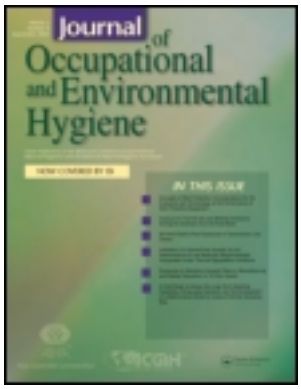

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The dermal route is important in many occupational exposures. Some materials may reduce the barrier function of the skin to enhance absorption and effect on internal organs. We have reported previously that kerosene cleaning following treatment with used engine oil increased DNA adduct levels in the lungs of mice compared with animals treated with used oil alone. To investigate what other physiological parameters might be affected by kerosene, we conducted in vitro and in vivo measurements of skin barrier function. We also topically applied ^{3}H-BAP(100 nM in 25 μL acetone) and washed half the mice with 25 μL kerosene 1 hr after carcinogen application. Groups of four mice were euthanized from 1 to 72 hr after treatment. Skin, lungs, and livers were harvested from each animal and stored separately. Kerosene application reduced the barrier function of the skin in vitro beyond the effect of the acetone vehicle and the vehicle plus BAP. In vivo studies indicated that kerosene treatment reduced the barrier function at 4 and 8 hr post application and that the barrier function recovered at 24 hr after a single treatment. The fraction of the radiolabel remaining in the skin of animals treated with ^{3}H-BAP and washed with kerosene was significantly less than those not washed, beginning at 24 hr ($p < 0.05$). Fractional distribution to the lungs and livers of these animals became significantly elevated at this time. Kerosene treatment compromises dermal barrier function and the ability of the skin to retain water, enhances carcinogen absorption, and alters organ distribution. This appears to contribute to the increase in BAP DNA adducts we reported earlier.

Keywords benzo(a)pyrene, dermal route, mixtures, organotropism

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INTRODUCTION

A major function of the skin is to maintain hydrodynamic balance within the body. However, the skin, particularly the epidermis, also serves as the major barrier to absorption of xenobiotics. The barrier function of the stratum corneum of the epidermis is largely due to the high protein (mainly keratins) and lipid content of skin cells upon terminal differentiation. As much as 15% of the dry weight can be sphingolipids, with long-chain ceramides predominating.[1] It is thought that hydrophobic chemical agents enter the stratum corneum by dissolving in the lipid components of the cell membranes and ultimately pass into the vascular dermis and are distributed through the body via the blood supply. Among the materials capable of being absorbed through the skin are aliphatic and cyclic hydrocarbons including the probable human carcinogens and the polycyclic aromatic compounds (PAC).

However, direct absorption is not the only concern, as agents with fairly low systemic toxicity may damage the epidermal barrier and facilitate absorption of more toxic materials. It has been shown that removal of sphingolipids greatly reduces the barrier function of the skin.[2] The drying and redness associated with exposure to lipophilic agents such as petroleum distillates and halogenated solvents are thought to be associated with removal of these lipids from the epidermis.

Humans are frequently exposed to multiple chemical agents, sequentially or simultaneously. While the practice has been discouraged, it is not uncommon for individuals to use organic solvents to remove greasy or oily materials from their skin. Classic examples include the removal using kerosene of PAC-containing oils and greases from skin by workers such as pavers, coke oven workers, and auto mechanics.

Several million workers have potential occupational exposure to PACs. For example, it is estimated that there are slightly fewer than 1,000,000 auto mechanics/technicians in the United States, and a significant fraction of these may be exposed to PAC in used gasoline engine oil (UGEO) that coats the engine parts being repaired.[3] While only a fraction of all mechanics are working regularly with these oils, the occupation as a whole has been shown to be at increased risk for lung and urinary bladder tumors.[4,5] The lung is the most serious site of PAC

carcinogenicity in humans,[6] but recently, more attention has been paid to dermal exposure as means of exposing the lung.[7]

It has been demonstrated that the skin is a major source of exposure for occupations such as roofers, pavers, and coke oven and aluminum workers and that these occupations are at increased risk of lung cancer.[8–11] Blood flow from the skin favors distribution of absorbed materials to the lung. Blood returning from the skin enters the right side of the heart and is pumped diffusely throughout the entire lung for oxygenation. Lung cells may absorb PAC from the blood and metabolize it to reactive species that can bind to DNA and have serious genetic consequences.[12]

