Contam. Amb. 1, 35-43, 1985

GENETIC EFFECTS OF THINNER, BENZENE AND TOLUENE IN *Drosophila melanogaster*

1. SEX CHROMOSOME LOSS AND NON-DISJUNCTION

ROSARIO RODRIGUEZ ARNAIZ * AND RAFAEL VILLALOBOS-PIETRINI **

* Laboratorio de Genética, Facultad de Ciencias, Universidad Nacional Autónoma de México, México, D. F. 04510 México.
** Laboratorio de Citogenética y Mutagénesis Ambientales, Centro de Ciencias de la Atmósfera, Universidad Nacional Autónoma de México, México, D. F. 04510 y Centro de Investigación y Reproducción Animal, Universidad Autónoma de Tlaxcala.

ABSTRACT

Some genetic effects of thinner, benzene and toluene were studied in *Drosophila melanogaster,* namely sex-chromosome loss and non-disjunction in males and females. The order of toxicity was benzene > toluene > thinner. Viability was reduced by benzene and toluene but not by thinner. Sex chromosome loss and non-disjunction were induced in males only at the highest concentrations of benzene and toluene. Thinner did show genetic effects.

RESUMEN

Se estudiaron algunos efectos genéticos de tiner, benceno y tolueno en *Drosophila melanogaster,* tales como la pérdida de los cromosomas sexuales y la no disyunción de los mismos en machos y en hembras. El orden de toxicidad fue benceno > tolueno > tíner. La viabilidad se redujo con el benceno y el tolueno pero no con el tíner. Solamente las concentraciones más altas de benceno y tolueno indujeron la pérdida de los cromosomas sexuales y la no disyunción en los machos. El tíner no produjo dichos efectos genéticos.

INTRODUCTION

Widely used solvents have been involved in environmental pollution. This is the case of thinner, used by several industries, which is a balanced mixture of active solvents, latents and diluents. Although commercial mixtures of thinner are diverse,

most of them contain: toluene, benzene, n-hexane, n-heptane and some alcohols.

Thinner is a component of paints, lacquencs and varnishes. It control viscosity, consistancy, as well as evaporation (Gutiérrez-Flores 1975). Thinner stimulates the central nervous system in cats, disturbing perception and inducing hallucinations. It also decreases conscious levels and provokes indequate emotional responses to environmental simulation (Guzmán-Flores 1975).

Benzene is a solvent used to prepare dyes, artificial leather, varnishes and lacquers. It is also a solvent of waxes, resins and oils. As this compound is highy toxic, acute exposure by inhalation or ingestion produces mucous irritation, convulsions and depression, whereas chronic exposure produces aplasia, anemia and antibody deficiency with increased sensitivity to bacterial infections. It also depletes the activity of phagocytes and alters spleen, liver, kidneys and endocrin glands (Barroso-Moguel 1975). Benzene diminishes iron incorporation in rat reticulocytes, but the effect is neutralized or inhibited when it is mixed with toluene in adequate proportions (Andrews 1977). No teratogenic effects have been observed in rats (Green 1978). *Drosophila* larvae treated with benzene showed a decrease in survival (Nylander *et al.* 1978).

Toluene is used in benzonic acid synthesis, as a solvent for the extraction of active constituents from plants, as a mover in pesticide elaboration and as component of hair dyes. It produces irreversible cerebral degeneration in man (Grabski 1961). In rats, LD_{50} increases with age, a fact that can be related to changes in biotransformation activities (Kimura, 1971). Toluene induces equilibrium loss, ataxia and salivation in rats (Contreras *et al.* 1978) but in treated mice neither teratogenic effects (Hudak 1978), nor developmental alterations have been recorded (Newrot 1979).

In this paper we report the genetic effects of thinner, benzene and toluene on the induction of sex chromosome loss and non-disjunction in *Drosophila-melanogaster.*

MATERIALS AND METHODS

The genetic scheme designed by Oster (1958) was followed: "Oster" females have the X chromosome marked with yellow (y = body yellow) and the inversions scute s_1 inversion^{49} scute^{8} (scute = hairs scute), "Oster" males have a ring X chromosome (X^{C2}) with the genetic markers yellow and Bar (B = eye B) and in the Y chromosome a translocation of the X chromosome with a small fragment of X chromosome which contains the wild allele of the locus yellow.

