

Occupational Vitiligo Due to Unsuspected Presence of Phenolic Antioxidant Byproducts in Commercial Bulk Rubber

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We investigated the occurrence of cutaneous depigmentation (vitiligo) among employees of a company that manufactured hydraulic pumps. The interiors of these pumps were injection-molded with rubber. We identified a small but significant cluster of vitiligo cases among a group of employees who frequently handled the rubber used in this injection molding process. Although none of the additives specified in the rubber formulations was a phenolic or catecholic derivative, known to be potential causes of chemically induced vitiligo, gas chromatographic analysis identified a para-substituted phenol (2,4-di-tert-butylphenol, DTBP) in solid samples of the most frequently used rubber. Surface wipe analysis confirmed that workers could be exposed to DTBP from simple handling of the rubber. We subsequently established that the solid bulk rubber used as the base in these stock rubber formulations contained both DTBP and smaller quantities of p-tert-butylphenol. Both had formed as unsuspected byproducts during chemical synthesis of two antioxidants added to the solid bulk rubber by a major rubber supplier. We conclude that the unsuspected presence of potential chemical depigmenting agents in solid bulk rubber, from which industrial rubber products are formulated, may contribute to the occurrence of occupational vitiligo, and that a simple review of ingredients in rubber formulations is inadequate to detect their presence.

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Vitiligo is a cutaneous disorder, characterized by progressive macular depigmentation due to loss of pigment-producing melanocytes, which affects approximately 0.5% of the general US population.¹ Clinical lesions most frequently occur on the face, dorsal hands, wrists, body folds, and orifices (eg, nose, mouth, nipples, umbilicus, perianal skin, and external genitalia). The condition tends to affect symmetrical and bilateral areas. Advanced lesions are usually completely depigmented, but early lesions may show only partial hypopigmentation. The borders of lesions may be irregular and hyperpigmented, whereas perifollicular skin within patches of vitiligo may retain its normal pigmentation. In strictest usage, the term "vitiligo" refers to an acquired cutaneous disorder for which no specific etiology has been identified, although autoimmune, neurotoxic, and melanotoxic hypotheses have been advanced.² However, both clinical and experimental studies have firmly established that certain phenolic and catecholic chemicals may produce a toxic, cutaneous depigmentation which is clinically and histopathologically indistinguishable from idiopathic vitiligo.³ Epidemiologic investigation and industrial hygiene analysis may therefore be required to establish whether observed cases of vitiligo are idiopathic or induced by occupational exposure to chemical depigmenting substances.

In October, 1985, researchers for the National Institute for Occupational Safety and Health (NIOSH) and the Industrial Commission of Ohio initiated an investigation of vitiligo cases among employees of a company which manufactured specialized, cylindrical hydraulic pumps. A consulting dermatologist who had diagnosed vitiligo in one employee had also noted that this employee worked in the company's rubber department; chemically induced vitiligo was suspected, which prompted the company to request further investigation.

We report below the results of this investigation, which implicated 2,4-di-*tert*-butylphenol (DTBP) as a probable causal agent of chemically induced vitiligo among employees who had frequently handled rubber in the rubber department, where injection molding of the pump interiors was performed.

Methods

Review of Manufacturing Process

The company manufactured cylindrical hydraulic pumps designed for specialized industrial uses. In the general production area, pump cylinders were ground and finished according to design specifications. Following this initial grinding and finishing, the cylinders were shipped for injection molding to the rubber department, where the interior cavities were coated with various types of rubber, formulated to withstand specific corrosive industrial liquids. The rubber used in this operation was purchased from an outside contractor, who custom-formulated solid blocks of bulk rubber (purchased from a major rubber manufacturer) with various additives, as specified by the company. The unprocessed bulk rubber made up approximately 40% to 60% by weight of the final custom-formulated stock rubber. The injection molding process itself required the milling of the formulated stock rubber at temperatures ranging from 140 to 180°F, following which it was loaded into an industrial "gun" and injected through a mold into the pump cavities. After injection molding and curing of the rubber in an autoclave, the pump cylinders were returned to the general production area for further grinding, finishing, and final pump assembly.