The skin is capable of biotransforming xenobiotics; however, its total activity is a small fraction on a per weight basis of more active tissues such as lung and, particularly, liver.[13–15] The skin can metabolize PAC to electrophilic species, evidenced by the formation of carcinogen-DNA adducts and tumors after topical application.[16] However, that these lesions are seen only at the site of application and not at distal skin sites indicates that skin is not a selective target for these materials.[17] The relatively limited metabolic capacity of the skin may allow a fraction of a topically applied dose to traverse the skin unmetabolized, enter the venous blood, and be returned to the heart and then to the lungs and other organs.

The metabolic activity of the lung is greater than that of the skin.[18] Several workers have demonstrated the formation of high levels of DNA damage in the lung following topical application of PAC.[19,20] This laboratory is concerned with the co-exposure circumstances that favor increased or decreased passage of PAC through the skin to affect the lung and other organs. For example, it is not uncommon that workers will use solvents intended for cleaning tools to clean their contaminated skin. While the use of materials like gasoline and kerosene is not recommended because of case study reports of skin irritation and dermatitis, workers persist in using these materials because they are effective.[21,22]

We recently found that kerosene cleaning following topical application of UGEO significantly increased the levels of carcinogen-DNA adducts in the lungs of mice as compared with animals that were treated with UGEO but cleaned with a commercially available cleaner.[7] The purpose of the research reported here was to investigate which other physiological parameters of the skin may be affected by kerosene treatment and contribute to the increased levels of DNA adducts we see following "cleaning" with kerosene.

MATERIALS AND METHODS

We purchased C57 black (C57B6) male mice aged 6–8 weeks from Jackson Laboratories (Bar Harbor, Maine) and quarantined them for 1 week before use in the study. Animals were housed in an Association for the Assessment and Accreditation of Laboratory Animal Care International-accredited facility with controlled temperature and humidity. Animals' backs were shaved with clippers 24 hr prior to treatment. A 100 nM BAP (Sigma Chemical Co., St. Louis, Mo.) solution was prepared in acetone (Fisher Scientific Co., Fair Lawn, N.J.), and 0.0074 GBq of ^{3}H-BAP was added to each sample. The treatment groups included acetone only, kerosene only, and (^{3}H) BAP in acetone.

A single topical application of 25 μL BAP was applied to the shaved intrascapular region of the animals. This area was approximately 20 mm by 12 mm. One hour after treatment, the backs of one-half of the animals in each of the three treatment groups were cleaned with 25 μL of kerosene (Sunnyside Corp., Wheeling, Ill.), followed by a water rinse. Protocol included the application of kerosene with a pipette and then gently swabbing with a cotton ball, followed by a rinse with a water-saturated cotton ball. Animals not treated with kerosene were swabbed (water rinsed) only.

Animals were then euthanized at 1, 4, 12, 24, 48, or 72 hr. Each animal was injected IP with 0.4 mL of a solution of pentobarbital sodium (200 mg/kg nembutal sodium solution; Abbott Laboratories, Abbott Park, Ill.) Lungs, livers, and skin were harvested for tritium radioactivity determinations. Each tissue sample was placed into a labeled cryovial (Nalge Co., Rochester, N.Y.) and immediately placed on dry ice. After harvesting procedures, all samples were stored at –80°C. In vivo measurements of transepidermal water loss (TEWL) were made before and following the exposure and before the animals were euthanized. Measurements were made 2 hr before then and 3, 6, and 22 hr after the application of the test materials.

The integrity of the skin as a barrier was assessed in two ways: (1) by measuring transepidermal water loss in vivo; and (2) by measuring the flux of tritiated water across the skin in vitro of animals treated in vivo. Damage to the skin barrier was assessed in vivo by measuring TEWL over the dorsal skin surface following light CO_2 anesthesia. TEWL data were collected over a 60-sec interval using a DermaLab evaporimeter (Cortex Technology, Hadsund, Denmark) connected to a Compaq Armada 4120 laptop computer (Compaq Computer Corporation, Houston, Texas). The device was controlled using cyberDERM software (CyberDERM, Inc., Media, Pa.).

