Toxicology and Industrial Health (2000) 16, 203–210

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Effects of metal working fluids on B6C3F1 mouse skin[1]

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Over 10 million workers in the United States are exposed to metal working fluids (MWFs) through dermal contact and/or inhalation of aerosolized fluids. The objective of this study was to elucidate the response of skin to dermal exposure to MWFs. Four- to six-week-old B6C3F1 mice of both sexes were divided into eight groups ($n=5$/group) and exposed to 200 μl of 0%, 5% (pH 7 and 9.7) and 100% (pH 10.4), unused MWFs/H_2O by topical application to the unshaven back (cervical to sacral region), twice a week for 6 weeks. Skin-mast cell number in females of most treated groups and of two male groups (100% and 5%, pH 7) were significantly higher than the control groups. Eventhough both males and females (treated with 100% MWF/H_2O) showed an increase in the skin-histamine levels (38% and 41%, respectively), this increase was significant in females only (ANOVA, $P\leq0.05$). Dermal exposure to 100% MWFs increased liver weight significantly in both sexes. Ulcers and associated inflammation were seen in the skin of mice treated with 100% unused MWFs and sacrificed at 6 weeks, but not in the recovery groups. Hypertrophy of the sebaceous gland epithelium is present in all mice treated for 6 weeks and sacrificed immediately. However, only the mice treated with 100% MWF retained the hypertrophy of this epithelium after a 6-week recovery period. In conclusion, dermal exposure to unused semi-synthetic MWF penetrates the normal skin, induces mast cell accumulation in the skin, produces hypertrophy of the sebaceous glands, and may affect females more than males. *Toxicology and Industrial Health* (2000) **16,** 203–210.

Keywords: *histamine, kidney, liver, mast cells, metal working fluid, mice, skin, ulcer.*

Introduction

In the early 1900s, metal working fluids (MWFs) were used for the first time to prolong the tool life of equipment (Newhouse, 1982). These fluids have been used to reduce friction between the cutting tool and the work surface, reduce wear and galling, protect surface characteristics, reduce surface adhesion or welding, carry away generated heat, and flush away swarf, chips, fines, and residues. It has been estimated that over 10 million workers in the United States are exposed to MWFs (Jackson, 1992). Such workers are involved in numerous industries and they perform a wide range of operations including cutting, drilling, grinding, and milling. Four major types of MWFs are available for use in the workplace: the traditional straight oils, soluble fluids, semi-synthetic fluids, and synthetic fluids (no oil). Refined petroleum oils may be used as base oils in all MWFs except the synthetics. These refined oils are complex mixtures of hydrocarbons (aromatics, naphthenes, paraffins, and cycloparaffins) and other organic compounds containing sulfur, oxygen, and nitrogen.

The two principle routes of occupational exposure to MWFs are dermal contact and inhalation. Dermal exposure to MWFs occurs through skin contact from splashes and aerosols produced during immersion or flooding of the machine tools or work site, or by handling parts, tools, and equipment that are covered with MWFs. Emulsifiers in and alkalinity of MWFs have been proposed to be causes of irritant contact dermatitis (Pryce et al., 1989). Population-based prevalence studies reported that irritant and allergic contact dermatitis was more frequent in women than men (Agrup, 1969; Coenraads et al., 1983; Meding, 1990).

MWFs are complex mixtures of eight or more chemicals. Semi-synthetic MWFs contain the following: water, mineral oils, emulsifiers, chelating agents, coupling agents, antiweld agents, surfactant wetting agents, antifoaming agents, alkaline reserve, dyes, corrosion inhibitors (anti-rust), biocides (bioresistant compounds), and extreme pressure additives (Key et al., 1983; Howell, 1996). Toxicities of some individual components of MWFs have been reported. Two-year dermal exposure of B6C3F1 mice to diethanolamine (DEA), a component of MWFs, caused an increased incidence of hepatocellular adenomas and carcinomas, and hepatoblastomas in both males and females (NTP, 1994). Formaldehyde-releasing antimicrobial agents like triazine can cause allergic or contact dermatitis (Zugerman, 1986). Semi-synthetic MWFs were found to

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[1]Part of the data in this research has been published in the Proceedings of the Metal Working Fluid Symposium II, September 15–18, 1997, Detroit, Michigan. Please refer to the publisher information on page number ii.

be the primary cause of irritant contact and occasionally allergic contact dermatitis (Fisher, 1986).

