

STUDY ABOUT THE INFLUENCE OF THE SAMPLING AND STORAGE CONDITIONS, ON THE RESULT OF THE ANALYSIS OF INSPECTION SAMPLES OF FUNGICIDE *MANCOZEB*

(*Estudio de la influencia de las condiciones del muestreo y almacenamiento sobre los resultados del análisis en muestras de inspección del fungicida Mancozeb*)

H. Seltzer*, C. Fernández-Armijos**, H. Gaese***, R. Prisco****

*GTZ – Sociedad Alemana de Cooperación Técnica y Financiera
Postfach 5180, D-65760 Eschborn, Germany

**Universidad Nacional Mayor de San Marcos, Facultad de Química e Ingeniería Química
Departamento de Análisis y Diseño de Procesos
Av. Venezuela s/n Lima-1, Perú

***Instituto de Tecnología de los Trópicos, Univ. Téc. de Colonia
Betzdorfer Str. 2, D-50679 Köln, Germany

**** Ministerio de Agricultura de São Paulo, Brasil
Coordenadoria de Assistência Técnica Integral (CATI)
Campinas, São Paulo, Brazil

Abstract : The use of pesticides is an important part of the crop protection system world-wide. In the 1990's, Brazil is not only a pesticide consumer but an important pesticides producer in the southamerican region. The production, marketing and application of pesticides involve risks to the users and to the environment. Therefore is important the quality control of pesticides at all stages and a close collaboration of all the involved groups. The fungicide Mancozeb has developed problems due to its breakdown products as Ethylene-thiourea (ETU), which is reported to inhibit the functions of the Thyroid gland, interfering in iodine metabolism. This study appears as a contribution to clarify matters concerning the stability and degradation of ethylenebisdithiocarbamates under different conditions of temperature, moisture, storage time and sun radiation exposition for a plant protection practice without hazardous side effects.

Key words : Pesticides, fungicides, ethylene-bis-dithio-carbamates, stability, degradation, breakdown products, Ethylene-thiourea, metabolism, crop protection, quality control, environmental impact assessment.

Resumen : El uso de pesticidas constituye una parte importante de la protección de cultivos agrícolas en todo el mundo. En la década de los '90s, Brasil no solamente es un consumidor, sino que también es un importante productor de pesticidas en la región sudamericana. La producción, mercadeo y aplicación de pesticidas involucra riesgos para el usuario y para el medio ambiente. Por tales razones, es importante el control de calidad de los pesticidas en todas las etapas, así como una estrecha colaboración entre todos los grupos involucrados. El fungicida Mancozeb ha desarrollado problemas debido a sus productos de degradación, como la Etileno-tiourea (ETU), la cual es reportada como inhibidora de las funciones de la glándula Tiroides, interfiriendo en el metabolismo del yodo. Este estudio aparece como una contribución en el esclarecimiento de aspectos concernientes a la estabilidad y degradación de etilenobisditiocarbamatos bajo diferentes condiciones de temperatura, humedad, tiempo de almacenamiento y exposición a la radiación solar para la protección de cultivos agrícolas sin efectos secundarios peligrosos.

Palabras Clave : Pesticidas, fungicidas, etileno-bis-ditio-carbamatos, estabilidad, degradación, productos de descomposición, Etileno-tiourea, metabolismo, protección de cultivos, control de calidad, estudio de impacto ambiental.

INTRODUCTION

In Brazil, the pesticides formulation industry was developed in the 1950's. After two decades, in the 1970's, with the creation of an industrial park for pesticides, Brazil become an important manufacturer for active ingredients. Since 1979 Brazil is selling his formulated pesticides in the international market. The new "*Pesticides Federal Law 7.802*" of 1989 and its regulations in 1990 – substituting the older one of 1934-, gave more autonomy to each state.

In the mid-1990's, the pesticide industry in Brazil appears as a result of the governmental industrialization policies by import-substitutions of the mid-1970's, and is expanding as exporter into the world market. The pesticides use in the agricultural sector against pests, diseases and weeds, helps to protect and improve the productivity, assuring the Brazilian internal supply and the large-scale crop production for export.

By 1994 the total sales of pesticides in Brazil was nearly 1.000 millions US \$, as shown in **Table 1**.

