Dithiocarbamate residues in soils and crops from garlic-growing areas of Lombok, Indonesia

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DITHIOCARBAMATE RESIDUES IN SOILS AND CROPS FROM GARLIC-GROWING AREAS OF LOMBOK, INDONESIA

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Dedication

For my wife Nunuk, my two lovely daughters Fina and Riri, my parents and for my brothers and sisters who never stop to encourage me
I hereby certify that, the experimental work described in this thesis was performed by me and has not been submitted previously for a degree at this or any other university.

Surya Hadi
28/04/95
This present study has shown that residues of dithiocarbamate fungicides persisted on/in soils and garlic bulbs on garlic-growing areas of Sembalun, Lombok island, Indonesia. The technique of analysis used was USEAP-Method S 15, based on evolution of carbon disulfide which was measured as a xanthate. The study indicated that the dithiocarbamate residues mainly persisted on soil surface (0-1 cm); ranging from 1.82 to 4.82 mg/kg dry matter and only a low concentration was found at a depth of 1-10 cm. The analysis of garlic bulbs revealed that all sampled garlic bulbs were contaminated with dithiocarbamate residues, with concentrations ranging from 8.95 to 31.26 mg/kg dry matter. Some localities showed consistently higher concentration than other localities. However, the residues tended to decrease exponentially, and within 21 days of harvesting were 0.78 and 1.73 mg/kg dry matter. This study also proved that the 'xanthate technique' was precise and reproducible for analysing dithiocarbamates from soil and garlic bulbs, even though garlic bulbs contain an unknown compound producing carbon disulfide in a relative low concentration (0.17±0.01 mg/kg dry matter).
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CHAPTER ONE

INTRODUCTION

1.1 General

Products of commercial dithiocarbamate fungicide are used intensively for the control of fungus diseases of certain crop such as vegetables, fruits and ornamental crops. However, Graham and Hansen (1972) have reported that ethylenethiourea (ETU), a possible degradation product of the dithiocarbamate fungicides, is carcinogenic to rats. Khera (1973) also studied ETU administration to rats in varying doses and on a daily basis and found that a teratogenic effect was seen at 10 mg g\(^{-1}\). These indicate that dithiocarbamate fungicides may also pose health risks for humans and other organisms who consume products coated with the fungicides.

At Sembalun, the largest garlic-producing area of Lombok, Indonesia, the dithiocarbamate fungicide products are major pesticides heavily used by the farmers in order to suppress the fungus *Alternaria porii* (ELL.) Clif., the causal agent of purple blotch disease. Parman (1993) reported that the use of fungicides in the Sembalun areas has been at far higher concentrations than the recommended levels. For example, the use of Antracol 70 WP, a commercial fungicide product, has been applied two to three times a week at concentration two to three times higher than the
recommended dosage so that the use of Antracol 70 WP has reached a hundred kilogram per hectare per planting.

Based on overuse of the fungicide, it was suspected that dithiocarbamate residues may persist in soils and garlic. Because of concern about this problem, residue analysis, based on the evolution of carbon disulfide, was carried out to monitor the residues throughout the Sembalun areas.

1.2 Application of Fungicide at Sembalun Areas

In 1983 at Sembalun, pesticides were first introduced by the government to the farmers following intensively used fertilizers. By 1985 most farmers were using the fungicides and fertilizers on their land. As a result, garlic production had increased, which in turn led to increased farm income. The highest production per hectare (8.75 tonne dry bulb) was reached in 1987 (Mustiadi et al., 1994).

In order to maximize production and price offered, the farmers have continued to cultivate the crop intensively and monoculturally. The intensive and monocultural cultivations led to an explosive population growth of the insect Thrips tabaci Lind., a key pest of garlic, and of the fungus Alternaria porii (Ell.) Clif., the causal agent of the purple blotch disease, a destructive disease of garlic. As a consequence, pesticide/fungicide demand has gone up dramatically. For example, in 1985 the need of pesticide/fungicide was about 75 tonnes, in contrast, the requirement reached 800
tonnes for the Sembalun villages in 1993 (Mustiadi et al., 1994). This condition has been worsened by the farmers not having been trained in the correct use of pesticides/fungicides so that the use of these chemicals is largely uncontrolled.

At Sembalun, many types of commercial products have been marketed. However, there are six fungicide products commonly used by the farmers namely Dithane M-45 (Mancozeb 80%), Antracol 70 WP (Propineb 70.5%), Delsen MX-200 (Carbandazim 6.2% and Mancozeb 73.8%), Manzate 200 (Mancozeb 80%), and Phycozan 70 WP (Mancozeb 70%). It can be seen that all of them contain dithiocarbamate compounds as their active ingredients.

In general, the farmers applied the fungicides three weeks after planting and thereafter until a week before harvesting at various intervals, two to three times a week. In application, most of them used more than a single product and usually mixed with other chemical farm products such as insecticides, foliar fertilizers and commercial growth regulator products. In other words, they have no standard technique for application of pesticides/fungicides. Thus, it is quite difficult to record the exact rates and concentrations applied by the farmers.

1.3 Description of Sembalun Areas

Sembalun areas are part of the Aikmel district of Eastern Lombok. These areas are bordered with northern Sajang village, southern
Sapit village, eastern Sambalia and Pringgabaya districts, and western west Lombok region. Sembalun areas are divided into two villages; Sembalun Lawang (125.86 km square) and Sembalun Bumbung (55.17 km square) (WKPP, 1991).

Sembalun Lawang has five garlic-planting locations namely Dusun Karya, Dapur Belek, Lendang Luar, Orong Telaga Tengak, and Nyumbo. At Sembalun Bumbung, also there are five central garlic-planting areas; Sembalun Bumbung I, Sembalun Bumbung II, Dayan Desa, Bebante, and Orok (see Figure 1.1).

1.3.1 Climate and Hydrology

Sembalun Areas, based on the classification of Smith and Ferguson (WKPP, 1991), are C and D type climates with weather typical of mountainous regions. The average temperature is from ten to eighteen degrees Celsius for Sembalun Bumbung and from ten to thirty degree Celsius for Sembalun Lawang. The amount of rainfall in both of villages ranged between 2000-3000 millimetre per year with 100-200 millimetre a day of rainfall (WKPP, 1991). The rainfall is distributed unevenly over the year and normally, most of rain falls in November-March and the rest is dry season. Unfortunately, in 1994 these months were unusually dry (see Figure 1.2). However, the crops was still planted at normal time.
Scale 1: 100,000

= Areas of garlic plantation
1. Dusun Karya
2. Dapur Belek
3. Lendang Luar
4. Sembalun Bumbung I
5. Sembalun Bumbung II
6. Orong Telaga Tengak
7. Dayan Desa
8. Nyumbo
9. Bebante
10. Orok

Figure 1.1 Study area of Sembalun, Lombok island, Indonesia
There are five and twelve water resources in *Sembalun Lawang* and *Sembalun Bumbung* respectively. Mostly, the resources are effectively used for irrigation and drinking water. *Segara Anak* lake (1,350 metres above see level), which is in *Sembalun Lawang*, is a main water resource for *Lombok* Island (*Sembalun Lawang* Council, 1987).

### 1.3.2 Soils

According to a monograph of *Sembalun Lawang* Council (1987), the *Sembalun* areas have two types of soil; Alluvial and Regosol. Alluvial soils develop on alluvium of recent origin and have very weakly developed profiles. In many of them the colour change from the A to C horizon is hard to see or is nonexistent. They are, in large part, soils which have been transported from elsewhere and are usually characterised by lateral stratification. The texture is related to the rate at which the water deposited the alluvium. For this reason, they tend to be coarse-textured near the stream and fine-textured near the outer edges of the flood plain. Mineralogically, they related to the soil which served as a source for the alluvium (Miller *et al.*, 1966).

The alluvial soil characters could be seen in *Orong Telaga Tengak, Nyumbo, Dayan Desa, Bebante*, and *Orok*. Dahlan *et al.*, (1991) have reported the texture on the areas was silt loam with macro element status of nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and sulfur (S) from low to medium levels and
the organic content was 6.65 percent. The pH of the soil was about 6-6.5 with cation exchange capacity 6.50 meq/100 g.

Regosol are underlain by soft rather than hard rock. All zonal and interzonal soils that developed in unconsolidated deposits serving as parent material were regosol when they were young. Steep slopes where erosion occurs rapidly, and insufficient length of time, or movement of the material, as in the case of sand dunes, are the major causes for their existence (Miller et al., 1966).

Regosol soil appeared on Dusun Karya, Dapur Belek, Lendang Luar, Sembalun Bumbung I, and Sembalun Bumbung II. They have a
loamy sand texture. Organic content was about five percent with macro element status of N, P, K, Mg, and S from very low to low. The pH of the soil was about 5.5-6.0 with a cation exchange capacity of 30 meq/100 g (Baharuddin et al., 1992; Dahlan et al., 1991).

1.3.3 Garlic and Its Planting Seasons
In the 1950s garlic began to be planted by Sembalun farmers as a secondary crop. The crops were first introduced by Chinese who came from Santung and bartered the garlic in exchange for local agricultural products. After a long period of adaptation to local condition, the garlic is now claimed as a local variety called Sangga or Sembalun Variety. Nowadays, this variety is still promoted to become national variety (Mustiadi et al., 1994).

As a member of the Alliaceae family, Sembalun garlic (Allium sativum cv. Sangga) is 70-85 cm tall with superficial adventitious roots (Benson, 1979). The bulb is composed of a disclike stem, thin dry scales which are the bases of foliage leaves, and smaller bulbs or cloves formed from axillary buds of the younger foliage leaves (Figure 1.3). Each clove consists of a protective cylindrical sheath, a single thickened storage leaf sheath and a small central bud. The bulb size is 4.5-5.5 cm diameter and 3.4-4.5 cm length with violet-white colour. One bulb comprises 12-14 cloves, each being 2.3-2.7 cm in length and 1.2-1.3 cm wide.
Garlic cloves contain approximately: water 63 percent, protein 7 percent, fat 0.2 percent, carbohydrate 28 percent, fibre 2.8 percent, ash 1.0 percent. The uninjured bulb contains a colourless, odourless, water-soluble amino acid, alliin; on crushing the enzyme, alliinase, breaks down alliin to produce allicin, of which the principal ingredient is the odoriferous diallyldisulphide (Purseglove, 1972). Unfortunately, information is not yet available on the ingredients of the Sembalun variety.

Figure 1.3 The *Sembalun* garlic (*Allium sativum* cv. Sangga) hung up before analysis.
The garlic is always propagated vegetatively by single clove planted at a spacing of 15x15 cm, 10x15 cm, or 15x20 cm and can be harvested 95-110 days after planting. Approximately, 1000-1250 kg of sets are required to plant one hectare and produce nine tonnes dry bulb (Mustiadi et al., 1994; Purseglove, 1972).

On Sembalun, there are two garlic planting seasons; wet season between February and May and a dry season between June and October, in which the garlic has been planted on two types of different soils. During the wet season, the garlic has been cultivated on dry land which is mainly regosol and during the dry season, the plantation has been held on wet land which mostly consists of Alluvial soil.

1.4 Aims of the Project

The overall aims of this project were to monitor dithiocarbamate residues in garlic bulbs and to monitor the residues on soil surfaces and their vertical movement in soil throughout Sembalun areas, the main garlic-growing areas of Lombok, Indonesia.
CHAPTER TWO
LITERATURE REVIEW

2.1 Fungicide

2.1.1 History
Clear evidence regarding the first use of fungicides could not be gleaned from the literature. Although many references in the Old Testament mentioned blights, blast, mildews, rusts, and smuts, it seems there were no efforts to control the diseases and these tribulations were likely accepted as an expression of the wrath of the deity. However, McCallan (1967) divided the history of fungicides into eras, based on the discovery and development of new kinds of pesticides. The first era is Sulfur from ancient times to 1882, the second era is Copper Era from 1882 to 1934, and the last era is called Organic Fungicide Era beginning in 1934.

