

# Mutagenicity Study of Workers Exposed to Alkylene Oxides (Ethylene Oxide/Propylene Oxide) and Derivatives

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The mutagenic effect of ethylene oxide/propylene oxide is known from previous studies. In 1975 Embree<sup>1</sup> demonstrated the initiation of point mutation in *Salmonella typhimurium*. Ethylene oxide has been found to have the same mutagenic effect in *Drosophila*,<sup>2,6</sup> barley, rice, maize<sup>4,6-9</sup> and *Neurospora crassa*,<sup>10-11</sup> and in the Ames test.<sup>12-15</sup> Embree found an increased rate of gaps and breaks in the bone marrow of rats after they had been exposed to 250 ppm ethylene oxide for three 7-hour periods. He was not able to relate the dose to the effect. The increased incidence of micronuclei in the bone marrow of rats exposed to ethylene oxide<sup>1-16</sup> also indicates a mutagenic effect of ethylene oxide.<sup>1-6, 16-17</sup> Ehrenberg et al<sup>18-21</sup> and Embree<sup>16-17</sup> estimate the genetic risk of exposure to ethylene oxide to be as high as that of exposure to ionizing radiation. Kalling<sup>22</sup> found an increased chromosome aberration rate in the lymphocytes of industrial workers 18 months after an accident involving ethylene oxide. He reported that two hours of acute exposure also produced a slightly significant increase in the number of cells with chromosome aberrations. No details were given regarding the methods employed and the measurement of the concentration at the workplace.

The purpose of this study was to ascertain whether or not BASF employees who were exposed for several years to alkylene oxides and their secondary products and employees who had been subjected to an acute ethylene oxide intoxication several years ago now display an increased chromosome aberration rate in comparison with that of controls. In this study, the term "alkylene oxides" includes ethylene oxide, propylene oxide, butylene oxide, dioxane, epichlorohydrin, dichloropropane, ethylene-

chlorohydrin and propylenechlorohydrin. The subjects were also exposed to a number of other products (e.g., benzene).

## Methods

The mutagenicity studies were carried out on lymphocytes of 43 males aged 27 to 63 years (average age, 47.1) and divided into four groups, as follows: (1) Long-term exposure, more than 20 years; (2) exposure for less than 20 years; (3) long-term exposure plus accident<sup>23-24</sup>; and (4) accident, i.e., short-term high<sup>23-24</sup> exposure to ethylene oxide.

The subjects in Groups 1, 2 and 3 all work in plants in which ethylene oxide is manufactured or further processed. Fire department and maintenance workers are included in Group 4. The control group comprises male employees from the Occupational Medicine and Health Protection Department and the office staff, and also includes maintenance workers, none of whom had been exposed to radiation at the time of testing. The controls were aged 24 to 58 years (average age, 38.6).

The concentration of ethylene oxide at the workplace of Groups 1, 2 and 3 was measured only recently. It is thus impossible to quote accurate figures for past exposure, but a higher exposure is to be assumed, in accordance with the level of technology at that time.

The chromosome investigations were carried out on lymphocytes in peripheral blood.<sup>25</sup> A 0.2 ml heparinized venous blood sample was incubated for 70 to 72 hours at 37° C in 4 ml TC Chromosome Microtest Medium and Reconstituting Fluid (Difco). For the final two hours, 0.25 µg/ml Colcemid (Ciba-Geigy) was added to the culture. Following hypotonic treatment with 0.0075 molar KCl, the cells were fixed in a 3:1 mixture of methanol and acetic acid. The air-dried preparations were stained with a 10% Giemsa solution. One hundred metaphases from

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**Table 1. — Group 1. Long-Term Exposure (More Than 20 Years) to Alkylene Oxides and Secondary Products.**

No.	Age	Years of Exposure	No. of Cells 1st / 2nd Eval.	1st Eval. Incl. Gaps	% Aberrant Cells		
					Oct. 78 Excl. Gaps	2nd Eval. Incl. Gaps	Aug. 79 Excl. Gaps
498	60	40	100/100	5	3	3	3
499	48	31	100/100	6	1	6	2
500	52	30	100/100	12	5	8	3
502	56	28	100/100	7	3	4	2
507	42	25	100/100	7	4	5	2
508	59	28	100/100	5	4	6	4
509	41	25	100/100	5	4	5	3
510	59	28	100/100	7	4	4	3
513	63	26	100 —	6	4	—	—
527	56	28	100/100	6	5	6	4
528	42	23	100/100	4	2	4	1
11 Total	52.54*	28.36	1100/1000	6.4*	3.5*	5.7*	2.7*

\*Mean

each subject and each control were examined for chromosome aberrations. The slides were coded and a total of 7800 metaphases were evaluated. Each aberration was recorded photographically. Statistical analysis was carried out using the t-test, the Mann-Whitney u-test and contingency tables. The percentages of certainty quoted in the results were calculated using the Fisher-Yates exact test.

