Metalworking Fluid Effects on B6C3F1 Mouse Skin

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ABSTRACT

It has been estimated that over 10 million workers in the United States are exposed to metalworking fluids (MWF) either by inhalation or through the skin. We have applied an unused semisynthetic MWF to the dorsal skin of unshaven B6C3F1 mice of both sexes twice a week for six weeks. Body weights, the morphological changes seen in the skin and the draining lymph nodes, and their possible health implications for workers' health will be presented.

INTRODUCTION

Over 10 million industrial workers performing cutting, drilling, grinding, milling and other operations are exposed to metal working fluids (MWFs), which are used as lubricants and coolants. MWFs are available as straight oils (petroleum- based oils), soluble fluids (30-85% v/v oil), semi-synthetic fluids (5-30% v/v oil), and synthetic fluids (no oil). The main routes of occupational exposure to MWFs are through skin and respiratory tract. The objective of this study is to determine the effects, if any, of a new (unused) semisynthetic MWF on mice skin. The emulsifiers present in and alkalinity of MWF are considered as causative agents of irritant and contact dermatitis.

Materials and Methods:

The experimental design is given in Table 1. B6C3FI mice of both sexes from Harlan Sprague Dawley and Charles River Laboratories were used after a two-week acclimatization period. Mice were housed one per cage and kept in a room in the West Virginia University animal facility, at 75 (± 5) degrees F, with 40%-60% relative humidity and 12-hour light/dark cycle. Municipal water and feed (Harlan Teklad Rodent Diet # 8604) were given with no restrictions. Using 1 ml tuberculin syringes, 0.2 ml of the respective fluids were dispensed on the unshaven back skin (from the cervical to sacral region) of treated mice twice a week for six weeks. The fluids were evenly distributed with the help of curved gavage needle throughout the back. Animals were weighed soon after receiving and every two weeks thereafter and at sacrifice. Recovery groups were kept for six more weeks without any more treatment after six weeks of treatment. At necropsy the liver, kidney, lung, spleen, and thymus weights were recorded after euthanization with carbon dioxide and exsanguination. The organs and tissues saved for histopathology are given in Table 2.

Table 1. Skin Application of Metalworking Fluid to B6C3F1 Mice: Experimental Design

Treatment[a]	Number of Mice[b]	
	Males	Females
Control	10	10
Control (Recovery)	10	10
100% MWF[c]	10	10
100% MWF (Recovery)	10	10
1/20 MWF/H_2O[d]	5	5
1/20 MWF/H_2O (Recovery)	5	5
1/20 MWF/H_2O pH7	5	5
1/20 MWF/H_2O pH7 (Recovery)	5	5
1/1 MWF/DMSO[e]	5	5
1/1DMSO/H_2O	10	5

a: All were not exposed at the same time. 0.2 ml of respective fluid was dispensed on the unshaven back for six weeks
b: B6C3F1 mice
c: Metal (Machining) Working Fluid
d: Distilled water
e: Dimethyl Sulfoxide

Table2. Skin Application of Metalworking Fluid to B6C3F1 Mice: Organs and Tissues Saved at Necropsy

Brown Fat
Kidneys
Liver
Lungs
Lymph Nodes: Axillary and Inguinal
Skin
Spleen
Thymus

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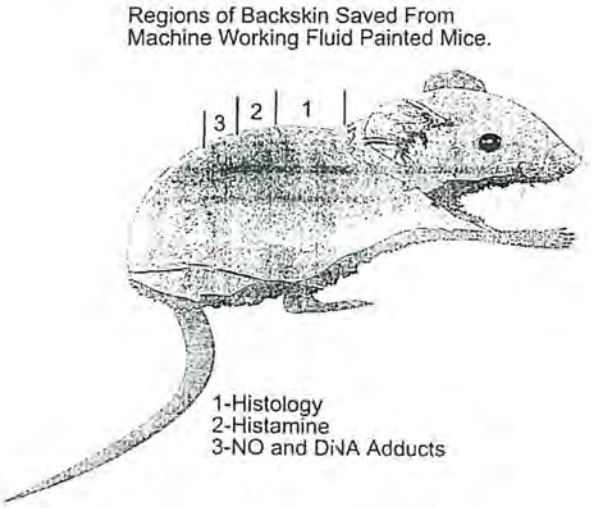
The area of the skin used for evaluation is detailed in Figure 1. Skin total histamine was measured by an absorbance method (Immunotech Inc., Westbrook, Maine). Forty milligrams of wet skin was extracted with 0.2 N perchloric acid and 1 M potassium borate. The samples were diluted (1:2000) with PBS (phosphate buffer solution) pH 7.4. Histamine levels were measured, using a Dynatech Immuno Assay System (Dynatech Inc.).