Cutaneous barrier integrity was evaluated in vitro by measuring tritiated water flux across whole skin specimens using Franz diffusion cells. These analyses were performed on the animals treated above immediately after sacrifice. The diffusion cells (Dana Enterprises, West Chester, Ohio) comprise two compartments, an upper donor compartment and a lower receptor compartment with a diffusional surface area of 0.79 cm^2. Excised dorsal whole skin was mounted between the two compartments, with the dermal surface facing the receptor compartment and the stratum corneum surface facing the donor compartment. The receptor compartment was filled with phosphate buffered saline, pH 7.4 and was thermo-controlled at 37°C in a Reacti-Therm heating/stirring module (Pierce Chemical Company, Rockford, Ill.), resulting in a skin surface temperature between 30–32°C. A small magnetic stirrer was used to mix the contents of the receptor compartment.

Following an hour equilibration, the stratum corneum surface was treated with 150 μL of ^3H_2O, i14,800 Bq/mL (New

England Nuclear, Boston, Mass.). After 5 min, unabsorbed ^3H_2O was removed by wicking the skin surface with a dry cotton swab. Receptor fluid was collected at precisely 1 hr. The amount of ^3H_2O that penetrated through the barrier was determined by adding 12 mL of Ultima-Gold scintillation cocktail (Packard Bioscience Company, Meriden, Conn.) to the receptor solution, mixing well, and counting the radioactivity using a Beckman scintillation counter LS-600 (Beckman Instruments, Inc., Palo Alto, Calif.).

^{3}H-BAP levels in the tissues were determined by mincing the tissue, solubilizing in 600 μL of Scintigest (Fisher Scientific) at 60°C for 1 hr before neutralizing with acetic acid (Fisher Scientific). Then, 5 mL of ScintiLene (Fisher Scientific) was added to each vial, and samples were counted on a Packard Model 2200TR Liquid Scintillation Analyzer (Packard Instruments Co,. Downers Grove, Ill.). Disintegrations/min (DPM) were automatically determined from the transformed spectral index coupled with automatic efficiency correction and correction for luminescence and quenching. The data are reported as the percentage of the DPM in each tissue divided by the total DPM in all measured tissues for that animal, treatment, and time.

Differences between groups and treatments were evaluated by student t-test using a p-value of 0.05 as the indicator of significance on the raw or log-transformed data. Comparisons are made pairwise as indicated in the captions of the particular figures.

RESULTS

Figure 1 shows the results of studies measuring the passage of tritiated water through the skin of animals treated in vivo and measured in vitro. The impact of kerosene cleaning on this parameter is indicated by the significant differences seen when kerosene treatments were performed. BAP had a small, insignificant effect when added to the acetone. Washing the skin sample with kerosene significantly increased the permeability of the skin to tritiated water in vitro. When BAP treatment preceded kerosene cleaning, a greater reduction of barrier function was noted, although this reduction was not statistically significant. Because these measurements were performed after the animals were euthanized, they indicate that there was some residual damage even 24 hr after exposure.

Figures 2–4 show data for in vivo TEWL to determine how various treatments affected the skin. In Figure 2, the time course of TEWL is shown for groups of animals treated with the acetone vehicle or acetone-BAP. There was no difference between these groups at any time, suggesting that the effect was due to the acetone. A pattern of an early increase in water loss, followed by recovery at 24 hr, is seen. Figure 3 compares the TEWL in groups treated with acetone alone or acetone, then washed with kerosene. In this case the kerosene clearly exacerbates the effect of the vehicle, as significant differences were seen 4 hr after the application followed by a much slower return to baseline conditions. TEWL of groups treated with acetone-BAP and acetone-BAP kerosene are compared in Figure 4. Again, kerosene application results in an earlier peak in TEWL and a slower return to baseline conditions. The strong similarity of the in vitro and in vivo data taken together suggest that kerosene has a deleterious effect on the barrier function of the skin, significantly reducing the ability of the skin to prevent permeation of external water and simultaneous loss of internal water, a critical function.

