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Evaluation of propylene oxide for mutagenic activity in 3 in vivo test systems

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Summary

Propylene oxide (CAS No. 75-56-9) was tested for mutagenic activity following vapor exposure using 3 in vivo test systems. Rat dominant lethal and mouse sperm-head morphology assays were conducted using males exposed to propylene oxide at 300 ppm in a dynamic exposure chamber for 7 h per day on 5 consecutive days. A sex-linked recessive lethal test in *Drosophila melanogaster* employed a 24-h static exposure to propylene oxide at 645 ppm. Male mice were killed 1, 3, 5, 7, and 9 weeks post-exposure for evaluation of sperm-head morphology. Propylene oxide exposure did not result in an increase in abnormal forms. Male rats were mated with 2 virgin females per week for 6 weeks following exposure. A statistically significant increase in preimplantation losses and a statistically significant reduction in the number of lving implants in the first post-exposure week did not appear to be treatment related. A highly significant increase in sex-linked recessive lethal mutations was observed in two germ cell stages (mature sperm and developing spermatocytes). These results warrant continued caution in potential human exposure to propylene oxide.

Propylene oxide (PO, 1,2-epoxypropane) is a volatile liquid used extensively as a chemical intermediate. It has also been used as a fumigant, herbicide, preservative, and solvent (Hine and Rowe, 1963). PO is chemically reactive and its toxic properties are similar to, but less severe than, those of ethylene oxide. The National Institute for Occupational Safety and Health (NIOSH) estimates that about 270 000 workers are occupationally exposed to PO in the U.S.A. The current permissible

exposure limit enforced by the Occupational Safety and Health Administration is 100 ppm (240 mg/m^3) as an 8-h time-weighted average.

The acute toxicity of PO has been investigated in some detail (Jacobson et al., 1956; Rowe et al., 1956; Weil et al., 1963), but its chronic toxicity has not been reported. PO is the subject of a current NIOSH chronic toxicity study in which rats and monkeys have been exposed to PO vapor for 24 months. Schalet (1954) observed an increased incidence of recessive lethal mutations in Drosophila following postcopulatory vaginal douches with 100% PO. Mutagenic activity in *Salmonella typhimurium* strains TA98 and TA1535, without metabolic activation, was reported by Pfeiffer and Dunkelberg (1980). Bootman et al. (1979) reported that PO was mutagenic in *Salmonella typhimurium* and *Escherichia coli*, and that it caused chromosomal damage to human lymphocytes in vitro. PO was not active in the mouse dominant lethal or micronucleus tests when administered orally, but a highly significant increase in micronuclei was seen when it was injected intraperitoneally (Bootman et al., 1979). These authors suggested that PO was rapidly detoxified by conversion to propylene glycol following oral dosing, and proposed that it might present a mutagenic hazard 'following parenteral exposure — particularly by inhalation'. The work reported here investigated the in vivo mutagenic potential of PO following inhalation exposures of rats and mice at 300 ppm (720 mg/m^3), the higher of 2 concentrations employed in the ongoing NIOSH chronic study. Fruit flies were exposed to PO vapor for 24 h at an empirically established maximally tolerated concentration.

Materials and methods

Inhalation exposures

Male rats and mice were jointly exposed to filtered air or to PO in 4.5-m^3 stainless steel and glass exposure chambers. Control (filtered air) and PO-exposed animals were individually housed in stainless steel wire mesh cages inside their respective exposure chambers throughout the 5-day exposure period. Food and water were available ad libitum except during exposures, when both were removed. Exposures were for 7 h per day on 5 consecutive days.

The PO used was manufactured by Eastman Kodak * (No. 2068) and had a stated minimum purity of 98%. Chemical analyses were not performed to verify that purity. Exposure atmospheres were generated by metering liquid PO directly into the tangential filtered air intake. The chamber concentration was monitored with an infrared analyzer (Wilks Miran 1A) at a wavelength of 12.0 μm with a pathlength of 20.25 m and a 2-mm slit. The average measured concentration was 305 ppm, with daily time-weighted averages ranging from 290 to 323 ppm.