The cross is:

P "Oster" females	×	"Oster" males
$y\ sc^{S1}$ Inv 49 sc^{8}		$X^{C2}\ y\ B/sc^{8}\ Y$

The first generations (F_1) is:

F_1 normal progeny: females $y\ sc^{S1}$ Inv 49 sc^{8}/X^{C2}
$y\ B$ (*y B/+*)
males $y\ sc^{S1}$ Inv 49 $sc^{8}/sc^{8}Y$
(*wild type*)

exceptional:

a) non-disjunction in females:
XXY females: sc^{s1} Inv 49 sc^{8}/y sc^{s1} Inv 49 sc^{8}/sc^{8} Y (*wild type*)
XO males: X^{c2} y B (*y B*)

b) non-disjunction in males:
XXY females: y sc^{s1} Inv 49 sc^{8}/X^{c2} y B/sc^{8}Y (*B*/+)
XO males: y sc^{s1} Inv 49 sc^{8} (*y*)

c) X-loss in females:
XO males: X^{c2} y B (*y B*)

d) X-loss in males:
XO males: y sc^{s1} Inv 49 sc^{8} (*y*)

e) Y-loss in males:
XO males: y sc^{s1} Inv 49 sc^{8} (*y*)

Two experimental blocks were done: treated males and treated females, in order to determine non-disjunction and X-loss in females and non-disjunction and X-Y loss in males. In preliminary experiments LD_{50} was determined. The solvents were administrated orally with food. Males and females were allowed to mate for three days after treatment. Fifteen days later the F_1 emerged flies were recorded.

TABLE I. NON DISJUNCTION AND X CHROMOSOME LOSS IN "OSTER" MALES TREATED AS ADULTS WITH DIFFERENT CONCENTRATIONS OF THINNER

Treatment Concentration %	*Females*				*Males*			
	Normal y B	*Exceptional*			*Normal ++*	*Exceptional*		
		Proceed from non disjunction in females wild type [1]	*Proceed from non disjunction in males B/+* [2]	*Frequency %*		*Proceed from non disjunction or X chromosome loss yellow Bar* [3]	*Proceed from non disjunction or (X or Y) chromosome loss in males yellow* [4]	*Frequency %*
Control	475				503			
0.5	495				529			
1.0	452				468			
1.5	429				495			
2.0	413		1	0.24	463		1	0.21
2.5	408		1	0.24	438		2	0.45
3.0	369		2	0.54	447		2	0.45

Genotypes:
[1] y sc^{s1} In^{49} sc^{8}/y sc^{s1} In^{49} sc^{8}/sc^{8} Y
[2] X^{c2} y B/sc^{8} Y/ysc^{s1} In^{49} sc^{8}
[3] X^{c2} y B
[4] y sc^{s1} In^{49} sc^{8}.

TABLE II. NON DISJUCTION AND X CHROMOSOME LOSS IN "OSTER" FEMALES TREATED AS ADULTS WITH DIFFERENT CONCENTRATIONS OF THINNER

Treatment	*Females*				*Males*			
		Exceptional				*Exceptional*		
Concentration %	*Normal y B*	*Proceed from non disjunction in females wild type* [1]	*Frequency %*	*Proceed from non disjunction in males B/+* [2]	*Normal ++*	*Proceed from non disjunction or X chromosome loss yellow Bar* [3]	*Proceed from non disjunction or (X or Y) chromosome loss in males yellow* [4]	*Frequency %*
Control	439	1	0.22		536		4	0.74
0.5	524	1	0.19		664		5	0.75
1.0	384	1	0.25		583		4	0.69
1.5	367				495		4	0.80
2.0	371	1	0.26		467		4	0.85
2.5	225	1	0.44		292		3	1.02
3.0	287				289		3	1.04

Genotypes:
[1] y sc^{s1} In^{49} sc^{s}/y sc^{s1} In^{49} sc^{s}/sc^{s} Y
[2] X^{c2} y B/sc^{s} Y/ysc^{s1} In^{49} sc^{s}
[3] X^{c2} y B
[4] y sc^{s1} In^{49} sc^{s}.