**Measuring the Concentrations of Ethylene and Propylene Oxide at the Workplace**

Measurement of the concentrations of ethylene and propylene oxide at the workplace in order to estimate the exposure of workers in plants where these substances are processed was carried out as follows: It was possible to measure propylene oxide levels using personal samplers over periods of up to 10 hours. The results obtained thus correspond to the average exposure of the particular employee over the period of a working shift (12-hour alternating shifts), and in each case were far below the MAK value of 100 ppm (maximum allowable concentration at the workplace). Higher propylene oxide concentrations were measured for brief periods, depending upon what the particular employee was doing.

It was not possible to use personal samplers to measure ethylene oxide in the ethylene oxide plant and thus spot samples or local collective samples were taken over approximately two hours under normal working conditions and under conditions of plant breakdown. It was found that the concentration of ethylene oxide which is below 5 ppm under normal working conditions could rise to as much as 1900 ppm for several minutes in exceptional cases during plant breakdown (MAK: 50 ppm).

Measurements carried out in other production areas in the various sections of the plant revealed ethylene oxide concentrations of below 1 ppm with trouble-free production, although the buildings concerned were enclosed. In one plant, propylene oxide concentrations of up to 1 ppm were measured simultaneously.

**Results**

Tables 1 to 5 show the results of the investigations, and the numbers of employees involved. The mean frequency of aberrant metaphases in Control Group 1 is 4% including gaps (1.4% excluding gaps); in Control Group 2 it is 3.5% including gaps (1% excluding gaps).

The subjects in Group 1 (long-term exposure, i.e., more than 20 years, 28.36 years on average), when first examined in October 1978, displayed a mean aberration frequency of 6.4% including gaps (3.5% excluding gaps) which is significantly increased in comparison with that of the control group ( $p < 0.005$  including gaps;  $p < 0.002$  excluding gaps). Post examination of the subjects in Group 1 in August 1979 showed that the group had an aberration rate of 5.7% including gaps (2.7% excluding gaps). In comparison with an enlarged control group (Control Groups 1 and 2), the men in Group 1 were found to have a significant increase in the chromosome aberration rate on evaluation of changes excluding gaps ( $p < 0.05$ ).

Employees in Group 2 (exposure of less than 20 years, average 12.5 years) displayed an average of 6.0% aberrant cells including gaps (2.3% excluding gaps). Although there was an increase in the rate of chromosome aberrations, this was not significant ( $p > 0.05$ ).

The members of Group 3 (average period of exposure 17.6 years, plus at least one high exposure to ethylene ox-

**Table 2. — Group 2. Exposure (Less Than 20 Years) to Alkylene Oxides and Secondary Products.**

No.	Age	Years of Exposure	No. of Cells	% Aberrant Cells	
				Incl. Gaps	Excl. Gaps
128	48	10	100	6	0
492	31	13	100	8	2
493	56	9	100	6	2
512	48	15	100	4	3
517	59	18	100	7	4
524	58	10	100	5	3
6 Total	50*	12.5*	600	6*	2.33*

\*Mean

**Table 3. — Group 3. Long Term Exposure and Acute Exposure to Alkylene and Secondary Products Due to Accident (EO).**

No.	Age	Years of Exposure	No. of Cells	% Aberrant Cells	
				Incl. Gaps	Excl. Gaps
70	38	20	100	5	4
491	42	19	100	2	1
494	45	31	100	5	3
496	56	42	100	5	2
501	37	10	100	9	1
502	46	22	100	4	2
505	45	10	100	3	1
511	46	7	100	5	2
515	43	22	100	6	2
522	41	19	100	3	0
523	41	13	100	6	4
529	50	15	100	7	3
538	41	23	100	3	2
543	39	14	100	3	3
567	52	9	100	6	2
568	49	19	100	5	3
569	36	22	100	2	1
574	42	11	100	2	1
587	50	27	100	5	2
589	32	7	100	6	4
590	45	8	100	7	4
21 Total	43.6*	17.6*	2100	4.71*	2.23*

\*Mean

ide caused by an accident) have an average aberration frequency of 4.7% including gaps (2.2% excluding gaps). The aberration rates are increased; however this increase is insignificant when compared with the rate of the control group.

The members of Group 4, who were only exposed once for a brief period following an accident, also displayed no significant increase in the aberration rate (5.2% including gaps; 1.6% excluding gaps) ( $p > 0.05$ ).

#### Discussion

The significantly increased chromosome aberration rate (excluding gaps) in employees exposed to alkylene oxides for more than 20 years indicates a mutagenic effect. Some of the subjects in Group 1 were engaged in production of ethylene oxide by the ethylene chlorohydrin process. (Propylene oxide is still produced by this method.) Other substances with which the members of this group were in contact were chlorine, ethylene chlorohydrin, ethylene cyanohydrin, hydrogen sulfide, hydrocyanic acid, chlorinated hydrocarbons, phenol, cresol, ethylenimine, propylene and ethylene urea, propylene oxide, propylene chlorohydrin, aniline and dioxane. As the members of Group 1 were exposed to a number of products, the test results do not support the assumption that risk is specific to one product.

