A Health Hazard Evaluation of Nitrosamines in a Tire Manufacturing Plant

J. D. McGLOTHLIN, T. C. WILCOX, and J. M. FAJEN
National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, OH 45226

G. S. EDWARDS
New England Institute for Life Sciences, Waltham, MA 02154

In a preliminary study we have recently reported (1) that the air in three rubber and tire industry plants was contaminated with several N-nitroso compounds. The compounds included N-nitrosomorpholine (NMOR), N-nitrosodimethylaminne (NDMA), and N-nitrosodiphenylamine (NDPhA). The latter compound is used as a vulcanization retarder, so its presence near processes employing it was not surprising. Bismorpholine-carbamylsulfonamide, a cross-linking accelerator used in rubber tires, was found in tire factories and may be contaminated with NMOR. The source of the NDMA was not identified, but it could arise from nitrosation of amines which may be decomposition products of diamine based accelerators, as pointed out by Yeager, et al (2).

Our present work, reported here, covers the results of four separate survey visits made to a single tire plant. It strongly suggests that NMOR may be generated by transnitrosation of morpholine by NDPhA when these two chemicals are used together. Although such transnitrosation has been shown to occur experimentally (3,4,5), this is the first instance we are aware of where this occurrence may result in human exposure to NMOR, a known animal carcinogen (6). The chemical structures of four nitrosamine compounds found in this tire plant and three typical vulcanization accelerators and stabilizers used in the tire industry are in Figure 1.

Efforts to improve the worker environment through engineering controls and chemical substitution, and the results of a brief survey of biological samples (blood, urine, and feces) obtained from the workers during two of the NIOSH visits are also reported.

Background. The tire plant in Maryland produces bias-ply passenger, truck, and off-road tires 24 hours per day, 7 days per week. On the average, the tire company mixes approximately

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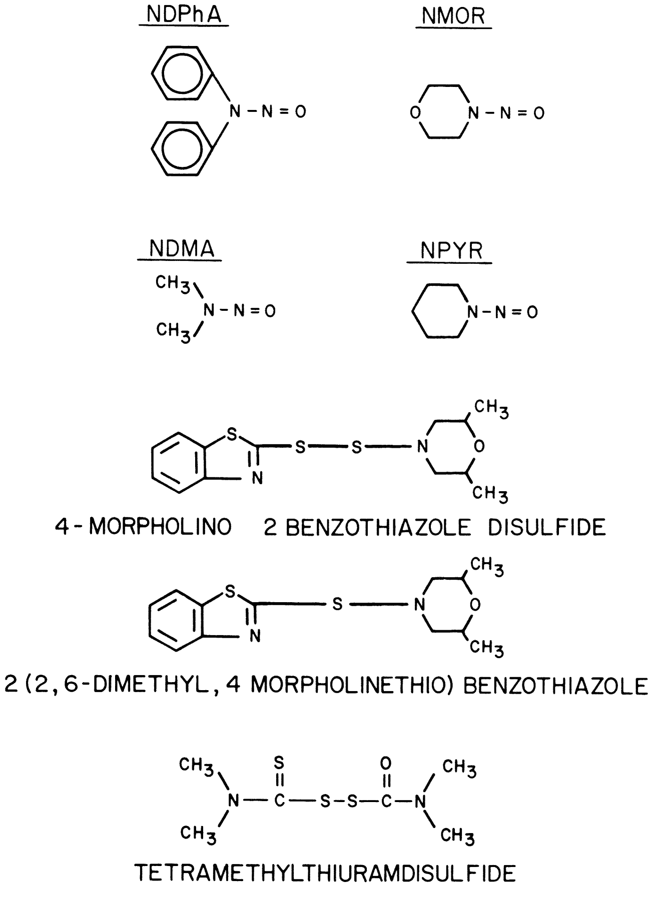


Figure 1. Volatilized N-*nitrosamines found in this tire plant and typical vulcanization accelerators and stabilizers used in the tire industry*