The dermal effects of semi-synthetic MWFs have not been completely elucidated. Therefore, the objective of this study was to determine the effect of dermal exposure of B6C3F1 mice to unused semi-synthetic MWF on the dermal histology, total skin histamine levels and mast cell numbers, organ weight and anatomical changes that may suggest the possibility of a systemic response.

Materials and methods

Preparation of MWFs

The 5% concentration of unused MWF was prepared in municipal water (v/v) to represent the concentration of MWFs used in metal cutting industry, and the 100% concentration of unused MWF was used as obtained without any additives.

Animal Exposure

Four- to six-week-old B6C3F1 mice of both sexes from Harlan Sprague Dawley and Charles River Laboratories were divided into eight groups as shown in Table 1. All groups were used after 2 weeks of acclimatization. Mice were housed one per cage and kept at 75 (±5)°F, 40–60% relative humidity, with a 12-h light/dark cycle in an AAALAC accredited facility. Municipal water and feed (Harlan Teklad Rodent Diet #8604) were provided *ad libitum*. Sany Chips Bedding (Harlan Teklad) was used and changed once a week. Control mice were left without any treatment and unshaven. The semi-synthetic MWF used in our study is similar to the one presented in a NIOSH publication (NIOSH, 1998). Exposure groups were treated with 200 μl of the respective fluids twice a week (Monday and Thursday) for 6 weeks. Fluids were dispensed on the unshaven (to avoid any injury) back skin from the cervical to the sacral region (Figure 1) using a 1 ml tuberculin syringe. The fluids were evenly distributed with the help of a curved gavage needle throughout the back. Recovery groups were kept for 6 weeks post-exposure without further treatment. Animals were weighed every 2 weeks and at sacrifice.

Table 1. Skin application of metal working fluid to B6C3F1 mice: experimental design.

Treatment[a]	Number of mice[b]	
	Males	Females
Control	10	10
Control (Recovery)[c]	10	10
100% MWF[d], pH 10.4	10	10
100% MWF (Recovery)	10	10
5% MWF/H_2O[e], pH 9.7	5	5
5% MWF/H_2O (Recovery)	5	5
5% MWF/H_2O, pH 7	5	5
5% MWF/H_2O, pH 7 (Recovery)	5	5

[a]Exposure was twice a week with 200 μl of the respective fluid dispensed on the unshaven back for 6 weeks. All groups were not exposed at the same time.
[b]B6C3F1 mice.
[c]Recovery groups were kept for 6 weeks post-exposure without further treatment.
[d]Metal (machining) working fluid.
[e]Distilled water.

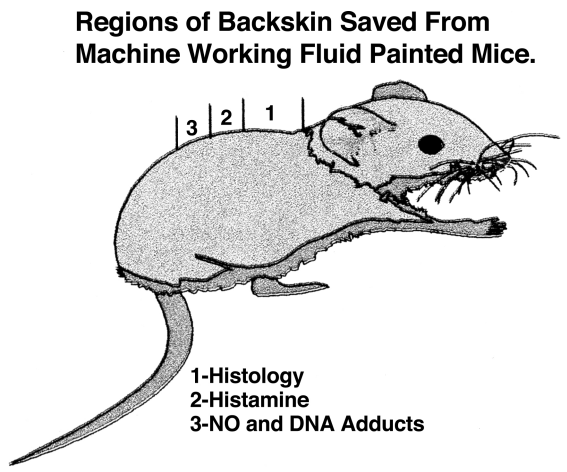

Figure 1. Areas of the skin used for histopathological evaluation and histamine determination.

Tissues

Animals were euthanized with carbon dioxide and exsanguinated. Complete necropsy was done on each mouse. All organs except brown fat were weighed immediately. The organs and tissues saved for histopathology were: brown fat, kidneys, liver, lungs, lymph nodes (axillary and inguinal), skin, spleen, and thymus. The skin was divided into two areas for consistency (Figure 1). One part was kept in the freezer at −80°C and used for total histamine measurements and the second part was immersed in the Davidson fixative and used for histology. After fixation with Davidson fixative, the tissue samples were embedded and sectioned at 5 μm. The sections were stained with hematoxylin and eosin. The skin sections to detect mast cells were stained with 0.1% toluidine blue. Mast cell counts were made using a light microscope (Olympus BX 40) with a high dry objective (40×). Five random fields were examined for mast cells. Cumulative counts from these five fields were recorded as the relative number of mast cells for the sample. Always, the epidermis was clearly within the field of observation.