Figure 1

The Areas of the Skin Used for Evaluation.



Results:

Mice showed almost identical weight gains during the experiment (data not shown). The terminal mean body weights of male mice treated with 100% unused MWF and 1/20 MWF pH7 are higher than the controls (Tables 3 and 4). The absolute and the relative weights of the liver of the male and only the relative liver weight of female mice treated with 100% unused MWF are higher than the controls. In addition, the relative weight of the liver of males treated with 1/1 MWF/DMSO is increased. These weight changes have not translated into morphological changes that could be discerned with light microscopic examination. The absolute and relative weights of the kidneys of both sexes do not show any significant differences. Among the mice in the recovery groups there are no differences in body weight or organ weights. The lung, spleen, and thymus do not show any variation in weight among the groups (data not shown).

Table 3. Mean Terminal Body Weights: Male B6C3F1 Mice

Treatment	Body Weight (gm)	Liver		Kidney	
		Absolute Weight (gm)	Relative Weight (%)	Absolute Weight (gm)	Relative Weight (%)
Control	27.42	1.62	5.86	0.54	2.03
Control (Recovery)	31.67	1.90	5.93	0.70	2.22
100% MWF[b]	30.32[a]	2.18	7.20[a]	0.65	2.21
100% MWF (Recovery)	30.99	2.00	6.46	0.69	2.18
1/20 MWF/H_2O	26.66	1.62	6.06	0.54	2.03
1/20 MWF/H_2O (Recovery)	30.46	1.77	5.78	0.66	2.15
1/20 MWF/H_2O pH7	30.24[a]	1.80	5.95	0.64	2.11
1/20 MWF/H_2O pH7 (Recovery)	30.04	1.67	5.57	0.64	2.13
1/1 MWF/DMSO[c]	27.64	1.98	7.30[a]	0.59	2.10
1/1DMSO/H_2O	29.17	1.62	5.64	0.56	2.06

a: Significantly different at $p < 0.05$
b: Metalworking fluid
c: Dimethyl Sulfoxide

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Table 4. Mean Terminal Body Weights: Female B6C3F1 Mice

Treatment	Body Weight (gm)	Liver		Kidney	
		Absolute Weight (gm)	Relative Weight (%)	Absolute Weight (gm)	Relative Weight (%)
Control	26.40	1.61	5.69	0.44	1.65
Control (Recovery)	31.77	1.90	5.97	0.70	1.66
100% MWF[b]	27.25	1.92	7.07[a]	0.49	1.80
100% MWF (Recovery)	28.40	1.85	6.52	0.50	1.77
1/20 MWF/H_2O	28.17	1.80	6.35	0.48	1.72
1/20 MWF/H_2O (Recovery)	29.43	1.73	5.89	0.50	1.69
1/20 MWF/H_2O pH7	27.00	1.66	6.15	0.47	1.73
1/20 MWF/H_2O pH7 (Recovery)	28.63	1.78	6.20	0.48	1.67
1/1 MWF/DMSO[c]	25.10	1.76	7.40[a]	0.43	1.80
1/1DMSO/H_2O	25.10	1.40	5.40	0.44	1.74

a: Significantly different at $p < 0.05$
b: Metalworking fluid
c: Dimethyl Sulfoxide

Table 5. Skin Application of Metalworking Fluid to B6C3F1 Mice: Histo-pathology Observations[a]

Treatment	Epidermis-One Cell Thick	Epidermis-Two or More Cells Thick	Sebaceous Gland Epithelial Cell Hypertrophy	Ulcer	Inflammation of Dermis	Draining Lymph Nose-Reactive Hyperplasia[b]
Control	Yes	No	No	No	No	No
Control (Recovery)	Yes	No	No	No	No	No
100% MWF[c]	No	Yes	Yes	Yes	Yes	Yes
100% MWF (Recovery)	Yes	No	Yes	No	No	Yes
1/20 MWF/H_2O[d]	No	Yes	Yes	No	No	Yes
1/20 MWF/H_2O (Recovery)	Yes	No	No	No	No	Yes
1/20 MWF/H_2O pH7	No	Yes	Yes	No	No	Yes
1/20 MWF/H_2O pH7 (Recovery)	Yes	No	No	No	No	Yes
1/1 MWF/DMSO[e]	Yes	No	Yes	No	No	Yes
1/1DMSO/H_2O	Yes	No	No	No	No	No

a: Mast cell information is given elsewhere
b: Draining lymph nodes = Axillary and Inguinal
c: Metalworking Fluid
d: H_2O = Distilled water
e: DMSO = Dimethyl Sulfoxide

