

Adverse effects of gasoline on the skin of exposed workers

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Gasoline is widely used as a solvent in industry. To study its adverse effects on the skin and to understand their mechanisms, a matched epidemiological study (1:1, 52 exposed workers and 52 control subjects) was developed. Information about general conditions, history of dermatosis, changes in skin after exposure to gasoline, etc., was obtained. Ceramide, fatty acid and cholesterol collected from the backs of the hands were analyzed by high-performance thin-layer chromatography (HPTLC), because stratum corneum lipids play a predominant rôle in maintaining the physiological function of skin. The results showed that prevalences of hyperkeratosis, dryness, onychosis and dermatitis were clearly higher in exposed workers than in the control group, prevalence ratios being 3.33 ($p < 0.05$), 3.00 ($p < 0.001$), 11.25 ($p < 0.001$), 5.00 ($p < 0.001$), respectively. Fissures and onychorrhexis were the common symptoms in exposed workers. The stratum corneum lipid levels of ceramide, fatty acid and cholesterol were significantly lower in the exposed group than in the control group ($p < 0.05$). Findings indicated that prolonged or repeated contact with gasoline could cause fissuring of the skin and nail disorders, and that the mechanism was perhaps depletion of stratum corneum lipids.

Key words: gasoline; occupational; stratum corneum lipids; epidemiological; cross-sectional study; irritant contact dermatitis; nail disorders. © Munksgaard, 2002.

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The skin is one of the major organs in human beings that interfaces with the environment. Not only the barrier against chemicals, microorganisms, heat, cold, etc., but also one of the most important targets for industrial exposure. In many countries, skin disease ranks 1st among all notified occupational diseases, constituting up to 30% of the total (1, 2). In Germany, 34% of all registered occupational disease is skin disease, and in China about 60% (3, 4). The incidence rate of occupational skin disease is believed to be around 0.5–1.9 cases per 1000 full-time workers per year (5–7).

Accounting for more than 90% of all cases of occupational skin disease attributed to chemicals, weak (marginal) irritants, e.g. organic solvents and detergents, are the most common cause of occupational dermatoses (1, 4). Organic solvents are common industrial chemicals, and gasoline, as a solvent, is widely used in many industries, rubber, plastic and paint manufacture and mechanical repair. In spite of the widespread use and exposure to gasoline, its toxic effects on the skin have not been well studied (8). To our knowledge, though

there are some clinical and epidemiological reports on the adverse effects of gasoline on the skin, there is an absence of information on stratum corneum lipids in the skin of workers exposed to gasoline. The skin has been reported to show decreases in major lipid species with ageing (9, 10). Thus, to control for the effect of age on skin lipids, exposed and control workers from whom stratum corneum samples were collected were sampled stratified according to age, and the analytical results on lipids were also stratified for age in this study.

Materials and Methods

Subjects

A matched (1:1) epidemiological study was developed, the matched condition being age (± 1 year). Exposed workers, all female, were employed in a sewing machine factory, and were all included. The work process was to clean sewing machines and to moisten a transfer (a design of a flower, butterfly, etc., adherent to paper) with yarn wetted with no. 200 gasoline (98% decane, nonane and hendecane,

without additives), then apply the transfer to the sewing machine, thereafter removing the paper and leaving the design on the machine. Because this was a fiddly process, the workers did not wear gloves. They worked for 7 h per day, and got through 500 ml of gasoline per person per day. There were ventilation facilities in the workplace, and the ambient temperature was around 20°C. The level of gasoline in the air was below the maximum allowed concentration (MAC) of 300 mg/m³.

The control group were female workers in a spinning mill, their workload and salary being almost identical with the exposed workers⁹. They had no contact with industrial chemicals. Those women with atopic eczema, past or present, or other dermatoses on the hands before starting the work were excluded. 52 exposed workers and 52 control subjects were investigated. 30 pairs of exposed and control workers were sampled stratified according to age. Their stratum corneums were sampled and the content of ceramide, fatty acid and cholesterol measured by high-performance thin-layer chromatography (HPTLC) (10–12).

Questionnaire and examination of skin status

The study was based on an interview questionnaire, which included questions about general conditions, occupational history, frequency of hand washing during the working day, and use of skin-care products. The current skin status was examined by the same dermatologist in the occupational health departments of each factory, the doctor not knowing to what chemicals the workers were exposed.