Histamine Analysis

Total skin histamine was measured by ELISA (Immunotech, Westbrook, Maine), as per the manufacturer's

Table 2. Mean terminal body and organ weights: male B6C3F1 mice.

Treatment	Body weight (g)	Liver		Kidney	
		Absolute weight (g)	Percentage relative weight	Absolute weight (g)	Percentage relative weight
Control	27.42±0.55	1.62±0.02	5.86±0.11	0.54±0.02	2.03±0.04
Control (Recovery)[a]	31.67±0.68	1.90±0.05	5.93±0.07	0.56±0.02	2.22±0.03
100% MWF[b], pH 10.4	30.32[c]±0.60	2.18[c]±0.03	7.20[c]±0.18	0.65±0.01	2.21±0.01
100% MWF (Recovery)	30.99±0.37	2.00±0.05	6.46±0.13	0.69±0.01	2.18±0.04
5% MWF/H_2O, pH 9.7	26.66±0.79	1.62±0.06	6.06±0.12	0.54±0.02	2.03±0.01
5% MWF/H_2O (Recovery)	30.46±0.61	1.77±0.10	5.78±0.25	0.57±0.08	2.15±0.08
5% MWF/H_2O, pH 7	30.24[c]±0.48	1.80±0.03	5.95±0.04	0.64±0.02	2.11±0.02
5% MWF/H_2O, pH 7 (Recovery)	30.04±0.96	1.67±0.06	5.57±0.10	0.64±0.01	2.13±0.05

[a]Recovery groups were kept for 6 weeks post-exposure without further treatment.
[b]Metal working fluid.
[c]Significantly different at $P \leq 0.05$. ANOVA.
±SEM, Standard error of the mean.

instructions. Briefly, wet skin (40 mg) was extracted with 10 μl of 0.2N perchloric acid per milligram of tissue. Samples were homogenized using a sonifier cell disruptor (model 350, Branson Sonic Power, Danbury Conn, USA) with a frequency of 20 Hz and an output of 40% max, 25 pulses (75 pulses/min), on ice. Samples were then centrifuged at 10,000×g for 5 min at 4°C. Supernatant was collected and neutralized (pH 6.8) by addition of an equal volume of 1 M potassium borate, pH 9.25 (400 μl of 1 M potassium borate per 400 μl of 0.2N $HClO_4$). Samples were then centrifuged at 10,000×g for 1 min at 4°C. Supernatant was collected and diluted (1:2000) with PBS (phosphate buffer solution), pH 7.4. Analysis was performed using a Dynatech Immuno Assay System at a detection wavelength of 405 nm (Dynatech, Chantilly, VA). Standards range from 0 to 100 nM histamine.

Data Analysis

Data were analyzed using Sigma Plot (version 2.0, Jandel Scientific Software, San Rafael, CA) statistical software for Windows 95, NT and 3.1. An analysis of variance (ANOVA) was conducted. Values of $P \leq 0.05$ were considered statistically significant.

Results

Mice showed almost identical weight gains during the treatment period (data not shown). The mean body weights of male mice at sacrifice, treated with 100% unused MWF or 5% MWF (pH 7) were significantly higher than the controls (Tables 2 and 3). The absolute and the relative weights of the liver of the male and the female mice treated with 100% unused MWF were significantly higher than the

Table 3. Mean terminal body and organ weights: female B6C3F1 mice.

Treatment	Body weight (g)	Liver		Kidney	
		Absolute weight (g)	Percentage relative weight	Absolute weight (g)	Percentage relative weight
Control	26.40±1.25	1.65±0.03	5.69±0.31	0.47±0.02	1.65±0.04
Control (Recovery)[a]	31.77±1.13	1.90±0.06	5.97±0.08	0.70±0.02	1.66±0.04
100% MWF[b], pH 10.4	27.25±0.63	2.04[c]±0.05	7.07[c]±0.08	0.56±0.02	1.80±0.03
100% MWF (Recovery)	28.40±0.36	1.92±0.04	6.52±0.06	0.57±0.02	1.77±0.03
5% MWF/H_2O, pH 9.7	28.17±0.44	1.80±0.04	6.35±0.06	0.48±0.02	1.72±0.03
5% MWF/H_2O (Recovery)	29.43±0.59	1.73±0.02	5.89±0.07	0.50±0.01	1.69±0.01
5% MWF/H_2O, pH 7	27.00±0.52	1.76±0.11	6.15±0.16	0.47±0.01	1.73±0.03
5% MWF/H_2O, pH 7 (Recovery)	28.63±0.72	1.78±0.06	6.20±0.08	0.48±0.01	1.67±0.03