6000 batches of rubber per month for passenger and off-roadtires, and another 2000-2,500 batches per month for truck tires. Batch weights range between 400-500 pounds each. During the manufacture of truck tire tread and bias-ply rubber approximately two pounds of NDPhA, a retarding agent which controls the time of rubber cure, are added to each rubber batch. In August 1979, NIOSH took short term workplace air samples (approximately 2 to 3 hours) of truck tire rubber batches only. On subsequent NIOSH visits workplace air sampling was longer (approximately 5 to 7 hours), and included passenger tire rubber batches (65 to 75 percent of all batches sampled) which did not contain NDPhA. Nitrosamines found during NIOSH surveys were primarily in "hot process" areas where rubber is heated by friction and compression from milling, extruding, and curing operations. The milling and calendering temperatures range from 200-230^{O}F, while extruding and curing operations range from 300-350^{O}F. The term "process sampling" used in this report, refers to workplace air samples collected approximately one foot away from a tire manufacturing process. The word "calendering" refers to the sandwiching of rubber onto nylon fabric to make the plies for bias-ply tires.

Materials and Methods

Air Samples. Airborne nitrosamines were collected with a Thermo-Sorb/N* air sampler (7) connected to a battery-operated pump (DuPont, model P-4000)* which had been calibrated using a 500 ml bubble burett. The pumps were operated between 1.5 and 3.0 L/min. Air sampling ranged from 1 to 8 hours. The air collectors were tightly capped and returned to the laboratory for analysis of nitrosamines. They were eluted with 2 mL of methanol-dichloromethane (1:3, v/v)--and directly injected into a gas chromatograph (GC) and/or high performance liquid chromatograph (HPLC), each equipped with a TEA Thermal Energy Analyzer; (Thermo Electron*, Waltham, MA) detector.

The GC-TEA conditions used for the detection of volatile nitrosamines have been described by Fine and Rounbehler (8). A 14' x 1/8" stainless steel column packed with 5% Carbowax 20M containing 2% NaOH on Chromosorb W HP (80-100 mesh) was operated at 175^{O}C with argon gas as the carrier at a flow rate of 15 mL/min. A TEA was used as the detector with dry ice/ethanol as the cold trap. The HPLC-TEA was constructed by sequentially connecting a high pressure pump (Altex, model 110), an injector (Waters, model U6K), a μPorasil column (Waters), and a TEA. The operation of HPLC-TEA has been described by Fine, et al.(9).

The samples were screened using two different solvent systems: 4% acetone and 96% isooctane for NMOR quantitation, and 0.5% acetone in isooctane for the determination of NDPhA.

Biological Samples. There were three types of biological samples obtained from workers at the plant: urine, whole blood, and feces. All urine and blood samples were internally "spiked" at the factory with 1 µg/mL of a nitrosopiperidine (NPiP) standard. NPiP was used for spiking because it has a similar stability and recovery characteristic to nitrosomorpholine, and to provide a means of gauging the accuracy of the analytical methods. Due to the inability to perform homogeneous mixing on-site, the feces samples were not spiked until they were thawed upon return to the laboratory. Ethyl acetate extracts of urine samples were examined for the presence of N-nitrosodiethanolamine (NDEIA), a metabolite of NMOR, by HPLC-TEA. All samples were immediately frozen at the plant (-80°C) and kept at this temperature until analysis.

Urine Samples.

Analysis for NMOR: Ten mL of thawed urine were placed on a Preptube cartridge (Thermo Electron Corp.) and eluted with 60 mL of dichloromethane (DCM). The Preptube was pre-wet with DCM before receiving the sample. The resulting solution was concentrated to a volume of 1 mL at 55°C using a Kuderna-Danish apparatus. The concentrate was analyzed for NMOR by GC-TEA. Recoveries of the internal standard (NPiP) were typically 80-100%.

Analysis for NDElA: Ten mL of the thawed urine were placed on another Preptube, pre-wet with ethyl acetate. The sample was washed with 60 additional milliliters of ethyl acetate and the effluent dried (rotary evaporator) to 1 milliliter. It was then analyzed by HPLC-TEA using a μNH_2 column eluted with isooctane:dichloromethane:methanol (60:30:7). Recoveries for NDElA using this method were approximately 70%. The percent recovery was judged by an internal spike of N-nitrosodipropanolamine.

