Captan-Induced Reversions in Bacteria

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CAPTAN-INDUCED REVERSIONS
IN BACTERIA

by

Gayle M. Nii LoPiccolo

A Thesis
Submitted to the
Faculty of The College of Graduate
Studies in partial fulfillment
of the
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Gayle Nii LoPiccolo
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INTRODUCTION

For some time now, the public in general and the scientific community in particular became aware of the harmful effects of pesticides on man and on his ecosystem, especially in terms of their toxic albeit non-genetic effects.

With respect to the genetic effects of pesticides, a literature review by Wuu and Grant (1966) is informative. These authors found that a considerable number of pesticides, such as antibiotics, certain chlorinated hydrocarbons, organic phosphates, carbamates and acridine dyes may induce chromosomal aberrations. In their own work, Wuu and Grant (1966; 1967) used pesticides on meiotic and somatic cells of barley and found that several pesticides increased the frequency of chromosomal aberrations over control levels. The chromosomal abnormalities induced included anaphase bridges, chromosome fragmentations and pycnosis. Sax and Sax (1968) found that the commercial pesticide Vapona (active ingredient--gamma benzene hexachloride) and two other insecticides used in the home, increased chromosomal aberrations in onion root tips significantly over control levels.

In order to determine if certain pesticides that are readily available to the urban consumer as well as to agriculture are mutagenic, fourteen pesticides purchased from a local hardware store and eight pesticides received from Dr. W. F. Grant of McGill University were tested for mutagenicity in Escherichia coli and

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Salmonella typhimurium. Two of the pesticides tested, both containing captan as an active ingredient, were found to be mutagenic.

Captan in its commercial form as Captan 50WP and in its pure form as 98.8 percent technical captan was further tested to characterize its mutation kinetics and its probable mutagenic action in bacteria.
REVIEW OF LITERATURE

Fungitoxicity and Toxicity Studies

History of captan

Captan was discovered by A. R. Kittleson in 1952 as a result of a joint effort by the Standard Oil Development Corporation and Rutgers University. Subsequent testing of this organic fungicide was performed by R. H. Daines and L. E. Hagman (McCallan, 1967).

Physical and chemical properties

Technical captan (N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide) is an odorless, crystalline compound that can be easily synthesized by the reaction of sodium tetrahydrophthalimide with perchloromethyl mercaptan in basic aqueous solution and under good mixing conditions. Captan is precipitated out as a white solid which is then filtered.

\[
\text{N-NO + CISCICl}_3 \rightarrow \text{N-SCCICl}_3 + \text{NaCl}
\]

It has a melting point of 172° - 173° C, density of 1.69 g/ml and is slightly soluble in many organic solvents. It is stable, even in the presence of moisture, at room temperature. 200° C is where rapid decomposition of this compound is observed, while heating at 100° C for several days results in gradual decomposition.

Captan hydrolyzes in solution but its solubility in water varies due to differing degrees of hydrolysis in the samples. The solubility
of captan in water has been reported to be as low as 0.5 ppm and as high as 10 ppm (Lukens, 1969). Dugger et al. (1959) reported the solubility of 99 percent captan in water to be less than 5 ppm at 25°C.

Captan hydrolyzes slowly to non-toxic compounds in soil. The half life of captan mixed with moist silt loam was found to be 3 - 4 days while the half life of captan in an aqueous solution at pH 7 was found to be 2.5 hours (Burchfield and Schechtman, 1958). Burchfield (1959) found that the breakdown was slower in an air dried soil than in moist soil.

Uses of captan

Captan is usually used commercially in combination with inert ingredients such as wetting agents and diluents which help in the dispersal of the fungicide (Somers, 1967).

The annual consumption of captan or more generally the trichloromethylthio fungicides for agricultural purposes is approximately 10 million pounds (Lukens, 1969).

Captan has been used extensively as a preharvest and postharvest spray on fruits to control diseases. It has been shown to be one of the toxicologically safest and more effective fungicides on fruits and other crops (Daines, 1953; Stauffer Chemicals Product Guide, 1969). Its uses have not been confined to agricultural sprays but have been used as a germicide in soaps and as a microbial deterioration protectant for leather. It has also been found to be an effective antifouling agent to protect marine surfaces from microbes that cause
deterioration.

Captan uptake

Uptake of captan in Neurospora crassa was studied by Richmond and Somers. They first reported in 1962 that captan uptake was not a first order kinetics reaction. The energy of activation process could not be calculated but they concluded that uptake was dependent partially upon the cell's metabolic activity. Captan was also actively transported by the fungal cells.

Captan uptake was also found to decrease with the pre-treatment of the cells with thiol reagents such as iodoacetic acid (Richmond and Somers, 1966).

Electron microscopic studies on N. crassa revealed that captan produced convoluted forms of the nuclear membrane, however the cytoplasmic membrane was not affected. Therefore they suggested that captan reacted with the sulfhydryl groups of the nuclear protein leading to inhibition of cell division and that captan did not inhibit the cell's permeability facilities (Richmond and Somers, 1967).

Captan's mechanism of action

The sulfhydryl dependent enzymes or thiol groups have been considered as the ultimate site of action of all fungicides. There is no evidence of a relationship between captan uptake and toxicity, captan uptake and spore surface area or captan toxicity and sulfhydryl content (Richmond and Somers, 1963).
The toxicity of captan was at first attributed to the -NSCCl₃ group (Kittleson, 1953) but the nitrogen atom was found to be non-essential with the finding that fungicides with a sulfur, carbon or oxygen atom in place of the nitrogen atom were also found to be fungitoxic (Sosnovsky, 1956; Johnston et al., 1957; Fawcett, 1958). Therefore the -SCCl₃ group was considered the toxic reaction center or toxophore by Lukens (1966).

The -SCCl₃ group affects the fungitoxic activity in two ways, that of permeation of the fungicide into the fungal cells and that of the R-SCCl₃ molecule reacting with cellular components (Lukens, 1966).

The toxic effect of captan has been attributed to the depletion of sulfur in the fungi through the production of thiophosgene when captan comes in contact with the fungal tissue. Thiophosgene in turn reacts with free thiol groups in the cell. Owens and Novotny (1959) however attributed the basic toxic action of captan to be due to the reaction of the captan molecule and not to its decomposition products reacting with unprotected -SH groups in the cell.

**Site of action**

Horchstein and Cox (1956) found that the captan concentration needed to inhibit fungal growth also affected the degradation of carbohydrates in the cell. They did not preclude the possibility that other processes may be inhibited simultaneously. Inhibition was found to occur at the decarboxylation of pyruvate by yeast.
carboxylase. Thiophosgene was thought to interfere with the coenzyme-
 enzyme linkage necessary for carboxylation by Dugger (1969).

Owens and Blaak (1960) specifically showed the pathway between
acetate and citrate as the site of action of captan. They also sug-
gested that captan inactivated coenzyme A although inactivation of
other essential components in the pathway was not excluded.

Toxicity studies

Toxicity studies have been done on various organisms with varied
results.

Ackerson (1955) fed one day old male chicks with corn sprayed
with commercial captan. These chicks showed early signs of slow
growth but at the end of 28 days made equal gains with the controls.
He noticed no abnormalities in the experimental chicks outward
appearance.

Link et al. (1956) fed captan treated corn (580 ppm on corn) to
pigs and cattle and found low toxic effects. Hunter (1961) studied
the toxic effects of 50 percent captan on spotted spider mite
populations. He sprayed the host plants, lima bean and peas, with
two and four pounds of 50 percent captan dissolved in 100 gallons of
water and found no or only a slight inhibiting effect on the mite
population.

Hislop (1969) studying the effects of captan on non-parasitic
microflora of apple leaves found no conclusive data on the toxicity
of captan to leaf bacteria.
Captan has also been found to induce toxic effects. Poulsen (1964) using pigeons, administered 450 milligrams per kilogram body weight of technical captan or commercial captan via a stomach tube which resulted in three out of five pigeons dying of acute poisoning. Autopsy revealed inflammation of the esophagus and the intestine. Palmer (1963) investigated sheep's tolerance to captan by orally administering 250 and 500 milligrams per kilogram body weight with a dose syringe. The sheep showed signs of loss of appetite and at these doses, the effect was lethal with the autopsy results varying.