The movement of people and susceptible crops into new areas are causal factors for major outbreaks of new pests and diseases. Such outbreaks lead to the use of pesticides and fungicides as control measures and the consequent appearance of chemical residues in harvested crops. The detection of some fungicide residues in the environment prompted the development of non-persistent fungicides.

Moreover, the need of more selective fungicides and the interest in the potential of organic compounds resulted in the discovery of
the organic fungicide, dithiocarbamate in 1934. Previously, Fleming (1929) had invented penicillin and so ushered in a new era in medicine. Finally, an important moment noted, in which a new era began, is the discovery of the growth-regulating properties of 2,4-D by Zimmerman and Hitchcock in 1942.

2.1.2 Classification

In general, the fungicides, based on chemical structure, are grouped into two classes as follows (Kramer, 1983; Ware, 1978, Green et al., 1979). The following is a brief survey covering both inorganic and organic fungicides.

Inorganic Fungicide

The principle of how an inorganic works is based on the fact that a very small quantity of various inorganic components is required by fungi and other organisms for their growth and development and have to be contained in the culture medium. A deficiency of trace elements inhibits growth or activity and high concentration of the elements are also toxic. The heavy metal ions are the most important of these trace elements.

Sulfur

Sulfur in many forms is probably known as the oldest effective fungicide and is still applied as a garden fungicide with good broad-spectrum fungicidal properties which control a multitude of diseases. The main use of sulfur as a protective fungicide is against powdery mildews.
Sulfur used as fungicides has three formulations or physical forms. The first is finely ground sulfur dust which is made from 1 to 5 percent clay or talc. This form is produced to assist application in dusting qualities and is also used as a carrier for another fungicide or insecticide. The second is flotation or colloidal sulfur which must be formulated as wet paste in order to be mixed with water. The last form is wettable sulfur which is finely ground with a wetting agent so that the sulfur is able to mix with water for spraying.

Some forms of sulfur are vineyard sulfur (Kolodust®), calcium polysulfide and barium polysulfide (BaS₄.H₂O, Solbar®). Certain pathogenic organisms and most mites are killed by direct contact with sulfur and its fumigant action at temperature 70°F. The activity of polysulfide is due to the formation of the hydrosulfide (HS₂⁻). Sulfur interferes in electron transport along the cytochromes and is then reduced to hydrogen sulfide (H₂S), a toxic entity to most cellular proteins.

**Copper**

Inorganic copper compounds have a wide spectrum in which they are used on a large scale today in fruit, grape, potato, tomato, and banana growing and in other tropical plantation crops. They are employed especially against *Plasmopara viticola* and *Phytophthora infestans* as protective foliar fungicides and against damping-off diseases as seed dressings and soil fungicides.

The copper ions are available from both the highly soluble and relative insoluble copper salts. Some inorganic copper compounds
and their uses are given in Table 2.1. Since the copper compounds are relative insoluble in water, they are not easily washed from leaves by rain and so give longer crop protection from disease pathogens. They are relatively safe to use and require no special precautions during spraying. However, there is some danger in an accumulation of copper in agriculture soil due to frequent and prolonged use, even though, this element is essential for plants. Soluble copper compounds in higher concentration cause foliar damage, splitting, and malformation.

Table 2.1 Some inorganic copper compounds used as fungicides

<table>
<thead>
<tr>
<th>Name</th>
<th>Chemical Formula</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cupric sulfate</td>
<td>CuSO₄.5H₂O</td>
<td>Seed treatment and preparation of bordeaux mixture</td>
</tr>
<tr>
<td>Copper dihydrazine</td>
<td>CuSO₄(N₂H₄)₂SO₄</td>
<td>Powdery mildew and black spot of roses</td>
</tr>
<tr>
<td>Copper oxychloride</td>
<td>3Cu(OH)₂.CuCl₂</td>
<td>Powdery mildew</td>
</tr>
<tr>
<td>Copper oxychloride</td>
<td>3Cu(OH)₂.CuCl₂ and 3Cu(OH)₂.CuSO₄</td>
<td>Many fungal diseases</td>
</tr>
<tr>
<td>Copper zinc chromates</td>
<td>15Cu.10ZnO.6CrO₃.25H₂O</td>
<td>Disease of potato, tomato, cucurbits, peanuts, and citrus</td>
</tr>
<tr>
<td>Cuprous oxide</td>
<td>Cu₂O</td>
<td>Powdery mildews</td>
</tr>
<tr>
<td>Basic copper sulfate</td>
<td>CuSO₄.Cu(OH)₂.H₂O</td>
<td>Seed treatment and preparation of bordeaux mixture</td>
</tr>
<tr>
<td>Cupric carbonate</td>
<td>Cu(OH)₂.CuCO₃</td>
<td>Many fungal diseases</td>
</tr>
</tbody>
</table>

Source: Ware, 1978
**Mercury**

The most toxic of the inorganic fungicides is probably mercury due to its toxicity associated with divalent mercury ions which are toxic to all forms of life. As a result, mercury residues are prohibited in foods or feeds. The mode of action for the mercury is the non selective or nonspecific inhibition of enzymes, especially those containing iron and sulfhydryl sites. A typical example of this fungicide is ceresan (2-methoxyethylmercuric chloride) used as seed treatment and phenylmercury acetate applied for turf diseases and as dormant sprayer.

Although a host of organic mercury compounds was developed over 30 years, both inorganic and organic fungicides have been banned by the Environmental Protection Agency (EPA). The main reason is that mercury is toxic to warm-blooded animals and is accumulated in the environment.

**Organic Fungicides**

Over 30 years many organic fungicides have been developed in order to replace the more harsh, less selective inorganic materials. Fortunately, most of the organic fungicides have had no measurable build up effect on the environment after many years of use. In 1931, the first organic sulfur fungicide, thiram, was discovered and many others, such as zineb and captan introduced in 1943 and 1949 respectively, have followed the invention of thiram. Since then, dramatic advances in organic synthesis have occured, in which more than 200 fungicides of all classes have been developed.
Dithiocarbamates

In this group are thiram, maneb, ferbam, ziram, zineb, mancozeb, propineb. Such fungicides probably have greater popularity and use than all other fungicides combined, including the home gardens. The action of dithiocarbamate probably is caused by being metabolised to the isothiocyanate radical (-N=C=S), which inactives sulfhydryl groups in amino acids contained within the individual pathogen cells. A further explanation of this group of fungicides is given in point 2.2.

Thiazoles

The thiazoles, a class of heterocyclic sulfur compounds, offer a surprising chemical disposition. Under soil condition, the five-membered ring of the thiazoles is cleaved rather quickly to form either the fungicidal isothiocyanate (-N=C=S) or dithiocarbamate, depending on the structure of the parent molecule. Terrazole® is one of the member widely marketed and used only as a soil fungicide against Pythium, Rhizoctonia, and Fusarium spp. The mode of action is probably similar to that of the dithiocarbamates.

Triazines

The triazine structure is often found in herbicides, but is found only once in the fungicides. Anilazine (Dyrene®) introduced in
1955 has received wide use as a foliar fungicide for the control of Alternaria, Fusarium, Plasmofara, Puccinia, and Rhizoctonia spp. Burchfield and Storrs (1957) showed that this fungicide reacts with metabolic intermediates containing amino acid and sulfhydryl groups within cells of organism.

\[ \text{ANILAZINE} \]

\[ \begin{align*}
\text{Cl} & \quad \text{N} \\
\text{N} & \quad \text{N} \\
\text{Cl} & \quad \text{Cl} \\
\text{Cl} & \quad \text{Cl}
\end{align*} \]

2,4-dichloro-6-(p-chloroanilino)-s-triazine

**Substituted Aromatics**

The substituted aromatics belong in a somewhat discretionary classification assigned to the simple benzene derivates that possess long recognised fungicidal properties. Some of them are described as follows.

Hexachlorobenzene (HCB), introduced in 1945, is used as seed treatment and as a soil treatment to control stinking smut of wheat. Pentachlorophenol (PCP), used since 1936, is marketed widely by many firms as a wood preservative, insecticide, and herbicide. Pentachloronitrobenzene (PCNB) or quintozene was introduced in the 1930s as a fungicide for seed treatment and selected foliage applications. It is also used as a soil treatment to control the pathogens of certain damping-off diseases of seedlings. Chlorothalonil is a very useful, broad spectrum foliage-protectant
fungicide made available in 1964. And chloroneb, marketed as Demosan® or Tersan® and developed in 1965, is heavily used for cotton, beans, sugar beet, and soya seedling and used against *Rhizoctonia*, *Sclerotium*, and *Pythium spp.*

**HEXACHLOROBENZENE**

![1,2,3,4,5,6-hexachlorobenzene](image1)

**PCNB**

![pentachloronitrobenzene](image2)

**CHLORONEB**

![1,4-dichloro-2,5-dimethoxybenzene](image3)

**PCP**

![pentachlorophenol](image4)

**CHLOROTHALONIL**

![tetrachloroisophthalonitrile](image5)

Modes of action of substituted aromatics are different. Being generally fungistatic, they reduced growth rates and sporulation of fungi, probably by combining with -NH₂ or -SH groups of essential metabolic compounds.

**Dicarboximides**

There are three extremely useful foliage protectant fungicides within this group. The first is Captan which appeared in 1949, and is undoubtedly the most heavily used in fruit, vegetables, cereal, rice, grape, citrus, and ornamentals. The second is Folfet (Phaltan®), developed in 1962, and Captafol (Difolatan®) developed in 1961. They are protective and eradicative fungicides.
and mainly used as foliage dusts and sprays on fruits, vegetables, and ornamentals.

Fungitoxicity of the dicarboximides is apparently nonspecific and is not a result of a single mode of action. The dicarboximides' lethal effect on disease organism is probably due to the inhibition of the synthesis of amino compounds and enzymes containing -SH radical.

Systemic Fungicides

Nowadays systemic fungicides have been widely marketed and a large number are available. This type of fungicides works through absorption process by plant and the active ingredient is carried by translocation through the cuticle and across leaves to the growing points. Most systemic fungicides have eradicant properties that terminate the progress of existing infections. They are therapeutic so they can be used to cure plant diseases. A few of the systemics can be applied as soil treatment and are slowly absorbed through the roots to give prolonged disease control.
**Oxathiins**

There are two systemic fungicides in this group, carboxin and oxycarboxin, introduced in 1966. They were the first of the systemics to succeed in practice. They are used as seed treatment for the cereal crops, particularly those affected by embryo-infecting smut fungi. They are selectively toxic to smuts, rust and to *Rhizoctonia*, organisms belonging to the *Basidiomycetes*. The apparent mode of action of the oxathiins begins with their selective concentration in the fungal cells, followed by the inhibition of succinic dehydrogenase, an important enzyme for respiration in the mitochondrial systems.

![Chemical structures of Carboxin and Oxycarboxin](images)

**Benzimidazoles**

The benzimidazoles, represented by benomyl and thiabendazole (TBZ), were introduced in 1968 and have received wide acceptance as systemic fungicides against a broad spectrum of diseases. Benomyl has the widest spectrum of fungitoxic activity of all the newer systemics, including the *Sclerotinia*, *Botrytis*, and *Rhizoctonia* species, and the powdery mildews and apple scab. Thiabendazole has a similar spectrum of activity to that of benomyl. Introduced in 1969, thiophanate, although not a
benzimidazole in its original structure, is converted to that group by the host plant and the fungus through their metabolism. Thiophanate has a fungitoxicity similar to that of benomyl. All three compounds have been used in foliar applications, seed treatment, dipping of fruit or roots and soil application. Their mode of action appears to be the induction of abnormalities in spore germination, cellular multiplication, and growth, as a result of their interference in the synthesis of that vital nucleic material, deoxyribonucleic acid (DNA).

