION:

The procedure of sieving the soil to <250 µm changed the soil to a heavier textured soil due to the removal of the medium and coarse sand. Therefore, the results cannot be said to apply to a Hagerstown type silty clay loam soil. However, the data are useful in demonstrating the mobility of cycloheximide in soil.

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CHEMICAL: CYCLOHEXIMIDE, ACTI-DIONE

FORMULATION: 00 - Active Ingredient

FICHE/MASTER ID: None

CITATION: Gottlieb, D., P. Siminoff, and M.M. Martin. 1952. The production and role of antibiotics in soil. IV. Actidione and clavacin. Phytopath. 42(9):493-496.

DIRECT RVW TIME = 14 (MH) START-DATE END DATE

REVIEWED BY: D. Harper and R. Hebert
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SIGNATURE: Daniel Harper, Richard L Hebert DATE: May 28, 1981

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TITLE:
ORG:
LOC/TEL:

SIGNATURE: DATE:

CONCLUSIONS:

Metabolism - Aerobic Soil

1. This section of the study is scientifically valid.

2. Cycloheximide rapidly dissipated from a loam and an unidentified soil. At 11 days after treatment, only 8% of the cycloheximide applied to the loam soil remained and cycloheximide levels were below fungitoxic levels in the unidentified soil. Sterile loam and an unidentified soil contained 47 and 30% of the applied cycloheximide, respectively, 11 days after treatment.

Microbiological - Effects of Pesticides on Microbes

1. This section of the study is scientifically valid.

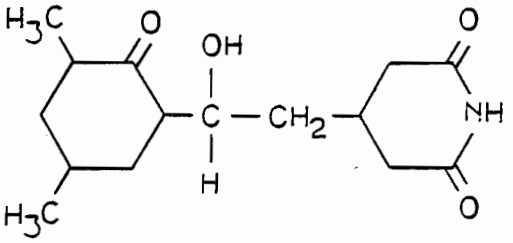
2. Cycloheximide at 5-20 ppm severely (>90%) inhibits the growth of Saccharomyces pastorianus and Pythium debaryanum in soil.

Mobility - Leaching

This section of the study is scientifically invalid because the experimental procedures and data were so poorly presented that no conclusions can be drawn.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

Soil Metabolism

Samples of unidentified sterile and nonsterile soil were treated with cycloheximide (obtained from cultures of Streptomyces griseus) at 10 ppm and maintained at 24 C. A second experiment was conducted with a prairie-loam soil that was maintained at 60% of moisture-holding capacity and 28 C for 7 days. This soil was then treated with cycloheximide and incubated for an unspecified period. Both soils were extracted with warm water. The extracts were tested for cycloheximide by a bioassay using Saccharomyces pastorianus (Whiffen, 1948, J. Bacteriol. 56:283-291). The sensitivity level of the method is 0.1 ppm.

Adsorption

Sterile soil, illite, or bentonite clay was added to an aqueous solution of cycloheximide (10 ppm) and shaken. Cycloheximide was extracted by saturating the soil with water, heating it to 80 C for 10 minutes, and centrifuging. The recovery level was ~90%.

Microbiological

S. pastorianus or Pythium debaryanum was cultured in the presence of extracts of soil, or in soil, treated with cycloheximide at 0-20 ppm by the method of Whiffen referenced above.

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REPORTED RESULTS:

Soil Metabolism

Cycloheximide levels in unidentified nonsterile soil were <0.1 ppm 11 days after treatment. In the unidentified sterile soil, 30% of the cycloheximide remained 11 days after treatment. In the loam soil, 15 and 8% of the cycloheximide remained at 9 and 11 days after treatment, respectively. In the sterile loam soil, 47 and 30% of the cycloheximide remained at the respective time intervals.

Adsorption

Cycloheximide was not readily adsorbed to sterile soil. A small amount (1 µg/mg) of cycloheximide was adsorbed to the illite and bentonite clays but cycloheximide did not flocculate the clays.

Microbiological

The growth of S. pastorianus was inhibited more than 90% in soil treated with cycloheximide at 5-20 ppm. However, growth was not completely inhibited by cycloheximide at 20 ppm. P. debaryanum did not grow in potato dextrose media containing cycloheximide at 2.5 ppm and did not grow in soil treated at 5 ppm.

DISCUSSION:

1. Complete characteristics (percent sand, silt, clay, or organic matter; and pH) were not given for the prairie-loam soil or the unidentified soil used in the study.

2. The amount of cycloheximide that desorbed from the clays reportedly was constant for concentrations ranging from 5 to 2,000 µg/mg. However, the constant value was not specified. It was also stated that a small amount (1,000 ppm) was adsorbed to the clays. However, the treatment rate(s) for which this value was obtained were not given. It cannot be determined if the treatment rate for the adsorption experiments was 5-2,000 µg/mg or some other amount. In addition, the amount of soil and volume of cycloheximide solution used for the adsorption experiments were not reported. Furthermore, 1,000 ppm is not a small amount, as was discussed. Because of these ambiguities, the mobility portion of this study is considered invalid.

3. The protocols used to test the sensitivity of the fungi to cycloheximide in soil were not clearly described. However, the available information indicates these studies were performed in a valid manner.

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CASE GS0038 CYCLOHEXIMIDE STUDY 26 PM 12/08/80

CHEM. 043401 Cycloheximide

BRANCH EFB DISC 30 TOPIC 05053b GUIDELINE 40 CFR 163.62-10b

FORMULATION 12 - EMULSIFIABLE CONCENTRATE (EC OR E)

FICHE/MASTER ID 00012843 CONTENT CAT 01

Petzold, E.N.; Neff, A.W.; Chapman, D.D. (1971) Effect of Repeated Applications of Water upon the Migration and Persistence of Cycloheximide in a Treated Plot of Florida Soil: Report No. 120-9760-52. (Unpublished study received Mar 22, 1972 under 2F1252; submitted by Upjohn Co., Kalamazoo, Mich.; CDL:095124-D)

FICHE/MASTER ID 00012844 CONTENT CAT 01

Petzold, E.N.; Neff, A.W.; Gosline, R.E. (1971) Observation on the Ecology of Microflora in Soil which was Heavily Contaminated with Cycloheximide: Report No. 120-9760-53. (Unpublished study received Mar 22, 1972 under 2F1252; submitted by Upjohn Co., Kalamazoo, Mich.; CDL:095124-E)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
SEC: EFB -30-050530

DIRECT RVW TIME = 16½ (MH) START-DATE END DATE

REVIEWED BY: R. Hebert and J. Caplan
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SIGNATURE: Richard L Hebert, J. Caplan DATE: June 25, 1981

APPROVED BY:
TITLE:
ORG:
LOC/TEL:

SIGNATURE: DATE:

CONCLUSIONS:

Metabolism - Aerobic Soil

1. This portion of the study is scientifically valid.

2. Cycloheximide had a half-life of about 2-3 days (determined by cylinder-plate bioassay analytical techniques) in nonsterile sandy soil treated at 0.13 ppm ai and incubated aerobically in Florida ground during January.

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Metabolism - Aerobic Aquatic

1. This portion of the study is scientifically valid.

2. Cycloheximide was present at 0.13 ppm (determined by cylinder-plate bioassay analytical techniques) after 5 days in nonsterile irrigation ditch water treate at 0.2 ppm ai and incubated aerobically outdoors in Florida during January.

Mobility - Leaching

1. This portion of the study is scientifically valid.

2. The leaching potential of cycloheximide cannot be assessed from this study because the period studied was too short and the amount of water applied was too low. Cycloheximide was not detectable (<0.017 ppm, determined by cylinder-plate bioassay analytical techniques) in the 4-12 inch layer of sandy soil for 5 days after treatment with cycloheximide at <0.19 lb ai/A and irrigation with 2 inches of water. Cycloheximide was also not detectable (<0.012 ppm) at any time in the groundwater (<20 inches from surface) sampled from the center of the treated area.

Field Dissipation - Terrestrial

1. This portion of the study is scientifically valid.

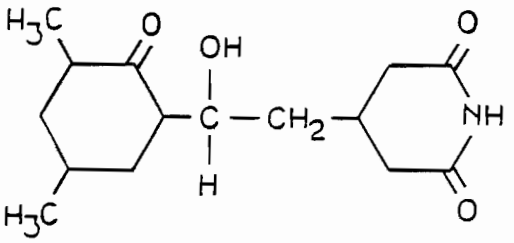
2. Cycloheximide is degraded rapidly in soil under field conditions. Cycloheximide had a half-life of 2 days (determined by cylinder-plate bioassay analytical techniques) in the top 4 inches of sandy soil treated at ~0.19 lb ai/A. Cycloheximide did not move below the 4-inch depth over a 5-da period in which the soil was irrigated from overhead with 2 inches of water.