Abedi (1967) found that one ppm of captan had drastic effects on Zebrafish larvae. At this concentration, the larvae were strongly excited after exposure for five minutes. After a total of 90 minutes exposure, all the larvae were dead due to captan poisoning associated with observable head injury; the heads were ruptured into lateral halves giving a bicephalous appearance. This effect was not found when the zebrafish were exposed to DDT, Dieldrin, Malathion, Parathion, Carbaryl-2,4-D and Warfarin.

Captan was found to strongly inhibit the photoautotrophic growth of 37 Chlorella strains by Soeder (1969). Three strains were found to be resistant. This resistance was not species specific.

Captan has been found to be toxic to rats deficient in protein (Boyd, 1968; 1969; Krenjnen and Boyd, 1970). LD$_{50}$ amounts of captan (12.5 ± 3.5 milligram per kilogram body weight) was given orally to overnight starved male albino rats with death resulting in 2.6 ± 1.4 days. The LD$_{50}$ was lowered for rats fed from weaning on low protein
diets. These rats were 25 times more sensitive to captan with the oral LD50 being 0.048 ± 0.11 grams per kilogram body weight. The rats showed signs of irritability, listlessness, soft stools, soiling of fur and loss of body weight.

Cellular and Molecular Basis of Mutations

Before an understanding of mutations could be attempted it was necessary to review the current literature concerning how the genetic information is stored, replicated, transcribed and translated into amino acid sequences of polypeptides and how the genetic information may be altered as a result of exposure to chemical mutagens.

DNA

Deoxyribonucleic acid (DNA) contains the hereditary information that is transferred to each generation. It is composed of a polysugar-phosphate backbone from which the nucleic acid bases, (purines and pyrimidines) extend. According to the Watson-Crick DNA model, the DNA is a double helix held together by hydrogen bonding between specific pairs of bases. These bases are guanine and adenine as the purines and thymine and cytosine as the pyrimidines. Adenine always pairs with thymine and cytosine always pairs with guanine.

DNA replicates semiconservatively (Meselson and Stahl, 1958) with each single strand serving as a template. During replication bases pair with their complementary bases in the template. These two strands, the template and the newly synthesized single strand,
are intertwined into a double helix as the new strand is being formed.

The genetic code

The four nucleic acid bases specify amino acid sequences. These bases are 'read' in a triplet sequence; three bases (codon) coding for a specific amino acid.

Many codons are contained within a gene and following the one gene, one polypeptide hypothesis each gene contains the code for a specific amino acid sequence resulting in the formation of a polypeptide.

Not all codons however, code for amino acids. Some are used as initiators to start the reading of the codons while others act as terminators.

Transcription

This is the process in which the genetic information is transferred from DNA to RNA (ribonucleic acid). The base sequences of RNA are complementary to those of DNA.

Only one strand of the DNA is transcribed. Messenger RNA (m-RNA) is formed that is the same length as the corresponding segment of DNA and containing complementary bases. The base sequence carried in the m-RNA determines the sequence of amino acids in the polypeptide chain.

Translation

This is the process involving the formation of a protein directed by the codons of the m-RNA in a ribosome. The ribosome starts the
machinery for protein synthesis as soon as the m-RNA moves through.

Transfer RNA (t-RNA) contain a specific "anticodon" which recognizes its corresponding complementary codon in m-RNA in the ribosome. It also contains the amino acid that the codon codes for. Reading of the m-RNA by t-RNA begins with the first codon of the m-RNA (chain initiation) and continues until the last codon of a particular m-RNA is reached (chain termination). As the first amino acid is attached to the m-RNA through complementary pairing between m-RNA and t-RNA, the second amino acid is brought into position by a similar process. A peptide bond is then formed between the first and second amino acids. Subsequently the t-RNAs release the amino acids and are themselves released from both the newly forming polypeptide and ribosomes. In this manner the polypeptide chain grows until the chain terminating codon is reached which signals the end of the polypeptide. Then the polypeptide chain is released into the cytoplasm. Punctuation or coding for polypeptide termination have been associated to three nonsense triplets, amber (UAG), ochre (UAA) and UGA with ochre as the regular chain terminator.

The mutation process involve changes in DNA that alter the amino acid sequence of proteins resulting in defective enzymes or structural proteins or it may affect the translation process by altering the anticodons of the t-RNA.

Types of mutations

There are two major classes of mutations, point mutations and large alteration mutations (Freese, 1963). The bacterial test
Point mutations

Point mutations are due to a change affecting a single nucleotide and could be due to (i) replacement of a nucleotide by another (ii) insertion of an extra nucleotide or (iii) deletion of a nucleotide (Davis et al., 1967).

The replacement of a nucleotide can be accomplished in two ways: through transitions or transversions.

In transitions a purine base on the DNA strand is replaced by another purine base or a pyrimidine is replaced by another pyrimidine (Freese, 1959). Transition mutations can be caused by base analogs, deamination, alkylating agents or can arise spontaneously.

Base analogs differ slightly in structure from normal purine and pyrimidine bases and are incorporated into the DNA without impairing its ability to replicate. The action of base analogs is dependent upon their incorporation into the replicated DNA strand in place of the normal base.

Alkylating agents act similar to that of base analogs but instead of inserting an altered base during replication the base is altered by the mutagen. The altered base can then lead to pairing errors (Fishbein, 1970).

Nitrous acid acts by deaminating the amino substituted bases. As a result of deamination adenine is converted to hypoxanthine which resembles guanine and pairs with cytosine instead of thymine;
cytosine is converted to uracil which resembles thymine and pairs with adenine.

Hydroxylamine specifically deaminates cytosine to a base that resembles thymine, therefore pairing with adenine instead of guanine.

Transition mutations arising spontaneously occur through tautomeric shifts in which bases in their more frequent keto form change to their rare enol form. The change, from enol to keto form also changes the steric pairing property of bases. Thus adenine (enol) may pair with cytosine (keto). These mistakes in pairing in turn lead to transition type mutations. This is accomplished through a change in the hydrogen bonding.

According to Drake (1970) transversion mechanisms can be divided into null-base schemes and mispairing schemes. Null-base schemes can be due to DNA modifications in which a base is removed entirely (depurination) leaving a gap in the DNA template and during replication, the depurinated base is ignored leading to deletions or insertions of a random base. Null-base schemes can also be due to extensive degradation of a base so that it does not resemble any base. Mispairing schemes are dependent upon two different processes: mispairing between normal bases or mispairing due to chemical modifications of the normal base.

In transversions, a purine base is replaced by a pyrimidine base or vice versa. The mechanism responsible for this base pair substitution is much less understood than those for frameshifts and transitions (Drake, 1970; Davis et al., 1967). Transversions are caused by chemical agents such as alkylating agents and low pH.
These chemicals remove bases from the DNA leaving a gap in the strand. During the following DNA replications any base may be incorporated opposite the gap resulting in both transversion and transition type mutations.

Exposure of DNA to low pH (below pH 4) causes the complete removal of adenine and guanine (Tamm et al., 1952) by changing the charge of the bases leading to transitions and transversions. Alkylating agents function similarly however the bases are first methylated or ethylated and then removed.

Point mutations due to an insertion or deletion of a nucleotide lead to frameshift mutations. The phenotypic anomaly brought about by a frameshift mutation is based on the assumption that at translation, the bases coding for a specific amino acid are read off in groups of three and in a given order from a fixed starting point. With the insertion or deletion of a base, the reading frame is altered and the transcription of every triplet from that point on is out of phase and only mutant proteins are formed.

Point mutations of this type are known to be induced by acridines in bacteriophage T2 and T4 (Orgel and Brenner, 1961; Lerman, 1964). ICR compounds which resemble acridines in structure are known to induce frameshift mutations in enteric bacteria (Ames and Whitfield, 1966).

Proflavine and acridine orange are examples of acridine dyes that exhibit mutagenic properties that seem to have direct influence on DNA. According to Lerman (1961) acridines insert themselves between neighboring bases in one DNA strand therefore causing an
insertion or deletion of a single nucleotide (Brenner et al., 1961).

