CHLOROPRENE: ADVERSE EFFECTS ON REPRODUCTION

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Chloroprene 2-chlorobutadiene is a colorless, flammable, volatile liquid with a pungent, ethereal odor. An estimated one billion pounds of chloroprene are produced annually in the United States.[1] All of the chloroprene produced is subjected to polymerization, either into polychloroprene, a synthetic rubber marketed in the U.S. under the trade name Neoprene, or into a liquid polymer, polychloroprene latex. An estimated 2,000 workers have direct exposure to this agent in the U.S. during the manufacture of chloroprene monomer and its polymerization into polychloroprene. The number of workers having indirect exposure through working with polychloroprene rubber or latex is not known. Because of the structural similarity of chloroprene to vinyl chloride, a known carcinogen and mutagen, a search and synthesis of chloroprene-related research addressing carcinogenesis and mutagenesis was undertaken. This review will emphasize the data bearing on potential mutagenic or reproductive hazards.

With regard to carcinogenesis, one experimental study has been reported as negative.[2] However, because of limited presentation of information and inadequate study design and methodology, interpretation of the study results is not possible.[3] In terms of epidemiologic observations, two studies have concluded that workers exposed to chloroprene are at an increased risk of developing lung and skin cancer.[4,5] A third study also is suggestive of an increased risk of lung cancer among a subset of maintenance mechanics exposed to chloroprene.[6] These studies, however, have not given adequate consideration to job classification, environmental concentrations, intensity and duration of exposure, or latency--factors known to influence the risk of cancer.[7] In two studies, no mention is made of age adjustment procedures nor of the criteria used to diagnose the cancers.[4,5] On the basis of these studies, it is difficult to develop valid inferences regarding the carcinogenicity of chloroprene.

With regard to mutagenicity or adverse effects on reproduction, as far back as 1936, Von Oettingen et al. reported infertility associated with chloroprene exposure to male mice.[8] The fertility rate (number of animals pregnant) in mice exposed to chloroprene levels ranging from 12 to 150 parts per million was 43% (6/14) versus 83% (5/6) for controls. Most chloroprene-exposed mice received doses at the relatively lower end of the dose range. In rats exposed to higher levels of chloroprene, the fertility rate was 32% (6/19) as compared to 100% (5/5) for the control group.

TABLE I shows the effects of chloroprene on testicular weight and on the sperm of rats. At atmospheric concentrations of 0.5 parts per million (ppm), five of the eight animals were reported to have atrophied testicles.[9,10]

Total embryonic mortality was 40.9% in the chloroprene-exposed group as compared to 9.9% in the control group. The results were reported as statistically significant. The authors stated that the embryonic mortality was due to preimplantation loss, not postimplantation loss. Also the number of dead spermatozoa for animals with non-atrophied testicles was reported to be 85% as compared to 32% in the control animals. Spermatozoan motility time in minutes was 91 for the chloroprene-exposed rats, as compared to 333 for control animals.

TABLE II shows the results of a dominant lethal test by Davtian et al.[11] Following male rat exposure to chloroprene at 11 ppm, and also at 1.1 ppm, a significant excess of total embryonic mortality was observed. Again, most of the excess is a reflection of preimplantation loss.

The authors state that 36 animals were used, but it is not possible to determine whether there were 36 total animals or 36 in each group. There also does not appear to be an increase with an increase in the dosage of chloroprene in this particular study.

TABLE III shows the study results of a dominant lethal test in the mouse.[12] Following 1.0 ppm atmospheric chloroprene exposure to male mice, preimplantation loss was reported to be 32%, as compared to 3% in controls. At 0.5 ppm, 27% preimplantation loss was observed versus 11% for controls. Total embryonic mortality was in significant excess at both chloroprene exposure levels.

TABLE IV shows data from analyses of bone marrow cells of some of the same mice that were exposed to chloroprene and used for the dominant lethal test.[12] At concentrations of 1 ppm or 0.5 ppm, a significant excess in the percentage of aberrant cells was observed. Ten percent of the cells had aberrations in each exposure group as compared to 2% and 3% in the control animals. In this study, it also appears that an average of 80-100 cells were analyzed per animal.