Clinical and Epidemiologic Investigation

Of 272 total workers currently employed in the rubber department and general production area, 200 participated in the medical survey (73%). Twenty-two refused participation, and the remaining 50 employees were absent due to vacations or illnesses. Of the 200 participants, 159 were male and 182 were white, ranging in ages from 25 to 64 years. Eighty-six were current or former employees of the rubber department.

We evaluated survey participants by questionnaire and complete dermatologic examination. On the questionnaire, we asked all survey participants whether they currently had any white spots anywhere on their skin. We examined the entire skin surface of positive responders for typical cutaneous lesions of vitiligo with a Wood's lamp, including the perianal skin and external genitalia. We also examined approximately every fifth negative responder in a similar fashion. The use of a Wood's lamp (which emits black light irradiation in the long wavelength spectrum of ultraviolet light) facilitates visualization of hypopigmented skin as well as the degree of hypopigmentation. Vitiligo was diagnosed only when hypopigmented but otherwise normal skin was

clinically consistent with such a diagnosis and also demonstrated complete depigmentation following visualization with a Woods' lamp in a darkened room.

The overall prevalence of vitiligo was compared to the prevalence of vitiligo in the general US population after adjusting for the age and sex of the employees, based on age- and sex-specific rates published in the first National Health and Nutrition Examination Survey NHANES I.¹ Survey participants also listed all jobs held since onset of employment at the company. We independently ranked the degree of potential exposure to rubber for each job category, based on a visual assessment of time during a usual workshift that employees in each job category would directly handle rubber, either cured or uncured (no employees wore gloves while handling rubber). We then compared the prevalence of vitiligo within the company to rubber exposure rankings based on job classification. We considered workers whose job classifications required them to frequently handle rubber to have high exposure, and considered those whose contact was minimal or infrequent to have low exposure. We performed only one-sided significance testing, since there was no rational basis upon which to assume that rubber handling might protect against the development of vitiligo.

Industrial Hygiene Analysis

We reviewed the formulations of all rubber stock and spray adhesives used in the injection molding operation for the presence of phenols or catechols known to cause chemical depigmentation.⁴ We obtained samples of both unprocessed solid bulk rubber (used as the base for the rubber formulations) and the custom-formulated rubber (both cured and uncured), and then subjected them to heating at 150°C for 30 min in a tube furnace, during which time air was continuously passed through the furnace and into a silica gel tube. Any effluent collected from the heated rubber samples was desorbed from the silica gel in ethanol and analyzed by gas chromatography and mass spectrometry (GC/MS). Quantitative analysis was performed by extracting a weighed portion of the rubber samples in 10 ml of carbon disulfide using an ultrasonic bath, analyzing by gas chromatography, and comparing with a weighed standard. Surface wipes of the custom-formulated rubber samples were obtained by rubbing with ethanol-soaked cotton swabs. The surface wipes were subsequently extracted in a sonicated ethanol bath, analyzed by GC/MS, and compared with a blank swab treated in the same manner. We also obtained samples of the two antioxidants added to the unprocessed solid bulk rubber by its commercial supplier for analysis by GC/MS.

Results

Clinical and Epidemiologic Investigation

Fifteen (7%) of the survey participants reported white spots on their skin. We examined all 15 positive

responders and 43 of 185 participants who did not report any cutaneous white spots on the questionnaire. Five of the 15 positive responders had vitiligo confirmed by clinical examination and Wood's lamp fluorescence (Fig. 1); two had extensive vitiligo on the genitals, trunk, and extremities, two had vitiligo confined to the genitals, and one had vitiligo affecting the genitals and arms. All except this latter case began after the start of employment at the company. The two employees with extensive vitiligo both reported onset of their conditions more than 10 years prior to the date of this survey; the other two people reported onset within the preceding 2 years. All cases occurred in white men. One employee with extensive vitiligo also had alopecia areata.

Clinical examination determined that cutaneous disorders other than vitiligo, which included melasma, welding chip burns, discoid lupus erythematosus, and dishydrotic eczema, accounted for the "white spots" noted on the questionnaire by the remaining 10 positive responders. No cases of vitiligo were detected among the 43 negative responders who also underwent complete dermatologic examination.