Sample collection

Skin samples were removed from the back of hand by sequential stripping with 8 pieces of tape strip (Tape 1601, Hadleigh Enterprise, England) on the last day before the weekend. All tapes were stored at -20°C until analysed. Corneocytes were removed from the tapes by sonication in methanol. The squames were then dried under nitrogen and the lipids extracted with chloroform/methanol (2:1) for 2 h.

Analysis of stratum corneum lipids

The lipid extracts were evaporated under nitrogen, reconstituted in chloroform (200 µl) and separated on amino-propyl bonded silica gel columns (100 mg Bond Elut NH2, Analytichem International, USA) preconditioned with hexane (2 ml). The samples were loaded onto the columns and cholesterol, ceramide and fatty acid fractions were eluted with 2-ml hexane/ethyl acetate (85:15), chloroform/isopro-

panol (2:1) and 2% acetic acid in methanol, respectively. All the lipids were then separated using high-performance thin-layer chromatography (HPTLC) on 20×10 cm plates (HSGF254, Boao, China). The ceramide species were separated according to the method of Wertz et al. (13–15), and the fatty acids and cholesterol were separated by eluting up the plate with hexane/ethyl acetate (85:15, then 70:30) to 95 mm. After development, the HPTLC plates were dried, stained (aqueous solution containing 10% copper sulfate, 8% orthophosphoric acid) and then charred at 160°C for 20 min. Finally, the HPTLC plates were cooled and quantified using a scanning densitometer at 420 nm (CS-910, Daojin, Japan). Lipids were identified by their comigration with cholesterol, palmitic acid and N-stearoyl DL-dihydrosphingosine standards (Sigma Chemical Company). Ceramide subclasses were also identified by their *rf* values obtained from the literature. The mass of each lipid was determined from the appropriate series of standards chromatographed on each plate.

Analysis of stratum corneum protein levels

To allow interpersonal comparisons, the mass of each lipid fraction was normalized to the amount of stratum corneum removed by tape stripping. Stratum corneum removal was estimated by quantification of the detergent-soluble protein of the corneocytes extracted under reducing conditions. The lipid-depleted corneocytes were extracted with 1% sodium dodecyl sulfate, 10 mM sodium phosphate buffer and 20 mM mercaptoethanol at 60°C for 2 h. Aliquots of the protein extracts were then dried, reconstituted in water and quantified using the Lowry protein microassay (16). All lipid levels were expressed as ng lipid per µg protein.

Statistical analysis

The results of the questionnaire and the lipids of the stratum corneum were evaluated using computer-based statistical programs (Epi-info 6.04c and SPSS 10.0 for Windows). Prevalence ratios (PR) and χ^2 were calculated, and the corneum lipids were reported as means±SD. The significance of the difference in lipids between the 2 groups was evaluated by the Student *t*-test, *p*<0.05 being considered significant.

Results

General characteristics of the study population

Age and job duration of exposed workers were 34.7±7.0 and 9.7±6.2 years; these parameters were

almost identical to those of control workers, 34.4 ± 7.0 and 7.0 ± 5.8 years.

Comparison of skin status between exposed and control groups

The prevalences of hyperkeratosis, dryness, onychosis and dermatitis were clearly higher in the exposed workers than in the control group, the PRs being 3.33 ($p < 0.05$), 3.00 ($p < 0.001$), 11.25 ($p < 0.001$), 5.00 ($p < 0.001$), respectively. Fissures were a common sign, present in 75.0% of exposed workers; most frequently this occurred at the tips of the fingers.

Nail disorders in exposed workers

Nail disorders in workers exposed to gasoline included onychorrhexis, leuconychia, onychauxis and schizonychia. Onychorrhexis (62.3%) and leuconychia (13.2%) were the major signs of onychosis (data not shown). Leuconychia mainly appeared as small white dots and in striations.

Exposure time and dryness of skin in exposed workers

There was no correlation between dryness of skin and exposure time ($r = 0.01$, $p > 0.05$). Dermatitis regularly occurred 1 month after exposure to gasoline began.

Lipid levels in both groups

Stratum corneum lipid levels of ceramide, fatty acid and cholesterol were significantly lower in the exposed group than in the control group (Table 1). Stratified by age, there were significant differences between exposed and control groups in each age stratum, except for the level of cholesterol in the 30–39 years group.