Another type of point mutation is that of suppression. This type of mutation occurs at a genetic locus distant from the original mutation but reverses the effect of the original mutation. It is in contrast to true reversion in which the original altered codons are restored to normal function.

A suppressor mutation may be located in the same cistron as the original mutation (intragenic suppression) or may be located in a different cistron of the same chromosome or even in a different chromosome (intergenic suppression). One suppressor mutation may also suppress several mutations at different codons in the same cistron or mutations of the same codon in different cistrons.

There are two types of intragenic suppression. The first deals with a deletion of a nucleotide with a subsequent insertion of another nucleotide nearby. The second type of intragenic suppression occurs when the suppressor mutation brings about the insertion of a new amino acid which compensates for the amino acid inserted by the original mutation in the same polypeptide chain. An example was found in the A gene of E. coli tryptophan synthetase. Two mutations were mapped approximately 100 nucleotides apart and when both mutations were present together, partial reversion of the mutant effect was found (Helinski and Yanofsky, 1963). In phage intragenic suppression by both addition and deletion of single bases has been demonstrated (Orgel and Brenner, 1961).

The understanding of intergenic suppressors grew with the greater understanding of the transcriptional and translational
processes of the cell. Yanofsky and St. Lawrence (1960) postulated that suppressors act at the translational level of protein synthesis. Evidence for this was put forth by Yanofsky and Crawford in 1959. They found that suppression brings about activity in the missing enzyme and that this activity is due to the synthesis of active enzyme protein (Crawford and Yanofsky, 1959). Such correction may be accomplished through the second mutation affecting t-RNA specificity with the result that an amino acid is inserted in the forming polypeptide that was not coded for by the m-RNA. The amino acid substitution may result in a polypeptide that is less functional than the wild type enzyme. The external suppressor may also adversely affect the function of other wild type enzymes by inserting the wrong amino acid. The total result is that suppressed cells are usually less functional than wild type cells or true revertants. In addition to intergenic suppression through t-RNA, intergenic suppression may also be accomplished by altering the specificity of the amino acid activating enzymes and the ribosomes (Drake, 1970).

Suppression may also be accomplished by indirect suppression which acts through substitution of an alternative mechanism for the defective one. These suppressions act primarily through intermediary metabolism. Thus for example mutational blocks in the biosynthesis of a metabolite may be suppressed by an external suppressor leading to a new metabolic pathway with the same end product. This type of indirect suppression was exhibited in several acetate mutants of *Neurospora crassa* (Lein and Lein, 1952; Strauss and Pierog, 1954) and in cysteine mutants of *Salmonella typhimurium* (Howarth, 1958).
In another example, the suppressor mutation substitutes a functional product of another gene that is capable of performing the missing function of the original mutation. A suppressor may also cytoplasmically activate an inactive enzyme produced by a mutant gene.

A change in the base sequence may lead to a missense mutation or to a nonsense mutation. Missense mutations are where a codon for one amino acid is substituted for another while nonsense mutations are substitutions of a codon not coding for any amino acid.

A mutation to missense, by definition, is the changing of a sense codon to another sense codon. Wherever the second sense codon is read in the genome, the new amino acid will be substituted. Suppression of this codon leads to translation of a sense codon to a missense codon. Therefore an effective missense suppressor may be harmful to the cell.

With the degeneracy of the code where two or more codons specify the same amino acid, most of the mutations induced would most likely be missense rather than nonsense.

Benzer and Champe (1962) proposed that the amber and ochre mutants of *E. coli* were responsible for the termination of protein synthesis at the site of the mutation. The altered codon was therefore presumed not to code for any amino acid. This assumption was confirmed by the demonstration that amber mutants of coat protein for T4 bacteriophage produced only fragments of that protein and that the fragment length was dependent upon the distance of the amber mutation from the original chain terminating codon for the gene.
The difference between ochre and amber mutants is based on the action of their suppressors. The amber triplet is UAG and the ochre triplet is UAA (Brenner et al., 1965; Weigart and Garen, 1965). An ochre mutant can be converted to an amber mutant by a base pair transition (adenine - thymine to guanine - cytosine), but the converse was not true (Gorini and Beckwith, 1966).

Separate genetic loci for both amber and ochre suppressors have been discovered in several laboratories (Signer, Beckwith and Brenner, 1965; Brenner and Beckwith, 1965; Garen et al., 1965; Henning et al., 1965; Eggertson and Adelberg, 1965).

Since nonsense codons terminate a polypeptide chain, nonsense suppressors allow the formation of the polypeptide chain beyond the termination point. This is accomplished by the insertion of an amino acid at the site of an ochre or amber mutation (Gorini and Beckwith, 1966).

**Large alterations**

These mutations are a result of a large deletion, duplication or rearrangement of genetic material with the resultant chromosome exhibiting inversions or translocations.

With rearrangements the number of base pairs have not been altered, but have either a chromosome segment that has been inverted or else two different segments have been exchanged (Drake, 1970). Duplications and deletions show a resultant increase or reduction, respectively in the number of loci on the chromosome. With
micro-organisms this type of macrolesion has been found to be due to exchanges between certain temperate phages and its bacterial hosts. The hybrids can be defective for certain genes. The chromosome portion exchanged was limited in locality and was not a 1:1 reciprocal exchange. That is, an equal region of viral DNA was not exchanged for an equal region of bacterial DNA.

Duplication mutations are less characterized than deletion mutations. No information was available to explain the mechanism of duplications however many models have been proposed (Drake, 1970).

Translocations and inversions have not been detected frequently enough in microbial systems to analyze their possible origins (Drake, 1970).

Deletions are known to occur spontaneously in bacteria (Coukell and Yanofsky, 1970) and bacteriophage. T4rII spontaneous mutations were found to be 12 percent deletions (Benzer, 1959; Folsome, 1962).

The mechanisms behind deletions may be due to errors in DNA replication, in genetic recombination and in DNA repair (Drake, 1970).

Errors in DNA replication are known to arise as a result of a disruption in the binding between the parental DNA template and the DNA polymerase. If the polymerase was removed from the template, there were two possible results (i) if the polymerase reattaches itself on the template at a point farther from its point of initial attachment, then a deletion mutation was formed (ii) if the polymerase reattaches itself on the template at a point closer than its point of initial attachment, then a duplication arose. This type of error
was not dependent upon homologous chromosomes (Drake, 1970).

Error in genetic recombination was dependent upon intrachromosomal and interchromosomal homology—large enough to exhibit aberrant recombinants. Recombination occurs as postulated by Campbell in 1962 for the release of an episome from a bacterial chromosome. It also occurs in transduction where interchromosomal homology exist between the phage and bacterial chromosome. The recombinants would be either a duplication or a deletion since exchanges between viral and bacterial chromosomes are not quantitatively equal.

Errors in repair was dependent upon intrachromosomal homology and repair mechanism deficiency. The mechanism could be due to the excision of a portion of the single DNA strand and during the repair processes the DNA opposite the excised portion forms a loop. This brings the two ends of the interrupted strand together and reannealing of these ends take place, thereby forming a deletion.

Mutagenic and Carcinogenic Studies

Legator et al. (1969) found a ten fold increase in mutation frequency in E. coli from streptomycin dependence to independence with captan concentration of 1,000 mcg per disc. Captan was also shown to induce chromosome breaks in heteroploid human embryonic lung cell line and a cell line derived from the kidney of the rat kangaroo (Protorus tridactylis).

Legator and coworkers (1970) using a histidine auxotroph of Salmonella typhimurium tested captan in in vitro and in vivo (Host
Mediated Assay) tests. Their results showed no increase in reversions for both tests.

Malling and deSerras (1970) using Neurospora crassa found that captan induces forward mutations in the ad-3 region of a two component heterokaryon but no reverse mutations in a series of tester strains.

Ficsor and Nii (1970) reported captan induced reversions in various auxotrophs of E. coli and S. typhimurium.

Epstein et al. (1968) using dominant lethal tests in mice found no positive results with captan when administered orally or intra-peritoneally.