Microbiological - Effects of Pesticides on Microbes

This portion of the study is scientifically invalid because the methods used to monitor changes in microbial populations within the soil were not adequate.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

Leaching and Dissipation in the Field

A 32-square-foot plot of sandy soil at the Upjohn Florida Substation was tilled and planed in January, 1971. A well drill point (2-inch diameter, 31-inch depth) was driven into the center of the plot. About 17 inches of water rose in the point within 20 minutes, leaving 13-14 inches of soil above the water table. Circles were scribed on the soil surface at radii of 10, 12, 14, and 16 feet from the well point. Numbered sampling points were placed 2 feet apart along most of the circumferences of the circles, except the outer two circles, where only short arcs were used for sampling points. A circular plastic cover (10-foot diameter) was centered over the well point and held down with soil.

The plot was sprayed with cycloheximide (Acti-Aid, 4.23% ai WP, Upjohn Co.) plus surfactant (unspecified) in water at ~0.19 lb ai/A. After spraying, the circular plastic cover was removed from the plot.

An oscillating sprinkler was used to irrigate the plot. The plot was irrigated four times as shown in Table 1.

Drill core water and soil samples were taken at the times shown in Table 1. One-quart water samples were collected, 2 ml of buffer (pH 4.5) was added, and the samples were frozen until analysis. Soil cores were collected, with a galvanized pipe, at depths of 0-4, 4-8, and 8-12 inches at each sampling point. The samples were placed in plastic bags and placed in a freezer within 30 minutes after collection. Samples were assayed for cycloheximide by the assay described in Study 38 (00012869, 00011224, 00011225, and 00011195), which is accurate to levels as low as 0.01-0.02 ppm, has a range in random error of ±0.01 ppm, and has a range in recovery levels of 99-106.5% for samples fortified at 0.02-1 ppm.

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Soil and Aquatic Metabolism

Fifty pounds of soil was taken from the top 4 inches (see above), fortified with cycloheximide at 0.13 ppm, and placed in a 5-gallon plastic container. Four gallons of water, taken from an irrigation ditch near the test site described in the previous section, was fortified with cycloheximide at 0.2 ppm and placed in a 5-gallon plastic container. Both containers were placed in the ground at the sprinkling site described in the previous section, and covered with plastic. Soil and water samples were taken daily for 5 days. The soil samples were frozen immediately, and 2-3 ml of buffer was added to the water, which was then frozen. The samples were assayed as described in the previous section.

Microbiological

One-gram aliquots of the soil samples from the field and the plastic container were analyzed for changes in microbial populations (00012844). An additional set of field samples were taken at 20 days. The samples were diluted to 10^{-1} and 10^{-2} in sterile water and spread-plated onto bacterial growth medium (oxgall-potato dextrose agar; PDA) and fungal growth medium (PDA plus the bacterial inhibitors dichloronitroaniline, streptomycin, and chloramphenicol; PDAI). The PDA plates were incubated at 32 C for 18 hours, and the PDAI plates were incubated at room temperature for 6 days. Following incubation, the plates were photographed for qualitative differences in growth characteristics. This procedure was also repeated with untreated soil extracts fortified with cycloheximide at 2, 4, 16, and 32 ppm.

REPORTED RESULTS:

Cycloheximide was not detectable (<0.012 ppm) in any of the drill core water samples, or in 58 of the 60 samples taken below the 4-inch depth over a 5-day period (limit of detection ranged from <0.014 to <0.017 ppm). The ranges of cycloheximide concentrations in the 0-4 inch depth at each time point are shown in Table 1. The half-life in the 0-4 inch layer was 2.1 days, as calculated from a least squares fit of the data.

The degradation of cycloheximide in the containers of soil and water is shown in Table 2. The respective half-lives were 2-3 days and >5 days.

The density of microflora in both treated and untreated soils decreased as the sampling depth decreased. However, no significant differences were noticed between untreated and treated soils from any source. Similar results were observed in untreated soil fortified before plating.

DISCUSSION:

1. The results of the microbiological studies are subject to misinterpretation. Photographs of culture plates alone cannot provide conclusive taxonomic evidence of microbial population changes. Morphological data must be supported with biochemical tests to identify different microorganisms, and counts must be taken to accurately assess the bacterial and fungal populations dwelling within a soil microcosm.

2. The field study was performed well, but it was not conducted long enough to adequately determine the potential for leaching of cycloheximide and its degradation products. However, the study was designed to measure only cycloheximide, which was degraded rapidly in the soil.

3. With one exception, only one sample at each time was taken from the containers of soil and water. Also, there were only four sampling times. Therefore, the half-life of cycloheximide in each case can only be approximated. In addition, neither the temperature of the soil around the containers nor that of the soil and water in the containers was measured.

Table 1. Irrigation and sampling schedules plus cycloheximide levels in soil of plot treated with cycloheximide at ~0.19 lb ai/A.

Day	Inches of water applied	Time of soil sampling	Cycloheximide (ppm) in the 0-4 inch layer of soil	Inches from soil surface to water in drill core	Time of drill core water sampling
-2	--	Pretreatment	NR[a]	17	Pretreatment
0	--	Posttreatment, prewatering	0.068-0.204[b]		Posttreatment, prewatering
0	0.65	Postwatering- 2 hours	0.072-0.130	11	Postwatering-0, 2, and 24 hours
		24 hours	0.048-0.140	16	
1	0.55	Postwatering- 0 hours	0.044-0.115	4.5	Postwatering-0, 2, 8, and 24 hours
		24 hours	<0.014-0.085	15	
2	0.49	Postwatering- 0 hours	0.042-0.125	2	Postwatering-0, 2, and 8 hours
		8 hours	0.034-0.073		
5	0.49			20	Prewatering
		Postwatering- 2 hours	0.014-0.020	5	Postwatering-0, 2, and 8 hours
		8 hours	<0.017-0.034	14	

[a]Not reported.

[b]Range of 18 samples; all others are range of 6 samples.

Table 2. Cycloheximide dissipation in fortified soil and irrigation water.

Time after treatment (hours)	Cycloheximide (ppm)	
	Soil	Water
Pretreatment	NR[a]	<0.016
0	0.130, 0.147	0.200
24	0.133	0.155
48	0.098	0.139
120	0.036	0.123

[a]Not reported.

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(TDR038) DATA EVALUATION RECORD PAGE 1 OF

CASE GS0038 CYCLOHEXIMIDE STUDY 27 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 30 TOPIC 05

FORMULATION 12 - EMULSIFIABLE CONCENTRATE (EC OR E)

FICHE/MASTER ID 00012842 CONTENT CAT 01

Petzold, E.N.; Chapman, D.D. (1971) Cycloheximide Residues in a Citrus Orchard and Adjoining Soil and Lake Water after Spraying by Helicopter: Report No. 120-9760-50. (Unpublished study received Mar 22, 1972 under 2F1252; submitted by Upjohn Co., Kalamazoo, Mich.; CDL:095124-C)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
PRIM: RCBR-25-10310010
SEC: EFB -30-050530 EFB -30-050520 EFB -30-051025
EFB -30-1005 EFB -30-101015

DIRECT RVW TIME = 4 (MH) START-DATE END DATE

REVIEWED BY: R. Hebert
TITLE: Staff Scientist
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APPROVED BY:
TITLE:
ORG:
LOC/TEL:
SIGNATURE: DATE:

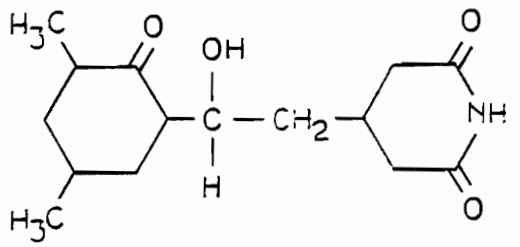
CONCLUSION:

Field Dissipation - Terrestrial

This study is invalid because the soil sampling depth was not provided, and thus the results cannot be interpreted.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-(2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

An orange grove having a leading edge 200 feet from a 10-acre lake in Winter Garden, Florida, was sprayed by helicopter on January 13, 1971, with 1 quart of cycloheximide (Acti-Aid, 4.23% ai WP, Upjohn Co.) plus 1.5 quart of Adsee 775 (an adjuvant) in 15 gallons of water. The treatment rate was 15 gal/A. The 6-acre plot contained an unspecified number of 17-foot trees spaced 20 x 30 feet apart in sandy soil. A trace of rain fell 4 days after treatment and 3 inches fell on the 25th day. Lake water and soil from the area between the orchard and the lake were collected by unspecified methods 0, 5, 12, and 26 days after treatment. Lake water was also sampled prior to treatment. Soil samples were taken at the point of runoff into the lake (0 feet) and at 10, 100, 200, and 300 feet from the lake. This area consisted of sandy soil with occasional weeds and grasses. The lake water samples were taken at the point of runoff. The samples were assayed for cycloheximide by the assay described in Study 38 (00012869, 00011224, 00011225, and 00011195), which is accurate for levels in soil as low as 0.01-0.02 ppm, has a range in random error of ±0.01 ppm, and has a range in recovery levels of 99-106.5% for soil samples fortified at 0.02-1 ppm. Samples were shipped frozen by air from the field to the laboratory, where they were frozen until they were assayed.