The members of Groups 2 and 3 were also exposed to a number of other substances in addition to ethylene oxide/propylene oxide. These substances are in some cases the same as those to which the members of Group 1 were exposed, but there is the additional possibility that these individuals were exposed to benzene. In contrast to the finding of Ehrenberg and Hallström<sup>22, 26</sup> that there was an increased aberration rate in seven employees 18 months after an accident whereby ethylene oxide was inhaled or came into contact with the skin, in the employees in Group 4 it was not possible to detect any significant increase in the aberration rate in comparison with that of the control group. In comparison with the control group, Groups 2 and 3 did not display a significantly increased chromosome aberration rate.

One possible explanation for this could be that, over the past 20 years, great improvements have been carried out in regard to technology in general. Improved worker safety conditions and changes in processing could explain the differences between Groups 1 and 3, as the subjects in Group 3 were exposed, for an average of about ten years less, to a reduced concentration of alkylene oxides and secondary products at the workplace, following changes in the method of manufacture.

Three members of the control group were conspicuous by having an increased chromosome aberration rate as

**Table 4. — Group 4. Brief Exposure to Ethylene Oxide Due to Accident.**

No.	Age	Years of Exposure	No. of Cells	% Aberrant Cells	
				Incl. Gaps	Excl. Gaps
497	48	0	100	7	1
537	27	0	100	6	0
539	39	0	100	6	2
579	56	0	100	6	5
581	47	0	100	1	0
5 Total	43.4*		500	5.2*	1.6*

\*Mean

Table 5. — Exposure to Alkylene Oxides and Secondary Products.

No.	Age	Years of Exposure	No. of Cells	% Aberrant Cells	
				Incl. Gaps	Excl. Gaps
<b>Control Group 1</b>					
331	37	0	100	2	0
482	40	0	100	8	3
483	32	0	100	5	0
484	36	0	100	3	2
485	24	0	100	2	1
488	36	0	100	3	2
495	39	0	100	6	2
514	28	0	100	3	1
520	52	0	100	3	1
541	32	0	100	3	1
549	58	0	100	3	2
563	54	0	100	6	3
564	36	0	100	8	2
573	26	0	100	8	4
575	52	0	100	2	0
576	44	0	100	3	2
577	43	0	100	2	0
578	33	0	100	1	0
582	43	0	100	4	2
583	29	0	100	6	2
584	36	0	100	3	0
21 Total	38.6*		2100	4.0*	1.4*
<b>Control Group 2</b>					
C	29	0	100	4	2
F	37	0	100	2	1
I	43	0	100	3	0
O	29	0	100	5	1
4 Total	34.5*		400	3.5*	1*

\*Mean

compared with the other controls. However, thorough clinical examinations revealed no ill health. One of the controls was subjected to more detailed chromosome diagnosis by the Institute for Human Genetic Research and Anthropology of Heidelberg University, where the results of the initial examination were confirmed. Further examinations of controls are to be carried out.

### Summary

Employees of plants where alkylene oxide is manufactured or processed were subjected to mutagenicity studies carried out on lymphocyte cultures in accordance with the methods of Moorhead et al,<sup>25</sup> de Jong and Anders. The employees were divided into four groups, according to their periods of exposure: (1) Long-term exposure for more than 20 years; (2) exposure for less than 20 years; (3) long-term exposure and accident (ethylene oxide inhalation or skin contact); and (4) accident, i.e., brief high exposure to ethylene oxide.

Measurement of the concentrations in various sections of the plant yielded values of up to 3 ppm under conditions of normal operation. However, this figure rose briefly to 1900 ppm under plant breakdown conditions. In the authors' experience, it is to be expected that workers were subjected to higher exposure in the past.

One hundred metaphases per person were analyzed for chromosome aberrations. The results are given in Tables 1 through 4. A significant increase in the aberration rate was found only in employees in Group 1. This was confirmed by a control examination carried out one year later. The employees of Groups 2, 3 and 4 displayed no significant increases. However, in evaluating these findings, it should

be noted that the employees had been in contact with a wide range of substances and products in the course of their occupation, which means that the increased aberration rate found cannot be attributed unequivocally to exposure to a particular substance.

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## The Real Person

There is an unfortunate prevalent tendency to think of the inner person as the real person and the outer as an illusion or pretender. Psychoanalytic data, which should never be viewed as other than supplementary information, have unfortunately come to be seen as an alternative (and superior!) view of human behavior. While psychoanalysis supplies us with an incredibly useful tool for explaining motives, purposes underlying human behavior, most of this has little bearing on the moral nature of that behavior and therefore has little to say about the essential judgments of a human individual. Like x-rays, psychoanalysis is a fascinating but relatively new technical tool in helping to illuminate certain aspects of the person. Few of us would want to enclose the x-rays of the principal parties with the photos in their wedding albums.

The inside of a man represents another view, not a truer one. A person may not always be what he appears to be, but what he appears to be is always a significant part of what he is. A man is the sum total of all of his behavior. To probe for the unconscious nature of an individual and to think that that defines the person exclusively, ignoring overt behavior, leads to a greater distortion than would ignoring the unconscious completely.

— From *Feelings* by Willard Gaylin, M.D., published by Ballantine Books, New York, June, 1980.