**Fumigants**

The fumigant action of these fungicides is due to their high volatility and small molecular size. Chloropicrin, for instance, controls fungi, insects, nematodes, and weed seeds in soil through fumigant action. Methyl bromide, also listed as a fumigant insecticides, is equally effective against fungi, nematodes, and weeds. Methylisothiocyanate (MIT) is closely related to the dithiocarbamates and has a similar mode of action against fungi, nematodes, and weeds.
**Antibiotics**

The antibiotic fungicides are substances produced by microorganisms, which in very low concentrations are able to inhibit growth and even destroy other microorganisms. Now, several hundred antibiotics are reported having fungicidal activity, and most of their chemical structures are already known.

The largest source of antifungal antibiotics is the *Actinomycetes*, a group of the lower plant. There are two antibiotics, streptomycin and cycloheximide, isolated from *Streptomycetes griseus* which is a species of *Actinomycetes*. The investigation of new antibiotics from this source has been continuously conducted.

Streptomycin is applied as dust, spray, and seed treatment for controlling most bacterial diseases such as blight on apples and pears, soft rot on leafy vegetables, and some seedling diseases. The mode of action of streptomycin probably interferes in the synthesis of proteins. Despite the evidence of antibiotic-resistant strains, streptomycin has a place in the control of some bacterial diseases.

![Streptomycin Structure](image)

**STREPTOMYCIN**

2,4-diguanidino-3,5,6-trihydroxycyclohexyl-5-deoxy-2-O-(2-deoxy-2-methylamino-β-glucopyranosyl)-3-formyl pentofuranoside
Cyclohexamide, introduced as a fungicide in 1949 and known commercially as Actidione®, has a wide range of toxicity to organisms and is a smaller, less complicated antibiotic, about which more is understood. First, cyclohexamide is toxic to a wide range of organisms, including yeast, filament-forming fungi, algae, protozoa, higher plants, and especially mammals. Surprisingly, it is inactive against bacteria. This is possibly due to the failure of bacteria to absorb it. Cycloheximide causes growth inhibition in yeasts and filament-forming fungi by inhibiting protein and RNA synthesis. Nowadays, cycloheximide has become popular in the control of powdery mildew, rusts, turf diseases, and certain blights.

**CYCLOHEXIMIDE**

\[
\begin{array}{c}
\text{H}_3\text{C} \text{O} \\
\text{CH} - \text{CH}_2 - \text{NH} \text{O} \\
\text{H}_3\text{C} \\
\end{array}
\]

\[\beta\text{2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl} \text{glutamimide}\]

**Dinitrophenols**

Dinitrophenols are commonly used as insecticide-ovicides and herbicides. Their mode of action as fungicides is the same, uncoupling oxidative phosphorylation in cells with an attendant upset of the energy systems within the cells. One dinitrophenol, dinocap (Arathane®, Karathane®), has been used since the late 1930s, both as an acaricide and for powdery mildew on a number of fruit and vegetable crops. Dinocap undoubtedly acts in the
vapor phase, since it is quite effective against powdery mildews whose spores germinate in the absence of water.

DINOCAP

\[
\begin{align*}
\text{DINOCAP} & \\
\text{O=O} & \\
\text{O} & \\
\text{CH\textsubscript{1}} & \\
\text{CH\textsubscript{1}} & \\
\text{CH\textsubscript{1}} & \\
\text{C-C\textsubscript{11}} & \\
\text{NO\textsubscript{2}} & \\
\end{align*}
\]

2,4-dinitro-6-(2-octyl)phenyl crotonate

Quinones

The quinones are a fascinating chemical group, because there are practically unending numbers of molecules that are potential fungicides. The one example presented briefly here is the most popular of that group, dichlone. It is used on a number of fruit and vegetable crops and for treatment of ponds, to control blue-green algae. Dichlone affects respiration in many fungi and acts by attaching to the -SH groups in enzymes, thus inhibiting their action and indirectly uncoupling oxidative phosphorylation.

DICHLONE

\[
\begin{align*}
\text{DICHLONE} & \\
\text{O} & \\
\text{Cl} & \\
\text{Cl} & \\
\end{align*}
\]

2,3-dichloro-1,4-naphthoquinone
**Organotins**
In general, the trialkyl derivatives of organotins are highly fungicidal, but also phytotoxic. The triaryl three-ring compounds are suitable for protective use, and also have acaricidal properties. Although it is not yet proved, the trisubstituted tin compounds probably block oxidative phosphorylation. The organotins were first introduced in the mid-1960s, ten years after their fungicidal properties had been discovered.

![Fentin Hydroxide](attachment:image)

**Aliphatic Nitrogen Compounds**
Dodine, a fungicide introduced in the mid-1950s, has proved effective in controlling certain diseases such as apple and pear scab and cherry leaf spot. It has disease specificity and slight systemic qualities. Its mode of action is not totally clear, but it is taken up rapidly by fungal cells, causing leakage in these cells, possibly by alteration in membrane permeability. The guanidine nucleus of dodine is also known to inhibit the synthesis of RNA.
2.1.3 Toxicity of Fungicide

Among many chemicals encountered daily by humans, pesticides occupy a rather unique position, because they are deliberately supplied to the environment in order to kill or injure some forms of life. Ideally their injurious action would be highly specific for undesirable target organism and noninjurious to desirable, non-target organisms (Martin, 1959). In fact, however, most of the pesticides that are currently used are not highly selective but are generally toxic to many desirable form of life that coinhabit the environment.

Fungicides like other classes of pesticides comprise a heterogeneous group of chemical compounds. With a few exception, the fungicides have not attracted the detailed toxicological research that insecticides have. The mercury-containing fungicides contain the group that has been of great concern for hazard to health, and they have been responsible for many deaths or permanent neurologic disability resulting from the misdirection of mercury fungicide-treated seed grains into human and animal food.
Wagner (1983) stated that pentachloronitrobenzene marketed under the names of Quintozene, Pentagen, Folosan, and Terrachlor was reported teratogen and carcinogen in mice at 2500 mg kg$^{-1}$ and 135 mg kg$^{-1}$ respectively. The chemical has oral LD$_{50}$ in rats of 1650 mg kg$^{-1}$ and in mice of 2500 mg kg$^{-1}$. Karathane (2,4-dinitro-6-octylphenyl crotonate) used in the Pacific Northwest in the treatment of fruits, mainly pears and apples also caused ducks to develop cataracts when fed 50 ppm.

Some toxic cases of dithiocarbamate fungicide are discussed in point 2.3.

2.2 Dithiocarbamate Fungicides

2.2.1 Introduction

Dithiocarbamates are mainly used in agricultural areas as insecticides, herbicides, and fungicides. The other uses are as biocides for industrial or other commercial applications, and in household products. Some forms of dithiocarbamates are used for vector control in public health.

The dithiocarbamates were discovered as a class of chemical compounds early in the history of organo-sulfur chemistry. The characteristic of this compound is the insolubility of its metal salts, with the exception of sodium and other alkali and the alkaline earth metals, and the capacity of the molecules to form chelate complex (Thorn and Ludwig, 1962).
The history of the development of dithiocarbamates as field fungicides and their role in sparking the organic era began with Tisdale and Williams patent issued in 1934. Guy (1936; 1937) was the first to report on the insecticidal activity of the dithiocarbamate, in which he showed that certain of the thiuram disulfides strongly repelled a variety of leaf-feeding insects. Previously, Montgomery et al. (1935) developed these compounds initially as practical field fungicides.

Recent reviews of the chemistry and mode of action of dithiocarbamate fungicides suggest chemical degradation of these compounds into organic compounds including ethylenediamine, carbon disulfide, ethylenethiourea (ETU), ethylenethiuram disulfide, ethylenethiuram monosulfide, isothiocyanates, metallic sulfides, element sulfur, and carbonyl sulfide (Kaufman, 1977). Unfortunately, ETU, the principle degradation product, has been studied and found to be carcinogenic and teratogenic in both mice and rats (Graham and Hansen, 1972; Khera, 1973). Other toxicological properties of these degradation products have been partially evaluated.

2.2.2 Chemical and Physical Properties

The general formula of the dithiocarbamate, for which the patent was issued to Tisdale and Williams (1934) of E.I. duPont de Nemours & Co

\[
\begin{align*}
X & \quad N-CS_2-Z \\
\uparrow & \\
Y &
\end{align*}
\]
where X is hydrogen or an alkyl radical, Y is hydrogen, alkyl or aryl and Z may be a metal or a salt-forming group such as ammonium etc.

The properties of dithiocarbamate are changed by replacement of the sulphur hydrogen with metals or other substituents. Extending the alkyl chain leads to a loss of activity. The optimum is reached with dithiocarbamate having two methyl groups on the nitrogen. Chain extension beyond two carbon causes a 20-200-fold drop in activity. Monomethyl derivates are less active, as are derivates in which the nitrogen is replaced by alkyl, oxygen, or sulphur. Depending on the metal cation, dimethyldithiocarbamate salts are more less unstable at pH values below 6, decomposing back into their corresponding starting materials (Tisdale and Flenner, 1942, Thorn and Ludwig, 1962; Kramer, 1983).

Dithiocarbamate with hydrophilic groups, such as OH- and COOH, form water-soluble metal complexes. However, dithiocarbamate metal complexes used as fungicides are all insoluble in water, though they are soluble in non-polar solvents (WHO, 1988).

2.2.3 Classification
Dithiocarbamates are divided into two groups of compound; dialkyldithiocarbamates and bisdithiocarbamates, which although related, have clearly different properties. The main differences are caused by the bisdithiocarbamates having a reactive hydrogen on the nitrogen. The differences are also shown in a number of observations of differences in biological behavior (Ludwig and

**Dialkyldithiocarbamates**
This group includes the dialkyldithiocarbamates proper and their oxidation product, tetramethylthiuram disulfide (Thiram). Sodium salt of dimethyldithiocarbamate is not found as a practical fungicide due to its solubility and only used in laboratory studies but the insoluble iron salt (ferbam) and zinc salt (ziram) have been continuously developed for commercial fungicides.

**Thiram**
Probably the most important compound of this class, tetramethylthiuramdisulfide is the first metal free organic compound to be described as a foliar fungicide. Thiram is widely active seed dressing and soil treatment for control damping-off and gives protective control of scab, shot-hole, and *Botrytis cinerea*.

