

**ROBUST SUMMARY
OF INFORMATION ON**

Substance Group:

ASPHALT

Summary prepared by: American Petroleum Institute

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NB. Reliability of data included in this summary has been assessed using the approach described by Klimisch, et al.

Klimisch, H. J., Andreae, M. and Tillman, U. (1997)

A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.

Regulatory Toxicology and Pharmacology 25, 1-5.

1. General Information

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1.1.1 GENERAL SUBSTANCE INFORMATION

Substance type : Petroleum product

Remark : Asphalt (Bitumen in Europe) is the residuum produced from the non-destructive distillation of crude petroleum at either atmospheric pressure or under reduced pressure in the presence or absence of steam. Asphalt may also occur as a natural deposit

Asphalts are complex mixtures of hydrocarbons with molecular weights ranging from 500 to 2000. They have high boiling ranges (400-500°C; 752-932°F) and carbon numbers predominantly higher than C25.

Two samples of asphalt that have been used in some of the mammalian toxicity studies were characterized as follows:

| Test | API Sample | |
|----------------------------|------------|-------|
| | 81-13 | 81-14 |
| Gravity (°API) | 6.6 | 11.8 |
| Sulfur (wt%) | 4.46 | 0.72 |
| Nitrogen (wt%) | 0.51 | 0.43 |
| Carbon (wt%) | 90+ | 90+ |
| Nickel (ppm) | 18 | 16 |
| Copper (ppm) | <1 | <1 |
| Iron (ppm) | 33 | 15 |
| Vanadium (ppm) | 39 | 5 |
| Initial boiling point (°F) | 650 | 662 |
| Aromatic (%) | - | - |
| Asphaltenes (%) | 6.5 | 1.2 |

This robust summary does not include any information on studies in man since most of them have been studies designed to assess exposure by biomonitoring methods to bitumen and its fumes during use.

03.12.2004

1.13 REVIEWS

Memo : IARC

Remark : IARC reviewed the evidence for the carcinogenicity of bitumen to animals and man and published their evaluation in 1985.

IARC concluded that

There is sufficient evidence for the carcinogenicity of extracts of steam-refined bitumens, air-refined bitumens and pooled mixtures of steam- and air-refined bitumens in experimental animals.

There is limited evidence for the carcinogenicity of undiluted steam-refined bitumens and for cracking-residue

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bitumens in experimental animals.

There is inadequate evidence for the carcinogenicity of undiluted air-refined bitumens in experimental animals.

There is inadequate evidence that bitumens alone are carcinogenic to humans.

Subsequently, IARC carried out a further review of newer studies and published their new evaluation in 1987. In this new review IARC concluded:

Bitumens are not classifiable as to their carcinogenicity to humans (Group 3).

Extracts of steam-refined and air-refined bitumens are possibly carcinogenic to humans (Group 2B).

04.12.2003

(33) (34)

Memo : CONCAWE

Remark : CONCAWE reviewed the available information on the health and environmental effects of bitumen and bitumen derivatives.

08.12.2003

(25)

2. Physico-Chemical Data

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2.1 MELTING POINT

Method : Softening Point of Bitumen; ASTM D36

Remark : Asphalts are viscous semi-solid to solid materials at ambient temperatures and do not have sharply defined melting points. They gradually become softer and less viscous as the temperature rises. For this reason, softening points are determined as a means of measuring the flow characteristics under closely defined test conditions. ASTM Standard Method D36 (ASTM 2000) is customarily used to determine the softening points of asphaltic materials. In this method, two horizontal discs of asphalt, each supporting a steel ball are heated under controlled conditions. The softening point is reported as the mean of the temperatures at which the two disks soften enough to allow each steel ball, enveloped in asphalt, to fall a distance of 25 mm (1.0 in.).

Result : Value:
30 - 60 °C Penetration Grade (CAS No. 8052-42-4)
60 - 75 °C Hard Grade (CAS No. 8052-42-4)
60 - 130 °C Oxidized Grade (CAS No. 64742-93-4)

08.12.2003

(13) (25)

2.2 BOILING POINT

Value : > 450 °C

Remark : Asphalt and vacuum residue are obtained as the residues from the vacuum distillation of crude oil.

23.05.2003

(27)

2.4 VAPOUR PRESSURE

Method : Calculated: Computer model subroutine MPBPWIN, V1.40; EPIWIN model V3.10 (U.S. EPA 2000)

Test substance : Bitumen/Asphalt

Remark : Asphalt and vacuum residue are obtained as the residues from the vacuum distillation of crude oil. They consist of high molecular weight hydrocarbon molecules having 25 or more carbon atoms. As such they have negligible vapor pressure.

However, small molecular weight polyaromatic compounds that constitute minute fractions of total bitumen may volatilize from the solid bitumen matrix when heated.

| Result | Molecular Weight | No. C Atoms | Estimated Value, hPa |
|--------------------------|-------------------------|--------------------|-----------------------------|
| Representative Component | | | |
| Paraffinic | 353 | 25 | 2×10^{-6} |
| | 703 | 50 | 2×10^{-9} |
| Naphthenic (1-ring) | 351 | 25 | 5×10^{-6} |
| | 701 | 50 | 2×10^{-15} |
| Naphthenic (3-ring) | 345 | 25 | 3×10^{-6} |
| | 695 | 50 | 2×10^{-16} |
| Aromatic (1-ring) | 345 | 25 | 3×10^{-6} |

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| | | | |
|-----------------------|-----|----|---------------------|
| | 695 | 50 | 2×10^{-16} |
| Aromatic (5-ring) | 391 | 25 | 4×10^{-12} |
| | 643 | 50 | 7×10^{-19} |
| Other polyaromatics: | | | |
| Naphthalene | 128 | 10 | 1×10^{-1} |
| Acenaphthene | 154 | 12 | 6×10^{-3} |
| Fluorene | 166 | 13 | 9×10^{-4} |
| Phenanthrene | 178 | 14 | 2×10^{-4} |
| Anthracene | 178 | 14 | 4×10^{-6} |
| Fluoranthene | 202 | 16 | 1×10^{-5} |
| Pyrene | 202 | 16 | 9×10^{-6} |
| Chrysene | 228 | 18 | 2×10^{-9} |
| Benzo(a)pyrene | 252 | 20 | 8×10^{-9} |
| Dibenz(a,h)anthracene | 278 | 22 | 1×10^{-10} |
| Benzo(g,h,i)perylene | 276 | 22 | 1×10^{-10} |
| Coronene | 300 | 24 | 3×10 |

Reliability : (2) valid with restrictions
Estimated vapor pressures were obtained using a validated computer model or reliable reference database.

08.09.2004

(48) (49)

2.5 PARTITION COEFFICIENT

Log pow : ≥ 10

Remark : Partition coefficients of various hydrocarbon isomers having 25 carbon atoms were estimated using the computer program EPIWIN (EPA 2000). This range of estimated Log Kow values indicates they are too high to be empirically determined using standard testing methodologies (OECD 1993).

08.06.2003

(41) (49)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Method : Dutch Normalisation Institute NEN 7345
Year : 1995
GLP : no data
Test substance : Asphalt/bitumen

Remark : A standardized test was conducted to determine the leaching of polyaromatic hydrocarbon (PAH) compounds from bitumen and asphalt (asphalt defined here as bitumen plus aggregate) materials. Nine different bitumens and an asphalt were tested, covering a representative range of commercially available products. The leaching test consisted of a 140 g layer of bitumen contained in a covered glass dish with purified water having a pH of 4. The liquid/water ratio was fixed at 4.5:1. The asphalt sample was tested as a cylindrical block placed on glass rods in a covered glass dish. The amount of water was chosen to keep the amount of water comparable to that of the tests with the bitumens (4 ml/cm^2). Leachate water was removed for analysis and replaced with fresh water after 0.25, 1, 2.25, 4, 9, 16, and 36 days. CONCAWE (2001) states that asphalt and vacuum residue consist of high molecular weight hydrocarbon molecules (m.w. 500 to 15,000) having 25 or

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Result : more carbon atoms. As such they have extremely low water solubilities. Products are widely used in waterproofing applications.