The assayed solvents were: Thinner * at concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5%; benzene (Baker) at 0.10, 0.25, 0.50, 0.75, 1.00 and 1.25%; and toluene (Baker) at 0.10, 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50%. For all concentrations, parallel controls were run. All experiments were carried out at 25°C ± 1. Statistical significance tests were done en the basis of X^2 and a z modified (Spiegel 1961).

RESULTS AND DISCUSSION

Tables I to VI summarize the results obtained. A decrease in viability induced by benzene and toluene was observed which is in agreement with the reports of Nylander *et al.* (1978) and Donner *et al.* (1981). Thinner did not reduce viability, due possibly to the interaction of the different solvents in the mixture.

The spontaneous frequency of non-disjunction in females was between 0.12 and 0.24%, while the spontaneous frequency of X loss in females was 0.22% and the spontaneous frequency of X-Y loss and non-disjunction in males varied from 0.16 to 0.74%. This difference can be explained on the basis of the higher

* Gas chromatogram run in the Centro Mexicano de Salud Mental showed that thinner constituents are: toluene 52.0%, n-hexane 25.5%, ethanol 12.5%, ethyl acetate 6.0%, isopropanol 1.0% benzene and n-heptane 1.0%.

spontaneous loss of ring chromosomes (Vogel and Natarajan 1979), as compared with normal chromosomes. It has been also suggested that the alteration could be the result of a different frequency of breacks in X and Y chromosomes (Lindsey and Grell 1968). Meanwhile Brink (1969) has related it to X-chromosome loss and the induction of dominant lethal mutation in the spermatozoa.

High concentrations of benzene induced sex-chromosome loss and non-disjunction less in females than in males (Tables III and IV). It has been shown that benzene in humans also produced stable chromosomes like delations, pericentric inversions, and non-disjunction of the G group (Forni *et al.* 1971; Koizumi *et al.* 1974) leukemia (Kahn and Kahn 1973, Vigliani and Forni 1976, Teleshi *et al.* 1981) mainly of the lymphatic type, even afther long latent periods (McMichael *et al.* 1975). Benzene has been classified as occupational carcinogen in the USA, and epidemiological studies have demonstrated the risk of leukemia to workers occupationally exposed to benzene (Tareff *et al.* 1963; Aksal *et al.* 1972; Browing 1975, Infante *et al.* 1977). Benzene also interferes with red blood cell and lymphocyte production in bone marrow (Moeschlin and Speck 1967, Tice *et al.* 1980), and with DNA and proteins (Lutz and Schlatter 1977, Tunek *et al.* 1978). Benzene induces fetal mortality in rats (Ungváry *et al.* 1978, Tetai 1980) and lack of ossification (Kuna and Kapp 1981).

Toluene induces non-disjunction and X-Y loss in males in the highest con-

TABLE III. NON DISJUCTION AND X CHROMOSOME LOSS IN "OSTER" MALES TREATED AS ADULTS WITH DIFFERENT CONCENTRATIONS OF BENZENE

Treatment	*Females*				*Males*			
Concen-	*Normal*	*Exceptional*			*Normal*	*Exceptional*		
tration %	*y B*	*Proceed from non disjunction in females wild type* [1]	*Proceed from non disjunction in males B/+* [2]	*Frequency %*	*++*	*Proceed from non disjunction or X chromosome loss yellow Bar* [3]	*Proceed from non disjunction or (X or Y) chromosome loss in males yellow* [4]	*Frequency %*
Control	466		1	0.21	479		2	0.41
0.10	418		1	0.24	393		2	0.50
0.25	417		1	0.24	425		2	0.47
0.50	401		1	0.25	478		2	0.41
0.75	452		1	0.22	410		3	0.73*
1.00	359		1	0.27	370		3	0.81*
1.25	282		2	0.70	277		3	1.08*

* $p < 0.05$.

