COMPARATIVE STUDIES TO EVALUATE THE EQUIVALENCY OF GENERIC PESTICIDES WITH SPECIAL REFERENCE TO THEIR IMPURITIES

By

DYAA MOHAMMED NASSAR

B.Sc. Agric. Sci. (Plant Production), Fac. Agric., Sababasha, Alexandria Univ., 2011

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

In

Agricultural Sciences

(Pesticides)

Department of Economic Entomology and Pesticides
Faculty of Agriculture
Cairo University
EGYPT

2018

Name of Candidate: Dyaa Mohammed Nassar **Degree: M.Sc**

Title of Thesis: Comparative Studies to Evaluate the Equivalency of Generic Pesticides with Special Reference to Their Impurities

Supervisors: Dr. Mohamed Abdelhady Kandil
: Dr. Yasmin Imam Abdelmobdy
: Dr. Mohammed Abdullah Saleh

Department: Economic Entomology and pesticide **Branch**: Pesticides

Approval: 3 / 12 / 2018

ABSTRACT

The aim of the study was directed to compare the effect of accelerated storage on the stability of brand and generic fungicides (carbendazim and chlorothalonil). In this respect two technical and three formulations from carbendazim and three formulations from chlorothalonil were selected in this studies. In addition, associated impurities for carbendazim and chlorothalonil were determined. Also, Subchronic effects of both fungicides on male albino rat's reproductive system were investigated. Pesticides were stored at temperatures (72±2°C for 3 days, 54±2°C for 14 days and 35±2°C for 3 months). Results indicated that all tested carbendazim were relatively stable under accelerated storage except for the formulation (Kemazid 50% WP) was markedly deteriorated when storage at 72±2°C for 3 days and 35±2°C for 3 months. Regarding the carbendazim impurities (2-amino-3-hydroxy-phenazine and 2,3diaminophenazine) were not detected in all samples.

Chlorothalonil active ingredient it is stability not affected after storage stability test where the percentage loss of chlorothalonil was about 0.11% to 1.64% in all tested formulations. The percentage of hexachlorobenzene in chlorothalonil formulations, Open 72% SC formulation before and after accelerated storage was above the FAO limit.

Concerning the subchronic effect of Carbendazim and chlorothalonil which, were orally administered to male rats daily for 65 successive days. Results revealed that Carbendazim significantly reduced the fertility index, testis weight, the sperm counts and motility. Also, LH and FSH were decreased. Histopathological examination showed testicular damages and severe changes in seminiferous tubules. The results indicated that carbendazim produce reproductive toxic effect, resulting a reduced fertility in male rats. In contrast, there were no adverse effects attributable to chlorothalonil were noted under the ingestion of chlorothalonil on the fertility index, testis weight,

the sperm counts and motility. Also, Chlorothalonil appear to have negative feedback effects on LH and FSH hormones.

Key words: Carbendazim, accelerated storage, Chlorothalonil, Fertility, sperms, Reproductive

CONTENTS

SUMMARY

The aim of this study was to investigate and compare the effect of accelerated storage on carbendazim (two technical and three formulations) and chlorothalonil (three formulations) and their contents of impurities .Also, to study the effect of subchronic exposure of carbendazim (Kemazed 50% WP) before and after storage at 72±2°C for 3 days and chlorothalonil (daconil 72% SC) before storage on albino rat males.

1. Effect of accelerated storage procedure on active ingredient.

a. The active ingredient of carbendazim

Data showed that the stability of carbendazim active ingredient of the tested two technical and the three formulations was relatively influencing during storage at 72±2°C for 3 days , 35±2°C for 3 months and 54±2°C for 14 days.

1. The first technical of (Carbendazim technical 98% elwatania company) was 97.77% at the beginning of experiment than became 95.20, 96.02 and 96.93% after storage at 72, 35 and 54±2^{o} C, respectively.
2. The second technical (Carbendazim technical 98% barigat company) was 98.05% at the beginning of experiment and we than became 95.89, 96.76 and 97.16% after storage at 72, 35 and 54±2°C, respectively.
3. The first Carbendazim formulation (Bendazin 50% WP) was 49.89% at the beginning of experiment than became 48.02,

49.63 and 49.81% after storage at 72, 35 and 54±2°C, respectively.

4. The second Carbendazim formulation (Kemazid 50% WP) was The most degraded formulation when stored at 72±2°C for 3 days and 35±2°C for 3 months , the percentage loss of carbendazim after 72±2°C of storage was 26.7% and after 35±2°C of storage was 18.03% .
5. Third Carbendazim formulation (Fusion 50% WP) was 50.60% at the beginning of experiment than became 49.71, 49.81 and 49.88% after storage at 72, 35 and 54±2°C, respectively.