We next determined whether kerosene application after carcinogen treatment would change the kinetics of BAP loss

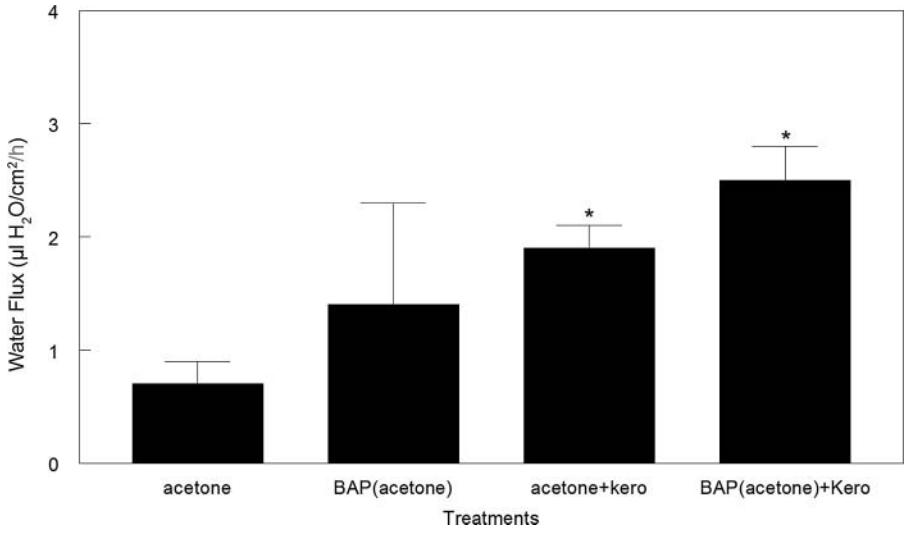

FIGURE 1. Movement of tritiated water into the receptor fluid of Franz cells with mouse skin in vitro with indicated treatments. Bars indicate the standard error of the mean. Asterisks indicate effects that are significantly different ($p<0.05$) from the acetone-treated group.

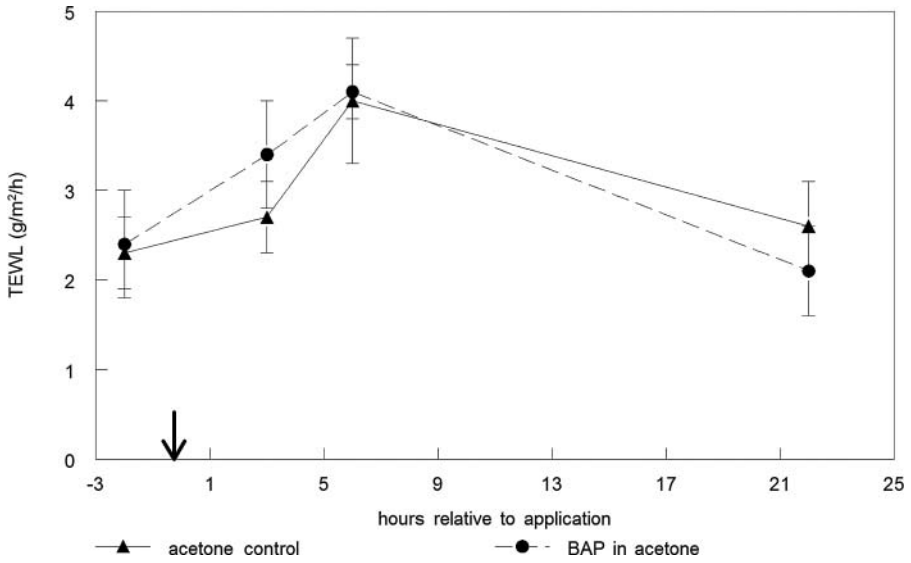

FIGURE 2. Time course of TEWL across skin of mice treated as indicated. Bars show the standard error of the mean with each animal considered the experimental unit. There were no differences between these groups of animals. The arrow indicates time of material application. Pretreatment measurements were taken 2 hr prior to application and then at 3, 6, and 22 hr following application of the test compound.

from the skin and into internal organs. Figure 5 shows the distribution of tritiated BAP on the skin of mice treated once either with BAP-acetone or BAP-acetone and washed with kerosene. No significant difference is seen in the first 12 hr after application of the BAP, although at 12 hr there is a non-significant increase in the fraction distributed to liver and lung for the BAP (no kerosene) treated animals. Beginning at 24 hr, a significant and consistent decrease in the fraction of the radiolabeled BAP remaining in the skin is noted in the animals whose treatment included kerosene. At the same time, the fraction of BAP in the liver and lungs of the kerosene-treated animals increases significantly as compared with those treated with BAP-acetone (Figures 6 and 7).