Blood Samples. Ten mL blood samples were analyzed for NMOR, using the method described for urine samples. Recoveries of the NPiP standard were more variable, ranging from 32 to 87%.

Feces Samples. Twenty to 45 grams of samples were weighed out and ground to a fine powder in a blender containing liquid nitrogen. The resulting homogenate was placed in a 500 mL distillation flask with 50 milliliters of mineral oil (containing 1 mg/mL of α-tocopherol [to prevent nitrosation during distillation]) and allowed to thaw. The contents of the flask were mixed with 500 ng of NPiP to determine the recovery of the method. The feces samples were distilled under a vacuum of 2.2 torr at up to 130°C for 1 hour. Recoveries of NPiP were low, approximately 20%.

Results

Air Samples: NMOR, NDMA, and NPYR were found during the first NIOSH visit in air samples collected at a tire manufacturing plant in Maryland. One process sample, collected at a feedmill, contained 250 $\mu g/M^3$ of NMOR, a level several times higher than has been reported for any airborne nitrosamine at any industrial site (1). Maximum concentrations of NDMA and NPYR found in the hot process areas were 4.4 $\mu g/M^3$ and 3.4 $\mu g/M^3$, respectively. Over the following 7 months, ventilation improvements and changes in chemical formulation of the rubber resulted in a 200-fold reduction in NMOR levels and elimination or reduction of other nitrosamines at most sites. Results are shown in Figure 2, and Table I.

Personal (breathing zone) air samples obtained in October 1979, showed feed mill and calendering operators to be most heavily exposed to nitrosamines; one worker had a time-weighted average NMOR exposure of 25 $\mu g/M^3$. Workers in other hot process such as warm-up mills, extruding machines, and curing processes were determined to have substantial personal exposure to airborne nitrosamines (Table II). Personal exposures to airborne NMOR and NDMA were also detected in the truck tire building and tire shipping area. Although these nitrosamine levels are not very high (1.9 $\mu g/M^3$ NMOR, 0.1 $\mu g/M^3$ NDMA), they demonstrate the residual effect of nitrosamines still volatilizing off from storage of freshly cured tires. When compared to passenger tire builders, truck tire builders on the average had 3 times the NMOR exposure even though 4 to 5 passenger tires could be built to every truck tire.

By November, 1979, there were strong indications that the source of the high levels of airborne NMOR was the thermal decomposition of the retarding agent NDPhA, and the subsequent reaction of its nitroso group with other rubber additives (preformed morpholino compounds). The results were most striking when two short term air samples were collected from the feedmill and calendering area - one rubber batch contained NDPhA, and the other did not. NMOR levels from the NDPhA batch were 14 times higher (120.3 $\mu g/M^3$) than the rubber batch without NDPhA (NIOSH Interim Report No. 2, HE 79-109). In December 1979, ventilation improvements (3-sided canopy enclosures and new fan motors) to the feed mills which process rubber for the calendering of bias-plies, and installation of local exhaust on the top and bottom of the tire tread extrusion

Table I. Area and Process Samples in $\mu g/M^3$[a]

