EFFECT OF SOME MUTAGENS ON DROSOPHILA AND MICE

Salama, S.A.; A.M.A. Hablas and M.I.A. Sherif

Department of Genetics, Faculty of Agriculture, Minufiya Univ.

تأثير بعض المطفرات على الدروسوفيلا والفئران

صلاح الدين أحمد سلامة ـ عبد المنعم محمد حبلص ـ محمد ابراهيم عبد البارى شريف

قسم الوراثة ـ كلية الزراعة ـ جامعة المنوفية

ملخص البحث

الكائنات المختبرة كانت سلالة الفئران السويسرية الالبينو والدروسوفيلا ميلانوجستر وكانت المطفرات المستخدمة هى الميثيل ميثان سلفونات والكافيين والماليك هيدرازيد ·

أدت معاملة ذكور الفئران بتركيز ١٠٠ مجم / كجم ميثيل ميثان سلفونات الى زيادة ملحوظة فى تكرارات المميتات السائدة خاصة فى الأسبوع الأول بعد المعاملة ثم بدأت تقل هذه الزيادة بدرجة خفيفة فى الأسبوع الثانى ثم انحدرت هذه الزيادة بشدة من الأسبوع الثالث الى الثامن · ولقد أظهرت المعاملة بـ ١٠٠ مجم / كجم كافين زيادة طفيفة فى تكرارات المميتات السائدة المشاهدة فى الأسبوع الأول حتى الثالث بعد المعاملة ثم ارتفعت هذه الزيادة فى التكرارات بدرجة ملحوظة فى الأسبوع الخامس ثم انحدرت فى الأسبوع الثامن · من ناحية أخرى ، لم يتحصل على زيادة معنوية فى تكرارات المميتات السائدة عند أى مرحلة فحص عند المعاملة بـ ٤٠٠ مجم / كجم ماليك هيدرازيد · وبالنسبة لمعاملة ذكور الدروسوفيلا ميلانوجستر ، بينت النتائج أن المعاملة بتركيزات مختلفة من الميثيل ميثان سلفونات أدت الى الحصول على زيادة ملحوظة فى المميتات السائدة ، بينما أعطت المعاملة بالكافيين زيادة طفيفة فى تكرارات المميتات السائدة · من ناحية أخرى فان المعاملة بتركيزات مختلفة من الماليك هيدرازيد لذكور الدروسوفيلا ميلانوجستر لم تعط زيادة معنوية فى تكرارات المميتات السائدة · ولقد أستخلص أن الميثيل ميثان سلفونات كان فعالا فى استحداث المميتات السائدة فى الفئران والدروسوفيلا وأن أكثر المراحل حساسية للميثيل ميثان سلفونات فى الفئران هى مرحلة الحيوان المنوى الناضج والاسبرماتيد المتأخر · أيضا أستخلص أن الكافيين كان تأثيره طفيفا فى استحداث المميتات السائدة فى الفئران والدروسوفيلا وأن أكثر المراحل حساسية للكافيين هى مرحلة الاسبرماتوسيت المبكر ·

ABSTRACT

Test organisms were swiss albinomice and Drosophila melanogaster. Mutagens were MMS, caffeine and MH. Treatment of male swiss albino mice with 100 mg/kg MMS resulted in marked increases in frequencies of DLS, particulary at the first week post-treatment, then decreased slightly after the second week and dropped sharply at weeks 3-8. Treatment with 100 mg/kg caffeine resulted in slight increases in frequencies of DLS observed during weeks 1-3 post-treatment then rose sharply at week 5 and dropped at week 8. On the other hand, no significant increase in frequencies of DLS at any scoring times have been obtained with 400 mg/kg MH. With respect to treatment of Drosophila melanogaster males, the results showed that treatment with various concentrations of MMS resulted in marked increases in DLS, while treatment with caffeine resulted in slight increases in DL-frequencies. On the other hand, treatment of D. melanogaster males with various concentrations of MH resulted in no significant increase in DL-frequencies. It was concluded that MMS is effective for inducing DLS in mice and Drosophila and the most sensitive stages to MMS in mice were epididymal sperm and late spermatids. Also it can be concluded that caffeine is slightly effective for inducing DLS in mice and Drosophila and the early spermatocytes in mice were the most sensitive stage.

