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In vivo cytogenetic effects of 2-trans hexenal on somatic and germ cells of laboratory mice

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Abstract

The *in vivo* cytogenetic effects of 2-trans hexenal were evaluated by investigating chromosomal aberrations and sperm head abnormalities in the bone marrow cells of laboratory bred Swiss albino mice. Single intraperitoneal injections of 8, 16 or 32 µl per kg bodyweight resulted in dose-dependent decreases in the mitotic index, significantly so at the higher doses and earlier times. Chromosomal aberrations per cell and the percentage of aberrant metaphase cells increased with dose, again fading with time. The percentage of abnormal sperm heads also showed a dose-related increase, with statistical significant again following the same pattern. The overall result suggests a weak but positive dose-response relationship between treatment and induction of chromosomal aberrations in the somatic cells, and induction of abnormal sperm head morphology in germ cells.

Keywords: chromosomal aberrations, bone marrow cells, sperm head abnormality

Introduction

2-trans hexenal is an oily liquid, used as food additive to make flavor. 2-trans hexenal occurs naturally in fruits such as apples, grapes, blueberries, apricot, peach, bananas, pear as well as in tea and coriander. Its vapor causes eye and skin irritation and is harmful if absorbed through skin. Prolonged skin contact may cause serious health effects. It may cause gastrointestinal irritation with nausea, vomiting and diarrhea. Its vapor or mist is irritating to the mucous membrane and upper respiratory tracts. Oral ingestion may cause irritation and ulceration of the stomach and eyes. Humans are exposed regularly to this compound via vegetable food.

We found no data on humans describing carcinogenicity associated with 2-trans hexenal exposure, but there were a few reports on its genotoxicity *in vitro*. 2-trans hexenal induced highly significant increased frequencies of micronuclei (Ditterberner et al. 1990, 1992). It induced significant and dose-related increase in frequencies of sister-chromatid exchange and micronuclei in human blood lymphocytes and cells of the permanent Namalva lines (Ditterberner et al. 1995). A low concentration of 2-trans hexenal showed evidence of a genotoxic effect *in vivo* in mucosal cells lining the mouth (Ditterberner et al. 1997). Mutagenic effects of 2- trans hexenal have also been reported in strains of *Salmonella typhimurium* and *Escherichia coli* (Kato et al. 1989). Trans-2-hexenal exerted a mutagenic effect in a slightly modified pre-incubation Ames test with *Salmonella typhimurium*TA100 (Eder et al. 1992). 2-trans hexenal forms exocyclic 1,N^2 propanodeoxyguanosine adducts like other carcinogenic compounds (Eder & Schuler, 2000), causes DNA adducts and genomic damage (Gölzer et al. 1996; Schäferhenrich et al. 2003a,b), and causes DNA damage in genes relevant for human colon cancer (Glei et al. 2007). Hexenal-derived DNA adduct formation and cell proliferation in the fore-stomach were observed by Stout et al. (2008) in male F344 rats.

Due to lack of direct evidence, this study was undertaken to assess the genotoxicity of 2-trans hexenal in the somatic and germ cells of mice by analysing chromosomal aberrations in bone marrow cells and sperm-head abnormalities in laboratory mice.

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Materials & Methods

Laboratory-bred Swiss albino mice *(Mus musculus)* were used. The animals were housed in wire-topped polypropylene cages with rice-husk bedding and maintained under standard laboratory conditions and temperature (Jha & Kumar 2006). Apart from 2-trans hexenal (CAS Registry No. 6728-26-3; molecular weight 90.1, molecular formula $C_6H_{10}O$, obtained from Sigma-Aldrich) and mitomycin C (Kyowa Hakko Kohyo Co., Ltd., Tokyo, Japan; marketed in India by Biochem Pharmaceuticals, Mumbai), all other chemicals were of analytical grade. Levels of 8, 16 and 32 µl per kg-bodyweight of 2-trans hexenal were selected for treatment of animals on the basis of toxicity assays. 1.5 mg per kg bodyweight of mitomycin C is the recommended dose for use as a positive control for animals in the bone-marrow chromosomal aberration assay.

