Rev. Int. Contam. Ambient. 5, 41-48, 1989.

CYTOGENETIC EFFECTS OF SOME CELLOSOLVES

Rafael VILLALOBOS-PIETRINI [1], Sandra GÓMEZ-ARROYO [1], Mario ALTAMIRANO-LOZANO [2], Patricia OROZCO [3] and Patricia RÍOS [1]

[1] Laboratorio de Citogenética y Mutagénesis Ambientales, Centro de Ciencias de la Atmósfera, UNAM, Ciudad Universitaria, Coyoacán 04510 D.F., México.

[2] Laboratorio de Citogenética y Mutagénesis, Escuela Nacional de Estudios Profesionales-Zaragoza, Campo II, UNAM, Apartado Postal 9-020, México 15000 D.F.

[3] Laboratorio de Genética, Facultad de Ciencias, UNAM, Ciudad Universitaria, Coyoacán 04510 D.F., México.
(Recibido marzo 1989, aceptado diciembre 1989)

ABSTRACT

Several concentrations of methyl, ethyl and butyl cellosolves were applied to cultures of human lymphocytes in order to investigate their effect in the induction of chromosomal aberrations and sister chromatid exchanges. When metaphase cells were observed, it was noted that these solvents did not increase significantly the frequency of aberrations, but they did make a difference in the case of sister chromatid exchanges. The effectiveness order was: ethyl > butyl > methyl. Ethyl cellosolve was the most toxic because 2000 ppm inhibited the cell division, which did not happen with the other two cellosolves.

RESUMEN

Se aplicaron diferentes concentraciones de metil, etil y butil celosoves a cultivos de linfocitos humanos con el fin de investigar su efecto en la inducción de aberraciones cromosómicas y de intercambios de cromátidas hermanas. La observación de las células en metafase permitió notar que estos disolventes no aumentaron significativamente la frecuencia de aberraciones, pero si la de intercambios de cromátidas hermanas, cuyo orden de efectividad fue el siguiente: etil > butil > metil. El etil celosolve fue el más tóxico, ya que a partir de 2000 ppm inhibió la división celular, lo que no sucedió con los otros celosolves.

INTRODUCTION

The ethylene glycol ethers are compounds specially useful as solvents for the coating industry. The combination of characteristics such as blush resistance, flow-out and

leveling, handling and gloss, causes them to be widely used (Smith 1984). Also they exhibit the polar properties of alcohols as well as the non polar properties of ethers. Their applications in the industry go from the manufacture of plastics and thinners, bases for inks, dyes, cleaners, soaps, perfums, cosmetics, conditioners and desiccants, degreasing and deicing agents, to solubilizing agents for plants, lacquers, enamels, waxes, resins and cellulose (Savolainen 1980, Andrew and Hardin 1984, House *et al.* 1985, Lorenzana *et al.* 1988). It was from this last usage that the name cellosolve was derived (Savolainen 1980). In general, they are soluble in water, ethers, alcohols, ketones and aliphatic hydrocarbons (Andrew and Hardin 1984).

Just 12 chemicals account for over 98 % of the glycol ethers produced (House *et al.* 1985) and among the methyl, ethyl and butyl ethers, the first cellosolve seems to be the most toxic (Savolainen 1980).

The standard of reference in determining the evaporation rate of solvents is n-butyl acetate, in comparison with the above mentioned cellosolves are considered as having slow evaporation rates of less than 0.8 (Smith 1984).

The over-exposure of individuals to methyl cellosolve causes hematologic system and central nervous system alterations (Nitter-Hauge 1970, Ohi and Wegman 1978). The teratogenic effect of methyl cellosolve was observed in newborns when pregnant rats and rabbits inhaled it (Doe 1984, Hanley *et al.* 1984, Nelson and Brightwell 1984). Testes of rats and hamsters are also damaged (Doe 1984, Hanley *et al.* 1984, Miller *et al.* 1984, Nagano *et al.* 1984).

Exposure of rats and rabbits to ethyl cellosolve vapours induced high mortalty in embryos at maternal toxic concentrations (Andrew and Hardin 1984, Doe 1984) and also teratogenic effects (Doe 1984, Nelson and Brightwell 1984).

