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Sister-Chromatid Exchanges and Chromosome Aberrations in Lymphocytes from Monkeys Exposed to Ethylene Oxide and Propylene Oxide by Inhalation[1,2]

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Sister-Chromatid Exchanges and Chromosome Aberrations in Lymphocytes from Monkeys Exposed to Ethylene Oxide and Propylene Oxide by Inhalation. LYNCH, D. W., LEWIS, T. R., MOORMAN, W. J., BURG, J. R., GULATI, D. K., KAUR, P., AND SABHARWAL, P. S. (1984). *Toxicol. Appl. Pharmacol.* 76, 85–95. The ability of long-term exposures to inhaled ethylene oxide (EO) and propylene oxide (PO) to induce sister-chromatid exchanges (SCEs) and chromosome aberrations in peripheral lymphocytes of monkeys was investigated. Five groups of adult male cynomolgus monkeys were exposed at 0 (shared control), 50, or 100 ppm EO, and at 100 or 300 ppm PO (7 hr/day, 5 days/week) for 2 years. EO exposures at 50 and 100 ppm resulted in statistically significant increases in sister-chromatid exchange rates and in the incidence of chromosome aberrations in monkey lymphocytes. Both EO-exposed groups had increased numbers of SCEs/metaphase compared to controls, with the SCEs/metaphase of the EO 100 ppm group also significantly elevated versus the EO 50 ppm group. Variability of SCEs/metaphase within each monkey increased even more than the increase in total SCEs/metaphase group with increasing EO exposure. Chromatid-type aberrations were also significantly increased for both EO 50 and EO 100 ppm groups compared to controls. Statistically significant increases in the number of chromosome-type aberrations (excluding gaps) were found only in the EO 100 ppm group. Combined chromatid- and chromosome-type aberrations were increased in both EO 50 and EO 100 ppm groups. No group differences in the number of gaps were found. In lymphocytes from monkeys exposed at 100 and 300 ppm PO, there were no group differences compared to controls for any variable-chromatid or chromosome-type aberrations, gaps, or SCEs/metaphase. These results indicate that EO is a more potent clastogen than PO and demonstrate, for the first time, statistically significant effects of EO on both SCEs and chromosome aberrations in lymphocytes of nonhuman primates.

Ethylene oxide (EO) and propylene oxide (PO) are reactive alkylating agents used extensively as chemical intermediates, with minor usages in sterilization and fumigation. The toxicology of these simple epoxides has recently been reviewed (Glaser, 1979; Hine *et al.*, 1981; Lynch *et al.*, 1984). The National Institute for Occupational Safety and Health (NIOSH, 1977) estimates that about 150,000

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and 270,000 workers are occupationally exposed to EO and PO, respectively, in the United States. The current permissible exposure limits enforced by the Occupational Safety and Health Administration (OSHA) are 50 ppm (90 mg/m^3) for EO and 100 ppm (240 mg/m^3) for PO as 8-hr time-weighted averages. Because of recent reports of adverse mutagenic, reproductive, and tumorigenic effects, the standard for EO is currently being reevaluated by OSHA.

The mutagenic potential of EO and PO has been reviewed (Wolman, 1979; Bootman *et al.*, 1979). EO is a direct-acting mutagen by base pair substitution in *Salmonella* (Embree and Hine, 1975; Pfeiffer and Dunkelberg, 1980). PO induced a dose-dependent increase in the number of revertant mutations in *Salmonella* strains TA 100 and TA 1535 (Pfeiffer and Dunkelberg, 1980). Both EO and PO are mutagenic to *Neurospora* (Kilbey and Kolmark, 1968; Kolmark and Giles, 1955) and to *Drosophila* (Bird, 1952; Fahmy and Fahmy, 1956; Hardin *et al.*, 1983).