Mice weighing approximately 25-30 g (5-6 weeks) were divided into groups of five animals (three males and two females) and treated with one of the three levels of 2-trans hexenal by intraperitoneal injection diluted in olive oil. A negative control group received just the carrier, 0.2 ml olive oil, and a positive control group received 1.5 mg per kg-bodyweight of mitomycin C. Animals were sacrificed 6, 12 and 24 h after treatment by cervical dislocation. Separate negative and positive control animals were used for each period of exposure. Two hours before sacrifice, mice were injected with 0.3 ml of freshly prepared colchicine solution (4 mg per kg-bodyweight). Bone marrow preparations for metaphase cells were obtained using the standard technique (Preston et al. 1981). The slides were stained in 10% buffered giemsa (pH 7.0) for 20 min, air-dried and mounted in DPX adhesive.

Slides were analyzed blind. The mitotic index was obtained by counting the number of mitotic cells in 1000 cells per animal, expressed as percentage. Two hundred well-spread metaphases per animal (1000 metaphases per treatment) were scored for presence of chromosomal aberrations. Data were evaluated as the number of chromosomal aberrations per cell (excluding gaps, and stickiness and pulverizations) and percent aberrant metaphase cells (excluding gaps). Chromosomal aberrations were classified into categories, such as chromatid and isochromatid gaps, chromatid and isochromatid breaks, fragments, and stickiness and pulverization of chromosomes. Gaps were not considered for statistical analysis because of their controversial genetic significance (Preston et al. 1981, WHO 1985).

To assay for sperm-head abnormalities, adult male mice 6-8 weeks old were treated with the same set of treatments as above (ie three levels of 2-trans hexenal plus negative control), but the animals were sacrificed 1, 3 and 5 weeks after treatment (n=5 in each case). Both testes of each animal were removed and placed in 60 mm petri dishes containing 2.2% sodium citrate at room temperature. After removing fatty tissues, each testis was quickly transferred to another petri dish containing fresh 2.2% sodium citrate solution. A sperm suspension obtained from the cauda epididymis was dropped onto clean glass slides and smears prepared. The slides were dried and stained in buffered giemsa, coded, and the morphology of sperm heads observed under 100x oil immersion lens. 2500 sperm heads per dose (500 from each of five animals) were scored as having either normal or abnormal morphology, adopting the criteria of Wyrobek & Bruce (1975).

Results

Table 1 records the fact that 2-trans hexenal treatment resulted in a dose-dependent decrease in the mitotic index, as compared to negative control. Statistically significant reductions in the mitotic index were recorded in the animals treated with 32 µl after 6 and 12h, and 16 and 32 µl for 24h. The inhibitory effect of the treatment on mitotic activity thus persisted up to 24 h after treatment.

Table 1: Influence of three durations of 2-trans hexenal treatments on the cell division in the bone marrow cells of Swiss albino mice.
Doses are per kg body weight; the negative control is the olive oil carrier. * = significantly different from the negative control with Student's t-test at $p < 0.05$

duration	treatments		number of dividing cells	Mitotic Index (mean ± s.e.)
6 h	negative control	0.20 ml	311	6.22 ± 0.69
	2-trans hexenal	8 µl	300	6.00 ± 0.54
	2-trans hexenal	16 µl	267	5.28 ± 0.34
	2-trans hexenal	32 µl	211	4.22 ± 0.31*
12 h	negative control	0.20 ml	314	6.28 ± 0.97
	2-trans hexenal	8 µl	287	5.74 ± 0.48
	2-trans hexenal	16 µl	253	5.06 ± 0.39
	2-trans hexenal	32 µl	189	3.76 ± 0.34*
24 h	negative control	0.20 ml	307	6.14 ± 0.97
	2-trans hexenal	8 µl	272	5.44 ± 0.28
	2-trans hexenal	16 µl	253	4.38 ± 0.37*
	2-trans hexenal	32 µl	208	4.16 ± 0.49*

There was a dose-dependent increase relative to the control in the mean chromosomal aberrations per animal was recorded in 2-trans hexenal treated animals (Table 2).

