SUMMARY OF ANIMAL SKIN CARCINOGENICITY TEST RESULTS WITH BITUMEN FUME CONDENSATE SAMPLES

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ABSTRACT

Laboratory-generated fume condensates prepared from oxidized roofing bitumens (Types I and III built-up roofing asphalt (BURA)) were shown by NIOSH to elicit skin tumors in mouse skin in the 1980's. These results were recently replicated for a different laboratory-generated Type III BURA fume condensate. This finding was not inconsistent with the knowledge that residual polycyclic aromatic compounds (PAC) may remain in finished bitumens following distillation from crude oil. However, significant industry research has since demonstrated that laboratory-generated fumes are not compositionally representative of typical worker exposures. Additional industry research resulted in identification, collection and validation of bitumen fumes that were compositionally similar to fumes found in the workplace. This was accomplished for both paving and roofing bitumens. Two "field-matched" samples have now been tested by industry for potential carcinogenicity. Screening tests showed that field-matched fume condensate from Type III BURA had greater mutagenic potential than fume from paving bitumens. A field-matched fume condensate sample from a straight-run paving asphalt was not carcinogenic in a 2-year skin cancer bioassay in mice. In contrast, a field-matched fume condensate sample of Type III BURA was found to be weakly carcinogenic to mouse skin, and a subsequent study indicated that the carcinogenic action involved a genotoxic mechanism. Acknowledgements: research funding was from either Asphalt Institute (AI) (straight-run fume testing) or AI/ARMA/NRCA (Type III BURA fume studies).

Keywords: Bitumen, fume, carcinogenicity

1. INTRODUCTION

Bitumen products may contain polycyclic aromatic compounds (PAC), some of which are carcinogens, as residual contaminants following petroleum refining. Certain PAC-containing petroleum streams are carcinogenic to skin in humans and have been demonstrated to cause skin cancer in mice. Thus, PAC-containing petroleum streams have continued to be of interest from a cancer hazard perspective, and the skin painting bioassay has been the primary tool to identify potential skin carcinogens.

Cancer testing of bitumens in animals presents difficulties. The physical properties of bitumen have made it difficult to test, historically precluding inhalation studies and providing technical challenges to the more straight-forward skin route of application. For skin studies, the bitumen properties required manipulation to facilitate skin administration. This most often was accomplished with the use of solvents, but has historically even included procedures such as applying the bitumen to the skin hot. Such approaches resulted in confounded studies, for example by compromising the integrity of the skin or through the use of solvents that are themselves carcinogenic or co-carcinogenic. Most of the historical studies of any relevance utilized skin painting because of its relative ease, low cost and established relevance for petroleum streams to skin cancer. However, the skin carcinogenicity bioassay has been used for hazard identification purposes; its design does not lend itself to quantitative risk assessment.

More recently, attention has focused on bitumen fumes. Under typical use conditions under which bitumen is heated, PAC may partition into the fumes fraction, the most likely medium for human exposure. As such, bitumen fume condensate samples have been tested for carcinogenic potential. This paper will overview the mouse cancer bioassays conducted with bitumen fume condensates, and the conclusions that can be drawn from such studies.

2. NIOSH STUDIES

NIOSH is the US National Institute for Occupational Safety and Health. NIOSH initially reported results of mouse skin cancer bioassays of bitumen fume condensates in 1988 (Niemeier et al.). Follow-up studies (Sivak et al.) were reported in 1997.

Prior to the NIOSH studies, experimental animal carcinogenicity studies with bitumens had largely utilized whole bitumens, often diluted in solvents, using the skin painting protocol. The NIOSH studies established the precedent for collecting and testing that fraction of the bitumen (commonly referred to as “fume”) that is the most relevant to human exposures. This approach of relating potential human exposure to experimental toxicity studies has since been used again for both skin painting studies as well as an animal carcinogenicity study conducted by the inhalation route of exposure.

The NIOSH studies were very complex studies that assessed a range of physical, chemical and biological variables including, in two sets of studies, comparisons of Types I and III BURA to coal tar pitch, three strains of mice, fume generation temperatures (232 and 316^{o}C), effect of ultraviolet (B) light exposure, evaluation for co-carcinogenicity and tumor promotion and chemical characterization / fractionation to identify proximate carcinogenic fractions.

The key learnings from the NIOSH studies can be summarized as follows.