We show that the fraction of BAP transferred to the lung is significantly increased beginning at 24 hr following exposure and that the difference remains significant through 72 hr following treatment following a single in vivo treatment with kerosene.

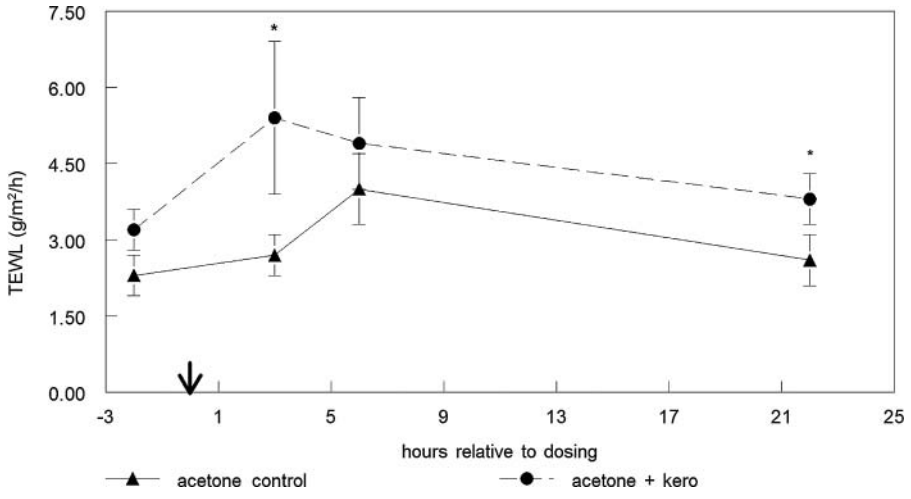

FIGURE 3. Time course of TEWL across skin of mice treated with either the vehicle (acetone) or acetone then washed 1 hr later with kerosene. Bars show the standard error of the mean with each animal considered the experimental unit. Measurements were taken as indicated in the legend for Figure 2. Asterisks indicate where there was a statistically significant difference ($p<0.05$) between the groups at the same time point. The kerosene-treated group was significantly different at 3 and 22 hr after the treatment. The arrow indicates the time of acetone application.