![Chemical Structure of Thiram](image)

Thiram; LD$_{50}$: 750 mg/kg rat, oral, acute

**Ziram**
Bis(dimethylcarbamodithioato-S,S')zinc precipitates as an insoluble salt when an aqueous solution of sodium dimethylcarbamodithioate is mixed with a water-soluble zinc salt. This product has been used world-wide as an important fungicide.
since the 1930s. Ziram finds use as a protective fungicide in fruit, wine, grape, and vegetable growing.

\[
\begin{array}{c}
\text{(H}_3\text{C)}_2\text{N}-\text{C}^\text{S} \text{S}_2 \text{Zn}
\end{array}
\]

Ziram; LD$_{50}$: 1400 mg/kg rat, oral, acute

**Ferbam**

Tris (dimethylcarbamodithioato-S,S')iron is used in fruit, vegetables, citrus, and tobacco, particularly in the United States, and, like ziram, is active against scab, rust, and damping-off disease.

\[
\begin{array}{c}
\text{(H}_3\text{C)}_2\text{N}-\text{C}^\text{S} \text{S}_3 \text{Fe}
\end{array}
\]

Ferbam; LD$_{50}$: 1000 mg/kg rat, oral acute

**Bisdithiocarbamates**

Included in this group are the ethylene bisdithiocarbamates, derived from alkylenediamines, such as nabam, zineb, manebei, and mancozeb as well as propineb which is the propylene derivative. They are characterised by the presence of a reactive hydrogen on the nitrogen which impart properties lacking in the dialkylamines.
Nabam

The product of reaction between ethylene diamine and carbon disulfide in the presence of sodium hydroxide solution, disodium 1,2-ethanediylbis(carbamodithioate). This finds application as a soil fungicide owing to its water solubility and stability.

\[ \text{Nabam; LD}_{50}: 395 \text{ mg/kg rat, oral, acute} \]

**Maneb, Zineb and Mancozeb**

Zineb is used against *Phytophthora infestans* and other downy mildews, *Alternaria spp.*, rusts, and scabs. Maneb is used on large scale as a foliar fungicide for control of *Phytophthora infestans*, *Peronospora* of hops and vine, *Pseudopeziza tracheiphila* (red fire disease of grapevine), scab, rust, and downy mildew species in fruit, vegetables, maize, rice, cereals, tobacco, grapevine, and ornamentals, as well as a seed dressing and soil treatment.

Combinations of maneb and zineb (Mancozeb, Dithane M-45) have an even better stability and activity. Mancozeb in combination with 2,4-dinitrophenyl esters is marketed as a foliar fungicide with additional acaricidal action.

Zineb and maneb form stable addition compounds with ammonia and amines. \( N,N'-(1,1\text{-methanediyl})\text{bis(}[1,2\text{-ethanediylbis(carbamodithioato)](2-)}\text{manganese/zinc)} \), the
product of maneb and zineb and formaldehyde, is used in hops and grapes.

\[
\text{M} = \text{Zn}, \text{Zineb}; \text{LD}_{50} = > 5200 \text{ mg/kg rat, oral acute}
\]

\[
\text{M} = \text{Mn}, \text{Maneb}; \text{LD}_{50} = 6750 \text{ mg/kg rat, oral, acute}
\]

**Propineb**

Propineb, the product from reaction of 1,2-diaminopropane with carbon disulfide/sodium hydroxide and subsequent precipitation with zinc sulfate, \([1\text{-methyl-1,2-ethanediyl]bis(carbamodithioato)]-(2-)zinc\), is used in orchard fruit and vegetables.

Oxidation of zineb, maneb and propineb leads to the thiuram sulfides and disulfides. The thiurammonosulfides of monoalkylcarbamodithioic acids are more active than the disulfides, in contrast to the thiuram derivates of dialkylcarbamodithioic acids (Kramer, 1983).

\[
\text{Propineb, Antracol}^\circledR, \text{LD}_{50}: 8500 \text{ mg/kg rat, oral, acute}
\]
2.2.4 Behaviour in Soil and Plant

Soil

Dithiocarbamates, like all pesticides, can reach the soil through many routes, ranging from direct application to drift from foliage treatment. These compounds, in general, are not persistent and undergo different types of degradation.

Helling et al. (1974) have stated that the mobility of ethylene bisdithiocarbamates (EBDCs) in soil considerably depends on water solubility and type of soil. They are generally more mobile in wet and in sandy soil than in dry soil or soil rich in organic matter (peat or muck). In addition, the use of thin-layer chromatography in studies of the mobility has shown that nabam is more mobile than maneb, which in turn is more mobile than zineb, zineb being almost immobile.

Thiram and dimethyldithiocarbamic acid give rise in soil to methyl isothiocyanate and sulphur, and, under acidic conditions, to carbon disulfide, hydrogen disulfide, methylamine, methylisocyanate, and the bisdisulfide of methyldithiocarbamic acid. Two of the products, carbon disulfide and dimethylamine, evaporated from soil (Raghu et al., 1975). Dimethyldithiocarbamic acid also binds with heavy metals in soil to form complexes (Ludwig and Thorn, 1960).

Lyman and Lacoste (1974) have studied the leaching of radio active $^{14}$C-mancozeb and its degradation products in five different
soils. The organic content ranged from 0.4 to 15%, while the pH ranged 4.7 to 7.4. An aqueous slurry of $^{14}$C-mancozéb (15.6 mg) was mixed with a soil sample and applied to the top of the column and water was then added (2.5 cm) once a week for 9 weeks. The water was collected and its radioactivity measured and, after 9 weeks, the columns were cut into 2.5 cm sections. The result showed that no radioactivity leached through four of five columns (only 2-5% of the activity leached through the Cecil clay column; the reason for this is not known). Losses of the radioactivity by volatilization or by metabolism to carbon dioxide were significant in all soils.

The various metal derivatives of ethylene bisdithiocarbamic acid appear to be converted in the soil to 5,6-dihydro-3H-imidazo (2,1-C)-1,2,4-dithiazole-3-thione) or DID, ETU, carbon disulfide, hydrogen disulfide, and carbonyl sulphide (Moje et al., 1964; Kaufman, 1977). The conversion by soil bacteria and fungi of DID into ETU has been demonstrated Vonk and Kaars Sijpesteijn (1976). Even though, ETU was slowly converted into ethyleneurea (EU) in soil, pure cultures of soil bacteria and fungi were unable to effect this transformation (Kaufman, 1977).

**Plants**

ETU is one of several metabolites found when EBDCs are applied to plants (Vonk and Kaars Sijpesteijn, 1970). In plants, nabam, manebé, and zineb are transformed to ETU, DID, EU, 2-imidazoline, a diisothiocyanate (EDI), and other metabolites (Figure 2.1).
Nash and Beall (1980) have studied the fate of maneb and zineb in microagroecosystem chambers (enclosed glass chamber), under the following conditions: pH, 6.7; organic matter content, 5.2%; soil type, Gelastown sandy loam; soil water content, 15.6%. The fungicides were applied twice to tomato plants at 2 kg/ha, and the residual fungicides (measured as EDA and ETU) were monitored.

Figure 2.1 Metabolic pathways for the decomposition of ethylene bisdithiocarbamates and reactions leading to ETU (Source: WHO, 1988 adapted from: Engst and Schnaak (1967, 1970a,b); Freudenthal et al., (1977), and Aldridge and Magos (1978).
on the fruits, leaves, and in the soil, water, and air for 100 days after treatment. ETU was detected at < 20 ug/kg on whole fruit after 3 days, but had completely disappeared after 3 weeks. Maneb and zineb were present on whole fruit at 1 mg/kg and were still present in measurable amounts, as ethylenediamine (EDA), after 10 weeks. Both had half-concentration times (C$_{1/2}$) of 14 days on leaves. Half concentration times for ETU, maneb, and zineb in soil were 3, 36, and 23 days respectively, and that for ETU in air was 9 days.

Kaars Sijpesteijn et al. (1977) have stated that a gradually increasing amount of radioactivity occurred after application of $^{14}$C-propineb to apples and grapes. The breakdown of propineb starts with the formation of oligomers. They have also revealed that in plants treated with mancozeb, after two weeks, were found residues of ETU, ethylenediamine, DIDT, 2-imidazolene, EU, 1-(2'-imidazolin-2'-yl)-2-imidazolinethionine, elemental sulfur, and sulfur ion.

### 2.2.5 Analytical Methods

Residue analysis for environmental matrices, in general, consists of sampling the environmental material or matrix, extracting the pesticide/fungicide residue, removing interfering substances from the extract, and identifying and quantifying the pesticide residue. The manners in which the matrix material is sampled, stored, and handled should be considered because they can affect the results of analysis. Samples should be truly representative, and their
handling and storage must not further contaminate and degrade the residue being measured.

The dithiocarbamates are conveniently determined on the basis of their decomposition by mineral acids to the amine and carbon disulfide. The hydrolysis products can also be measured iodometrically or colorimetrically. This method was first published by Clarke et al. (1951) and Lowen (1951). The procedure based on acid decomposition, scrubbing the evolved carbon disulfide with lead acetate, absorbing in alcoholic potassium hydroxide and titrating with iodine. Clarke et al (1951) have found that the recovery of known amounts of disodium ethylenebis(dithiocarbamate) in water solution averages 79%. They have stated that the compound decomposes slowly in dilute solution.

Keppel (1969) made some modification in order to increase recovery of the method by adding a reducing agent (stannous chloride) to the sample before treatment with hot acid, substituting diluted sodium hydroxide for lead acetate to remove hydrogen sulfide and other interferences, and using boiling diluted hydrochloric acid. He found the recovery ranges 85.3-103.8% for ethylenebisdithiocarbamate, with the exception zineb (range 89.1-96.8%). Nowadays, this method is widely accepted and used as standard analysis by USEPA-Method S 15 (1979).

The basic principle of the carbon disulfide method evolution is that dithiocarbamate residues are decomposed by refluxing the material with boiling dilute acid. Evolved CS$_2$ is carried by gas
stream through a trap to remove H₂S and other volatile interferences. It then reacts with color reagent to form a yellow complex, the cupric salt of N,N-bis(2-hydroxyethyl) dithiocarbamic acid, which is measured. Dithiocarbamate is calculated from CS₂ found (Keppel, 1971). Using this technique, 50 µg of dithiocarbamate residue can be determined.

Gas chromatography is widely used in pesticide residue analysis due to its sensitivity and has been used a limited amount for analysis of dithiocarbamates (Nash, 1974 and Tetsumi et al., 1985). Bighi (1964) and Bighi and Saglietto (1965) have analysed CS₂ obtained from pure and commercial dithiocarbamates and bisdithiocarbamates after preliminary decomposition in hot acid. Newsome (1974) determined ethylenebis(dithiocarbamate) residues on food crops by ion exchange chromatography as bis(trifluoroacetamido)ethane. The use of HPLC was demonstrated by Gustafsson and Thomson (1981) and Gustafsson and Fahlgren (1983) and involved transforming iron, zinc, and manganese salts of dithiocarbamic acids into readily water-soluble sodium salts in an alkaline solution of EDTA and L-cystein.

A polarographic method has been used to estimate residues of maneb and zineb (detection limit, 0.5 mg/kg product) and ethylene bisthiuram monosulfide (detection limit, 0.02 mg/kg product (Engst and Schnaak, 1969a,b).
2.2.6 Environmental Levels

Currently, information on the possible environmental impact of dithiocarbamates and problems relating to persistence and bioaccumulation in various species and the food chain is limited. WHO (1988) based on the available information has stated that most dithiocarbamate fungicides are rapidly degradable in the presence of oxygen, moisture, etc. to form a number of compounds, in which some of them, ETU and propylenethiourea, are toxicologically important. In general, the residue should be below 0.1 mg/kg for ETU levels.

NHMRC (National Health and Medical Research Council) (1993) has recommended that the maximum residue limit (MRL) for dithiocarbamate should range between 0.01 and 5 mg kg\(^{-1}\) for various agriculture products. For instance, 0.01 mg kg\(^{-1}\) is MRL for potato, 0.2 mg kg\(^{-1}\) for onion bulb, and 5 mg kg\(^{-1}\) for celery.

2.3 Effects Dithiocarbamates on Organism in Environment

2.3.1 Microorganism

According to WHO (1988), there are some evidence that dithiocarbamates, at concentration 10 times that of normal field application, may reduce microbial biomass and increase the bacterial : fungal ratio.
2.3.2 *Mammals*

The toxicity of dithiocarbamates for mammals, in general, is relatively low. Table 2.1 indicates the acute toxicity of dithiocarbamates based on acute oral and dermal toxicity of several animals. It can be seen from the table that nabam and metham-sodium are the most toxic of the dithiocarbamates compared with the others which are relative low. Ivanova-Chemishanska (1969) found that rats treated with zineb, maneb, or mancozeb showed dose-dependent signs of depression, adynamia, decreased tonus, disturbances in coordination, paresis, and paralysis of extremities combined with general weakness, lack of appetite, and prostration.