EO was not active in a dominant lethal study following iv administration in mice (Appelgren *et al.*, 1977), but did have dominant lethal effects following ip (Generoso *et al.*, 1980) and inhalation exposures (Generoso *et al.*, 1983) in the same species. PO was not active in a mouse dominant lethal study following po administration (Bootman *et al.*, 1979) nor following inhalation exposures in rats (Hardin *et al.*, 1983). EO injected iv induced an increase in the number of micronuclei in bone marrow of mice (Appelgren *et al.*, 1978). Micronuclei in erythroblasts and polychromatic erythrocytes were elevated in workers exposed to inhaled EO *in vivo* (Hogstedt *et al.*, 1983). PO did not increase the incidence of micronuclei when administered po, but did increase micronuclei incidence following ip injection in mice (Bootman *et al.*, 1979). Sister-chromatid exchanges were increased in peripheral lymphocytes of rabbits (Yager and Benz, 1982), rats (Kligerman *et al.*, 1983) and humans (Ehrenberg and Hallstrom, 1967; Garry *et al.*, 1979; Abrahams, 1980) exposed to inhaled EO. Inhaled EO increased the incidence of chromosome aberrations in bone marrow cells of rats (Fomenko and Strekalova, 1973; Strekalova *et al.*, 1975; Embree *et al.*, 1977). The incidence of chromosome aberrations was increased in human lymphocytes exposed to PO *in vitro* (Bootman *et al.*, 1979), in a human cell line exposed to EO *in vitro* (Poirier and Papadopoulo, 1982), and in lymphocytes from workers exposed to EO *in vivo* (Hogstedt *et al.*, 1983).

EO and PO have been reported to induce tumors at the site of administration (sc and po) in mice and rats (Dunkelberg, 1979; 1982). EO exposure resulted in an increase in the incidence of mononuclear cell leukemia in Fischer 344 rats exposed to inhaled EO for 2 years (Snellings *et al.*, 1982; Lynch *et al.*, 1982). Results of two separate 2-year inhalation studies to evaluate the tumorigenic potential of inhaled PO in rodents are currently being reviewed and evaluated (Lynch *et al.*, 1982; NTP, 1983). Epidemiological evidence (Hogstedt *et al.*, 1979*a*, 1979*b*) has suggested a link between EO exposure and an increased incidence of leukemia in humans.

The current study was designed to investigate the mutagenic potential of EO and PO in monkeys following 2-year exposures under controlled experimental conditions. The two indices used to evaluate mutagenicity were the induction of SCEs and chromosome aberrations in peripheral lymphocytes. Peripheral lymphocytes were chosen for evaluation since they are readily accessible and frequently employed to screen human worker exposures to suspected mutagens (Garry *et al.*, 1979; Lambert and Lindblad, 1980). Monkeys are ideal experimental animals for cytogenetic studies due to their larger size and blood volume and because cytogenetic data from nonhuman primates may be more readily extrapolated to humans (McClure, 1975).

METHODS

Animals. Sixty adult male cynomolgus monkeys (*Macaca fascicularis*; Primate Imports Corp., Port Washington, N.Y.)[4] were randomly assigned to five groups, each consisting of 12 monkeys. A shared common control group was employed. Monkeys weighed 5.31 ± 0.82 kg ($\bar{x}$ ± SD) at the start of the study. Monkeys were housed in stainless-steel housing cages with automatic watering in animal rooms except during the exposure period. A 12 hr on:12 hr off (7 AM:7 PM) lighting system was maintained. Monkeys were fed a standard pellet diet of Purina Monkey Chow-Jumbo (No. 5037, Ralston Purina Co., St. Louis, Mo.) once daily at the end of the exposure day, with fresh fruit provided two to three times per week. Water was provided *ad libitum* except during exposures.

Experimental design. Monkeys were exposed at 0 (shared control), 50 or 100 ppm EO, and at 100 or 300 ppm PO for about 7 hr/day, 5 days/week for 2 years. Control monkeys were exposed in chambers to filtered air. EO 50 ppm and PO 100 ppm concentrations were selected since they were the current federal workplace standards for EO and PO, respectively, at the time the exposures were started. EO 100 ppm and PO 300 ppm concentrations were chosen since they approximate maximum tolerated airborne concentrations for both rats and monkeys.