REPORTED RESULTS:

Cycloheximide was not detectable at any time in the lake water (concentrated samples, <0.0016 ppm) and in the soil at the lake and 10 feet from the lake (<0.016). The soil sampled 100-300 feet from the lake contained cycloheximide at >0.02 ppm at 0 and 5 days after treatment (Table 1). By the 12th day the compound was undetectable (<0.014 ppm) in all samples but one (100 feet), in which it was present at 0.014 ppm.

DISCUSSION:

The soil sampling depth was not provided, and the sampling procedure was neither described nor referenced. Therefore, no conclusions can be drawn from the results.

Table 1. Cylcoheximide levels in soil between a lake and an orange grove treated at 40 mg ai/A.

Distance from lake (feet)	Days after treatment of orange grove[a]	Cycloheximide concentration (ppm)
0	0	<0.016
100	0	0.025
200	0	0.105
300	0	0.029
10	5	<0.016
100	5	0.028
200	5	0.050
300	5	0.057

[a]The orange grove was sprayed with 1 quart of 4.23% ai WP cycloheximide in 15 gallons of water per acre.

STUDY 28

CHEMICAL: CYCLOHEXIMIDE, ACTI-DIONE

FORMULATION: 06 - Wettable Powder

FICHE/MASTER ID: 00011201

CITATION: Buttram, J.R. 1970. Residue determination for cycloheximide on oranges, leaves and soil (Florida, 1970): Report No. 120-9760-28. (Unpublished study submitted by Upjohn Co., Kalamazoo, MI)

DIRECT RVW TIME = 4 (MH) START-DATE END DATE

REVIEWED BY: R. Hebert
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SIGNATURE: Richard L Hebert DATE: June 22, 1981

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TITLE:
ORG:
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SIGNATURE: DATE:

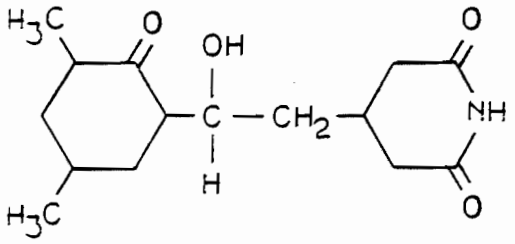
CONCLUSION:

Field Dissipation - Terrestrial

The results of this study are invalid for the following reasons. The samples were stored at room temperature prior to assay, and the available information indicates this may have been a period of several days (cycloheximide is known to be degraded rapidly in soil). Also, the soil sampling depth was not provided, and thus the results cannot be interpreted.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

An orange grove in Polk City, Florida, was sprayed on April 16, 1970, with 1 quart of cycloheximide (Acti-Aid 4.23% ai WP, Upjohn Co.) plus 1.5 quart of Adsee 775 (an adjuvant) in 500 gallons of water. This resulted in a 20 ppm spray applied at 5.96 gal/tree. The grove contained sandy soil and 168 trees planted in 1950. No other pesticides were used and no rain fell between treatment and sampling. At 0, 3, and 5 days after treatment soil was sampled as described in Upjohn Co. Report No. 120-9760-27. The samples were assayed for cycloheximide by the assay described in Study 38 (00012869, 00011224, 00011225, and 00011195), which is accurate for levels as low as 0.02 ppm, has a range in random error of ±0.01 ppm, and has a range in recovery levels of 99-106.5% for samples fortified at 0.02-1 ppm.

REPORTED RESULTS:

Cycloheximide was undetectable (<0.016 ppm) in all samples.

DISCUSSION:

1. The soil sampling depth was not provided; therefore no conclusions can be drawn from the results. The soil sampling procedure was not described, but a reference was cited (Sampling in the Field for Cycloheximide Assay, Upjohn Co. Report No. 120-9760-27, May 19, 1970). The soil sampling depth may be described there, but the reference was unavailable for review.

2. The samples were stored at room temperature prior to assay. This may have involved a period of several days, based on the interval between sampling and the dates on the bioassay data tables. In other reports, cycloheximide was shown to be degraded very rapidly in soil (Study 4, 00011196; Study 5, 00012845; Study 25, no MRID; Study 26, 00012843).

STUDY 29

CHEMICAL: CYCLOHEXIMIDE, ACTI-DIONE

FORMULATION: 06 - Wettable Powder

FICHE/MASTER ID: 00012826

CITATION: Buttram, J.R. 1970. Residue determination for cycloheximide on oranges, leaves and soil (Florida, 1970): Report No. 120-9760-29. (Unpublished study submitted by Upjohn Co., Kalamazoo, MI)

DIRECT RVW TIME = 4 (HH) START-DATE END DATE

REVIEWED BY: R. Hebert
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LOC/TEL: 468-2500

SIGNATURE: Richard L. Hebert DATE: June 22, 1981

APPROVED BY:
TITLE:
ORG:
LOC/TEL:

SIGNATURE: DATE:

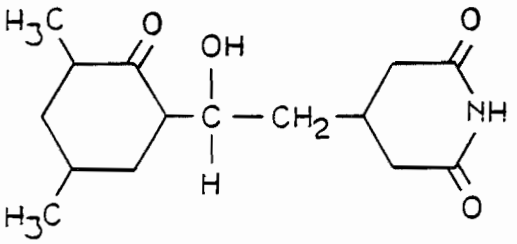
CONCLUSION:

Field Dissipation - Terrestrial

The results of this study are invalid for the following reasons. The samples were stored at room temperature prior to assay, and the available information indicates this may have been a period of several days (cycloheximide is known to be degraded rapidly in soil). Also, the soil sampling depth was not provided, and thus the results cannot be interpreted.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

An orange grove in Dade City, Florida, was sprayed on May 1, 1970, with 1 quart of cycloheximide (Acti-Aid, 4.23% ai WP, Upjohn Co.) plus 1.5 quart Adsee 775 (an adjuvant) in 500 gallons of water. This resulted in a 20 ppm spray applied at 4.8 gal/tree. The grove was about 1 acre in size and contained sandy soil. Liquid lime sulfur (calcium polysulfides) was also applied at an unspecified time. No rain fell between treatment and sampling. At 0 and 6 days after treatment, soil was sampled as described in Upjohn Co. Report No. 120-9760-27. The samples were assayed for cycloheximide by the assay described in Study 38 (00012869, 00011224, 00011225, and 00011195), which is accurate for levels as low as 0.02 ppm, has a range in random error of ±0.01 ppm, and has a range in recovery levels of 99-106.5% for samples fortified at 0.02-1 ppm.

REPORTED RESULTS:

Cycloheximide was present at 0.023 and 0.028 ppm at 0 and 6 days after treatment, respectively.

DISCUSSION:

1. The soil sampling depth was not provided; therefore no conclusions can be drawn from the results. The soil sampling procedure was not described, but a reference was cited (Sampling in the Field for Cycloheximide Assay, Upjohn Co. Report No. 120-9760-27, May 19, 1970). The soil sampling depth may be described there, but the reference was unavailable for review.

2. The samples were stored at room temperature prior to assay. This may have involved a period of several days, based on the interval between sampling and the dates on the bioassay data tables. In other reports, cycloheximide was shown to be degraded very rapidly in soil (Study 4, 00011196; Study 5, 00012845; Study 25, no MRID; Study 26, 00012843).

(TDR03B) DATA EVALUATION RECORD PAGE 1 OF

CASE GS0038 CYCLOHEXIMIDE STUDY 30 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 30 TOPIC 100520

FORMULATION 12 = EMULSIFIABLE CONCENTRATE (EC OR E)

FICHE/MASTER ID 00012846 CONTENT CAT 02

Buttram, J.R. (1970) Residue Determination for Cycloheximide on Oranges, Leaves and Soil (Florida, 1970): Report No. 120-9760-32. (Unpublished study received Mar 22, 1972 under 2F1252; submitted by Upjohn Co., Kalamazoo, Mich.; CDL:095124-Q)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
PRIM: RCBR-25-10310010 EFB -20-259928036

DIRECT RVW TIME = 4 (MH) START-DATE END DATE

REVIEWED BY: R. Hebert
TITLE: Staff Scientist
ORG: Enviro Control, Inc., Rockville, MD
LOC/TEL: 468-2500
SIGNATURE: Richard L Hebert DATE: June 23, 1981

APPROVED BY:
TITLE:
ORG:
LOC/TEL:
SIGNATURE: DATE:

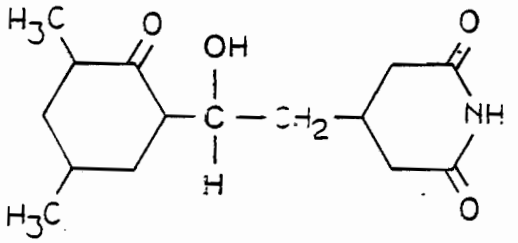
CONCLUSION:

Field Dissipation - Terrestrial

This study is invalid for the following reasons. The soil sampling depth was not provided; thus the results cannot be interpreted. The soil samples were shipped unfrozen by air to the laboratory, where they were frozen until analysis. However, the preshipment condition of the samples was not given, and this is necessary for validation since cycloheximide is known to be degraded rapidly in soil.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HITAROCIN



3-[2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-]glutarimide

An orange grove in Winter Garden, Florida, was sprayed on June 11, 1970, with 1 quart cycloheximide (Acti-Aid, 4.23% ai WP, Upjohn Co.) plus 1.5 quart Adsee 775 (an adjuvant) in 500 gallons of water. This resulted in a 20 ppm spray applied at 16 gal/tree. The plot contained 74 trees (25-30 feet high) spaced 25 feet apart. Copper was also applied to the grove at an unspecified time. No rain fell between treatment and sampling. Soil was sampled prior to treatment and at 0, 5, and 104 days after treatment. Samples were collected as described in Upjohn Co. Report No. 120-9760-27. The samples were assayed for cycloheximide by the assay described in Study 38 (00012869, 00011224, 00011225, and 00011195), which is accurate for levels as low as 0.02 ppm, has a range in random error of ±0.01 ppm, and has a range in recovery levels of 99-106.5% for samples fortified at 0.02-1 ppm.

