EFFECT OF MMS, CAFFEINE AND MH ON SPERMHEAD AND BONE MARROW MITOTIC CELLS IN SWISS ALBINO MICE

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تأثير كل من الميثايل ميثان سلفونات والكافين والماليك هيدرازيد على رأس الحيوان المنوى والخلايا المنقسمة ميتوزيا فى نخاع سلالة الفئران الألبينو السويسرية

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ملخص البحث

أظهرت المعاملة بالميثايل ميثان سلفونات أو الكافين عند أى تركيز من التركيزات المستعملة زيادة ملحوظة فى تكرارات الحيوانات المنوية ذات شكل الرأس الشاذ . هذه الزيادة ارتفعت من الأسبوع الأول حتى الأسبوع الثالث ثم انخفضت الى أدنى مستوى عند الأسبوع الثامن فى حالة المعاملة بالميثايل ميثان سلفونات ولكنها وصلت الى هذا المستوى المنخفض فى الأسبوع الخامس وكذا الأسبوع الثامن فى حالة المعاملة بالكافين بينما لم تؤد المعاملة بالماليك هيدرازيد بأى تركيز الى أى زيادة ملحوظة فى تكرارات الحيوانات المنوية ذات الرؤوس الشاذة . وقد تم الحصول على تكرار عالى من خلايا الوضع المتوسط الميتوزى الشاذة (١٥ ضعفا بالمقارنة بغير المعامل) وذلك فى حالة المعاملة بـ ١٠٠ مجم / كجم ميثايل ميثان سلفونات وكان الفراغ الكروماتيدى أكثر الشذوذات شيوعا هذا ولم تعط المعاملة بالكافين أو الماليك هيدرازيد أى زيادة معنوية فى تكرارات أدوار الوضع المتوسط الشاذة . وقد أستخلص أن الميثايل ميثان سلفونات فعال بدرجة كبيرة فى احداث شذوذات فى رأس الحيوانات المنوية فى الفئران وأن أكثر المراحل حساسية لهذا المركب هى مرحلة الاسبرماتيد . أيضا أستخلص أن الكافين كان مؤثرا وأن أكثر المراحل حساسية هى مرحلة الحيوان المنوى الناضج والاسبرماتيد المبكر .

ABSTRACT

Treatment with MMS or caffeine, at any of the concentrations used, resulted in marked increases in frequencies of abnormal spermahead. Such frequency rose from week 1 to week 3 then dropped reaching the lowest level at the eighth week in case of treatment with MMS, but reaching the lowest level at the fifth and eighth weeks in case of treatment with caffeine. On the other hand, treatment with MH, at any of the concentrations used, resulted in no increase in frequencies of abnormal spermheads at all scoring times. High frequency of abnormal metaphases (15-fold increase above control) have been obtained in case of treatment with 100 mg/kg MMS. Many of the abnormalities were chromatid gaps. Treatment with caffeine or MH, did not result in significant increases in frequencies of aberrant metaphases. It was concluded that MMS is highly effective in causing spermhead abnormalities in mice and the most sensitive stage was spermatids. Caffeine was affective and the most sensitive stages were epididymal sperm and early spermatids.

INTRODUCTION

Over the past thirty years or so thousands of published research articles have added tremendously to the wealth of information in mutation research. Mutagenesis, the artificial induction of mutations, has also developed considerably during the past 20 years. Consequently, many assays or test systems have been developed, and are still being developed, to asses the possible damage natural and industrial chemical pollutants can cause to the genetic material. A simple test is one which does not involve too much labour or time to perform. Optimum accuracy requires expression of maximum number of lesions actually induced in the genetic material. Among the test systems most widely used in mutagensis research is the dominant lethal (DL) assay in mammals and/or insects. Another assay which has been suggested more recently, is the mammal spermhead abnormality test (Bruce et al., 1974). This technique is relatively simple to conduct and inexpensive to perform. The aim of this study was to

investigate the effect of a well known powerful mutagen, namely methyl methanesulfonate (MMS) in inducing end points, using the test systems spermhead abnormality in mice and chromosome aberrations in mice bone marrow cells. Also the study aimed to 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; using the two test systems.