Table 2.1 Acute toxicity (LD$_{50}$) of dithiocarbamates for experimental animals

<table>
<thead>
<tr>
<th>Compound</th>
<th>Animal</th>
<th>Dose (mg/kg body weight)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Oral</td>
<td>Dermal</td>
</tr>
<tr>
<td>Ferbam</td>
<td>mouse</td>
<td>1000</td>
<td>FAO/WHO (1965)</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>&gt;4000</td>
<td>Hodge <em>et al.</em> (1956)</td>
</tr>
<tr>
<td></td>
<td>guinea-pig</td>
<td>450-2000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rabbit</td>
<td>2000-3000</td>
<td></td>
</tr>
<tr>
<td>Metham-sodium</td>
<td>mouse</td>
<td>285</td>
<td>Worthing and Walker (1983)</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>1700-1800</td>
<td>Worthing and Walker (1983)</td>
</tr>
<tr>
<td></td>
<td>rabbit</td>
<td>1300</td>
<td>Worthing and Walker (1983)</td>
</tr>
<tr>
<td>Ziram</td>
<td>rat</td>
<td>1400</td>
<td>Hodge <em>et al.</em> (1952)</td>
</tr>
<tr>
<td></td>
<td>guinea-pig</td>
<td>100-150</td>
<td>Hodge <em>et al.</em> (1952)</td>
</tr>
<tr>
<td></td>
<td>rabbit</td>
<td>100-1020</td>
<td>Hodge <em>et al.</em> (1952)</td>
</tr>
<tr>
<td>Thiram</td>
<td>mouse</td>
<td>1500-2000</td>
<td>Worthing and Walker (1983)</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>865-1300</td>
<td>Van Esch (1956)</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>780-865</td>
<td>Worthing and Walker (1983)</td>
</tr>
<tr>
<td>Substance</td>
<td>Species</td>
<td>LD₅₀ (mg/kg)</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------</td>
<td>---------</td>
<td>-------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Zineb</td>
<td>cat</td>
<td>230</td>
<td>Lehman (1951)</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>&gt;4000</td>
<td>VanEsch (1956)</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>&gt;5200</td>
<td>Blackwell-Smith (1953)</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>9000</td>
<td>Ivanova-Chemishanska (1969)</td>
</tr>
<tr>
<td>Maneb</td>
<td>mouse</td>
<td>4100</td>
<td>Engst <em>et al.</em> (1971)</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>4500</td>
<td>Engst <em>et al.</em> (1971)</td>
</tr>
<tr>
<td></td>
<td>rat (male)</td>
<td>6750</td>
<td>Worthing and Walker (1983)</td>
</tr>
<tr>
<td>Nabam</td>
<td>rat (male)</td>
<td>395</td>
<td>Blackwell-Smith <em>et al.</em> (1953)</td>
</tr>
<tr>
<td>Mancozeb</td>
<td>rat (female)</td>
<td>12800</td>
<td>Ivanova-Chemishanska (1969)</td>
</tr>
<tr>
<td></td>
<td>rat (male)</td>
<td>14000</td>
<td>Ivanova-Chemishanska (1969)</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>&gt;8000</td>
<td>Worthing and Walker (1983)</td>
</tr>
<tr>
<td>Propineb</td>
<td>rat</td>
<td>8500</td>
<td>Worthing and Walker (1983)</td>
</tr>
<tr>
<td></td>
<td>rabbit</td>
<td>2500</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cat</td>
<td>2500</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ham</td>
<td>2500</td>
<td></td>
</tr>
<tr>
<td>Metiram</td>
<td>mouse</td>
<td>5400</td>
<td>Worthing and Walker (1983)</td>
</tr>
<tr>
<td></td>
<td>rat (female)</td>
<td>&gt;10000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>guinea-pig</td>
<td>2400-4800</td>
<td></td>
</tr>
</tbody>
</table>

Source: WHO, 1988

Minor *et al.* (1974) have conducted experiment by daily feeding of ferbam at 23, 66, or 109 mg/kg body weight to male rats for 13 weeks. The results show that the death and weight loss occurred at the highest dose, but the ferbam did not have any effect on reproduction. Daily feeding (equivalent to 15 or 51 mg/kg body weight) to female for 2 weeks caused severe weight loss at the highest dose level.
A dog was given ferbam and ziram together for one month, each at a dose of 5 mg/kg body weight per day. She remained healthy except for slight anaemia. The same result was found when ferbam was given alone for one month at a dose of 25 mg/kg body weight per day, or for one week at 50 mg/kg body weight. Raising the dose to 100 mg/kg body weight per day, however, provoked severe vomiting and malaise immediately (Hodge et al., 1952).

Wedig et al. (1968) have also reported that thiram (99.9% purity) caused decreasing egg production for the domestic chicken (Gallus domesticus), pigeon (Columbia livia), and pheasant (Phasianus colchicus torquatus). A 50% reduction in egg-laying in bobwhite quail (Colinus virginianus) was effected by a dose level of 8.8 mg/kg body weight per day. During this period of reduced egg laying, it seems that an alteration of hormone levels took place resulting in significant weight body losses of ovary and oviduct, decrease in serum calcium levels, which is controlled by oestrogen and alteration in normal maturation of the ova.

2.3.3 Man

The acute toxicity of dithiocarbamates is relatively low so that acute intoxication in human beings is inconclusive (IARC, 1976). The most extensive studies of exposure to dithiocarbamates were reported by Gowers and Gordon (1980) and Charkes et al. (1985). Forty two currently exposed and 112 previously exposed workers were compared with equal size control group matched for age, period of employment, race, and type of job. Detail questionnaires and interviews were conducted by personal interviewer to get
health history and family health. Thyroid parameters measured were total T₃, T₃ resin uptake, T₄, TSH, free T₄ index, thyroglobulin antibodies, and microsomal antibodies. Moreover, urine was analysed consisting of ETU, EBDC, zinc, manganese, creatine, iodide, specific gravity, and pH. Blood parameters determined were glucose, urea nitrogen, sodium, potassium, calcium, chloride, carbon dioxide, cholesterol, total protein, albumin, bilirubin, uric acid, creatinine, inorganic phosphate, lactic dehydrogenase, and serum glutamic oxaloacetic transaminase. As in the earlier studies, the occurrence of unusually high levels of ETU in the urine of currently exposed workers confirmed their exposure. However, statistical analysis showed that no differences in thyroid function, urine, and blood, indicators for liver and kidney functions. In other words, the general health between exposed and control groups was non-significant.
CHAPTER THREE

EXPERIMENTAL SECTION

3.1 Sampling

In order to obtain representative sampling areas, ten garlic-growing locations throughout Sembalun were selected as sampling areas during the 1994 garlic-plantation season. On each location, the largest field was selected to take soil and garlic samples with an assumption that the heaviest application of fungicides had occurred on those fields. The ten fields selected varied in area from 0.8 to 1.5 hectare (see Table 3.1).

Table 3.1 The owner names of selected field for collecting soil and garlic samples and the areas

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Area (ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. Suhirman</td>
<td>Sembalun Bumbung I</td>
<td>1.5</td>
</tr>
<tr>
<td>H. Nursaid</td>
<td>Sembalun Bumbung II</td>
<td>0.9</td>
</tr>
<tr>
<td>H. Mahmud</td>
<td>Dusun Karya</td>
<td>1.2</td>
</tr>
<tr>
<td>H. Mustiadi</td>
<td>Lendang Luar</td>
<td>1.0</td>
</tr>
<tr>
<td>Abdurrachman</td>
<td>Dapur Belek</td>
<td>0.8</td>
</tr>
<tr>
<td>H. Nursaid</td>
<td>Orong Telaga Tengak</td>
<td>1.4</td>
</tr>
<tr>
<td>Wahidin</td>
<td>Nyumbo</td>
<td>1.3</td>
</tr>
<tr>
<td>H. Umar Sindih</td>
<td>Dayan Desa</td>
<td>1.5</td>
</tr>
<tr>
<td>H. Wildan</td>
<td>Bebante</td>
<td>1.0</td>
</tr>
<tr>
<td>H. Sainur</td>
<td>Orok</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The sample collections and analyses of dithiocarbamate residue were carried out in two terms of February-May and June-September 1994 of the garlic-planting seasons. On the first-five locations, Sembalun Bumbung I, Sembalun Bumbung II, Dusun
Karya, Lendang Luar and Dapur Belek, the residue in the soils and garlic were monitored in May-June 1994 and the last five locations, Orong Telaga Tengak, Nyumbo, Dayan Desa, Bebante and Orok, were monitored in September-October 1994.

3.2 Collection and Preparation of Soil and Garlic Samples


3.2.1 Soils

To study vertical movement of dithiocarbamate fungicides, soil samples were taken from four depth intervals; surface (< 1 cm), 1-10 cm, 10-20 cm, and 20-30 cm with spades for soil surface and corers for other levels. The sampling sites were spread evenly across the field diagonally. Twenty to thirty cores were made, from which approximately three to five kilogram of composite soil sample was collected at each depth. The soils were put in plastic bags closed tightly and then were transported by car equipped with air conditioning to our laboratory in the University of Mataram, Lombok-Indonesia with the journey taking about two and half hours.

In the laboratory, the field samples were mixed thoroughly and stones, roots, and the like were removed. Approximately 500 g of analytical samples were taken from the sieved soil and put into glass bottles fitted with plastic seals and stored at -5°C in a refrigerator until analysed.
3.2.2 *Garlic*

The garlic samples were collected close to the soil sample sites, from which three to four individual garlic plants were taken to get two to three kilogram of total sample at each selected location. The samples collected were tied into a bundle and transported with the soil samples. However, the garlic samples were stored at room temperature i.e. the same as the farmers store for their garlic. The bundles were hung from a cord until analysed. The bulbs of garlic to be analysed were prepared by separating their leaves and roots. The soil coating the bulbs was carefully brushed off. Finally, the samples were analysed 1, 3, 7, 14, and 21 day(s) after collecting or harvesting.

3.3 Chemicals and the Preparation

The chemicals used in the analysis were AR and PA grades which consist of carbon disulfide standard (CS$_2$), methanol (CH$_3$OH), sulfuric acid 95-97% (H$_2$SO$_4$), sodium hydroxide pellets (NaOH), tin(II)chloridedihydrate (SnCl$_2$.2H$_2$O) were from Riedel-de Haens; potassium hydroxide pellets (KOH), hydrochloric acid fuming 37% (HCl), ethylenediaminetetraaceticacid disodium salt dihydrate (C$_{10}$H$_{14}$N$_2$Na$_2$O$_8$. 2H$_2$O) or disodium EDTA were from E. Merck, and mancozeb standard was donated by Analchem Bio assay, Sydney.

The solutions required in analyses were prepared as follows: 6 M HCl was made by dilution of 37 % H Cl with distilled water; 40% (w/v) SnCl$_2$ was diluted in concentrated HCl; 0.5 M tetrasodium EDTA was made up by mixing equal volumes of 1 M disodium
EDTA and 2 M NaOH; and 2 M KOH was prepared by dissolving KOH pellets in methanol.

3.4 Apparatus

A gas burner was used to heat a three-neck, round-bottom 1000 mL boiling flask fitted with Liebig condenser with 3 absorption tubes connected by spherical socket joints and teflon pipe and connected to a vacuum pump equipped with flowmeter (see Figure 3.1 for diagram illustrating the setup of the apparatus); volumetric flask, 2000-mL, 1000-mL, 250-mL, 50-mL, 25-mL; electronic balances, Ohaus, model galaxyTm160 and galaxyTm400; and UV-VIS spectrophotometer, Varian DMS-70, with 1-cm quartz cell.

