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Informal Report

Acute Toxic Effects of Two Crude Shale Oils and Two Standard Petroleum Crude Oils Applied to Mouse Skin

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ABSTRACT

The acute dermal toxicity of two crude shale oils and two crude petroleums was studied, in an effort, to establish threshold tolerance levels for use in a subsequent epidermal carcinogenesis experiment. Five dose levels of each test oil were applied daily to the skin of C3Hf/He mice for two weeks. At the end of the two week treatment period all mice were humanely sacrificed and histological sections of the skin were examined for inflammatory changes. Representative tissues from major organ systems were also examined for signs of systemic toxicity. Neither of the reference petroleum crude oils caused severe inflammation, while both shale oils caused marked inflammation accompanied by ulceration at the higher dose levels.

I. INTRODUCTION

Prior to the full scale development of oil shale deposits, several questions related to the toxicity of associated effluents and the final crude product should be investigated. To this end we initiated an acute toxicity experiment using two crude shale oils and two standard petroleum crude oils in a skin painting study designed to probe the effects of these agents during short-term, high-dose exposures and to establish threshold damage levels for a more extended study of carcinogenicity. Assaying toxicity through skin exposure correlates with the most probable human exposure. It is not sufficient to study the toxicity of the shale crude oils alone; they must be compared with standard petroleum crudes, to which humans have been exposed for many years. Based on chemical analysis it is recognized that crude shale oils contain many organic compounds known to have toxic, carcinogenic, mutagenic, and teratogenic effects, and that many of these same compounds are present in crude petroleums now being refined.[1,2,3] The relative toxicity of process specific crude shale oils when compared to each other and to reference petroleum crude oils has not been adequately defined. Further, if epidermal damage caused by shale oil is elevated significantly above that caused by petroleum crude, by understanding the mechanisms of the toxicity, reasonable control technology measures may be applied in a way that will allow economical development of the resource.

II. MATERIALS AND METHODS

The two crude shale oils used for this study were from different retorting processes. The first was from an above ground gas combustion retort (Type A), and the second was from a modified *in situ* (MIS) process (Type B). The standard petroleum crude oils were American Petroleum Institute (API) reference oils No. 1 and 2. All undiluted oils were stored in dark, polyethylene containers at $4^{\circ}C$, and subsequent working solutions were stored at room temperature (22°-$27^{\circ}C$) in glass vials. Each oil was diluted into 5 dose levels using a 30% cyclohexane-70%

acetone vehicle which was freshly made for each set of dilutions. Final doses represented weight-volume percentages of 50%, 25%, 10%, 5%, and 1%. Each animal was painted with 50 microliters of the appropriate oil-solvent mixture daily, Monday through Friday, for two weeks and then sacrificed for histological study. Since the vials containing undiluted oils were stored at 4°C prior to dilution they were warmed gently to achieve an even solution. Once room temperature was reached, no more manipulation was necessary.

C3Hf/He mice, supplied to us by Dr. J. M. Holland of Oak Ridge National Laboratory, were used for these studies. The mice were about 180 days old at the initiation of the experiment. Three males and three females were used for each dose level of each oil (Table I). Experimental and control mice were maintained three to a cage on aspen shavings with water and Teklad food (Teklad Mouse/Rat Diet, ARS/Spraque-Dawley, Winfield, IA) provided *ad lib*.

Each mouse was shaved once along the spine from hips to neck with Oster small animal clippers utilizing a #40 blade. The solutions were applied in the intrascapular region using a 50 microliter MLA precision pipette as described by Holland.[4]

TABLE I

EPIDERMAL ACUTE TOXICITY STUDY

Agent	Dose (%)	Number of Males	Number of Females	Total Acute (14 days)
API #1	50	3	3	250 mg
	25	3	3	125
	10	3	3	50
	5	3	3	5
API #2	50	3	3	250
	25	3	3	125
	10	3	3	50
	5	3	3	25
	1	3	3	5
Type A Shale	50	3	3	250
	25	3	3	125
	10	3	3	50
	5	3	3	25
	1	3	3	5
Type B Shale	50	3	3	250
	25	3	3	125
	10	3	3	50
	5	3	3	25
	1	3	3	5

Following the two week exposure period, all mice were sacrificed by cervical dislocation. The entire skin of the back from the pelvis to the base of the skull was removed and fixed for pathology and histology. Additionally, internal organs were observed for changes and representative tissues saved for further study. All mice were weighed at the initiation of treatment and at sacrifice.

III. RESULTS

Within two days after initiation of treatment, the higher dose levels (50 and 25%) of all oils stained the area of application in a uniform way. The lower doses (10 to 1%) stained only slightly or not at all. By the end of the first week it was apparent the two shale oils had a more profound effect than the standard petroleum crudes, and that the Type A shale oil was more severe than the Type B. The treated area displayed a mild, persistant reddening, and some scaling and patchy hair growth. In some cases the hair in the exposure area regrew quickly and in others the area became steadily denuded. At the termination of treatment the animals exposed to the shale oils exhibited a denuded, sometimes scaling area, and the response to the Type A oil was more severe than that seen with the Type B. In several cases large scabs and frank ulcers were observed. The Type A and B shale oils elicited these more severe lesions at concentration levels above 10% and only slight inflammation below that level. Animals exposed to the two petroleum crudes had no severe lesions at the 10% level, and few at the higher doses.