Table 2: Number of chromosomal aberrations (CAs) per animal, total chromosomal aberrations and mean chromosomal aberrations per animal in the bone marrow of Swiss albino mice recorded after 2-trans hexenal treatment.
Doses are per kg body weight; the negative control is the olive oil carrier; the positive control is mitomycin C, a known carcinogen; asterisks mean significantly different from the negative control with Student's t-test at * = $p < 0.05$, *** = $p<0.001$

			Number of cells with CAs					
duration	**treatments**		**A1**	**A2**	**A3**	**A4**	**A5**	**mean ± s.e.**
6 h	negative control	0.2 ml	5	7	3	4	3	4.40 ± 0.74
	2- trans hexenal	8 µl	4	6	5	5	4	4.80 ± 0.91
	2- trans hexenal	16 µl	7	7	5	5	5	6.00 ± 0.22
	2- trans hexenal	32 µl	6	7	7	8	9	7.40 ± 0.41*
	positive control	0.15 mg	59	43	63	37	50	50.80 ± 4.69***
12 h	negative control	0.2 ml	4	5	3	6	6	4.80 ± 0.58
	2- trans hexenal	8 µl	4	6	5	7	6	5.60 ± 0.91
	2- trans hexenal	16 µl	7	5	7	9	6	6.80 ± 0.48
	2- trans hexenal	32 µl	7	12	10	11	8	9.60 ± 0.64*
	positive control	0.15 mg	51	70	78	50	75	64.80 ± 5.97***
24 h	negative control	0.2 ml	3	3	0	4	3	2.60 ± 0.24
	2- trans hexenal	8 µl	4	4	2	3	1	2.80 ± 0.29
	2- trans hexenal	16 µl	4	3	6	4	4	4.20 ± 0.26
	2- trans hexenal	32 µl	4	7	6	3	9	5.80 ± 0.43
	positive control	0.15 mg	51	56	65	64	61	59.40 ± 2.61***

Table 3 records the numbers and percentages of cells with different types of chromosomal aberrations. Statistically significant increases in the number of chromosomal aberrations per cell, and the percentage of aberrant metaphase cells were recorded 12 h after treatment with the intermediate and high doses of 2-trans hexenal, an effect which persisted 24 h after treatment with the high dose. The maximum frequency of chromosomal aberrations per cell and percentage aberrant metaphase cells was recorded at 12h, and had decreased by 24 h after treatment. 2-trans hexenal treatment resulted in the induction of structural chromosomal

aberrations viz. chromatid and isochromatid gaps and breaks, fragments and stickiness and pulverization (Table 3).

Table 3: Chromosomal aberrations induced by 2-trans hexenal in bone marrow cells of Swiss albino mice

Doses are per kg body weight; the negative control was the olive oil carrier; the positive control was mitomycin C, a known carcinogen; 1000 metaphase cells were analyzed per animal, and hence 5000 per treatment; G = chromatid and isochromatid gaps; C' = chromatid breaks, C" = isochromatid breaks; Fg = fragments, St+Pl = stickiness and pulverizations; CAs per cell = mean number ± s.e. excluding gaps, stickiness and pulverization; %abnormal cells = mean number ± s.e. including stickiness and pulverization wherever found; asterisks mean significantly different from the negative control with Student's t-test at * = p< 0.05, *** = p<0.001

			Number and types of CAs						
duration	**treatment**		**G**	**C'**	**C"**	**Fg**	**St+Pl**	**CAs per cell**	**%abnormal cells**
6 h	negative control	0.2 ml	7	7	0	8	0	0.015 ± 0.010	1.20 ± 0.20
	2- trans hexenal	8 µl	4	9	0	11	0	0.020 ± 0.011	1.60 ± 2.26
	2- trans hexenal	16 µl	5	12	0	11	2	0.023 ± 0.011	1.80 ± 0.34
	2- trans hexenal	32 µl	5	13	0	13	3	0.026 ± 0.023	2.10 ± 0.38
	positive control	0.15 mg	59	60	58	77	0	0.195 ± 0.540	17.20 ± 2.25***
12 h	negative control	0.2 ml	7	9	0	8	0	0.017 ± 0.016	1.20 ± 0.20
	2- trans hexenal	8 µl	5	14	5	5	0	0.024 ± 0.190	2.20 ± 0.45
	2- trans hexenal	16 µl	6	16	6	6	0	0.028 ± 0.014*	2.50 ± 0.46*
	2- trans hexenal	32 µl	10	15	5	14	4	0.034 ± 0.026*	3.40 ± 0.38*
	positive control	0.15 mg	61	80	88	95	0	0.263 ± 0.310	24.60 ± 2.17***
24 h	negative control	0.2 ml	7	3	0	3	0	0.060 ± 0.016	0.40 ± 0.061
	2- trans hexenal	8 µl	3	0	6	5	0	0.011 ± 0.011	1.00 ± 0.28
	2- trans hexenal	16 µl	3	0	9	5	4	0.014 ± 0.015	1.60 ± 0.45
	2- trans hexenal	32 µl	3	0	12	8	6	0.020 ± 0.026*	2.10 ± 0.38*
	positive control	0.15 mg	61	66	85	85	0	0.236 ± 0.245	21.60 ± 2.10***

Table 4: Effect of 2-trans hexenal treatments on the incidence of sperm abnormality in Swiss albino mice at different stages of spermatogenesis.

