Genotoxicity of Twelve Pesticides in White Mouse, *Mus musculus*

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The use of pesticides in both the farmer's fields and storage godowns has contributed greatly to the boon of agriculture. As a consequence, more new pesticides are released into the market. While the practice of pesticide application seems to be indispensable in this modern technology for improved crop production, it is quite ironical that these pesticides have been found to be sources of potentially hazardous substances to man.

Monitoring of environmental genotoxic substances therefore is very important in order to help curb environmentally-induced diseases. While extensive toxicologicall evaluation are conducted and acceptable daily intake (ADI) values are set before any pesticide can be released into the market, studies on genotoxicity of pesticides are wanting.

This research work was therefore conducted to evaluate the genotoxic activity of twelve pesticides *in vivo* in the white mouse, *Mus. musculus*. Results of this study would add to the limited information on the genotoxicity of pesticides which serve as basis for providing warning to many people (i.e. farmers, etc.) on the possible long term effects posed by these toxicants.

Materials and Methods

The twelve pesticides (Table 1) belonging to three categories, namely: insecticides, fungicides and herbicides, purchased from various agricultural supplies in Iligan City were used in the present study.

Randomly-bred Japanese Namru strain of white mouse, *Mus musculus*, seven to twelve weeks old, were used in this study. Preliminary toxicity studies were conducted to establish the sublethal dosages for each of the different pesticides tested. Distilled water was used as diluent in making the different working concentrations. Three dose levels (given in mg/kg body weight) were set for each test pesticides with distilled water as negative

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control. Three replications for each treatment were made. The genotoxic potential of these pesticides were evaluated in vivo using the white mouse, *musculus*, based on their capacity to induce breakage on the chromosomes following micronucleus test (Schmid, 1976) and bone marrow chromosome analysis (Medina, 1988).

The test pesticides were administered to the mice intraperitoneally (acute treatment) 24 hours before sacrifice. For micronucleus test, about 1,000 polychromatic erthrocytes were screened for the presence of

Table 1. List of pesticides investigated for genotoxic activity.

NAME	BRAN D NAME	FAMILY	MODE OF ACTION
Insecticides			
1. Endosulfan	Thiodan	organochlorine	non-systemic insecticide and acaricide with contact and stomach action
2. Parathion methyl	Follidol M50	organophosphorous nitrocompound	- same as above - in addition, cholinesterase inhibitor
3. Metamidophos	Tamaron	organophosphorus	- same as Follidol M50 -
4. Monocrotophos	Azodrin 202R	organophosphorus	- same as Follidol M50 -
5. Azinphos-methyl	Bionex	triazine, organophosphorous	- same as Follidol M50 -
Fungicides			
1. Mancozeb	Dithane M45	dirthiocarbamate organomaganese organozinc	foliar fungicide with protective action
2. Propineb	Antracol	carbamate organozinc	- same as Dithane M45 -
3. Thiophanante methyl	Fungitox	carbamate benzimidazole	systemic fungicide with protective and curative action
4. Maneb	Maneb 80	dithiocarbamate organomanganese	- same as Dithane M45
5. Benomyl	Benlate	benzimidazole	- same as Fungitox -
Herbicides			
1. 2,4-D	2,4-D ester	phenoxy	selective systemic herbicide; acts as growth inhibitor
2. Butachlor	machete	acetamide	selective systemic herbicide; acts by inhibition of protein synthesis

micronucleus per animal under the high power objective. Fifty well-spread metaphases were scored per animal in the cytogenetic assay. Data gathered from both micronucleus test and bone marrow chromosome analysis were treated statistically using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT).

Results and Discussion

Data obtained from the micronucleus test are presented in Tables 2 and 3. A photograph of a micronucleated polychromatic erythrocyte is shown in Figure 1. All the insecticides tested were equally capable of inducing micronucleated polychromatic erythrocytes. Four of the five fungicides registered positive results while the herbicides yielded negative results.

