factors affecting the skin penetration and carcinogenic potency of petroleum products containing polycyclic aromatic compounds

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ABSTRACT

A review is made of the factors which affect the skin penetration of petroleum products containing polycyclic aromatic compounds (PAC).

The bioavailability of petroleum products in contact with the skin is determined by penetration of the product through the stratum corneum into the deeper layers consisting of living cells. This in turn is dependent on a number of factors including viscosity of the petroleum product, the matrix composition of the product, the thickness of the layer of the product on the skin and the duration of skin contact. Notwithstanding these factors, the PAC content of the product applied to the skin has an overwhelming influence for those products studied which ranged in viscosities from 6 to 415 cSt.

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SUMMARY

The potential of oils to produce skin cancer depends on their polycyclic aromatic compound (PAC) content and on the bioavailability of the PAC; i.e. penetration of the PAC into the cells of the viable epidermis. Residual aromatic extracts are less carcinogenic than their PAC content would imply. It has been suggested that this is due to their lack of bioavailability. Factors influencing bioavailability include the barrier properties of the skin, the partition coefficient between the oil and the skin, concentration of the specific PAC in the oil, duration of contact, the size and shape of specific PAC, and the metabolism of PAC to a form that can interact with the DNA of epidermal cells. The interaction of all these factors is not fully understood.

Results from chemical analysis, dermal penetration, and dermal carcinogenicity studies of oils with viscosities between 6 and 415 cSt show that the carcinogenic activity is highly correlated with the concentration of 3 to 7 ring PAC in the oil, and suggest that viscosity has a minimal effect on carcinogenic activity. Over the viscosity range of 6 to 415 cSt, the dermal penetration rate of benzo-a-pyrene from oils was inversely proportional to the log of the viscosity. No data are available which relates oil composition to carcinogenic activity of oils with viscosities above 415 cSt; although the viscosity of these high boiling oils may have no greater effect on dermal penetration, further work is needed to substantiate this. Therefore, the viscosity of the mixture is not expected to be a major factor in determining carcinogenic potency.

Further work on high viscosity oils is needed to investigate to what extent the viscosity of the oil affects its carcinogenic activity and to what extent the carcinogenic activity is governed by composition.

1. INTRODUCTION

Some petroleum oils which have not been solvent refined can produce skin cancer in both laboratory animals and man because of the polycyclic aromatic compounds (PAC) that they contain (1). Oils of high viscosity, however, appear to be less carcinogenic than expected from their total PAC content and it has been suggested that this might be because the viscous oil retards PAC penetration through the stratum corneum.

This review discusses the structures of skin that affect penetration, the relationship between bioavailability in the skin and in the rest of the body, the chemical and physical factors that affect skin penetration by materials, and the penetration and bioavailability of PAC from petroleum oils. Bioavailability refers to the degree to which substances become available to target tissue after administration. For substances applied to the skin, bioavailability describes the phenomenon of penetration through the dead layer of cells (stratum corneum) to the living epidermal cells beneath.

2. STRUCTURE OF SKIN

Because of its structure and composition , the skin forms a barrier to the external environment, regulating loss of body fluids from within and limiting the penetration of chemicals from without. The effects within the skin and in internal organs that result from the contact of chemicals with the skin are dependent, in part, on the rate of penetration of these chemicals into and through the skin.

The skin consists of three layers: the outer stratum corneum, the underlying viable epidermis, and, the inner layer, dermis, as depicted in Fig. 1.