[a]Recovery groups were kept for 6 weeks post-exposure without further treatment.
[b]Metal working fluid.
[c]Significantly different at $P \leq 0.05$. ANOVA.
±SEM, Standard error of the mean.

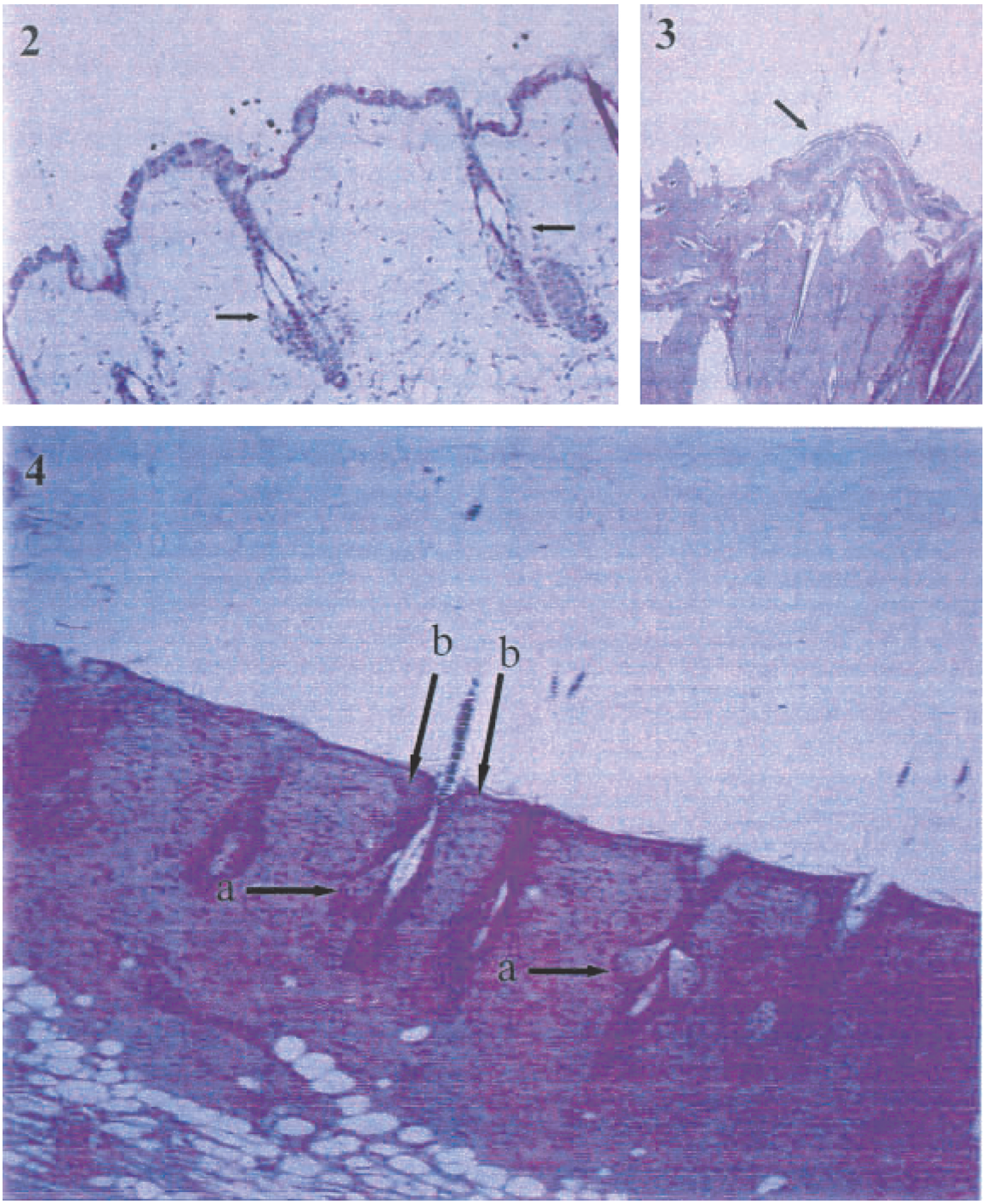

Figure 2. Control skin of female mouse showing one-cell-thick epidermis and normal size sebaceous glands (arrows) and dermis. Original magnification 10×. **Figure 3.** Skin from a female mouse treated with 100% MWF twice a week for 6 weeks. Note the large ulcer with cellular debris (arrow), serous fluid, and acute inflammatory cells. Original magnification 25×. **Figure 4.** Skin from a female mouse treated as in figure 3 but away from any skin ulcers. Note the enlarged sebaceous glands in the dermis (a) and slightly thickened epidermis (b). Original magnification 50×.

