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Asphalt fume dermal carcinogenicity potential: II. Initiation–promotion assay of Type III built-up roofing asphalt

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ABSTRACT

Clark et al. (accepted for publication) reported that a sample of field-matched fume condensate from a Type III built-up roofing asphalt (BURA) resulted in a carcinogenic response in a mouse skin bioassay, with relatively few tumor-bearing animals, long tumor latency and chronic skin irritation. This mouse skin initiation/promotion study was conducted to assess possible mechanisms, i.e., genotoxic initiation vs. tumor promotion subsequent to repeated skin injury and repair. The same Type III BURA fume condensate sample was evaluated in groups of 30 male Crl:CD1® mice by skin application twice per week (total dose of 50 mg/week) for 2 weeks during the initiation phase and for 26 weeks during the promotion phase. Positive control substances were 7,12-dimethylbenz(a)anthracene (DMBA, 50 μg applied once) as an initiator and 12-O-tetradecanoyl-13-acetate (TPA, 5 μg, applied twice weekly) during the promotion phase. During the 6 months of study with the asphalt fume condensate, eight skin masses were observed when tested for initiation, five of which were confirmed microscopically to be benign squamous cell papillomas. Only two papillomas were observed when tested for promotion. There was no apparent relationship between skin irritation and tumor development in this study. These results are more indicative of genotoxicity rather than a non-genotoxic mode of action.

1. Introduction

Asphalt (or bitumen in the European Union) is a residual product from the non-destructive distillation of crude oil. Asphalt treated by blowing air through it at elevated temperatures is referred to as oxidized, blown, semi-blown or air-rectified (depending on the degree of oxidation) asphalt (CASRN 64742-93-4). Oxidized asphalts are used most commonly in roofing and other industrial applications, whereas the softer air-rectified or semi-blown asphalts are typically used for paving. Because asphalts are semi-solid or solid at ambient temperature, heating is required for end-use and typical occupational exposures during roofing or road paving activities would be to the resulting fumes.

Laboratory-generated fume condensates prepared from oxidized roofing asphalts (Types I and III built-up roofing asphalt (BURA)) have been shown to elicit skin tumors in mouse dermal carcinogenicity studies (Niemeier et al., 1988; Sivak et al., 1997; Clark et al., , accepted for publication). However, laboratory fumes are not compositionally representative of typical worker exposures (Reinke et al., 2000; Kriech et al., 2007). In the two-year dermal carcinogenesis study in mice reported by Clark et al. (accepted for publication), a laboratory fume sample generated at 232 °C was compared with a fume sample from a storage tank that was considered to be "field-matched" because it had a similar analytical profile to fumes found in an occupational roofing setting using the same asphalt. Both the laboratory and the field-matched fumes from the Type III BURA were carcinogenic to mouse skin. However, the field-matched sample resulted in fewer tumor bearing animals and had a longer latency compared to the laboratory fumes. The field-matched sample had low mutagenic activity; its mutagenicity index in an optimized Salmonella assay was 1.2 (Kriech et al., 2007) on a scale in which values greater than 1.0 raise concern

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about carcinogenic potential in the validated domain for this test (ASTM, 2004).[1] By comparison, the mutagenic index for the laboratory sample was 3.3 (Kriech et al., 2007). Skin irritation including moderate hyperplasia was also noted during the course of the two-year bioassay with the field matched fume condensate sample. Similar patterns of low mutagenic potential, weak carcinogenic activity with a long latency and prolonged/repeated skin irritation have also been observed with a class of materials referred to as petroleum middle distillates, which includes products such as kerosene, jet fuel and diesel. The skin carcinogenic potential of such middle distillates has been attributed to a non-genotoxic mechanism involving repeated skin injury and repair, as evidenced in part from studies of initiation and promotion in mouse skin (Freeman et al., 1990; Przygoda et al., 1994; Nessel et al., 1998, 1999). Thus, it was hypothesized that the field-matched fume condensate may have acted on mouse skin in a similar manner, resulting in tumors via promotion caused by repeated skin injury and repair.

Presented here are the results of a six-month mouse skin tumor initiation and promotion study conducted with the field-matched fume condensate collected from a storage tank of an oxidized Type III BURA. This material is the same as that used by Clark et al. (accepted for publication) to conduct their mouse carcinogenicity bioassay.