In pregnant rats, inhalation of butyl cellosolve produced maternal toxicity, embryotoxicity and fetotoxicity, and in rabbits it induced maternal toxicity and embryotoxicity, but no fetal toxicity; no teratogenicity was seen in either species (Tyl *et al.* 1984).

To estimate cellosolves uptake by all routes of exposure, mainly respiratory and skin absorption, Smallwood *et al.* (1984) proposed that the environmental monitoring should be supplemented with biological monitoring, so the compound can be measured in blood or their metabolites in urine.

Although the results of most studies on mutagenic effects of these cellosolves has been negative, namely in *Escherichia coli* (Szybalski 1958), *Salmonella typhimurium* (Kawalek and Andrews 1980, McGregor 1984) and *Schizosaccharomyces pombe* (Abbondandolo *et al.* 1980), it was interesting to confirm them at chromosomal level looking for alterations and sister chromatid exchanges in human lymphocytes *in vitro*.

MATERIAL AND METHODS

Methyl cellosolve (ethylene glycol monomethyl ether, 2-methoxi ethanol of methyl glycol) is an almost odourless liquid (Browning 1965, Ohi and Wegman 1978) with a bitter taste. Ethyl cellosolve (ethylene glycol monoethyl ether, 2-etoxy ethanol or ethyl ethylene glycol) is a colourless liquid with a bitter taste and nearly odourless (Browning 1965). Butyl cellosolve (ethylene glycol monobutyl ether, 2-butoxy ethanol or butyl oxitol) is a colourless liquid with a slightly rancid odour and a bitter taste followed by burning sensation of the tonge (Browning 1965).

The solutions of the cellosolves (Quimivan) were prepared with distilled water and sterilized by means of "Millipore" membranes of 0.45 μm. After some preliminary experiments the concentrations selected were as follows: Methyl cellosolve: 500, 1000 and 3000 ppm for chromosomal aberrations (CA) and sister chromatid exchanges (SCE) and 1500 ppm only for SCE. Ethyl cellosolve: 500, 1000 and 3000 ppm for CA and 250, 500, 750, 1000 and 1500 ppm for SCE, these lowest concentrations being used for the latter because above 2000 ppm produced cellular damage. Butyl cellosolve: 500, 1000, 2000 and 3000 ppm were used for both types of observation.

Cultures were made of peripheral blood of healthy subjets extracted by vein puncture with heparinized syringes. Eight drops were put in a culture glass containing 3 ml of McCoy's 5A medium (Microlab) and 0.2 ml of phytohemagglutinin solution (Gibco). These were incubated at 37 °C during 48 hours for chromosomal aberrations analyses in which most of the metaphase are first division cells and 72 hours for sister chromatid exchanges, the most of the metaphases are second division cells.

For SCE, 24 hours after the begining of the culture, 5-bromodeoxyuridine (Sigma) was added to the culture medium at final concentration of 5 μg/ml simultaneously with the corresponding concentrations of cellosolves.

Cochicine (Merk) was added after 46 h (for CA) and 70 h (for SCE) for two hours. Then cells were harvested by centrifugation and the pellet was resuspended in hypotonic solution of 0.075M KCl for 20 min. Cells were again centrifuged and finally fixed in methanol-acetic acid (3:1). The slides were made by dropping and air-drying. In the case of preparations for SCE, the slides were immersed in a solution of 0.05 μg/ml of Hoechst-33258 in deionized water in the dark, then rinsed and mounted with a coverslip in a phosphate buffer (pH 6.8), irradiated with UV for 20-24 h and removed the coverglass. In both cases (CA and SCE) the cells were stained in Giemsa-distilled water (1:50) for 10 min.

For each concentration (an experiment and its replicate), 200 first division metaphase cells for CA and 50 of second-division cells for SCE were analyzed.

RESULTS AND DISCUSSION

Chromosomal Aberrations

All experiments were done with blood supplied by a single healthy donor. Cells with fragments were observed and scored (Table I). All the resulting values χ^2 for chromosomal breaks produced by cellosolves had $P > 0.001$, which means that the differences between the control and experimental values were not significant. The cellosolves used were non-clastogens to human lymphocytes exposed *in vitro*. The results were in agreement with that of McGregor *et al.* (1983) and McGregor (1984) who found no clastogenic effects in bone marrow cells of rats that inhaled methyl cellosolve. However, this disagreed with those of Galloway *et al.* (1987) who found chromosomal aberrations induced by ethyl cellosolve in Chinese hamster ovary cells (CHO) in absence of S9 mix but not in its presence.