Doses are per kg body weight; the negative control is the olive oil carrier. asterisks indicate significant differences from the negative control with Student's t-test, * = p< 0.05, ** = p<0.01

duration	**treatments**		**Sampled population**	**Number of abnormal sperm heads**	**Abnormal sperm heads (mean ± s.e.)**
1 week	negative control	0.2 ml	Spermatozoa	58	2.32 ± 0.89
	2- trans hexenal	8 µl		71	2.84 ± 0.85
	2- trans hexenal	16 µl		137	5.48 ± 0.34*
	2- trans hexenal	32 µl		156	6.24 ± 0.31**
3 weeks	negative control	0.2 ml	Spermatids	63	2.57 ± 2.25
	2- trans hexenal	8 µl		99	3.96 ± 0.48
	2- trans hexenal	16 µl		158	6.32 ± 0.39**
	2- trans hexenal	32 µl		179	7.16 ± 0.34**
5 weeks	negative control	0.2 ml	Preleptotene spermatogonia	56	2.24 ± 0.38
	2- trans hexenal	8 µl		59	2.36 ± 0.37
	2- trans hexenal	16 µl		67	2.68 ± 0.25
	2- trans hexenal	32 µl		92	3.68 ± 0.34

Table 4 and 5 record abnormalities of the sperm induced by 2-trans hexenal treatment, measured 1, 3 and 5 weeks after treatment. There was a dose-related increase in the percentage of abnormal sperm heads (Table 4), statistically significant at the higher doses 1 and 3 weeks after treatment. Common types of abnormality (Table 5) were sperm with short hooks or without hooks, giant amorphous sperm, and sperm of the 'banana' type. Short-hooked sperm heads followed by sperm heads without hooks were found to be the most prevalent.

Table 5: Frequency and spectrum of abnormal type of sperm heads observed in Swiss albino male mice after 2-trans hexenal treatments.
Doses are per kg body weight; the negative control is the olive oil carrier.

			Types and percentages of abnormal sperm heads			
duration	**treatments**		**short hook**	**no hook**	**giant amorphous**	**banana type**
1 week	negative control	0.2 ml	0.48	0.60	0.24	1.00
	2-trans hexenal	8 µl	1.24	1.00	0.60	0.00
	2-trans hexenal	16 µl	2.40	1.28	0.60	1.20
	2-trans hexenal	32 µl	2.00	2.00	1.00	1.24
3 weeks	negative control	0.2 ml	0.60	0.88	0.56	0.48
	2- trans hexenal	8 µl	2.00	0.80	0.44	0.56
	2-trans hexenal	16 µl	3.00	2.08	0.80	0.44
	2-trans hexenal	32 µl	3.40	2.32	0.92	0.52
5 weeks	negative control	0.2 ml	0.60	0.80	0.20	0.64
	2- trans hexenal	8 µl	1.00	0.56	0.40	0.40
	2-trans hexenal	16 µl	1.00	0.80	0.44	0.44
	2-trans hexenal	32 µl	2.08	1.00	0.32	0.28

Discussion

There are very few published reports on the potential *in vivo* cytogenetic effects of 2-trans hexenal. All the tested intraperitoneal doses of 2-trans hexenal induced increases in the frequency of chromosomal aberrations in the bone marrow cells, significant at the higher doses, and fading with time. They also resulted in mitotic inhibition as is evident by the reduction in the mitotic index. They induced several structural chromosomal aberrations, such as chromatid and isochromatid breaks, fragments and gaps. The effect was both dose and time dependent. Decreases in the number of chromosomal aberrations per cell and percentage of aberrant metaphase cells in the bone marrow cells after 24 h suggests a reduced survival rate of the affected cells and their subsequent elimination. The induction of chromosomal aberration is a complex cellular process and the mechanisms are not yet fully understood (Brewen & Preston 1974). Structural chromosomal aberrations may result from: (i) direct DNA breakage, (ii) replication on a damaged DNA template, (iii) inhibition of DNA synthesis, and other mechanisms such as topoisomerase II inhibition.