Steady state concentrations in leachate water

| Bitumen code/ PAH analysis | (ng/l) | |
|-------------------------------|-------------|--------------------|
| | Naphthalene | Sum of 2+ rings |
| A | 35 | 8.8 |
| B | 371 | 263 |
| C | 51 | 68 |
| D | 175 | 10 |
| E | 30 | 5.9 |
| F | n.v. | 51 |
| G | 120 | 17 |
| H | 0.9 | 5.4 |
| I | 168 | 172 |
| Asphalt | 33 | 2.4 |

n.v. = not valid

Reliability : The range of bitumens tested showed the same leaching behavior against time. In the first days the concentrations increase and reach steady state between day 3 and day 6. Generally, only the polyaromatic hydrocarbon (PAH) compounds with 4 rings or less were found in concentrations above 0.1 ng/l. As shown in the above table, naphthalene dominated the concentrations when compared to the PAHs having 3 or more rings

30.11.2004 : (2) valid with restrictions

A well documented publication which meets basic scientific principles

(23) (27)

Method : U.S. EPA SW846-1311, Toxic Characteristic Leaching Procedure (TCLP)

GLP : no data

Test substance : Asphalt cements

Method : Leachate samples were extracted according to U.S. EPA SW846-1311 for Toxic Characteristic Leaching Procedure (TCLP). All asphalt samples were heated then frozen as a thin layer on aluminum at -24°C. This thin layer was rolled to break the asphalt cement into pieces and then pulverized with a mortar and pestle (also at -24°C) into pieces small enough to fit through a 9.5 mm sieve. Approximately 100 g of the crushed asphalt cement was tumbled in two liters (50,000 mg/l loading rate) of a pH 2.88 solution of acetic acid in water for 18 hours. The solution was analyzed by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS).

Remark : This study surveyed the leachability of metals from six paving asphalt cements from the Strategic Highway Research Program and four commercial roofing asphalt cements to provide a cross-section of various asphalt sources that are used in the United States. The results should be qualified in that the severe extraction procedure used to leach metals from the asphalt matrix may not represent constituents in leachate in environmental applications.

Result :

| Element | Detection Limits (µg/l) | No. Detects | Average Result (µg/l) |
|-----------|-------------------------|-------------|-----------------------|
| Silver | 5.0 | 0/10 | <5.0 |
| Aluminum | 5.0 | 10/10 | 56 |
| Arsenic | 10 | 0/10 | <10 |
| Barium | 5.0 | 8/10 | 5.7 |
| Beryllium | 5.0 | 0/10 | <5.0 |

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| | | | |
|------------|-----|-------|------|
| Cadmium | 5.0 | 0/10 | <5.0 |
| Cobalt | 5.0 | 0/10 | <5.0 |
| Manganese | 5.0 | 3/10 | 4.2 |
| Mercury | 4.0 | 0/10 | <4.0 |
| Molybdenum | 5.0 | 0/10 | <5.0 |
| Nickel | 5.0 | 4/10 | 5.2 |
| Lead | 5.0 | 4/10 | 4.0 |
| Antimony | 5.0 | 1/10 | 2.9 |
| Selenium | 10 | 1/10 | 5.5 |
| Tin | 5.0 | 7/10 | 6.7 |
| Strontium | 5.0 | 1/10 | 4.4 |
| Thorium | 5.0 | 0/10 | 5.0 |
| Titanium | 5.0 | 10/10 | 23 |
| Thallium | 5.0 | 0/10 | <5.0 |
| Uranium | 5.0 | 0/10 | <5.0 |
| Vanadium | 5.0 | 0/10 | <5.0 |
| Zinc | 5.0 | 10/10 | 64 |

When this study was conducted, aluminum was not of a regulatory concern and aluminum foil was used in the preparation process. Therefore, values for aluminum may include contamination. With the exception of zinc, there was no statistically significant difference between paving and roofing asphalts in terms of what leaches out. Mean values of zinc in leachate from paving and roofing asphalts were 40 µg/l and 102 µg/l, respectively

Reliability : (2) valid with restrictions
A well documented publication which meets basic scientific principles
01.12.2004 (35)

2.14 ADDITIONAL REMARKS

Memo : Additional comments on water solubility

Remark : Brandt and De Groot (2001) provided the following data for various individual polyaromatic hydrocarbon compounds measured at steady-state in aqueous leachate of bitumen. For details of leachate preparation see section 2.6.1. Data provided below show measured PAH concentrations in leachate from one of the bitumen test samples and the corresponding measured or estimated water solubility value of the pure compounds (SRC, 2004). Aqueous concentrations of individual compounds in complex mixtures are a function of their pure-substance aqueous solubilities and their mole fraction in the mixture. Therefore, for complex mixtures such as bitumen, the concentrations of the PAH compounds in aqueous leachate are substantially less than the respective measured or estimated concentrations for their pure state.

| PAHs Detected in Leachate | Concentration in Leachate (ng/l) | Water Solubility of Pure Component (ng/l) |
|---------------------------|----------------------------------|---|
| Naphthalene | 35 | 3 x 10 ⁷ |
| Acenaphthene | 1.3 | 4 x 10 ⁶ |
| Fluorene | 2.1 | 2 x 10 ⁶ |
| Phenanthrene | 4.1 | 1 x 10 ⁶ |
| Anthracene | 0.09 | 7 x 10 ⁴ |
| Fluoranthene | 0.4 | 3 x 10 ⁵ |
| Pyrene | 0.4 | 1 x 10 ⁵ |
| Chrysene | 0.3 | 3 x 10 ³ |

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| | | |
|-----------------------|------|-----------------|
| Benzo(a)pyrene | bdl* | 4×10^3 |
| Dibenz(a,h)anthracene | bdl* | 5×10^2 |
| Benzo(g,h,i)perylene | bdl* | 6×10^3 |
| Coronene | 0.03 | 1×10^2 |

* bdl= below detectable limit

Reliability : (2) valid with restrictions
Estimated water solubility values of pure components were obtained from a validated computer model or reliable reference database.

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Memo : Asphalt Institute Leachability Study

Remark : The Asphalt Institute (2003) analyzed 17 polycyclic aromatic hydrocarbon compounds and eight metals in aqueous leachate from fresh hot mix asphalt. Naphthalene measured 0.25 µg/l, while all other PAH compounds were below the detection limits (detection limits ranged from 0.015 µg/l to 0.194 µg/l). Benzene also was below the detection limit concentration in the leachate. Of the metals, only chromium was measured at a concentration of 0.1 mg/l, but was considered a sample contaminant. Concentrations of all other metals were below detection limits.

Reliability : (2) valid with restrictions
The report was based on sound scientific principles

28.02.2005 (10)

3. Environmental Fate and Pathways

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3.1.1 PHOTODEGRADATION

- Deg. product** :
Method : Calculated: Computer model subroutine AOPWIN, V1.90; EPIWIN model V3.10 (U.S. EPA 2000)
Test substance : Bitumen/Asphalt
Remark : Under ambient conditions, substances in the asphalt and vacuum residue are semi-solid to solid materials having negligible vapor pressure and water solubility. Hence, they do not disperse when released in the environment. However, when used in road-building and roofing applications, these substances may be heated, creating fumes and vapors that could potentially disperse in the atmosphere. Asphalt fume condensate generated in this fashion has been determined to consist of 1% aromatic and 99% aliphatic components (NIOSH, 2000).

Individual constituents in these substances have the capacity to undergo various direct or indirect photodegradation pathways, although the extent to which these substances engage in such reactions depends upon their dispersal and transport where these reactions may take place. For example, polyaromatic compounds can absorb light in the 290 to 800 nm range where direct photolytic reactions can occur, although absorption is not always sufficient to effect a chemical change. Other saturated hydrocarbons and mono and di-aromatic hydrocarbons have the ability to indirectly photodegrade through interaction with OH or NO₃ radicals in the troposphere (Atkinson 1990).