TABLE I. CHROMOSOMAL BREAKS INDUCED BY VARIOUS CONCENTRATIONS OF DIVERSE CELLOSOLVES IN HUMAN LYMPHOCYTES *IN VITRO* AND ITS CORRESPONDING X^2 VALUES

	CELLOSOLVES					
CONCENTRATION	*METHYL*		*ETHYL*		*BUTYL*	
(ppm)	*Breaks*	X^2 *values*	*Breaks*	X^2 *values*	*Breaks*	X^2 *values*
0	15		13		11	
500	12	0.636*	11	0.044*	15	0.370*
1000	19	0.289*	18	0.559*	8	0.884*
2000	21	0.763*	15	0.038*	16	0.636*
3000	25	2.250*	11	0.044*	15	0.370*

* $P > 0.05$, $df = 1$, Not-significant.

Sister chromatid exchanges

All experiments were also with blood of only one healthy donor. All tested concentrations of methyl cellosolve produced significant increases in SCE over the frequency found in the controls (Table II), but the response was asymptotic.

The higher concentration of ethyl cellosolve, the higher frequency of SCE (Table II). The difference was significant from the lowest concentrations upon comparison with the control values. This was in agreement with Galloway *et al.* (1987) who described SCE with and without S9 mix in CHO cells exposed to ethyl cellosolve, besides S9 mix increased the response.

Butyl cellosolve in this study also increased SCE frequency with increasing con-

TABLE II. SISTER CHROMATID EXCHANGES INDUCED BY CELLOSOLVES IN HUMAN LYMPHOCYTES *IN VITRO*. AVERAGE PER METAPHASE ($\bar{X}$), STANDARD ERROR (S.E.) AND THE "t" STUDENT TEST

	CELLOSOLVES					
CONCEN-TRA-TION (ppm)	*METHYL*		*ETHYL*		*BUTYL*	
	$\bar{X}$ ± S.E.	"t" value	$\bar{X}$ ± S.E.	"t" value	$\bar{X}$ ± S.E.	"t" value
0	4.08 ± 1.21		4.08 ± 1.21		4.26 ± 1.50	
250	----	---	13.62 ± 1.73	8.67*	----	-----
500	8.66 ± 1.47	6.18*	20.02 ± 2.26	10.61*	10.94 ± 1.61	8.43*
750	----	---	19.82 ± 1.77	14.36*	----	-----
1000	6.82 ± 1.41	3.97*	23.74 ± 1.22	18.88*	8.72 ± 1.54	6.71*
1500	10.34 ± 1.60	6.63*	28.04 ± 1.81	21.69*	----	-----
2000	6.58 ± 1.72	2.81*	NO METAPHASES		16.56 ± 2.18	12.37*
3000	6.72 ± 1.58	7.05*			18.50 ± 2.46	12.11*

* $P < 0.001$; ---- No tested.

centration from the lowest concentration used (Table II). Tyler (1982), however, obtained negative results in CHO cells in both the presence and absence of S9 mix.

In this investigation, the order of effectiveness of cellosolves to produce SCE was ethyl > butyl > methyl. Also, ethyl cellosolve was the most toxic because it inhibited cell division from 2000 ppm, which did not happen with the other two cellosolves. In a short-term reproductive toxicity assay in mice, Schuler *et al.* (1984) showed that the LD_{10} estimated from non-pregnant mice reduced the proportion of viable litters to zero when pregnant mice were dosed on days 7-14 of gestation with either methyl or ethyl cellosolves, whereas 77 % of the litters were viable after treatment with butyl cellosolve.

It has been shown that mutagenic and carcinogenic agents can produce SCE (Sugiyama 1971, Latt 1974, Kihlman 1975, Perry and Evans 1975) and sometimes at concentrations ten times smaller than those producing chromosomal aberrations (Kato 1974, Latt *et al.* 1981). With the cellosolves the concentrations that induced SCE did not produce CA possibly the higher concentrations which would produce it were also cytotoxic, masking in this way the effect or on the other hand because both fenomena were induced by different mechanisms.