Wyrobek & Bruce (1975) developed the mouse sperm morphology assay, and its relevance in evaluating mammalian germ cell mutagens is well accepted. The presence of abnormal sperm heads suggests induction of genetic damage in the male germ cells. Sperm head abnormalities may arise due to small deletions or point mutations, physiological, cytotoxic or genetic mechanisms (Odeigah 1997), or alteration in testicular DNA which in turn disrupts the process of differentiation of spermatozoa (Bruce & Heddle 1979). In the present experiment 2-trans hexenal induced a dose-dependent increase in the frequency of abnormal sperm and a significantly higher percentage of abnormal sperm during the first and third weeks of treatment, decreasing five weeks after treatment. The time interval chosen for sampling sperm represents pre-meiotic (29-35 days) and post-meiotic (1-21 days) stages of

spermatogenesis. 1-7 days post-treatment, sperm are regarded as spermatozoa, after 15-21 days they are early spermatids, and after 29-35 days they are spermatogonial cells (Adler 1982). The lower percentage of abnormal sperm heads five weeks after treatment indicates that pre-meiotic cells are less sensitive to the treatment of 2-trans hexenal than the post-meiotic cells.

Wyrobek et al. (1983) emphasized that chemicals which are mutagenic to somatic cells, could also affect germ cells. The dose-dependent increase in the frequency of abnormal sperm observed in the present study suggests that 2-trans hexenal has a definite effect on the differentiation of male germ cells, inducing mild genetic damage in the germ cells during the later stage of sperm maturation (as reported earlier for crotonaldehyde (Jha & Kumar 2006, Jha et al. 2007). Thus we conclude that 2-trans hexenal (an unsaturated aldehyde) is mildly clastogenic and mutagenic to the somatic and germ cells of the mouse.

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References

Adler ID (1982) Male germ cell cytogenetics. pp. 249-276 in Hall TC (ed.) Cytogenetic Assay of Environmental Mutagens. Totowa, NJ: Allenheld Osmum.

Brewen JG & Preston RJ (1974) Cytogenetic effects of environmental mutagens in mammalian cells and the extrapolation to man. Mutation Research 26: 297-305.

Bruce W & Heddle J (1979) The mutagenicity of 61 agents as determined by the micronucleus, *Salmonella* and sperm abnormality assays. Canadian Journal of Cytology & Genetics 21: 319-334.

Ditterberner U, Eisenbrand G & Zankl H (1990) Cytogenetic effects of the alpha, beta-unsaturated aldehydes crotonaldehyde and E-2 hexene-1-al. Mutagenesis 5 (6): 618.

Ditterberner U, Eisenbrand G & Zankl H (1992) Frequency and classification of micronuclei in Namalva cells induced by treatment with the alpha, beta-unsaturated aldehydes crotonaldehyde, 2-trans-hexenal and 2-trans, 6-cis nonadienal. Mutagenesis 7 (2) : 154.

Ditterberner U, Eisenbrand G & Zankl H (1995) Genotoxic effects of the alpha, beta-unsaturated aldehydes 2-trans-butenal, 2-trans-hexenal and 2-trans, 6-cis-nonadienal. Mutation Research 335: 259-265.

Ditterberner U, Schmetzer B, Goolzer P, Eisenbrand G & Zankl H (1997) Genotoxic effects of 2-trans hexenal in human buccal mucosa cells in vivo. Mutation Research 390-391: 161-165.

Eder E, Deininger C, Neudecker T & Deininger D (1992) Mutagenicity of beta-alkyl substituted acrolein congeners in the *Salmonella typhimurium* strain TA100 and genotoxicity testing in the SOS chromotest. Environmetal & Molecular Mutagenesis 19 (4): 338-345.

Eder E & Schuler D (2000) An approach to cancer risk assessment for the food constituent 2-hexenal on the basis of 1, N 2-propanodeoxyguanosine adducts of 2-hexenal *in vivo*. Archives of Toxicology 74: 642-648.

Glei M, Shäferhenrich A, Claussen U, Kuechler A, Liehr T, Weise A, Marian B, Sendt W & Pool-Zobel BL (2007) Comet fluorescence *in situ* hybridization analysis for oxidative stress–induced DNA damage in colon cancer relevant genes. Toxicological Sciences, 96(2): 279-284

Gölzer P, Janzowski C , Pool-Zobel BL & Eisenbrand G (1996) Hexenal-induced DNA damage and formation of cyclic 1,N^2-(1,3-Propano)-2'-deoxyguanosine adducts in mammalian cells. Chemical Research in Toxicology 9:1207–1213

Jha AM & Kumar M (2006) *In vivo* evaluation of induction of abnormal sperm morphology in mice by an unsaturated aldehyde crotonaldehyde. Mutation Research 603:159-163.