- Laboratory-generated fume condensates of BURAs were carcinogenic to mouse skin **(Table 1).** Compared to coal tar, bitumen emitted far less fume which had much lower carcinogenic activity than fume condensed from coal tar.
- The carcinogenic activity of the lab fumes could be identified in only two of the five HPLC fractions prepared **(Table 1)**.
- UV light did not synergistically increase tumor yields.
- Chemical analysis of the fractions did not elucidate specific causative carcinogens, that is, biologically relevant concentrations of carcinogenic PACs were not identified in the active fractions
- Neither the whole fumes nor any of the fractions exhibited co-carcinogenic or tumor promoting properties in C3H mice.

Table 1: Selected tumor results from NIOSH studies of Type III bitumen fumes

Percent Tumor Bearing Animals (C_3H mice) - / 316°C Fume		
Study 1	Coal Tar Fume	93
	Bitumen Fume	80
Study 2	Raw Bitumen	13
	Heated Bitumen	0
	Heated Bitumen + Fume	0
	Neat Fume	70
	Fume Fraction A	0
	Fume Fraction B	0
	Fume Fraction C	37
	Fume Fraction D	67
	Fume Fraction E	0
	Recombined Fume	0

Data selected from Niemeier et al. (1988) and Sivak et al. (1997).

From the outset, it was recognized that there was an opportunity to clarify some of the confounders noted in the NIOSH studies since the relatively robust skin tumor response was contrary to conventional wisdom and many questions arose about the relevance of the fume generation protocol. Nevertheless, the NIOSH studies impacted years of research on the possible carcinogenicity of bitumen and re-focused the hazard investigation onto the volatile fraction of bitumen that is emitted with heating.

3. GENERATON OF REPRESENTATIVE FUMES

Significant research programs subsequent to the NIOSH studies demonstrated that the laboratory fume generation system produced a test material that was not representative of typical worker exposure (McCarthy et al., 1999; Kurek et al., 1999; Reinke et al., 2000). The laboratory fume generation procedure utilized conditions such as relatively high (vs. the field) temperatures, stirring and reduced pressures, and resulted in fumes with chemical and biological properties different from fumes collected in the workplace. Further research ultimately resulted in techniques to collect bitumen fumes that were validated to be representative ("field-matched") of fumes collected at actual worksites (Pohlmann et al., 2006; Preiss et al., 2006; Kriech et al., 2007). This availability of field-matched fumes then facilitated the conduct of state-of-the art toxicity studies to evaluate carcinogenic potential in a manner believed to be more relevant to humans than any preceding studies.

4. ASPHALT INSTITUTE SKIN PAINTING STUDIES

With the development of validated procedures to collect field-matched fumes, new toxicity studies were initiated. In the USA, these consisted of new skin painting studies of field-matched fume condensates, under the management of the Asphalt Institute. The studies were conducted with guidance from an independent Scientific Advisory Committee (SAC) comprised of scientists from government (NIOSH), academia and private consulting. New skin carcinogenicity bioassays were considered necessary in part due to guidance by the SAC that skin was a more sensitive target tissue for PAC-containing streams than other tissues, including the other likely (and primary) target organ for human exposures, the lung. These studies were specifically designed to answer two questions that remained outstanding since the completion of the NIOSH studies:

Q1. Are relevant field-matched fumes carcinogenic to mouse skin?

Q2. Are there differences in carcinogenic activity between field-matched fume condensates from paving and roofing bitumens?

Fume condensates were collected from heated storage tanks and analyzed as described by Kriech et al. (2007). Field-matched fume condensates were collected at 147°C for a straight-run PG 64-22 paving bitumen and at

201°C for severely oxidized Type III built-up roofing asphalt (BURA). For a comparison evaluation, a lab-generated fume using the NIOSH protocol was also produced from the BURA.

4.1 Chemical characterization

PAC concentrations in the three sets of fume condensates increased in the order of field-matched paving < field-matched BURA < lab-generated BURA (**Table 2**). Biological activity as assessed by the modified Ames mutagenicity test paralleled PAC concentrations.