In mammals, cellosolves are hydrolysed to the corresponding alcohol and ethylene glycol (Flanagan and Licke 1964, Nitter-Hauge 1970, Ohi and Wegman 1978, Pedersen *et al.* 1980) and the oxidised to the corresponding acid and oxalic acid (Stenger *et al.*

1971). Also, methyl, ethyl and butyl cellosolves can be oxidised by hepatic alcohol and aldehyde dehydrogenases to the corresponding acids (Miller *et al.* 1982, 1983)in agreement with Browing (1965) findings the oxidation of methyl and butyl cellosolves to methoxyacetic and butoxyacetic acid respectively. Both had been detected in the urine of exposed individuals (Jönsson and Steen 1978). Similarly, Miller *et al.* (1983) had found methoxy acetic acid 48 h after methyl cellosolve was taken orally by male rats and is considered its active metabolite but both compounds had a negative response in the Ames test (Kawalek and Andrews 1980, McGregor *et al.* 1983, McGregor 1984). Although, there were other negative results in *Escherichia coli* (Szybalski 1958) and *Schizosaccharomyces pombe* (Abbondandolo *et al.* 1980), to those positive by Galloway *et al.* (1987) for chromosomal aberrations in absence of the metabolic fraction but not in the presence and the most effective SCE production without the S9 mix than with the mix, indicated that the effect was produced mainly by the direct action of the substances rather than by its metabolites.

REFERENCES

Abbondandolo A., Bonatti S., Corsi C., Corti G., Fiorio R., Leporini C., Mazzaccaro A., Nieri R., Barale R. and Loprieno N. (1980). The use of organic solvents in mutagenicity testing. Mutat. Res. *79*, 141-150.

Andrew F. D. and Hardin B. D. (1984). Developmental effects after inhalation exposure of gravid rabbits and rats to ethylene glycol monoethyl ether. Environ. Health Perspect. *57*, 13-23.

Browing E. (1965). *Toxicity and metabolism of industrial solvents*. Elsevier, London, pp. 390-415.

Doe J. E. (1984). Ethylene glycol monoethyl ether and ethylene glycol monoethyl ether acetate teratology studies. Environ. Health Perspect. *57*, 33-41.

Flanagan P. and Libcke J. H. (1964). Renal biopsy observations following recovery from ethylene glycol nephrosis. Amer. J. Clin. Path. *41*, 171-174.

Galloway S. M., Armstrong M. J., Reuben S., Colman S., Brown B., Cannon C., Bloom A. D., Nakamura F., Ahmed M., Duk S., Rimpo J., Margolin B. H., Resnick M. A., Anderson B. and Zeiger E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluation of 108 chemicals. Environ. Mol. Mutat. *10*, 1-175.

Hanley T. R. Jr., Young J. T., John J. A. and Rao K. S. (1984). Ethylene glycol monomethyl ether (EGME) and propylene glycol monomethyl ether (PGME): inhalation fertility and teratogenicity studies in rats, mice and rabbits. Environ. Health Perspect. *57*, 7-12.

House R. V., Lauer L. D., Murray M. J., Ward E. C. and Dean J. H. (1985). Immunological studies in B6C3F1 mice following exposure to ethylene glycol monomethyl ether and its principal metabolite methoxyacetic acid. Toxicol. Appl. Pharmacol. *77*, 358-362.

Jönsson A. K. and Steen G. (1978). n-butoxyacetic acid, a urinary metabolite from inhalated n-butoxyethanol (butyl cellosolve). Acta Pharmacol. Toxicol. *42*, 354-356.

Kato M. (1974). Spontaneous sister chromatid exchanges detected by a BrdU labelling methods. Nature *251*, 70-72.

Kawalek J. C. and Andrews A. W. (1980). The effect of solvents on drug metabolism *in vitro*. Drug Metab. Dispos. *8*, 380-384.

Kihlman B. A. (1975). Sister chromatid exchanges in *Vicia faba*. II. Effects of thiotepa, caffeine and 8-ethoxy caffeine on the frequency of SCEs. Chromosoma *51*, 11-18.

Latt S. A. (1974). Sister chromatid exchanges, indices in human chromosome damage and repair: detection by fluorescence and induction by mytomicin-C. Proc. Nat. Acad. Sci. (USA) *71*, 3162-3164.