MATERIALS AND METHODS

I. Experimental organism:

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

II. Chemicals:

The following chemical compounds were used:

1. Methyl methanesulfonate (MMS), $CH_3So_2OCH_3$, MW. 110.13.
2. Caffeine (1,3,7-trimethylxanthine), $C_8H_{10}N_4O_2$, MW. 194.19.
3. Maleic hydrazide (MH), $C_4H_4N_2O_2$, MW. 112.09.

All were obtained form Eastman Kodak Organic Chemicals Rochester, N.Y. 14650 U.S.A.

III. Breeding mice:

Mice were housed in suspended, wire top cages in an air-conditioned room. They were fed on wheat seed and bread in milk twice daily.

IV. Treatment of male mice with chemical mutagens:

Animals were group housed (up to 15 mice per cage) with diet unless contra indicated by the particular experimental design. Animals were assigned for study at random. Prior to study initiation, animals were weighed, and weight of test article to be administered per animal established using a mean weight. Each weight of chemical was then dissolved in distilled water and used for intraperitoneal (i.p.) injection of mice.

V. Biological criteria:

a) Spermhead abnormalities:

Adult male mice were obtained and kept until they reached an age of 11-14 weeks. Before being used, mice were injected (i.p.) by the chemical in solution. The animals were sacrified by cervical dislocation at 1,3,5 and 8 weeks following treatment. Caudae epididmides were removed and dissected and placed in a centrifuge tube containing 3 ml of 0.9% saline solution. The contents of caudae were cut into several small pieces by scissors. The resulting suspensions were gently pipetted five to six times up and down in a 5 or 10 ml pipette. The sperm suspension was then filtered through an 80 micro-m silk mesh to remove tissue fragments, and 0.5 ml of the filtrate was transferred to a centrifuge tube to which 0.05 ml of 1% Eosin-Y was added. The solutions were gently agitated and slides were made by placing a drop of the stained solution on a slide and spread by three passes of another slide. The slides were allowed to dry on a mildly hot plate. At least 2000 sperms were examined per treatment by observing under a light microscope at 40 x. Three males were used for each treatment, and three replications were made.

b) Cytological examination:

Male mice, 11-14 days old, were injected i.p. with a single dose of chemical 24 hours post-treatment males were injected with metaphase

blocking agent (i.e. 0.6 mg/kg of colchicine). Two hours later, animals were sacrificed by cervical dislocation. Both femurs were immediately removed by using small syringe needle (26 guage) and femoral contents were flushed with phosphate saline into a small tube. The cell suspension was centrifuged at 1000 rpm for 5 minutes, the supernatant was discarded and cell pellet disrupted by flicking the base of the tube. A hypotonic solution (0.075 M KCl) was added to give a slight cloudy solution, and let stand for 12 minutes at 37°C, then centrifuged. The supernatant was discarded and the pellet disrupted. For final fixation about 1-2 ml fresh fixative was added, and slides were made immediately by dropping three drops on clean dry slide. Check cell density through microscope, then slides were immersed in 4% Giemsa for six minutes, then rinsed in water and dried before examination by light microscope at 100 x. Three animals were used for each treatment and 20 metaphase plants per animal were analysed.

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).