* *Disclaimer:* Mention of a company name or product does not constitute endorsement by the National Institute for Occupational Safety and Health.

Dominant lethal test

Virgin female and proven breeder male Sprague–Dawley rats (200–225 g and 300–350 g, respectively, when received) were purchased from Harlan Industries, Inc., Indianapolis, IN. Each rat was individually identified by toe-clipping. Males were randomly assigned to control or treatment groups (10 males per group), and females were randomly selected for breeding. Exposures began about 5 weeks following receipt of the rats. After the 5th day of exposure, males were transferred to individual suspended wire mesh cages for the breeding phase of the study. In the morning of the 2nd day after the final exposure (approximately 40 h post-exposure), 2 randomly selected virgin females were caged with each male. Females remained with males for 5 consecutive days. Another pair of virgin females was introduced following a 2-day rest period. Breeding continued in this fashion until each male had been exposed to 6 pairs of females. Females were killed and uterine contents evaluated in the middle of the 2nd week following the week of cohabitation. Statistical analyses were conducted in accordance with the procedures of Vollmar (1977).

Sperm-head morphology test

6-week-old C3H-He male mice were purchased from Harlan Industries, Inc., Indianapolis, IN. Each mouse was toe-clipped for individual identification and was randomly assigned to a control or exposure group for 1 of 5 scheduled post-exposure sacrifice weeks (10 mice in each of 10 groups). Exposures began 4 weeks after receipt and were concurrent with exposures of rats in the dominant lethal study. Following the 5 days of exposure, mice were housed in groups of 10 in solid-bottom polycarbonate cages with food and water ad libitum until killed as scheduled in post-exposure weeks 1, 3, 5, 7, and 9.

Mice were killed by cervical dislocation. Cauda epididymides were removed and minced separately in 2 ml phosphate-buffered saline. Suspensions were filtered through 75-μm stainless steel mesh to remove tissue fragments. Sperm were stained with 1% Eosin Y and, after 30 min, 2 smears were made from each suspension (4 slides per mouse). Slides were air dried and coverslipped with Permount®. Slides were coded and read without knowledge of the treatment. Approximately 500 sperm per slide (2000 sperm per mouse) were scored for abnormal morphologies using the categories of Wyrobek and Bruce (1975).

Sex-linked recessive lethal test

About 200 adult males of wild-type *Drosophila melanogaster* (Oregon-R) were exposed to PO vapor for 24 h. This static exposure took place in a 1-l stoppered glass bottle into which PO was injected (2.0 μl in 1.085 l, resulting in a concentration of 1530 mg/m^3 or 645 ppm). The 645 ppm represents a maximum tolerated concentration based upon previously conducted toxicity tests that showed approximately 10% mortality at that concentration following a 24-h exposure period. Following equilibration, flies anesthetized with CO_2 were introduced into the bottle. A similar bottle to which no PO was added was used to hold a control group of about 200 flies.

TABLE I

RAT DOMINANT LETHAL TEST [a] FOLLOWING INHALATION EXPOSURE TO PROPYLENE OXIDE

Week group	1		2		3		4		5		6	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Number pregnant	14	15	16	18	18	18	19	17	19	19	18	17
Number mated	20	20	19	20	20	20	20	19	20	20	20	20
Number of corpora [b] lutea per female	14.7 ± 2.4	14.7 ± 2.7	14.1 ± 1.5	13.9 ± 2.0	13.7 ± 1.6	13.3 ± 2.2	13.5 ± 2.9	12.9 ± 1.8	13.4 ± 2.4	13.5 ± 2.3	14.0 ± 2.1	14.4 ± 2.8
Living implants [c] as % of corpora lutea	95.0 87.2–99.2	85.9 [d] 78.8–91.7	78.4 66.2–88.5	84.9 73.5–93.5	91.9 85.1–96.8	92.1 86.0–96.6	92.1 83.4–97.7	84.8 66.5–96.7	90.6 84.7–95.2	85.5 74.6–93.7	88.3 81.6–93.6	91.3 85.0–96.0
Preimplantation [c] loss as % of corpora lutea	1.0 0.002–3.7	8.6 [e] 3.8–15.0	10.6 3.2–21.5	9.1 2.6–19.0	3.3 0.5–8.3	2.2 0.4–5.5	3.1 0.3–8.5	4.0 0.2–12.2	3.3 0.9–7.3	5.5 1.0–13.4	2.9 0.4–7.5	2.0 0.2–5.8
Dead implants [c] as % of implants	2.4 0.1–7.9	3.4 0.9–7.4	5.2 0.8–12.8	2.9 0.6–6.9	2.1 0.3–5.4	3.6 1.0–7.7	2.7 0.4–6.9	6.4 0.3–19.1	2.8 0.5–6.8	6.5 2.5–12.1	5.8 2.6–10.4	5.1 2.0–9.6