TABLE V shows a summary of experimental studies indicating cytogenetic, mutagenic, or reproductive effects of chloroprene. In 1972, Davtian reported a dominant lethal effect, effects on the sperm, and testicular atrophy in the rat.[9] In 1973, Davtian et al. reported dominant lethality and chromosomal aberrations in bone marrow cells in the rat.[11] Again, in 1974, Bagramyan and Babayan reported chromosomal aberrations to be in significant excess in bone marrow cells in the rat.[13] Davtian[9] and Volkova[10] also reported dominant lethality and effects on the sperm. In 1976, Sanotskii reported dominant lethality and chromosomal aberrations in mice.[12] In 1975, Bartsch et al. reported that chloroprene was mutagenic in Salmonella Typhimurium, TA-100.[14] Brusick has also indicated that chloroprene is slightly mutagenic in Salmonella Typhimurium.[15] Vogel has demonstrated sex-linked recessive lethal mutations in Drosophila.[16]

Thus, reports indicate that chloroprene is mutagenic in bacteria, is sex-linked recessive lethal in Drosophila, and causes both dominant lethality as well as chromosomal aberrations in bone marrow cells in the mouse and rat. In 1936, chloroprene had been associated with sterility and later with decreased numbers and motility of sperm as well as testicular atrophy in mice and rats at very low concentrations.

With regard to cytogenetic or reproductive effects in humans, TABLE VI shows data for the frequency of chromosomal aberrations in lymphocyte cultures from workers in the Soviet Union.[12] The control group contained nine subjects that were not exposed to chloroprene, while the study group consisted of 18 workers indicated as having an average chloroprene exposure concentration of 5 ppm. The percentage of aberrant cells with chromosomal aberrations was 4.9% in the study group versus 0.65% in the concurrent control group and 1.2% in the historical control group. The frequency of gaps per 100 cells is 3.7 in the exposed workers versus 1.1 in the control group. This observation also was indicated as statistically significant.

In terms of epidemiologic considerations, from the data in TABLE VI, it is noteworthy that there is an age difference of 6 years between the study and control group. Data not shown in TABLE VI indicate that the number of cells analyzed per individual in the study group ranged from 45-185 cells while the number of cells analyzed per individual in the control group ranged from 19-43 cells. Also, 5 out of 18 in the study group were women, while 1 out of 8 in the control group was a woman.

TABLE VII shows data for the frequency of chromosomal aberrations in lymphocytes of female workers exposed to chloroprene at two concentrations as contrasted with historical controls. A significant excess of cells with aberrations is demonstrated for the group of 20 women whose age ranged between 19-23 years. A second group of eight women exposed to lower levels of chloroprene and whose ages ranged between 19-50 years also had a significantly elevated number of cells with aberrant chromosomes. The mean number of cells analyzed per exposure group is 87 and 81, respectively.

TABLE VIII shows study results by Bochkov.[17] Again, a significant excess of cells with aberrant chromosomes is apparent for workers exposed to chloroprene. The mean number of cells analyzed per individual was 100 for the chloroprene-exposed group, while an average of 137 cells was analyzed for subjects in the control group.

TABLE IX shows a summary of human studies indicating cytogenetic or reproductive effects of chloroprene. In addition to chromosomal aberrations, Sanotskii has reported that the examinations of chloroprene workers revealed functional disturbances in spermatogenesis after 6 to 10 years of work in chloroprene production, and morphological disturbances after 11 or more years.[12] The reproductive function in the wives of 143 workers exposed to chloroprene indicated a threefold excess of spontaneous abortion, as compared to wives of 118 controls consisting of factory and office workers in an electrical engineering plant.