Test materials. EO, 99.7% pure, was obtained from Union Carbide Corporation, Chicago, Illinois, in cylinders under its own vapor pressure. PO, 98% pure, was obtained as a liquid from Matheson, Coleman and Bell, Norwood, Ohio. The purity of both epoxides was verified by gas chromatography at intervals throughout the exposure period. Purity was greater than 99% for both epoxides at each analysis period.

Inhalation exposures. Animal exposures were conducted using 4.5-m^3 stainless-steel and glass inhalation chambers. Chambers were operated under dynamic flow conditions with tangential airfeed manifolds maintained at 40 liters/min with a pressure of −0.25 cm water. Chamber airflows provided 12 to 15 air changes/hr. Temperature and humidity were maintained at 24 ± 3°C and 50 ± 10%, respectively. PO exposure atmospheres were generated by metering liquid PO directly into the tangential filtered air intake. EO was vaporized using an Atinco EO vapor generator (Atlas Instrument and Manufacturing Co., Tulsa, Okla.), and the vapors were metered into the filtered air intake of the chamber. Chamber concentrations were monitored two to four times per hour in an infrared analyzer (Wilks Miran 1A or Miran 801, Foxboro Analytical, S. Norwalk, Conn.). Infrared analyzers were calibrated with certified gas standards obtained from Liquid Carbonic Corporation, Specialty Gas Division, Chicago, Illinois. Charcoal tube samples of the test atmospheres analyzed by gas chromatography verified that the chamber concentrations were within 10% of the target values, and that the control chamber atmosphere was free of EO and PO.

[4] Mention of a product or company name does not constitute endorsement by NIOSH.

Blood collection. About 4 ml blood was collected from the femoral vein of each monkey while the animal was tranquilized following im ketamine hydrochloride. The phlebotomy site was shaved and cleansed with isopropyl alcohol, and then blood was withdrawn. Blood was immediately placed in sterile stoppered tubes, two per animal, containing 0.1 ml sodium heparin, mixed thoroughly for at least 15 min, and stored at room temperature until cultured. Blood collections were initiated during the final month of exposure and were completed prior to the termination of the 2-year exposures. If the initial attempt to culture the peripheral lymphocytes from individual monkeys was not successful, then additional blood samples were drawn, with phlebotomies at least 1 week apart. No preexposure blood was collected for cytogenetic assays at the start of the study.

Sister-chromatid exchange studies. Whole blood samples were cultured in 25-cm^2 flasks for the SCE assay within 6 hr of collection. Culture medium consisted of RPMI 1640 (GIBCO, Grand Island, N.Y.); fetal bovine serum (GIBCO), final concentration equivalent to 15% (v/v); penicillin and streptomycin (GIBCO) at 50 units/ml and 50 μg/ml, respectively; phytohemagglutinin at 2% (Burroughs-Wellcome, Research Triangle Park, N.C.); and sodium heparin (Sigma, St. Louis, Mo.), 100 units/ml. Replicate cultures were established. Each flask contained 7.5 ml of the culture medium and 0.5 ml whole blood. Immediately prior to establishing the cultures, each blood sample was gently agitated. Approximately 18 hr after initiating the cultures, 5-bromodeoxyuridine (BrdUrd) was added to each flask at a final concentration of 50 μM/ml. The duration of culture varied between 68 and 74 hr. Four hours prior to harvest, Colcemid[5] (GIBCO) was added at a final concentration of 0.1 μg/ml. To harvest the peripheral lymphocytes, the cell suspension was centrifuged for 10 min at 1000 rpm. The cell pellet was suspended in 0.075 M KCl and incubated at 37°C for 10 min. Cells were collected by centrifugation, fixed in ice-cold Carnoy's fixative (methanol–acetic acid, 3:1), and held at 4°C for 20 min. After two successive changes of fixative, the cells were dropped on ice-cold, wet slides and air-dried overnight. Cells were stained by the Hoechst–black light–Giemsa method described by Goto *et al.* (1978). Slides were stained in Hoechst 33258 (0.50 μg/ml in distilled water) for 15 min, blotted, and covered with MacIlvaine's buffer. While on a 50°C slide warmer, the slides were subjected for 22 min to black light irradiation. They were rinsed, dried at 37°C for at least 1 hr, and then stained in 6% Giemsa in phosphate buffer (Gurr's R66, Biomedical Specialties, Santa Monica, Calif.) for 10 min. Coverslips were mounted using Pro-Texx mounting medium.