REPORTED RESULTS:

Cycloheximide was undetectable (<0.012 ppm) prior to sampling and after 104 days (<0.014 ppm). Cycloheximide was present at 0.033 and 0.056 ppm 0 and 5 days after treatment, respectively.

DISCUSSION:

1. The soil sampling depth was not provided. Therefore, no conclusions can be drawn from the results. The soil sampling procedure was not described, but a reference was cited (Sampling in the Field for Cycloheximide Assay, Upjohn Co. Report No. 120-9760-27, May 19, 1970). The soil sampling depth may be described there, but the reference was unavailable for review.

2. The samples were shipped unfrozen by air to the laboratory, where they were frozen until analysis. No information was given on the preshipment condition of the samples, which may have involved a period of several days, based on the interval between samplings and the dates on the bioassay data tables. In other reports, cycloheximide was shown to be degraded very rapidly in soil (Study 4, 00011196; Study 5, 00012845; Study 25, no MRID; Study 26, 00012843).

(TDR038) DATA EVALUATION RECORD PAGE 1 OF

CASE GS0038 CYCLOHEXIMIDE STUDY 31 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 20 TOPIC 259928036

FORMULATION 12 - EMULSIFIABLE CONCENTRATE (EC OR E)

FICHE/MASTER ID 00014438 CONTENT CAT 02

Buttram, J.R. (1970) Residue Determination for Cycloheximide on Oranges, Leaves and Soil (Florida, 1970): Report No. 120-9760-33. (Unpublished study received Mar 22, 1972 under 2F1252; submitted by Upjohn Co., Kalamazoo, Mich.; CDL:095124-X)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
PRIM: RCBR-25-10310010 EFB -30-100520

DIRECT RVW TIME = 4 (MH) START-DATE END DATE

REVIEWED BY: R. Hebert
TITLE: Staff Scientist
ORG: Enviro Control, Inc., Rockville, MD
LOC/TEL: 468-2500

SIGNATURE: Richard L Hebert DATE: June 23, 1981

APPROVED BY:
TITLE:
ORG:
LOC/TEL:

SIGNATURE: DATE:

CONCLUSION:

Field Dissipation - Terrestrial

This study is invalid for the following reasons. The soil sampling depth was not provided; thus the results cannot be interpreted. The soil samples were shipped unfrozen by air to the laboratory, where they were frozen until analysis. However, the preshipment condition of the samples was not given, and this is necessary for validation since cycloheximide is known to be degraded rapidly in soil.

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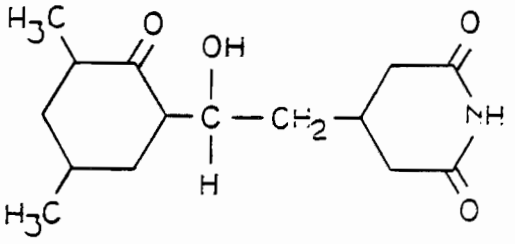
MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

An orange grove in Orlando, Florida, was sprayed on June 25, 1970, with 1 quart cycloheximide (Acti-Aid, 4.23% ai WP, Upjohn Co.) plus 1.5 quart Adsee 775 (an adjuvant) in 37.5 gallons of water. The treatment rate was 37.5 gal/A. The 19.8-acre plot contained 90 trees 18-20 feet high spaced 22 feet apart in sandy soil. Soil was sampled prior to and just after treatment. Samples were collected as described in Upjohn Co. Report No. 120-9760-27. The samples were assayed for cycloheximide by the assay described in Study 38 (00012869, 00011224, 00011225, and 00011195), which is accurate for levels as low as 0.02 ppm, has a range in random error of ±0.01 ppm, and has a range in recovery levels of 99-106.5% for samples fortified at 0.02-1 ppm.

REPORTED RESULTS:

Cycloheximide was undetectable (<0.012 ppm) in all samples.

DISCUSSION:

1. The soil sampling depth was not provided; therefore no conclusions can be drawn from the results. The soil sampling procedure was not described, but a reference was cited (Sampling in the Field for Cycloheximide Assay, Upjohn Co. Report No. 120-9760-27, May 19, 1970). The soil sampling depth may be described there, but the reference was unavailable for review.

2. The samples were shipped unfrozen by air to the laboratory, where they were frozen until analysis. No information was given on the preshipment condition of the samples, which may have involved a period of several days, based on the interval between samplings and the dates on the bioassay data tables. In other reports, cycloheximide was shown to be degraded very rapidly in soil (Study 4, 00011196; Study 5, 00012845; Study 25, no MRID; Study 26, 00012843).

(TDR038) DATA EVALUATION RECORD PAGE 1 OF

CASE GS0038 CYCLOHEXIMIDE STUDY 32 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 30 TOPIC 10052D

FORMULATION 12 - EMULSIFIABLE CONCENTRATE (EC OR E)

FICHE/MASTER ID 00012847 CONTENT CAT 02

Buttram, J.R. (1970) Residue Determination for Cycloheximide on Oranges, Leaves and Soil (Florida, 1970): Report No. 120-9760-34. (Unpublished study received Mar 22, 1972 under 2F1252; submitted by Upjohn Co., Kalamazoo, Mich.; CDL:095124-R)

FICHE/MASTER ID 00012847 CONTENT CAT 02

Buttram, J.R. (1970) Residue Determination for Cycloheximide on Oranges, Leaves and Soil (Florida, 1970): Report No. 120-9760-34. (Unpublished study received Mar 22, 1972 under 2F1252; submitted by Upjohn Co., Kalamazoo, Mich.; CDL:095124-R)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
PRIM: RCBR-25-10310010 EFB -20-259928036

DIRECT RVW TIME = 4 (MH) START-DATE END DATE

REVIEWED BY: R. Hebert
TITLE: Staff Scientist
ORG: Enviro Control, Inc., Rockville, MD
LOC/TEL: 468-2500
SIGNATURE: Richard L Hebert DATE: June 24, 1981

APPROVED BY:
TITLE:
ORG:
LOC/TEL:

SIGNATURE: DATE:

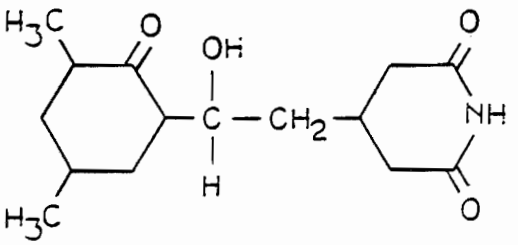
CONCLUSION:

Field Dissipation - Terrestrial

This study is invalid for the following reasons. The soil sampling depth was not provided; thus the results cannot be interpreted. The soil samples were shipped unfrozen by air to the laboratory, where they were frozen until analysis. However, the preshipment condition of the samples was not given and this is necessary for validation since cycloheximide is known to be degraded rapidly in soil.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-[2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide

A portion of an orange grove in Plant City, Florida, was sprayed on June 5, 1970, with 1 quart cycloheximide (Acti-Aid, 4.23% ai WP, Upjohn Co.) plus 1.5 quart Adsee 775 (an adjuvant) in 500 gallons of water (00012847). This resulted in a 20 ppm spray at 780 gal/A. Another portion of the grove was sprayed with the same formulations but in 30 gallons of water (00014439). This resulted in a 333 ppm spray at 35 gal/A. The plot contained 45 trees (70 trees/A) 20-22 feet high spaced 25 feet apart in sandy soil. No rain fell between treatment and sampling. Prior to treatment and 3 days after treatment, soil samples were collected as described in Upjohn Co. Report No. 120-9760-27. The samples were assayed for cycloheximide by the assay described in Study 38 (00012869, 00011224, 00011225, and 00011195), which is accurate for levels as low as 0.02 ppm, has a range in random error of ±0.01 ppm, and has a range in recovery levels of 99-106.5% for samples fortified at 0.02-1 ppm.

REPORTED RESULTS:

Cycloheximide was undetectable (<0.012 ppm) in all samples.