Table 2: Characterization of fume condensate samples

PAC Concentration (mg/kg)	Paving	Built-up Roofing	
	Field Matched	Field Matched	Lab
Naphthalene	250	110	39
Acenaphthene	240	16	18
Anthracene	76	23	33
Fluorene	320	110	82
Phenanthrene	500	130	250
Benz(a)anthracene*	<0.08	12	20
Chrysene*	0.5	14	20
Fluoranthene	10	7	22
Pyrene*	33	25	150
Triphenylene	0.2	4	6
Benzo(k)fluoranthene*	<0.08	2	3
Benzo(a)pyrene*	<0.08	3	6
Benzo(b)fluoranthene*	<0.08	3	6
Benzo(e)pyrene*	<0.08	8	17
Indeno(1,2,3-cd)pyrene*	<0.08	1	1
Benzo(g,h,i)perylene*	<0.08	2	3
*Total 4-6 ring PAC	34	74	232
Fluorescence (EU/g)	30	157	336
Mutagenicity index	0.7	1.2	3.3
Simulated distillation (50% - degrees C)	269	342	364

Note: Benzo(k)fluoranthene and benzo(j)fluoranthene co-elute (Kriech et al., 2007).
* Used in summing total 4-6 ring PAC.

From Clark et al. (2011).

4.2 Two-year carcinogenicity bioassays

Results of the two-year carcinogenicity bioassays were reported by Clark et al. (2011). Paving bitumen field-matched fume condensate was diluted to 19% in mineral oil and applied to the skin of C3H mice 7 days per week for a total dose of 50μg/week. Preliminary 4 week studies showed that the fume condensate was irritating to mouse skin and that this dosing regimen, in this short-term study, precluded the occurrence of skin irritation, which would possibly confound the two-year carcinogenicity bioassay. Microscopic evaluations confirmed the absence of treatment-related tumorigenic effects in the treated skin regions of the test mice (**Table 3**). One tumor was observed in the skin of each of the fume-treated and control groups; this incidence is within control range and below statistical significance.

The field-matched and lab-generated BURA fume condensates were applied twice per week in a volume of 37.5 μl for a total weekly dose of 50 μg. Mineral oil and benzo(a)pyrene served as vehicle and positive controls, respectively. Both BURA samples produced skin tumors. The relative order of potency, based on tumor incidence, was field-matched << lab-generated = B(a)P (**Table 3**). The lab generated fume also resulted in a

shorter latency to tumor development compared to the field-matched fume. Both bitumen fume groups exhibited significant skin irritation.

Table 3: Bioassay results – field-matched fume condensates

Tumor Type	Mineral Oil	Field-matched paving	Field-matched BURA	Lab BURA	BaP
Papilloma, squamous cell	0	0	4	3	2
Carcinoma, squamous cell	0	0	8	35	34
Keratoacanthoma	0	0	1	2	3
Mean Latency (weeks)	NA	NA	90	76	58
Range	NA	NA	64-104	57-104	28-83

From Clark et al. (2011).

It is noteworthy that the relative biological activity of the three fume condensates, as measured by carcinogenic response in mouse skin, paralleled both the mutagenic activity and the 4-6-ring PAC concentrations: field-matched paving < field-matched BURA < lab-generated BURA.

Thus, the questions that remained unresolved from the earlier NIOSH studies were answered by these studies:

Q1. Are relevant field-matched fumes carcinogenic to mouse skin? Yes, field-matched fume condensates from BURA were found to have carcinogenic activity in mouse skin, albeit much less than lab-generated fume condensates

Q2. Are there differences in carcinogenic activity between field-matched fume condensates from paving and roofing bitumens? Yes, field-matched fumes from BURA were carcinogenic to mouse skin, field-matched fumes from paving bitumen were not carcinogenic.

4.3 Tumor initiation / promotion

The results of the two-year bioassay of field-matched fume condensate from BURA led to a follow-up question regarding potential mechanism of action. More specifically, the relatively long latency, relatively low tumor yield and occurrence of significant skin irritation in mice treated with field-matched BURA fume group suggested that tumor promotion may have contributed to tumor development. Thus, resolution of a third question was pursued

Q3. Is the carcinogenic response observed in mouse skin with BURA fume condensate likely to result from tumor promotion?

Similar responses related to tumor promotion have been observed with other petroleum streams (Nessel et al., 1998). This question was not completely novel; Robinson et al. reported on the testing of four cutback bitumens for tumor initiation and promotion activity in mouse skin in 1984, and NIOSH tested selected HPLC fractions of BURA fume condensate for co-carcinogenicity and tumor promotion (Table 2, Sivak et al., 1997). Neither bitumen-related investigation resulted in evidence of tumor promotion.