INTRODUCTION

Mutation research is a rapidly expanding field of study. Interest in environmental pollution and the risks involved to the human population at large and to the genetic make up in particular have enhanced mutagenesis research tremendously. Consequently, many assays on test systems have been developed, to asses the possible damage natural and industrial chemical pollutants can cause to the genetic material. Among the test systems most widely used in mutagenesis research is the dominant lethal (DL) assay in mammals and/or insects. Although this system has been widely used for some time to screen for the potential mutagenicity of large numbers of chemical substances, many of which are present in man's environment, yet

a controversy as to the accuracy of this system exists. Some regard induced DLS as being due to true genetic causes. Others attribute DL-inducation to non-genetic factors. Probably there is some truth to both beliefs. Moreover, the question remains as to whether the DL-assay provides accurate information as to the potential mutagenicity of chemicals tested in this system. The present investigation was initiated to: 1. Study the effect of a well known powerful mutagen methyl methanesulfonate (MMS) in inducing end points in each of the test systems DLS in mice and DLS in Drosophila. 2. Test the mutagenic potential of caffeine, whose mutagenicity has been a controversy for more than 30 years, and MH a herbicide, which is known to be very effective in damaging plant chromosomes yet hardly effective on mammalian or insect chromosomes.

MATERIALS AND METHODS

I. Experimental organisms:

a) Mice:

Swiss albino mice were used as experimental organism. Stocks were obtained from the High Institute of Public Health, Alexandria University.

b) Drosophila:

Drosophila melanogaster was used as experimental organism. Wild type flies were obtained from the Department of Genetics, Faculty of Agriculture, Alexandria University.

II. Chemicals:

The following chemical compounds were used:

1. Methyl metahanesulfonate (MMS), $CH_3\ SO_2\ OCH_3$, MW. 110.13.
2. Caffeine (1,3,7-trimethylxanthine), $C_8H_{10}N_4O_2$, MW. 149.19.
3. Maleic hydrazide (MH), $G_4H_4N_2O_2$, MW, 112.09.

All were obtained from Eastman kodak organic chemicals Rochester, N.Y. 14650 U.S.A.

III. Biological criteria:

a) Dominant lethals (DLS) in mice:

Males mice, 11-14 weeks old, were injected i.p. with a single nonlethal dose of chemical. Dose was adjusted according to the weight of animals (which ranged from 22-24 gms). Immediately after injection, each male was caged separately with 2 virgin females for 5 days, after a mating period of 5 days, the females were removed and housed in other cages untill sacrificed. The males were introduced into each cage with one male. Each male was used for mating at periods of 1,2,3,5 and 8 weeks post-treatment with chemical. Females were sacrified by cervical dislocation at 14 days after mid week of mating. At necropsy the uteri were examined and the number of corporalutea and living implantations were counted for each pregnant female. Three males were used for each treatment, and four replicaitons were made. The percentage of dead implants, taken as an index of mutagenicity, was calculated as:

$$\% \text{ dead implants } \frac{\text{Number of dead implants}}{\text{Total number of implants}} \times 100$$

b) Dominant lethals in Drosophila:

The methods suggested by Sankoranarayanan (1969), with minor modifications, was adopted. Following treatment of males with chemicals, 15 adult males were mated to about 20 virgin females (or excess of males) in specially labelled fifth-pint bottles. Each bottle opening was covered with dacron netting held tightly by a rubber band to the neck of the bottle. Each bottle was then inverted and placed firmly on the surface of a large petridish containing egg-laying medium and a thin layer of fresh yeast.

Females were permitted to oviposit for about 24 hr. The bottles were removed, their dacron netting brushed free of adhering eggs or food and transferred, after about 60 minutes, to another fresh nutrient, containing petri-dish. Observations of the petri-dishes were made, using transmitted light and a dissecting microscope soon after the bottle was removed. At this time total egg counts were made and 40-48 hrs. later another count was made for unhatched eggs only. Throughout bottles and petri-dishes were kept at 25 ± 1°C.

VI. Statistical analysis:

Statistical analysis of the present data was carried out using the standard complete randomized blocks design illustrated by Cochran and Cox (1957).