Fig. 1: Schematic representation of the skin

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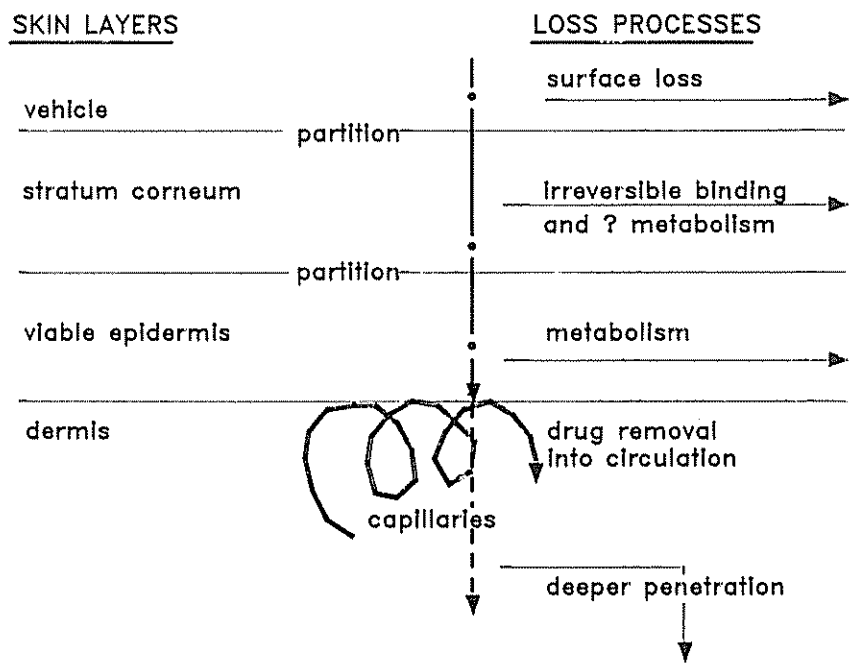


The stratum corneum, or horny layer, is an aggregate of tightly adherent non-living cells, densely packed with lipid-enriched keratin fibrils (3,4). The intercellular spaces of the stratum corneum are filled with lipid (5). Under normal circumstances water diffuses from the underlying tissue through the stratum corneum to the outside environment. The stratum corneum usually contains little water but occlusion or water soaking can greatly increase hydration such that it can absorb three to five times its own weight in water (6). The degree of hydration of the stratum corneum

can affect the penetration of materials. Both the viable epidermis (the site of cutaneous metabolism) and the dermis (the site of vascular uptake) contain larger amounts of water and lower levels of lipid than the cells of the stratum corneum, and therefore, present a different barrier to penetration than does the stratum corneum.

In the basal layer of the epidermis (nearest the dermis), the cells undergo mitosis to provide new squamous cells that are continuously pushed toward the skin surface. As they move further from the dermis, they die and become part of the stratum corneum. Skin cancer can only start by alteration of the genetic material of basal cells which is expressed in daughter squamous cells or by turning on mitosis in normally non-mitotic squamous cells.

3. LOCAL VS SYSTEMIC BIOAVAILABILITY

Molecules in a mixture applied to the skin, partition between the application medium and the stratum corneum, diffuse through the stratum corneum, and partition between the stratum corneum and the viable epidermis. At this point, the material is bioavailable to the viable epidermis (local bioavailability). Molecules in the viable epidermis may pass into the dermis, where they can be absorbed through blood capillaries into the blood stream (systemic bioavailability).

For many materials, passage through the stratum corneum becomes the rate limiting step for bioavailability because passage through it presents the greatest resistance (2). Some investigators believe that passage through the stratum corneum is the rate limiting step for all materials.

Others believe that for lipophilic materials, the lipid-saturated stratum corneum is more like a sink or sponge; the amount of material that is absorbed into the stratum corneum is limited only by the solubility for the chemical in epidermal lipids (7). For these materials, they believe that the rate limiting step is the passage from the stratum corneum into the viable epidermis. Partitioning between the viable epidermis and the dermis has little, if any, effect on the systemic bioavailability of a material. The rate of passage of materials from the epidermis to the dermis is dependent on the concentration of the solute in the epidermis. The concentration of a material in the epidermis, where it can be metabolized to carcinogenic components, is determined by the rate of penetration through the stratum corneum and subsequent removal by passage into the dermis.