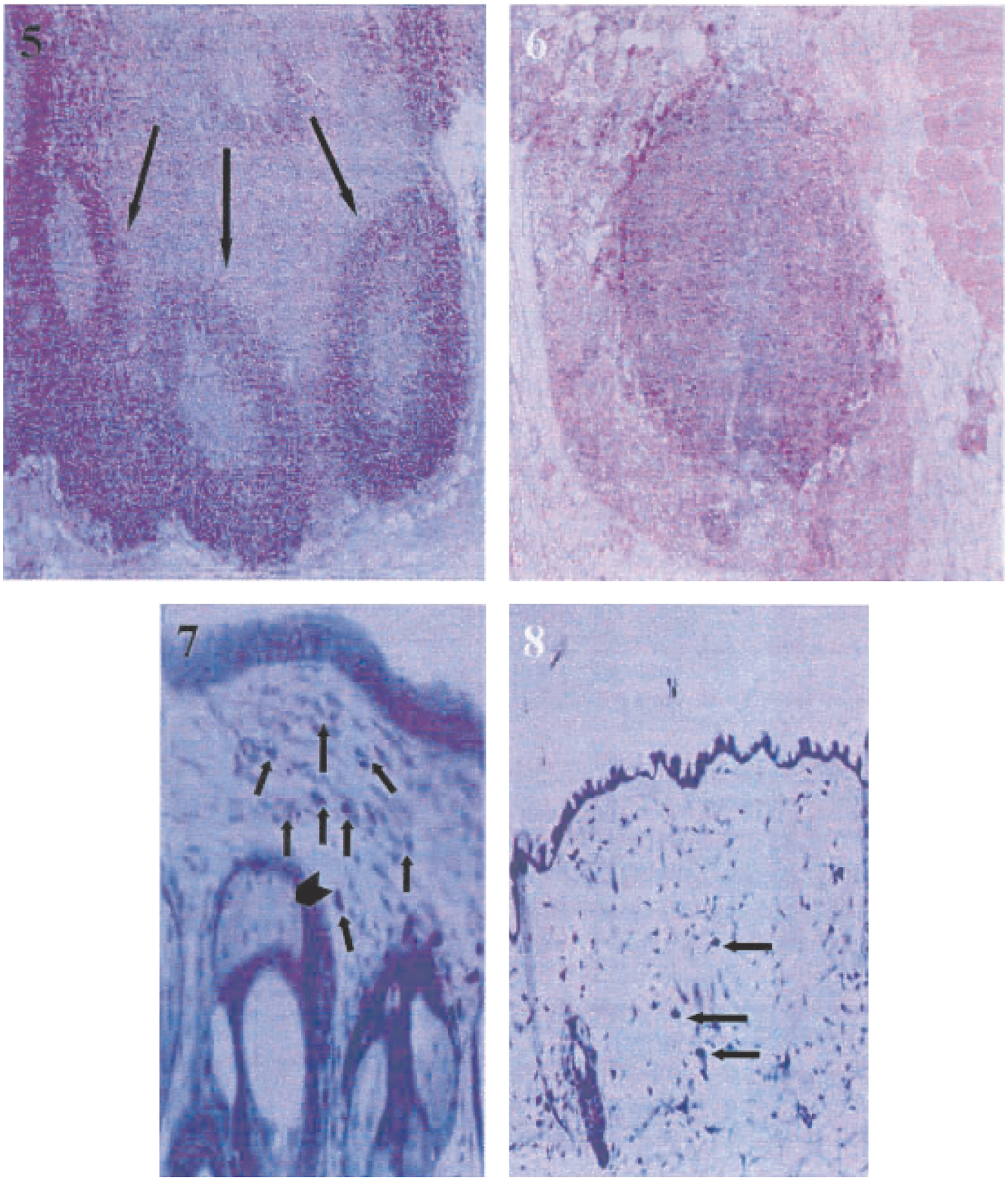

Figure 5. Axillary lymph node from a female mouse treated as in figure 3. Note the formation of germinal follicles in the cortex (arrows) and increased cellularity in the medulla. Original magnification 25×. **Figure 6.** Axillary lymph node from a female control mouse. Normal lymph node with indistinct follicles. Original magnification 25×. **Figure 7.** Skin from a female mouse treated as in figure 3. Note the presence of increased numbers of mast cells (arrows) and hypertrophy of sebaceous glands (arrow head) in the dermis. Original magnification 50×. **Figure 8.** Skin from a female control mouse. Note the presence of sparse number of mast cells in the dermis (arrows). Original magnification 25×.

Table 4. Skin application of metal working fluid to B6C3F1 mice: histo-pathology observations[a] (for both sexes).

Treatment	Epidermis—one cell thick	Epidermis—two or more cells thick	Sebaceous gland epithelial cell hypertrophy	Ulcer	Inflammation dermis	Draining lymph nodes—reactive hyperplasia[b]
Control	Yes	No	No	No	No	No
Control (Recovery)	Yes	No	No	No	No	No
100% MWF[c], pH 10.4	No	Yes	Yes	Yes	Yes	Yes
100% MWF (Recovery)	Yes	No	Yes	No	No	Yes
5% MWF/H_2O[d], pH 9.7	No	Yes	Yes	No	No	Yes
5% MWF/H_2O (Recovery)	Yes	No	No	No	No	Yes
5% MWF/H_2O, pH 7	No	Yes	Yes	No	No	Yes
5% MWF/H_2O, pH 7 (Recovery)	Yes	No	No	No	No	Yes

[a]Mast cell information is given in Table 5.
[b]Draining lymph nodes=axillary and inguinal.
[c]Metal working fluid.
[d]Distilled water.

controls. Weight changes were not accompanied by discernable morphological changes in the liver. No changes were seen in brown fat when examined by light microscope. The absolute and relative weight of the kidney of both sexes did not show any significant differences. Among the mice in the recovery groups, there were no differences in body weight or organ weights. There were no significant differences in absolute and relative weight (data not shown) of the lung, spleen, and thymus between the control and the treated groups.