Although any single study cited in the summary of the literature does not allow one to definitively conclude that chloroprene is mutagenic, the consistency of positive response, and the number of test systems indicating a positive mutagenic response, as well as additional observations indicating that chloroprene affects the sperm, testicles, and reproductive outcome as a result of male exposure only, it would seem that a prudent approach would be to control chloroprene as an agent which possesses a potential mutagenic risk to humans.

In view of the data presented in this summary of reports on the mutagenicity of chloroprene, several points need to be addressed initially in terms of methodologic considerations. They are as follows: How sensitive is the dominant lethal test, and which is the most sensitive species for use in conducting the test? What is the significance of preimplantation versus postimplantation loss? In cytogenetic studies, what is the significance of gaps versus chromatid or chromosome breaks? How many cells should be analyzed per individual in order to identify with reasonable probability an aberrant cell? What is the appropriate sample size of individuals needed? (These latter two questions are dealt with in two recent publications.)[18,19] Are sex, race, and age variables that are related to the prevalence of cytogenetically abnormal cells?

In addition, when conducting human studies, is it possible to measure either the agents or metabolites of the agents being studied in human tissue, in order to biologically verify that those individuals with presumptive exposure were, in actuality, exposed to the agent under study? It is not uncommon to review the results of studies where there is no confirmation that individuals in the study group were exposed to the agent under study, while subjects in the control group were exposed to other known mutagens or carcinogens. This is particularly the situation when the control group consists of other industrially exposed workers. Such methodology would presumably result in an underestimate or dilution of the effects of the agent being studied.

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TABLE I

Effects on testicular weight and sperm of rats exposed to chloroprene

Atmospheric chloroprene concentration (ppm)	No. male animals exposed	No. with atrophied testicles	Total embryonic mortality	No. dead spermatozoa[a] (%)	Spermatozoan motility time (min.)
0.5	8	5	40.9±11.5*	85±13.8**	91±44*
0	8	1	9.9±1.8	32±9.9	333±15

SOURCE: Davtian (1972); Volkova et al. (1976).

[a]For animals with non-atrophied testicles

*p<0.05.
**p<0.01.

TABLE II

Frequency of embryonic mortality following male rat exposure to chloroprene

Atmospheric chloroprene concentration (ppm)	Preimplantation loss (%)	Postimplantation loss (%)	Total embryonic mortality (%)
11.0	26.0±4.4[a]	8.4±3.4	32.01±7.4[b]
1.1	27.2±6.0[b]	4.7±1.5	30.08±5.7[b]
0	11.7±2.9	2.2±1.1	12.88±2.7

SOURCE: Davtian, Fomenko, and Andreyeva (1973). (Number of animals in each group not stated.)

[a]All values are means ± S.E.

[b]$p<0.05$.

TABLE III

Frequency of dominant lethal mutations following male mice exposure to chloroprene

Atmospheric chloroprene concentration (ppm)	No. of animals M	F	Fert. capacity (%)	Preimplantation deaths (%)	Postimplantation deaths (%)	Total embryonic mortality (%)
1.0	15	30	52±8[a]	32±10*	35±3	63±10*
0	14	31	54±8	3±2	26±10	28±10
0.5	11	31	80±10	27±4*	27±4	42±6**
0	10	25	80±11	11±4	10±?	19±6

SOURCE: Sanotskii (1976).

[a]Values are means ± S.E.

*p<0.05.
**p<0.01.

TABLE IV

Metaphase analysis of bone marrow cells
of mice exposed to chloroprene
for two months

Atmospheric chloroprene concentration	No. of animals	No. cells analyzed	Aberrant cells (%)
1.0	8	799	10.0±0.7*
0	8	750	3.0±0.5
0.5	10	910	10.9±1.3*
0	6	488	2.0±0.6

SOURCE: Sanotskii (1976).

*$p<0.001$.

