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Mutagenicity Assessment of Airborne Particles From Three Polyurethane Foam Manufacturing Facilities

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In conjunction with industrial hygiene surveys, mutagenicity studies were performed for the airborne contaminants in three polyurethane manufacturing plants. Airborne particles were collected on glass-fiber filters with Hi-Vol pumps from different locations in each plant. Gases were collected in multiple-sorbent cartridges. The collected airborne particles and sorbent cartridges were extracted with organic solvents. Each extract was tested for mutagenic activity using *Salmonella typhimurium* TA98 and TA100. The results showed that airborne particles from all three plants are mutagenic to TA98 with or without S9 activation. The mutagenicity with S9, however, was two to four times higher than that without S9. None of the samples of gases collected on sorbent cartridges showed mutagenic activity.

Key words: polyurethane manufacturing industry, toluene diisocyanate, workplace environment, mutagenicity, airborne particles, *Salmonella typhimurium*

INTRODUCTION

In the flexible polyurethane foam manufacturing industry, toluene diisocyanate (TDI) is a primary starting material. Both monomeric isomers of TDI as well as prepolymeric isocyanate-containing compounds may be released during foam production and out-gassing of the finished product [NIOSH, 1984; Rando et al, 1984]. In addition to TDI, various secondary and tertiary aliphatic polyamine compounds such as triethylenediamine, N-methylmorpholine and triethylamine are used to initiate the polymerization of polyurethane resin [Beling et al, 1983]. Secondary and tertiary amines may react with nitrogen oxides, which may be present in the polyurethane manufacturing industry if thermal decomposition of foam occurs [Dodson, 1975; NRC, 1981] or if unvented combustion sources exist, to form N-nitrosamines [Rounbehler, 1983]. The flame retardant compounds, tris 2,3-dibromopropyl phosphate and 2,3- or 1,3-dichloro-2-propanol phosphate, are often added to polyurethane resin [IARC, 1979a]. Some of the foams can be thermally decomposed to release hydrogen

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cyanide, acetonitrile, acrylonitrile, pyridine and benzonitrile during production, [Chambers, 1976; Napier, 1972; Wooley and Mifire, 1973]. For cleaning machinery, a variety of volatile organic compounds including methylene chloride, chlorofluoroethanes, 2-methyoxyethanol, and 1,1,1-trichloroethane are also used in this industry.

In the United States, hundreds of workers are employed by the polyurethane manufacturers. These workers, therefore, are potentially exposed to dust, smoke, and the above-mentioned chemical agents. Some of these chemicals are known to be mutagenic and/or carcinogenic [Andersen et al, 1980; CIB, 1977; IARC, 1979b; Matsuoka et al, 1979; Nakamura et al, 1979; Parent, 1979] and may pose potential hazards to workers. In 1984, researchers from the National Institute for Occupational Safety and Health initiated a cohort mortality study and industrial hygiene study in this industry. In conjunction with industrial hygiene surveys, mutagenicity studies of airborne particles in three polyurethane manufacturing plants were performed. This paper reports the results of exploratory workplace environmental studies where airborne particles and gases were collected and organic solvent soluble extracts were tested for mutagenic activity using the Ames Salmonella assay.

METHODS AND MATERIALS

Sample Collection and Extraction

Airborne particles were collected on glass-fiber filters (type A/E, 4" in diameter) with Hi-Vol pumps (General Metal Works, Cleves OH) at flow rates between 18 and 25 cfm [Whong et al, 1981]. Sampling time was 8, 16, or 24 h depending on conditions. Each filter was used for 8 h sampling. Gases and vapors were collected in sorbent cartridges each containing 5 ml each of charcoal, Tenax-GC, XAD-2, and magnesium silicate. The sampling was conducted at a flow rate of 2.5 l/min using P-4000 pumps (Dupont, Wilmington DE) simultaneously as that with filters. During sampling, new cartridges were installed after every 8 h. Sampling sites were the same as those for airborne particles.

Samples of airborne particles were extracted with 150 ml methylene chloride (DCM) and then with 150 ml of acetone plus methanol (A+M) [Krishna et al, 1983; Whong et al, 1981]. Samples of sorbent cartridges were extracted only with 150 ml of acetone because DCM can dissolve Tenax-GC. The extraction was carried out by shaking overnight (16 h) at 25°C. DCM and A+M extracts from each air particle sample were combined. Each extract was filtered and concentrated to 3 ml at 45°C under a N_2 stream. Five ml of dimethyl sulfoxide (DMSO) was then added to each extract which was reconcentrated to 5 ml.