Genotypes:
[1] y sc^{s1} In^{49} sc^{8}/y sc^{s1} In^{49} sc^{8}/sc^{8} Y
[2] X^{c2} y B/sc^{8} Y/ysc^{s1} In^{49} sc^{8}
[3] X^{c2} y B
[4] y sc^{s1} In^{49} sc^{8}.

TABLE IV. NON DISJUCTION AND X CHROMOSOME LOSS IN "OSTER" FEMALE TREATED AS ADULTS WITH DIFFERENT CONCENTRATION OF BENZENE

Treatment Concentration %	*Females*				*Males*				
	Normal y B	*Exceptional Proceed from non disjunction in females wild type* [1]	*Exceptional Frequency %*	*Exceptional Proceed from non disjunction in males B/+* [2]	*Normal ++*	*Exceptional Proceed from non disjunction or X chromosome loss in females yellow Bar* [3]	*Exceptional Frequency %*	*Exceptional Proceed from non disjunction or (X or Y) chromosome loss in males yellow* [4]	*Exceptional Frequency %*
Control	472				532			2	0.37
0.1	456				541	1	0.18		
0.25	478	1	0.20		521	1	0.19		
0.50	474				530			2	0.37
0.75	382	1	0.26		539			2	0.37
1.00	448	1	0.22		495	1	0.20	2	0.40
1.25	141				167			2	1.18*

* $p < 0.05$.

Genotypes:
[1] y sc^{s1} In^{49} sc^{8}/y sc^{s1} In^{49} sc^{8}/sc^{8} Y
[2] X^{c2} y B/sc^{8} Y/ysc^{s1} In^{49} sc^{8}
[3] X^{c2} y B
[4] y sc^{s1} In^{49} sc^{8}.

centrations assayed. In humans it neither provokes chromosomal aberrations nor sister chromatid exchanges (Funes-Craioto *et al.* 1977), althoughit does inhibi protein synthesis in *Bacillus subtilis* (Winston and Matsuhita 1975) and is teratogenic to fishes (Stoss and Haines 1979).

REFERENCES

Andrews L. S. (1977). Effects of toluene on the metabolism and hemopoietic toxicity of H-benzene. Biochem. Pharmacol *26,* 292-300.

Aksay M., Dincol D., Erdem S., Akgun T. and Doncol G. (1972). Details of blood changes in 32 patients with pancytopenia associated with long-term exposure to benzene. Brit. J. Ind. Med. *29,* 56-64.

Barroso-Moguel R. (1975). Alteraciones morfológicas producidas por inhalantes. Cuadernos Científicos CEMEF *2,* 97-106.

Brink N. G. (1969). The mutagenic activity of the pyrrolizidine alkaloid heliotrine in *Drosophila melanogaster.* II. Chromosome rearrangements. Mutat. Res. *4,* 138-146.

Browing E. (1975). *Toxicity and metabolism of industrial solvents.* Elsevier, Amsterdan.

Contreras C. M., González-Estrada T., Zarabozo D. and Fernández-Guardiola A. (1978). Alteraciones electrocorticográficas, electromiográficas y conductuales producidas por inhalación experimental de tolueno en ratas. Cuadernos Científicos CEMESAM *9,* 27-40.