TABLE V

Laboratory studies indicating cytogenetic, mutagenic, or reproductive effects of chloroprene

Laboratory test system	Observation	Investigators	Reference
Mice & rats	Sterility	Von Oettingen, Hueper, Deichmann-Gruebler, Wiley (1936)	
Rat	1. Dominant lethal 2. Effects on sperm 3. Testicular atrophy	Davtian (1972)	10
Rat	1. Chromosomal aberrations 2. Dominant lethal	Davtian, Fomenko, Andreyeva (1973)	
Rat	Chromosomal aberrations	Bagramian and Babaian (1974)	
Rat	1. Dominant lethal 2. Effects on sperm	Davtian (1972); Volkova (1976)	
Mice	1. Dominant lethal 2. Chromosomal aberrations	Sanotskii (1976)	
S. Typhimurium	Mutagenic	Bartsch, Malaveille, Montesano, Tomatis (1975)	
S. Typhimurium	Mutagenic	Brusick (unpub. 1977)	
Drosophila	Recessive lethal	Vogel (1975)	

TABLE VI

Frequency and types of chromosomal aberrations in lymphocyte cultures from workers in the Soviet Union

Group	Subjects studied	Mean age (yrs.)	No. cells analyzed	Aberrant cells (%)	Rate per 100 cells	
					Aberrations	Gaps
Chloroprene[a] workers	18	39	1,666	4.77±0.57+	4.9	3.71±0.59*
Controls[a]	9	33	572	0.65±0.56	1.0	1.14±0.43
Spontaneous[b]	181	--	28,386	1.19±0.06	1.2	

Average atmospheric chloroprene concentrations = 5.0 ppm.

[a]Data from L. D. Katosova (1973).

[b]Data from N. P. Bochkov (1972) reported by Sanotskii (1976).

+$p<0.001$ all values are means ± S.E.

*$p<0.01$.

TABLE VII

Frequency of chromosomal aberrations in lymphocyte cultures from workers in the Soviet Union

Atmospheric chloroprene concentration	Duration employment (years)	Subjects studied (women)	Age range (years)	No. cells analyzed	Aberrant cells (%)
0.8-1.9[a]	1-4	20	19-23	1,748	3.49±0.51+
0.3-1.1[b]	1-20	8	19-50	648	2.50±0.49*
0[c]	----	181	-----	28,386	1.19+0.06

[a]Volkova et al. (1976).

[b]Data from Fomenko and Katosova (1973) reported by Sanotskii (1976).

[c]Data from N. P. Bochkov (1972) reported by I. V. Sanotskii (1976).

+$p<0.001$ all values are means ± S.E.

*$p<0.01$.

TABLE VIII

Frequency of chromosomal aberrations in lymphocyte cultures from workers in the Soviet Union

Group	Subjects studied	No. cells analyzed	Aberrant cells (%)
Chloroprene workers	57	5,720	2.90
Controls	437	60,020	1.19

SOURCE: N. P. Bochkov (1976).

TABLE IX

Human studies indicating cytogenetic or reproductive effects of chloroprene

Human studies	Observation	Investigators
Workers	Chromosomal aberrations	Katosova (1973)
Workers	Chromosomal aberrations	Bochkov (1976)
Workers	Chromosomal aberrations	Sanotskii (1976) reporting data of Fomenko and Katosova (1973)
Workers	Chromosomal aberrations	Volkova et al. (1976)
Workers	1. Decrease in motility and number of sperm 2. Three-fold excess of miscarriages in wives of male workers	Sanotskii (1976)