	Location	Nitrosamines	Aug. '79 Highest	Aug. '79 Avg.	Oct. '79 Highest	Oct. '79 Avg.	Dec. '79 Highest	Dec. '79 Avg.	Feb. '80 Highest	Feb. '80 Avg.
Hot Processes	Banbury	NMOR	2.1	1.8	N.S.[2]	--	N.S.	--	0.3	--
		NDMA	0.1	--	N.S.	--	N.S.	--	0.1	--
		NPYR	N.D.[1]	--	N.S.	--	N.S.	--	N.D.	--
		NDPhA	N.D.	--	N.S.	--	N.S.	--	N.S.	--
	Feed Mill & Calender	NMOR	250	160	120	64	63	25	1.3	1.0
		NDMA	1.9	1.5	2.9	1.6	1.1	0.4	0.8	0.3
		NPYR	3.4	2.3	3.9	2.0	1.0	0.6	N.D.	--
		NDPhA	N.D.	--	12	--	N.S.	--	N.D.	--
	Warm-up Mills	NMOR	5.2	3.5	N.S.	--	N.S.	--	14	4.6
		NDMA	0.7	0.4	N.S.	--	N.S.	--	5.5	2.7
		NPYR	N.D.	--	N.S.	--	N.S.	--	N.D.	--
		NDPhA	N.D.	--	N.S.	--	N.S.	--	N.D.	--
	Extruders	NMOR	32	18	N.S.	--	N.S.	--	N.S.	--
		NDMA	4.4	--	N.S.	--	N.S.	--	N.S.	--
		NPYR	N.D.	--	N.S.	--	N.S.	--	N.S.	--
		NDPhA	N.D.	--	N.S.	--	N.S.	--	N.S.	--
	Curing Room	NMOR	6.4	5.3	2.0	--	N.S.	--	N.S.	--
		NDMA	0.2	0.2	0.1	.07	N.S.	--	N.S.	--
		NPYR	N.D.	--	N.D.	--	N.S.	--	N.S.	--
		NDPhA	N.D.	--	N.D.	--	N.S.	--	N.S.	--

Other Processes	Tire Storage	NMOR	N.S.	--	0.6	--	N.S.	--	N.S.	--
		NDMA	N.S.	--	0.1	--	N.S.	--	N.S.	--
		NPYR	N.S.	--	N.D.	--	N.S.	--	N.S.	--
		NDPhA	N.S.	--	N.D.	--	N.S.	--	N.S.	--
	Lunch Room	NMOR	N.S.	--	Trace	--	N.S.	--	N.S.	--
		NDMA	N.S.	--	.02	--	N.S.	--	N.S.	--
		NPYR	N.S.	--	N.D.	--	N.S.	--	N.S.	--
		NDPhA	N.S.	--	N.S.	--	N.S.	--	N.S.	--
	Outside Plant	NMOR	N.S.	--	N.D.	--	N.S.	--	N.S.	--
		NDMA	N.S.	--	N.D.	--	N.S.	--	N.S.	--
		NPYR	N.S.	--	N.D.	--	N.S.	--	N.S.	--
		NDPhA	N.S.	--	N.D.	--	N.S.	--	N.S.	--

NMOR = N-nitrosomorpholine, NDMA = N-nitrosodimethylamine, NPYR = N-nitrosopyrrolidine, NDPhA = N-nitrosodiphenylamine

[1]N.D. = Not Detected
[2]N. = Not Sampled

Trace: <.002 µg/M^3

Detection Limit: 1 part per billion

[a] µg/M^3 = micrograms per meter cubed.

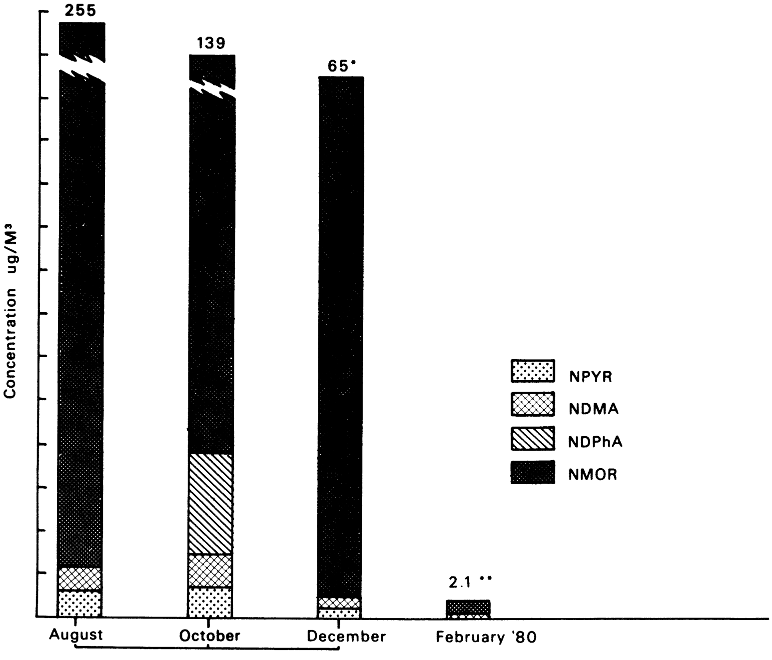
Table II. Personal Samples in $\mu g/M^3$ [a]