Sampling sites

Plant A. This facility produces molded foam items for the automobile industry. A large single building contains one hot foam cured line, and four cold foam cured lines. Three locations (foam crusher, foam line, and outside the plant) were selected for the experiments. In the crusher area, samplers were set 3 ft behind the crusher and 1½ ft above the floor. In the foam line, samplers were set within the oval loop, which formed the production operation, 6 ft above the floor. The outside samplers were located upwind of the building, 6 ft away and 3 ft above the ground.

Plant B. This facility also produces molded foam items for the automobile industry. Being the smallest facility, this plant used only two hot cured foam lines to

produce molded foam parts. Three sites (curing oven, pouring station, and outside the plant) were selected. In the oven area, samplers were set 6 ft beyond the oven exit point and 2 ft above the floor. In the pouring area, samplers were set 2 ft behind the pourer and 3 ft off the floor. The outside samplers were located on the roof upwind of the factory stacks.

Plant C. This facility produces slab foam stock from which finished products, such as carpet cushioning and automobile padding, are obtained. This facility employed the largest number of workers. Four locations (foam slitter, foam production line, hot wire cutting, and outside the plant) were sampled during two visits to this facility. In the slitter area, samplers were set 3 ft beside the slitter and 1.5 ft above the floor. At the pouring line, samplers were set 10 ft to the side of the cutting station and 6 ft above the floor. At the hot wire cutting area, they were located 8 ft above the floor. The outside samplers were located on the roof upwind of the exhaust from the plant and at the back of the plant 4 ft above the ground and 3 ft away from the building, also upwind, for the first and second surveys, respectively.

Mutagenicity Assay of the Collected Samples

All extracts were tested for mutagenic activity with the Ames Salmonella/microsome assay system [Ames et al, 1975]. The plate incorporation test, with and without S9 activation, was employed using tester strains TA98 and TA100. Mutations were scored from histidine dependence to histidine independence. The procedure of the assay followed that described by Ames and coworkers [Ames et al, 1975]. In brief, 0.1 ml of tester cells from overnight culture and 0.1 ml of test extract or DMSO (negative control) were added to 2 ml molten soft agar containing biotin and a trace amount of histidine. The soft agar was overlayed onto a VB salt minimum agar plate. For metabolic activation, 0.5 ml of S9 mix (0.05 ml S9) was also added to the soft agar. The S9 was prepared from the livers of male Wistar rats pretreated with Aroclor 1254 (500 mg/kg body wt). At least three samples were collected, combined, and extracted from each site. Four concentrations of each extract were tested. The solvent extract of new filters and 2-aminoanthracene (2.5 μg/plate) were used as filter and positive controls, respectively. All the overlayed plates were scored for revertants after 2 days of incubation. An extract was considered mutagenic if the number of revertants in any of the four concentration tested was twofold or greater than the control and showed a dose-related response.

RESULTS

Airborne particles collected on glass-fiber filters from all three plants were found to be mutagenic (Tables I–V). At plant A, mutagenic activity was found only with samples collected from inside the plant. Although more particles were obtained from the foam production area, the mutagenic activity, based on air volume, of samples collected near a cold cure production line was not much different from those collected in the crusher area. Approximately 40 to 48 revertants per m^3 air were found for samples from both areas (Tables I, II). Mutagenic activity, with and without S9 activation, was found only in TA98. The activity with S9, however, was 5 to 11 times higher than without S9 (Table I)

A very weak mutagenic activity was detected for samples collected outside plant B. Samples from inside the plant had higher mutagenic activites. Based on air volume,

TABLE I. Mutagenicity of Airborne Particle Extracts From Polyurethane Manufacturing Plant A Tested With *S. typhimurium* TA98 and TA100

Site	Air vol (m^3/plate)	Particle (mg/plate)	Revertants/plate[a] TA98 −S9	TA98 +S9	TA100 −S9	TA100 +S9
Outside	2.66	0.07	29	40	236	281
	5.33	0.139	33	42	252	291
	10.65	0.277	41	47	235	301
	21.31	0.554	45	61	238	308
Crusher	2.25	0.59	44	109	276	341
	4.49	1.18	49	217	283	377
	8.99	2.35	64	471	324	379
	17.97	4.69	105	962	325	474
Foam line	1.29	0.4	27	88	243	306
	2.58	0.8	38	135	251	307
	5.16	1.59	59	250	232	302
	10.33	3.18	67	455	228	364
Filter control			23	36	216	271
Negative control			21	50	206	267
Positive control				828		990

[a]The number is an average of three plates.