Table 1: Pesticides sales in Brazil over the period 1989-1994 in million US \$ (ANDEF [1], BFAI [2]).

Year	Pesticides Sales
1989	980,5
1990	1084,3
1991	988,1
1992	947,4
1993	1049,8
1994	998,7

The agro-pesticides consume in Brazil over the period 1970 – 1991 in tons of active ingredient is shown at **Table 2**. Since 1970 the participation volume of the pesticides local production has growth up to reach in the 1990's decade 80% of the brazilian pesticides market.

Table 2: Agro-pesticides consume (tons) in Brazil 1970-1991, and the participation of the local manufacturers as a percentage (ANDEF [1]).

Year	Agro-pesticides consume (ton.)	Participation of the local production (%)
1970	27.728	27,3
1975	60.592	31,5
1980	80.968	52,2
1985	43.081	76,4
1991	60.188	83,0

In general, the pesticides consume in Brazil, after a dramatically increased in the 70's, reached its maximum by 1980 (80.968 tons), and in the early 90's stabilised by 60.000 tons. By 1994 it was expected an annual growth rate of 4% for the Brazilian pesticide's branch (BFAI [2]).

By March 1994 were registered in the state of São Paulo 280 different active ingredients, belonging to 750 different pesticide trade formulations; being 603 of them commercialised by the about 1600 selling stores. The **Diagram 1** shows the change of pesticide trade brands registered in São Paulo by 1995, with a decreasing tendency.

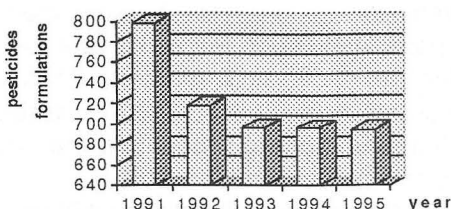


Diagram 1 : Quantity variation of the pesticide trade brands registered in São Paulo, between 1991 and March 1995 (ANDEF, 1994, [1]).

By 1995, the pesticide manufacture company *ROHM AND HAAS DO BRAZIL LTDA*, in its facilities located in Jacareí, São Paulo, produces about 14.000 tons of *DITHANE* yearly (trade name for a Mancozeb formulation); 60% of them is being exported overall in the world. The **Diagram 2** shows the production process of Dithane-PM.

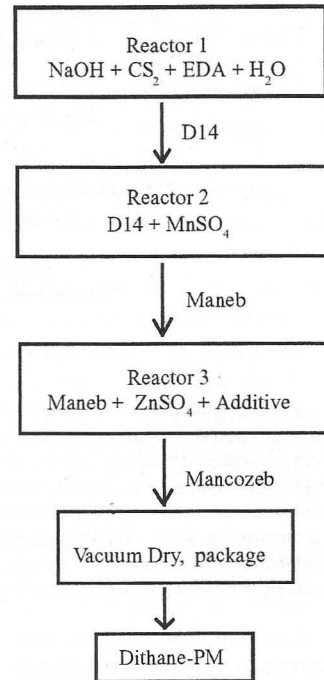


Diagram 2: Flow chart of the production process of Dithane-PM (Courtesy of *ROHM AND HAAS DO BRASIL LTDA*, Jacareí, São Paulo).

The fungicide Mancozeb is a member of the carbamates group, it is a ethylene-bis-dithiocarbamate (EBDC) of Manganese and Zinc, and has a low toxicity level for humans. According to BARRAT and HOSSFALL [7], THORN and LUDWIG [8], ENGST and SCHNAAK [9], one of its trace contaminants and breakdown products is Ethylene-thiourea (ETU). SOBOTKA and PETROVA [10], GRAHAM and MATOLCSY [11] have found that EDBC's and ETU action in the human body is centred on the Thyroid gland, inhibiting its functions and interfering the iodine metabolism. GRAHAM [12] reports carcinogenic effects on mammals due to ETU exposition. **Diagram 3** shows typical breakdown reactions for Mancozeb.