Donner M., Husgatvel-Pursianinen K., Maki-Paajjanen J., Sorsa M. and Vainio H. (1981). Genetic effects of in-vivo exposure to toluene. Mutat. Res. *85,* 293-294.
Forni A. M., Capellani A., Pacifico E. and Vigliani C. (1971). Chromosome changes and their evolution in subjects with past exposure to benzene. Arch. Environ. Health *23,* 385-391.
Funes-Cravioto F., Kolmodin-Hedman B., Lindstein J., Norden-Skojol M., Zapata-Gayón C., Lambert B., Norberg E., Olin R. and Swensson A. (1977). Chromosome aberations and sister chromatid exchanges in workers in chemical laboratories and a rotoprinting factory and in children of woman laboratory workers. Lancet *2,* 322-325.
Grabski D. A. (1961). Toluene sniffing producing cerebeler degeneration. Amer. J. Psychiat, *118,* 461-466.
Green J. P. (1978). Inhaled benzene fetotoxicity in rats. Toxicol. Appl. Pharmacol. *11,* 55-63.
Gutiérrez-Flores R. R. (1975). Solventes industriales. Cuadernos Científicos CEMEF *2,* 35-48.
Guzmán-Flores C. (1975). Neurobiología del tíner. Alteraciones conductuales producidas a largo plazo. Cuadernos Científicos CEMEF *2,* 49-58.
Hudak A. (1978). Embryotoxic effects of benzene and its methyl derivates: toluene and xylene. Toxicology *11,* 55-63.
Infante P. F., Rinsky R. A., Wagner J. K. and Young R. J. (1977). Leukemia in benzene workers. Lancet *2,* 76-78.
Kahn D. and Kahn M. H. (1973). Cytogenetic studies following chronic exposure to benzene. Arch Toxicol. *31,* 39-49.
Kimura E. T. (1971). Acute toxicity and limits of solvent residue for 16 organic compounds. Toxicol. Appl. Pharmacol. *19,* 699-704.

TABLE V. NON DISJUCTION AND X CHROMOSOME LOSS IN "OSTER" MALES TREATED AS ADULTS WITH DIFFERENT CONCENTRATION OF TOLUENE

Treatment Concentration %	*Females*				*Males*			
	Normal y B	*Exceptional*			*Normal ++*	*Exceptional*		
		Proceed from non disjunction in females wild type [1]	*Proceed from non disjunction in males B/+* [2]	*Frequency %*		*Proceed from non disjunction or X chromosome loss yellow Bar* [3]	*Proceed from non disjunction or (X or Y) chromosome loss in males yellow* [4]	*Frequency %*
Control	818		1	0.12	830		2	0.24
0.10	561				672		3	0.44
0.25	585				612		3	
0.50	466				566		3	0.53
0.75	481				552		3	0.54
1.00	423		1	0.23	478		4	0.83*
1.25	398		1	0.25	401		4	0.99*
1.50	301		1	0.33	348		5	1.41*

* $p < 0.05$.

Genotypes:
[1] y sc^{s1} In^{49} sc^{8}/y sc^{s1} In^{49} sc^{8}/sc^{8} Y
[2] X^{c2} y B/sc^{8} Y/ysc^{s1} In^{49} sc^{8}
[3] X^{c2} y B
[4] y sc^{s1} In^{49} sc^{8}.

TABLE VI. NON DISJUCTION AND X CHROMOSOME LOSS IN "OSTER" FEMALE TREATED AS ADULTS WITH DIFFERENT CONCENTRATION OF TOLUENE

Treatment Concentration %	*Females*				*Males*				
	Normal y B	*Exceptional Proceed from non disjunction in females wild type* [1]	*Exceptional Frequency %*	*Exceptional Proceed from non disjunction in males B/+* [2]	*Normal ++*	*Exceptional Proceed from non disjunction or X chromosome loss in females yellow Bar* [3]	*Exceptional Frequency %*	*Exceptional Proceed from non disjunction or (X or Y) chromosome loss in males yellow* [4]	*Exceptional Frequency %*
Control	434	1	0.23		462	1	0.22		
0.10	456				493			2	0.40
0.25	420				477			2	0.41
0.50	416	1	0.24		532			2	0.37
0.75	341				413			2	0.48
1.00	294				305			2	0.65
1.25	256				232	1	0.42	2	0.85
1.50	174				224	1	0.44	1	0.45

Genotypes:
[1] y sc^{s1} In^{49} sc^{8}/y sc^{s1} In^{49} sc^{8}/sc^{8} Y
[2] X^{c2} y B/sc^{8} Y/ysc^{s1} In^{49} sc^{8}
[3] X^{c2} y B
[4] y sc^{s1} In^{49} sc^{8}.

Koizumi A., Dobashi Y., Tachibana Y., Tsuda K. and Katsunuma H. (1974). Citokinetic and cytogenetic changes in cultured human leukocytes and HeLa cells induced by benzene. Ind. Health *12*, 23-29.