[a] 10 male Sprague–Dawley rats per group exposed to 300 ppm propylene oxide (test) or to filtered air (control), 7 h/day for 5 days.

[b] Mean ± standard deviation. No significant differences by Wilcoxon test corrected for ties.

[c] Mean and 95% confidence interval. Proportions (p) expressed as arcsine transformation (θ = arcsine $\sqrt{p}$) and analyzed by Wilcoxon test corrected for ties.

[d] Significantly less than corresponding control ($P < 0.05$).

[e] Significantly greater than corresponding control ($P < 0.01$).

Following treatment, males were maintained for 24 h in shell vials with instant Drosophila medium, after which 100 males from each group were individually brooded at 25°C with 2 virgin Muller-5 (Basc) females for 48 h on days 2–3 post-exposure (brood I) and again on days 7–8 post-exposure (brood II). 10 virgin F_1 females (heterozygous for the control or treated wild-type X-chromosome) were collected from each brood of 70 males, to yield 700 vials representing 700 X-chromosomes per brood. These F_1 females were individually mated with Muller-5 males and their cultures were scored for the presence or absence of wild-type F_2 males. A sex-linked recessive lethal mutation was scored if no wild-type males were seen in a culture containing at least 10 F_2 males, but cultures were not scored if there were no wild-type males and fewer than 10 F_2 males. Cultures with one or more wild-type males were scored as non-lethal regardless of the total number of F_2 males in the culture. Lethal cultures were not confirmed in the F_3 generation, but with the scoring criteria used the probability of falsely scoring a non-lethal culture as lethal was $P = 0.5^{10} < 0.001$.

Results

Dominant lethal test

Male rats tolerated the PO exposures reasonably well, although there was a statistically significant ($P < 0.01$ by paired t test) weight loss (421 ± 19 g to 413 ± 18 g) from the day before exposure began to the 2nd post-exposure day (the day breeding began). The body weights of control rats increased slightly (404 ± 36 g to 408 ± 35 g) during this interval, but the change was not statistically significant. The mean body weight of the PO-exposed group was consistently greater than that of the control group, but the difference was not statistically significant at any time during the study.

Breeding results are summarized in Table 1. The weekly number of pregnant females per number of females cohabited was tested by Fisher's exact test. All other comparisons were made using the Wilcoxon 2-sample test, corrected for ties. Control and experimental groups differed significantly only in the first week of breeding. In that week, females mated with PO-exposed males had significantly higher preimplantation losses ($p < 0.01$) and fewer living implants (as a percentage of corpora lutea) ($P < 0.05$) than the corresponding control females. There were no other statistically significant differences.

Sperm-head morphology test

Male mice exhibited no obvious signs of toxicity during exposure. Results of evaluating sperm-head morphology at various times post-treatment are summarized in Table 2. Only in week 5 did exposed mice have more abnormal sperm recorded than did the corresponding control group. Control data were first examined by analysis of variance and were seen to differ significantly ($P < 0.001$) across weeks. Therefore, control data could not be pooled for comparisons with exposed groups, so a test comparing each control versus the corresponding exposed group (within