3.5 Principle and Procedure of Analysis

The procedure of analysis followed was USEPA-Method S 15 (1979) modified by the use of air instead of nitrogen as the sparging gas (Analchem Bioassay, Sydney). The outline of analysis is that the dithiocarbamates react with the solution of stannous chloride and hydrochloric acid to produce carbon disulfide which is distilled, purified in the solution of sodium hydroxide and concentrated sulfuric acid and is then collected in methanolic potassium hydroxide as a xanthate:

\[
\begin{align*}
S=\underset{\text{KOH/CH}_3\text{OH}}{\text{C=S}} & \quad \rightarrow \quad \text{CH}_3\text{O-C-SK} \\
& \quad \text{II} \\
& \quad \text{S}
\end{align*}
\]

The absorbance of the xanthate solution is then measured at 302 nm using a 1-cm quartz cell.
Figure 3.1 Setup of distillation and decomposition apparatus

Figure 3.2 UV-VIS spectrophotometer, Varian DMS-70
The steps of the analytical procedure were as follows; into the first and second absorption tubes of the decomposition apparatus, 25 mL of 0.5 M potassium hydroxide and 25 mL of concentrated sulfuric acid were added respectively, an accurately determined mass (∼17 g) of 2 M methanolic potassium hydroxide (xanthates are quantified gravimetrically) was put into third or last tube. For the decomposition and distillation, 40 g of soil sample or certain mass of unchopped garlic (10-40 g) was put into the flask and 200 mL of 6 M HCl and 10 mL of 40% (w/w) stannous chloride were added and the flask was connected to the condenser. To run the analysis, the Liebig condenser was turned on and the vacuum pump was connected to the last absorption tube so that the rate of flow on the flowmeter was approximately 300 mL min⁻¹. The flask was then heated immediately using a gas burner to boil the solution for 60 min. At the end, gas burner and vacuum pump were switched off and the third absorption tube was disconnected. The first and second tubes were also disconnected to avoid a possible backflow of the solutions.

The third tube was then weighted to obtain the exact weight of the solution. Finally, the solution was transferred into the cell and the absorbance measured at 302 nm against the blank solution made up from methanolic potassium hydroxide.

The samples were analysed in triplicate, from which the standard deviation (s) can be obtained. Coefficient of variation (CV) was only calculated for the recovery of mancozeb from soils and garlic.
bulbs. Both calculations were done by Lotus 1-2-3 package program based on the following equations.

\[ s = \sqrt{\frac{(x_1-x)^2 + (x_2-x)^2 + (x_3-x)^2}{n - 1}} } \]  
(3.1)

\[ CV = \left(\frac{s}{\bar{x}}\right) \times 100\% \]  
(3.2)

where \( s \) is standard deviation, \( CV \) is coefficient variation, \( x_{1,2,3} \) and \( \bar{x} \) are results and means of analysis respectively, and \( n \) is the number of replications.

### 3.6 Standard Solution and Calibration Curve

Standard stock solution of carbon disulfide was prepared by weighing a 50 mL flask (previously dried in an oven and placed in desiccator) (a g), and weighing the flask plus 0.6 mL of carbon disulfide (b g), then weighing the flask after it was made up to 50 mL with 2 M methanolic potassium hydroxide (c g). The exact concentration of stock solution (Cs) was obtained by calculating the mass of carbon disulfide and the mass of total solution stated as

\[ Cs \ (g/g) = \frac{b-a}{c-a} \]  
(3.3)

The working standard of carbon disulfide was freshly prepared by the dilution of stock standard solution in a 50 mL flask same as the stock standard procedure. The first dilution was prepared by weighing 1 mL of the stock solution into a 50 mL flask (of known mass), and then weighing the flask made up to 50 mL with
methanolic potassium hydroxide. The second was done with same technique to obtain a concentration of approximately 7 μg g\(^{-1}\) (the exact concentration is obtained by calculation). This was then made up to 0.5, 1, 2, 3, 4, 5, 6, and 7 μg g\(^{-1}\) by mixing varying amount of the carbon disulfide with methanolic potassium hydroxide solution. Each concentration was measured at 302 nm using a UV-VIS spectrophotometer, Varian DMS-70 with 1 cm-quartz cell.

Absorbance was plotted against carbon disulfide concentration and the simple regression procedure was used to obtain the concentration of carbon disulfide trapped. The simple regression is stated as

\[ y = mx + b \]  

(3.4)

where \( y \) is absorbance, \( x \) is concentration of carbon disulfide, \( m \) is slope of the line, and \( b \) is intercept.

3.7 The Determination of Water Content

The procedure used to determine water content of soils and garlic bulbs followed that of Hidayat (1978) and Rangana (1979) which were prepared by placing the weighing bottles with the tight-fitting lids in an oven, and drying them at 105°C for 2h, putting them into a desiccator about 20 min and weighing them. The next step was employed by weighing 5 g of soil or 2 g of garlic bulb in the bottle with the lid off and drying them to a constant mass at 105°C. Finally, after constant mass was obtained, the bottles were removed from the oven, lids replaced, and the bottles were put
into the desiccator. After 20 min, the bottles were reweighed. The following is the calculation to obtain dry matter content:

\[
\text{Dry matter content (\%) } = \frac{(W - w)}{Ws} \times 100\% \quad (3.5)
\]

where \(W\) is the total mass of the sample and weighing bottle after drying (g), \(w\) is mass of the weighing bottle, and \(Ws\) is the mass of the sample.

### 3.8 Calculation of Residues

The residue of carbon disulfide, in mg/kg oven dry matter, was calculated from the following equation:

\[
R = \frac{W_{AR}}{G \times Wd} \quad (3.6)
\]

where \(G\) is the mass of sample (g), \(Wd\) is dry matter content (%), and \(W_{AR}\) is the amount of carbon disulfide from calibration curve (µg).

### 3.9 Evaluation of the Technique

The detection limit and the recovery of mancozeb from soils and garlic bulbs were determined in order to evaluate the analytical technique used. To obtain the detection limit, a calibration curve was made as described in 3.6, then the detection limit was calculated based on the following equation (Nur et al., 1992),

\[
D_l = \frac{3}{m} \sqrt{SB^2 + Si^2 + (i/m)^2 Sm^2} \quad (3.7)
\]

where \(D_l\) is limit of detection, \(m\) is slope, \(i\) is intercept, \(SB\) is standard deviation of blank, \(Si\) is standard deviation of \(i\), and \(Sm\) is standard deviation of \(m\). \(m\), \(i\), \(SB\), and \(Si\) were calculated from the following equations prior to calculated \(D_l\):
\[ m = \frac{1}{\Delta} (n\Sigma cx - \Sigma c\Sigma x) \]  
(3.8)

\[ i = \frac{1}{\Delta} (\Sigma c^2\Sigma x - \Sigma c\Sigma cx) \]  
(3.9)

\[
SB = \frac{\SigmaxB^2 - (\SigmaxB)^2}{\frac{n}{n - 1}}
\]  
(3.10)

\[
Si = s\sqrt{\frac{\Sigma c^2}{\Delta}}
\]  
(3.11)

\[
Sm = s\sqrt{\frac{n}{\Delta}}
\]  
(3.12)

s and \( \Delta \) were obtained from the following equations:

\[
s = \frac{1}{n-2} [\Sigma x^2 - \frac{1}{\Delta} \left( \Sigma c^2 (\Sigma x)^2 - 2\Sigma c\Sigma x\Sigma cx + n(\Sigma cx)^2 \right)]
\]  
(3.13)

\[\Delta = n\Sigma c^2 - (\Sigma c)^2\]  
(3.14)

The calculation of determination limit from the calibration curve was achieved using a BASIC program written for IBM compatibles (see Appendix 1).

The recovery of mancozeb (Rm) from soils and garlic bulbs was obtained by comparing a recovered concentration from the analysis (Ca) and an injected concentration into soil or garlic free of dithiocarbamate fungicides (Ci), which is stated as

\[ Rm = \frac{Ca}{Ci} \times 100\% \]  
(3.15)
For this purpose, garlic of free dithiocarbamate fungicides had been planted at Sembalun during the June-September garlic-planting season in 1994.

3.10 Confirmation

For comparison and to study the effect of matrix interference, a standard addition technique was carried out for both soil and garlic samples collected from Orok.

The procedure employed was similar to the procedure described in point 3.5 and involved the addition of various concentrations of mancozeb standard solution, diluted in 0.5 M tetrasodium EDTA. Sample sizes used were 40 g and 2 g for soils and garlic respectively. The mixture of the sample and tetrasodium EDTA solution was treated immediately with hydrochloric acid and SnCl₂ and then heated. The absorbances against the concentrations of mancozeb added were then plotted. The concentration of carbon disulfide as xanthate was obtained from the curve through calculating the x-intercept where x is the concentration of added mancozeb converted to carbon disulfide (the conversion factor is 1.776) and the calculation of residues was mentioned in point 3.8.
CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Calibration Curve
A calibration curve was generated by plotting the absorbance measured at 302 nm versus disulfide concentration. The standard solutions were prepared by weighing the solutions in stoppered bottles. This technique is to avoid the loss of the volatile solvent from methanolic potassium hydroxide solution and the human error involved in determining volumes during preparation.

![Calibration Curve Image]

Figure 4.1 The calibration curve for the absorbance at 302 nm against the series of carbon disulfide concentration

By plotting ug carbon disulfide, diluted in methanolic potassium hydroxide, per mixture on the abscissa (x) against the absorbance
value on the ordinate (y), a linear calibration curve was obtained with a correlation coefficient (r) 0.9999.

To differentiate between the absorbance signals due to blank solution and the lowest contaminant determined, the detection limit is calculated statistically (see point 3.9) and discussed in the following point.

4.2 Recovery and Detection Limit

The overall recoveries of mancozeb spiked into soil and garlic bulb samples are shown in Table 4.1. The recovery obtained from soils is between 81 and 90 percent, which is similar to the recovery mentioned in USEPA-method S 15 (1979). The recovery from garlic was found to be between 102 and 110 percent. The over-recovered percentage from garlic may arise from decomposition of alline disulfide and/or organic sulfur compounds present in garlic. The analysis of garlic bulb without mancozeb spiking solution was found to contain an amount of carbon disulfide equal to 0.17±0.01 mg/kg dry matter.

Since the over-recovery contributed by the garlic should be a fixed amount, one would expect a larger percentage over-recovery for small additions of CS2 spike and a smaller percentage over-recovery for larger additions of spike. In Table 4.1 this expected effect is barely apparent. The smallest spike did give the largest percentage over-recovery, but no systematic trend was seen for the remaining spikes. The coefficient of variation (~3%) is sufficient to obscure any such trend.
Coefficient of variation or CV \((n = 3)\) calculated from the recovery of mancozeb from both soil and garlic was relatively low, between 1.11 and 5.25 for soils and from 0.68 to 4.11 for garlic bulbs. A low standard deviation was also obtained from the results of each analysis of samples. This proved that the 'xanthate technique' was precise and reproducible.