These observations made at the time of sacrifice and during exposure are relative ones, leading to the general impression that the shale oils are indeed more damaging than the two standard petroleum crudes, and that Type A shale crude appears to be more damaging than Type B. None of the animals in any of the dose groups died during exposure and there were no significant weight changes in any of the groups.

Histological examination of the treated skin areas revealed a graded dose response to all of

the test oils. The response to both crude shale oils was more profound at equivalent doses than the response to either of the reference petroleums. At all dose levels of the reference petroleums and at the 1% and 5% levels of both shale oils, the observed changes were slight and included an inconsistent thickening of the epidermis and a reduction in hair follicle numbers (Fig. 1). At the three higher dose levels of both shale oils (10, 25, and 50%) focal thickening of the epidermis associated with hypercellularity of the dermis was observed in many animals. Absence of viable hair follicles was observed in many, but not all of the treated animals. The highest dose level of Type A shale oil caused a severe inflammatory response consisting of thickened epidermis, sub-dermal edema, crust formation and ulceration with an influx of mono- and poly-nuclear cells (Fig. 2). Frank ulceration occurred in 4 of the 6 exposed animals. Ulcers were also observed in 2 of the 6 animals treated with the 25% dose of Type A shale oil. Two of the mice treated with 25% Type B shale oil exhibited ulceration of the epidermis while two animals from the 50% dose group, and one from the 25% dose group exhibited surface crusts associated with discrete wheals, but no disruption of the epidermal layer.

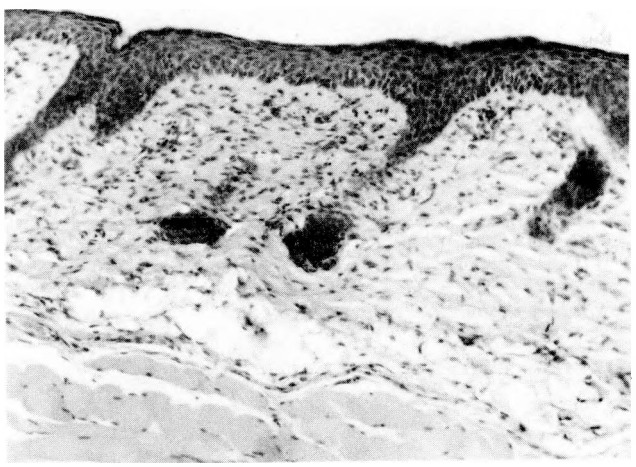

Fig. 1. Histological section of skin from a mouse painted with a 5% solution of API Reference Oil #2. Epidermal thickness is within normal limits.

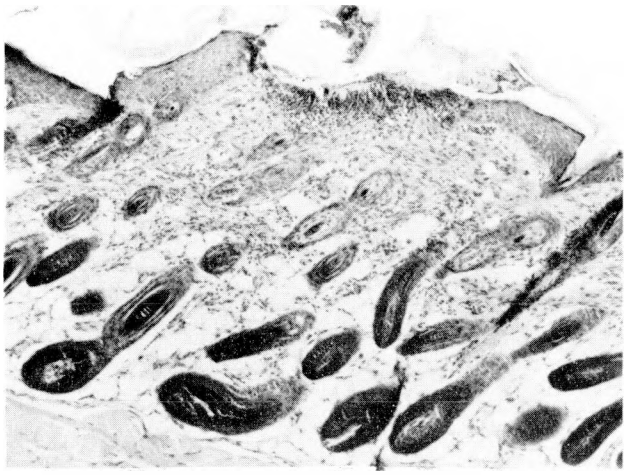

Fig. 2. Histological section through an ulcer of the epidermal layer of a mouse painted with a 25% solution of shale oil A.

Histological examination of major internal organ systems, from mice exhibiting gross lesions, revealed no alterations in micro-anatomy that could be attributed to exposure to crude oil.

IV. CONCLUSIONS

Both of the shale oils tested in the experiment caused moderate to severe inflammatory responses at doses above 10%. This included breakdown of the epidermal layer, and involvement of dermal structures. Neither of the reference petroleums caused severe damage at any of the dose levels used. As a result of this experiment, a two year carcinogenicity assay using doses below the 10% level was initiated.

The question of systemic toxicity as a result of epidermal application of crude oils cannot be answered by a study of this small scope. Localization of certain organic constituents of natural and synthetic oils in the sebaceous glands of treated mice has been demonstrated,[5] but clear evidence of selective absorption resulting in systemic mobilization of these materials is lacking. Because of shared and individual grooming by caged mice, it is difficult to identify the true mechanism of systemic intake (i.e. alimentary tract vs skin penetration). None of the tissues related to major organ systems

other than the skin exhibited changes that could be attributed to the test substances.

The major purpose of this study, to establish a threshold of acute skin damage for each test oil, was accomplished. In addition, the results revealed a more profound irritative action by the shale-derived oils than that observed with the reference petroleums. This observation underscores the need for a pragmatic approach to protective measures, including adequate personal hygiene and appropriate protective clothing, by higher risk industrial populations.

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