Chromosome aberration studies. The chromosome aberration assay was identical to the SCE assay except that BrdUrd was not added, cultures were harvested 48

[5] Trademark of Ciba-Geigy Corporation, Summit, N.J.

to 52 hr after initiating the experiment, and slides were stained in Giemsa only.

Evaluation for sister-chromatid exchanges. Fifty metaphases per monkey were evaluated for the number of sister-chromatid exchanges. Metaphases were randomly selected. Only cells with the diploid number (42 ± 4) of chromosomes were evaluated. The average number of SCEs per cell and the associated standard deviation were calculated for each animal. After all SCEs were scored, the data were decoded and the average number of SCEs per cell in each treatment group was calculated using the average value for each animal.

Evaluation for chromosome aberrations. Two hundred metaphases were evaluated for chromatid- and chromosome-type aberrations from each monkey. Cells were screened for the incidence of the following chromatid-type aberrations and chromatid interchanges: chromatid breaks, terminal deletions, fragmentations, triradials, and quadriradials. Chromosome-type aberrations, which were scored (if present), included chromosome breaks, acentric fragments, dicentrics, tricentrics, rings, and minutes. The incidence of chromatid and chromosome gaps was also recorded and analyzed separately. Only well-spread metaphases with 42 ± 4 chromosomes were evaluated. The percentage of abnormal cells was calculated for each monkey. After all cells were scored, the animal numbers were decoded and the percentage abnormal lymphocytes in each treatment group was calculated.

Statistical analyses. For the number of aberrations and gaps, frequency tables were created using the number of aberrations as columns and exposure groups as rows. The association of exposure with increasing aberrations was tested by the chi-square test (Conover, 1980) with a Bonferroni adjustment for multiple comparisons (Worsley, 1982). The number of percent abnormal cells was compared by a Kruskal–Wallis test (Conover, 1980), followed by pairwise comparisons if significant. The mean number of SCEs/metaphase per animal was compared among groups by a Kruskal–Wallis test followed by pairwise comparisons. All significant differences reported reflect an experimentwise α-level for a given variable.

RESULTS

Animal Observations

Monkeys, with the exception of the EO 100 ppm group, tolerated the exposures well. The EO 100 ppm group had a statistically significant reduced mean body weight compared to the control group beginning at Week 19 and continuing through Week 104. Five monkeys died during the 2-year exposures, one each in the EO 50 and EO 100 ppm groups, and three in the PO 300 ppm group. These deaths did not appear to be related to oxide exposure. In addition, 10 monkeys, 2 per group, were killed at the termination of exposures. The remaining 44 monkeys are still being maintained and are being examined quarterly for body weight and hematological changes.

Effect of EO and PO on SCE

Data from the individual animals are summarized in Fig. 1. The group statistics are shown in Table 1. Toxic effects of the EO exposure were encountered during attempts to culture blood samples from EO-exposed monkeys. In 1 out of 11 animals exposed at 50 ppm EO and 3 out of 12 monkeys exposed at 100 ppm EO, there were minimal (if any) mitosis. In one of these three EO 100 ppm monkeys, where only two metaphases could be evaluated, the SCEs/metaphase were 20 and 42, respectively. Further attempts employing different culture times and varying phytohemagglutinin concentrations and sources were also unsuccessful.