Table 4.1 Recovery of mancozeb, converted to carbon disulfide, spiked to soils and garlic bulbs

<table>
<thead>
<tr>
<th>Sample</th>
<th>Spike conc. (mg of CS₂)</th>
<th>Recovery, %</th>
<th>Mean±s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rep. 1</td>
<td>Rep. 2</td>
</tr>
<tr>
<td>Soils</td>
<td>1.80</td>
<td>90</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>3.61</td>
<td>85</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>5.41</td>
<td>85</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>9.02</td>
<td>80</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>12.62</td>
<td>89</td>
<td>91</td>
</tr>
<tr>
<td>Garlic bulbs</td>
<td>1.63</td>
<td>111</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>3.25</td>
<td>108</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>4.88</td>
<td>104</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>8.13</td>
<td>104</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>11.38</td>
<td>105</td>
<td>104</td>
</tr>
</tbody>
</table>

From the calculation of detection limits mentioned in point 3.9, it was found that the detection limit for the technique was 0.1 ug/g carbon disulfide. This means that the technique can be applied in determining the lower concentrations of dithiocarbamate contamination or for small sample sizes.
4.3 Dithiocarbamate Residues in Soils and Garlic Bulbs

4.3.1 Soils

The results of dithiocarbamate analysis found in soils from the February-May and June-September terms of the 1994 garlic-planting season are shown in Table 4.2 and 4.3 respectively.

Table 4.2 Dithiocarbamate residues found from various depth of soils collected two weeks before harvesting, the term of February-May of the 1994 garlic planting season.

<table>
<thead>
<tr>
<th>Location</th>
<th>Depth of soil (cm)</th>
<th>Residues (as CS₂ in mg/kg dry matter)</th>
<th>Rep. 1</th>
<th>Rep. 2</th>
<th>Rep. 3</th>
<th>Mean±s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sembalun Bumbung I</td>
<td>&lt; 1</td>
<td>5.14</td>
<td>4.72</td>
<td>4.59</td>
<td>4.82±0.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 - 10</td>
<td>0.42</td>
<td>0.43</td>
<td>0.44</td>
<td>0.43±0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 - 20</td>
<td>0.21</td>
<td>0.16</td>
<td>0.12</td>
<td>0.16±0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 - 30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sembalun Bumbung II</td>
<td>&lt; 1</td>
<td>2.86</td>
<td>3.01</td>
<td>2.87</td>
<td>2.91±0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 - 10</td>
<td>0.64</td>
<td>0.65</td>
<td>0.44</td>
<td>0.58±0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 - 20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 - 30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Lendang Luar</td>
<td>&lt; 1</td>
<td>1.82</td>
<td>1.73</td>
<td>1.90</td>
<td>1.82±0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 - 10</td>
<td>0.22</td>
<td>0.21</td>
<td>0.19</td>
<td>0.21±0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 - 20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 - 30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Dusun Karya</td>
<td>&lt; 1</td>
<td>4.49</td>
<td>4.48</td>
<td>4.04</td>
<td>4.34±0.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 - 10</td>
<td>1.81</td>
<td>1.44</td>
<td>1.51</td>
<td>1.59±0.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 - 20</td>
<td>1.08</td>
<td>0.78</td>
<td>0.97</td>
<td>0.94±0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 - 30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Dapur Belek</td>
<td>&lt; 1</td>
<td>4.24</td>
<td>4.07</td>
<td>4.33</td>
<td>4.21±0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 - 10</td>
<td>0.39</td>
<td>0.37</td>
<td>0.42</td>
<td>0.39±0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 - 20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 - 30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*) not detected

The dithiocarbamate residues were present in all sampling sites not only on the surface, but also in the depth of 1-10 cm. Indeed at Sembalun Bumbung I and Dusun Karya, the residues exist at 10-20 cm depth. The mean of residues ranged from 1.82 mg/kg
to 4.82 mg/kg dry matter and from 0.21 mg/kg to 1.59 mg/kg for the surface of soil and the depth of 1-10 cm respectively, low concentrations of 0.16-0.21 mg/kg dry matter were found in the 10-20 cm level. This shows that the vertical movement of the residue was relatively slow. Although the soil types of the first five locations and the others, alluvial and regosol respectively, are different, the vertical movement of residues was not significantly different. Helling et al. (1974) stated that the mobility of EBDCs in soil varies considerably, depending on water solubility and soil type. They are generally more mobile in wet and in sandy soils than in dry soil or soil rich in organic matter. The very low rainfall during the 1994 garlic-planting season (see Figure 1.2) probably contributed to reducing the residue penetration in the soil.

It can be seen that the residues mainly persisted on the surface of soil. These residues might have the effect of reducing the availability of some elements required by plants, in which dimethyldithiocarbamic acid binds with heavy metals in soil to form complexes (WHO, 1988). This would lead to an increased fertilizer requirement for plant growth to continue.

However, the residues tended to decrease compared with the post harvesting residues. Residue monitoring performed on the soil surface a day after harvesting showed that the residues were relatively lower than that of the two weeks prior to harvesting with a range from 1.20 mg/kg to 2.69 mg/kg dry matter (see Table 4.4).
Table 4.3 Dithiocarbamate residues obtained from various depth of soils collected two weeks before harvesting, the term of June-September of the 1994 garlic-planting season

<table>
<thead>
<tr>
<th>Location</th>
<th>Depth of soil (cm)</th>
<th>Residues (as CS$_2$ mg/kg dry matter)</th>
<th>Mean±s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rep. 1</td>
<td>Rep. 2</td>
</tr>
<tr>
<td><strong>Orong Telaga Tengak</strong></td>
<td>&lt; 1</td>
<td>3.41</td>
<td>3.36</td>
</tr>
<tr>
<td></td>
<td>1 - 10</td>
<td>1.17</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>10 - 20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20 - 30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Dayan Desa</strong></td>
<td>&lt; 1</td>
<td>4.55</td>
<td>4.59</td>
</tr>
<tr>
<td></td>
<td>1 - 10</td>
<td>0.37</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>10 - 20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20 - 30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Nyumbo</strong></td>
<td>&lt; 1</td>
<td>2.77</td>
<td>2.76</td>
</tr>
<tr>
<td></td>
<td>1 - 10</td>
<td>1.09</td>
<td>1.19</td>
</tr>
<tr>
<td></td>
<td>10 - 20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20 - 30</td>
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<td>-</td>
</tr>
<tr>
<td><strong>Bebante</strong></td>
<td>&lt; 1</td>
<td>4.46</td>
<td>4.63</td>
</tr>
<tr>
<td></td>
<td>1 - 10</td>
<td>1.49</td>
<td>1.58</td>
</tr>
<tr>
<td></td>
<td>10 - 20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20 - 30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Orok</strong></td>
<td>&lt; 1</td>
<td>4.24</td>
<td>4.07</td>
</tr>
<tr>
<td></td>
<td>1 - 10</td>
<td>0.39</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>10 - 20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20 - 30</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*) not detected

Table 4.4 Dithiocarbamate residues in surface soils collected a day post harvesting for both of the terms of February-May and June-September of the 1994 garlic planting season

<table>
<thead>
<tr>
<th>Location</th>
<th>Residue (as CS$_2$ mg/kg dry matter)</th>
<th>Mean±s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rep. 1</td>
<td>Rep. 2</td>
</tr>
<tr>
<td><strong>Term of Feb.-May</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sembulan Bumbung I</td>
<td>2.42</td>
<td>2.29</td>
</tr>
<tr>
<td>Sembulan Bumbung II</td>
<td>1.32</td>
<td>1.39</td>
</tr>
<tr>
<td>Lendang Luar</td>
<td>1.86</td>
<td>1.82</td>
</tr>
<tr>
<td>Dusun Karya</td>
<td>2.65</td>
<td>2.82</td>
</tr>
<tr>
<td>Dapur Belek</td>
<td>1.36</td>
<td>1.25</td>
</tr>
<tr>
<td><strong>Term of June-Sep.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orong Telaga Tengak</td>
<td>1.12</td>
<td>1.21</td>
</tr>
<tr>
<td>Dayan Desa</td>
<td>1.98</td>
<td>1.70</td>
</tr>
<tr>
<td>Nyumbo</td>
<td>2.21</td>
<td>2.09</td>
</tr>
<tr>
<td>Bebante</td>
<td>2.52</td>
<td>2.32</td>
</tr>
<tr>
<td>Orok</td>
<td>1.39</td>
<td>1.49</td>
</tr>
</tbody>
</table>

61
4.3.2 Garlic

The residues of dithiocarbamate found in garlic bulbs during the two terms of the 1994 garlic planting season are given in Table 4.5 and 4.6.

Table 4.5 Dithiocarbamate residues in garlic bulb analysed 1, 3, 7, 14, and 21 day(s) after harvesting, the term of February-May of the 1994 garlic planting season

<table>
<thead>
<tr>
<th>Location</th>
<th>Day(s) After Harvesting</th>
<th>Residues (as CS₂ in mg/kg dry matter) *</th>
<th>Mean±s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rep. 1</td>
<td>Rep. 2</td>
</tr>
<tr>
<td>Sembalun Bumbung I</td>
<td>1</td>
<td>14.19</td>
<td>12.84</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.85</td>
<td>7.92</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2.47</td>
<td>2.52</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.28</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>0.74</td>
<td>0.87</td>
</tr>
<tr>
<td>Sembalun Bumbung II</td>
<td>1</td>
<td>17.14</td>
<td>16.56</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.78</td>
<td>5.33</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2.48</td>
<td>2.80</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.76</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>0.87</td>
<td>0.94</td>
</tr>
<tr>
<td>Lendang Luar</td>
<td>1</td>
<td>16.89</td>
<td>15.92</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>11.06</td>
<td>9.48</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>6.59</td>
<td>5.82</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1.25</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>1.13</td>
<td>0.92</td>
</tr>
<tr>
<td>Dusun Karya</td>
<td>1</td>
<td>33.24</td>
<td>30.39</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>13.31</td>
<td>13.05</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>5.28</td>
<td>3.76</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>2.49</td>
<td>2.94</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>0.65</td>
<td>0.95</td>
</tr>
<tr>
<td>Dapur Belek</td>
<td>1</td>
<td>13.44</td>
<td>13.29</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.90</td>
<td>7.31</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>4.82</td>
<td>4.72</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>2.29</td>
<td>2.66</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>0.89</td>
<td>0.91</td>
</tr>
</tbody>
</table>

*) Garlic grown without fungicides gave an apparent concentration of 0.17 ± 0.01 mg/kg of dry matter. This value has been deducted from the data in the table.

The dithiocarbamate residues were detected in all garlic bulbs collected from ten sample locations. Overall monitoring showed residues to be greater than the Maximum Residue Limit (MRL)
recommended by National Health and Medical Research Council (NHMRC) of Australia which allow 0.2 mg/kg for onion (no mention for garlic). Residues should be low in root crops such as carrots, potatoes, onions or garlic, as dithiocarbamates are not systemic and tend to remain on the external part of the crops.

Table 4.6 Dithiocarbamate residues in garlic bulb analysed 1, 3, 7, 14, and 21 day(s) after harvesting, the term of June-September of the 1994 garlic planting season

<table>
<thead>
<tr>
<th>Location</th>
<th>Day(s) After Harvesting</th>
<th>Residues (as CS₂ in mg/kg dry matter) *)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rep. 1</td>
</tr>
<tr>
<td>Orong Telaga Tengak</td>
<td>1</td>
<td>15.17</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12.59</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>8.11</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>3.96</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>0.85</td>
</tr>
<tr>
<td>Dayan Desa</td>
<td>1</td>
<td>10.29</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8.90</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>7.49</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>3.16</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>0.48</td>
</tr>
<tr>
<td>Nyumbo</td>
<td>1</td>
<td>13.49</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9.32</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>5.97</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1.99</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>1.83</td>
</tr>
<tr>
<td>Bebante</td>
<td>1</td>
<td>9.02</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.38</td>
</tr>
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<td>7</td>
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<td>2.22</td>
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<td></td>
<td>21</td>
<td>1.27</td>
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<tr>
<td>Orok</td>
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<td>15.81</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9.67</td>
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<tr>
<td></td>
<td>7</td>
<td>7.36</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>3.10</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>2.24</td>
</tr>
</tbody>
</table>

*) Garlic grown without fungicides gave an apparent concentration of 0.17 ± 0.01 mg/kg of dry matter. This value has been deducted from the data in the table.