Although component hydrocarbons may undergo direct or indirect photodegradation, the physicochemical characteristics of asphalt and vacuum residue under ambient conditions will not facilitate these reactions.

| Result | Molecular Weight | No. C Atoms | Estimated Half-Life (days) |
|---------------------------------|-------------------------|--------------------|-----------------------------------|
| <u>Representative Component</u> | | | |
| Paraffinic | 353 | 25 | 0.3 |
| | 703 | 50 | 0.2 |
| Naphthenic (1-ring) | 351 | 25 | 0.3 |
| | 701 | 50 | 0.2 |
| Naphthenic (3-ring) | 345 | 25 | 0.4 |
| | 695 | 50 | 0.1 |
| Aromatic (1-ring) | 345 | 25 | 0.4 |
| | 695 | 50 | 0.2 |
| Aromatic (5-ring) | 391 | 25 | 0.1 |
| | 643 | 50 | 0.1 |
| <u>Other Polyaromatics</u> | | | |
| Naphthalene | 128 | 10 | 0.6 |
| Acenaphthene | 154 | 12 | 0.2 |
| Fluorene | 166 | 13 | 1.2 |
| Phenanthrene | 178 | 14 | 0.8 |
| Anthracene | 178 | 14 | 0.3 |
| Fluoranthene | 202 | 16 | 0.2 |

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| | | | |
|-----------------------|-----|----|-----|
| Pyrene | 202 | 16 | 0.2 |
| Chrysene | 228 | 18 | 0.2 |
| Benzo(a)pyrene | 252 | 20 | 0.2 |
| Dibenz(a,h)anthracene | 278 | 22 | 0.2 |
| Benzo(g,h,i)perylene | 276 | 22 | 0.2 |
| Coronene | 300 | 24 | 0 |

Reliability : (2) valid with restrictions
Estimated Atmospheric Oxidation Potentials were calculated using a validated computer model or obtained from a reliable data base.
09.02.2006 (14) (39) (48) (49)

3.1.2 STABILITY IN WATER

Remark : Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters. Materials in the asphalt category are not subject to hydrolysis, as they lack these reactive groups.

Reliability : (1) valid without restriction
08.12.2003 (32)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Remark : See Section 3.8.
08.06.2003

3.5 BIODEGRADATION

Type : Aerobic

Remark : There are no known studies of the biodegradation of bitumen/asphalt using standard guideline methodologies. However, from many years of experience in their use in roadway and roofing applications, they are clearly persistent materials, the absence of biodegradation being a key property (CONCAWE 2001). However, substances in this category are not completely exempt from mechanisms of biodegradation. Various microorganisms have been isolated that are able to utilize asphalt as a source of carbon for growth. For example, Phillips and Traxler (1963) demonstrated that species of Pseudomonas, Chromobacterium, and Bacillus were capable of degrading thin films of asphalt painted on culture flasks. Degradation between 3 and 25% were measured after one week of incubation, and in one experiment measured 90% after one month. Fluctuations in temperature, pH, and oxygen tension affected to a greater or lesser degree the ability of these microorganisms to biodegrade asphalt (Phillips and Traxler 1963; Cundell and Traxler 1973). Although hydrocarbon components in asphalt appear capable of being biodegraded by specific bacteria, the rate is exceedingly slow and may take decades to effect changes in such materials in commercial use (ZoBell and Molecke

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1978). Under realistic exposure conditions where the bulk properties of asphalt limits dispersion and the available surface area for microbial exposure, biodegradation is expected to be minimal.

(27) (28) (43) (51)

3.8 ADDITIONAL REMARKS

Remark

: Due to their high molecular weights (C25 and higher) and physicochemical properties, asphalt and vacuum residue will tend to remain intact and within the medium in which they were released (CONCAWE 1992; US EPA 1985). Although substances in this category would not be expected to disperse in the environment, their use in road surfacing and roofing products are widespread. This has generated an interest and concern for the fate and effects of hydrocarbons in fugitive emissions and runoff/leachate during their manufacture and use (NIOSH 2000; NIOSH 2001; Buckler and Granato 1999). Almost exclusively, the interest and concern has been in the content of polyaromatic hydrocarbons generated under these conditions. Although the vast majority of hydrocarbon molecules are C25 and higher, small amounts of low molecular weight polyaromatic hydrocarbons (PAHs) have been measured in solid matrix materials (API 1987; CONCAWE 1992). While the concentrations of these low molecular weight substances in asphalt and vacuum residue are slight (typically <0.001%) and under normal ambient conditions trapped in the solid matrix, when heated as occurs in road building and roofing applications, asphalt products emit fumes and vapors that contain mixtures of aliphatic and aromatic groups (NIOSH 2000). As fumes and vapors cool, they condense onto local surfaces or collide and stick together with further precipitation from the air (NIOSH 2000), which limits the transport from the site of origin. Vapors of aliphatic and aromatic hydrocarbons which remain suspended have the potential to undergo direct and/or indirect photodegradation in accordance with the molecule's capacity and the conditions that permit those reactions to occur.

Chemical analysis of runoff from "in place" asphaltic materials have found a wide variety of inorganic and organic compounds. However, these substances are attributed to vehicle emissions, spills/droppings of crankcase oil, deicers, nutrients, pesticides/herbicides, fuel additives, maintenance materials and catalytic converter emissions (Buckler and Granato 1999). Bench-scale laboratory leaching studies of fresh bituminous materials have found few measurable quantities of PAHs. In one such study only trace amounts of naphthalene were found in leachate from fresh asphalt (Asphalt Institute 2003) Brandt and De Groot (2001) also determined that naphthalene dominated the PAHs leached from nine different bitumens, with substantially lesser amounts of 3 and 4 ring PAHs occurring. However, even maximum concentrations did not exceed ng/l levels in the leachate water. In a study of in-place asphalt pavement, samples of weather pavement were brought into the laboratory, crushed, and subjected to leachability trials. That study, of the various PAHs measured, only naphthalene was detected slightly above the detection limit (Asphalt Institute, 2003)

08.02.2006

(8) (10) (12) (23) (24) (25) (39) (40) (50)

4. Ecotoxicity

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4.1 ACUTE/PROLONGED TOXICITY TO FISH

Remark : See Section 4.9.
08.06.2003

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Remark : See Section 4.9.
08.06.2003

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Remark : See Section 4.9.
08.06.2003

4.9 ADDITIONAL REMARKS

Remark : Asphalt and vacuum residue are not expected to cause acute or chronic toxicity to aquatic organisms due to the extremely low water solubility of these materials. This is supported by aquatic toxicity data from other petroleum products having similar types of hydrocarbon constituents (i.e., saturate and aromatic fractions). For example Aromatic Extracts, which contain highly aromatic hydrocarbons of C15 and higher, showed no acute or chronic toxicity in aquatic organisms. Those data were referenced in CONCAWE (2001) and are illustrated in the following table.

| Test Species | Value | | |
|----------------------------|------------------------|-------|---------|
| | Endpoint | mg/l | Source |
| Oncorhynchus mykiss | 96-H LL ₅₀ | >1000 | BP 1994 |
| Daphnia magna | 48-H EL ₅₀ | >1000 | BP 1994 |
| Scenedesmus subspicatus | 96-H LL _{50r} | >1000 | BP 1994 |
| | 96-H LL _{50b} | >1000 | BP 1994 |
| Daphnia magna | 21-D EL _{50S} | >1000 | BP 1995 |
| | 21-D EL _{50R} | >1000 | BP 1995 |

Similarly, lubricating oil basestocks, which contain saturate as well as aromatic hydrocarbons of C15 and higher, showed no acute or chronic toxicity in aquatic organisms.

Those data were submitted to the U. S. EPA in support of the Lubricating Oil Basestocks HPV Category (API 2003) as well as referenced in CONCAWE (1997) and are summarized in the following table.

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| Test Species | Endpoint | Value, mg/l | Source |
|-------------------------|------------------------|-------------|------------|
| Oncorhynchus mykiss | 96-H LL ₅₀ | >1000 | BP 1990 |
| Daphnia magna | 48-H EL ₅₀ | >10000 | Shell 1988 |
| Scenedesmus subspicatus | 96-H LL _{50r} | >1000 | BP1990 |
| | 96-H LL _{50b} | >1000 | |
| Daphnia magna | 21-D EL _{50S} | >1000 | BP 1995 |
| | 21-D EL _{50R} | >1000 | |

Asphalt and vacuum residue, which contain saturate and aromatic hydrocarbon molecules of C25 and higher, also would not be considered sufficiently water soluble to elicit acute or chronic toxicity in aquatic animals and plants.

Fish hatchery ponds lined with hot-mix asphalt are operated by the Oregon Department of Fish and Wildlife and the Washington State Department of Fisheries who have said to produce millions of high quality fish each year (Asphalt Institute 2003).