Siebert et al. (1968) tested 14 fungicides for genetic activity in Saccharomyces cervisiae. The test systems employed were the induction of mitotic gene conversion at two different loci and the induction of cytoplasmic respiratory deficient mutants. Captan in its commercial form was one of the fungicides tested and was found to weakly induce mitotic gene conversion but did not induce cytoplasmic respiratory deficient mutants.

Steckeral et al. (1965) found captan to inhibit mouse ascites tumor. 0.15 grams per kilogram body weight was injected intra-peritoneally for 14 days in white Swiss mice. The day before inoculation with captan began the mice were injected with $2 \times 10^6$ ascites tumor cells. With an injection of captan the mean survival of mice inoculated with the tumor cells were increased from 26 to 80 days. The investigators also discovered that all mice treated with captan had a solid tumor on autopsy. This was attributed to
the injection site in the abdominal wall.

Teratogenic Studies

Kennedy et al. (1968) using captan in its commercial preparation found it to be nonteratogenic in two strains of rabbits selected for detecting embryotoxic effects of thalidomide. No abnormalities were found in offsprings of albino rats given captan either during gestation only or over three consecutive generations. Captan given to hamsters throughout gestation did not produce any abnormal young.

Robens, in 1970 studied the teratogenic effect of Captan, Folpet, Difolatan and Hercules 14503 in the golden hamster. The effects of single administration were compared with the effects of repeated administration throughout organogenesis. Single administration was found to be a more sensitive method of detection of teratogenic effects than multiple administrations. Multiple administrations of captan at toxic levels produced maternal mortality or fetal resorption.
METHODS AND MATERIALS

Bacterial Strains

Strains used throughout this experiment with their relevant characteristics are listed in Table I. Following the identification of captan as a mutagenic agent further quantitative studies were done with a set of six *Salmonella typhimurium* tester strains obtained from B. Ames, Department of Biochemistry, University of California at Berkeley. These *Salmonella typhimurium* strains were chosen for their ability to detect mutations by base substitution, positive and negative frameshift mutations in a background of normal or excision repair deficient mechanisms (Table I).

Neurospora Strain

*Neurospora crassa* wild type was obtained from The Fungal Genetics Stock Center, Dartmouth College, Department of Biological Sciences, Hanover, New Hampshire.

Media

Bacterial media

The media used for growth of cultures was nutrient broth containing 8 grams Difco Nutrient Broth in one liter of water.

The minimal media contained a salt solution, 2 percent Difco agar and supplemented with 0.2 percent glucose or 0.2 percent lactose.
One liter of minimal salt solution contained 9.85 g K$_2$HPO$_4$, 4.22 g KH$_2$PO$_4$, 0.94 g (NH$_4$)$_2$SO$_4$, 0.44 g sodium citrate and 0.05 g MgSO$_4$.

Those strains obtained from B. Ames were plated on enriched minimal media containing a trace (0.1 $\mu$ mole per plate) of histidine and an excess (0.1 $\mu$ mole per plate) of biotin (1971) and 0.2 percent glucose. Survival was assayed on nutrient agar (Difco) plates. The top agar used contained 0.6 percent agar and 0.5 percent NaCl.

**Neurospora media**

One liter minimal salt solution contained 1.0 g KH$_2$PO$_4$, 0.5 g MgSO$_4$, 0.1 NaCl, 0.01 g CaCl$_2$ and 1.0 g KNO$_3$. The minimal plates also contained 2 percent agar, 0.02 percent glucose and 0.005 g biotin.

**Methods of Treatment and Scoring of Survival and Mutations**

A list of pesticides tested are given on Tables II and III. Those listed in Table II were purchased from a local hardware store. Chemicals listed in Table III were supplied by Dr. W. F. Grant and technical captan (98.8 percent) was supplied by Stauffer Chemical Company.

**Bacterial plate assay**

The pesticides were tested by the bacterial plate assay as described by Iyer and Szybalski (1958). They developed two methods (paper disc and membranes) for testing chemical mutagenicity in
bacteria. These methods surpass the Demerec method of liquid culture treatment in ease of application (Demerec et al., 1951; Hemmerly and Demerec, 1955). Szybalski subsequently omitted the use of paper discs and membranes, and placed the test chemical directly on the agar medium with no decrease in mutagenic response.

Quick screening of the pesticides was done through the method of direct application of the pesticide to the agar medium. Sterile distilled water or one percent dimethylsulfoxide (DMSO) was added to the pre-weighed pesticide and agitated for about two minutes. A suspension was used in most instances, however, emulsions, solutions of the pesticide, crystals, powder or drops of the undiluted pesticides were also used.

An overnight culture of cells were grown in nutrient broth at 37°C in a water bath shaker. 0.1 ml amount of this cell suspension was spread over the surface of the appropriate medium to form a lawn of bacteria containing about 1 - 2 x 10^8 cells. The plates were dried and spotted with a drop of the pesticide. Depending on experimental runs, 1 - 4 0.05 ml drops of the pesticide were placed. The control plate contained a drop of sterile distilled water or a drop of the solvent employed in the test.

If only one spot of the pesticide or mutagen was used per plate, mutagenic effect was determined by counting the number of colonies per plate less the number of spontaneous revertants. If more than one spot was used per plate, mutagenic effect was determined by a halo of revertants around the spot. The plates were incubated at...
37° C and the revertants scored at 24, 48 and 72 hours of incubation.

If a pesticide was found to induce reversions quantitative studies were done through liquid culture treatment.

Liquid culture treatment

The pesticide suspension was prepared as follows: pyrol was added to the pre-weighed pesticide and agitated till the pesticide was uniformly wet with pyrol. To this slurry minimal salt solution was added for a final one percent pyrol solution. Further dilutions of the mutagen was done under continuous stirring until a treatment media containing the desired amount of pesticide was produced.

Cells were grown in nutrient broth overnight in a water bath shaker at 37° C. 5 ml samples were harvested by centrifugation and were resuspended in sterile nutrient broth. The desired amount of pesticide was then added to the suspended cells and the treatment mixture was then incubated at 37° C for 4 hours under continuous shaking. Control treatment media contained 0.1 percent pyrol as a final concentration but no pesticide. Following treatment the cells were washed once and were concentrated five fold in minimal salt solution. 0.1 ml samples of this suspension were inoculated in top agar and plated on appropriate media. Revertant colonies were scored as 24, 48 and 72 hours of incubation.

Induced mutation frequency was calculated as \( M_i - M/N_i \) where \( M_i \) are the number of mutant colonies per plate for treated cells, \( M \) the number of spontaneous mutations and \( N_i \) denotes the number of
surviving colonies plated (Kondo et al., 1970).

Determination of the Effect of Temperature on Mutagenicity and Fungitoxicity of Captan

A suspension was prepared by adding sterile distilled water to the pre-weighed captan and agitated for about two minutes. The suspension was divided into three parts of which one was allowed to stand at room temperature; the second sample was steam sterilized for 15 minutes (at about 100° C); and the third was autoclaved for 15 minutes at 15 pounds pressure (at about 121° C). After cooling, captan was spot tested on bacteria and conidia of N. crassa.

Mutation frequency in bacteria was determined as described for bacterial plate assay.

N. crassa was used in the fungitoxic determination. The conidia was suspended in minimal media. The plates were dried and the chemical was spotted in the middle of the plate. Fungitoxicity was determined by measuring the diameter of clearing around the spot in millimeters.