Chronic inhalation exposures to EO produced a dose-related increasing trend ($p \leq 0.05$) in the incidence of SCEs (Table 1). As can be seen in Fig. 1, both SCEs/metaphase and SCE variability were increased for individual monkeys exposed to EO. The group mean for the control group was significantly different from the mean of the EO 50 ppm and EO 100 ppm groups. The EO 100 ppm group mean was also significantly increased compared to the mean of the 50 ppm group.

The inhalation of PO at both 100 and 300 ppm, however, did not significantly alter the incidence of SCEs in monkey lymphocytes compared to the control group (Table 1). As seen in Fig. 1, the mean SCE values from individual monkeys and the variability reflected by the SD were not different from the control group monkeys.

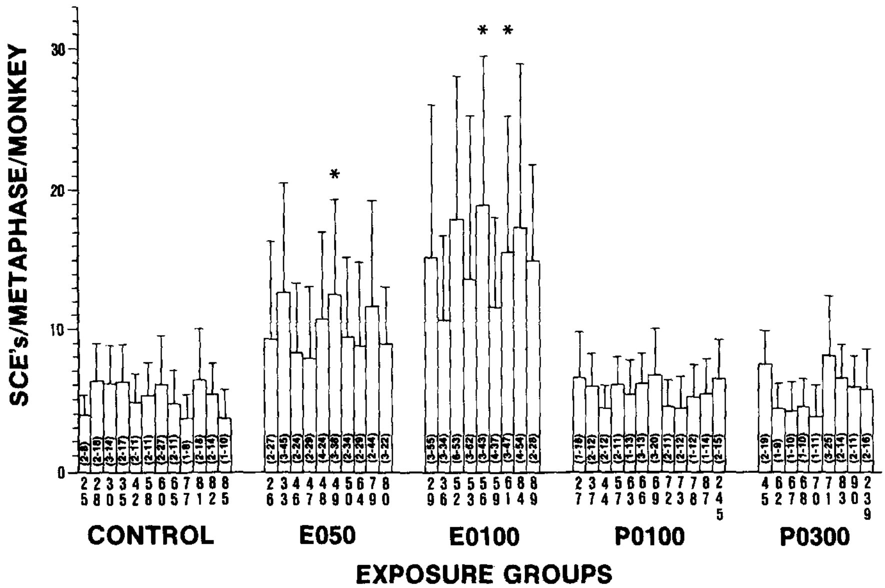

FIG. 1. SCEs/metaphase per animal in lymphocytes from monkeys exposed intermittently to ethylene oxide and propylene oxide for 2 years. Each bar represents x̄ ± SD (range) of 50 cells scored per animal. Monkey numbers are indicated below each bar. Asterisks indicate groups statistically different from controls, $p < 0.05$. Two asterisks indicate groups statistically different from the control and the EO 50 ppm group, $p < 0.05$.

Effects of EO and PO on Chromosome Aberrations

These data are summarized by individual animals in Table 2, and for groups in Table 3. As with the SCE cultures, EO exposure affected the chromosome aberration cultures and, in 1 out of 11 animals exposed at 50 ppm EO and 4 out of 12 monkeys exposed at 100 ppm EO, there were minimal (if any) mitosis. In the control group, no multiple aberrations for a single cell were found. In the 50 ppm EO group, two monkeys each had one cell with two aberrations, while in

TABLE 1

SISTER-CHROMATID EXCHANGES IN PERIPHERAL LYMPHOCYTES IN GROUPS OF MONKEYS INTERMITTENTLY EXPOSED TO ETHYLENE OXIDE AND PROPYLENE OXIDE FOR 2 YEARS

		SCEs/group[a]				
Treatment group	Number of animals	Mean	Standard deviation	Minimum	Maximum	Median
Control	12	5.4	1.04	3.9	6.6	5.6
EO 50 ppm	10	10.2*	1.71	8.1	12.8	9.6
EO 100 ppm	9	15.1*,**	2.77	10.8	19.0	15.2
PO 100 ppm	12	5.8	0.82	4.6	6.9	5.8
PO 300 ppm	9	5.8	1.54	4.0	8.3	5.8

[a] 50 metaphases/monkey.
* Statistically significant difference versus controls, $p < 0.05$.
** Statistically significant difference versus the EO 50 ppm group, $p \leq 0.05$.