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(9) (11) (16) (17) (18) (19) (20) (21) (22) (26) (27) (47)

5.1.1 ACUTE ORAL TOXICITY

| | | |
|--------------------------|---|--|
| Type | : | LD ₅₀ |
| Value | : | > 5000 mg/kg bw |
| Species | : | Rat |
| Strain | : | Sprague-Dawley |
| Sex | : | male/female |
| Number of animals | : | 5 |
| Vehicle | : | Corn oil |
| Doses | : | 5 g/kg |
| Year | : | 1982 |
| GLP | : | Yes |
| Test substance | : | Vacuum residue API sample 81-13 (See section 1.1.1.) |
| Method | : | <p>Test material was administered as a suspension in corn oil to five male and five female Sprague-Dawley rats.</p> <p>Each animal was given a single oral dose of 5 g test material /kg (at a dose volume of 20 ml/kg). The animals were observed for clinical signs at hourly intervals for the first six hours after test material administration and twice daily thereafter.</p> <p>Body weights were recorded before test material administration and again 7 and 14 days after administration. At study termination (day 14) all animals were killed and were subjected to a gross necropsy when any abnormalities observed were recorded.</p> |
| Result | : | <p>There were no mortalities in the study.</p> <p>Clinical signs included hypoactivity, diarrhea, dark brown and black-stained anal region. Growth was normal during the 14 day observation period. There were no significant treatment-related abnormalities observed at necropsy.</p> |
| Reliability | : | (1) valid without restriction |
| 04.12.2003 | | (1) |

5.1.2 ACUTE INHALATION TOXICITY

| | | |
|--------------------------|---|---|
| Type | : | LC ₅₀ |
| Value | : | > 94.4 mg/m ³ |
| Species | : | Rat |
| Strain | : | Wistar |
| Sex | : | male/female |
| Number of animals | : | 5 |
| Vehicle | : | Air |
| Exposure time | : | 4.5 hour(s) |
| Method | : | OECD Guide-line 403 "Acute Inhalation Toxicity" |
| Year | : | 2000 |
| GLP | : | Yes |
| Test substance | : | Fume generated from a sample of bitumen condensate |
| Method | : | <p>Five male and five female Wistar rats (aged approximately 7 wks) were exposed to either clean air (control) or bitumen fume (100mg/m³ as Total hydrocarbon concentration) for 4.5 hours. The extra 30 minutes was necessary in order to achieve the correct exposure concentration for 4 hours.</p> <p>Exposure was by means of a nose-only inhalation system and the animals</p> |

were individually housed during the remainder of the study. Apart from the exposure period, food and water were available ad libitum. All animals were observed for clinical signs during the exposure period, several times after the exposure on the same day and daily thereafter.

Records were maintained of the following:

General condition, fur, grooming activity

Visible mucous membranes

Behavior and locomotor activity (lethargy, coma, convulsions, diarrhea and salivation)

Central nervous system symptoms

Breathing pattern

Reflexes (at least 1, 24, 48 hr after cessation of exposure, the following reflexes were assessed -

visual placing, climbing reflex, pinna reflex, vibrissae reflex, auditory startle response, pain sensitivity and seizures)

Rectal temperature, once after cessation of exposure.

Body weights were recorded before exposure and again on days 3, 7 and 14.

At the end of the study, each animal was subjected to a necropsy.

A t-test was used to determine the statistical significance of differences between treated and control animals for: rectal temperature, body weight and body weight gain.

Remark : The report states that the exposure concentration chosen was five times the indoor German occupational exposure limit of 20 mg/m³ THC and contains a comment that in the workplace this level is only likely to be exceeded under grossly abnormal conditions.

Result : The exposure conditions are summarized in the following table.

| | <u>Clean air control</u> | <u>Exposure group</u> |
|---------------|--------------------------|------------------------------|
| Exposure time | 4.5 hrs | 4.5 hrs |
| Temperature | 22.7 ± 0.7 °C | 23.8 ± 0.5 °C |
| Humidity | 53.3 ± 3.2 % | 48.2 ± 2.1 % |
| Air inflow | 20.4 l/min | 20.3 l/min |
| Air outflow | 11.8 l/min | 8.6 l/min |
| Conc. THC* | - | 65 mg/m ³ |
| Conc THC ** | - | 94.4 ± 7.7 mg/m ³ |
| NMAD*** | - | 85/1.7 nm |

* Measured during the 30 minute pre-exposure period

** Measured during the 4 hr exposure period

*** Number median aerodynamic diameter.

No clinical signs of intoxication were observed during or after the exposure period.

No body weight differences were observed.

Body temperature was significantly lower for both males and females at the end of the exposure period.

| | Body temperature (°C) | |
|-----------------|------------------------------|----------------|
| | Males | Females |
| Control | 37.3 | 37.7 |
| Exposed animals | 35.6 | 36.6 |

There were no effects on any of the reflexes examined.

There were no gross abnormalities in either the control or treated groups at necropsy.

Test condition : The fume was generated using an evaporation condensation generator.

The bitumen fume condensate was fed via a peristaltic pump to a nitrogen operated dispersion nozzle. A droplet spray was generated and the droplets were evaporated in a heating tube. The hot vapor issued through a nozzle into a slowly flowing cool air stream surrounding the jet. The fume was subsequently diluted with clean air to achieve the intended concentration and the diluted fume was delivered to the nose-only system at a flow rate of about 20 l/min.

Fume concentration was determined by sampling the nose-only unit using a combination of a glass filter and an XAD absorption tube. The material collected on the filter and the XAD tube was extracted and analyzed separately by IR spectroscopy.

In addition the fume was analyzed once for PAHs.

For continuous monitoring of the total hydrocarbon exposure concentration a flame ionization detector with heated sampling line was used.

Particle size distribution was determined using a scanning mobility particle sizer.

Test substance : The PAH content of the exposure atmosphere was as follows:

| PAH | ng/absolute | ng/m³ |
|------------------------|--------------------|-------------------------|
| Naphthalene | 6497.56 | 4709.40 |
| Acenaphthylene | * | * |
| Acenaphthene | 132.41 | 95.97 |
| Fluorene | 58.48 | 42.39 |
| Phenanthrene | 153.59 | 111.32 |
| Anthracene | 58.48 | 42.39 |
| Fluoanthene | 54.25 | 39.32 |
| Pyrene | 131.75 | 95.49 |
| Benz(a)anthracene | 41.36 | 29.98 |
| Chrysene | 42.75 | 30.99 |
| Benzo(b)fluoranthene | 15.27 | 11.07 |
| Benzo(k)fluoranthene | * | * |
| Benzo(e)pyrene | 31.12 | 22.56 |
| Benzo(a)pyrene | 6.11 | 4.43 |
| Indeno(1,2,3-cd)pyrene | * | * |
| Dibenz(ah)anthracene | * | * |
| Benzo(ghi)perylene | 5.84 | 3.23 |

Reliability : (1) valid without restriction
18.11.2004

(30)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD₅₀
Value : > 2000 mg/kg bw
Species : rabbit
Strain : New Zealand white
Sex : male/female
Number of animals : 2
Vehicle : None
Doses : 2 g/kg
Year : 1982
GLP : yes

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| | |
|----------------------------------|---|
| Test substance | : Vacuum residue API sample 81-13 (See section 1.1.1.) |
| Method | : Four male and four female New Zealand White rabbits were used for each dosage level. The skin area designated for treatment was abraded in two males and two females whilst the skin of the other animals remained intact. Undiluted test material was applied to the skin of each rabbit at a dose level of 2000 mg/kg. [The test material was warmed overnight in a water bath to reduce its viscosity]. The treated skin was covered with gauze and an occlusive dressing. The dressings were removed after 24 hours and the treated skin site wiped to remove residual test material. Collars were fitted to the rabbits throughout the study to prevent ingestion of test material. Rabbits were observed for clinical signs, hourly for the first six hours after dosing and twice daily thereafter for 14 days. Body weights were recorded just prior to dosing and again at 7 and 14 days after dosing. At study termination all animals were killed and subjected to a gross necropsy. Any observed abnormalities were recorded. |
| Result | : After the 24 hour exposure period, it was not possible to remove all of the applied test material due to its tar-like nature. Mucoid diarrhea was exhibited by one female on day 1 of the study and diarrhea was exhibited by one female on days 6 and 7. No other clinical signs were observed and the growth of the rabbits was normal following dosing. There were no mortalities and no visible lesions at necropsy. |
| Reliability 04.12.2003 | : (1) valid without restriction |