Stability of carbendazim active ingredient in the tested two technical and the three formulations during storage at 72±2°C for 3 days, 35±2°C for 3 months and 54±2°C for 14 days was relatively influencing.

Kemazid 50% WP was the most degraded formulation when stored at 72±2°C for 3 days and 35±2°C for 3 months and this could be attributed to the variation of manufactures and diversity of additives used in each manufacture.

b. The active ingredient of chlorothalonil

Results showed that stability of chlorothalonil active ingredient in the three formulations was relatively influencing during storage at 72±2°C for 3 days, 35±2°C for 3 months and 54±2°C for 14 days.

1. The first formulation (Open 72% SC) was 71.86% at the beginning of experiment then became 71.43, 71.78 and 71.68% after storage at 72, 35 and 54±2°C, respectively.

2. Second formulation (Daconil 72% SC) was 71.54% at the beginning of experiment and we then became 70.16, 71.51 and 71.41 after storage at 72,35 and 54±2°C , respectively.
3. The third formulation (Clortosip 50% SC) was 50.04% at the beginning of experiment then became 49.22, 49.85 and 49.65 after storage at 72, 35 and 54±2°C , respectively .

These results showed that the stability of chlorothalonil active ingredient in the three formulations was relatively influencing during storage at 72±2°C for 3 days, 35±2°C for 3 months and 54±2°C for 14 days. It can be noticed that chlorothalonil is more stable at 72, 35 and 54±2°C.

2. Effect of accelerated storage procedure on impurities

a. Effect of accelerated storage procedure on impurities of carbendazim

Carbendazim have two impurities, 2-amino-3-hydroxyphenazine and 2, 3 diaminophenazine. According to FAO (1991) the amount of 2-amino-3-hydroxyphenazine Maximum: 0.0005 g/kg of the carbendazim content and the amount of 2, 3 diaminophenazine Maximum: 0.003 g/kg of the carbendazim content.

Over the storage period impurities in carbendazim of the five of tested commercial formulations were not detected. However, 2-amino-3-hydroxyphenazine and 2, 3 diaminophenazine could occur as a result of certain manufacturing processes.

b. Effect of accelerated storage procedure on impurities of chlorothalonil

1. The first formulation (Open 72 % SC) hexachloro-benzene was 0.0069% of chlorothalonil content at the beginning of

experiment and became 0.0015, 0.0066 and 0.0067% of chlorothalonil content after storage at 72, 35 and 54±2°C, respectively.

2. The second formulation (Daconil 72% SC) hexachloro-benzene was 0.001% of chlorothalonil content at the beginning of experiment than were 0.00096 and 0.00096 after storage at 35±2°C and 54±2°C, respectively. Also, it was not detected after storage at 72±2°C.
3. In the third formulation (Clortosip 50% SC) hexachlorobenzene in the beginning of experiment was 0.0015% of chlorothalonil content then were 0.0015 and 0.0016 after storage at 35±2°C and 54±2°C , respectively. Also, it was not detected after storage at 72±2°C.

Data showed that hexachlorobenzene was more stable when stored at 54±2°C for 14 days and 35±2°C for 3 months.Also, Hexachlorobenzene was not affected when chlorothalonil was exposed to different temperatures which indicated that the chlorothalonil found together with related manufacturing impurities.

3. Subchronic effect of carbendazim and chlorothalonil on spermatogenesis and fertility male albino rats

a. Subchronic effect of carbendazim on spermatogenesis and fertility male albino rats

1. Accelerated storage procedure

After the storage of carbendazim (Kemazid 50% WP) at 72 ±2 °C for 3 days which determinated by HPLC, carbendazim decreased to 36.80%.

2. Clinical Signs and Body Weight

During the experimental period there were no death has been occurred and there were no other signs of general toxicity were observed in Albino male rats treated orally with carbendazim for 65 days .

There were no significant difference in body weights between carbendazim five different exposed groups and the control group.

Testes weight was substantially different in rats treated carbendazim before and after accelerated storage compared to the control group, which were 1.7 g and 2.03 g in rats treated with 1/10 and 1/30 of the LD_{50} before accelerated storage. Testes weight were 1.99 g and 2.43 g in rats treated with 1/10 and 1/30 of the LD_{50} after accelerated storage compared to testes weight in control rats which were 3.13 g.