RESULTS AND DISCUSSION

Treatment of male swiss albino mice with 100 mg/kg MMS resulted in a marked increase in frequencies of DLS (Table 1). The highest frequency of DLS was observed at the first week Post-treatment then decreased slightly after the second week and dropped sharply at weeks 3-8. If a male is mated, for successive weeks, following treatment with a chemical, the results of each week's mating represent the response of a specific maturation stage to the chemical. Each week's mating represents the response of the following germ cell stage: week 1: epididymal sperm; week 2: late spermatids; week 3: early spermatids; week 4: late spermatocytes; week 5: early spermatocytes; week 6: five generations of definitive spermatogonia and week 7 or more represent stem cells (Bateman, 1971). Results in Table (1) show that the most sensitive stage to MMS was epididymal sperm (Week 1) and late spermatids were less sensitive. With respect to treatment of male swiss albino mice with 100 mg/kg caffeine, treatment resulted in a slight increase in frequencies of DLS (Table 2). The

Table 2- Frequencies of dominant lethals (DLs) induced by Caffeine in male Swiss albino mice.

Week	1			2			3			5			8		
Caf.concent. mg/Kg	Total implants	DLs No.	DLs %	Total implants	DLs No.	DLs %	Total implants	DLs No.	DLs %	Total implants	DLs No.	DLs %	Total implants	DLs No.	DLs %
0	182	14	7.7	178	11	6.2	198	8	4.0	196	6	3.1	186	8	4.3
100	153	19	12.4	186	16	8.6	180	10	5.6	168	18	10.7**	199	13	6.5

** = Highly significant at 0.01
* = Significant at 0.05

Table 1- Frequencies of dominant lethals (DLs) induced by Methyl methanesulfonate (MMS) in male Swiss albino mice.

Week	1			2			3			5			8		
MMS concent. mg/Kg	Total imp-lants	DLs		Total imp-lants	DLs		Total imp-lants	DLs		Total imp-lants	DLs		Total imp-lants	DLs	
		No.	%		No.	%		No.	%		No.	%		No.	%
0	182	14	7.7	178	11	6.2	198	8	4.0	196	6	3.1	186	8	4.3
100	91	51	56.0**	106	56	52.8**	114	21	18.4**	147	10	5.7	178	13	7.3

** = Highly significant at 0.01
* = Significant at 0.05

lowest frequency of Dls was observed during weeks 1 and 3 and at week 8. The results showed a significant increase in Dl-frequency at the fifth week. Furthermore treatment of mice with MH Led to no significant increases in DL-frequencies (Table 3).

Treatment of Drosophila melanogaster males with various concentrations of MMS, caffeine, and MH resulted in marked increases in frequencies of DLS for treatment with MMS (Table 4), slight increases in frequencies of DLS for treatment with caffeine (Fig. 1 and Table 5) and non effective effect for inducing DLS by MH (Table 6). For MMS, the DL-frequencies increased gradually with increase in cocnentration (Fig. 2). The first period (1-3 days) showed a slightly higher frequency of DLS than the second period (4-6 days) at all concentrations used.