(1)

5.2.1 SKIN IRRITATION

| | |
|--------------------------|--|
| Species | : Rabbit |
| Concentration | : Undiluted |
| Exposure | : Occlusive |
| Exposure time | : 24 hour(s) |
| Number of animals | : 6 |
| Vehicle | : Undiluted |
| Year | : 1982 |
| GLP | : Yes |
| Test substance | : Vacuum residue API sample 81-13 (See section 1.1.1.) |
| Method | : Undiluted test material (0.5 ml) was applied to two areas of the skin of six young male New Zealand White rabbits. One area of skin on each rabbit had been abraded whilst the other was intact. The treated skin sites were covered with an occlusive dressing which remained in place for 24 hours. Body weights were recorded prior to material application and at weekly intervals throughout the study. After the 24 hours exposure, the coverings were removed and the skin was wiped from the area as thoroughly as possible without irritating the skin. A record was made of the degree of erythema and edema (using the Draize scale) immediately after dressing removal and again at 72 hours, 96 hours, 7 and 14 days. At study termination all animals were killed and subjected to a gross necropsy. Any observed abnormalities were recorded. |

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Result : The primary dermal irritation scores* were:

| Observation | Erythema | | Edema | |
|-------------|----------|---------|--------|---------|
| | Intact | Abraded | Intact | Abraded |
| 24 hrs. | 0.2 | 0 | 0 | 0 |
| 72 hrs. | 0 | 0.2 | 0.2 | 0.2 |
| 96 hrs. | 0 | 0.02 | 0.2 | 0.2 |
| 7 days | 1 | 1 | 0.3 | 0.2 |
| 14 days | 0.8 | 0.8 | 0 | 0 |

Primary dermal irritation index**: 0.2

* Primary dermal irritation score is the sum of the irritation scores for each site divided by the number of animals at each observation period.

** Primary dermal irritation index is the sum of the 24 and 72 hour primary dermal irritation scores for intact and abraded skin (8 values) divided by 4 and rounded to the nearest tenth.

Growth was unaffected by treatment and there were no visible lesions at necropsy.

Reliability : (1) valid without restriction
04.12.2003

(1)

5.2.2 EYE IRRITATION

Species : Rabbit
Concentration : Undiluted
Dose : 0.1 ml
Comment : rinsed after 30 seconds for 3 rabbits. Eyes not rinsed for 6 rabbits
Number of animals : 9
Vehicle : None
Year : 1982
GLP : Yes
Test substance : Vacuum residue API sample 81-13 (See section 1.1.1.)

Method : 0.1 ml of undiluted test material was placed into the conjunctival sac of one eye of each of nine rabbits. The eyelids were held together for one second to prevent loss of test material.
30 seconds after instillation of the test material the eyes of three rabbits were flushed with lukewarm water for one minute.
Body weights were recorded just prior to test material instillation and weekly thereafter throughout the study. Eyes were examined for ocular lesion 1, 24, 48, and 72 hours and 7 days after treatment. Scoring of lesions was according to the Draize scale and was recorded for each observation time. Sodium fluorescein and an ultraviolet light was used to assist in the examination of the cornea for possible damage at the 72 hour and 7 days observation times.
At study termination all animals were killed and were subjected to a necropsy. Any abnormalities were recorded.

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Result : The primary eye irritation scores* were:

| | Unwashed eyes (mean of 6 rabbits) | Washed eyes (mean of 3 rabbits) |
|----------|--|--|
| 1 hour | 2.0 | 1.3 |
| 24 hours | 4.0 | 5.3 |
| 48 hours | 4.2 | 2.0 |
| 72 hours | 1.8 | 0.7 |
| 7 days | 0 | 0 |

* Primary eye irritation score is the total eye irritation score for all animals, divided by the number of animals in each group at each observation period (i.e. average irritation score).

One rabbit exhibited hypoactivity, and was possibly anorexic. It had a bloated appearance and diarrhea at the 7 day observation time. These clinical signs were not considered to be treatment-related.

With the exception of the animal referred to above, body weights were normal throughout the study and there were no abnormalities observed at necropsy.

Reliability : (1) valid without restriction
28.02.2005

(1)

5.3 SENSITIZATION

Type : Buehler Test
Species : guinea pig
Concentration : 1st: Induction undiluted occlusive epicutaneous
2nd: Challenge undiluted occlusive epicutaneous
Number of animals : 10
Vehicle : None
Result : not sensitizing
Year : 1984
GLP : yes
Test substance : Vacuum residue API sample 81-13 (See section 1.1.1.)

Method : A group of ten young adult male guinea pigs were used for this study. 0.4 ml of test material was applied to the shorn dorsal skin of the guinea pigs using Hilltop chambers. The applied material was covered with an occlusive dressing. After six hours the patch was removed and any residual test material was removed from the skin using liquid paraffin as a solvent.
The animals received one treatment each week for three weeks. Two weeks following administration of the third dose, a challenge dose of test material was applied to a virgin skin site on the opposite flank of the animal. This test site was occluded as before.

24 and 48 hours after each skin application an assessment of reaction to the dose was made and recorded.

The positive control group (20 animals) were treated in a similar manner to

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the animals in the test group except that 2,4-dinitrochlorobenzene was used at a concentration of 0.3% in 80% ethanol for the sensitizing doses. The challenge dose of positive control was 0.1% in acetone.

A group of 10 animals was used as naive controls. This group of animals received challenge dose only.

In a previously conducted range finding study, it was established that the test material should be administered undiluted for both sensitizing and challenge doses.

The criteria for evaluating the response:
Determination of sensitization was based on reactions to the challenge dose. Grades of 1 or greater in the test animals indicate evidence of sensitization, provided grades of less than 1 are seen in the naive control animals.

If grades of 1 or greater are noted in the naive control animals, then the reactions of the test animals that exceeded the most severe naive control reactions are considered sensitizing reactions.

Result : No skin reactions were observed in any of the naive control animals or in the animals in the test group. In contrast, skin reactions 1 or greater for erythema were observed in 17/20 animals and for edema in 8/20 animals. These data demonstrate that the test material was not sensitizing.

Reliability : (1) valid without restriction (4)
04.12.2003

Type : Buehler Test
Species : guinea pig
Concentration : 1st: Induction undiluted occlusive epicutaneous
2nd: Challenge undiluted occlusive epicutaneous
Number of animals : 10
Vehicle : None
Result : not sensitizing
Year : 1984
GLP : yes
Test substance : Vacuum residue API sample 81-14(See section 1.1.1.)

Reliability : (1) valid without restriction (5)
04.12.2003

5.4 REPEATED DOSE TOXICITY

| | |
|-----------------------------|--|
| Type | : Sub-acute |
| Species | : rabbit |
| Sex | : male/female |
| Strain | : New Zealand white |
| Route of admin. | : dermal |
| Exposure period | : 6 hours |
| Frequency of treatm. | : once per day, three times each week for four weeks |
| Doses | : 200, 1000 & 2000 mg/kg/day |
| Control group | : yes |
| Year | : 1983 |
| GLP | : yes |
| Test substance | : Vacuum residue API sample 81-13 (See section 1.1.1.) |

Method : Groups of five male and five female young adult New Zealand White rabbits were used for this study.
The dose groups employed were: control, 200, 1000 and 2000 mg/kg/day

Application was by weighing the appropriate quantity of undiluted test material onto a 4x4 inch patch which was applied to the shorn dorsal skin of each rabbit. The patch was covered by an occlusive dressing. Six hours after administration of the test material, the patches were removed and any residual test material was removed from the skin by gentle wiping with a dry gauze.

This procedure was repeated once daily, three times weekly until a total of 12 applications of test material had been made. Sham-treated controls underwent the same procedure except that no test material was applied. Clinical observations were made twice daily. Body weights were recorded just before the first application of test material and once weekly throughout the study thereafter. The skin exposure site was examined and reactions recorded daily during the test period. Degree of erythema and edema were assessed using the standard Draize method.