TABLE 2

SPECIFIC CHROMATID- AND CHROMOSOME-TYPE ABERRATIONS IN PERIPHERAL LYMPHOCYTES FROM INDIVIDUAL MONKEYS EXPOSED INTERMITTENTLY TO ETHYLENE OXIDE AND PROPYLENE OXIDE FOR 2 YEARS

		Aberrations[a,b]								Abnormal cells[c]		
		Chromatid-type				Chromosome-type						
Treatment group	Animal number	Breaks	Triradials	Quadriradials	Complex rearrangements	Breaks	Acentric fragments	Dicentrics	Total	Total	Percentage	Gaps
Controls	25	1							1	1	0.5	2
	28					1			1	1	0.5	
	30	1					2		3	3	1.5	
	35	1						1	2	2	1.0	
	42						2	1	3	3	1.5	
	58						1		1	1	0.5	1
	60								0		0.0	
	65								0		0.0	
	77								0		0.0	
	81								0		0.0	
	82	1				1			2	2	1.0	
	85							1	1	1	0.5	
											0.6 ± 0.3[d]	
Ethylene oxide (50 ppm)	26	3				1	2	3	9	9	4.5	
	21			ND[e]								
	33	3				3		2	8	7	3.5	
	46	1				1			2	2	1.0	2
	47					1		1	2	2	1.0	3
	48			2					2	2	1.0	
	49	2					2		4	4	2.0	
	50	3				1	2		6	5	2.5	1
	64			1					1	1	0.5	
	79		1	1				1	3	3	1.5	
	80	1						1	2	2	1.0	1
											2.0 ± 0.4*	
Ethylene oxide (100 ppm)	29	1		1		5	5	1	13	12	6.0	
	36	3							3	3	1.5	1
	39				ND							
	52					1		1	2	2	1.0	
	53			1		2	2		5	5	2.5	2

	56				ND							
	59	3		1		1	2		7	6	3.0	1
	61	2			1				3	3	1.5	5
	76				ND							
	84	4	1	1		1		2	9	7	3.5	
	89	6	4	5				2	17	15	7.5	7
	242				ND							
											3.7 ± 0.8*	
Propylene oxide (100 ppm)	27	1							1	1	0.5	
	37							2	2	2	1.0	
	44						1		1	1	0.5	
	57	1							1	1	0.5	
	63	1							1	1	0.5	
	66	1				1			2	2	1.0	
	69	1							1	1	0.5	
	72								0		0.0	
	73								0		0.0	
	78								0		0.0	
	87	1							1	1	0.5	
	245						1		1	1	0.5	
											0.5 ± 0.1	
Propylene oxide (300 ppm)	45							1	1	1	0.5	
	62	1							1	1	0.5	
	67							1	1	1	0.5	
	68	1							1	1	0.5	
	70								0		0.0	1
	71					1			1	1	0.5	
	83	1		1				1	3	2	1.0	
	90								0		0.0	1
	239						1				0.5	
											0.5 ± 0.1	

[a] 200 metaphases per monkey were evaluated for aberrations.
[b] No entry indicates aberration was not detected.
[c] Excluding gaps.
[d] $\bar{x} \pm SD$.
[e] No mitogenic activity.
* Statistically significant difference versus controls, $p < 0.05$.