	Location	Nitrosamines	Oct. '79		Dec. '79		Feb. '80	
			Highest	Avg.	Highest	Avg.	Highest	Avg.
Hot Processes	Banbury	NMOR	N.D.[1]	--	N.S.[2]	--	N.S.	--
		NDMA	N.D.	--	N.S.	--	N.S.	--
		NPYR	N.D.	--	N.S.	--	N.S.	--
		NDPhA	N.D.	--	N.S.	--	N.S.	--
	Feed Mill & Calender	NMOR	25	17	18	17	1.0	0.1
		NDMA	0.4	0.3	0.1	0.1	0.2	0.1
		NPYR	0.8	0.5	0.2	0.2	N.D.	N.D.
		NDPhA	N.D.	N.D.	13	9.9	N.S.	N.S.
	Warm-up Mills	NMOR	0.8	0.8	N.S.	--	1.3	1.0
		NDMA	0.2	0.1	N.S.	--	0.5	0.4
		NPYR	N.D.	--	N.S.	--	N.D.	--
		NDPhA	N.S.	--	N.S.	--	N.D.	--
	Extruders	NMOR	1.0	--	N.S.	--	N.S.	--
		NDMA	0.1	--	N.S.	--	N.S.	--
		NPYR	N.D.	--	N.S.	--	N.S.	--
		NDPhA	N.D.	--	N.S.	--	N.S.	--
	Curing Room	NMOR	1.8	--	0.4	--	N.S.	--
		NDMA	0.1	--	0.3	--	N.S.	--
		NPYR	N.D.	--	N.D.	--	N.S.	--
		NDPhA	N.S.	--	N.S.	--	N.S.	--

Other Processes	Tire Building	NMOR	1.9	1.6	N.S.	--	N.S.	--
		NDMA	0.1	0.1	N.S.	--	N.S.	--
		NPYR	N.D.	--	N.S.	--	N.S.	--
		NDPhA	N.S.	--	N.S.	--	N.S.	--
	Shipping	NMOR	0.6	--	1.7	1.3	N.S.	--
		NDMA	.04	--	0.1	0.1	N.S.	--
		NPYR	N.D.	--	N.D.	--	N.S.	--
		NDPhA	N.S.	--	N.S.	--	N.S.	--
	Receiving	NMOR	N.D.	--	0.3	--	N.S.	--
		NDMA	N.D.	--	N.D.	--	N.S.	--
		NPYR	N.D.	--	N.D.	--	N.S.	--
		NDPhA	N.S.	--	N.S.	--	N.S.	--
	Tire Storage	NMOR	N.S.	--	0.7	--	N.S.	--
		NDMA	N.S.	--	0.1	--	N.S.	--
		NPYR	N.S.	--	N.D.	--	N.S.	--
		NDPhA	N.S.	--	N.S.	--	N.S.	--

NMOR = N-nitrosomorpholine, NDMA = N-nitrosodimethylamine, NPYR = N-nitrosopyrrolidine, NDPhA = N-nitrosodiphenylamine