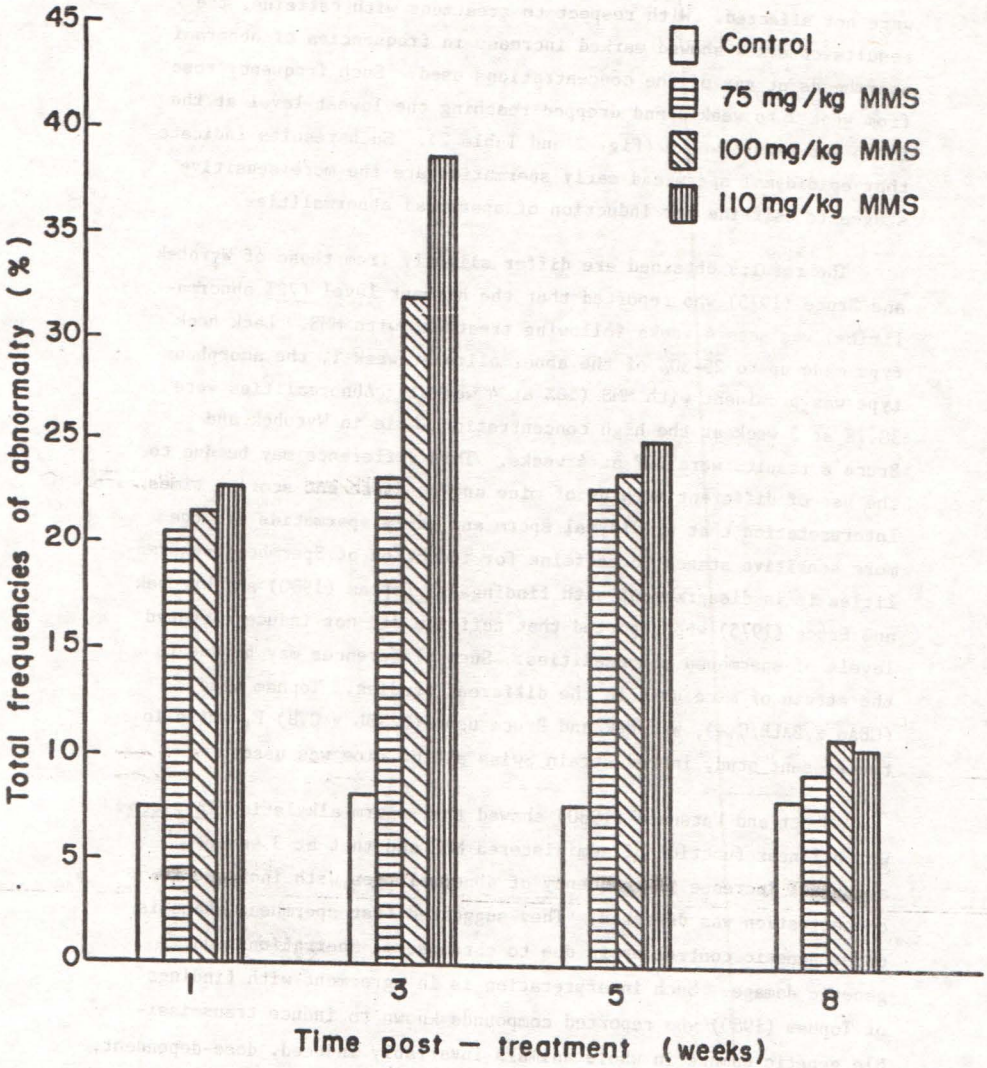
RESULTS AND DISCUSSION

Treatment with MMS at any of the concentrations used, resulted in marked increases in frequencies of abnormal spermheads. Such frequency rose from week 1 to week 3 then dropped reaching the lowest level at the eight weeks (Table 1 and Fig. 1). Most of the abnormalities scored were either lack hook or amorphous with few banana like abnormalities. The results show that the most sensitive stage to MMS was early spermatids, and that epidiymal sperm and early spermatocytes were the least sensitive stages to MMS, while the other stages

Table 1- Frequencies of abnormal spermhead induced by different concentrations of Methyl methanesulfonate (MMS) in male Swiss albino mice.

Abnormality	1 week							3 weeks							5 weeks							8 weeks						
		Abnormal							Abnormal							Abnormality							Abnormality						
Concent. mg/Kg	No. scored	HL	BL	A	O	Total	%	No. scored	LH	BL	A	O	Total	%	No. scored	LH	BL	A	O	Total	%	No. scored	LH	BL	A	O	Total	%
0	6540	197	22	262	3	484	7.4	6342	161	19	318	1	499	7.8	6447	186	3	308	-	492	7.6	6510	197	7	324	1	529	8.1
75	6115	605	10	636	-	1254	20.5**	6133	883	22	516	15	1436	23.4**	6350	628	7	775	8	1418	22.8**	6530	237	17	351	2	605	9.3
100	6427	565	61	747	-	1373	21.4**	6730	1311	14	818	11	2154	32.0**	6288	722	8	744	-	1474	23.4**	6523	291	13	411	9	724	11.1
110	6332	625	32	772	3	1432	22.6**	6151	1548	80	750	-	2378	38.7**	6430	730	15	871	5	1621	25.2**	6370	260	16	386	-	662	10.4

LH = Lack hook
BL = Banana like
A = Amorphous
O = Other
** = Highly significant at 0.01
* = Significant at 0.05