4. METABOLISM IN THE SKIN

PAC are metabolized in the viable epidermis of laboratory animals and humans to a variety of derivatives including phenols, quinones, and dihydrodiols. Enzymes involved include aryl hydrocarbon hydroxylase and epoxide hydrase. Reports in the literature indicate that these enzyme systems become saturated at comparatively low doses of PAC. Yang et al (8), found no significant difference in the flux of benzo(a)pyrene (BaP) through the skin of whole animals and excised skin sections (non-viable epidermis) at doses of 10-25 $\mu g/cm^3$ suggesting that cutaneous metabolism was essentially overwhelmed at this dose. Metabolism does not appear to contribute significantly to the bulk diffusion of PAC through the skin but it is the metabolism of PAC in the epidermis to certain stereoselective isomers of dihydrodiol-epoxides that is responsible for the carcinogenic activity of PAC (9). It is the concentration of carcinogenic PAC in the epidermis resulting from percutaneous absorption that drives the production of carcinogenic metabolites.

5. FACTORS AFFECTING SKIN PENETRATION

Each compound has a unique skin "permeability constant" which is a function of molecular size, shape and polarity. For solutes dissolved in a complex mixture, the rate of skin penetration is further dependent on diffusion through the applied sample to the stratum corneum. Diffusion of solute through the application medium is a function of solute concentration, size, shape and polarity as well as chemical composition and viscosity of the medium.

Thus, the rate of skin penetration of a component from a mixture is dependent not only on the intrinsic "permeability constant" of the compound but also on a complex interaction of a number of other factors. Additionally, the rate of penetration measured in a dermal penetration experiment may also be influenced by specific test procedures, such as occlusion of the application site, the rate of application, the duration of exposure, etc. The nature and impact of these many variables on skin penetration are detailed below.

5.1. INTRINSIC FACTORS AFFECTING SKIN PENETRATION

The intrinsic factors affecting skin penetration rate include the molecular size and shape, and polarity of the applied compounds. These factors determine the diffusivity, solubility and partition coefficient of a molecule.

Diffusion is the movement of a chemical from an area of higher concentration to an area of lower concentration. Within a homologous series, the diffusion rate decreases with increasing molecular size (10). Differences among isomers that cause significant changes in molecular shape can be expected to cause differences in diffusion and, therefore, to cause differences in the rate of skin penetration. Roy et al.(11) determined the *in vitro* skin penetration of a series of polycyclic aromatic compounds and showed that isomers of different molecular shapes (e.g. chrysene and benz(a)anthracene; perylene, benzo(a)pyrene, and benzo(e)pyrene) had different rates of penetration.

The partition coefficient is the ratio of the concentrations of a chemical at equilibrium in two immiscible phases, such as an organic liquid (octanol, heptane, isoamyl alcohol) and water. The relative solubility of the chemical in the two phases will determine the proportion which remains in each phase at equilibrium. Because the skin contains phases or regions that are primarily lipid and phases that are primarily water, rates of penetration are to some extent related to partition coefficients. For most materials, the partition coefficient between vehicle and the stratum corneum is of primary importance. Those with high octanol/water partition coefficients penetrate faster than those with low partition coefficients (12,13,14). However, a number of

other factors relating to chemical structure affect penetration, and so the octanol/water partition coefficient by itself is not predictive of skin penetration (6).

The in vitro penetration of a series of three- through five-ring polycyclic aromatic compounds (PAC) through rat skin has been determined and related to a mathematical structure-activity model (11). The mathematical model showed that dermal penetration of the PAC correlated with molecular surface area, molar reactivity (related to logarithm of octanol/water coefficient), molecular symmetry and molecular moment of inertia (related to dipole moment).

5.2. PHYSICAL FACTORS AFFECTING SKIN PENETRATION

The physical factors affecting the rate of skin penetration of a chemical include the thickness of the layer of material applied, the viscosity of the vehicle (or mixture), the concentration of the given chemical within the mixture, and the duration of contact with the skin.