DISCUSSION:

1. The soil sampling depth was not provided; therefore no conclusions can be drawn from the results. The soil sampling procedure was not described, but a reference was cited (Sampling in the Field for Cycloheximide Assay, Upjohn Co. Report No. 120-9760-27, May 19, 1970). The soil sampling depth may be described there, but the reference was unavailable for review.

2. The samples were shipped unfrozen by air to the laboratory, where they were frozen until analysis. No information was given on the preshipment condition of the samples, which may have involved a period of several days, based on the interval between samplings and the dates on the bioassay data tables. In other reports, cycloheximide was shown to be degraded very rapidly in soil (Study 4, 00011196; Study 5, 00012845; Study 25, no MRID; Study 26, 00012843).

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CASE GS0038 CYCLOHEXIMIDE STUDY 33 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 30 TOPIC 100520

FORMULATION 12 - EMULSIFIABLE CONCENTRATE (EC OR E)

FICHE/MASTER ID 00012848 CONTENT CAT 02

Buttram, J.R. (1970) Residue Determination for Cycloheximide on Oranges, Leaves and Soil (Florida, 1970): Report No. 120-9760-35. (Unpublished study received Mar 22, 1972 under 2F1252; submitted by Upjohn Co., Kalamazoo, Mich.; CDL:095124-S)

FICHE/MASTER ID 00012853 CONTENT CAT 2

Buttram, J.R. (1970) Residue Determination for Cycloheximide on Oranges, Leaves and Soil (Florida, 1970): Report No. 120-9760-35. (Unpublished study received Mar 22, 1972 under 2F1252; submitted by Upjohn Co., Kalamazoo, Mich.; CDL:095124-Y)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
PRIM: RCBR-25-10310010 EFB -20-259928036

DIRECT RVW TIME = 4 (MH) START-DATE END DATE

REVIEWED BY: R. Hebert
TITLE: Staff Scientist
ORG: Enviro Control, Inc., Rockville, MD
LOC/TEL: 468-2500

SIGNATURE: Richard L Hebert DATE: June 24, 1981

APPROVED BY:
TITLE:
ORG:
LOC/TEL:

SIGNATURE: DATE:

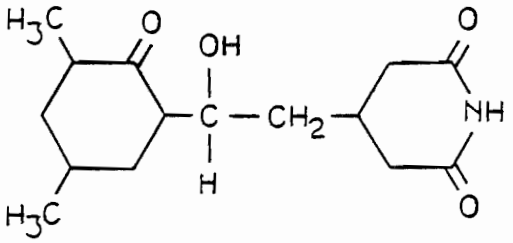
CONCLUSION:

Field Dissipation - Terrestrial

This study is invalid for the following reasons. The soil sampling depth was not provided; thus the results cannot be interpreted. The soil samples were shipped unfrozen by air to the laboratory, where they were frozen until analysis. However, the preshipment condition of the samples was not given, and this is necessary for validation since cycloheximide is known to be degraded rapidly in soil.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-(2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-)glutarimide

A portion of an orange grove was sprayed on May 13, 1970, with 1 quart cycloheximide (Acti-Aid, 4.23% ai WP, Upjohn Co.) plus 1.5 quart Adsee 775 (an adjuvant) in 500 gallons of water (00012848). This resulted in a 20 ppm spray at 6.5 gal/tree. Another portion of the grove was sprayed with the same formulations in 30 gallons of water (00012853). This resulted in a 333 ppm spray at 70 gal/A. The plot contained 55 trees (95 trees/A) 10-12 feet high spaced 15 x 30 feet apart in a sandy soil with some clay. No other pesticides were used and no rain fell between treatment and sampling. Soil was sampled prior to treatment and at 0 and 5 days after treatment. Samples were collected as described in Upjohn Co. Report No. 120-9760-27. The samples were assayed for cycloheximide by the assay described in Study 38 (00012869, 00011224, 00011225, and 00011195), which is accurate for levels as low as 0.02 ppm, has a range in random error of ±0.01 ppm, and has a range in recovery levels of 99-106.5% for samples fortified at 0.02-1 ppm.

REPORTED RESULTS:

Cycloheximide was undetectable in all samples; levels were <0.016 ppm prior to and just after treatment and <0.021 ppm 5 days after treatment.

DISCUSSION:

1. The soil sampling depth was not provided; therefore no conclusions can be drawn from the results. The soil sampling procedure was not described, but a reference was cited (Sampling in the Field for Cycloheximide Assay, Upjohn Co. Report No. 120-9760-27, May 19, 1970). The soil sampling depth may be described there, but the reference was unavailable for review.

2. The samples were shipped unfrozen by air to laboratory, where they were frozen until analysis. No information was given on the preshipment condition of the samples, which may have involved a period of several days, based on the interval between samplings and the dates on the bioassay data tables. In other reports, cycloheximide was shown to be degraded very rapidly in soil (Study 4, 00011196; Study 5, 00012845; Study 25, no MRID; Study 26; 00012843).

CASE GS0038 CYCLOHEXIMIDE STUDY 34 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 20 TOPIC 259928036

FORMULATION 12 - EMULSIFIABLE CONCENTRATE (EC OR E).

FICHE/MASTER ID 00012852 CONTENT CAT 02

Buttram, J.R. (1971) Residue Determination for Cycloheximide on Oranges, Leaves and Soil (Florida, 1970): Report No. 120-9760-43. (Unpublished study received Mar 22, 1972 under 2F1252; submitted by Upjohn Co., Kalamazoo, Mich.; CDL:095124-W)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
PRIM: RCBR-25-10310010 EFB -30-100520

DIRECT RVW TIME = 4 (MH) START-DATE END DATE

REVIEWED BY: R. Hebert
TITLE: Staff Scientist
ORG: Enviro Control, Inc., Rockville, MD
LOC/TEL: 468-2500

SIGNATURE: Richard L Hebert DATE: June 25, 1981

APPROVED BY:
TITLE:
ORG:
LOC/TEL:

SIGNATURE: DATE:

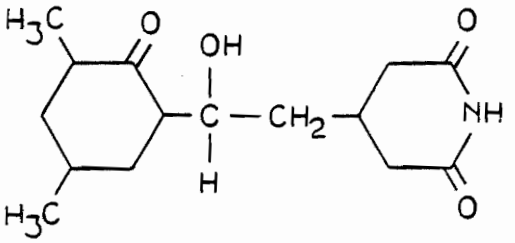
CONCLUSION:

Field Dissipation - Terrestrial

This study is invalid for the following reasons. The soil sampling depth was not provided; thus the results cannot be interpreted. The soil samples were shipped unfrozen by air to the laboratory, where they were frozen until analysis. However, the preshipment condition of the samples was not given, and this is necessary for validation since cycloheximide is known to be degraded rapidly in soil.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

An orange grove at Indian River Exchange, Florida, was sprayed by air on January 10, 1971, with 0.75 quart cycloheximide (Acti-Aid, 4.23% ai WP, Upjohn Co.) plus 1.13 quart Adsee 775 in 15 gallons of water. The treatment rate was 15 gal/A. The plot size was 5 acres, the number of trees was unspecified, and the soil type was sandy with some clay. No rain fell between treatment and sampling. At 4 days after treatment, soil was sampled as described in Upjohn Co. Report No. 120-9760-27. The sample was assayed for cycloheximide by the assay described in Study 38 (00012869, 00011224, 00011225, and 00011195), which is accurate for levels as low as 0.02 ppm, has a range in random error of ±0.01 ppm, and has a range in recovery levels of 99-106.5% for samples fortified at 0.02-1 ppm.

REPORTED RESULTS:

Cycloheximide was not detectable (<0.016 ppm).

DISCUSSION:

1. The soil sampling depth was not provided; therefore no conclusions can be drawn from the results. The soil sampling procedure was not described, but a reference was cited (Sampling in the Field for Cycloheximide Assay, Upjohn Co. Report No. 120-9760-27, May 19, 1970). The soil sampling depth may be described there, but the reference was unavailable for review.

2. The sample was shipped unfrozen by air to the laboratory, where it was frozen until analysis. No information was given on the preshipment condition of the sample, which may have involved a period of several days, based on the interval between samplings and the dates on the bioassay data tables. In other reports, cycloheximide was shown to be degraded very rapidly in soil (Study 4, 00011196; Study 5, 00012845; Study 25, no MRID; Study 26, 00012843).