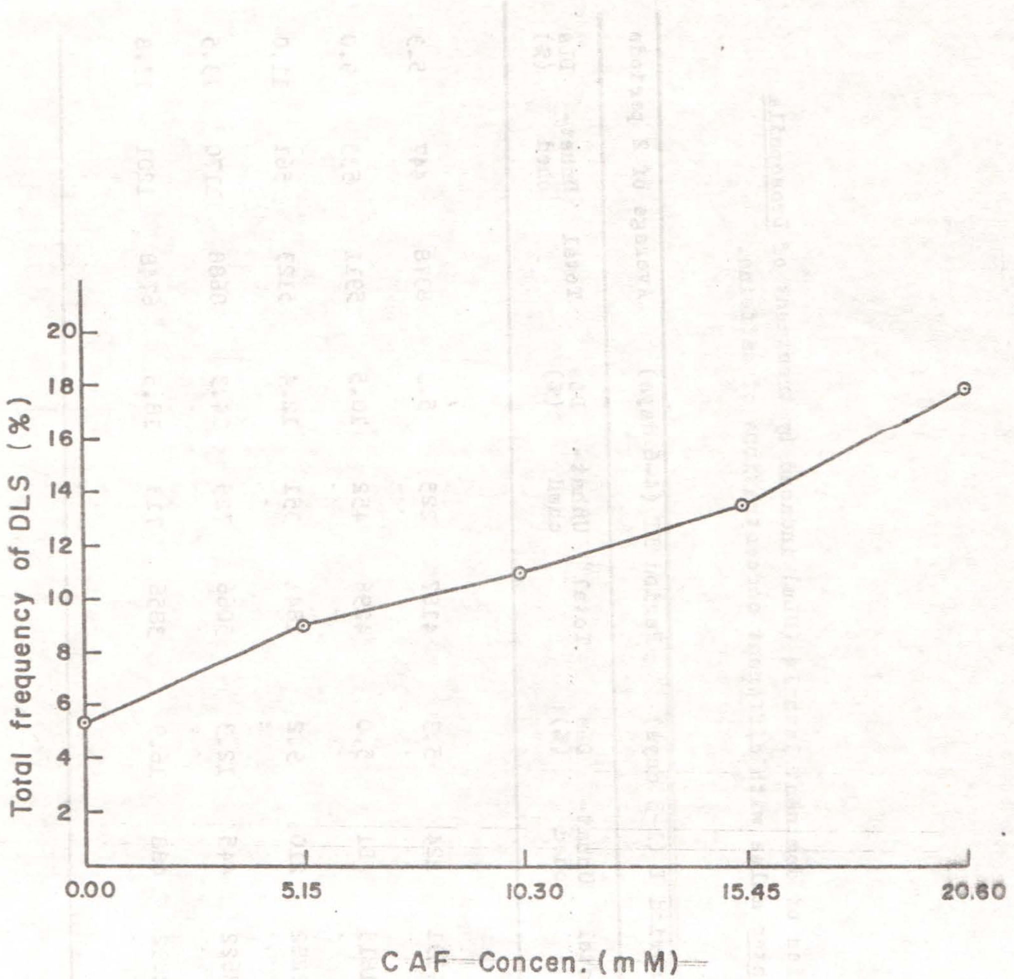
The results obtained are in agreement with findings by Ehling et al. (1967) who reported that MMS induced a high frequency of DLS in mouse spermatozoa of Vas and epididymis, testicular sperm, and late spermatids, and low frequency in early spermatids. Ehlin (1970) reported that MMS induced high frequencies of DLS in spermatozoa and late spermatids. Furthermore no mutations were induced in spermatocytes. In another study Ehling, 1977 reported that the most sensitive mating interval for the induction of DLS was 5-8 days post-treatment with MMS. The present results obtained agree also with those of Lang and Adler (1977) who found that the sensitive period of post-meiotic spermatogenesis for MMS was spermatozoa and spermatids. With respect to treatment of mice with caffeine, the results obtained agree with those of Rohrborn (1972) who reported that treatment of (C_3H males) with caffeine, as drinking water gave no significant evidence of the induction of DLS. Also results obtained by Epstein et al. (1970) which showed that caffeince administered to male mice, produced no mutagenic effect in the DL assay. These results indicate that caffeine is non-mutagenic to

Table 3- Frequencies of dominant lethals (DLs) induced by Maleic hydrazide (MH) in male Swiss albino mice.

Week	1			2			3			5			8		
		DLs			DLs			DLs			DLs			DLs	
MH concent. mg/Kg	Total	No.	%	Total	No.	%	Total	No.	%	Total	No.	%	Total	No.	%
0.00	182	14	7.7	178	11	6.2	198	8	4.0	196	6	3.1	186	8	4.3
4.00	181	16	8.8	191	17	8.9	184	9	4.9	174	10	5.7	188	8	4.3

Table 4- Frequencies of dominant lethals (DLs) induced by treatment of *Drosophila melanogaster* males with different concentrations of methyl methanesulfonate (MMS)