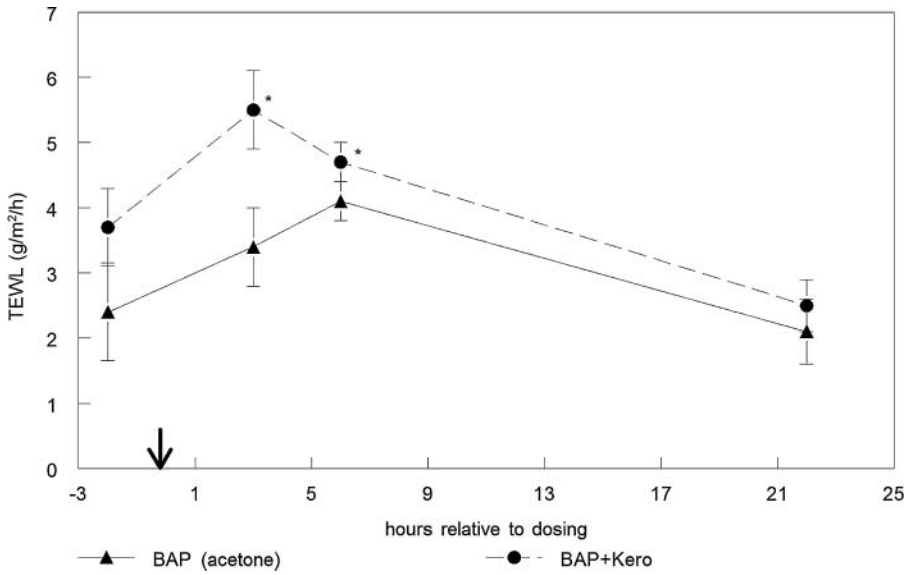

FIGURE 4. TEWL in groups of animals treated with BAP in acetone or BAP in acetone and then washed 1 hr later with kerosene. Measurements were taken as indicated in the legend for Figure 2. Asterisks indicate where there was a statistically significant difference ($p < 0.05$) between the groups at the same time point. The kerosene-treated group was significantly different at 3 and 7 hr after the treatment. The arrow indicates the time of BAP application.

DISCUSSION

Exposure to complex mixtures is more the rule rather than the exception in both occupational and environmental exposures.[23–28] PAC are highly lipophilic and can be absorbed through intact skin to an appreciable extent.[29] However, once the skin is contaminated with PAC the exposed person may take actions to remove materials from the skin that may have a positive or negative impact on the PAC absorption. The data we present corroborate our earlier findings that kerosene

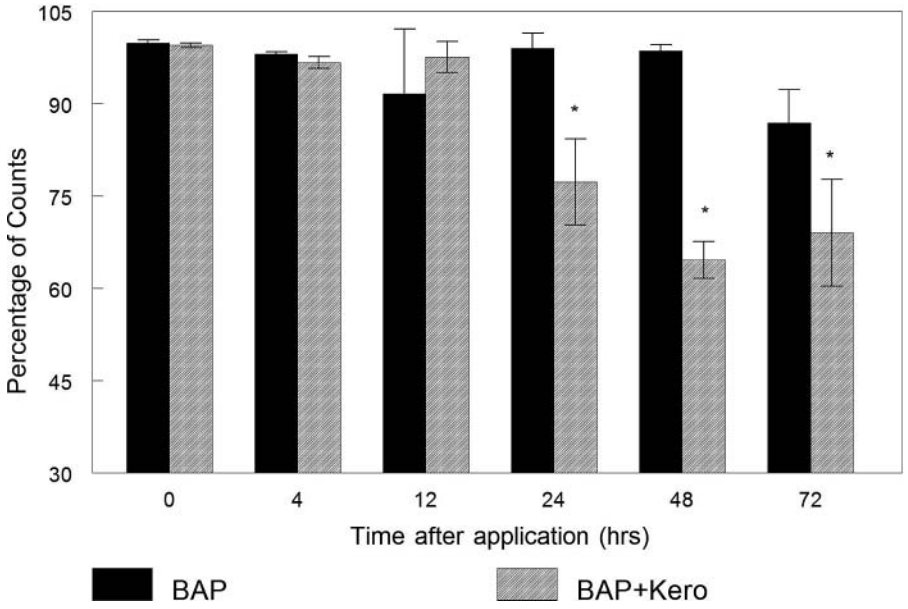

FIGURE 5. Time course of the tritiated BAP in the skin for groups of animals treated either with BAP in acetone or BAP in acetone followed 1 hr later by a wash of the area with 25 μL of kerosene. The y-axis indicates the percentage of counts in the skin divided by the total counts in skin, liver, and lung for that time period. There were four animals in each group. Error bars indicate the standard error of the mean. Differences between the groups are statistically different ($p > 0.05$) at 24, 48, and 72 hr after treatment as indicated by the asterisks.