However, Philips et al. (1977) showed that washing the agricultural products prior to processing removed 33-87% of the EBDCs and the majority of the ETU residues.
Statistically analysis using Analysis of Variance (ANOVA) conducted on the residue values where samples collected a day post harvesting showed that the residues found in garlic taken from miscellaneous location differ significantly. This is probably because of the different various techniques (including type and concentration and dosage of fungicides applied) used by the farmers to suppress plant diseases.

However, further analyses of dithiocarbamate residues showed that the residues had reduced exponentially (see Figure 4.2 and 4.3). Interestingly, although the residues of a day post harvesting at each location significantly differ, the patterns of the reduction in residues at each location are similar. In general, the residues decreased to less than 2.00 mg/kg within 21 days of harvesting. Nash and Beall (1980) showed that of the residual fungicides maneb and zineb (measured as EDA and ETU) applied at 2 kg ha\(^{-1}\) to tomato plants less than 20 µg kg\(^{-1}\) of ETU remained after 3 days and completely disappeared after three weeks. EDA was still present on whole fruits at 1 mg kg\(^{-1}\) after 10 weeks.

Moreover, Kaars Sijpesteijn et al. (1977) have reported that potato plants sprayed in the field with \(^{14}\)C-Mancozeb contained very small amount of ETU and ethyleneurea in the tubers. It has been suggested that mancozeb is ultimately degraded into glycine via ethylene diamine.
4.4 Standard Addition Method

As a confirmation analysis, the standard addition method mentioned in point 3.10 was carried out and the results for both soils and garlic are respectively given in Table 4.7 and 4.8.

While the simple calibration curve method of determining dithiocarbamate as xanthate gave good precision, the accuracy of the data is another question. Positive error have been found previously for rape seed, cauliflower, and savoy cabbage (USEPA, 1979). In the present work, garlic grown without fungicides gave an apparent carbon disulfide concentration 0.17±0.01 mg/kg dry matter. This value has been deducted from the data in Table 4.5, 4.6, and 4.8. This source of systematic positive error will be present both in the simple method and the standard addition technique.

Table 4.7 Dithiocarbamate residues in soil collected from Orok 2 weeks before harvest analysed by standard addition method (1) and calibration curve method (2)

<table>
<thead>
<tr>
<th>Depth of soil (cm)</th>
<th>Residue (as CS₂ mg/kg dry matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1)</td>
</tr>
<tr>
<td>&lt; 1</td>
<td>4.96</td>
</tr>
<tr>
<td>1-10</td>
<td>2.71</td>
</tr>
<tr>
<td>10-20</td>
<td>0.64</td>
</tr>
<tr>
<td>20-30</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*) not detected

Systematic negative errors can arise from incomplete conversion of Mancozeb to CS₂ and from losses of CS₂ via incomplete transfer to the KOH/CH₃OH absorber solution or incomplete reaction of CS₂ with this solution. In particular, if the distillation flask is not heated quickly enough, cyclic intermediates may form from which
CS$_2$ does not cleave quantitatively (USEPA, 1979). Depending on matrix, recoveries of Mancozeb were 76-91%. In the present work, it is noteworthy that results determined from the Orok area by the standard addition method are higher than the corresponding data obtained by simple calibration curve procedure. The standard addition technique corrects for these systematic losses.

Table 4.8 Dithiocarbamate residues in garlic bulb collected from Orok analysed by standard addition method (1) and calibration curve method (2)

<table>
<thead>
<tr>
<th>Day(s) post harvesting</th>
<th>Residue (as CS$_2$ mg/kg dry matter) $^*$</th>
<th>(1)</th>
<th>(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21.06</td>
<td>15.68</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>15.96</td>
<td>9.73</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>12.56</td>
<td>6.19</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>5.42</td>
<td>2.87</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>3.94</td>
<td>1.73</td>
<td></td>
</tr>
</tbody>
</table>

$^*$ Garlic grown without fungicides gave an apparent concentration of 0.17 ± 0.01 mg/kg of dry matter. This value has been deducted from the data in the table.

For example, suppose the sample contained a Mancozeb concentration equivalent to 1.0 ug/g of CS$_2$ in the final xanthate solution. If the conversion of Mancozeb to CS$_2$ was 100% efficient, the absorbance (Figure 4.1) of the xanthate solution would be 0.233. If the conversion was only 50% efficient, the absorbance would be 0.121, and the concentration of Mancozeb determined by the simple procedure would be only half the true value. If standard addition of Mancozeb equivalent to CS$_2$ concentrations of 0.5 ug/g and 1.0 ug/g respectively are made to separate samples prior to distillation, the absorbance of the final solution will rise to 0.345 and 0.457 respectively (100% conversion) or 0.289 and 0.345 (50% conversion). When three absorbance data points for
100% conversion (0.233, 0.345, 0.457, original + two standard additions) are plotted, the x axis intercept = minus 1.0 ug/g i.e. the original concentration inferred is Mancozeb equivalent to 1.0 ug/g of CS$_2$ in the final solution. Hence provided exactly the same experimental procedure is followed in every case, the standard addition method compensates for systematic incomplete conversion of Mancozeb to CS$_2$. There is an assumption that the Mancozeb contained within the soil or garlic reacts as readily and completely as does the EDTA solution containing the Mancozeb spike.

Thus, in term of accuracy, the standard addition method is the preferred technique. However, if two standard additions are made, each sample requires three times the analysis time of the simple procedure. Even the latter is quite lengthy (> 1.5h per sample), and so it was not feasible to analyse all samples in trilplicate by the standard addition method. Even though the simple method understates the concentration of dithiocarbamate in soil or garlic, because exactly the same procedure was followed in each case, the data are valid for comparison purposes, e.g. when looking for trend such as concentration versus soil depth, length of storage of garlic etc.
Figure 4.2. Patterns of the decrease of dithiocarbamate residues in garlic taken from the first five location throughout Sembalun (Series 1 = Sembalun Bumbung I, series 2 = Sembalun Bumbung II, series 3 = Lendang Luar, series 4 = Dusun Karya, and series 5 = Dapur Belek).

Figure 4.3. Patterns of the decrease of dithiocarbamate residues in garlic collected from the second five of sampling sites throughout Sembalun (series 1 = Orong Telaga Tengak, series 2 = Dayan Desa, series 3 = Nyumbo, series 4 = Bebante, and series 5 = Orok).
CHAPTER FIVE

CONCLUSION

The monitoring of residues of dithiocarbamate fungicides (conducted during the 1994 garlic planting season at Sembalun, Lombok Indonesia) indicated the following:

1. The technique of carbon disulfide evolution followed by conversion to xanthate was precise and reproducible with the limit of detection 0.1 ug/g. The technical recovery from soils and garlic was found the range between 81-90% and 102-110% respectively. Garlic especially contained compounds producing a carbon disulfide signal of 0.17±0.01 mg/kg dry matter which caused minor interference with the analysis. Although it is more time-consuming, the standard addition method is preferred to the calibration curve method.

2. Monitoring of dithiocarbamate residues two weeks before harvesting found that the residues mainly persisted on surface of both types of soil with the range of from 1.82 mg/kg to 4.82 mg/kg dry matter, in which the vertical movement of the residues was relatively slow. The residues generally persisted until the depth of 1-10 cm. Further monitoring one day post harvesting indicated that the residues tended to decrease.

3. All sampled garlic bulbs analysed a day after harvesting were contaminated with dithiocarbamate residues ranging from 8.95
mg/kg to 31.26 mg/kg dry matter, in which the residues significantly differ among locations selected. However, the residues tended to decrease exponentially to the range 0.78 to 1.73 mg/kg dry matter within 21 days of harvesting.

4. While the garlic bulbs used in this study were analysed unwashed (in the same state as those sent to market by farmers), it is likely that most of the fungicide is on the outer surface. It may therefore be possible to lower the fungicide residue concentration by husking or by washing the bulbs with water prior to sending them to market.
ACKNOWLEDGMENT

I would like to express my appreciation to my supervisor Associate Professor John Ellis for his kind guidance and assistance during the course of the research. I especially acknowledge the skilful technical assistance given by Dr. John Korth who always encourage me so that this research kept going. I also acknowledge AUSAID that funded this study.

I would like to thank to Mr. H. Mustiadi (ex. head of Sembalun Lawang village), Mr. Hery Haryanto (lecturer of Faculty of Agriculture, Mataram University), and Mr. Taspin (technical laboratory of Laboratory of Chemistry, Mataram University) for their assistance during experiment.
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Appendix 1. Basic Program of PC-IBM for the calculation of detection limit

```
5  PRINT "ANALYSIS"
7  PRINT "DETECTION LIMIT"
10 A = 0: B = 0: C = 0: D = 0: E = 0
20 FOR N = 1 TO 6
30 INPUT X
40 INPUT Y
50 A = A + X
60 B = B + Y
70 C = C + X * Y
80 D = D + X ^ 2
90 E = E + Y ^ 2
100 NEXT N
110 F = A ^ 2
120 K = 6 * D - F
130 m = (6 * C - A * B) / K
140 i = (D * B - A * C) / K
150 PRINT "m="; m
160 PRINT "i="; i
170 INPUT J
180 T = (J - i) / m
190 IF J = 99 THEN 220
200 PRINT "SAMPLE CONCENTRATION"'; T
210 GOTO 170
220 G = B ^ 2
230 H = C ^ 2
240 L = D * G - 2 * A * B * C + 6 * H
250 O = (E - L / K) / 4
260 S = O ^ .5
270 PRINT "STANDARD DEVIATION SLOPE (Sm)="; R
280 Q = S * (D / K) ^ .5
290 PRINT "STANDARD DEVIATION INTERCEPT (Si)="; Q
300 INPUT P
310 W = 3 / m * (P ^ 2 + Q ^ 2 + (i / m) ^ 2 * R ^ 2) ^ .5
320 PRINT "LIMIT DETECTION"'; W
330 END
```
Appendix 2. Analysis of variance (ANOVA) of dithiocarbamate residues among location

<table>
<thead>
<tr>
<th>Location</th>
<th>Rep.1</th>
<th>Rep. 2</th>
<th>Rep. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sembalun Bumbung I</td>
<td>14.36</td>
<td>13.01</td>
<td>13.34</td>
</tr>
<tr>
<td>Sembalun Bumbung II</td>
<td>17.31</td>
<td>16.73</td>
<td>16.88</td>
</tr>
<tr>
<td>Lendang Luar</td>
<td>17.06</td>
<td>16.09</td>
<td>14.12</td>
</tr>
<tr>
<td>Dusun Karya</td>
<td>33.41</td>
<td>30.56</td>
<td>30.32</td>
</tr>
<tr>
<td>Dapur Belek</td>
<td>13.61</td>
<td>13.46</td>
<td>14.81</td>
</tr>
<tr>
<td>Orong Telaga Tengak</td>
<td>15.34</td>
<td>14.17</td>
<td>16.06</td>
</tr>
<tr>
<td>Dayan Desa</td>
<td>10.46</td>
<td>11.61</td>
<td>11.31</td>
</tr>
<tr>
<td>Nyumbo</td>
<td>13.66</td>
<td>11.56</td>
<td>13.68</td>
</tr>
<tr>
<td>Bebante</td>
<td>9.19</td>
<td>9.19</td>
<td>8.97</td>
</tr>
<tr>
<td>Orok</td>
<td>15.98</td>
<td>14.19</td>
<td>17.39</td>
</tr>
</tbody>
</table>

Anova: Single Factor

**SUMMARY**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sembalun Bumbung I</td>
<td>3</td>
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**ANOVA**

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