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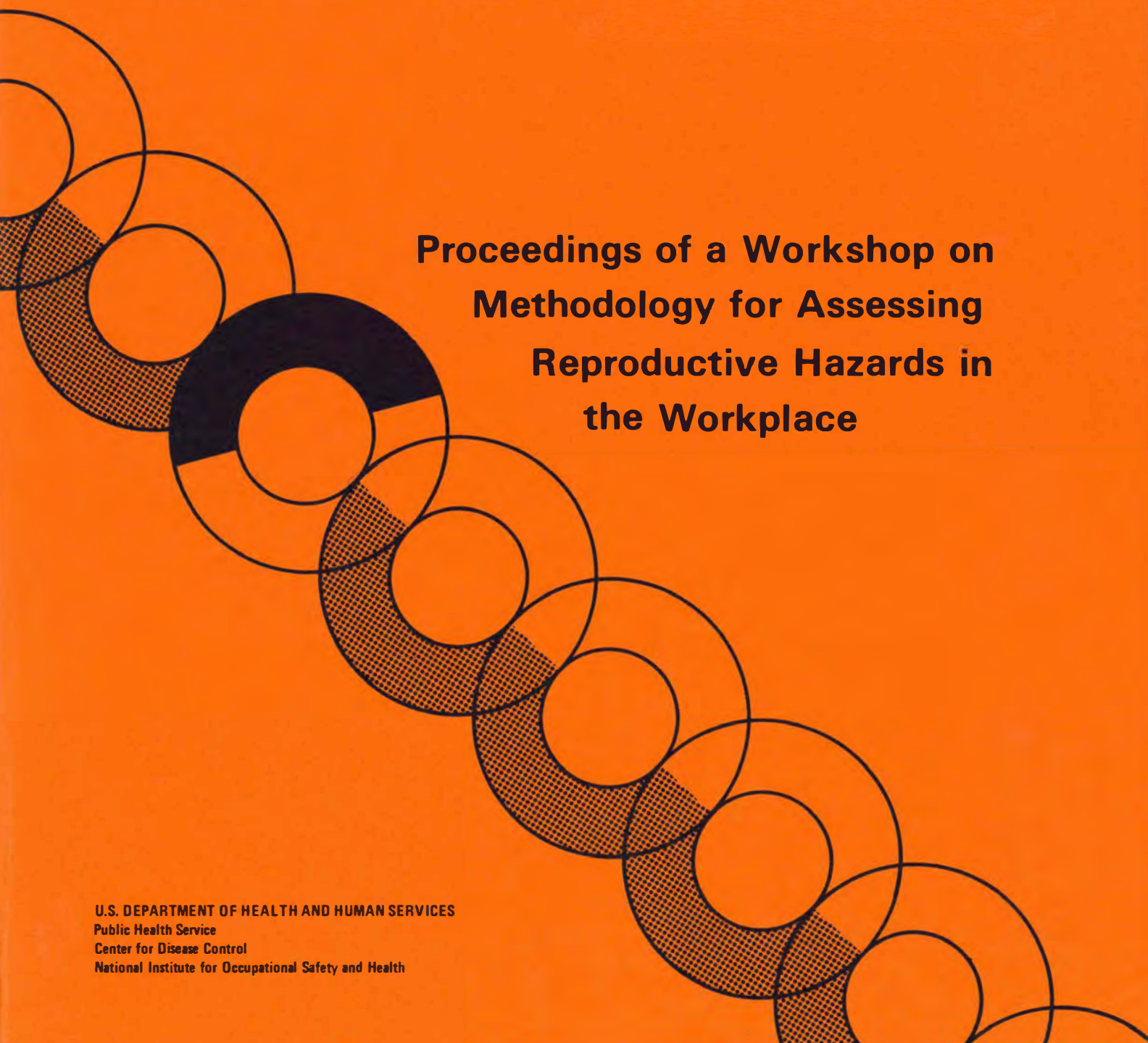

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Center for Disease Control
National Institute for Occupational Safety and Health

PROCEEDINGS OF

A WORKSHOP ON METHODOLOGY

FOR ASSESSING

REPRODUCTIVE HAZARDS IN THE WORKPLACE

April 19-22, 1978

Edited by

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and

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Center for Disease Control
National Institute for Occupational Safety and Health
Division of Surveillance, Hazard Evaluations and Field Studies
Cincinnati, OH 45226

October 1980

SPONSORED BY: National Institute for Occupational Safety and Health

Society for Occupational and Environmental Health

Contract 210-77-0106

NIOSH Project Officer: Philip J. Bierbaum

SOEH Project Officer: Sandra Zimmerman

DHHS (NIOSH) Publication No. 81-100

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
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