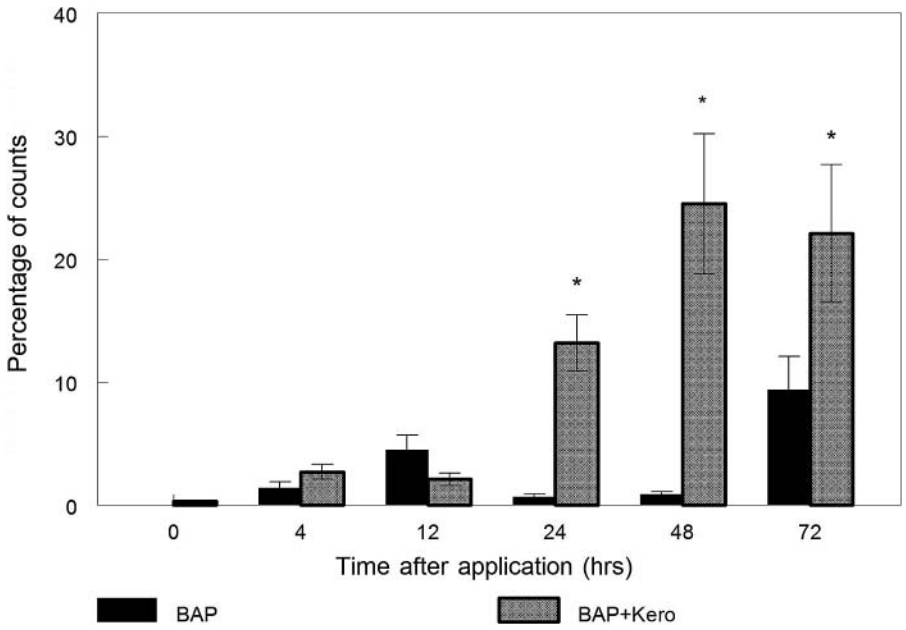

FIGURE 6. Time course of the distribution of tritiated BAP into the liver of groups of mice treated either with BAP in acetone or BAP in acetone and then washed 1 hr later with 25 μL of kerosene. The y-axis indicates the percentage of counts in the liver divided by the total counts in skin, liver, and lung for that time period. There were four animals in each group. Bars indicate the standard error of the mean. There were significant differences(indicated by asterisks) between the groups at 24, 48, and 72 hr in the fraction of the total counts in the liver of the BAP- + kerosene-treated animals compared with those treated with BAP alone.

application as a cleaner increases the levels of DNA damage in the lungs of mice treated topically with used gasoline engine oils (UGEO).[7,30] We have shown previously in mice that prompt use of commercially available cleaners reduces the DNA adduct levels in the lungs of mice treated topically with UGEO.[31] However, we also saw that while cleaning with kerosene reduced the levels of UGEO DNA adducts in the skin, adduct levels in the lung were significantly elevated with

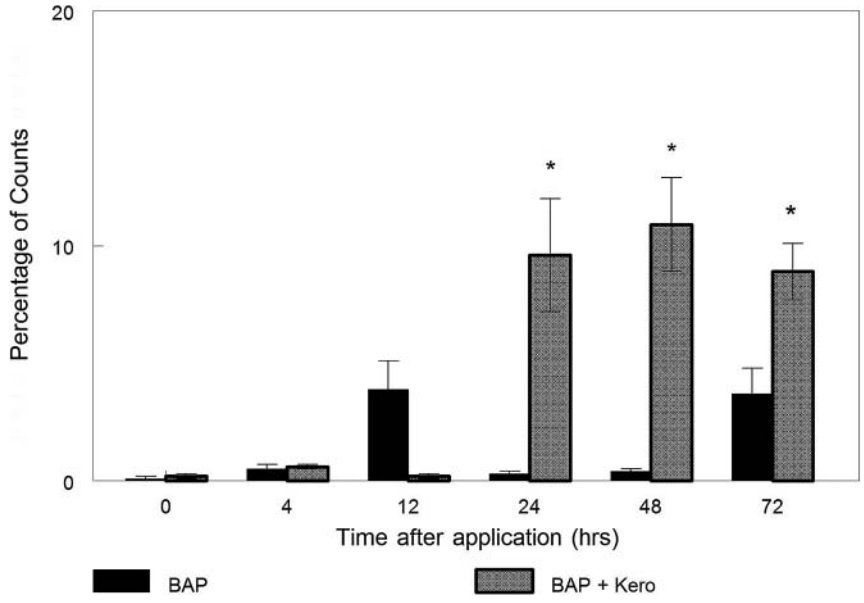

FIGURE 7. The time course of the distribution of tritiated BAP into the lungs of groups of animals treated topically with either BAP in acetone or BAP in acetone followed 1 hr later by a wash with kerosene. The y-axis indicates the percentage of counts in the lung divided by the total counts in skin, liver, and lung for that time period. Bars indicate the standard error of the mean. There were four animals in each group. There were statistically significant differences (indicated by asterisks) in the groups at 24, 48, and 72 hr after the treatment.

this cleaning regimen. The current work explores potential mechanisms for this activity using a single-model PAC to avoid the potential for interaction between PAC in mixtures.