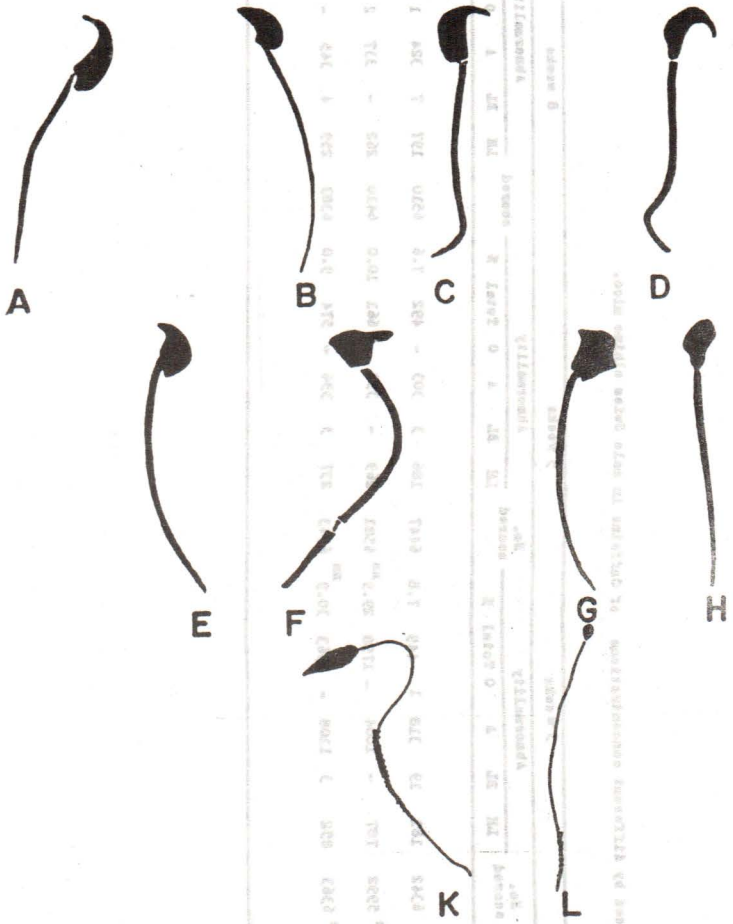


(Fig. 1) Frequencies of abnormal spermhead induced by different concentrations of MMS in male Swiss albino mice.

were not affected. With respect to treatment with caffeine, the results obtained showed marked increase in frequencies of abnormal spermheads at any of the concentrations used. Such frequency rose from week 1 to week 3 and dropped reaching the lowest level at the fifth and eighth weeks (Fig. 2 and Table 2). Such results indicate that epididymal sperm and early spermatids are the more sensitive stages to caffeine for induction of spermhead abnormalities.

The results obtained are differ slightly from those of Wyrobek and Bruce (1975) who reported that the highest level (72% abnormalities) was seen 4 weeks following treatment with MMS. Lack hook type made up to 25-30% of the abnormality at week 1, the amorphous type was prominent with MMS (58% at 4 weeks). Abnormalities were 38.7% at 3 week at the high concentration while in Wyrobek and Bruce's results were 72% at 4 weeks. This difference may be due to the use of different strains of mice and/or different scoring times. Interpretation that epididymal sperm and early spermatids are the more sensitive stages to caffeine for induction of Spermhead abnormalities is in disagreement with findings by Topham (1980) and Wyrobek and Bruce (1975) who reported that caffeine did not induce elevated levels of spermhead abnormalities. Such differences may be due to the strain of mice used in the different studies. Topham used (CBAo x BALB/C +), Wyrobek and Bruce used ($G_{57}BL$ x C_3H) F_1 while in the present study inbred strain Swiss albino mice was used.

Stott and Watanabe (1980) showed that sperm alkylation *in vitro* was a linear function of administered MMS and that at 3 weeks a gradual increase in frequency of abnormalities with increase in concentration was detected. They suggested that spermhead shape is under genetic control or is due to chromosomal aberrations and genetic damage. Such interpretation is in agreement with findings of Topham (1980) who reported compounds known to induce transmissible genetic damage in whole animals invariably induced, dose-dependent,



(Fig. 2) Normal and abnormal spermhead. Judgement was based on shape of spermhead:

(A) Normal
(B) Lack hook
(D) Banana like

(C-E-F-G-H-K-L) Amorphous.