Compared with the expected number of cases of vitiligo (based on data from the NHANES I survey, after adjusting for age- and sex-specific rates), a significantly higher prevalence existed at the company (5 observed, 1.1 expected; standardized morbidity ratio (SMR) = 4.5; $P = .005$; 90% lower confidence limit (CL) = 1.8, using a one-sided exact test for a Poisson variate⁶). Since inclusion of the initial case which prompted this investigation could have biased the results in favor of significance, we repeated the analysis after excluding this case; the prevalence still remained significantly higher (SMR = 3.6; $P = .03$; 90% lower CL = 1.2).

Within the rubber department, only the gun and mill operators, mold assemblers (who trimmed excess rubber injected through the molds), and group leaders (who performed all job tasks) directly handled rubber on a frequent basis. Other job classifications within the rubber department, as well as all job classifications outside the rubber department, had minimal or infrequent contact with the rubber in either its cured or uncured state.

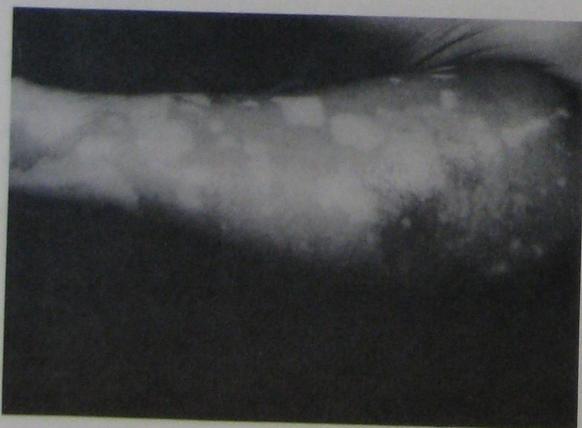


Fig. 1. Vitiligo on the arm of a gun and mill operator in the rubber department.

After excluding the one case of vitiligo in which onset began before employment at the company (ie, before exposure to rubber), current employees who had worked in these job classifications with relatively high exposure to rubber had a significantly greater risk for developing vitiligo than employees who had worked in job classifications judged to have relatively low exposure (Table 1) (risk ratio = 22.0; 90% lower CL = 3.4; one-sided Fisher's exact $P = .006$).

Industrial Hygiene Analysis

Our review of the ingredient lists for the rubber formulations and spray adhesives identified phenol-formaldehyde resin in a few products. However, we could not find any para-substituted phenolic or catecholic derivatives among these ingredients which were recognized or suspected causes of cutaneous depigmentation.⁴

Analysis of formulated stock rubber samples by GC/MS disclosed the unexpected presence of the para-substituted phenol DTBP in both cured and uncured samples. DTBP was present in rubber samples formulated with or without phenol-formaldehyde resin, suggesting that its presence in the formulated rubber was unrelated to the use of phenol-formaldehyde resin as an additive. Surface wipe samples from formulated stock rubber also demonstrated the presence of DTBP in both cured and uncured samples. No substituted phenolics of any type could be detected by GC/MS in the spray adhesives containing phenol-formaldehyde resin. *p*-tert-Butylphenol (PTBP) was detected in unprocessed bulk rubber used as the base for the rubber formulations, but was below the analytical limits of detection in the formulated stock rubber.

Quantitative analytical results for DTBP and PTBP are presented in Table 2. Concentrations of DTBP in solid stock rubber formulations ranged between 0.01% and 0.03% by weight (100 to 300 ppm). DTBP in unprocessed solid bulk rubber ranged between 0.05% and 0.06%; smaller quantities of PTBP (0.01%) were also detected. When commercial samples of the two antioxidants used in the bulk rubber were analyzed, DTBP was detected at 7.4% and 0.88% concentrations by weight, respectively; both antioxidants contained approximately 1% PTBP by weight.

TABLE 1
Prevalence of Vitiligo and Exposure to Rubber

Exposure*	Vitiligo†	No Vitiligo	Totals	Case Rate, %
High	3	21	24	12.5‡
Low	1	174	175	0.6
Totals	4	195	199	

* All current employees who presently or had ever worked as gun and mill operators, group leaders, or mold assemblers in the rubber department were considered to have high exposure to rubber, relative to the remaining survey participants.

† Excludes one case of vitiligo whose onset occurred prior to employment at the company.

‡ Risk ratio (90% lower confidence limit) = 22.0 (3.4); $P = .006$ (one-sided Fisher's exact test).