Latt S. A., Allen S. E., Bloom A., Carrano E., Falke E., Kram D., Schneider E., Schreck R., Tice R., Whitfield B. and Wolff S. (1981). Sister-chromatid exchanges: a report of the geno-tox program. Mutat Res. *87*, 17-62.

Lorenzana-Jiménez M., Capella S., Labastida C., Magos G. A. and Amancio-Chassin O. (1988). Determinación de la composición de varias muestras de tíner por cromatografía en fase vapor. Contam. Ambient. *4*, 65-71.

McGregor D. B., Willins M. J., McDonald P., Holmström M., McDonald D. and Niemeier W. (1983). Genetic effects of 2-methoxyethanol and bis (2-methoxyethyl) ether. Toxicol. Appl. Pharmacol. *70*, 303-316.

McGregor D. B. (1984). Genotoxicity of glycol ethers. Environ. Health Perspect. *57*, 97-103.

Miller R. R., Ayres J. A., Calhcun L. L., Young J. T. and McKenna M. J. (1981). Comparative short term inhalation toxicity of ethylene glycol monomethyl ether and propylene glycol monomethyl ether in rats and mice. Toxicol. Appl. Pharmacol. *61*, 368-377.

Miller R. R., Carreon R. E., Young J. T. and McKenna M. J. (1982). Toxicity of methoxyacetic acid in rats. Fundam. Appl. Toxicol. *2*, 158-160.

Miller R. R., Hermann E. A., Languardt P. W., McKenna M. J. and Schwertz B. A. (1983). Comparative metabolism and disposition of ethylene glycol monomethyl

ether and propylene glycol monomethyl ether in male rat. Toxicol. Appl. Pharmacol. *67*, 229-237.

Nagano K., Nakayama E., Oobayashi H., Nishizawa T., Okuda H. and Yamasaki K. (1984). Experimental studies on toxicity of ethylene glycol alkyl ethers in Japan. Environ. Health Perspect. *57*, 75-84.

Nelson B. K. and Brightwell S. (1984). Behavioral teratology of ethylene glycol monomethyl and monoethyl ethers. Environ. Health Perspect. *57*, 43-46.

Nitter-Hauge S. (1970). Poisoning with ethylene glycol monoethyl ether. Acta Med. Scand. *188*, 277-280.

Ohi G. and Wegman D. H. (1978). Transcutaneous ethylene glycol monomethyl ether poisoning in the work setting. J. Occup. Med. *20*, 675-676.

Pedersen L. M., Nielsen G. D. and Cohr K. H. (1980). Alcohol elimination rate after inhalation of oxitol (2-ethoxyethanol). Z. Rechtsmed. *85*, 199-203.

Perry P. and Evans H. J. (1975). Cytological detection of mutagen carcinogen exposure by sister chromatid exchange. Nature *258*, 121-125.

Savolainen H. (1980). Glial cell toxicity of ethylene glycol monomethyl ether vapor. Environ. Res. *22*, 423-430.

Schuler R. L., Hardin B. D., Niemeier R. W., Booth G., Hazalden K., Piccirillo V. and Smith K. (1984). Results of testing fifteen glycol ethers in a short-term *in vivo* reproductive toxicity assay. Environ. Health Perspect. *57*, 141-146.

Smallwood A., Debord K. E. and Lowry L. K. (1984). Analyses of ethylene glycol monoalkyl ethers and their proposed metabolites in blood and urine. Environ. Health Perspect. *57*, 249-253.

Smith R. L. (1984). Review of glycol ether and glycol ether ester solvents used in the coating industry. Environ. Health Perspect. *57*, 1-4.

Szybalski W. (1958). Special microbiological system. II. Observations on chemicals mutagenesis in microorganisms. Proc. N.Y. Acad. Sci. *76*, 475-489.

Tyl R. W., Millicovsky G., Dodd D. E., Pritts I. M., France K. A. and Fisher L. C. (1984). Teratologic evaluation of ethylene glycol monobutyl ether in Fischer 344 rats and New Zaeland white rabbits following inhalation exposure. Environ. Health Perspect. *57*, 47-68.

Tyler T. R. (1982). Review of ethylene glycol monobutyl ether toxicity testing. Union Carbide Corporation Report. In: *The toxicology of ethylene glycol monoalkyl ethers and its relevance to man* (ECETOC Technical Report No. 4), ECETOC, Brussels.