A new tumor initiation / promotion study in mouse skin was thus conducted with field-matched fume condensate from BURA (Freeman et al., 2011). A standard skin initiation/promotion protocol in CD-1 mice was utilized. Thus, the initiation phase was defined as the first two weeks of the study, followed by the promotion phase until study termination. Field-matched fume condensate from Type III BURA was tested as both an initiator and as a promoter. Positive control materials were 7,12-dimethylbenzanthracene as a tumor initiator, and 12-o-tetradecanoylphorbol-13-acetate as a promoter. The field-matched fume condensate was applied diluted in mineral oil at a dose of 25 µg twice per week. Again, the data indicate that tumor promotion is not operative in the carcinogenic response in mouse skin to BURA fume condensate. However, the data were indicative of tumor initiation (**Table 4**), a presumptive genotoxic mechanism. Sivak et al. (1997) had not

evaluated for tumor initiation. Robinson et al. (1984) tested for tumor initiation using cutback bitumens; tumor yields were similar to slightly increased versus solvent controls.

Table 4: Percent of mice with tumors – initiation/promotion study of field-matched Type III BURA fume condensate

Initiator	Promoter	% with Tumors
Mineral Oil	Mineral Oil	**0**
DMBA*	TPA*	**97%**
DMBA	Mineral Oil	**0**
DMBA	BURA fume condensate	**7%**
Mineral Oil	TPA	**3%**
BURA fume condensate	TPA	**27%**
BURA fume condensate	Mineral Oil	**0**

*DMBA:7,12-dimethylbenzanthracene; TPA:12-o-tetradecanoylphorbol-13-acetate
From Freeman et al. (2011).

Thus, the third experimental question was answered:

Q3. Is the carcinogenic response observed in mouse skin with BURA fume condensate likely to result from tumor promotion? No, and further, the mechanism of tumor formation appears to be consistent with tumor initiation, a presumptive genotoxic effect.

5. SUMMARY AND CONCLUSIONS

The mouse skin carcinogenicity data distinguish the carcinogenic potential of field-matched fume condensates from straight-run paving and oxidized BURA bitumens. The biological activity of the tested samples, measured either by mutagenicity or skin carcinogenicity, directionally parallels PAC content. The carcinogenic activity of field-matched BURA fume is less than that observed with lab generated fumes, but likely involves a genotoxic mechanism. Field-matched fume from straight-run paving bitumen was not carcinogenic. These results are consistent with the results of an inhalation carcinogenicity bioassay (i.e., not carcinogenic) conducted in rats using fume prepared from a paving bitumen that was a pooled sample of straight-run and air-rectified bitumens (Fuhst et al., 2007).

The International Agency for Research on Cancer (IARC) assessed the available hazard data data for bitumen and bitumen fumes in October 2011. IARC (Lauby-Secretan et al. (2011) concluded:

- There was, in experimental animals, limited evidence for the carcinogenicity of oxidized bitumens and sufficient evidence of carcinogenicity for fume condensates of these oxidized bitumens. In humans, there was limited evidence for the carcinogenicity of occupational exposures to bitumens and bitumen emissions during roofing. DNA damage was noted to occur in roofers. Overall, IARC concluded that occupational exposures to oxidized bitumens and their emissions during roofing were probably carcinogenic to humans (IARC Group 2A).
- "Studies in experimental animals also provided "inadequate" evidence for the carcinogenicity of straight-run bitumens and fume condensates from straight-run bitumens". Further, "none of the studies with straight-run bitumens ("Class 1") or their fume condensates, including the inhalation study in rats, showed an increased risk". In humans, "evidence for the carcinogenicity of occupational exposures to bitumens and bitumen emissions during road paving was 'inadequate'". However, IARC evaluated that there was strong evidence for mutagenic and genotoxic effects in exposed pavers, and evaluated that occupational exposures to straight-run bitumens and their fume condensates during road paving as possibly a carcinogenic to humans (IARC Group 2B).

- No data in experimental animals was reviewed for mastic. Based on data from humans, IARC concluded that occupational exposures to hard bitumens and their emissions during mastic work was possibly carcinogenic to humans (IARC Group 2B).

The evaluations and interpretations by IARC of the toxicology and epidemiology data appear to be consistent with the industry interpretation of the data. The one area of discordance may be the assessment of the mechanism data. Industry's view prior to IARC was that the mechanistic data was inconclusive or confounded by potential co-exposures, e.g., to diesel exhaust or coal tar residues. The emphasis and strength of the conclusions by IARC on the mechanisms data indicates that the mechanisms data should be re-assessed by industry scientists to better understand its significance, and to assure updated communications of any re-assessed findings, as appropriate.

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