DL frequencies / MMS concent. (mM)	Period I (1-3 days)			Period II (4-6 days)			Average of 2 periods		
	Total	Unhatched	DLs (%)	Total	Unhatched	DLS (%)	Total	Unhatched	DLs (%)
0	4370	167	3.8	7093	217	3.1	11463	384	3.4
0.27	2872	173	6.0	5822	327	5.6	8694	500	5.8
0.54	3242	292	9.0	5820	519	8.9	9063	811	9.0
1.09	4003	731	18.3	6010	938	15.6	10013	1669	16.7
2.27	4284	1745	40.7	8195	2881	35.2	12479	4626	37.1



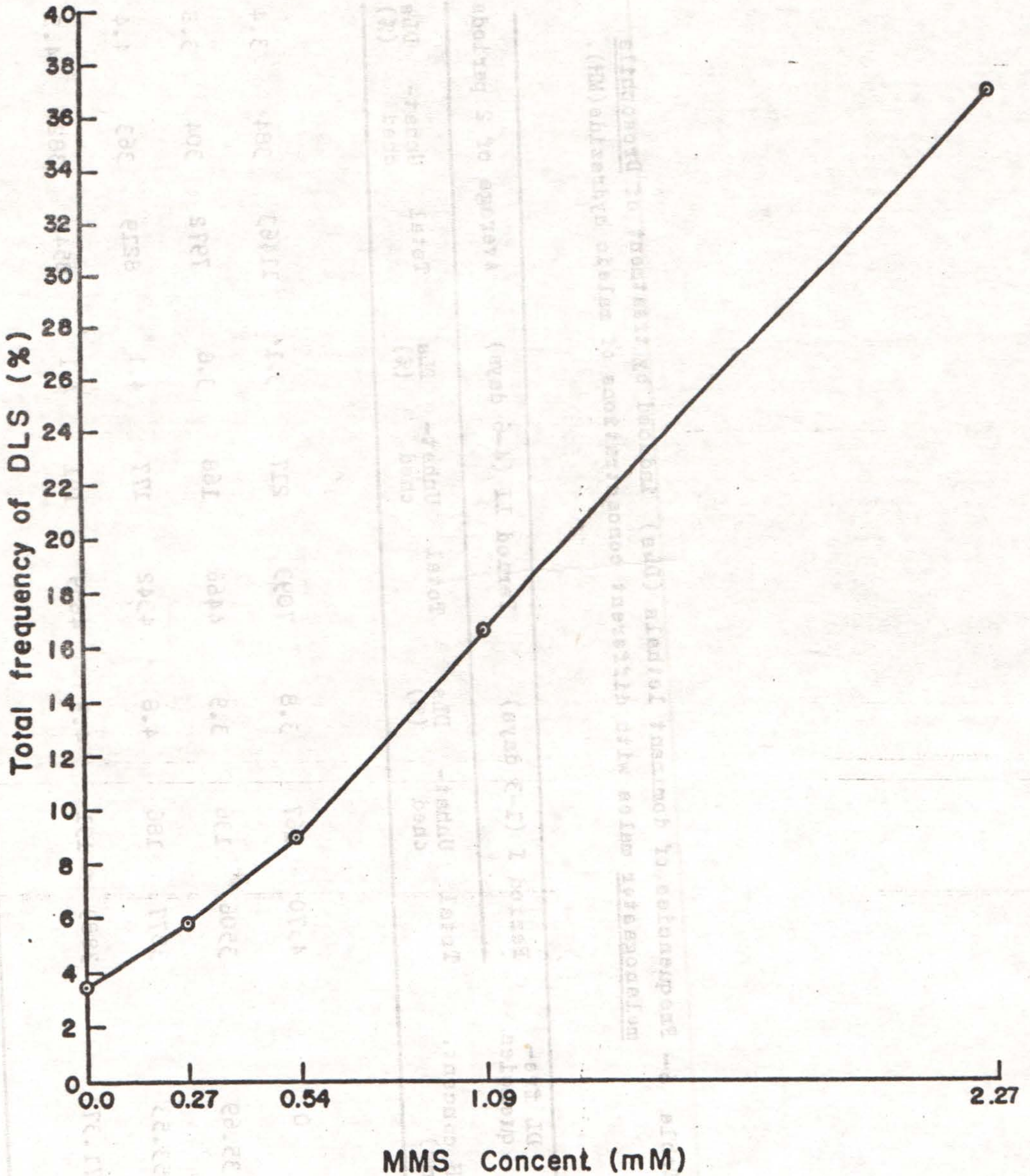
(Fig. 1) Frequency of DLs induced by different concentrations of caffeine in Drosophila melanogaster males.