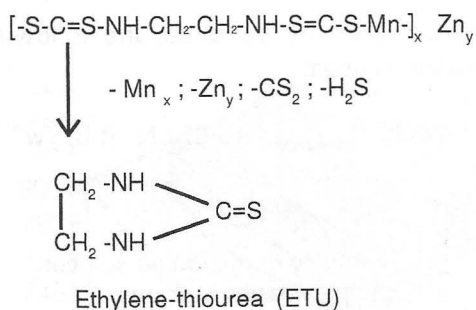


Diagram 3: Typical breakdown reactions of Mancozeb active ingredient according to MATOLCSY et al. [13].

To guarantee and maintain a safe pesticide use, the Brazilian laws dictate an Environmental Impact Assessment (EIA) to determine the active ingredient behavior and effects on nature and the humans. An EIA involves a close collaborative research work between legislators, politicians, chemists, biologists, engineers, users and citizens.

OBJECTIVE OF LABORATORY STUDY ABOUT THE DEGRADATION OF MANCOZEB

The purpose of this research, is to determine the influence of sampling and storage conditions, to which the Mancozeb commercial formulations may be subjected during the inspection process in São Paulo and thus to clarify matters concerning the active ingredient stability and its degradation.

In the planning of the experimental study following points must be considered:

In the containers to be used (high density polyethylene bags, coextruded plastic bottles and glass bottles)

In the analysis method (CS₂-evolution)

In the analysis intervals (0, 7, 21 and 42 days)

In the storage conditions (temperature, moisture)

In the amount of material and necessary reserve.

The changes in properties and characteristics of a pesticide formulation are not only time dependant, but also environmental factors such as temperature, moisture, sun radiation, light, etc. are probable to affect the stability of the active ingredient.

SAMPLE IDENTIFICATION

For identification purposes at the laboratory, where the real work-field conditions will be simulated, each individual sample gets a four digit number "XXXX", with the following meaning:

1 st . Digit: container type	1= polyethylene bag 2= glass bottle 3= plastic bottle
2 nd . Digit: temperature	1= +5°C 2= +23°C 3= +50°C 4= sun exposure
3 rd . Digit: moisture	1= 0% (weight) 2= 2% (weight) 3= 4% (weight)
4 th . Digit: storage time	0= initial 1= 7 days 2= 21 days 4= 42 days

For example, the sample with the code "2134" was in a glass bottle, with a storage temperature of + 5°C, 4% moisture and a storage time of 42 days. The sample with the code "3412" was in a plastic bottle, under sun radiation, 0% moisture and an exposure time of 21 days.

ANALYTICAL METHOD

According to the international standard specifications for pesticide evaluation and to chemical literature, the method suggested for the determination of ethylenebis(dithiocarbamates) is known as "Carbon disulphide evolution method" (CIPAC, [3]). For the purpose of this study, FAO specifications [14] and WHO reference guidelines reports [15] were regarded, too.

This method was mentioned for the first time by CALLAN and STRAFFORD in 1924 [4], as an analysis method for identification and quantitative determination of the many different trade name substances used at that time in the rubber industry as vulcanising accelerators, the first industrial use of EDBC's.

CS₂-evolution method overview: A Mancozeb sample (about 0,2 to 0,3 g of Dithane-PM), dissolved in tetrasodium EDTA

solution, is decomposed by boiling with sulphuric acid to ethylenediamine sulphate and carbon disulphide. The latter is first passed through a lead sulphate scrubber to remove any hydrogen sulphide, and then into an absorption train containing methanolic potassium hydroxide to afford potassium methyl xanthate. The apparatus is assembled as shown in **Diagram 4**. After a digestion time of 1 hour and 45 minutes, the excess of the methanolic KOH solution with the xanthate is neutralized with dilute acetic acid, and titrated with standard iodine solution. **Diagram 5** shows a scheme of the degradation reaction of Mancozeb.

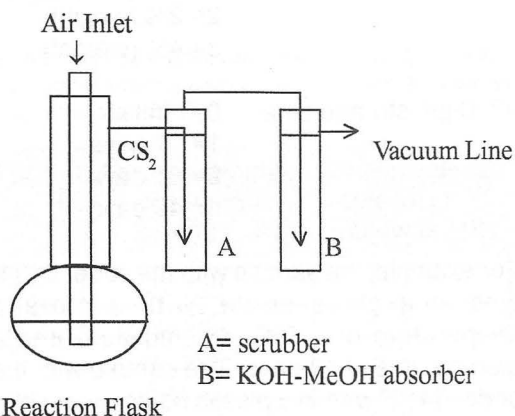


Diagram 4: Glass apparatus for CS₂-evolution process (FONSECA ALMEIDA et al. [5]).