TABLE 2

Results of Gas Chromatography/Mass Spectrometry Analysis of Stock Rubber, Unprocessed Bulk Rubber, and Antioxidant Samples for 4-*tert*-Butylphenol (PTBP) and 2,4-Di-*tert*-butylphenol (DTBP)*

Sample	% by Weight	
	PTBP†	DTBP
Formulated stock rubber no. 1	ND‡	0.01
Formulated stock rubber no. 2	ND‡	0.03
Unprocessed bulk rubber no. 1	0.01	0.05
Unprocessed bulk rubber no. 2	0.01	0.06
Antioxidant A	0.98	7.4
Antioxidant B	1.02	0.88

* Limits of detection: 0.003% by weight, limits of quantification: 0.008% by weight.

† Estimated from standard absorption curve for 2,4-di-*tert*-butylphenol.

‡ ND = none detected.

Discussion

Clinical and experimental observations have identified the following chemical substances which may cause cutaneous depigmentation: hydroquinone; monomethyl, monoethyl, and monobenzyl ethers of hydroquinone; *p*-cresol; *p*-isopropylcatechol; *p*-methylcatechol; *p*-nonyl phenol; *p*-octylphenol; *p*-phenylphenol; *p*-*tert*-amylphenol; *p*-*tert*-butylcatechol; PTBP; β -mercaptoethylamine hydrochloride; *N*-(2-mercaptoethyl)-dimethylamine hydrochloride; diisopropyl fluorophosphate; and physostigmine.⁴ Optimal depigmenting action from phenols and catechols is seen when the para position is occupied by a substituted nonpolar side chain.⁶ The mechanisms of action may involve interference with synthesis of melanin from its precursor amino acid tyrosine, which these substances structurally resemble (Fig. 2), leading to the formation of semiquinone free radicals and death of the melanocyte through peroxidation of its lipoprotein membranes.³ Rubber formulations frequently contain para-substituted phenols as antioxidants or plasticizers, and exposure to rubber has often been implicated in clinical occurrences of chemical depigmentation.

Although our ingredient review failed to disclose the presence of any potentially depigmenting para-substituted phenols or catechols, we found an excessive prevalence of vitiligo among company employees compared with data from the NHANES I survey, after adjusting for age and sex. Dermatologic diagnoses established in this survey were based on clinical findings of examining dermatologists in a national statistical probability sample of 20,749 participating subjects, ages 1 to 74 years, selected from US households to represent the non-institutionalized civilian population during the survey period (1971 to 1974); diagnoses in this survey have been further stratified by age and sex. NIOSH has previously demonstrated the potential usefulness of data from the NHANES I survey in epidemiologic studies for comparisons of prevalence rates of chronic diseases where occupational exposure is a distinguishing feature.⁷ Such analysis may be a useful preliminary step in occupational studies involving chronic prevalent cutaneous diseases such as vitiligo.

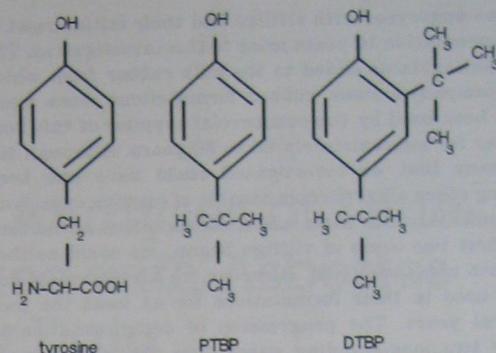


Fig. 2. Structural similarities of *p*-*tert* butylphenol (PTBP) and 2,4-di-*tert*-butylphenol (DTBP) to tyrosine, the amino acid precursor of melanin.

Subsequent analysis of vitiligo cases by job classifications within the company supported a causal relationship between the occurrence of vitiligo and the handling of rubber. After excluding one employee whose onset of vitiligo occurred prior to employment at the company, we found an excessive prevalence in those job classifications judged to have relatively high exposure to stock rubber formulations prior to our data analysis. Industrial hygiene analysis confirmed the presence of a potential chemical depigmenting agent (DTBP) in both cured and uncured samples of the most frequently handled rubber formulations, and positive wipe samples demonstrated that skin exposure could occur from simple handling of the rubber. The concentration of PTBP in samples of the rubber formulations actually handled by the workers was below our analytical limits of detection, but it was detected in samples of the bulk rubber used in these formulations. Although its concentration level was only approximately 15% to 20% of DTBP, a primary depigmenting role for PTBP cannot be absolutely excluded.