2. Materials and methods

2.1. Sample collection and selection

Fume condensate from the oxidized Type III BURA used in the study was generated, collected and characterized by the Heritage Research Group, Indianapolis, IN, as previously described (Kriech et al., 2007; Clark et al., accepted for publication). An eight-member scientific advisory committee (see Acknowledgments) comprised of scientists from government, academia and private consulting provided scientific oversight, assisted with the sample acceptance criteria, and review of the test material selection process.

2.2. Animal species selection and acclimation

Male Crl:CD1® mice (approximately 6 weeks of age) were received from Charles River Laboratories (Portage, Michigan) and were allowed an acclimation period of 2 weeks prior to randomization and selection for the various study groups (30 per group). During the study animals were housed individually under fluorescent yellow lighting (12 h/d) in suspended, stainless steel, wire mesh cages equipped with an automatic watering system. Cage-side tags provided a source of environmental-enrichment to the animals.

2.3. Application site preparation and administration procedure

Prior to administration, the hair was clipped from the back of the animal, in an area comprising not less than 10% of the total body surface area. The body surface area was estimated from the following equation: $A = 9.0^{*}W^{2/3}$ where A was the estimated area in square centimeters and W was the body weight in grams (Derelanko and Hollinger, 1995). Hair was reclipped as necessary, and the 10% area of application was adjusted weekly based upon the mean body weight. If it was deemed necessary to reclip any animal, then all animals in all groups were re-clipped to ensure consistent procedural handling in the event of any potential irritation from the clipping. Clipping always occurred the day before dosing.

2.4. Test material application

Initiation phase: the initiation phase was defined as weeks one and two of the study, and the promotion phase of the study was defined as weeks three through study termination. The asphalt fume condensate was administered in a 37.5 μL volume of a 67% solution in mineral oil (MO, a total dose of 25 mg) twice per week for a total weekly dose of 50 mg. 7,12-Dimethylbenzanthracene (DMBA) served as a positive control initiating agent and was administered once only on day one at a dose of 50 μg. 12-0-tetradecanoyl-13-acetate (TPA) was the positive control promoting agent and was administered twice weekly at a dose of 5 μg (0.01% in acetone) during the promotion phase starting on week three and ending at study termination. Table 1 summarizes the experimental groups and initiation/promotion treatments for the asphalt fume condensate (AFC) and the concomitant vehicle and positive controls.

The test substances were administered dermally using a calibrated positive displacement micropipette. After application of the total dosing volume the pipette tip was used to spread the material. The application area was not occluded, washed off, or wiped clean of any residual test materials.

2.5. Experimental evaluations

During the studies, observations for morbidity, mortality, injury, and the availability of food and water were conducted at least twice daily for all animals. Observations for signs of toxicity and masses were conducted weekly. Dermal irritation examinations and scoring were conducted (as described by Clark et al., accepted for publication) and body weights were measured and recorded weekly. When visible, dermal growths were assigned a tentative tumor type (papilloma, carcinoma or mass). Tentative identification as a papilloma was the basis for identifying an animal as "tumor-bearing." At study termination or upon incidental death, necropsy examinations were performed and selected tissues were collected.

2.6. Microscopic evaluations

Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections was performed on the skin. Gross lesions and stomach were examined in all animals.

2.7. Statistical analysis

Non-tumor data were analyzed by analysis of variance (Snedecor and Cochran, 1989), and if indicated, pair-wise comparisons were performed using a Tukey adjustment (Steel and Torrie, 1980). Tumor incidence data were analyzed for both grossly identified tumors and microscopically-confirmed tumors. Carcinogenicity tests of petroleum hydrocarbons typically focus on the number of tumor bearing animals as the unit of measurement for statistical analysis (Freeman and McKee, 1993). Consistent with this, the number of tumor bearing animals, not total number of tumors, was used as the basis for statistical evaluation. Fisher's exact test (Zar, 1999) was used to compare each treatment group with the corresponding control group. A survival adjusted poly-3 test (Bieler–Williams) of trend was performed according to the methods described in Peigorsch and Bailer (1997).

[1] The validated domain for the ASTM mutagenicity test is virgin base oils, for which carcinogenic potential is dependent upon the occurrence of polycyclic aromatic hydrocarbons (PAHs). While asphalt fumes are outside of this domain, the test is considered useful as a screening tool for possible carcinogenicity of petroleum-derived materials that may contain polycyclic aromatic hydrocarbons.