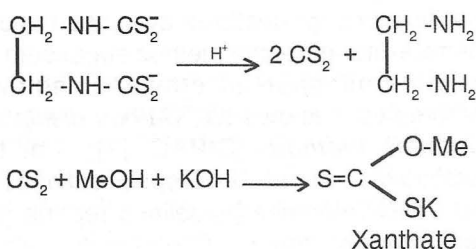


Diagram 5 : Scheme of the degradation reaction of Mancozeb in the CS₂-evolution process (BAUER and MOLL [6]).

For the xanthate titration a Metrohm-716 DMS-TITRINO potentiometer was used. It allows an extremely small volume increments of titration reagent (ml), providing an accurate end point. For the calculations on the concentration of

Mancozeb in the samples, the following equation is used:

$$\%(\text{weight})_{\text{MANCOZEB}} = 13,55 \cdot N \cdot (t-b) \cdot w^{-1}$$

where:

N = normality of the iodine solution
w = sample-weight in grams
t = ml of iodine solution, titration
b = ml of iodine solution, blank titration.

The calculation of the analytical error was based on several analysis (%-weight) using the same Dithane-PM sample, under the same general conditions of the present study, giving for the method a precision of $\pm 0,22$ absolute, or $\pm 0,31\%$ when expressed as a percentage.

EXPERIMENTAL RESULTS

The practical execution of this study was realized in Campinas city at the laboratories for Pesticide Formulations Control of the Brazilian state São-Paulo. After the specified storage time under the mentioned experimental conditions, each sample was analysed two times on the same day, by the same analyst.

For the simulation of the storage conditions under different temperatures, the samples were exposed to $+5^\circ\text{C}$, $+23^\circ\text{C}$ and $+50^\circ\text{C}$, with an accuracy of $\pm 2^\circ\text{C}$. The analytic results for samples at lower temperature are given in **Diagram 6**.

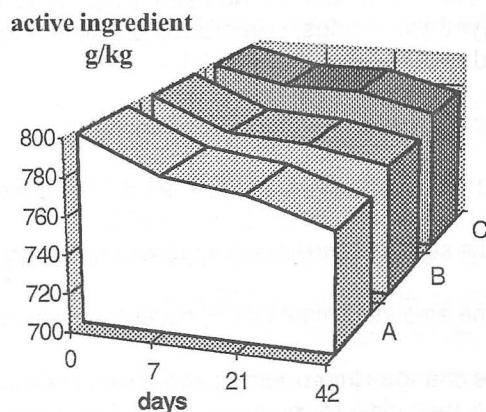


Diagram 6: Comparative degradation curves for Mancozeb, when stored at $+5^\circ\text{C}$ without water addition (A= plastic bag, B= plastic bottle, C= glass bottle).

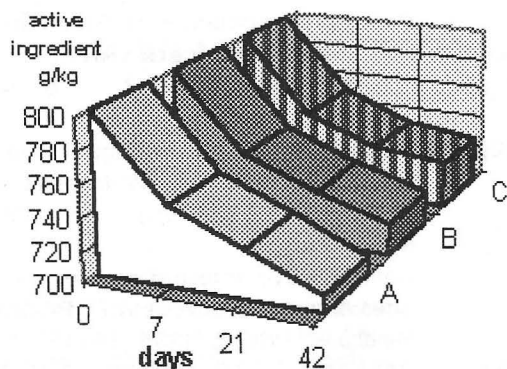


Diagram 7: Comparative degradation curves for Mancozeb, when stored at +50°C with 4% water addition (A= plastic bag, B= plastic bottle, C= glass bottle).

The outdoors placed containers received direct sun radiation with maximal temperatures of +48°C (during the day), and minimal temperatures of -5°C (at night), according to measures of the author. This Diagram shows the combined effect of high temperatures, moisture and sun radiation, which is the case in the daily application of Mancozeb in the plantations.