Table 5- Frequencies of dominant lethals (DLs) induced by treatment of *Drosophila melanogaster* males with different concentrations of caffeine.

DL frequencies / CAF concent. (mM)	Period I (1-3 days)			Period II (4-6 days)			Average of 2 periods		
	Total	Unhatched	DLs (%)	Total	Unhatched	DLs (%)	Total	Unhatched	DLs (%)
0	4191	224	5.3	4187	223	5.3	8378	447	5.3
5.51	1615	81	5.0	4296	452	10.5	5911	533	9.0
10.30	2282	210	9.2	2841	351	12.4	5123	561	11.0
15.45	3622	445	12.3	5066	725	14.3	8688	1170	13.5
20.60	2892	488	16.9	3856	713	18.5	6748	1201	17.8

Table 6- Frequencies of dominant lethals (DLs) induced by treatment of <u>Drosophila melanogaster</u> males with different concentrations of maleic hydrazide(MH).

DL frequencies	Period I (1-3 days)			Period II (4-6 days)			Average of 2 periods		
MH concent. (mM)	Total	Unhatched	DLs (%)	Total	Unhatched	DLs (%)	Total	Unhatched	DLs (%)
0	4370	167	3.8	7093	217	3.1	11463	384	3.4
35.69	3506	136	3.9	4466	168	3.8	7972	304	3.8
53.53	3877	186	4.8	4342	177	4.1	8219	363	4.4
71.37	3889	191	4.9	4659	191	4.1	8548	382	4.5



(Fig. 2) Frequencies of DLs induced by different concentrations of methyl methanesulfonate in *Drosophila melanogaster* males.

mice. Such interpretation is in agreement with the findings of Aeschbacher et al. (1978) who reported that caffeine did not accumulate in the testicular tissue of mice. The maximum concentration of caffeine found was below 10 micro gm./g testicular tessue, which is about 100 times lower than concentrations that cause chromosome aberrations in cultured mammalian cells. The significant increase in DL-frequency at the fifth week may indicate that early spermatocytes are more sensitive to caffeine than other stages.

For treatment of Drosophila melanogaster males with different mutagenic agents, the results obtained are in agreement with results obtained by Nawar and Hamza (1978) and Nagaty (1981) who showed that EMS is very effective for increasing DLS in Drosophila melanogaster particularly when young spermatids were treated. Also the results are in agreement with the findings of Mittles et al. (1967) who found that injection of Drosophila with caffeine did not induce XO males, non-disjunction or DLS. Also, Nawar and Hamza (1978) and Nagaty (1981) reported that when adult Drosophila melanogaster males were fed on caffeine no increase in frequency of DLS was observed, and treatment with saturated aqueous solutions of MH Lead to no significant increases in DL frequencies in Drosophila.

It is suggested that the high frequency of obtained DLS is due to chromosome aberrations. This is in agreement with finding of Sega and Owens (1983) who showed that methylation of protamine did increase in germ cell stages most sensitive to MMS, and showed an excellent correlation with incidence of DLS produced by MMS in the different germ cell stages. The occurrence of S-methyl-L cysteine as the major reaction product in sperm protamine after MMS exposure supports the interpretation of how DLS are induced in mouse germ cells by MMS. Alkylation of cysteine sulfhydryl groups contained in mouse-sperm protamine blocks normal disulfide-bone formaiton, preventing proper chromatin condensation in the sperm protamine

blocks normal disulfide-bone formation, preventing proper chromoatin condensation in the sperm nucleus. Subsequent stresses produced in the chromatin structure eventually lead to chromosome breakage. The same interpretation is in agreement with findings of Brewen et al. (1975) who reported that the type of aberrations seen following treatment with MMS were predominantly isochromatid interchange, and some chromatid deletions, as well as shattering effects on the male complement. These aberrations were observed at a concentration of 100 mg/kg body weight and at the time of peak sensitivity to DL induction. When the frequency of cells containing a cytologically visible aberration was compared to DL frequency an excellent correlation was obtained.