Dilutions of the pesticide were made to indicate the effective range of the pesticide.
<table>
<thead>
<tr>
<th>Strain*</th>
<th>Genotype</th>
<th>Mutation</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NG422</td>
<td>lac&quot;</td>
<td>amber</td>
<td>A. Newton</td>
</tr>
<tr>
<td>YA482</td>
<td>lac&quot;</td>
<td>amber</td>
<td>A. Newton</td>
</tr>
<tr>
<td><strong>S. typhimurium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B12</td>
<td>cys&quot;</td>
<td></td>
<td>P. Hartman</td>
</tr>
<tr>
<td>AP517</td>
<td>leu&quot;</td>
<td></td>
<td>S. Friedman</td>
</tr>
<tr>
<td></td>
<td>ara9&quot;</td>
<td></td>
<td>S. Friedman</td>
</tr>
<tr>
<td>5BU</td>
<td>leu&quot;</td>
<td></td>
<td>S. Friedman</td>
</tr>
<tr>
<td></td>
<td>ara9&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G46</td>
<td>his&quot;</td>
<td>missense</td>
<td>B. Ames</td>
</tr>
<tr>
<td>TA1530</td>
<td>his&quot;</td>
<td></td>
<td>B. Ames</td>
</tr>
<tr>
<td></td>
<td>gal&quot;</td>
<td></td>
<td>contains G46</td>
</tr>
<tr>
<td></td>
<td>bio&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>uvrB&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C207</td>
<td>his&quot;</td>
<td>frameshift(-)</td>
<td>B. Ames</td>
</tr>
<tr>
<td>TA1531</td>
<td>his&quot;</td>
<td></td>
<td>B. Ames</td>
</tr>
<tr>
<td></td>
<td>gal&quot;</td>
<td></td>
<td>contains C207</td>
</tr>
<tr>
<td></td>
<td>bio&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>uvrB&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3076</td>
<td>his&quot;</td>
<td>frameshift(+)</td>
<td>B. Ames</td>
</tr>
<tr>
<td>TA1532</td>
<td>his&quot;</td>
<td></td>
<td>B. Ames</td>
</tr>
<tr>
<td></td>
<td>gal&quot;</td>
<td></td>
<td>contains C3076</td>
</tr>
<tr>
<td></td>
<td>bio&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>uvrB&quot;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations in the strain designations indicate that the mutant was induced by: NG=nitrosoguanidine, YA=nitrogen mustard, AP=amino purine, 5BU=5 bromo uracil, B12=uv, G46=spontaneous, C207=x-rays.
### TABLE II.
Trade names, composition and manufacturers of pesticides tested.

<table>
<thead>
<tr>
<th><strong>Trade Name</strong></th>
<th><strong>Active Ingredients(%)</strong></th>
<th><strong>Manufacturer</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotox Garden Spray</td>
<td>Lindane (5) Malathion (10) Dichloro Diphenyl trichloroethane (5) Tetrachloro (3) Aromatic petro solvent (20)</td>
<td>Ortho</td>
</tr>
<tr>
<td>Borer Spray</td>
<td>Lindane (20) Aromatic petro solvent (59)</td>
<td>Ortho</td>
</tr>
<tr>
<td>Fatsco Ant Poison</td>
<td>Sodium arsenate (3)</td>
<td>Fatsco</td>
</tr>
<tr>
<td>Ortho-Klor 74 Chloride Spray</td>
<td>Octachloro-4,7-methano-tetrohydridiane (44.4) Related cpds. (29.6) Petroleum distillate (22)</td>
<td>Ortho</td>
</tr>
<tr>
<td>OFF Insect Repellent</td>
<td>N,N-diethyl toluamide (50)</td>
<td>Raid</td>
</tr>
<tr>
<td>Malathion 50 Spray</td>
<td>Malathion (50) Aromatic petro deriv. (33)</td>
<td>Ortho</td>
</tr>
<tr>
<td>Chickweed and Clover Killer</td>
<td>Iso-octyl ester of Silvex [2-(2,4,5-trichlorophenoxy) propionic acid] (13.8)</td>
<td>Ortho</td>
</tr>
<tr>
<td>Pest-B-Gon</td>
<td>DDT (50)</td>
<td>Ortho</td>
</tr>
<tr>
<td>Weed-B-Gon</td>
<td>Dimethyl amine salt of 2,4-dichlorophenoxy acetic acid (12) Dimethyl amine salt of dicamba (2.4)</td>
<td>Ortho</td>
</tr>
<tr>
<td>Dormant Spray</td>
<td>Calcium polysulfides (4.6) Petroleum oils (58)</td>
<td>Ortho</td>
</tr>
<tr>
<td>Trade Name</td>
<td>Active Ingredients(%)</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>-----------------</td>
<td>------------------------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Liquid Crab</td>
<td>Octyl ammonium methyl arsonate (8)</td>
<td>Ortho</td>
</tr>
<tr>
<td>Grass Killer</td>
<td>Dodecyl ammonium methyl Arsonate (8)</td>
<td></td>
</tr>
<tr>
<td>Home Orchard</td>
<td>Captan (15)</td>
<td>Ortho</td>
</tr>
<tr>
<td>Spray</td>
<td>Malathion (7.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methoxychlor, technical (15)</td>
<td></td>
</tr>
<tr>
<td>Tomato Vegetable</td>
<td>Captan (5)</td>
<td>Ortho</td>
</tr>
<tr>
<td>Dust</td>
<td>Methoxychlor, technical (5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rotenone from cube (.75)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other resins from cube (.75)</td>
<td></td>
</tr>
<tr>
<td>Isotox Insect</td>
<td>Carbaryl (5)</td>
<td>Ortho</td>
</tr>
<tr>
<td>Spray</td>
<td>0,0-Dimethyl S-2-(ethyl sulfinylethyl) phosphorothioate (5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,1-bis (p-chlorophenyl)-2,2,2-trichloroethanol (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aromatic petro solvent (18)</td>
<td></td>
</tr>
<tr>
<td>Trade Name</td>
<td>Active Ingredients (%)</td>
<td>Source</td>
</tr>
<tr>
<td>------------</td>
<td>------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Banvel D</td>
<td>2-methoxy-3,6-dichloro-benzoic acid; dimethyl-amine salt of related acids (57)</td>
<td>Velsicol Chem. Corp.</td>
</tr>
<tr>
<td>Cytrol</td>
<td>3-amino-1,2,4-triazole (38.2 oz. per Imperial gallon)</td>
<td>Cyanamid of Canada Ltd.</td>
</tr>
<tr>
<td>Hyvar X</td>
<td>5-bromo-3-secbutyl-6-methyl uracil (80)</td>
<td>DuPont of Canada Ltd.</td>
</tr>
<tr>
<td>Atrazin</td>
<td>2-chloro-4-ethylamino-6-isopropyl amine-S-triazine (50)</td>
<td>Fisons (Canada) Ltd.</td>
</tr>
<tr>
<td>Simazin</td>
<td>2-chloro-4,6-bis (ethyl-amino)-S-triazine (50)</td>
<td>Fisons (Canada) Ltd.</td>
</tr>
<tr>
<td>Botran</td>
<td>2,6-dichloro-4-nitroaniline (50)</td>
<td>Upjohn Co. of Canada</td>
</tr>
<tr>
<td>Lorox</td>
<td>3-(3,4-dichlorophenyl)-(methoxyyl-1-methylurea) (50)</td>
<td>DuPont of Canada Ltd.</td>
</tr>
</tbody>
</table>

Pesticides received from Dr. W. F. Grant, McGill University at McDonald College, Quebec, Canada.

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RESULTS

Identification of Mutagenic Pesticides

Testing of the fourteen pesticides and of the mutagen nitroso-guanidine (NG) with the bacterial plate assay method have shown (Table IV) that two (Tomato Vegetable Dust and Ortho Home Orchard Spray) of the fourteen randomly picked pesticides were mutagenic. Since Ortho Home Orchard Spray reverted only one strain in one experiment there was some doubt as to whether it was mutagenic or not. A later experiment (below) however confirmed the mutagenicity of this product. As expected NG was mutagenic in all strains and in all experiments.

Identification of the Mutagenically Active Ingredients of Tomato Vegetable Dust

Of the four active ingredients of Tomato Vegetable Dust (Table IV), three compounds, captan, Methoxychlor and Rotenone were obtained. Another pesticide, Ortho Home Orchard Spray, which contained captan, was retested in cys B12 of Salmonella typhimurium. The results of this experiment are summarized in Table V. At least four aspects of Table V should be mentioned. The first is that, of the ingredients of Tomato Vegetable Dust, only captan was mutagenic. The second observation is that Ortho Home Orchard Spray was definitely mutagenic. The third observation is that per milligram of captan per plate Tomato Vegetable Dust (15 percent captan) induced the most
mutations followed by Captan 50WP (50 percent captan) and Ortho Home Orchard Spray (15 percent captan). This difference is perhaps due to captan being more or less soluble in the different products. Finally the fact that in Tomato Vegetable Dust and Ortho Home Orchard Spray, captan is the only common ingredient (aside from Methoxychlor which was not mutagenic when tested alone) indirectly confirmed captan as their mutagenic component.