Following the application of a layer of a material to the skin, the rate of transfer of solute or solutes through the skin increases and reaches a steady rate. This steady rate is maintained indefinitely provided that a constant concentration of solute(s) is maintained on the skin (15). In actuality, as the concentration of any single component in a finite dose decreases, so too does the penetration rate in accordance with Fick's laws of diffusion (2). Penetration rates can be sustained by increasing the thickness of the layer of the applied dose above a prescribed section of skin. The steady-state penetration rate attained with large doses at any single concentration is referred to as the "flux rate at infinite dose", i.e. the penetration rate attained when the change in concentration of solute on the skin is essentially zero over the time course of the experiment (10).

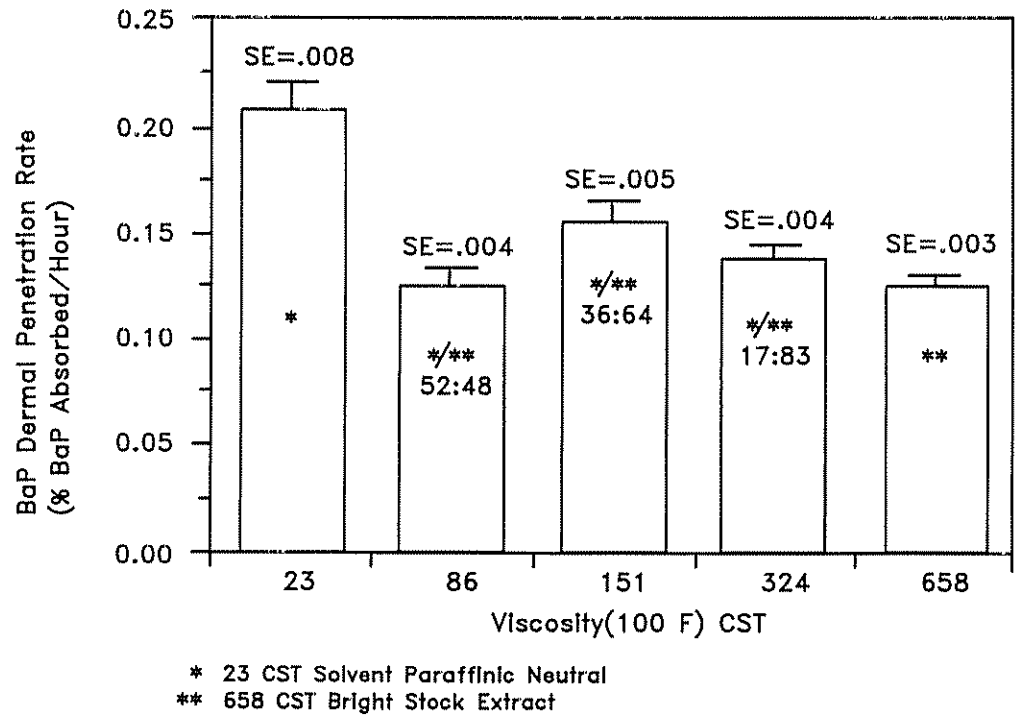
When a mixture (including solutions and formulations) is applied to the skin, some molecules are in close contact with the skin surface while the remainder are contained above the skin. Those molecules near or at the skin surface may pass through the stratum corneum but, before other molecules of the material of interest can pass through the skin, they must diffuse through the overall matrix until they can reach the skin surface. Diffusion through the matrix is dependent on the molecular size of the solute and the viscosity of the mixture (16,17).

Generally, increasing the viscosity of a mixture slows diffusion and decreases skin penetration (18). A recent study by Roy et al (manuscript in preparation) also investigated the magnitude of the effect of viscosity on penetration rate. The results in Fig. 2 show that for two oils differing in viscosity by a factor of 30 (23 cSt to 658 cSt), the penetration rate for benzo(a)pyrene varied by about 30%. It should be pointed out that viscosity is not an

independent property; in the case of complex mixtures, like refinery streams, changes in the chemical composition of the stream which make it more viscous may also significantly affect the partitioning of a solute between the matrix and the stratum corneum and thus counteract or add to the effect of increased viscosity. In the case of the oils in Fig. 2 a low viscosity paraffinic oil was blended with high viscosity residual aromatic oil; thus, the increased aromaticity of the oils may have had as much effect on limiting penetrations as did the increased viscosity.