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Table 6. Skin Application of Metalworking 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	56	0.276	0.533
Control (Recovery)	—	—	0.450	1.075
100% MWF[c]	92[c]	167[c]	0.381	0.752[c]
100% MWF (Recovery)	71	99	0.474	1.006
1/20 MWF/H_2O[d]	33	80[c]	0.320	0.448
1/20 MWF/H_2O (Recovery)	41	101	0.490	0.858
1/20 MWF/H_2O pH7	45	81[c]	0.350	0.620
1/20 MWF/H_2O pH7 (Recovery)	46	96	0.434	0.680
1/1 MWF/DMSO[e]	25	61	0.264	0.692[c]
1/1DMSO/H_2O	35	104[c]	0.257	0.943[c]

a: Mean number of mast cells present in five contiguous fields of skin examined (40 x 10 magnification)
b: Total histamine was measured using ELISA (Immunotech) micro mole/mg wet skin
c: Significantly different at $p < 0.05$

Ulcers and associated inflammation are seen in the skin of mice treated with 100% unused MWF (Table 5). Similar lesions are not present in the skin of these mice from the recovery group. Hypertrophy of the sebaceous gland epithelium is present in all mice sacrificed at 6 weeks and treated with MWF irrespective of its concentration and pH. However, only the mice treated with 100% MWF and left to recover retained the hypertrophy of this epithelium.

Consistent increase in the number of mast cells is seen in both sexes treated with MWF, except in the males treated with 1/1 MWF/DMSO. The female mice seem to have been affected more than the males. These increased numbers of mast cells are reflected in the increases seen in the skin total histamine concentration (Table 6). The other organs, like the brown fat, kidney, liver, lungs, spleen and thymus do not show any microscopic changes related to treatment.

Discussion and Conclusions:

The organ weight changes probably are adaptive responses to the MWF application to the skin. The skin ulcers and the inflammation are due partly to the alkalinity and partly due to the concentration of the MWF. The diluted MWF (1/20 MWF/H20) failed to have similar effects, although the alkalinity is still very high (pH 9.7). The response of mice to this and to the 1/20 MWF/H20 (pH 7) is almost identical, suggesting that at this concentration the pH (alkalinity) does not have any effect.

The hypertrophic response of the sebaceous gland epithelium suggests that there is transdermal penetration of the MWF constituents and that these glands acted as depots. The increased numbers of mast cells in the skin suggest that they are in response to the material applied to the skin. The persistence of mast cells in the skin after 6 weeks recovery indicates that the material stored in the sebaceous glands has played an important role. The mast cell response may be a harbinger of serious immunological perturbation in these mice.

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. These preliminary studies do not confirm that these changes are due to MWF. However, suggestion of such a possibility is hard to ignore. The increased susceptibility of treated females suggests that perhaps the female hormones (estrogen?) predispose these mice.

In conclusion, the unused semisynthetic MWF, when applied to the unshaven skin of B6C3FI for six weeks, twice a week, penetrates the normal skin; induces mast cell accumulation in the skin; accumulates in the sebaceous glands; affects females more than males; and possibly perturbs the immunological homeostasis.

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The reader will notice a variety of nomenclature differences among authors when referring to these fluids which were the subject of the Symposium and of this volume. Indeed, even the Symposium title reflected some of this variation: "*Metalworking* Fluids Symposium II," and "The Industrial Metalworking Environment: Assessment and Control of *Metal Removal* Fluids." Lest we add to the confusion, our use of the term *metalworking* in the title "Metalworking Fluids Symposium II" was a conscious decision based on nothing more than to maintain continuity with the title from the first Symposium. It was for that reason that "Assessment and Control of Metal Removal Fluids" was added in recognition of, and to call attention to the fact that the vast majority of research and data to date has been generated on a subset or class of metalworking fluids known as **metal removal fluids**. In addition to metal removal fluids, the very general term 'metalworking' fluids also encompasses the large and general classes of *metal protecting* fluids, *metal forming* fluids, and *metal treating* fluids. Besides functional differences between metalworking fluid classes, there are substantial compositional differences both between and within classes. So while it is somewhat sloppy though quite common and generally harmless to use generic terms such as metalworking fluids, or machining fluids, or coolants, the reader should be well aware of these important distinctions and that in virtually all instances where there is a connection with purported health effects, the person is really referring to that subclass of metalworking fluids known as *metal removal fluids*.

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