TABLE 2

MOUSE SPERM HEAD MORPHOLOGY TEST [a] FOLLOWING INHALATION EXPOSURE TO PROPYLENE OXIDE

	Post-exposure week of sacrifice				
	1	3	5	7	9
Control					
Number of mice scored	10	8	10	10	8
Abnormal sperm (%)					
Average per mouse	20.3	25.7	17.0	16.7	16.7
Standard error	1.6	2.3	0.6	2.1	0.4
Test					
Number of mice scored	10	9	10	9	9
Abnormal sperm (%)					
Average per mouse	18.8	21.5	17.3	15.1	16.6
Standard error	1.0	1.1	0.6	0.7	0.9

[a] 50 male C3H-He mice per group exposed to 300 ppm propylene oxide (test) or to filtered air (control). 7 h/day for 5 days and sacrificed in groups on alternate post-exposure weeks.

weeks) was done. This test indicated that the control and exposed groups did not differ significantly within weeks.

Sex-linked recessive lethal test

Results of scoring F_2 cultures for sex-linked recessive lethality are summarized in Table 3. The overall incidence of sex-linked recessive lethal mutations was significantly ($P < 0.01$) increased in PO-exposed flies (4.28% versus 0.25% in controls). Individual broods also differed significantly ($P < 0.01$) from their corresponding controls.

TABLE 3

DROSOPHILA SEX-LINKED RECESSIVE LETHAL TEST [a] FOLLOWING EXPOSURE TO PROPYLENE OXIDE VAPOR

Brood	Control	Test
	Lethal/total scored	Lethal/total scored
I	2/668 (0.30%)	31/666 (4.65%) [b]
II	1/515 (0.19%)	11/315 (3.49%) [b]
Total	3/1183 (0.25%)	42/981 (4.28%) [b]

[a] Male Oregon-R *D. melanogaster* exposed for 24 h to a static concentration of 645 ppm propylene oxide (test) or to room air (control) in a sealed glass bottle.

[b] Significantly greater than controls ($P < 0.01$) by Kastenbaum–Bowman table.

Discussion

In these experiments, mutagenic activity was not detected in two mammalian tests, but PO was clearly mutagenic in the sex-linked recessive lethal test. The statistically significant differences seen in week 1 of the dominant lethal test (Table 1) appear to be due to an unusually low rate of preimplantation loss in the control group that week. In comparison with data for succeeding weeks, the week 1 experimental group does not appear to be exceptional. In post-exposure weeks 4 and 5, the PO-treated groups differed from controls as they would in the presence of dominant lethal mutations (reduced live implants, increased preimplantation losses and dead implants) but the differences were not statistically significant. No treatment-related effects were detected in the sperm-head morphology test.

Several explanations can be proposed for the discrepancy between the Drosophila and mammalian test results. The sex-linked recessive lethal test is statistically more powerful than either mammalian test because of the much larger sample size involved, and it is capable of detecting smaller induced changes against the lower spontaneous frequency of its observational endpoint. The likelihood of detecting significant effects in the mammalian tests may have been further reduced by the use of an exposure that was not a 5-day maximally tolerated concentration (MTC). However, 300 ppm was already in use as the MTC in a chronic toxicity study, and these data are useful in providing a point for comparison of results in the different kinds of study. Furthermore, 300 ppm was toxic to the exposed males, as evidenced by the statistically significant weight loss during exposure. Results of the sperm-head morphology test are particularly difficult to evaluate since the incidence of abnormal morphology was exceptionally high in all groups, even considering the fact that the strain used has a higher background frequency than do hybrid lines such as B6C3F1.

Failure to demonstrate mutagenic effects in mammals may well be related to the reactivity of the PO molecule. As an epoxide, PO may itself be the proximate mutagen in Drosophila. However, even at toxic concentrations the actual dose of epoxide delivered to the mammalian testis may have been quite low following these inhalation exposures or the oral dosing as reported by Bootman et al. (1979). Another possibility is that PO induces primarily point mutations, which are detected in the sex-linked recessive lethal test, but which were not screened in the mammalian systems. In view of the clearly mutagenic activity of PO in the Drosophila sex-linked recessive lethal test, continued caution in potential human exposure to PO is warranted.

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