[1] N.D. = Not Detected
[2] N.R. = Not Sampled



* Local exhaust improved-canopy inclosed on 3 sides

** Rubber formulation change n-nitrosodiphenylamine not used.

Figure 2. Highest reported airborne N-*nitrosamine levels in feedmill and calendering area*

machines, significantly reduced airborne nitrosamine exposure (Tables I & II). By February 1980, a phthalimide derivative was substituted for NDPhA. In the feed mill and calendering area, this resulted in a 99.5% NMOR reduction in process sample concentrations when compared to the August 1979, results, and a 96% NMOR reduction in the highest personal exposure when compared to the October results. In the same area, NPYR, which had measured 3.4 $\mu g/M^3$ in August and 3.9 $\mu g/M^3$ in October, was not detectable after removal of NDPhA. In general, the NPYR levels usually were detected when high levels of NMOR were found and were not detectable when NMOR levels diminished. The appearance and disappearance of NPYR seemed to be related to NDPhA, but the source of NPYR in these samples is not yet known. NDMA levels did not decrease substantially between August 1979 and February 1980. In fact, NDMA and NMOR levels increased slightly in the tire tread warm-up mill area. We suspect that the reason for the increase is due to a higher percentage of rubber stock containing NDMA additives on the day we sampled, and also due to the continued use of NDPhA in truck tire tread.

NDPhA was detected in one process sample on the second survey and in two personal samples on the third survey. The fact that NDPhA was not detected more frequently was probably due to its high molecular weight and relatively low volatility.

On the fourth NIOSH survey, process and personal nitrosamine samples were collected from rubber batches made at a Banbury (rubber batch mixer) where only starting ingredients such as natural and synthetic rubber, and waxes, were mixed. No retarding, accelerating, or stabilizing additives were added during this phase of mixing. The purpose of this environmental sampling was to determine if nitrosamines were being generated without rubber additives. Four samples were collected; three process, one personal. NMOR and NDMA were detected in two of the four samples, but at very low levels. The highest NMOR process sample was 0.27 $\mu g/M^3$, and 0.09 for NDMA. The personal sample was 0.24 $\mu g/M^3$ and 0.12 $\mu g/M^3$ for NMOR and NDMA, respectively. Thus, it seems that the majority, if not all of the nitrosamines are derived from rubber additives, either as a raw chemical contaminant, and/or from transnitrosation of various compounds during the final mixing and tire manufacturing stage.

In summary, nitrosamines were detected in every area of the tire plant where NIOSH sampled. Only outside the plant, next to the guard house, nearly 400 yards away, were nitrosamines not detected. Generally, highest nitrosamine levels were in the hot process areas, in particular, the feedmill and calendering area. With the exception of the guard house, the lowest nitrosamine levels found were in the receiving area (0.3 $\mu g/M^3$ NMOR) and lunch room (trace <.002 $\mu g/M^3$ NMOR). Results from the nitrosamine levels found throughout the plant, and over the past four NIOSH surveys are in Figure 3. The tire building and tire storage areas were the only locations where appreciable NMOR levels were still off-gassing, after the tires had been cured. Personal NMOR exposures of truck tire builders were 3 times higher than personal NMOR exposures of passenger tire builders. NDMA, however, did not decrease in the tire tread warm-up mill area because there were no significant ventilation improvements and NDPhA continued to be used for truck tire tread. Nitrosamine results from rubber batches without additives seems to indicate that natural and synthetic rubber, and other basic ingredients are not sources of significant levels of nitrosamines. Finally, over a 7 month period, process sample NMOR levels decreased 200 fold and personal sample NMOR exposure in the feedmill and calendering area decreased by 96%. NPYR was reduced to non-detectable levels. The reduction was caused by improved ventilation and substitution of NDPhA with a phthalimide derivative.

Biological Samples. In December 1979, urine, and either blood, or stool specimens were collected from 15 non-smoking workers at the end of their work shift. Based upon environmental results from the previous two surveys, workers were selected according to high, medium and low nitrosamine exposure. The high exposure area was the feedmill and calendering area. The medium exposure areas were the truck tire tread warm-up mills, truck tire tread extrusion area, truck tire curing presses, and truck tire building area. The low exposure areas were receiving, shipping, passenger tire building, and Banbury area. Workers donating specimens for nitrosamine analysis were selected from at least one of these varied exposure areas. Results of analyses for nitrosamines were negative for all samples (detection limit = 1 ppb). Breathing-zone nitrosamine measurements were taken simultaneously for all workers volunteering biological specimens. The time-weighted average (TWA) exposure for these workers ranged from 18.2 $\mu g/M^3$ to 0.78 $\mu g/M^3$ for NMOR; from 0.180 to non-detectable for NDMA; and from 0.228 to non-detectable for NPYR.