Results of the cytogenetic assay (Tables 5 and 6) confirmed the genotoxicity of all the insecticides and most of the fungicides. Most of the structural changes observed were predominantly deletions as indicated by dots, rods and some acentric fragments (Figure. 2A, B and C). As a consequence of chromosome breakage and subsequent restitution at sticky ends, dicentrics (Figure 2D) were observed. Some chromatic type gaps were also noted.

Extensive studies employing a battery of tests using both prokaryotic and eukaryotic systems both *in vivo* and *in vitro* were conducted by Waters et al. (1982). Sylianco (1990) in her review revealed that a great number of pesticides have been found to display genotoxic activity in different test systems. Short term bioassay showed that three types of genetic damages including gene or point mutations, DNA damage and chromosomal aberrations.

The observed chromosome-breaking capacity of the pesticides can be attributed to their reactivity with the chromosomal materials. Pesticides are reactive, primarily electrophilic. They often form ever more reactive eletrophiles as intermediate products during environmental or metabolic degradation (Grosby, 1982). This reactivity property of pesticides can account for their genotoxicity. Some pesticides especially those that bear organophosphate triesters show alkylating reactivity (Hutson and Roberts, 1985). Lofroth (1970) postulated that some pesticides like dichlorvos may methylate and cause damage to the mammalian gene leading to mutation and/or carcinogenesis.

Previous studies (Bartels and Hilton, 1973; Liang et al., 1969) have shown that some pesticides act as spindle poisons causing stickiness of the

Table 2. Effects of the various insecticides on the induction of micronucleus in the bone marrow cells of the white mouse, *Mus musculus*.

INSECTICIDE	DOSE RATE (mg/kg body weight)	NO. OF MICRONUCLEATED POLY-CHROMATIC ERYTHROCYTES PER 1,000 CELLS*	
		Mean	S.E.
Control (Distilled water)			
Endosulfan**	1.50	4.66a	0.97
	3.00	9.00a	1.72
	5.00	15.66b	1.82
		13.33b	1.82
Parathion-methyl**	0.50	11.00b	1.47
	1.50	12.00b	1.28
	3.00	15.33b	1.92
Methamidophos**	0.50	16.50b	0.70
	1.50	18.00b	0
	3.00	13.00b	1.41
Monocrotophos**	0.50	13.00b	0
	1.00	18.00b	1.00
	2.00	14.50b	2.35
Azinphos-methyl**	0.50	13.00b	1.00
	1.50	5.50a	0.71
	300	8.50a	0.71

chromosomes, delaying chromosomal disjunction and inhibiting crosswall formation during cell division. Mann (1981) and Grover and Malhi (1988) likewise reported that some breaks on the chromosomes may have resulted from radiomimetic action of some pesticides. Vijaya and Janardhan (1987; 1988) demonstrated the mutagenic potential of monocrotophos using micronucleus test and sperm abnormality assay.

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Table 3. Effects of the different fungicides and two herbicides on the induction of micronucleus in the bone marrow cells of the white mouse, *Mus musculus*.

PESTICIDE	DOS RATE (mg/kg body weight)	NO. OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES PER 1,000 CELLS*	
		Mean	S.E.
Control (Distilled water)	15	3.33a	0.09
Mancozeb **	30	3.00a	0
	45	5.50a	1.43
		9.00b	0.90
Propineb**	15	4.50a	1.10
	30	7.50b	0.64
	60	8.50b	0.64
Thiophanate methyl**	15	4.00	0.90
	30	6.00	0
	60	6.00	1.47
Maneb**	15	5.33a	1.37
	30	7.50b	0.64
	60	7.50b	1.60
Benomyl**	15	5.50a	0.64
	30	9.00b	0.90
	60	9.00b	0
2,4-D**	15	2.00	0.09
	30	4.00	0
	60	4.00	0.09
Butachlor**	15	3.00	0.09
	30	3.50	1.10
	60	no data obtained	

* Mean values with dissimilar letters show significant difference based on DMRT.
** Analysis o variance shows highly significant difference compared to the control.
ns Analysis of variance shows no significant difference compared to the control.