Compared to control group (Figure 2), ulcers and associated inflammation were seen in the skin of mice treated with 100% unused MWF (Figure 3) and (Table 4). Similar lesions were not present in the skin of these mice from the recovery group. Hypertrophy of the sebaceous gland epithelium (Figure 4) and hyperplasia of the draining lymph nodes (Figures 5 and 6) were noted in all mice treated with MWF for 6 weeks and examined immediately after discontinuation of treatment irrespective of the concentration or pH of the MWF compared to control groups. However, only the mice treated with 100% MWF retained the hypertrophy of the sebaceous gland epithelium after a 6-week recovery period, while reactive hyperplasia of the lymph node remained after the recovery period for all MWF treatments. The other organs (brown fat, kidneys, liver, lungs, spleen and thymus) did not show any treatment-related microscopic changes.

Mast cell numbers and histamine level in the skin of the control females (56 and 0.533 μM, respectively) were higher than in males (30 and 0.276 μM, respectively) (Table 5). Females and males treated with 100% MWF showed a significant increase in the number of mast cells (Figure 7) compared to the control (Figure 8) (Table 5). These increased numbers of mast cells were reflected by the significant increase of the skin-histamine content in the 100% MWF-treated female group only (Table 5). Furthermore, mast cell numbers were significantly increased in both male and female groups treated with 5%

Table 5. Skin application of metal working fluid to B6C3F1 mice: mean mast cell numbers and total histamine.