We anticipated that kerosene could act via one or more mechanisms. Kerosene could solubolize the PAC and allow it to pass more readily through the skin without damaging the epidermal barrier. This did not seem to be the case as kerosene did, in fact, damage the epithelial barrier and allow more water to diffuse out of the skin of treated animals. Alternatively, kerosene could damage the barrier, allowing the PAC easier access to the internal environment. It is speculated that defatting agents like kerosene act by creating lacunae and voids in the epidermis allowing lipophilic materials shorter paths into the dermis.[32,33]

The third possibility, not explored here, is that kerosene alters epidermal metabolism, which reduces local activation of the PAC and allows more of reactive metabolites to reach or be formed in distal organs including the lung. Our data indicate that kerosene does indeed damage the epithelial layer and strongly suggests that this is at least one mechanism of its enhancing the lung genoxicity of PAC. We cannot rule out an interaction between solubolizing and damaging effects of kerosene in this work.

We report that the time course of ^{3}H-BAP disappearance from the skin and its concomitant increase in lung and liver followed a different kinetic pattern than the time course of the damage kerosene caused to the skin in vivo (Figures 5–7). The proportion of the BAP in the skin decreased more quickly in the kerosene-treated animals, and the fraction of BAP in the liver and lung increased concommitantly. These data are consistent with our earlier reports that there are higher levels of used gasoline engine oil DNA adducts in the lungs of animals when the animals' skin is washed with kerosene following application.[7,30]

It might be anticipated that BAP levels in the skin would decrease quickly if the increased water flux caused by kerosene occurs within 4 hr and then seems to disappear at 24 hr (Figure 4). However, there is a literature indicating that while passage through the epidermis is rate limiting for dermal absorption of agents, the whole skin, including dermal elements, acts to slow systemic delivery.[34–36] Indeed, Payan and co-workers[37] showed that there was a tripling of time between peaks of dermal absorption and systemic distribution that they attributed to the dermis acting as a "reservoir." Our data are consistent with this effect. Thus, it would appear that kerosene damage to the skin increases uptake of BAP into the skin as a whole (as opposed to on the surface) and this BAP is released into the systemic circulation over a period of 12–16 hr.

Maintenance of the barrier function is the primary role of the skin. In the usual course of events, retention of body water is the most important role of the skin. Severe dehydration is a significant complication in clinical states, e.g., thermal or chemical burns, where the barrier is severely compromised. We have seen that the ability of the skin to maintain body water is measurably and significantly impacted by treatment with kerosene with or without co-exposure to PAC.

There are significant differences between mouse skin and human skin that limit direct extrapolation of these results to workers. Mouse skin contains more hair follicles, fewer sweat glands, and a thinner stratum corneum than human skin.[38] Nonetheless, the mouse skin model has been used effectively used to predict human lung carcinogenicity.[39] So, while the data presented are not directly applicable to humans, they strongly suggest that caution be used when humans may be exposed topically to PAC carcinogens and kerosene.

CONCLUSIONS

We show that a single application of kerosene as a cleaner enhances the uptake and distribution of BAP into liver and lungs of mice. The differences in tissue distribution are congruent with the time course of dermal damage as measured by transepidermal water loss and the increased levels of carcinogen DNA adduct levels we reported earlier. These data indicate that an important mechanism for the enhanced absorption is direct damage to the epidermal barrier. However, we cannot rule out solvation of BAP by kerosene as an added mechanism and uptake enhancer. Workers are often told not to use solvents to clean their skin, and this work indicates why that might be and the possible results if the warning is ignored.

RECOMMENDATIONS

Workers should not use kerosene (or likely any related solvent) to clean their skin after exposure to PAH-containing materials. Our earlier data indicated that citrus-based cleaners were effective in this regard and did not increase DNA damage in internal organs.

ACKNOWLEDGMENTS

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