With the identification of captan as a likely mutagenic agent, further studies were done only with captan. The form of captan used was either its commercial preparation (Captan 50WP) or as 98.8 percent pure technical captan.

The Effect of Temperature on the Mutagenicity of Captan in Bacteria

A lac mutant of E. coli and a cys mutant of Salmonella typhimurium were used. In Table IV the lac mutant showed a five fold decrease in revertants following steam sterilization of Captan 50WP at 100\(^\circ\) C for 15 minutes when compared to captan that was left at room temperature for the same length of time. During autoclaving for 15 minutes (121\(^\circ\) C) the mutagenicity of captan decreased 21 folds over the room temperature treatment. The cys B12 mutant also exhibited a decrease in revertants with increasing heat treatment of captan. The decrease was not as great as that shown by the lac mutant, however. There was a three fold decrease when captan was steam sterilized and a two fold decrease when it was autoclaved as compared to the captan left at room temperature.
In both strains all treatment conditions increased the number of revertants over control levels.

The Effect of Temperature on the Fungitoxicity of Captan in *Neurospora*

Figure 1a and 1b show the fungitoxic relationship of heat treated captan and captan left at room temperature (25° C) after 20 hour incubation and 48 hour incubation, respectively. Three concentrations (in ppm) of captan was also compared.

Room temperature was designated as control with 100 percent fungitoxic effect.

As the temperature of the heat treated captan increases the fungitoxicity decreases. It also shown that the lower the concentration of captan the greater the decrease in fungitoxic effect irrespective of the time of incubation. The 100 percent fungitoxic effect of 100° C treated $5 \times 10^2$ ppm captan for both incubation times has been attributed to experimental error.

There is also a recovery of the inhibitory effects from 20 hours incubation to 48 hours incubation. After 48 hours incubation with 121° C (autoclaved) treated captan while at 20 hours incubation the inhibitory effects were still considerably high.

In both incubation times and with all heat treatments, 50 ppm captan showed no fungitoxic effects (not shown on graph).
Mutagenic Response of Known Mutagenic
Pesticides to his\(^{\ast}\) Tester Strains

The first eight pesticides in Table VII have been previously shown to induce chromosomal aberrations in meiotic cells of barley (Wuu and Grant, 1967). They were now tested in six Salmonella typhimurium tester strains with no positive mutagenic response.

The cys mutant was used as a control since captan induced reversions in this strain previously. Cys B12 did not give a positive mutagenic response with these pesticides.

The only tester strains giving positive mutagenic response to all three forms of captan were G46 and TA1530. His G46 is a missense mutant while TA1530 contains G46 and is gal\(^{-}\), bio\(^{-}\) and uvrB\(^{-}\).

Technical captan (98.8 percent) was used for the first time in this experiment. It was found that technical captan is very insoluble in minimal salt solution or Tris Buffer but the commercial preparations of captan appeared to be more soluble in these solvents. An attempt was made to increase the solubility of technical captan using various organic solvents but at the same time insuring that the solvent was not lethal to the bacterial system. Dimethyl sulfoxide (DMSO) was used by Legator et al. (1969) in their work with captan. A one percent solution of this solvent was used during the studies up to this point i.e. spot testing the pesticides but with unsatisfactory results in the DMSO did not increase mutation frequency over technical captan applied with water or buffer alone. M-pyrol (methyl pyrrolidone) was reported to solubilize 30 percent captan (Benson,
personal communication). M-pyrol was generously supplied by GAF Corporation and preliminary experiments showed that pyrol had no effect on survival and mutation frequency at those pyrol concentrations used in further experiments. It is not known whether pyrol increased the solubility of captan in the bacterial test system but it helps to obtain a fine suspension to work with.

Further quantitative studies were done using His G46 and a one percent solution of pyrol.

Mutation and Survival Kinetics of Technical Captan and Captan 50WP

Figure 2 shows that as the amount of technical captan in the treatment media increases, the mutation frequency increases linearly. Survival however, remains at essentially 100 percent.

Captan 50WP (Figure 3) exhibits a different response to increasing amounts of captan in the treatment media. There is an increase in mutation frequency with a plateau between 50 ppm and 75 ppm followed by a gradual decrease. Survival is decreased to approximately 50 percent and remains at this level up to 150 ppm beyond which there is a further decrease.
TABLE IV.
Mutagenic response of _S. typhimurium_ and _E. coli_ mutants to twelve pesticides with the bacterial plate assay.

<table>
<thead>
<tr>
<th>Trade Name</th>
<th>lac- NG422</th>
<th>lac- YA482</th>
<th>cys- B12</th>
<th>leu- AP517</th>
<th>leu- 5BU504</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotox Garden Spray</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Borer Spray</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fatsco Ant Poison</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ortho-Klor 74 Chlordane Spray</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>OFF Insect Repellent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Malathion 50 Spray</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chickweed and Clover Killer</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pest-B-Gon</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Weed-B-Gon</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dormant Spray</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Home Orchard Spray</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tomato Vegetable Dust</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Isotox Insect Spray</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Liquid Crab Grass Killer</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = halo of revertants around spot of pesticide.
- = no halo of revertants on lawn of bacteria around spot.
TABLE V.
Mutagenic response of \( \text{cys}^- \) Bl2 to three captan containing and two non-captan pesticides with the bacterial plate assay.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mg. of captan per plate</th>
<th>Total No. of revertants per plate*</th>
<th>Percent of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.00</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Captan 50WP</td>
<td>2.50</td>
<td>175</td>
<td>500</td>
</tr>
<tr>
<td>Rotenone</td>
<td>0.00</td>
<td>27</td>
<td>77</td>
</tr>
<tr>
<td>Methoxychlor</td>
<td>0.00</td>
<td>23</td>
<td>66</td>
</tr>
<tr>
<td>Tomato Vegetable Dust</td>
<td>0.25</td>
<td>259</td>
<td>743</td>
</tr>
<tr>
<td>Ortho Home Orchard Spray</td>
<td>0.75</td>
<td>112</td>
<td>320</td>
</tr>
</tbody>
</table>

*Number of \( \text{cys}^+ \) colonies counted after 4 days incubation. Approximately \( 2 \times 10^8 \) \( \text{cys}^- \) cells per plate.
TABLE VI.
Effect of temperature on mutagenicity of captan in bacteria.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment Condition</th>
<th>Total No. of Revertants per plate</th>
<th>Percent of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>lac&lt;sup&gt;-&lt;/sup&gt; NG422</td>
<td>Control</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Room</td>
<td>736</td>
<td>4090</td>
</tr>
<tr>
<td></td>
<td>100° C</td>
<td>152</td>
<td>845</td>
</tr>
<tr>
<td></td>
<td>121° C</td>
<td>34</td>
<td>188</td>
</tr>
<tr>
<td>cys&lt;sup&gt;-&lt;/sup&gt; B12</td>
<td>Control</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Room</td>
<td>947</td>
<td>6770</td>
</tr>
<tr>
<td></td>
<td>100° C</td>
<td>345</td>
<td>2460</td>
</tr>
<tr>
<td></td>
<td>121° C</td>
<td>457</td>
<td>3270</td>
</tr>
</tbody>
</table>

A suspension of captan was divided into three parts of which one was left at room temperature; the second was steam sterilized (100° C) for 15 minutes; the third was autoclaved (121° C) for 15 minutes at 15 pounds pressure. Upon cooling the three temperature treated samples were tested by the bacterial plate assay method.