Jha AM, Singh AC, Sinha U & Kumar M (2007) Genotoxicity of crotonaldehyde in the bone marrow and germ cells of laboratory mice. Mutation Research 632: 69-77.

Kato F, Araki, A. Nozaki, K & Matsushima T (1989) Mutagenicity of aldehydes and diketones. Mutation Research 216: 366-367.

Odeigah PGC (1997) Sperm head abnormalities and dominant lethal effects of formaldehyde in albino rats. Mutation Research 189: 141-148.

Preston RJ, Dean BJ, Galloway S, Holden H, McFee AF & Shelby M (1981) Mammalian *in vivo* cytogenetic assays: analysis of chromosomal aberrations in bone marrow cells. Mutation Research 189 :157–165.

Schäferhenrich A, Beyer-Sehlmeyer G, Festag G, Kuechler A, Haag N, Weise A, Liehr T, Claussen U, Marian B, Sendt W, et al. (2003a) Human adenoma cells are highly susceptible to the genotoxic action of 4-hydroxy-2-nonenal. Mutatation Research 526(1-2): 1–14

Schäferhenrich A, Sendt W, Scheele J, Kuechler A, Liehr T, Claussen U, Rapp A, Greulich KO, Pool-Zobel BL. (2003b) Endogenously formed cancer risk factors induce damage-of p53 in human colon cells obtained from surgical samples. Food Chemical Toxicology 41: 655–664.

Stout MD, Bodes E, Schoonhoven R, Upton PB, Travlos GS & Swenberg JA (2008) Toxicity, DNA binding, and cell proliferation in male F344 rats following short-term gavage exposures to trans-2-hexenal. Toxicological Pathology 36(2): 232-246

WHO (1985) Guidelines for the study of genetic effects in human populations: in: Environmental Health Criteria 46. World Health Organization, Geneva.

Wyrobek AJ & Bruce WR (1975) Chemical induction of sperm abnormalities in mice. Proceedings of the National Academy of Sciences USA 72: 4425-4429.

Wyrobek AJ, Gordon LA, Burkhart JG, Francis MW, Kapp RW, Letz G, Malling HV, Topham JC & Whorton MD (1983) An evaluation of the mouse sperm morphology test and other sperm tests in non-human mammals. Mutation Research 115: 1–72.

الملخص العربى

التأثيرات السيتولوجية-الوراثية لمركب 2- ترانز هيكسانال على الخلايا الجسدية والتناسلية فى فئران التجارب المعملية

أناند جها - ميثيليش كومار - أبها
معمل السموم الوراثية – قسم البيوتكنولوجى – كلية سامستيبور للدراسات العليا – سامستيبور - الهند

تم دراسة التأثير السيتولوجى - الوراثى لمركب الـ 2- ترانزهيكسانال من خلال متابعة التشوهات الكروموسومية وروؤس الحيوانات المنوية فى خلايا النخاع العظمى للفئران البيضاء وذلك تحت الظروف المعملية فى سويسرا. أوضحت الدراسة أن الحقن تحت الغشاء البريتونى لمرة واحدة بالجرعات 8، 12، 16، 32 ميكروليتر/كيلوجرام من وزن الحيوان تقلل من مؤشرات عملية التحول الميتوزى وذلك عند الجرعات الكبيرة والأوقات القصيرة. أيضا أتضح أن التشوهات الكروموسومية لكل خلية ونسبة تشوهات خلايا الميتافيز تتزايد بزيادة الجرعات، وأيضا تتلاشى مع الوقت. أتضح أيضا أن نسبة التشوهات برؤوس الحيوانات المنوية تزداد مع زيادة الجرعات وتسلك نفس الإتجاه كما فى الخلايا السابق ذكرها.
توضح الدراسة بشكل عام أن هناك تأثير ضعيف فى إحداث تشوهات كروموسومية فى الخلايا الجسدية وأيضا إلى حدوث تشوهات فى رؤوس الحيوانات المنوية ولكن هذا التأثير يعتمد على الجرعات المحقونة.