At study termination, blood samples were taken from the animals for the following hematological and clinical chemical determinations.

| <u>Hematology</u> | <u>Clinical chemistry</u> |
|------------------------------|---------------------------|
| Erythrocyte count | Glucose |
| Total leukocyte count | Blood urea nitrogen |
| Differential leukocyte count | Alkaline phosphatase |
| Hemoglobin | SGOT |
| Hematocrit | SGPT |
| RBC morphology | Total protein |

All animals were then sacrificed and underwent a gross necropsy. The following organs were weighed: Heart, Liver, spleen, kidneys, thyroid, pituitary, testes, ovaries and brain. The following tissues were removed, preserved and prepared for histological examination.

Heart, lungs, bronchi, trachea, thyroid, parathyroids, cervical lymph nodes, salivary gland, tongue, esophagus, stomach, duodenum, jejunum, ileum, sacculus rotundus, colon, thymus, spleen, liver, pancreas, kidneys, adrenals, vagina, seminal vesicles, testes/ovaries, epididymides,

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prostate/uterus, mesenteric lymph nodes, urinary bladder, adipose tissue, mammary gland, brain (cerebrum, cerebellum, pons), pituitary, spinal cord (two sections), skeletal muscle, sciatic nerve, skin (treated and untreated), bone, bone marrow, eyes, gross lesions.

Statistical analyses

Body weights, clinical pathology and absolute and relative organ weight data of the control and treated groups were statistically compared using a two-tailed Student's t-test at the 5% probability level.

Result : Two animals died and two were sacrificed moribund during the study but none of these was considered to be compound-related.
Treatment-related clinical signs in animals that survived to day 28 included: thin appearance, decreased food intake, flaking skin and wheezing.

Erythema for animals exposed to test material could not be scored at most daily intervals because the test material could not be removed from the skin, thus obscuring the test site.

Edema was recorded in all groups except controls throughout the study. The severity ranged from very slight to slight.

The average total edema score for each group was as follows:

| | <u>Male</u> | <u>Female</u> |
|----------------|-------------|---------------|
| Control | 0 | 0 |
| 200 mg/kg/day | 27 | 18 |
| 1000 mg/kg/day | 31 | 36 |
| 2000 mg/kg/day | 37 | 38 |

A treatment-related suppression in body weight gain was recorded for the high dose male groups. The total weight gains (kg) over the course of the study are shown in the following table

| | <u>Male</u> | <u>Female</u> |
|----------------|-------------|---------------|
| Control | 0.6 | 0.5 |
| 200 mg/kg/day | 0.6 | 0.4 |
| 1000 mg/kg/day | 0.4 | 0.5 |
| 2000 mg/kg/day | 0.1* | 0.4 |

* P < 0.05%

There were no treatment-related trends in any of the hematological or clinical chemical parameters that were measured. Statistical analyses revealed differences between controls and the following groups. Although the differences for RBC and glucose were not regarded as treatment-related, the significance of the changes in alkaline phosphatase was not understood.

| <u>Parameter</u> | <u>Dose group</u> | <u>Sex</u> | <u>Difference</u> |
|------------------|-------------------|------------|-------------------|
| RBC | 200 mg/kg/day | M | + 12% |
| Alk Phos. | 2000 mg/kg/day | M | - 50% |
| Glucose | 200 mg/kg/day | F | - 16% |

There were significant differences in the following, all of which were considered to be incidental and not treatment-related.

1000 mg/kg/day

Males Absolute left kidney weight - 14%

2000 mg/kg/day

Males Absolute left kidney weight - 16%

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Males Absolute/relative right adrenal weight + 86/133%
Females Absolute pituitary weight + 63%
Females Relative spleen weight + 50%

Treatment-related gross necropsy findings were confined to the skin. In these cases the skin was reddened and thickened.

Treatment-related microscopic findings were also confined to the skin. Minimal to moderate subacute acanthotic dermatitis and minimal to moderate hyperkeratosis was observed in the high dose males and females (5/5 males, 3/5 females). Females appeared more severely affected.

Incidental findings were observed and were consistent with Encephalitozoon infection.

Reliability : (2) valid with restrictions (2)
21.06.2004

Type : Sub-acute
Species : rabbit
Sex : male/female
Strain : New Zealand white
Route of admin. : dermal
Exposure period : 6 hours
Frequency of treatm. : once per day, three times each week for four weeks
Doses : 200, 1000 & 2000 mg/kg/day
Control group : yes
Year : 1983
GLP : yes
Test substance : Vacuum residue API sample 81-14 (See section 1.1.1.)

Result : The results of this study were similar to those described above with sample 81-13, except that there were no reductions in body weight gain in any of the treated groups.

Reliability : (2) valid with restrictions (3)
08.02.2006

Type : Sub-chronic
Species : Rat
Sex : male/female
Strain : Wistar
Route of admin. : Inhalation
Exposure period : 6 hours per day
Frequency of treatm. : 5 days per week for 14 weeks
Doses : 4, 20 & 100 mg/m³
Control group : Yes
NOAEL : = 20 mg/m³
LOAEL : = 100 mg/m³
Method : OECD Guide-line 413 "Subchronic Inhalation Toxicity: 90-day Study"
Year : 2001
GLP : Yes
Test substance : Bitumen fume from bitumen condensate

Method : Groups of sixteen Wistar rats of each sex (approximately 7 weeks of age) were exposed either to clean air or bitumen fumes at concentrations of 4, 20 or 100 mg/m³. Exposures were by nose only for six hours each day, five days a week for 14 weeks. The animals were individually housed with free access to food and water in between exposure periods.

All animals were observed daily for clinical signs. Additionally all animals were removed from their cages once each week and were examined for abnormalities. Body weights and food intakes were recorded weekly starting before exposure to test material had begun.

Of the 16 animals in each group, 10 were designated for the 90 day study and six for Broncho alveolar lavage (BAL).

At the end of the study, animals were fasted overnight and were then killed and subjected to a detailed post-mortem examination. Blood samples were taken for the following clinical chemical and hematological examinations.

| Clinical chemistry | Hematology |
|---|---|
| Aspartate aminotransferase | Erythrocyte count |
| Alanine aminotransferase | Hemoglobin |
| Gamma glutamyl transferase | Mean erythrocyte volume |
| Alkaline phosphatase | Mean erythrocyte hemoglobin (mass and concentration) |
| Total bilirubin | Total leukocyte count |
| Urea | Differential leukocyte count |
| Creatinine | Platelet count |
| Total protein | Prothrombin time |
| Albumin | |
| Cholesterol | |
| Glucose | |
| Sodium | |
| Potassium | |
| Calcium | |
| Chloride | |
| Inorganic phosphate | |
| Globulin and albumin/globulin ratios were also calculated | |

Urine was collected prior to sacrifice for the following semi quantitative analyses: leukocytes, pH, protein, glucose, ketones, bilirubin, blood, nitrate and urobilinogen. Osmolality was measured quantitatively.

The following organs were weighed at necropsy: Lung (including 2/3 of trachea, liver, adrenals, kidneys and testes. Relative organ weights were calculated.

The following tissues were collected from each rat and fixed for subsequent histopathology.

Brain, pituitary, tongue, eyes, lacrimal glands, Harderian glands, nasal and pharyngeal cavities, larynx, pharynx, trachea, thyroid, parathyroids, lungs, thymus, heart, aorta, lung associated lymph nodes, salivary glands, mandibular lymph nodes, liver, pancreas, spleen, kidneys, adrenals, esophagus, forestomach, duodenum, jejunum, ileum, cecum, colon, rectum, mesenterium and lymph nodes, urinary bladder, testes, epididymis, prostate, seminal vesicles, ovaries, uterus, vagina, mammary glands, skeletal muscle, femur with bone marrow and joint, spinal cord, peripheral nerve (N. ischiadicus) and sternum with bone marrow.

A bronchoalveolar lavage (BAL) was performed on six rats from each group and the cell concentration was determined using a counting chamber. Cytoslides were prepared from the lavagate for differential cell count (macrophages, PMNs, lymphocytes).

After centrifugation of the lavage fluid the supernatant was used for the determination of some relevant biochemical indicators of lung damage.

The following parameters were measured:

cell number, differential cell count, total protein, lactic dehydrogenase, β -glucuronidase and gamma-Glutamyl-transferase

Formalin-fixed terminal bronchioles and lung parenchymal cells were examined for cell proliferation using the sensitive S-phase response method. Proliferating cells were labeled by 5-bromo-2'-deoxyuridine (BrdU) which was administered to five animals per group by a mini pump following 90 days of inhalation. The animals were kept for an additional seven days without inhalation of test material until sacrifice.