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Fig. 1. EFFECT OF TEMPERATURE ON FUNGITOXICITY OF CAPTAN IN NEUROSPORA. (la) the fungitoxic effect after 20 hrs. incubation; (lb) the fungitoxic effect after 48 hrs. incubation at various concentrations (ppm) of captan. Values are an average of 3 plates. ●—● $5 \times 10^4$ ppm; ○—○ $5 \times 10^3$ ppm; □—□ $5 \times 10^2$ ppm.
Fig. 2. EFFECT OF CONCENTRATION OF TECHNICAL CAPTAN ON SURVIVAL AND MUTATION FREQUENCY. Mutation frequency (total number of His\(^+\) revertants per 10\(^8\) survivors) and survival (in percent) as a function of treatment dosage, in ppm, of captan. Data from an average of two independent experiments. Average control survival per plate: 19 x 10\(^8\). Average control mutation frequency per 10\(^8\): 0. ●——mutation frequency. ○——survival.
**TABLE VII.**
Mutagenic response of *S. typhimurium* his" mutants to eleven pesticides with the bacterial plate assay.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>G46</th>
<th>TA1530</th>
<th>C207</th>
<th>TA1531</th>
<th>C3076</th>
<th>TA1532</th>
<th>B12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banvel D</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cytrol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hyvar X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Atrazin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Simazin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alanap-3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Botran</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lorox</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Technical captain</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Captan 50WP</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tomato Veg. Dust</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = halo of revertants around spot of pesticide.
- = no halo of revertants around spot of pesticide.
Fig. 2. EFFECT OF CONCENTRATION OF TECHNICAL CAPTAN ON SURVIVAL AND MUTATION FREQUENCY.
Mutation frequency (total number of His$^+$ revertants per $10^8$ survivors) and survival (in percent) as a function of treatment dosage, in ppm, of captan. Data from an average of two independent experiments. Average control survival per plate: $19 \times 10^8$. Average control mutation frequency per $10^8$: 0. ●—● mutation frequency. □—□ survival.
Fig. 3. EFFECT OF CONCENTRATION OF CAPTAN 50WP ON SURVIVAL AND MUTATION FREQUENCY.
Mutation frequency (total number of His$^+$ revertants per $10^8$ survivors) and survival (in percent)
as a function of treatment dosage, in ppm, of captan. Data from an average of three independent
experiments. Average control survival per plate: $12 \times 10^8$. Average control mutation frequency
per $10^8$: 0. •—• mutation frequency. □—□ survival.
DISCUSSION

Plate Assay for Quick Screening of Mutagenic Compounds

The data presented here supports the idea that measurement of reversion of auxotrophs or fermentation markers with the bacterial plate assay is a convenient method of screening chemicals for mutagenicity (Iyer and Szybalski, 1958; Ames, 1971).

In contrast to the liquid culture method the bacterial plate assay appears to be superior not only by its simplicity but also because a concentric concentration gradient is established in the agar medium part of which provides maximum mutagenic effectiveness (Iyer and Szybalski, 1958; Ames, 1971). With liquid culture treatment techniques a great degree of effort needs to be expended before a concentration giving maximum mutagenic effectiveness is determined. Fewer chemicals could be screened for mutagenicity with the liquid culture treatment because of the increased time and expense involved than with the plate assay method. Thus it can be concluded that the plate assay method is superior to the liquid culture treatment technique if the purpose is to screen a large number of chemicals for mutagenicity. On the other hand if the purpose is to determine survival and mutation kinetics for a chemical previously identified as a chemical mutagen then liquid culture treatments need to be performed since the plate assay technique is difficult to quantify. It should be noted however that negative results with these methods

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do not assure that all mutagens hazardous to higher organisms will be detected since higher organisms may take up, detoxify, excrete or repair initial genetic damage differently than bacteria.

Organismal differences to chemical mutagens do exist and is shown by the data presented in Table VII. The pesticides have been shown to induce chromosomal aberrations in mitotic and meiotic cells of barley (Wuu and Grant, 1966; 1967). Some of these pesticides also induced mutations that were transmissible to the $C_2$ generation when induced premeiotically in the $C_1$ generation. None of these pesticides proved to be mutagenic in the bacterial strains employed in this experiment (Table VII). The difference may be more apparent than real since in barley chromosomal aberrations were scored while in the bacterial systems only base substitutions were detected. In the case of transmissible mutations in barley, they may or may not be due to base substitutions. Thus one can only conclude that the two systems are not directly comparable.

The check chemical, nitrosoguanidine in Table IV induced mutations in strains that revert by base substitutions and is also known to induce mutations in barley, maize, Arabidopsis and many other plants (probably by base substitutions as well as chromosomal aberrations). In the case of nitrosoguanidine there is a positive correlation between the ability of this chemical to induce mutations in higher and lower organisms. This correspondence is not limited to nitrosoguanidine. In general strong mutagens such as x-rays, uv, ethyl methane sulfonate, methyl methane sulfonate and diethyl sulfate are mutagenic in many procaryotic and eucaryotic organisms (Fishbein
et al., 1970). In view of these considerations one can conclude that the chemicals included in Table VII and the non-mutagenic chemicals in Table IV are either non-mutagens or if mutagenic they are extremely mild mutagens.

Captan was found to be a mild mutagen thus it is not surprising that this chemical shows a poor consistency of mutagenicity in the different species tested. In Neurospora, captan was mutagenic as found by Malling and deSerres (1970). In tissue cultures of heteroploid human embryonic lung and rat kangaroo kidney (Legator, Kelly, Green and Oswald, 1969) captan induced a low frequency of chromosomal aberrations but in corn captan induced no mutations (Ficsor, Janca and VanHook, 1970). Even with the same organism some discrepancy may occur with the same chemical in detecting mutagenicity. For example Legator and coworkers using a his– auxotroph in their work in 1970 found that captan did not induce reversions in both in vitro and in vivo (Host Mediated Assay) tests. The same mutant has responded to captan in the present experiments.

Technical Captan Induces Low Mutation Frequency Without Killing

Technical captan does not have any bactericidal effect for the doses tested (Fig. 2). Technical captan also shows increasing mutation frequency as the amount of captan increases in the treatment media. Since mutation frequency is based on the number of surviving cells this shows the type of mutation kinetics expected if survival is not affected. The low mutation frequency following a first order
kinetics exhibited by captan in the dose-response experiments may be due to (i) that the \(-\text{SCCl}_3\) group or other metabolite(s) of captan are changed to nonmutagens (ii) that captan is relatively insoluble in the treatment media used.

Evidence for the \(-\text{SCCl}_3\) group being detoxified is available in fungi. Lukens (1966) reported that the \(-\text{SCCl}_3\) group is decomposed by the fungal cell thiols to thiophosgene which in turn can react with other cell thiols. Miller (1969) reports that much of the captan is detoxified before any sensitive receptor sites are reached in fungi. Thus high doses of captan are needed on a conidia weight basis to inhibit germination of the fungi. Thiol groups are also present in bacterial cells so if \(-\text{SCCl}_3\) is the alkylating moiety of captan it is possible that the bacterial cell thiols can decrease the mutagenicity of captan as well.

The solubility of captan has been reported to be as low as 0.5 ppm and as high as 10 ppm in water depending on differing degrees of hydrolysis (Lukens, 1969). Dugger (1959) reported the solubility of 99 percent captan in water to be less than 5 ppm at 25° C.

Legator and coworkers (1970) using the same \textit{Salmonella typhimurium} his\(^{-}\) G46 mutant used in these experiments detected no increase of reversions with captan in both \textit{in vitro} and \textit{in vivo} tests. This could be due to the poor solubility of the technical captan sample used.

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Mutagenicity and Fungitoxicity Decreases with Increasing Temperature

The results presented (Table VI, Fig. 1) show that steam sterilizing \((100^\circ C\) for 15 minutes) and autoclaving \((121^\circ C\) for 15 minutes and 15 pounds pressure) Captan 50WP decreases its mutagenic and fungitoxic effects. These temperature treated captan however still showed increased levels of mutation frequency over the controls. Zanardi (1959) autoclaved \((1.5\) atmospheres, 30 minutes) captan and found a one tenth decrease in efficiency of the fungicides' initial potential.

These results can be attributed to a greater degree of decomposition of captan by the increasing temperature. Kittleson (1953) reports that at \(200^\circ C\) rapid decomposition occurs while heating at \(100^\circ C\) for several days results only in gradual decomposition.