In February 1980, urine samples were obtained from nine workers for mutagenicity testing by the Ames Salmonella test (10). Four hundred milliliters from each specimen were put through an XAD-2 column and the adsorbed material was eluted. Methylene chloride extracts of the post-column urine eluate were made and tested for mutagenicity with the Salmonella/ Mammalian Microsome Mutagenicity test. The TWA breathing-zone concentrations for nitrosamines taken from six workers donating

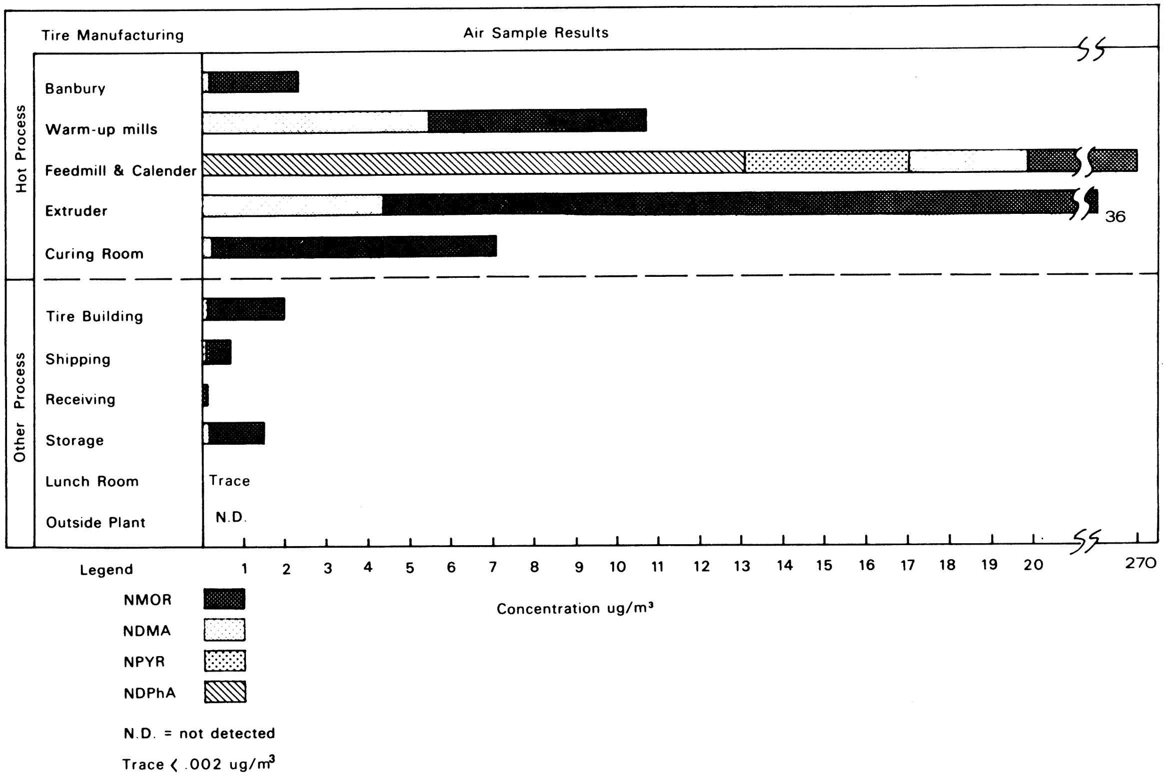


Figure 3. Highest reported N-*nitrosamine air samples during various stages of tire manufacturing*

urine specimens ranged from 1.3 $\mu g/M^3$ to 0.64 $\mu g/M^3$ for NMOR; and from 0.49 to 0.16 $\mu g/M^3$ for NDMA. NPYR was not detected in any environmental samples. The remaining three urine specimens were taken from truck tire builders for the purpose of monitoring mutagenic activity resulting from possible skin absorption (hands and forearms) of Nitrosamines. None of the samples tested were mutagenic.