TABLE 3

FREQUENCY OF CHROMATID- AND CHROMOSOME-TYPE ABERRATIONS IN PERIPHERAL LYMPHOCYTES IN GROUPS OF MONKEYS INTERMITTENTLY EXPOSED TO ETHYLENE OXIDE AND PROPYLENE OXIDE FOR 2 YEARS

Treatment Group	N^a	Frequency of aberrations/200 Cells[b]		
		0–1	2–3	≥4
Chromatid-type				
Control	12	12[c]	0	0
EO 50 ppm*	10	4	6	0
EO 100 ppm*	8	2	3	3
PO 100 ppm	12	12	0	0
PO 300 ppm	9	8	1	0
Chromosome-type				
Control	12	10	2	0
EO 50 ppm	10	5	3	2
EO 100 ppm*	8	2	4	2
PO 100 ppm	12	11	1	0
PO 300 ppm	9	9	0	0
Chromatid- plus chromosome-type				
Control	12	8	4	0
EO 50 ppm*	10	1	5	4
EO 100 ppm*	8	0	3	5
PO 100 ppm	12	10	2	0
PO 300 ppm	9	9	0	0

[a] Number of animals.

[b] Excluding gaps.

[c] Number of animals in group with given frequency of aberrations.

* Statistically significant difference in the number of aberrations/200 cells compared to controls $p \leq 0.025$.

the 100 ppm EO group, five monkeys each had one or more cells with multiple aberrations. Group differences in the percentage of abnormal cells were found between the control and the EO 50 ppm group and between the control and the EO 100 ppm group (Table 2).

Statistically significant increased numbers of chromatid-type aberrations ($p < 0.025$) were found for both EO 50 and EO 100 ppm groups compared to the control group (Table 3). Although not statistically significant, the increased numbers of aberrations with increasing exposure should be noted when comparing the EO 50 and EO 100 ppm groups. A statistically significant increase ($p < 0.025$) in the number of chromosome-type aberrations was found only in the 100 ppm group compared to the control group (Table 3). However, an increase, although not statistically significant, should be noted in the EO 50 ppm group compared to the controls. The frequency of the combined chromatid-plus chromosome-type aberrations (Table 3) were significantly increased ($p < 0.025$) for both the EO 50 and EO 100 ppm groups compared to the control frequency. No differences in the number of gaps were seen.

In addition to the various types of anomalies observed in the lymphocytes of control monkeys (Table 2), triradials, quadriradials, and complex chromosomal rearrangements were found in lymphocytes from EO-exposed monkeys. For the PO-exposed monkeys, no group differences were found for chromatid- or chromosome-type aberrations or for gaps compared to controls.

DISCUSSION

Chronic inhalation exposures of monkeys for 2 years to EO produced a dose-dependent increase in the incidence of SCEs in peripheral lymphocytes compared to controls. The incidence of chromatid- and chromosome-type aberrations was also increased in lymphocytes from monkeys exposed to EO. These findings further support the mutagenic and clastogenic potential of EO. EO exposure induced the formation of triradial and quadriradials in monkey lymphocytes. Abrahams (1980) and Kligerman *et al.* (1983) also reported the latter aberration in lymphocytes from humans and rats exposed to EO.

The finding that the variability of SCEs/metaphase within each animal increased significantly with increasing EO concentration is consistent with the findings of other investigators (Yager and Benz, 1982; Kligerman *et al.*, 1983).

Several others have reported cytogenetic effects of EO in both human and animal subjects. Yager and Benz (1982) reported statistically significant increases in SCEs in lymphocytes from rabbits exposed to EO for up to 12 weeks, and although SCEs decreased after termination of EO exposure, they remained elevated above baseline values 15 weeks after termination of the exposures. Kligerman *et al.* (1983) reported a concentration-dependent increase in SCEs following both 1 and 3 days of exposure to EO. No significant dose-dependent increases in chromosome breakage were noted by the latter investigators.

