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Development and application of a skin cancer slope factor for exposures to benzo[a]pyrene in Soil

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ABSTRACT

Humans may be dermally exposed to the carcinogenic substance benzo[a]pyrene (B[a]P) via contact with soil at contaminated sites. The potential for risk is typically assessed using the proportion of dose estimated to penetrate through exposed skin for comparison with an oral route slope factor. An alternate dermal slope factor of 25 $(mg/kg\ day)^{-1}$ was previously developed (Knafla et al., 2006) based on skin carcinogenicity, since skin painting studies with mice suggest the formation of epidermal tumors may be a more sensitive endpoint than systemic tumors following dermal exposure. An extension of this work resulted in a skin cancer slope factor derived on a per unit skin surface area basis of 3.5 $(\mu g/cm^2\ day)^{-1}$ that can be used to estimate risk as a function of exposed surface area. Various factors were examined for interspecies extrapolation of risks from mice to humans and for estimating skin exposures to B[a]P in soil. Using a nominal soil concentration of 1.0 mg/kg, a range of cancer risk values of 29–220 in 100,000 was calculated. Soil concentrations associated with a one in 100,000 risk ranged from 0.0046 to 0.035 μg/g, which are lower than those derived using an oral slope factor. These results suggest that B[a]P-related skin cancer (point of contact) risks should be considered at contaminated sites.

1. Introduction

B[a]P is a polycyclic aromatic hydrocarbon (PAH) that is lipophilic, of low solubility in water, and has a high affinity for organic matter in soil (United States Environmental Protection Agency, 1994, 2002). It is a product of incomplete combustion and its presence is widespread in the environment. Contamination of soil with B[a]P can occur from the combustion of fuels (Nesnow et al., 1983), volcanic activity (Abdel-Rahman et al., 2002), spills of hydrocarbon products (Shatkin et al., 2002), or vehicle exhaust processes (Grimmer et al., 1983; Schmahl et al., 1977; Saladi et al., 2003).

The routine assessment of cancer risks posed by dermal exposure to soil contaminated with B[a]P involves estimating the proportion of B[a]P that penetrates through a predetermined area of human skin in contact with soil, and then summating this (assumed) systemically absorbed dose with oral exposure for comparison with an oral slope factor and risk specific dose (RsD) expressed on a per kg-body weight-day basis. However, this typical approach does not account for skin cancer risk at the site of dermal contact.

We previously calculated an average dermal slope factor for B[a]P on a per kilogram body weight (BW) basis $(24.8\ (mg/kg\ BW\ day)^{-1}$; Knafla et al., 2006) using mouse skin painting cancer bioassays and reported incidences of epidermal tumors (Schmahl et al., 1977; Grimmer et al., 1983, 1985; Nesnow et al., 1983). This factor was developed using the US EPA benchmark dose software, a multistage model, and a 95th lower confidence interval point of departure at a 5% incidence level (*i.e.*, benchmark dose low (BMDL) of 0.05). Similar work was conducted by Hussain and Harris (1998) and LaGoy and Quirk (1994) where dermal slope factors on a per animal or per kg-body weight basis of 37.4 and 34.2 $(mg/kg\ BW\ day)^{-1}$, respectively, were developed. The LaGoy and Quirk (1994) limit was converted to per kilogram BW basis using a mouse weight of 45 g.

These dermal slope factors expressed on a body weight basis can be compared with oral slope factors derived by the US EPA (1994) $(7.3\ (mg/kg\ BW\ day)^{-1})$, Health Canada (1996) $(2.3\ (mg/kg\ BW\ day)^{-1})$, and more recently by Gaylor et al. (1998, 2000); $1.2\ (mg/kg\ day)^{-1}$). Of interest, the dermal slope factors are up approximately 15-fold more potent than the oral slope factors reported by the US EPA and Health Canada, highlighting the importance of having an assessment method for B[a]P induced skin cancer risks. Furthermore, the formation rate of DNA adducts has been shown to be orders of magnitude greater in the skin of mice exposed to B[a]P compared to internal organs, indicating the risk of skin tumors may exceed internal tumors following dermal exposure (Talaska et al., 1996; Schurdak and Randeranth, 1989).

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A practical extension of our previous work was to investigate parameters relevant to extrapolating mouse skin cancer bioassay results to human skin cancer risks (*i.e.*, interspecies extrapolation) associated with dermal exposure to B[a]P. Potential differences in cancer risk as a function of body part of application (*e.g.*, arms versus hands) were examined. The cancer slope factor was normalized to a per unit skin area basis (*i.e.*, $\mu g/cm^2$ skin instead of per kg BW) to allow for risk calculations on an exposed surface area basis, and an adjustment was made to account for interspecies differences in epidermal (target tissue) volume.

Furthermore, we investigated differences in skin absorption of B[a]P in acetone (used in the animal cancer bioassays) compared to soil for human exposures. The final goal was to provide an algorithm through which human skin cancer risks can be estimated as a function of dermal exposure to B[a]P in soil at contaminated sites. This was demonstrated using a nominal B[a]P concentration in soil, and results were compared to risks associated with systemically absorbed doses and use of an oral slope factor.

2. Interspecies extrapolation

Limited information is available for conducting interspecies extrapolations (*e.g.*, between mice and humans) for toxicants that exert their effect at the portal of entry (skin in the case of dermally applied B[a]P). A physiologically-based pharmacokinetic model could provide an informative evaluation of interspecies differences in terms of B[a]P related skin cancer risks. Alternately, a typical a body weight based scaling factor to the power of 2/3 (surface area correction) or 3/4 (allometric correction for biochemical and physiological processes) could be used to account for interspecies differences in whole body metabolism (and associated caloric intake), clearance rates, ventilation rates, *etc*. This type of extrapolation without further adjustment assumes the same dose delivered to a target tissue will elicit a similar toxicological response in humans and mice (toxicodynamic equivalence), although a lower associated exposure dose on a body weight basis would be estimated for humans. While the application of such a scaling factor is sensible for the oral route (US EPA, 2006; Kirman et al., 2003), it was not considered applicable for B[a]P skin cancer risks.