The previous interpretation is in agreement also with findings of Tanaka (1981) who reported that incidence of DLS induced by post-copulation treatment with MMS was about half of that induced in spermatozoa and late spermatids in males at the same dose. Furthermore, chromosomal aberrations observed in first cleavage were very high in pre-ovulatory Oocytes and sperm in oviducts (immediately after coupulation) by MMS treatment. The number of structural aberrations induced by MMS in the paternal chromosome was greater than in maternals.

The non effectiveness of MH can be attributed either to its inability to interact directly or indirectly with the genetic material of mammals or insects, or to its rapid metabolization to non-mutagenic products within cells of such organisms.

REFERENCES

Aeschbacher, H.U.; H. Milon and H.P. Wurzner (1978). Caffeine concentrations in mice plasma and testicular tissue and the effect of caffeine on the dominant lethal test. Mutat. Res., 57(2): 193-200.

Bateman, A.J. (1973). The dominant lethal assay in the mouse. Agents Actions, 3(2): 73-76.

Brewen, J.G.; H.S. Payne; K.P. Jones and R.J. Preston (1975). Studies on chemically induced dominant lethality. I- The cytogenetic basis of MMS-induced dominant lethality in post-meiotic male germ cells. Mutat. Res., 33(2-3): 239-250.

Cochran, W.C. and G.W. Cox (1957). Experimental designs 2nd. Ed. John Wiley and Sons, Inc. New York.

Ehling, U.H.; R.B. Cumming and H.V. Malling (1967). Induction of dominant lethal mutations by alkylating agents in male mice. Mutat. Res., 5: 417-428.

Ehling, U.H. (1970). Comparison of radiation and chemically induced dominant lethal mutations in male mice. Mutat. Res., 11: 35-44.

Ehling, U.H. (1977). Dominant lethal mutations in male mice. Arch. Toxicol. 38(1/2): 1-11.

Epstein, S.S.; W. Base; E. Arnold and Y. Bishop (1970). The failure of caffeine to induce mutagenic effects or to synergize the effect of known mutagens in mice. Fd. Cosmet. Toxicol., 8: 381-401.

Lang, R. and I.D. Adler (1977). Heritable translocation test and dominant lethal assay in male mice with methyl methanesulfonate. Mutat. Res., 48: 75-88.

Mittler, S.; J.E. Mittler; A.M. Tonetti and M.E. Szymczak (1967). The effect of caffeine on chromosome loss and non-disjunction in Drosophila. Mutat. Res., 4: 708-710.

Nagaty, H.H. (1981). Repair of chemically induced genetic damage in Drosophila. M.Sc. Thesis, Faculty of Agriculture, Minufiya University.

Nawar, M.M. and H.A. Hamza (1978). Influence of Maleic hydrazids mixed with ethyl methanesulfonate on mutation frequencies induced in Drosophila melanogaster. Egypt J. Genet. Cytol., 7(1): 69-78.

Nawar, M.M.; G. Morad; H. Hamza and A. Fahmy (1979). Differential sensitivity of Allium cepa and Drosophila melanogaster to the mutagenicity of Maleic hydrazide. Egypt J. Genet. Cytol., 8(1): 167-174.

Parry, P. and H.J. Evans (1975). Cytological detection of mutagen-carcinogen exposure by sister chromatide exchanges. Nautr. (London), 24: 121-125.

Rohrborn, Z. Enahrungswiss (1972). Study of mutagenicity in the mouse after prolonged treatment with caffeine. suppl. 14: 54-67.

Sankaranarayanan, K. (1969). The effect of nitrogen and oxygen treatment on the frequencies of X-ray induced dominant lethals and on the physiology of the sperm in Drosophila melanogaster. Mutat. Res. 4: 641-661.

Sega, G.A. and James G. Owens (1983). Methylation of DNA and protamine by methyl methanesulfonate in the germ cells of male mice. Mutat. Res., 111: 227-244.

Tanaka, N. (1981). Studies on chemical induction of chromosomal aberrations in post-copulation germ cells and Zygotes of female mice. I- Comparative studies on the frequency of first-cleavage chromosomal aberrations and dominant-lethal mutations. Jap. J. Genet., 56(2): 117-129.