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Figure 1. Photomicrograph of a micronucleated polychromatic erythocyte (1000x) (micronucleus with arrow)

Table 4. Date on mean number of bone marrow metaphase spreads in the white mouse, *Mus muculus*, containing chromosome aberrations following intraperitoneal treatment of some insecticides.

INSECTICIDE	DOSE RATE (mg/kg body weight)	NO. OF METAPHASE SPREADS CONTAINING ABERRANT CHROMOSOMES	
		Mean*	S.E.
control (Distilled Water)		4.66a	0.97
Endosulfan**	1.50	9.00a	1.72
	3.00	15.66b	1.82
	5.00	13.33b	1.82
Parathion Methyl**	0.50	11.00b	1.47
	1.50	12.00b	1.28
	3.00	15.33b	1.92
Methamidophos**	0.50	16.50b	0.70
	1.50	18.00b	0
	3.00	13.00b	1.41
Monocrotophos**	0.50	13.00b	0
	1.00	18.00b	1.00
	2.00	14.50b	2.35
Azinphos-methyl**	0.50	13.00b	1.00
	1.50	5.50a	0.71
	3.00	8.50a	0.71

* Mean values with dissimilar letters show significant difference based on DMRT.
** Analysis of variance shows highly significant defference compared to the control.

Table 5. Data on the mean number of bone marrow metaphase spreads of the white mouse, *Mus musculus*, containing aberrant chromosomes following intraperitoneal treatment of fungicides.

INSECTICIDE	DOSE RATE	NO. OF METAPHASE SPREADS CONTAINING ABERRANT CHROMOSOME	
		Mean*	S.E.
Control (Distilled water)	15	4.66a	0.97
	30		
Mancozeb**	45	8.50a	0.71
		8.50a	0.71
	15	12.50b	1.58
	30		
Propineb**	60	6.00a	1.41
		9.50b	1.88
	15	9.00a	1.41
	30		
Thiophanate methyl**	60	11.00b	1.00
		9.50b	0.71
	15	10.50b	1.22
	30		
Maneb**	60	6.50a	1.22
		10.50b	2.12
	15	11.00b	1.00
	30		
Benomyl**	60	6.50a	1.22
		11.00b	1.73
		10.50b	2.12

* Mean values with dissimilar letters show significant difference based on DMRT.
** Analysis of variance shows significant difference compared to the control.

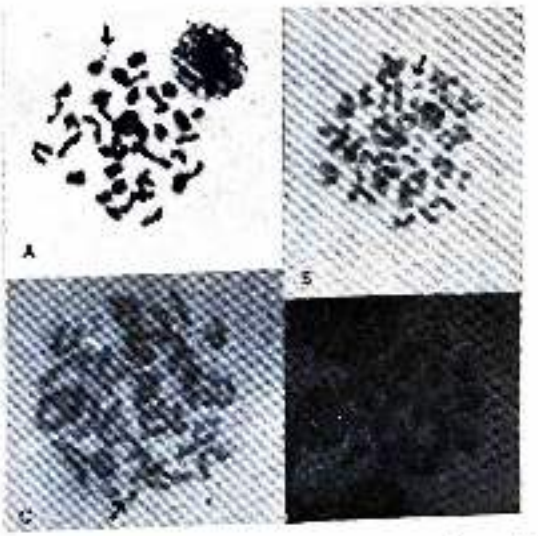

Figure 2. Representative photomicrographs of the metaphase spreads containing aberrant chromosomes (with arrow). (A. deletion [small dots]; B. deletion [large dots] C. acentric fragment; D. dicentric chromosome) (magnification 800x)

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