TABLE II. Mutagenicity of Airborne Particle Extracts From Different Polyurethane Manufacturing Plants*

Plant	Sampling site	Particle weight ($\mu g/m^3$ air)	Revertants/mg particle	Revertants/m^3 air
A	Outside	26	44.2	1.2
	Crusher	216	183.3	47.9
	Foam line	323	131.3	40.5
B	Outside	98	17.4	1.7
	Pourer	306	32.1	9.8
	Oven	204	36.0	7.4
C	Outside	105	26.9	2.8
	Slitter	92	18.8	1.7
	Foam line	210	67.1	14.1
	Hot wire	153	182.7	28.0
C[a]	Outside	168	19.8	3.3
	Foam line	137	445.2	61.2
	Hot wire	194	405.5	79.1

Numbers of revertants used for computations are based on the results in TA98 with S9 activation and have been subtracted by numbers from the filter controls. All the data from different concentrations were used for the calculation. Revertants/mg of particle = total revertants/total particle tested (mg). Revertants/m^3 of air = total revertants/total vol. of air tested (m^3).

*Results for different plants were obtained from different experiments.

[a]Data are from the second survey.

more particles were collected from the pourer than from the oven area. The mutagenic activity of samples from the pourer area was slightly higher than those from the oven area (Tables II, III). As in plant A, mutagenic activity of samples with and without S9 activation was found only in TA98. With S9 activation, however, the mutagenic activity was highly enhanced (Table III)

TABLE III. Mutagenicity of Airborne Particle Extracts From Polyurethane Manufacturing Plant B Tested With *S. typhimurium* TA98 and TA100

Site	Air vol (m^3/plate)	Particle (mg/plate)	Revertants/plate[a] TA98 −S9	TA98 +S9	TA100 −S9	TA100 +S9
Outside	3.1	0.30	25	40	169	188
	6.1	0.61	31	32	188	233
	12.2	1.21	34	46	188	253
	24.5	2.42	47	61	223	238
Pourer	4.1	1.25	27	70	166	161
	8.2	2.5	40	119	205	116
	16.3	4.99	47	196	226	126
	32.6	9.99	81	317	230	130
Oven	4.25	0.87	37	37	196	233
	8.5	1.74	35	39	245	271
	17.0	3.48	49	137	275	303
	34.0	6.95	65	357	304	333
Filter control			18	25	149	166
Negative control			18	30	187	184
Positive control				1,215		1,543

[a]The number is an average of three plates.

TABLE IV. Mutagenicity of Airborne Particle Extracts From Polyurethane Manufacturing Plant C Tested With *S. typhimurium* TA98 and TA100*

Site	Air vol (m^3/plate)	Particle (mg/plate)	Revertants/plate[a] TA98 −S9	TA98 +S9	TA100 −S9	TA100 +S9
Outside	2.7	0.28	28	39	172	196
	5.3	0.56	34	45	185	178
	10.6	1.12	34	58	180	209
	21.2	2.24	43	95	204	193
Slitter	4.4	0.41	29	40	173	201
	8.9	0.82	34	48	187	199
	17.7	1.63	33	59	182	187
	35.4	3.26	56	92	169	195
Foam	2.5	0.53	22	53	177	209
Line	5	1.05	41	91	181	246
	10	2.1	45	190	162	273
	20	4.2	66	319	178	272
Hot wire	4.1	0.63	45	102	203	206
	8.2	1.25	59	280	227	345
	16.4	2.51	92	586	209	358
	32.8	5.02	131	875	262	406
Filter control			22	31	190	202
Negative control			14	24	179	204
Positive control				1,302		1,287

*Data from the first survey (experiment).
[a]The number is an average of three plates.

TABLE V. Mutagenicity of Airborne Particle Extracts From Polyurethane Manufacturing Plant C Tested With *S. typhimurium* TA98 AND TA100*

Site	Air vol (m^3/plate)	Particle (mg/plate)	Revertants/plate[a] TA98 −S9	TA98 +S9	TA100 −S9	TA100 +S9
Outside	1.97	0.33	22	36	154	187
	3.94	0.66	24	31	161	200
	7.87	1.33	32	54	174	164
Foam	2.12	0.29	53	136	153	232
Line	4.24	0.58	79	287	152	286
	8.48	1.17	135	620	162	265
	17.0	2.34	165	1,007	150	349
Hot	1.93	0.38	31	183	146	240
Wire	3.85	0.75	27	334	176	277
	7.7	1.5	61	763	179	347
	15.4	3.0	61	1,103	185	427
Filter control			13	25	179	172
Negative control			12	24	140	174
Positive control				1,681		1,639

*Data from the second survey (experiment).
[a]The number is an average of three plates.