The rats were anesthetised and on the back of the rats an area of 5 x 10 cm was shaved. The area was disinfected and the skin was cut to allow implantation of the minipump.

After implantation, the incision was closed and disinfected again.

The lung slides were prepared and stained immunohistochemically following denaturation of the DNA (antibody technique). The slides were evaluated by analyzing an appropriate number of cells from the proximal regions of the pulmonary parenchyma for each rat. For an appropriate number of airways, the unit length labeling index was estimated.

Statistical evaluation

For comparisons between two treatment groups a two-sided t-test at the level of P = 0.05 was used.

Body weight, food and water consumption, hematology and clinical chemical data were analyzed using analysis of variance as a global test.

Pairwise comparisons of the means of the treatment groups with the controls were performed using the Dunnet's modification of the t-test.

Evaluation of histological findings: significance of differences of the frequencies were evaluated as pair wise comparison between clean air control and treatment groups using Fisher's exact test. These tests were performed at a level of P = 0.05.

Result

: The mean fume concentrations (total hydrocarbon content) and proportions of vapor and fume in the exposure chambers were:

| Nominal concentration (mg/m³) | Actual concentration (mg/m³) | Particulate/vapor (%) | Particle* size NMAD (nm) |
|---|--|------------------------------|---------------------------------|
| 4 | 5.53 | 24.6/75.4 | 105 |
| 20 | 28.17 | 42.9/57.1 | 82 |
| 100 | 149.17 | 68.1/31.9 | 86 |

* NMAD = number median aerodynamic diameter

No clinical signs of intoxication were observed. There was one mortality but this was not treatment-related.

Reduced body weights in the 100 mg/m³ males became apparent after one week of treatment and the difference increased during the study. At the end of the study the males in this group weighed 10% less than the corresponding controls.

Milder effects on body weight were noted in all female groups (-5%) exposed to bitumen fumes. Food consumption was also less in the 100 mg/m³ group males and this correlated with the reduced body weights. Water consumption was unaffected by treatment.

There were no toxicologically relevant findings in the hematological parameters measured.

In the 100 mg/m³ males the following differences were recorded in the clinical chemistry values:

20% Increase in mean urea

8% Increase in mean potassium
3% Decrease in calcium concentration
A 3% decrease in calcium concentration was also recorded for the 20 mg/m³ males
No other treatment-related changes were noted in the clinical chemical evaluations.

There were no differences in the urinalysis data.

There were small changes in the data from the BAL evaluations. These were as follows:

100 mg/m³ males

93% increase in lactic dehydrogenase
53% increase in gamma glutamyl transferase

100 mg/m³ females

cell concentration increased by 20%

4 mg/m³ males

42% increase in gamma glutamyl transferase

There were no treatment-related findings in organ weights at necropsy. However, there was a 7.6% higher relative kidney weight in the 100 mg/m³ males. This was attributed to the decreased body weights in this group.

Gross Pathology

There were no treatment-related gross abnormalities at necropsy.

Histopathology

The following treatment-related observations were recorded.

Nasal and paranasal cavities

Changes were only observed in the 100 mg/m³ groups.
The changes consisted of

Very slight to moderate eosinophilic cytoplasmic inclusions (hyalinosis) observed exclusively in epithelial cells of 8/10 males and 10/10 females.
This degenerative lesion affected the respiratory epithelium with olfactory involvement occurring primarily near the olfactory/respiratory transition area.

Occasionally, eosinophilic cytoplasmic inclusions were also seen in cells of the submucosal nasal glands. 1/10 females had moderate multifocal eosinophilic hyalinization of the submucosal glands.

There was focal/multifocal very slight to moderate mucous cell hyperplasia associated with the hyalinosis.
Incidences were 10/10 males, 9/10 females compared to 1/10 males of the control group.

Very slight to slight multifocal mucosal inflammatory cell infiltration was observed in 4/10 males and 3/10 females.

Kidneys

The incidence of multifocal very slight to slight tubular basophilia was

markedly increased in 8/10 males compared to controls or other bitumen treated groups (4-5/10 per group). This finding was not statistically significant, but a treatment-related effect cannot be excluded.

Other degenerative changes such as tubular cell degeneration, interstitial mononuclear cell infiltration and interstitial fibrosis occurred at incidences between 1/10 and 3/10 per group, but were also more common in groups exposed to bitumen fumes.

There were no other treatment-related histological changes in any other organ examined.

Results of pulmonary labeling studies with BrdU

There were no statistically significant differences for the parameters measured in the labeling studies.

However, the mean parenchymal labeling indices were slightly elevated in the males of the 20 and 100 mg/m³ groups compared to controls. In the female groups the labeling indices were higher in all the treated groups compared to controls.

| Group | Parenchymal labeling index |
|-----------------------|-----------------------------------|
| Control | 0.99 |
| 4 mg/m ³ | 0.87 |
| 20 mg/m ³ | 1.14 |
| 100 mg/m ³ | 1.32 |

Test condition : The fume was generated using an evaporation condensation generator.

The bitumen fume condensate was fed via a peristaltic pump to a nitrogen operated dispersion nozzle. A droplet spray was generated and the droplets were evaporated in a heating tube. The hot vapor issued through a nozzle into a slowly flowing cool air stream surrounding the jet. The fume was subsequently diluted with clean air to achieve the intended concentration and the diluted fume was delivered to the nose-only system at a flow rate of about 35 l/min.

Fume concentration was determined twice per week during the first week and weekly thereafter by sampling the nose-only unit using a combination of a glass filter and an XAD absorption tube. The material collected on the filter and the XAD tube was extracted and analyzed separately by IR spectroscopy.

In addition the fume was analyzed once each week for PAHs.

For continuous monitoring of the total hydrocarbon exposure concentration a flame ionization detector with heated sampling line was used.

Particle size distribution was determined 16-18 times using a scanning mobility particle sizer.

Test substance : The total hydrocarbon content for the aerosol and vapor phases at each dose concentration are shown in the following table.

| Dose Group | Mean | SD |
|----------------------|-------------|-----------|
| 4 mg/m ³ | | |
| Aerosol | 0.97 | 0.13 |
| Vapor | 2.98 | 0.25 |
| Total | 3.95 | 0.37 |
| 20 mg/m ³ | | |
| Aerosol | 8.63 | 0.91 |
| Vapor | 11.50 | 0.73 |

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Total 20.12 1.53

100 mg/m³

Aerosol 72.65 6.34

Vapor 33.90 1.76

Total 106.55 7.84

The mean PAH concentrations in the fumes for the various treatment groups were as shown in the following table.

| PAH | Mean concentration (ng/m ³) | | | |
|------------------------|---|------|------|---------|
| | 100 | 20 | 4 | Control |
| Naphthalene | 8304 | 1641 | 409 | 232 |
| Acenaphylene | nq | nq | nq | 7.37 |
| Acenaphthene | 4754 | 1046 | 222 | 31.9 |
| Fluorene | 11162 | 2296 | 505 | 33.1 |
| Phenanthrene | 15743 | 2450 | 449 | 22.4 |
| Anthracene | nq | nq | nq | nd |
| Fluoranthene | 631 | 150 | 26.2 | 1.65 |
| Pyrene | 1311 | 303 | 57 | nd |
| Benzo(a)anthracene | 217 | 45.8 | 7.86 | nd |
| Chrysene | 377 | 77.6 | 13.2 | 0.90 |
| Benzo(b)fluoranthene | 116 | 23.1 | 4.73 | nd |
| Benzo(k)fluoranthene | nd | nd | nd | nd |
| Benzo(e)pyrene | 222 | 45.5 | 8.8 | nd |
| Benzo(a)pyrene | 53.5 | 10.4 | 1.98 | nd |
| Indeno(1,2,3-cd)pyrene | nd | nd | nd | nd |
| Dibenzo(a)anthracene | 21 | 2.49 | nd | nd |
| Benzo(ghi)perylene | 49.9 | 9.83 | 1.82 | nd |

nd = not determined

nq = not quantified

Reliability : (1) valid without restriction
08.02.2006

(31)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test
System of testing : S. typhimurium, strains TA98 & TA100
Metabolic activation : with and without
Year : 1987
GLP : no data
Test substance : Penetration bitumen (3 samples)

Method : DMSO extracts of the bitumen samples were tested at increasing doses by means of the Ames test, using TA98 and TA100 strains, with and without rat-liver enzyme system (\pm S9 mix).