3. Fertility-related parameters

In testicular parameters sperm count, sperm motility and viable sperms examination, a statistically significant reduction in caudal epididymal sperm count was noted at dose levels of 1/10 and 1/30 of the LD_{50} before accelerated storage treatments more than dose levels of 1/10 and 1/30 of the LD_{50} after accelerated storage. Also, sperm counts in male rats were significantly reduced to about 23.24% in the carbendazim-treated males with 1/10 LD_{50} before accelerated storage compared with the control males. Sperm motility was altered primarily in those males ingested carbendazim for 65 day that exhibited very low sperm counts, which was significantly decreased parallel with the increasing of its dose. There was no motility detected in rat males

treated with 1/10 of the LD_{50} before accelerated storage. Although viable sperms were significantly decreased in the 1/10 and 1/30 of the LD_{50} before accelerated storage treated groups , it reach 5% in rat males treated with 1/10 of the LD_{50} before accelerated storage compared to control group.

4. Hormone levels

Hormone concentration levels of serum LH and FSH in treated male rats had a decreasing tendency with the increasing dose of carbendazim, which was statistically significant. The highest effect were observed in rats treated with 1/10 LD_{50} of carbendazim before storage compared to control. Although, there were slight decrease in serum T level.

5. Histopathological examination

Testicular histopathology examination showed a dose-dependent effect of carbendazim on spermatogenesis. Control rats showed a normal process of spermatogenesis, a regular arrangement of spermatogenic epithelium existed in seminiferous tubules. Rats which exposed to carbendazim provoked severe alterations in the seminiferous tubules namely the loss, derangement and necrotic germ cells appeared exfoliated into the tubular lumen, multinucleated giant cells appeared in the lumen, derangement and sloughing of spermatogenic cells, the vacuolization of sertoli cell cytoplasm and the disruption of sertoli cell cytoskeleton. The highest effect was in the dose 1/10 of the LD_{50} then 1/30 of the LD_{50} of carbendazim before storage and carbendazim 1/10 of the LD_{50} after storage. The lowest

effect was observed in the dose carbendazim 1/30 of the LD_{50} after storage.

The ingestion of carbendazim significantly reduced the fertility index, testis weight, sperm counts and motility. Also, LH and FSH had decreased. Histopathological evaluation showed testicular damages and severe changes in seminiferous tubules. The results indicated that carbendazim produce reproductive toxic effect, resulting a reduced fertility in male rats. Also, toxic effect of carbendazim before accelerated storage has oblivious effect on fertility in male rats than post storage.

It can be noticed that carbendazem is dangerous in terms of reproductive toxicity and should be scaled down to reduce its risk to humans and chlorothalonil is a safe reproductive health.

b. Subchronic effect of chlorothalonil on spermatogenesis and fertility male albino rats

1. Clinical Signs and Body Weight

During the experimental period there were no death was occurred and no other signs of general toxicity were observed in male rats treated orally with chlorothalonil (Daconil 72% SC) for 65 days. The effects of chlorothalonil on body weight gain for all the three different groups indicated that no significant difference in body weights between chlorothalonil exposure groups and control group. Testes weight was not different in rats treated with 1/10 and 1/30 of the LD_{50} of chlorothalonil compared with the control group.

2. Fertility-related parameters

There were no big difference on sperm count, sperm motility and viable sperms at dose levels of 1/10 and 1/30 of the LD_{50} of chlorothalonil (Daconil 72% SC) compared with the control males.

The sperm count reached 73% and 93% at dose levels of 1/10 and 1/30 of the LD_{50}, respectively. Sperm motility reached 81% and 89% at dose levels of 1/10 and 1/30 of the LD_{50}, respectively. The viable sperms reached 86% and 93% at dose levels of 1/10 and 1/30 of the LD_{50}, respectively compared to control group.

3. Hormone levels

Serum hormone levels, LH, FSH and T were studied .The concentration levels of serum LH , FSH and T were no significantly affected at dose levels of 1/10 and 1/30 of the LD_{50} of chlorothalonil (Daconil 72% SC) compared with the control male.

4. Histopathological examination

Testicular histopathology showed that the doses of chlorothalonil (1/10 and 1/30 of the LD_{50} of daconil 72% SC) on spermatogenesis was the same to control group , a regular arrangement of spermatogenic epithelium . Rats did not show a significant testicular damage and showed a normal process of spermatogenesis.

The ingestion of chlorothalonil had no effect on the fertility index, testis weight, the sperm counts and motility. Also, LH and FSH were not affected.