According to Fick's laws of diffusion, the rate of penetration of a material increases with increasing concentration in the application medium. As the duration of skin contact increases, the total amount absorbed increases as has been demonstrated for trichlorcarban (19). In both in vitro and and in vivo studies, the percent of applied doses of anthracene and benzo(a)pyrene which penetrated through skin of rats increased similarly over 5 days (8, 20); however, linear rates of absorption could only be maintained under conditions of "infinite dose".

Fig. 2: In vitro percutaneous absorption of ^{14}C-BaP from oil blends of increasing viscosities.



6. HOW THESE FACTORS MAY AFFECT THE BIOAVAILABILITY OF PAC IN PETROLEUM MIXTURES

Based on the foregoing discussion it can be concluded that a number of physical and chemical parameters affect the degree and rate of skin penetration by the aromatic components of a petroleum mixture. The skin penetration rate is dependent to some extent on the viscosity of the petroleum mixture. Increases in matrix viscosity will generally result in a decrease in the penetration rate of solutes of interest. Matrix composition or, more specifically, the aromatic content of the mixture will also affect the skin penetration. The larger the percentage of the mixture that is aromatic, the more each aromatic molecule will tend to remain in the aromatic matrix rather than partition into the stratum corneum (i.e., a high matrix/stratum corneum partition coefficient) (16,17,21). On the other hand, as the concentration of each individual PAC in the mixture increases, the penetration rate of that PAC increases according the Fick's laws of diffusion. Thus for any petroleum mixture, the degree of penetration of a particular PAC is directly related to its own concentration in the mixture and inversely related to the concentration of other aromatics in the mixture and to the viscosity of the mixture.

The degree to which these physical and chemical factors affect the potency of petroleum mixtures in standard mouse dermal carcinogenicity assays has been studied by Roy, et al. (22,23). 39 petroleum oils having viscosities of 6 to 415 cSt and 3-7 ring PAC content ranging from 0.2% to 19% were evaluated for correlation of mutagenic and carcinogenic activity with dermal penetration rate, viscosity and matrix composition (as measured by PAC content). Within each category of low, medium and high viscosity, a strong correlation was found to exist between 3-7 ring PAC content and mutagenic/carcinogenic activity (Table 1). However, neither the dermal penetration of the PAC nor the viscosity of the oil correlated with the mutagenic or carcinogenic potency of the oils. The dermal penetration rate (DPR) of PAC (measured by penetration of ^{14}C-BaP added to oils at 5 ppm) decreased modestly as the viscosity increased; the relationship is expressed by the equation DPR=1/log viscosity. It must be remembered that this was derived from a linear regression analysis and does not account for the concentration of aromatic components in the oil. The penetration of a specific aromatic component, such as added BaP, is expected to be proportional to its own concentration, but reduced by high concentrations of other aromatics.

In a second study by Roy et al.(1989), two oils, viscosities 23 and 658 cSt, were mixed in various proportions to look at the effect of viscosity on dermal penetration while minimizing changes in composition. Each of the blends was fortified with 50 000 ppm ^{14}C BaP in order that most of the PAC would be BaP. In this study dermal penetration decreased by 30% over the viscosity range of 23 to 658 cSt (Fig.2).

Table 1: Physicochemical and Biological Activity Parameters for Petroleum Oils (a)