At plant C, samples from outside the plant were also weakly mutagenic. The mutagenic activity of samples collected from slitter area inside the plant was similar to that of samples from outside the plant. Samples collected from foam line and hot wire areas inside the plant were highly mutagenic to TA98, particularly with S9 activation. In the first survey the mutagenic activity of samples from the hot wire area, based on air volume, was two times the activity of those from the foam line area (Tables II and IV). Mutagenicity, however, was similar for samples from both areas (Tables IV, V) in the second survey. With S9 activation, samples from hot wire area were found to be weakly mutagenic to TA100 (Table V).

None of the samples collected in any of the three plants on sorbent cartridges showed any mutagenic activity.

DISCUSSION

These studies clearly indicate that the solvent extracts of airborne particles collected from polyurethane manufacturing plants are mutagenic. The mutagenic activity varies from sample to sample, date to date, and plant to plant. The number of revertants range from 1.7 to 79.1 per m^3 air. The pattern of mutagenic activity appears to be similar in all three plants, i.e. the activity was found primarily in TA98 with S9 activation. This suggests that chemicals responsible for mutagenic activity are mainly promutagens inducing frameshift mutations. It has been shown that TDI, the basic material used for the production of polyurethane, is mutagenic in TA1538 and TA98 after metabolic activation [Andersen et al, 1980]. Therefore, the mutagenic activity found in this study may be due to the presence of TDI or other isocyanate derivatives in airborne particles. Some of the mutagenic activity, however, may be attributed to other chemicals used for or generated during the production of polyurethane foam. It should be indicated that the organic solvent DCM used for the extraction is mutagenic. The mutagenic activity found in this study is not likely due

to DCM because all DCM and other solvents used for the extraction were evaporated during the concentration procedure. It is possible, however, that DCM and other solvents used can interact with organic materials extracted from airborne particles and modify their mutagenic activity.

Mutagenic activity of airborne particles from workplaces has been reported by others using the Ames Salmonella/microsome assay. Extracts of airborne particles collected from a carbon electrode factory [Monarca et al, 1982], welding stations [Hedenstedt et al, 1977; Stern, 1983], an aluminum plant [Kørkje et al, 1985] and a steel foundry [Bryant and McCalla, 1982] were found to be mutagenic. Mutagenic airborne contaminants were also found in the work environment of a rubber factory [Donner et al. 1983].

Extracts of airborne particles collected outside two of the three polyurethane foam plants in this study showed mutagenic activity. This is not surprising as both plants are located in industrial/urban areas. Extracts of ambient airborne particles from industrial/urban areas are known to be mutagenic in *S. typhimurium* [Chrisp and Fisher, 1980; Hughes et al., 1980 for references]. No mutagenic activity was found in the outside samples from the third plant. This may also be expected because this plant is located in a rural area.

Without a repeated long-term mutagenicity testing, it is difficult to compare the mutagenic activity among different plants or different sites in the same plant. The mutagenic activity of air particles will depend on the operating conditions, type of chemical used, and ventilation conditions during the operation. Therefore, the differences in the mutagenic activities between the first and second surveys at plant C were probably due to the differences in operating conditions. The differences in the mutagenic activity of samples collected from different locations in plant C may not be seen in plants A and B, because both A and B are relatively small and all operations are conducted essentially in the same room.

In a laboratory study, we found that a multiple sorbent cartidge consisting of charcoal, Tenax-GC, XAD-2 and magnesium silicate was useful for the collection and acetone was useful for the recovery of gaseous mutagens (unpublished data). None of the samples collected in the three plants showed any mutagenic activity. It seems that no mutagens were collected and/or recovered from the sorbent cartridge or that the concentration of mutagens collected was too low to be detected by the assay used.

The airborne particle samples that were collected at polyurethane manufacturing plants were mutagenic. Whether exposure of workers to agents that are mutagenic to bacteria or the level of these agents to which workers are exposed pose any genetic, carcinogenic, and/or reproductive hazards is not known. A high proportion of carcinogens is known to be mutagenic in the Ames Salmonella assay system [Dunkel et al, 1985; McCann and Ames, 1976]. Limited information suggests that there is a correlation between the bacterial mutagenicity level of airborne particles and lung cancer incidence [Kaiser et al, 1981; Walker et al, 1982]. Index of the mutagenicity of air particles has been considered to be a more powerful measure of the human health hazard of air pollution than the traditional indices of particulate concentration [Walker et al, 1982]. This information is yet to be validated by further epidemiological studies where the mutagenic activity of collected air samples is known. In the meantime, it may be prudent to monitor and/or reduce mutagenically active contaminants whenever possible in the occupational setting.

A cohort mortality study of workers in the polyurethane manufacturing industry is in progress. Workers employed at the three facilities described are included in the study.

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