Table 2- Frequencies of abnormal spermhead induced by different concentrations of Caffeine in male Swiss albino mice.

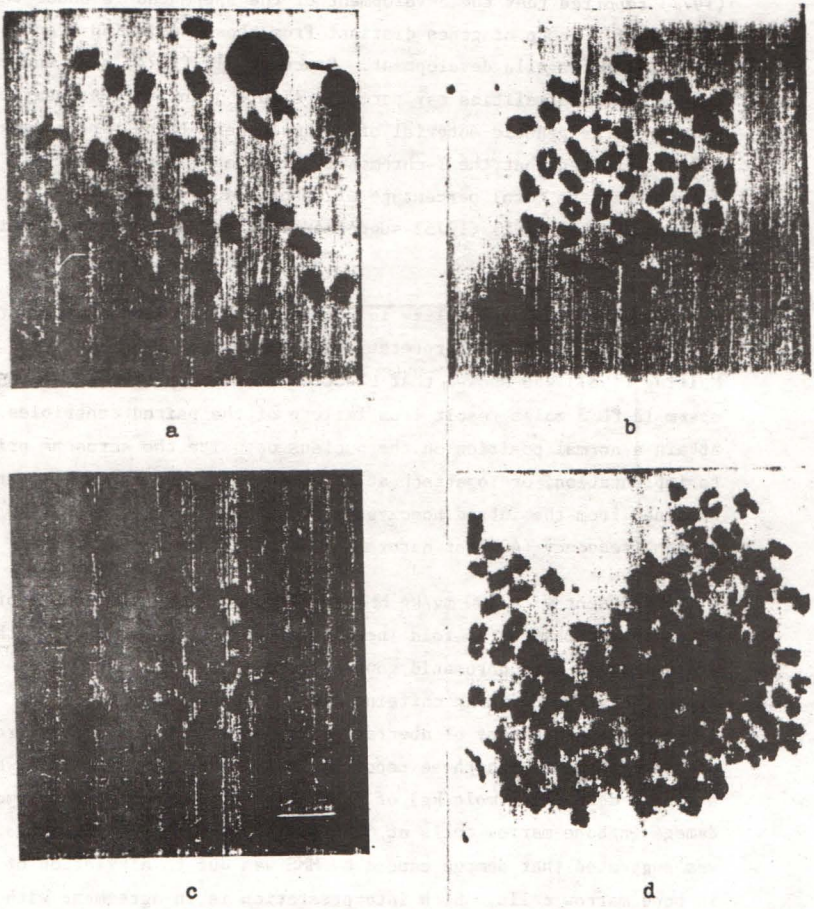
Abnormality	1 week							3 weeks							5 weeks							8 weeks						
Concent. mg/Kg	No. scored	Abnormality							No. scored	Abnormality						No. scored	Abnormality						No. scored	Abnormality					
		LH	BL	A	O	Total	%		LH	BL	A	O	Total	%		LH	BL	A	O	Total	%		LH	BL	A	O	Total	%
0	6540	197	22	262	3	484	7.4	6342	161	19	318	1	449	7.8	6447	186	3	303	-	492	7.6	6510	197	7	324	1	529	8.1
100	6199	798	1	1082	1	1882	30.4**	5992	787	-	1004	-	1798	29.9**	6581	269	-	392	-	661	10.0	6410	262	-	337	2	601	9.4
140	6078	785	8	991	-	1784	29.4**	6363	852	3	1108	-	1963	30.9**	6343	237	3	334	-	574	9.0	6383	259	4	345	-	608	9.5

LH = Lack hook
BL = Banana like
A = Amorphous
** = Highly significant at 0.01
* = Significant at 0.05

large increases in the incidence of abnormal spermheads. Bryan (1977) reported that the development of the spermhead is under the control of a group of genes distinct from those mediating events involved in flagella development. Bruce et al. (1974) also suggested that sperm abnormalities may permit a simple quantitative assay for damage to the genetic material of the male germ line. Krazanowska (1976) reported that the Y-chromosome plays an important role in determining the total percentage of abnormalities. Furthermore, Moutschen and Colizzi (1975) suggested that spermhead abnormalities are true mutants.