(TDR038) DATA EVALUATION RECORD PAGE 1 OF

CASE GS0038 CYCLOHEXIMIDE STUDY 35 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 30 TOPIC 100520

FORMULATION 12 - EMULSIFIABLE CONCENTRATE (EC OR E)

FICHE/MASTER ID 00012851 CONTENT CAT 02

Buttram, J.R. (1971) Residue Determination for Cycloheximide on Oranges, Leaves and Soil (Florida, 1970): Report No. 120-9760-44. (Unpublished study received Mar 22, 1972 under 2F1252; submitted by Upjohn Co., Kalamazoo, Mich.; CDL:095124-V)

FICHE/MASTER ID 00012850 CONTENT CAT 02

Buttram, J.R. (1971) Residue Determination for Cycloheximide on Oranges, Soil and Leaves (Florida, 1970): Report No. 120-9760-45. (Unpublished study received Mar 22, 1972 under 2F1252; submitted by Upjohn Co., Kalamazoo, Mich.; CDL:095124-AA)

FICHE/MASTER ID 00012849 CONTENT CAT 02

Buttram, J.R. (1971) Residue Determination for Cycloheximide on Oranges, Leaves and Soil (Florida, 1970): Report No. 120-9760-46. (Unpublished study received Mar 22, 1972 under 2F1252; submitted by Upjohn Co., Kalamazoo, Mich.; CDL:095124-T)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
PRIM: RCBR-25-10310010 EFB -20-259928036

DIRECT RVW TIME = 4 (MH) START-DATE END DATE

REVIEWED BY: R. Hebert
TITLE: Staff Scientist
ORG: Enviro Control, Inc., Rockville, MD
LOC/TEL: 468-2500
SIGNATURE: Richard L. Hebert DATE: June 25, 1981

APPROVED BY:
TITLE:
ORG:
LOC/TEL:
SIGNATURE: DATE:

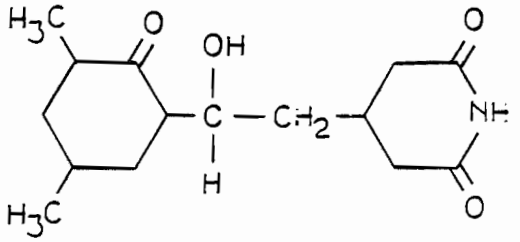
CONCLUSION:

Field Dissipation - Terrestrial

This study is invalid for the following reasons. The soil sampling depth was not provided; thus the results cannot be interpreted. The soil samples were shipped unfrozen by air to the laboratory, where they were frozen until analysis. However, the preshipment condition of the samples was not given, and this is necessary for validation since cycloheximide is known to be degraded rapidly

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

A portion of a 9.2-acre orange grove near Ocoee, Florida, was sprayed by air on December 8, 1970, with 1.5 quart cycloheximide (Acti-Aid 4.23% ai WP, Upjohn Co.) plus 2.25 quart Adsee 775 (an adjuvant) in 20 gallons of water per acre (00012851). Another portion of the grove was sprayed with the same formulations in 30 gallons of water at 26 gal/A (00012854). Another portion of the grove was sprayed with the same formulations in 750 gallons of water at 820 gal/A (00012849). The number of trees in the plot was unspecified and the soil type was sand. No rain fell between treatment and sampling. Soil was sampled prior to treatment and at 0, 7, and 38 days after treatment. The soil was sampled as described in Upjohn Co. Report No. 120-9760-27. The samples were assayed for cycloheximide by the assay described in Study 38 (00012869, 00011224, 00011225, and 00011195), which is accurate for levels as low as 0.02 ppm, has a range in random error of ±0.01 ppm, and has a range in recovery levels of 99-106.5% for samples fortified at 0.02-1 ppm.

REPORTED RESULTS:

Cycloheximide was undetectable prior to treatment (<0.013 ppm) and at 38 days after treatment with each mixture (<0.016 ppm). Cycloheximide was present at 0.04 and 0.07 ppm at 0 and 7 days, respectively, after spraying by air at 20 gal/A; at 0.03 and 0.04 ppm at 0 and 7 days, respectively, after spraying at 26 gal/A; and undetectable (<0.013 ppm) and at 0.07 ppm at 0 and 7 days, respectively, after spraying at 820 gal/A.

DISCUSSION:

1. The soil sampling depth was not provided. Therefore, no conclusions can be drawn from the results. The soil sampling procedure was not described, but a reference was cited (Sampling in the Field for Cycloheximide Assay, Upjohn Co. Report No. 120-9760-27, May 19, 1970). The soil sampling depth may be described there, but the reference was unavailable for review.

2. The samples were shipped unfrozen by air to the laboratory, where they were frozen until analysis. No information was given on the preshipment condition of the samples, which may have involved a period of several days, based on the interval between samplings and the dates on the bioassay data tables. In other reports, cycloheximide was shown to be degraded very rapidly in soil (Study 4, 00011196; Study 5, 00012845; Study 25, no MRID; Study 26, 00012843).

(TDR038) DATA EVALUATION RECORD PAGE 1 OF

CASE·GS0038 CYCLOHEXIMIDE STUDY 36 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 30 TOPIC 100520

FORMULATION 12 - EMULSIFIABLE CONCENTRATE (EC OR E)

FICHE/MASTER ID 00012850 CONTENT CAT 02

Buttram, J.R. (1971) Residue Determination for Cycloheximide on Oranges, Leaves and Soil (Florida, 1970): Report No. 120-9760-47. (Unpublished study received Mar 22, 1972 under 2F1252; submitted by Upjohn Co., Kalamazoo, Mich.; CDL:095124-U)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
PRIM: RCBR-25-10310010 EFB -20-259928036

DIRECT RVW TIME = 4 (MH) START-DATE END DATE

REVIEWED BY: R. Hebert
TITLE: Staff Scientist
ORG: Enviro Control, Inc., Rockville, MD
LOC/TEL: 468-2500

SIGNATURE: Richard L Hebert DATE: June 25, 1981

APPROVED BY:
TITLE:
ORG:
LOC/TEL:

SIGNATURE: DATE:

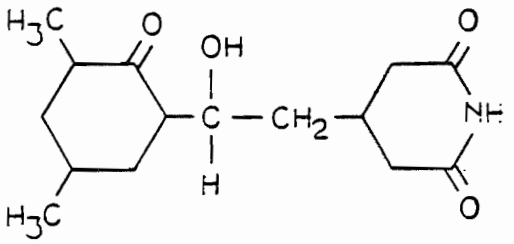
CONCLUSION:

Field Dissipation - Terrestrial

This study is invalid for the following reasons. The samples were stored at room temperature prior to assay, and the available information indicates this may have been for several days (cylcoheximide is known to be degraded rapidly in soil). Also, the soil sampling depth was not provided, and thus the results cannot be interpreted.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

An orange grove near Clermont, Florida, was sprayed on November 30, 1970, with 1 quart cycloheximide (Acti-Aid, 4.23% ai WP, Upjohn Co.) plus 1.5 quart Adsee 775 (an adjuvant) in 500 gallons of water at 500 gal/A. The 3.5-acre plot contained 15- to 17-foot trees spaced 25 feet apart in sandy soil. No other pesticides were used. Soil was sampled prior to treatment and at 0 and 7 days after treatment. Samples were collected as described in Upjohn Co. Report No. 120-9760-27. The samples were assayed for cycloheximide by the assay described in Study 38 (00012869, 00011224, 00011225, and 00011195), which is accurate for levels as low as 0.02 ppm, has a range in random error of ±0.01 ppm, and has a range in recovery levels of 99-106.5% for samples fortified at 0.02-1 ppm.

REPORTED RESULTS:

Cycloheximide was undetectable (<0.017 ppm) prior to treatment and was present at 0.03 and 0.15 ppm 0 and 7 days after treatment, respectively.

DISCUSSION:

1. The soil sampling depth was not provided; therefore no conclusions can be drawn from the results. The soil sampling procedure was not described, but a reference was cited (Sampling in the Field for Cycloheximide Assay, Upjohn Co. Report No. 120-9760-27, May 19, 1970). The soil sampling depth may be described there, but the reference was unavailable for review.

2. The samples were stored at room temperature prior to assay. This may have involved a period of several days, based on the interval between sampling and the dates on the bioassay data tables. In other reports, cycloheximide was shown to be degraded very rapidly in soil (Study 4, 00011196; Study 5, 00012845; Study 25, no MRID; Study 26, 00012843).

STUDY 37

CHEMICAL: CYCLOHEXIMIDE, ACTI-DIONE

FORMULATION: 00 - Active Ingredient

FICHE/MASTER ID: 00012880

CITATION: Petzold, E.N., and D.D. Chapman. 1971. Residues of ^{14}C-cycloheximide in bluegills from exposure via water for a month. Unpublished report submitted by Upjohn Co., Kalamazoo, MI. Report No. 120-9764-48.

FICHE/MASTER ID: 00012864

CITATION: Petzold, E.N., and D.D. Chapman. 1972. Residues of ^{14}C-cycloheximide in bluegills from exposure via water for a month. Unpublished report submitted by Upjohn Co., Kalamazoo, MI. Report No. 120-9764-48 (Revised).

DIRECT RVW TIME = 20 (HH) START-DATE END DATE

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SIGNATURE: Richard L Hebert J. Caplan DATE: June 18, 1981

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TITLE:
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LOC/TEL:

SIGNATURE: DATE:

CONCLUSIONS:

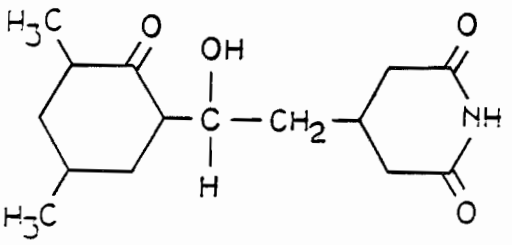
Accumulation - Fish

1. This section of the study is scientifically valid except that an unexplained factor added to data calculations may have increased the reported cycloheximide residue levels in whole fish by a factor of ~2.