Kuna R. A. and Kapp R. W. (1981). The embryotoxic teratogenic potential of benzene vapors in rats. Toxicol. Appl. Pharmacol. *57*, 1-7.

Lindsley D. L. and Grell E. H. (1968). *Genetic variations of Drosophila melanogaster*. Carnegie Inst. Wash. Uubl. 627. 427 p.

Lutz W. K. and Schlatter C. H. (1977). Mechanism of carcinogenic action of benzene. Irreversible binding to rat liver DNA. Chem. Biol. Interact. *18*, 241-245.

McMichael A. J., Spiritas R. and Kupper L. L. (1975). Solvent exposure and leukemia among rubber workers. An epidemiologic study. J. Occup. Med. *17*, 234-239.

Moeschlin S. and Speck B. (1967). Experimental studies on the mechanism of action of benzene on the bone marrow radioautographic studies using 3H-thymidine. Acta Haematol. *38*, 104-111.

Nawrot P. S. (1979). Embryofetal toxicity and teratogenicity of benzene and toluene in the mouse. Teratology *19*, 41A.

Nylander P. O., Olofson H., Rasmiuson B. and Syahlin H. (1978). Mutagenic effects of petrol in *Drosophila melanogaster*. I. Effects of benzene and y, 2 dichloroethane. Mutat. Res. *57*, 163-167.

Oster I. I. (1958). The spectrum of sensitivity of *Drosophila melanogaster* germ cells stages to irradiation. In: *Radiation Biology*. (J. H. Martin Ed.) Proc. Ind. Aust. Conf. Rad. Biol., pp. 253-267.

Spiegel M. R. (1961). *Theory and problems of statistics*. Schaums Publ. New York, pp. 168-171.

Stoss F. W. and Haines T. A. (1979). The effects of toluene on embryos and fry of the japanese medaka *Oryzias latipes* with a proposal for rapid determination of maximium acceptable toxicant concentrations. Environ. Pollut. *20,* 137-139.

Takeshi K., Santella R., Pulkrabek P. and Leffrey A. M. (1981). Benzene oxide: genetic toxicity. Mutat. Res. *91,* 99-102.

Tareeff E. M., Kontchalovkaya N. M. and Zorina L. A. (1963). Benzene leukemias. Acta. Unio. Int. Contra. Camerum *19,* 751-755.

Tátrai E., Ungaváry G., Hudak A., Rodics K., Lorinez M. and Barza G. (1980). Concentration dependance of the embryotoxic effects of benzene inhalation in CFY rats. J. Hyg. Epidemiol. Microbiol. Inmunol *24,* 363-371.

Tice R. R., Costa D. L. and Drew R. T. (1980). Cytogenetic effects of inhaled benzene in murine bone marrow. Induction of sister chromatid exchanges, chromosomal aberrations and cellular proliferation inhibition in DBA/2 mice. Genetics *77,* 2148-2152.

Tunek A., Platt K. L., Bentley P. and Oesch F. (1978). Microsomal metabolism of benzene to species irreversibility binding to microsomal protein and effects of modification of this metabolism. Mol. Pharmacol *14,* 920-929.

Ungváry G., Aranka H., Tratai E., Lorunez M. and Folly G. (1978). Effects of vinyl chloride exposure alone and in combination with tripan blue-applied systematically during all thirds of pregnancy on the fetuses of CFY rats. Toxicology *11,* 45-54.

Vigliani E. C. and Forni A. (1976). Benzene and leukemia. Environ. Res. *11,* 122-127.

Vogel E. and Natarajan A. T. (1979). The relation between reaction kinetics and mutagenic action of monofunctional alkylating agents in higher eukaryotic systems. II. Total and partial sex-chromosome loss in *Drosophila. Mutat.* Res. *62,* 102-123.

Winston S. and Matsuchita T. (1975). Permanent loss of chromosome initiation in toluene treated *Bacillus subtilis* cells. J. Bacteriol. *123,* 921-927.