In contrast, abnormality in sperm morphology may be epigenetic (non-genetic). This interpretation is suggested by Burkhart and Malling (1981) who showed that electron microscopy of the abnormal sperm in PL/S males result from failure of the paired centrioles to attain a normal position on the nucleus opposite the acrosome prior to implantation, or to attach at all. However, these results were obtained from the inbred homozygous mouse strain PL/J which show a high frequency (42%) of natural spermhead abnormality.

Treatment with 100 mg/kg MMS resulted in a high frequency of abnormal metaphases (15-fold increase above control). Many of the abnormalities were chromatid gaps (Fig. 3). On the other hand, treatment with 100 gm/kg caffeine did not result in significant increases in frequency of aberrant mataphases (Table 3). These result are in agreement with those reported by Frei and Venitt (1975),where a single dose (11 m.mole/kg) of MMS caused significant chromosome damage in bone marrow cells at 6,24 and 48 hr. after treatment. It was suggested that damage caused by MMS was due to alkylation of DNA in bone marrow cells. Such interprestation is in agreement with results obtained by Frei and Venitt (1975) and Cumming and Marva (1970) who reported that the amount of genetic damage done in the mouse (chromosome breakage) was correlated with in vivo alkylation



(Fig. 3) Bone marrow metaphase from Swiss albino mice:

(a) Normal metaphase
(b) Chromatid gap.
(c) Numerous aberrations.
(d) Polyploidy.

of macromolecules. Present results are also in agreement with findings of Anderson and Richardson (1981) who suggested that chromosome and chromatid gaps could be useful and sensitive indicators of chemically-induced genetic damage. The results obtained for treatment with caffeine are in agreement with findings of Hanssonk (1978) who reported that caffeine did not induce any significant chromosome damage. However, these results are in disagreement with findings of Dulout et al., (1981) who reported that caffeine exhibited a significant increase in the frequencies of gaps at doses 100 and 150 mg/kg and in frequencies of breaks at all doses used. Differences between the present results and those of Dulout et al. (1981) may be due to different time of sampling (24 hr. post-treatment in this study, 8 hr. in Dulout's) and/or to different strains; whereas Dulout et al., used BALB/C mice, Swiss albino mice were used in this study.

Treatment of mice with MH led to no significant increases in spermhead abnormality or chromosome aberrations (Tables 3 and 4). These results are in agreement with results obtained by Manna and Das (1976), who reported that MH was not effective in causing chromosomal aberrations in bone marrow. Also, the results agree with those of Parry and Eavns (1975), that MH was not effective in causing chromosomal aberrations in rat liver cells. The non-effectiveness of MH can be attributed to : 1- its inability to interact directly or indirectly with the genetic material of mammals or 2-MH is rapidly metabolized to non-mutagenic products within cells of such organism.

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Table 3- Frequencies of abnormal metaphases induced by methyl methanesulfonate (MMS) caffeine (CAF) and maleic hydrazide (MH) in bone marrow cells of male Swiss albino mice.

Treatment	No. abnormal metaphases with			
	Chromatid gaps	Other	Total	(%)
Control	5	-	5	2.80
100 mg/Kg MMS	49	22	71	39.40
100 mg/Kg CAF	6	1	7	3.90
300 mg/Kg MH	6	1	7	3.90

No. metaphase figures scored for each treatment of Control = 180.

Table 4 - Frequencies of abnormal spermhead induced by different concentrations of Maleic hydrazide (MH) in male Swiss albino mice.

Abnormality	1 week							3 weeks							5 weeks							8 weeks						
	No. scored	Abnormality						No. scored	Abnormality						No. scored	Abnormality						No. scored	Abnormality					
Concent. mg/Kg		LH	BL	A	O	Total	%		LH	BL	A	O	Total	%		LH	BL	A	O	Total	%		LH	BL	A	O	Total	%
0	6540	197	22	262	3	484	7.4	6342	161	19	318	1	499	7.8	6447	186	3	303	-	496	7.6	6510	197	7	324	1	529	8.1
300	6413	187	65	284	-	536	8.4	6305	205	33	398	-	636	10.1	6320	242	5	304	-	551	8.7	6759	215	5	293	5	518	7.7
400	6507	207	90	310	1	608	9.3	6403	199	30	381	11	621	9.7	6469	239	2	326	-	567	8.8	6449	228	11	302	2	543	8.4

LH = Lack hook
BL = Banana like
A = Amorphous
O = Other

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