2. Cycloheximide does not accumulate in bluegill sunfish. The maximum bioaccumulation factor was <10 for bluegills in a static system exposed to two treatments of [^{14}C]cycloheximide, 10 days apart, at 0.09 ppm. Under these conditions, maximum ^{14}C residue levels in tissues of whole fish were <0.14 ppm (cycloheximide equivalents), and chloroform extractable ^{14}C residues were 0.035 ppm over a 1-month exposure period. ^{14}C residue levels declined to ≤0.01 ppm after 3 days of depuration.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

The water in an aquarium was treated with randomly labeled [^{14}C]-cycloheximide (Upjohn Co.) at 0.090 ppm (determined by a fungal bioassay; Study 38, 00012869, 00011224, 00011225, and 00011195). Sixty-nine bluegill sunfish ranging in weight from 0.4 to 5.2 g were added to the aquarium and fed sparingly three times daily. The water was maintained at 62-64 F and was treated again with [^{14}C]cycloheximide, 10 days after the first treatment. One or two fish were removed for analysis each day during the 31-day exposure period. The fish remaining after the exposure period were transferred to an aquarium containing untreated water for 9 days. The fish were sampled on days 3-9 of the depuration period. Water samples were collected daily during the exposure period.

Water samples were partitioned three times with chloroform. The chloroform was evaporated and the residue was dissolved in hexane and water. The aqueous fraction was examined for cycloheximide by the bioassay referenced above, which is accurate for levels as low as 0.02 ppm, has a range in random error of ±0.01 ppm, and has a range in recovery levels of 99-106.5% for samples fortified at 0.02-1 ppm.

Each fish sample was homogenized in water. An aliquot was removed and allowed to dry for 24 hours. The aliquot was combusted by the Schoniger combustion method and analyzed by liquid scintillation counting (LSC).

The remainder of the fish sample was lyophilized, extracted with chloroform, and filtered. The filter cake was reextracted with chloroform. The chloroform extract was evaporated and the residue was dissolved in scintillation solution and analyzed by LSC.

REPORTED RESULTS:

Cycloheximide had a short half-life in the water (Table 1); therfore, the water was treated twice. The first half-life was 4.5 days. Cycloheximide was at 0.095 ppm on day 11, and declined with a half-life of 4.75 days, resulting in a 90% loss 15 days later.

There were no fish mortalities. ^{14}C residue levels in fish reached 0.085 ppm, expressed as cycloheximide, 10 days after the first treatment. The maximum ^{14}C level in fish, 0.14 ppm, was measured 2 days after the second treatment. Residues were present at 0.08-0.1 ppm at the end of the exposure period. Less than 0.035 ppm of the ^{14}C residues were extractable into chloroform at any time point. ^{14}C residue levels were below background levels during the first 7 days of the depuration period. On days 8 and 9 of the depuration period, residue levels were 0.02 and 0.01 ppm, respectively. However, these ^{14}C residue levels were not considered significant.

DISCUSSION:

1. This study initially was reported in 1971 (00012880) and was revised in 1972 (00012864). Different residue levels in whole fish were presented in each report. The formula used to calculate residue levels in the initial report was as follows:

$$\frac{\text{DPM in homogenate}}{\text{Fish weight (g)}} \times \frac{\text{Homogenate weight (g)}}{\text{Aliquot weight (g)}} \times \frac{1}{1{,}600 \text{ DPM/}\mu\text{g cycloheximide}}$$

This formula is erroneus because homogenate and aliquot weights were used to calculate DPM in homogenate, so they should not have been included in the formula. The proper formula is the one used in the revised report:

$$\frac{\text{DPM in homogenate}}{\text{Fish weight (g)}} \times \frac{1}{1{,}600 \text{ DPM/}\mu\text{g cycloheximide}}$$

2. Another aberration in the data calculations was not explained in either report; i.e., the reported DPM/g homogenate values were too high by a factor of ~2. Thus it would appear that the correct DPM values for whole fish homogenates are ~50% lower than the reported DPM values.

Nevertheless, the data do provide some useful information because if the calculations were wrong, then they yielded residue levels consistently higher than the true levels. Thus it can be concluded that the bioaccumulation factors at all time points were <2 (see Discussion 4). Also, maximum ^{14}C residue levels in fish occurred on the 12th day and were <0.14 ppm, expressed as cycloheximide. Residue levels declined slowly but were ≤0.1 ppm at the end of the exposure period. Depuration was rapid, with ^{14}C levels at ≤0.01 ppm after 3 days of depuration. The latter value is determined by the fact that the ^{14}C counts during depuration were not significantly higher than background counts. The chloroform-extractable ^{14}C residues, which would represent cycloheximide and structurally related degradates (see Discusssion 4), never exceeded 0.035 ppm (one sample on day 29, not in Table 1 of this review).

3. The text of the study states that there was a 30-day exposure period, and Figure 1 in the study indicates that two water samples were assayed for cycloheximide on day 10, once before and once after re-treatment. However, the data tables show that water samples were taken daily during

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a 31-day exposure period and that the values for samples taken after the second treatment were plotted (in Figure 1 of the study) 1 day before actual posttreatment times.

4. Although the bioassay was acceptable for determining cycloheximide levels in the water, the data should not be compared with the ^{14}C residue values in fish to obtain bioaccumulation factors. If this is done, the factors should be higher than the actual values because of the specificity of the bioassay. In this case, the factors are <10 for all time points. A better estimate can be derived by using the values for chloroform extractable ^{14}C residues because the extraction procedure is known to yield excellent recovery levels in soil (Study 38, 00012869, 00011224, 00011225, and 00011195). When this is done, the factors are <2 until day 29, when cycloheximide concentrations in water dropped below the sensitivity level of the assay. The first number (<10) should be used, however, as a safe maximum estimate for a bioaccumulation factor.

5. The mechanisms of degradation of cycloheximide in the water cannot be determined because the aquarium was not in the dark and the water was not sterile. Therefore, degradation could have been due to hydrolysis, photolysis, metabolism, or any combination of these.

Table 1. Cycloheximide degradation in aquarium water and accumulation in bluegill sunfish exposed to [^{14}C]cycloheximide.

Days after initial treatment[a]	Cycloheximide in water (ppm)[b]	Chloroform extractable ^{14}C residues in fish (ppm)
0	0.090	--
1	0.063	0.0047[c]
3	0.055	0.0040
5	0.040	0.0164
8	0.024	0.0022
10	0.019	0.0237
11	0.095	0.0072
12	0.086	0.0263
14	0.070	0.0063
16	0.042	0.0054
21	0.020	0.0074
26	0.010	0.0024
30	<0.008	0.0120[c]

[a]Water was initially treated at 0.09 ppm and re-treated 10 days later.

[b]Data from pages B2-B6 of 00012880 were computed to yield data shown here.

[c]Average of two fish; only one fish sampled on other dates.

STUDY 38

CHEMICAL: CYCLOHEXIMIDE, ACTI-DIONE

FORMULATION: 00 - Active Ingredient

FICHE/MASTER ID: 00012869

CITATION: Petzold, E.N., and D.D. Chapman. 1969. A cylinder-plate assay for cycloheximide: Report No. 120-9760-1. (Unpublished study submitted by Upjohn Co., Kalamazoo, MI)

FICHE/MASTER ID: 00011224

CITATION: Petzold, E.N., and D.D. Chapman. 1969. A sensitive method for determining cycloheximide in oranges: Report No. 120-9760-3. (Unpublished study submitted by Upjohn Co., Kalamazoo, MI)

FICHE/MASTER ID: 00011225

CITATION: Petzold, E.N., D.D. Chapman, and W.M. Wright. 1969. Evaluation of a cylinder plate method for analysis of cycloheximide in oranges: Report No. 120-9760-4. (Unpublished study submitted by Upjohn Co., Kalamazoo, MI)

FICHE/MASTER ID 00011195 CONTENT CAT 01

Petzold, E.N.; Chapman, D.D. (1970) Evaluation of the Analytical Method for Cycloheximide on Florida Soil: Report No. 120-9760-26. (Unpublished study received Jul 26, 1970 under 1023-EX-27; submitted by Upjohn Co., Kalamazoo, Mich.; CDL:210033-C)

SUBST. CLASS = S.

DIRECT RVW TIME = 14 (HR) START-DATE END DATE

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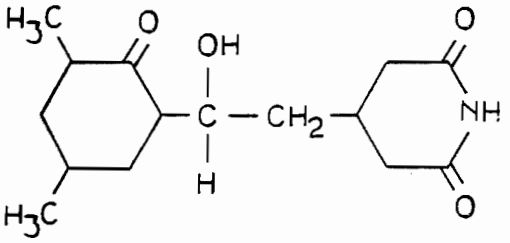
CONCLUSIONS:

1. This method study is scientifically valid.

2. An extraction procedure and a bioassay were developed by the Upjohn Co. to measure cycloheximide in extracts of whole oranges, orange process products, and soil. The assay accurately measures cycloheximide in soil at levels as low as 0.015-0.020 ppm, and has a random error level of ±0.01 ppm Recovery levels of ∿100% were obtained for soil extracts. The soil extraction procedure and bioassay were used in other Upjohn Co. reports dealing with the environmental fate of cycloheximide. No conclusions are derived regarding recovery from oranges.

MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclonexyl)-2-hydroxyethyl-}glutarimide

Preparation of Samples

Samples of whole oranges (macerated) or Florida soil (type unspecified) were fortified with cycloheximide at several concentrations (ranging from 0.02 to 0.25 ppm) with a 50-ppm standard solution of cycloheximide in chloroform. One hundred grams of uniformly mixed sample was placed in a Waring blender jar, and 200 ml of chloroform was added. The mixture was blended at a low rate for 10 minutes, poured into a 250-ml centrifuge bottle, and centrifuged at 2,000 rpm for 10 minutes (soil samples were not centrifuged). A 100-ml aliquot was transferred from the chloroform phase to a 500-ml round-bottom flask, and the solvent was removed by vacuum evaporation at 40 C. Twenty-five milliliters of hexane and 5.0 ml of water were added to the flask, and the mixture was swirled slowly for 10 minutes. The sample was poured into a 50-ml centrifuge tube and centrifuged at 1,000 rpm for 5 minutes (soil samples were not centrifuged). The hexane layer was drawn off and discarded, and the aqueous layer was transferred to a vial and refrigerated until it was assayed. No loss in activity occurred after refrigeration for at least 3 days.

Preparation of Cycloheximide Standards

One hundred milligrams of cycloheximide primary standard (purity not specified) was weighed into a 1,000-ml volumetric flask, dissolved with water, diluted to volume, and refrigerated. Working standards at 0.2, 0.4, 0.8, 1.2, 1.6, and 2.0 ppm were prepared by dilution of the refrigerated stock solution in water. Working standards were prepared fresh daily.

Preparation of Assay Plates

Three plates were prepared for each standard and sample to be analyzed. Each 100-mm sterile plate received 20 ml of an autoclaved base medium containing 1,000 ml water, 2.5 g yeast extract, 10 g glucose, 1.0 g KH_2PO_4, and 20 g agar. Plates were covered with absorbent disks in Brewer metal rings and allowed to cool to 65-85 C on a level surface. Seed medium (600 ml water, 1.5 g yeast extract, 6.0 g glucose, 0.6 g KH_2PO_4, and 12.0 g agar) was prepared, sterilized, and allowed to cool to 48-50 C. Thirty milliliters of a broth medium (same formula as base medium but without agar), which had been inoculated with Saccharomyces cerevisiae from a fresh agar slant and shaken at 36 C for 18 hours, was added to the cooled seed medium, and 5 ml of the mixture was added to each plate of molten base medium and swirled for confluence. Plates were allowed to cool to room temperature after inoculation.

Six stainless steel cylinders with a 6-mm inner diameter were placed on the surface of each plate. Alternate cylinders were filled with a 1-ppm cylcoheximide solution to serve as references. Remaining cylinders were filled with either the standard or the sample to be tested. Treated plates were incubated at 35 C for 16-18 hours, after which covers and cylinders were removed and zones of inhibition were read to ±0.1 mm with a Fisher-Lilly zone reader.

Calculations

For each set of three dishes, averages were calculated for reference readings and standard or sample readings. An overall reference average was also calculated. The difference between the reference average for each set and the overall reference average was then subtracted from the average standard or sample reading to adjust each standard or sample average for dish and environmental variations. A standard curve was established by plotting zone diameters versus the log concentration of cycloheximide. A best-fit curve was drawn, allowing sample concentrations to be read from measured zone diameters. Regression analysis of the results was performed to allow estimation of recovery.

REPORTED RESULTS:

Oranges

The threshold sensitivity for detection was reported as 0.015-0.020 ppm.

Background levels are well below this threshold, and apparently are artifacts of extrapolation of data by the least squares method. Error levels were smaller than the calculated standard error level at concentrations near the threshold level, and were greater than the standard error at levels above 0.25 ppm. Recovery levels ranged from 74.3 to 110.7%.

Soil

Results of four experiments with soil are summarized in Table 1. Untreated samples gave no response, indicating a negligible background response. Recovery levels were approximately 100%, and were higher and had a smaller range (99-106.5%) than those for oranges. The standard error was 0.004 ppm, and the expected range in random error at the 99% confidence level was ±0.01 ppm. The sensitivity level is 0.015-0.020 ppm.

DISCUSSION:

1. The bioassay was described in 00012869. The preparation of orange and soil extracts, and the application of the bioassay to these samples, were described in 00011224 and 00011225 for oranges and 00011195 for soil. This type of bioassay is routinely used to measure small concentrations of biologically active compounds. The assay and extraction methods used here indicate that the methods are highly efficient for the determination of cycloheximide in soil. The assay measures other compounds inhibitory to the test organism; such compounds may be in various types of soil. This assay was also used in several other Upjohn Co. reports that were reviewed. Adequate data were obtained in the appropriate studies to rule out the possibility of interference from other compounds.

2. The assay is accurate within the range of 0.02-0.25 ppm. Thus, soil samples known or suspected to contain higher amounts are diluted to obtain concentrations within the accurate range. This was done in the other Upjohn Co. studies that were reviewed.

Table 1. Summary of evaluation of the bioassay method for determination of cycloheximide in soil.

Experiment number	Number of samples	Percent recovery	Standard deviation (± ppm)
1	12	99.6 ± 2.4	0.005
2	12	98.9 ± 1.0	0.002
3	12	101.2 ± 1.8	0.004
4	12	106.5 ± 2.3	0.005
Total	48	101.6 ± 1.0	0.004

Data from 00011195.

CASE GS0038 CYCLOHEXIMIDE STUDY 39 PM 12/08/80

CHEM 043401 Cycloheximide

BRANCH EFB DISC 20 TOPIC 1005 GUIDELINE 40 CFR 163.62-8f3

FORMULATION 00 - ACTIVE INGREDIENT

FICHE/MASTER ID 05021055 CONTENT CAT 03

Ishizawa, K.; Enomoto, S.; Wada, S. (1979) Germination and photo-induction of polarity in the spherical cells regenerated from protoplasm fragments of "Boergesenia forbesii", Botanical Magazine 92(1027):173-186.

SUBST. CLASS = S.

DIRECT RVW TIME = 5 (MH) START-DATE END DATE

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CONCLUSIONS:

Microbiological - Effects of Pesticides on Microbes

1. This study is scientifically valid.

2. Cycloheximide, at 1 x 10^{-4} M, inhibits formation of rhizoids by spherical cells of the marine green alga Boergesenia forbesii.

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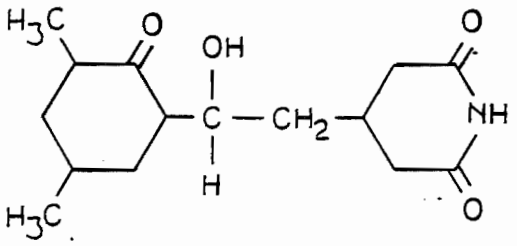
MATERIALS AND METHODS:

CYCLOHEXIMIDE, ACTI-AID, ACTI-DIONE, ACTISPRAY, HIZAROCIN



3-{2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl-}glutarimide

A pure culture of Boergesenia forbesii was grown in an autoclaved medium (ESP) prepared from sea water. The alga was grown for 2 months at 25 C with an 8-hour photoperiod, forming a cell 15 mm in length. The cell was immersed for 10 minutes in sea water containing 0.6 M mannitol, which induced plasmolysis. It was then transferred to ESP medium and cut into pieces to release protoplasm fragments into the medium. The fragments were maintained at 25 C for 4 hours in the dark; during this time the fragments became spherical and generated cell walls. These spherical cells were filtered through nylon meshes to obtain a population of cells homogenous in size. These cells were maintained in ESP medium for 3 days at 17 C prior to germination studies.

Twenty spherical cells were incubated in 3-cm dishes containing 2 ml ESP medium with or without metabolic inhibitors. Cycloheximide (Wako Chemical Industries, Ltd., Japan; purity unspecified) was added at 10^{-4} M. Four dishes were set on moist filter paper in a 9-cm dish and incubated for 6 days in the dark at 25 C. The development of rhizoidal protrusion from the cell (germination) was observed microscopically. The germination rate was determined based on a total of 80 cells (20 cells/dish).

REPORTED RESULTS:

None of the cells germinated in the presence of cycloheximide, whereas 69% of the control cells germinated.

DISCUSSION:

B. forbesii is a large unicellular marine green alga belonging to the order Siphonocladales. This order is a small group of algae characterized by the formation of spherical multinucleate cells that fragment from the parent cell. These spherical cells typically produce rhizoids to serve as holdfasts, or produce many branching projections. Cycloheximide was used in this study to determine that protein synthesis is required for rhizoid formation.