The recovery of *Neurospora* to the fungitoxic effects of captan after 20 hours incubation is probably due to further decomposition of captan on the plate and during incubation periods at \(37^\circ C\).

Captan 50WP More Mutagenic and Bactericidal than Technical Captan

As seen in Fig. 3 Captan 50WP exhibits an immediate decrease in percent survival and stabilizes at about 50 percent from 50 to 150 ppm of captan in the treatment media. This immediate decrease could be attributed to the toxic effects of captan and/or the inert ingredients.
Captan has been reported to be toxic not only to fungi, bacteria and higher plants but also insects (Susnovsky, 1958). It has been reported that ionic surface-active compounds that are present in fungicides can be toxic to microorganisms. It acts by physically disrupting the cell membrane or by inhibiting the enzymes present in the membrane (Newton, 1960).

The stabilization at approximately 50 percent from 50 to 150 ppm of captan in the treatment media could be the range in which the cell thiols are able to detoxify the captan entering the cell.

The mutation kinetics for Captan 50WP is drastically different from that obtained for technical captan (Fig. 3) in that with technical captan the dose-response is linear while it is bell shaped for Captan 50WP with a plateau of about 50 to 75 ppm. To interpret this mutation kinetics, detoxification, repair, mutation and selective killing of the his<sup>+</sup> phenotypes must be considered. At low concentrations of captan (under 25 ppm) the cell is able to repair initial DNA alkylations and thus the relatively slow increase of mutation frequency in response to increasing amounts of captan. At this concentration survival is nearly complete. This part of the curve is similar to the kinetics obtained for technical captan (Fig. 2). As the concentration of captan further increases a threshold level is reached beyond which the cell is unable to repair DNA alkylations as fast as captan enters the cell, hence the exponential increase in mutation frequency.

If lethality was unrelated to the recovery of his<sup>+</sup> revertants
one would expect a continued increase for his\textsuperscript{+} revertants as the concentration of the mutagen increased even at decreasing survival.

One finds, however, that at higher concentrations of captan the frequency of his\textsuperscript{+} revertants decreases. This indicates that with increased lethality his\textsuperscript{+} are killed selectively. Such selectivity may be due to uneven treatment of cells. Thus cells which receive the highest doses of the chemical are the most likely to mutate his\textsuperscript{-} to his\textsuperscript{+} and are also the ones most likely killed. In view of captans' poor solubility, such uneven treatment of the cell population is not altogether unexpected.

From comparing Figs. 2 and 3 it is apparent that the commercial form of captan (50 percent technical captan and 50 percent inert ingredient) is more mutagenic than technical captan. This comparison is based on equal amounts of captan in the treatment media. Since captan is insoluble and a suspension was worked with every precaution was taken to repeat the same conditions for both technical captan and Captan 50WP during the measurement into the treatment media. Therefore, the assumption of captan being present in equal amounts is valid to a certain degree.

This difference in mutagenicity may be due to (i) technical captan and the inerts acting synergistically (ii) the inerts themselves are mutagenic (iii) the presence of a more mutagenic form of captan in Captan 50WP than in technical captan.

Captan 50WP may be more mutagenic due to the synergism between technical captan and the inerts in uptake or mutagenicity and toxicity.
Inerts have been demonstrated in *in vitro* studies to act with fungicides in a synergistic relationship. They are postulated to act by affecting the cells permeability (Somers, 1967).

This synergism may also result in increased solubility of Captan 50WP. Fungicides are known for their low water solubility. Therefore inert ingredients such as attapulgite, kaolin, talc or wetting agents are used in commercial preparations to increase the water solubility of the fungicide (Somers, 1967).

An alternative possibility to explain the increased mutagenicity of Captan 50WP is that the inert ingredients themselves may be mutagenic. Inerts are generally of mineral composition and although these compounds are considered chemically "inert" (Somers, 1967) some have been shown to be toxic to foliage (Daines *et al.*, 1957). One then cannot exclude the possibility that they may be the mutagenic factor in Captan 50WP.

The third possibility is that the commercial product has a more mutagenic form of captan. The technical captan tested was not used in the formulation of the commercial product 50WP employed in the studies. It should be noted that technical captan contains some impurities that may be mutagenic and not captan. These impurities are a result of the synthesis of captan: the reaction between sodium $\Delta ^4$-tetrahydrophthalimide with perchloromethyl mercaptan, with captan precipitating out as a white solid. Further experiments are needed with chemically poor captan to exclude the possibility that some of the impurities in technical captan may be mutagenic.
Any one or a combination of the above conditions may contribute to the increased mutagenicity of Captan 50WP over technical captan.

**Mechanism of Action**

Since captan is equally mutagenic to replicating and non-replicating cells (Ficsor and Nii, 1970) and since it did not revert frameshift mutations (Table VII) it is concluded that captan is probably an alkylating agent. Mailing and deSerres (1970) also reported that captan is an alkylating agent in their work with *Neurospora*. Captan is a mild mutagen with relatively high levels of survival (Figs. 2 and 3). If captan is an alkylating agent it is most likely a monofunctional agent since these agents are known to produce less cytotoxicity and fewer lethal events per mutational event than the polyfunctional alkylating agents (Alderson, 1964; Fahmy and Fahmy, 1961).

Captan hydrolyzes into and -SCCl\textsubscript{3} groups, both of which may serve as an alkylating group. The rate of hydrolysis is dependent upon the stability of the sulfur bond. The ease of hydrolysis of this bond may be a measure of reactivity and stability of the compound since maximum fungitoxicity is associated with optimum stability of this bond (Lukens, 1966). Captan being an effective fungitoxic compound would therefore have relatively stable sulfur linkages.

Of the two hydrolysis products, it is likely that -SCCl\textsubscript{3} is the alkylating group since this group is also the toxophore. Direct chemical evidence of DNA alkylation by captan is not available.
CONCLUSIONS

1. Of the 22 pesticide products tested for mutagenicity with two species of enteric bacteria only two captan containing products were found to be mutagenic.

2. Technical captan induces low mutation frequency without killing.

3. Captan 50WP is more mutagenic and bactericidal than technical captan.

4. Mutagenicity and fungitoxicity decreases with increasing temperature.

5. Captan is probably a monofunctional alkylating agent.
LITERATURE CITED

Abedi, Z. H. and W. P. McKinley, 1967 Bioassay of captan (N-tri-
chloromethylthio-4-cyclohexene-1,2-dicarboximide) by zebrafish larvae

Ackerson, C. W. and F. F. Mussehl, 1955 Toxicity of treated seed

Alderson, T., 1964 Ethylation vs. methylation in mutation of E. coli

Ames, B. N. and H. J. Whitfield Jr., 1966 Frameshift mutagenesis in
Salmonella. Cold Spring Harbor Symposia on Quantitative Biology
31:221-225.

Ames, B. N., 1971 The detection of chemical mutagens with enteric
bacteria. pp. 267-282. In: Chemical Mutagens: Principles and
Methods for Their Detection, A. Hollaender (Ed.) Plenum Press,
New York.

Benson, A., Stauffer Chemical Company, personal communication.

Benzer, S., 1959 On the topology of the genetic fine structure.

Boyd, E. M., 1969 Dietary protein and pesticide toxicity in male

Boyd, E. M. and C. J. Krenjnen, 1968 Toxicity of captan and protein-

Brenner, S., A. W. W. Stretton and S. Kaplan, 1965 Genetic code; the
'nonsense' triplets for chain termination and their suppression.
Nature 206:994-998.

Brenner, S. and J. R. Beckwith, 1965 Ochre mutants, a new class of

Brenner, S., L. Barnett, F. H. C. Crick and A. Orgel, 1961 The

Benzer, S. and S. P. Champe, 1962 A change from nonsense to sense


Daines, R. H., 1953 Captan--the new discovery in fruit fungicides. Fruit Grower 73:16.


Malling, H. V., 1969 Ethylene dibromide: a potent pesticide with high mutagenic activity. Genetics 6:539 (abstr.).


Miller, P. M., 1957 Heat decomposition products of captan as phytotoxic agents. Phytopath. 47:245 (abstr.).