As indicated by the US EPA (2006) and by Rhomberg and Lewandowski (2004), while $BW^{3/4}$ is appropriate for toxicants that exert their action through an unmetabolized parent compound or stable metabolite and where clearance is first order, use of this scaling factor is not well supported for toxicants that exert their action through a highly reactive metabolite that is not removed from the site of formation and effects occur at the portal of entry. This is relevant for B[a]P where it exerts carcinogenic activity in the epidermis (portal of entry effect for dermal exposure), is rapidly absorbed into the target tissue, and within short order is metabolized into highly reactive metabolites that form stable adducts. Furthermore, there is evidence to suggest that skin metabolism of B[a]P to carcinogenic adduct forming metabolites appears to be nearly equivalent between humans and mice. These factors are discussed in greater detail in the following text and it is proposed that a slope factor for estimating human skin cancer risks from B[a]P mouse skin painting data incorporate the assumption that human and mouse skin are of nearly equivalent sensitivity in terms of tumor development, and body weight scaling is not applicable. An adjustment was considered for differences in epidermal (target tissue) thickness between humans and mice.

2.1. B[a]P carcinogenicity in the epidermis

The skin of mice and humans is composed of three general layers – epidermis, dermis, and adipose tissue. The viable epidermis (underlying the stratum corneum) was considered the primary site for B[a]P induced skin tumors based on results from animal complete carcinogenicity skin painting bioassays (Warshawsky et al., 1994; Habs et al., 1984; Cavalieri et al., 1983; Grimmer et al. 1983; Nesnow et al. 1983; Habs et al., 1980; Schmahl et al., 1977; Levin et al., 1977). Various tumor types have been observed including papillomas, sebaceous gland adenomas, malignant melanomas, basal cell carcinomas, and squamous cell carcinomas, with papillomas and carcinomas being the most frequently reported tumor types. Das et al. (1986) found an 8.7–15.4-fold greater binding of radiolabeled B[a]P metabolites *in vivo* in the epidermis compared to the dermis in neonatal mice, further suggesting the epidermis is a primary site for B[a]P induced tumor development.

In humans, both basal and squamous cell carcinomas can be locally invasive, but infrequently metastasize or lead to fatality (Canadian Cancer Society/National Cancer Institute of Canada, 2006). Regardless of the correlation between cancer type and risk of fatality, the formation of carcinomas as demonstrated in mice is considered an important endpoint in terms of regulatory toxicology and contaminated site guideline development for humans.

Although epidermal papillomas are associated with dermal exposure to B[a]P in mice and may develop into carcinomas over time, data for epidermal carcinomas were solely considered for cancer risk extrapolation to humans. A compilation of data from complete carcinogenicity studies that ranged in duration from 45 to 104 weeks (Schmahl et al., 1977; Grimmer et al., 1983, 1985; Cavalieri et al., 1983, 1988; Habs et al., 1984), where both papilloma and carcinoma data have been reported, is shown in Fig. 1. Each figure symbol represents the mean number of mice with tumors per dose level. Carcinoma incidence increased with dose, which was not observed for papillomas where the incidence failed to exceed 20%. Papillomas have a stronger correlation with dose in tumor initiation–promotion experiments with B[a]P, in comparison to complete carcinogenicity bioassays as demonstrated by studies reported by Nesnow et al. (1983).

In humans, melanomas can metastasize and thus pose a significant health risk (Canadian Cancer Society/National Cancer Institute of Canada, 2006). Melanomas have not been frequently observed in mice (Nesnow et al., 1983; Grimmer et al., 1983; Warshawsky et al., 1994; Cavalieri et al., 1983; Schmahl et al., 1977), possibly due to the restriction of melanocytes to hair follicle areas of the epidermis, whereas in humans melanocytes are present throughout the epidermis (Takeuchi et al., 2004). Skin painting of Syrian hamsters with B[a]P (initiation–promotion with B[a]P and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)) found a dose-dependent increase in melanoma surface area versus acetone controls (Bernfeld and Homburger, 1983), suggesting the possibility that melanomas may develop in humans exposed dermally to B[a]P. The available animal cancer bioassay data were insufficient for predicting melanoma risk in humans.

Tumor multiplicity was not directly considered in the assessment of skin cancer risks from B[a]P exposure. In complete carcinogenicity skin painting bioassays where epidermal carcinoma multiplicity was reported, the number of tumors per mouse did not exceed 1/mouse (or barely exceeded) at lower doses where the cancer incidence was less than 50%, although more than 1 tumor/mouse was observed at higher incidence levels (Fig. 2; Levin et al., 1977; Cavalieri et al., 1983). Similarly, in initiation–promotion experiments, doses of B[a]P associated with a 45% or lower carcinoma incidence were not associated with a tumor multiplicity of greater than 1/mouse (Habs et al., 1980; Nesnow et al., 1983; Ashurst et al., 1983; Cavalieri et al., 1988, 1991; LaVoie et al., 1993). A multiplicity of greater than 1/mouse was typically observed at tumor incidences of greater than 50% (Fig. 2), suggesting multiplicity may be a factor for consideration at higher doses, but has a lesser relevance for lower doses and thus for human