The negative findings for nitrosamines in these biological samples may reflect the fact that they were collected only during the last two visits, at which time the airborne nitrosamine levels had been greatly reduced. For example, assuming a respiration rate of 10 liters per minute, even the most exposed workers during the third survey (18 $\mu g/M^3$) would have breathed approximately 1 µg NMOR per kilogram body weight per shift. NMOR is relatively lipophilic, but even if all of this nitrosamine had been absorbed and evenly distributed in all body components, and none metabolized or excreted during the shift, its concentration in blood (a relatively aqueous medium) would be below our detection limit (1 ppb).

Discussion

These results are generally in agreement with previous reports (1) that NMOR and NDMA are present in tire factories. NDPhA and NPYR are reported here for the first time as being present in tire factory air. What is unique about this factory is that NMOR was found at 250 $\mu g/M^3$--a level higher than any nitrosamine ever reported in any industrial site--and that its formation depended upon the thermal decomposition of NDPhA and the subsequent reaction of its nitroso group with preformed morpholino compounds. Company management response was effective in reducing nitrosamine levels first through improved ventilation, then by reducing or eliminating the use of NDPhA. However, NDMA is still a problem since it has remained above 1 $\mu g/M^3$ despite process changes that reduced NMOR levels. Laboratory testing of commercial grade amines has shown NDMA to be present as a contaminant (11). Most recently, NDMA has been shown to be generated from heated rubber stock containing a rubber accelerator tetra-methyl thiuram disulfide (2). The precursor chemicals and bulk process samples from this factory environment have not, as yet, been tested to confirm that these are the sources of the NDMA found. Transnitrosation of the nitroso group from NDPhA is known to occur under a variety of conditions (3,4,5). The nitrosation of the accelerators and stabilizers used in vulcanization (which are designed to break apart at elevated temperatures yielding the amines) was expected.

A second source of nitrosamine formation could be nitrosation of amines by NO_x, which has been clearly demonstrated in laboratory experiments (12). In industrial processes, NO_x was shown to be responsible for nitrosation of amines in foodstuffs dried with gas-fired burners (13), and also for the appearance of nitrosamines in diesel engine crankcase emissions (14). It is possible the NO_x could be nitrosating the amine rubber additives. It has been speculated that the source of NO_x is from the combustion of gasoline-powered forklift trucks used to transport rubber from one work station to another. Unfortunately, NO_x levels were not measured during any of the surveys to test this hypothesis.

A number of epidemiological studies conducted in the tire industry have shown workers to be at excess risk for cancer (15,16,17). In particular, excess cancer of the stomach and lung has been found in "hot process" areas in the tire plants (17). Although the majority of N-nitroso compounds have been shown to be animal carcinogens, including all nitrosamines found in this plant, they have not been directly associated with human cancer because no definable exposed population groups have been identified. Assessing the cancer risk for nitrosamine exposure among tire workers is confounded by past exposure to potential carcinogenic agents such as asbestos, benzene, beta-naphthalamine, and polynuclear aromatic hydrocarbons from carbon black. Although some of these agents have been reduced or removed from the tire industry, their confounding effect may obscure the health effects of nitrosamine exposure in future epidemiological studies.

Despite the epidemiological pitfalls, the nitrosamine levels found in this study are highly significant. Initially, NIOSH found the worst case exposures were typically among the feed mill and calendering operators; one worker had a time-weighted average NMOR exposure of 25 $\mu g/M^3$, an NDMA exposure of 0.37 $\mu g/M^3$, and an NPYR exposure of 0.78 $\mu g/M^3$. By February 1980, however, exposure to a feed mill and calender operator was reduced to 1.00 $\mu g/M^3$ for NMOR, and 0.2 $\mu g/M^3$ for NDMA. Compared to the worst case exposure from the fourth NIOSH survey, a worker in this area now would inhale approximately 6.0 µg of nitrosamines per shift, an exposure equivalent to eating a few strips of bacon and drinking a liter of beer.

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