Discussion

It has been estimated that 75% of occupational dermatitis cases are caused by irritants (17). Such irritants are mostly chemicals, classified as acute

or chronic (cumulative) irritants (18). The irritancy of organic solvents, including gasoline, and soluble cutting fluids tends to be insidious, and is often not recognized by the workers as deleterious, because damage occurs over days, weeks or months of repeated exposure.

In this study, we found that gasoline could induce dermatitis in most exposed workers. This type of skin disease had a spectrum of clinical features, which ranged from a little dryness, reduction of sheen and elasticity, hyperkeratosis, "chapping" or leuconychia to fissuring and brittle nails. Fissuring was the most frequent symptom, and onychorrhexis another important disorder. No correlation between dryness of the skin and duration of exposure to gasoline was found, the most probable reason being that dryness was the most common sign of irritant contact dermatitis, and most of the workers had developed dermatitis within 1 month of starting to use gasoline as a solvent, while the job duration of exposed workers was at least 1 year, in the study.

Gasoline, as a solvent, has the ability to dissolve lipid or fatty materials and dissolves the lipids in the skin on contact. Analyses of stratum corneum lipids demonstrated that exposure to gasoline could significantly decrease ceramides, fatty acid and cholesterol. The unique morphology of the stratum corneum, often compared to "bricks and mortar", is essential to maintaining the barrier function of the skin (19, 20). The protein-enriched corneocytes are embedded in an intercellular lipid matrix, composed primarily of ceramides, cholesterol and fatty acids, together with smaller amounts of cholesterol sulfate, glucosylceramides and phospholipids. These lipids form multilamellar sheets within the intercellular spaces of the stratum corneum, the organization of which is essential to maintaining the functionality of the skin as an effective barrier to water loss (21, 22). Moreover, the lipids also play an important rôle in determining the mechanical, cohesive and desquamatory properties of the stratum corneum, and therefore play a key rôle in skin physiology (23, 24). Changes in stratum corneum lipid levels have been linked to aberrant skin conditions, such as xerosis (23, 25, 26). Depletion in lipid levels as a result of exposure to gasoline could lead to dis-

Table 1. Contents of ceramide, fatty acid and cholesterol in exposed workers and controls (means $\pm$ SD)

Age (years)	Ceramide (ng/ μ g protein)		Fatty acid (ng/ μ g protein)		Cholesterol (ng/ μ g protein)	
	control (n)	exposed (n)	control (n)	exposed (n)	control (n)	exposed (n)
19–29	178.4 $\pm$ 32.2 (10)	121.9 $\pm$ 2.9* (10)	173.4 $\pm$ 99.7 (10)	64.7 $\pm$ 25.3* (10)	29.4 $\pm$ 12.5 (10)	17.4 $\pm$ 7.7* (10)
30–39	148.6 $\pm$ 39.2 (10)	82.4 $\pm$ 3.6** (10)	160.9 $\pm$ 70.6 (10)	55.7 $\pm$ 11.1** (10)	22.1 $\pm$ 13.2 (10)	14.6 $\pm$ 4.9 (10)
40–50	111.0 $\pm$ 18.9 (10)	61.9 $\pm$ 30.4** (10)	159.0 $\pm$ 59.8 (10)	44.2 $\pm$ 14.4** (10)	18.3 $\pm$ 8.0 (10)	10.4 $\pm$ 4.4* (10)
mean	146.3 $\pm$ 40.9	88.1 $\pm$ 45.1**	164.1 $\pm$ 75.2	54.9 $\pm$ 18.6**	23.2 $\pm$ 12.1	14.2 $\pm$ 6.2**

vs control group *: $p < 0.05$; ** $p < 0.01$

turbances in skin function, for example, decline in lipid levels leads to increased water loss from the skin and influences the process of keratinocyte metabolism, so that the skin shows dryness, "chapping", and hyperkeratosis as clinical signs. The nail grows in the same way as the horny layer and is affected by similar pathogenic mechanisms to those responsible for diseases of epidermis (27). Thus, depletion in lipid levels was perhaps the mechanism of both skin and nail disorders induced by gasoline.

In summary, the present study demonstrated that prolonged or repeated skin contact with gasoline caused dryness and fissuring of the skin, as well as nail disorders. Decline in stratum corneum lipid levels was a likely contributor to the increased susceptibility of the skin to occupational skin disease caused by gasoline.

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