Lambert and Lindblad (1980) reported a positive correlation ($r = 0.7$) between exposure dose of EO and increased SCEs. Garry *et al.* (1979) and Garry *et al.* (1982) have reported increases in SCEs in workers exposed to EO and in human lymphocytes exposed to EO *in vitro.* Increased SCEs were also reported in workers exposed to an 8-hr time-weighted average of less than 1 ppm, with some brief exposures to much higher EO concentrations when sterilizers were first opened (Yager *et al.,* 1983). EO clearly has the capacity to induce SCEs in multiple species, including man.

Increased chromosome aberrations have been reported in workers exposed to mixtures of epoxides (including EO and PO) for more than 20 years (Thiess *et al.,* 1981). Ehrenberg and Hallstrom (1967) and Hogstedt *et al.* (1983) have reported an increase in the frequency of chromosome aberrations in workers exposed to EO.

The toxic effects encountered during attempts to culture the monkey lymphocytes in the current study are noteworthy. The cytotoxicity of EO to human cells *in vitro* was noted by Pero *et al.* (1981) and Poirier and Papadopoulo (1982).

Pero *et al.* (1981) reported that sterilizer workers exposed to a mixture of 50% EO and 50% methyl formate had an increased frequency of chromatid breaks and gaps compared to controls. In the same study, packers had reduced rates of unscheduled DNA synthesis (UDS) compared to controls but no difference in the incidence of chromosome aberrations. Poirier and Papadopoulo (1982) have reported dose-related increases in the frequency of chromosome aberrations in human amniotic cell lines treated with EO. Pero *et al.* (1982) have reported an inhibition of UDS induced by *n*-acetoxy-2-acetylaminofluorine in workers exposed to PO for up to 20 years. The reported effects on UDS may be related to brief, peak exposures to high concentrations of PO and/or to past exposures to PO in which occupational exposures to PO may have been much higher.

Contrary to the results obtained with lymphocytes from monkeys exposed to EO, PO did not demonstrate a capacity for the production of genetic alterations in monkey chromosomes following inhalation exposures at concentrations up to 300 ppm for 2 years. This finding is surprising in light of the known mutagenic potential of PO in both *in vitro* and *in vivo* systems (Bootman *et al.,* 1979). However, the absence of an effect at 300 ppm is in agreement with the negative dominant lethal and sperm head morphology results reported by Hardin *et al.* (1983). No body weight changes were detected in the monkeys exposed to PO during their 2 years of exposure compared to controls; however, the 300 ppm concentration was clearly irritating to the eyes and nasal passages of the monkeys. The absence of chromosome changes in lymphocytes of monkeys exposed to PO differs from results with human lymphocytes exposed *in vitro* to PO (Bootman *et al.,* 1979). Inhalation exposure to higher PO concentrations, i.e., greater than 300 ppm, or possibly longer duration exposures (greater than 2 years) to lower PO concentrations may be needed to produce *in vivo* increases in SCEs and/or chromosome aberrations as suggested by the findings of Pero *et al.* (1982) with UDS (discussed previously). Negative results for the SCEs and chromosome aberration assays suggest that, unlike EO, PO does not induce increases in the incidences of these two endpoints in peripheral lymphocytes of monkeys intermittently exposed at the permissible concentration for up to 2 years, and that PO is a less potent clastogenic agent than EO.

Based on results from the current cytogenetic study in which monkeys were exposed to known, documented concentrations of EO and PO, EO clearly increased the incidence of chromosome aberrations and sister-chromatid exchanges in monkey lymphocytes, while PO did not. Exposures to EO at the current permissible exposure concentration of 50 ppm clearly induce mutagenic and clastogenic effects in lymphocytes of nonhuman primates. These data, plus other recent data on possible adverse reproductive effects in humans and tumorigenic effects of EO in laboratory animals (NIOSH, 1981), strongly suggest that the permissible exposure concentration of 50 ppm of ethylene oxide is not adequate to prevent adverse human health effects.

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