Sample Identity	Viscosity (b) (cSt)	PAC (%) 3-7 ring	Skin Pen (c) (%-48hr)	MI (d)	CI (e)
Viscosity 0 to 30 cSt					
Shell 16	8.1	0.2	48	0	0
Shell 10	14.0	0.3	44	0	0
Shell 7	13.3	0.3	46	0	0
70" SPN	10.8	0.5	35	0	0
150" Paraffinic	15.0	0.5	40	0	3.9
Shell 9	19.5	0.6	29	0	0
Shell 11	19.9	0.7	38	0	3.3
Shell 15	28.7	0.7	30	0	3.5
Shell 8	17.6	0.9	31	0.9	4.2
Stock 619	20.2	1.2	36	0	0
Shell 13	18.9	3.1	28	2.4	5.9
Shell 18	13.7	3.1	33	4.0	14.3
Shell 17	19.7	3.7	29	3.9	16.0
Stock 615.5	18.7	4.6	28	4.5	46
Shell 19	17.7	4.9	24	3.6	21.7
Shell 20	26.5	5.2	26	6.5	23.4
API D4	5.91	6.1	35	5.2	21.7
Shell 21	19.9	7.7	23	9.2	138
Husos	21.4	7.8	26	5.9	74
Shell 14	20.3	10.0	22	9.1	71
API C4 A Aromatics	13.0	11.0	16	8.0	30.5
Parafinosa	10.3	12.0	27	10.0	154
Viscosity 31 to 70 cSt					
350" SPN	63.2	0.3	18	0	0
API D5 Saturates	(37) (f)	0.7	34	0	0
Refrigerator oil	57.5	0.7	26	0	2.1
Naphthenic Blend 2	32.7	1.2	26	1.2	6.2
Condor 350	57.0	1.3	22	1.6	2.7
Naphthenic Blend 3	43.1	1.5	23	2.1	38.3
Naphthenic Blend 1	48.3	4.1	21	4.1	104
Viscosity >70 cSt					
550" SPN	91	0.6	18	0	0
800" SPN	186	0.6	15	0	0
Shell 6	119	0.7	18	0	0
Shell 3	261	4.0	10	5.2	26.7
Shell 4	361	5.0	9.6	4.6	17.7
API D5	108.4 (g)	7.1	10	9.1	182
API C5	185.1 (g)	7.9	6.9	7.1	124
Shell 2	200	8.9	11	11	138
Shell 1	214	9.1	9.3	9.7	126
Shell 12	415	19.0	6.7	17	319

a) Oils are grouped by viscosity (0 to 30, >30 to 70, 71+) and ranked within each group according to increasing 3 to 7 ring PAC content.

b) Experimentally determined viscosities (in centistokes) at 40°C unless otherwise noted.

c) H-Benzo(a)pyrene (BaP) was added to the oils (5ppm) to serve as a surrogate for PAC. The dermal penetration rate of the PAC fraction of the oils was measured in vitro using 350 μm skin section, from rats, mounted on Franz diffusion cells. The receptor fluid was modified with a non-ionic surfactant to facilitate the dissolution of PAC. Skin permeation was determined by counting radioactivity in the receptor fluid at 24 hour intervals.

d) MI (mutagenicity index) is a ranking for relative mutagenic potency as determined in a modified annex assay. The MI is the slope of the dose response for mutagenesis (Cell Biol. Toxicol. 1, 67 1986).

e) CI (carcinogenicity index) is the percentage T/LP where percentage T is the number of mice in a two-year skin painting bioassay that develop tumours divided by the number of mice tests x 100 and 1/LP is the reciprocal of the latent period-the time in weeks for the first tumour to develop in a mouse (Cell Biol. Toxicol. 2, 63 1986).

f) An estimated viscosity based on the viscosity of the parent fraction, API D5.

g) Extrapolated 40°C viscosities based on a determination of viscosity at 100°C and 60°C and the calculated viscosity index.

Taken from Roy et al (22).

7. CONCLUSIONS/FUTURE WORK

For very high boiling oils (vacuum residues and residual aromatic oils) further work must be done to evaluate the parameters of viscosity, chemical composition and genetic interaction (e.g., DNA adducts) with regard to dermal carcinogenesis. Available evidence suggests that viscosity for these heavy oils may not be a significant factor in carcinogenesis but this is not proven. It is possible that the high boiling oils may simply contain a spectrum of aromatic substances of unknown, but perhaps lower carcinogenic potency. Small compositional differences would be extremely difficult to detect in these complex hydrocarbons mixtures, but as noted by Roy, et al (22), oils derived from vacuum residues (IBP > 1040°F) contain highly alkylated aromatics predominantly of 1 to 3 rings.

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