Although no direct toxicologic studies of the depigmenting properties of DTBP have been performed, its chemical structure differs from the potent chemical depigmenting agent PTBP only by the presence of an additional tertiary butylphenol side chain on the aromatic ring. Neither DTBP, PTBP, nor related alkyl phenols were used in the company's specified stock rubber formulations. We traced its unsuspected presence in the stock rubber formulations to the phenolic antioxidants added to the bulk rubber (used as a base for the stock rubber formulations) by a major rubber manufacturer who generically identified them as modified and butylated derivatives of bisphenol A. Both DTBP and PTBP had formed as unsuspected byproducts during chemical synthesis of these proprietary antioxidants, apparently the result of butylation and cleavage of the phenolic rings of bisphenol A. According to this manufacturer, antioxidants are routinely added to bulk rubber to prevent deterioration during storage and shipping, and phenolic antioxidants are popular choices among many commercial suppliers and manufacturers of nitrile and styrene butadiene rubber products.

Two employees with vitiligo had their initial onset of depigmentation 10 years prior to this investigation. The two antioxidants added to the bulk rubber from which the company's stock rubber formulations were made have been used by the commercial supplier of this bulk rubber for approximately 25 to 30 years. Although the company that we investigated could have also been adding other alkyl phenols capable of causing cutaneous depigmentation to their rubber formulations at the time the first two cases of vitiligo began, we could neither confirm nor assess this. However, no alkyl phenols had been used in their formulations for at least the past several years. The progression of depigmentation in these two long-standing cases over the past several years, as well as the detection of a relatively recent onset of vitiligo in an employee who also worked in the rubber department, suggest that the concentrations of DTBP within the formulated rubber associated with the occurrence of vitiligo in this investigation (100 to 300 ppm) were sufficient to cause depigmentation. European manufacturers using PTBP have set an airborne exposure threshold limit of 1 ppm for their workers, based on their experience with depigmentation from hydroquinone.⁸ Cutaneous exposure limits do not exist, since no "safe thresholds" have ever been determined. Gellin et al⁹ reported an outbreak of toxic vitiligo among tappet assemblers from cutaneous exposure to an oil containing only 0.005% (50 ppm) of *p*-tert-butylcatechol by weight.

The route of exposure by which chemical depigmenting agents produce toxic vitiligo is still a subject of debate, since hypopigmented patches often occur on body sites well removed from primary or obvious contact. James et al⁸ suggest that systemic absorption accounts for this, whereas Malten et al¹⁰ suggest that indirect transfer of material from the hands to more remote skin surfaces may be important. In our investigation, all four employees with vitiligo whose onset occurred after employment at the company had genital lesions, and in two the lesions were confirmed to this body site. Whether this occurred by transfer from the hands to the genitals or from systemic absorption (eg, accidental ingestion while eating with contaminated hands) remains speculative. One case was associated with another autoimmune condition, alopecia areata. Although James et al⁸ suggest that chemical depigmenting agents do not cause other autoimmune phenomena, this view has been disputed.¹¹ We made no attempt to measure autoantibodies (eg, thyroid antibody) in our investigation.

Persons with preexisting vitiligo may be more sensitive to the chemical depigmenting effects of alkyl phenols (James Nordlund, MD, Department of Dermatology,

University of Cincinnati Medical Center; personal communication). Pre-employment screening and routine medical surveillance for vitiligo, with complete dermatologic examinations and Wood's lamp fluorescence, should be performed wherever potential work exposures include these substances. Our experience suggests that surveillance for vitiligo among rubber handlers is appropriate, even when para-substituted alkyl phenols are not specifically added to rubber formulations, since these potential depigmenting agents may be unsuspectedly present in phenolic antioxidants added to bulk rubber by major rubber manufacturers.

Based on the findings in this investigation, the manufacturer of the two antioxidants containing DTPB and PTBP had decided to discontinue their use in any new rubber products and to label the material safety data sheets of these antioxidants with a warning on cutaneous depigmentation; the feasibility of synthesizing these antioxidants without the unwanted formation of DTBP and PTBP byproducts is currently under evaluation.

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