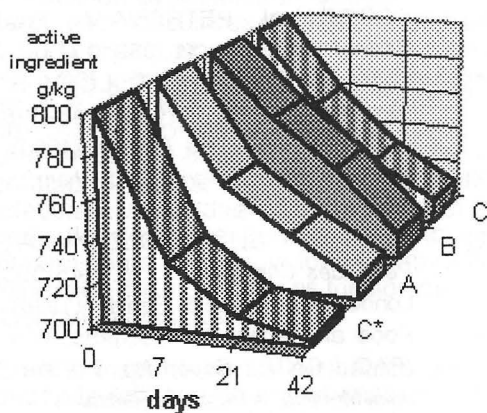


Diagram 8: Comparative degradation curves for Mancozeb, when stored under direct sun light exposure, A-B-C without additional moisture, C* with 4% water (A= plastic bag, B= plastic bottle, C= glass bottle).

CONCLUSIONS

The experimental simulation study about the degradation profile of Mancozeb commercial formulation DITHANE-PM, according to the **Diagrams 6, 7, and 8**, shows that there exists a dependence between the used container and the storage conditions.

For the same storage conditions, it is verified that the package material affects the stability

of the active ingredient. Plastic material is not recommendable for sampling with inspection purposes. Neutral glass bottles appear to be the most adequate package.

The temperature influence on the Mancozeb degradation profiles is stronger under increasing temperature values (**Diagram 9**), based on the fact that glass as package material is not affecting the Mancozeb stability.

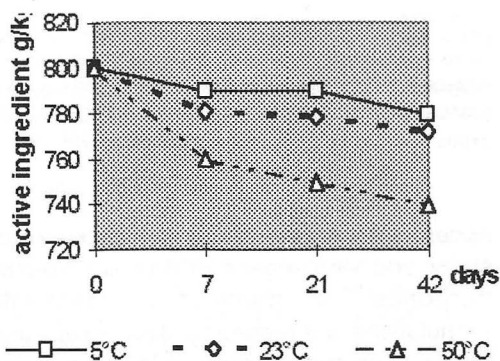


Diagram 9: Temperature effect over the Mancozeb active ingredient, when stored in glass bottles without additional moisture.

The additional moisture placed in the container for the simulation purpose of high air humidity, appears to be a determinant factor (**Diagram 10**) which accelerates the Mancozeb degradation, in spite of low temperatures.

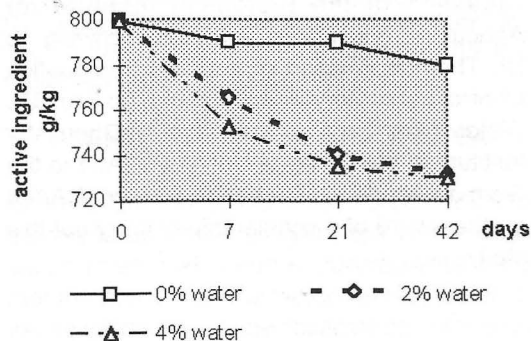


Diagram 10: Moisture effect over the Mancozeb active ingredient, when stored at +5°C in glass bottles.

Under simulated extreme weather conditions, the Mancozeb active ingredient appears to be very unstable (**Diagram 11**). The combination of varying temperatures and high moisture values outdoors, showed the greatest influence on the degradation rates for Mancozeb samples (compare **Diagrams 9 and 11**).

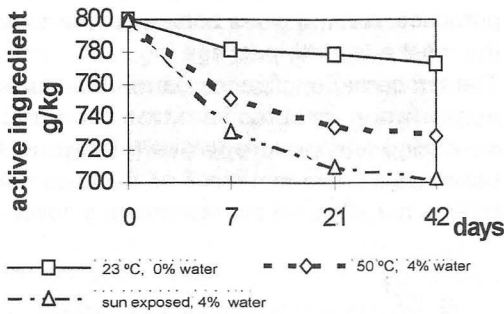


Diagram 11: Combined effect of normal temperatures (23°C); high temperature (50°C) and sun radiation exposition with additional moisture (4% weight).

According to the experimental results of this study and with regard to official inspection purposes of commercial pesticides formulations, it is recommendable to use glass bottles of an appropriate size for the sampling. After the collection, the pesticide samples must be protected from water, temperature variations and light, being analysed as soon as possible, which would be at least during the first seven days.

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