Treatment	Mast cells[a] # (males)	Mast cells[a] # (females)	Histamine[b] (males)	Histamine[b] (females)
Control	30±5.50	56±9.87	0.276±0.02	0.533±0.08
Control (Recovery)	32±2.4	77±15.97	0.450±0.04	1.075±0.09
100% MWF, pH 10.4	92[c]±25.20	167[c]±14.95	0.381±0.04	0.752[c]±0.05
100% MWF (Recovery)	71[c]±7.80	101±9.70	0.474±0.04	1.006±0.09
5% MWF/H_2O, pH 9.7	33±1.50	80[c]±4.23	0.320±0.05	0.448±0.05
5% MWF/H_2O (Recovery)	41±7.00	101±7.00	0.490±0.05	0.858±0.07
5% MWF/H_2O, pH 7	45[c]±5.70	81[c]±6.23	0.350±0.05	0.620±0.05
5% MWF/H_2O, pH 7 (Recovery)	46±3.56	96±17.92	0.434±0.03	0.680±0.09

[a]Mean number of mast cells present in five fields of skin examined (40×10 magnification).
[b]Total histamine was measured using ELISA (Immunotech.) μmol/mg wet skin.
[c]Significantly different at $P \leq 0.05$. ANOVA.
±SEM, Standard error of the mean.

MWF, pH 7 (Table 5). Females treated at 5% MWF at pH 9.7 had significant increase in the mast cell numbers.

Discussion and conclusions

The liver weight changes probably are adaptive responses to the MWF (or to one of its components like DEA) application to the skin. Early studies showed that DEA accumulates to high concentrations in certain tissues like the liver, kidney, spleen, and brain of the rats following repeated exposure to DEA (Mathews et al., 1995), and is not metabolized or readily eliminated from the liver or kidney (Knaak et al., 1997). It has been reported that alkalinity of MWF might be the cause of irritant as well as contact dermatitis (Pryce et al., 1989). The alkalinity and the high concentration of MWFs (100%) might be the cause of the skin ulcers and the inflammation observed in our study. Dilution of MWF to 5% with water did not produce similar effects, although it was very alkaline (pH 9.7). The skin responses of mice to this and to the 5% MWF/H_2O (pH 7) were not that different, suggesting that at this concentration, the pH (alkalinity) of MWF does not influence the skin response. Changing the pH to 7 did not reduce the toxicity of these fluids as reported by Gordon and Galdanes (1999). These authors used MWF inhalation exposures in guinea pigs.

The hypertrophic response of the sebaceous gland epithelium suggests that there is transdermal penetration of the MWF constituents and that these glands might have acted as depots (Figure 4). The increase in numbers of mast cells in the skin of the treated mice suggests that this reaction might be a characteristic response to the MWF applied to the skin. The persistence of mast cells in the skin after 6 weeks of recovery indicates that either the material (MWF constituents) stored in the sebaceous glands (Figure 4) has a prolonged effect or that the MWF components were released slowly over time. The mast cell response may be a harbinger of serious immunological perturbation in these mice (such as increasing the histamine and the induction of hypersensitivities).

The draining lymph nodes (axillary and inguinal) of mice of both sexes treated with MWF show mostly plasma cells suggesting activation and maturation of B cells. It has been suggested that Langerhans (dendritic) cells are central to the effective induction of contact sensitization and that they process and transport the chemical allergens in an immunogenic form from the skin to draining lymph nodes where it is presented to responsive T lymphocytes (Kimber and Cumberbatch, 1992). Studies to define the role of these antigen-presenting cells in mice exposed to MWFs are being planned. Our preliminary studies do not confirm that these lymph node changes are due to MWF. However, suggestion of such a possibility is hard to ignore. The increased susceptibility of treated females suggests perhaps the female hormones (estrogen?) predispose these mice to the effects of MWF. Estradiol has been reported to exacerbate allergic reactions (Wormald, 1977; Gibbs et al., 1984), augment the release of histamine *in vivo* (Conrad and Feigen, 1974; Vliagoftis et al., 1992), and enhance mast cell growth (Patra et al., 1995). In addition, estrogens have long been known to exacerbate autoimmune diseases (Grossman, 1984; Ahmed et al., 1985; Ahmed and Talal, 1989; Schuurs and Verheul, 1990; Grossman et al., 1991). In conclusion, B6C3F1 mice when dermally exposed to different concentrations of semi-synthetic MWFs showed concentration-dependent responses. Increases in the liver weight, hypertrophy of the sebaceous gland epithelium, hyperplasia of the draining lymph nodes, and ulcers and associated inflammation and increased mast cell numbers and histamine levels in the skin are all indications of the adverse responses to these fluids especially the 100% concentration.

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