The bitumen samples were also separated into four fractions by liquid chromatographic separation and these fractions were also tested in the Ames test. However, since it had been reported previously that petroleum distillates may have inhibitory effects on mutagenic activity, the derivatives in this study were tested in the presence of an increased concentration of S9 (50% instead of 10%).

5. Toxicity

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The ether and acetone extract of the fume samples were dissolved in DMSO. These solutions were tested as described above. Blank extracts of unloaded filters were also tested.

[It should be noted that the above summary contains all the information provided in the publication. No other experimental details were provided].

Result

: The results of the mutagenicity studies are given in the following table.

| Sample | DMSO ext. | Bitumen | TA98 | | TA100 | |
|-------------------------|-----------|---------|--------|--------|----------|--------|
| | | | -S9 | +S9 | -S9 | +S9 |
| * | ** | | | | | |
| Solid bitumen samples | | | | | | |
| 1 | 0.1 | 1.3 | 23±2 | 44±5 | 106±15 | 143±19 |
| | 5.0 | 65.2 | 34±5 | 62±9 | 165±24 | 207±31 |
| 2 | 0.1 | 1.1 | 22±3 | 36±4 | 127±16 | 138±18 |
| | 5.0 | 56.3 | 35±4 | 45±6 | 10±18 | 182±15 |
| 3 | 0.1 | 1.0 | 18±3 | 61±11 | 134±26 | 206±22 |
| | 5.0 | 43.3 | 37±4 | 50±7 | 145±18 | 167±19 |
| Negative control (DMSO) | | | 19±4 | 33±8 | 120±10 | 150±20 |
| Positive control*** | | | 572±41 | 261±31 | 1358±91 | 896±78 |
| Bitumen fume samples | | | | | | |
| Ethyl ether extracts | | | | | | |
| S1 | 0.1 | 0.2 | 23±2 | 29±4 | 119±14 | 148±20 |
| | 6.0 | 12.5 | 15±3 | 55±10 | 120±30 | 138±17 |
| S2 | 0.1 | 0.2 | 20±4 | 31±12 | 112±15 | 135±7 |
| | 6.0 | 12.3 | 31±6 | 36±4 | 131±12 | 129±11 |
| Acetone extracts | | | | | | |
| S1 | 0.05 | 5.0 | 19±6 | 25±2 | 105±19 | 137±22 |
| | 0.2 | 20.0 | 15±7 | 23±7 | 110±13 | 122±9 |
| S2 | 0.05 | 15.1 | 17±4 | 24±3 | 97±10 | 119±24 |
| | 0.2 | 60.0 | 16±2 | 22±6 | 104±12 | 140±23 |
| Negative control (DMSO) | | | 16±3 | 28±4 | 109±15 | 138±19 |
| Positive control*** | | | 531±82 | 280±44 | 1402±127 | 820±91 |

* DMSO extract residue (mg/plate)

** Corresponding dose of bitumen or airborne particulate (mg/plate)

*** Positive controls are:

TA98-S9 2-nitrofluorene (1µg)

TA98+S9 benzo(a)pyrene (1µg)

TA100-S9 sodium azide (1µg)

TA100+S9 benzo(a)pyrene (1µg)

The authors concluded that neither the solid bitumen samples nor the bitumen fume samples were mutagenic, with or without S9 activation, in the assays conducted.

5. Toxicity

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Test substance : Three different samples of solid penetration bitumens (80 to 100 penetration grade) were collected from road paving operations. The bitumen samples were dissolved in benzene. Asphaltenes were separated from the samples by precipitation with n-heptane. The heptane-soluble substances were weighed to constant weight and submitted to extraction with dimethylsulfoxide (DMSO), which concentrates mainly PAH. The DMSO extracts were divided in two, one half was used for PAH analysis and the other half was used for mutagenicity testing. The results of an analysis of PAH content of the samples is shown in the following table.

| PAH | Concentration ($\mu\text{g/g}$) | | |
|------------------------|-----------------------------------|-------|-------|
| | Sample | | |
| | 1 | 2 | 3 |
| Naphthalene | 28.7 | - | - |
| Acenaphthylene | - | - | - |
| Acenaphthene | 2.1 | 3.4 | 3.7 |
| Fluorene | - | 1.0 | 1.2 |
| Phenanthrene | 5.5 | 14.3 | 11.2 |
| Anthracene | 3.1 | - | 7.3 |
| Fluoranthene | 24.3 | 31.0 | 40.0 |
| Pyrene | - | 10.9 | 8.3 |
| Benzo(a)anthracene | 10.1 | - | 5.0 |
| Chrysene | 50.6 | 35.0 | 72.0 |
| Benzo(b)fluoranthene | - | 29.0 | 36.3 |
| Benzo(k)fluoranthene | 3.4 | 9.1 | 8.4 |
| Benzo(a)pyrene | 2.1 | 13.1 | 7.1 |
| Benzo(ghi)perylene | 2.7 | 4.5 | 1.9 |
| Dibenzo(a,h)anthracene | 3.2 | 5.4 | 8.6 |
| Indeno(1,2,3-cd)pyrene | 1.4 | 2.1 | 7.1 |
| TOTAL PAH | 137.2 | 158.8 | 218.1 |

- = Not detected

In addition, two bitumen fume samples were collected by high-volume sampler and glass filters (Gelman). The first sampling (S1) was performed during loading and pouring operations for two consecutive hours. The second sampling (S2) was performed for two hours but only during the periods of bitumen exposure. The filters were sonicated first with ethyl ether (30 mins.) and then with acetone (30 mins.). The ether extracts were divided into two portions, one for mutagenicity testing, the other for analysis.

The results of the analysis were as follows:

| PAH | Concentration ($\mu\text{g/m}^3$) | |
|--------------------|-------------------------------------|------|
| | Sample | |
| | 1 | 2 |
| Naphthalene | 0.18 | 0.24 |
| Acenaphthylene | - | - |
| Acenaphthene | 0.16 | 1.26 |
| Fluorene | 0.02 | 0.08 |
| Phenanthrene | 0.06 | 0.22 |
| Anthracene | 0.03 | 0.13 |
| Fluoranthene | 0.39 | 1.13 |
| Pyrene | 0.35 | 0.54 |
| Benzo(a)anthracene | 0.54 | 3.50 |

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| | | |
|------------------------|------|------|
| Chrysene | 0.16 | 0.20 |
| Benzo(b)fluoranthene | - | 1.03 |
| Benzo(k)fluoranthene | 0.09 | 0.67 |
| Benzo(a)pyrene | 0.03 | 0.61 |
| Benzo(ghi)perylene | 0.01 | 0.19 |
| Dibenzo(a,h)anthracene | 0.03 | 0.98 |
| Indeno(1,2,3-cd)pyrene | 0.02 | 0.05 |
| TOTAL PAH | 2.10 | 9.70 |

- = Not detected

Reliability : (2) valid with restrictions
Although the description of the assay was not complete, the authors cited Ames as the method used.
19.10.2003 (38)

Type : Ames test
System of testing : S. typhimurium strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100
Metabolic activation : with and without
Year : 1984
GLP : no
Test substance : Four samples of asphalt in xylene

Method : A standard plate assay was used.
Strains of S. typhimurium were TA 1535, TA1537, TA 1538, TA 98 and TA 100.
Assays were carried out in the presence and absence of a rat liver microsomal activation system.
The activation assays included 50 µl of S-9 fraction per plate as well as the required co-factors.
Assays were conducted at six dose levels of the asphalt paint: 0.005, 0.01, 0.1, 1.0, 5.0 and 10.0 µl per plate.
These concentrations were attained by adding dilutions of the paints in DMSO at a constant volume of 50 µl per plate.
Negative (solvent) and positive controls were assayed concurrently with each test sample.
The positive controls in assays without S-9 activation were sodium azide for TA1535 and TA 100, 2-nitrofluorene for TA 1538 and TA 98 and 9-aminoacridine for TA 1537. For the activation assays, 2-aminoanthracene was used for all five strains.

The criteria for assessing the response were the observation of twice the number of histidine-independent revertants per plate and a dose-related increase in the response. Mutagenic activities were quantitatively estimated using the number of revertants per µl of sample from the linear portion (initial slope) of the dose-response curves.

Result : No toxicity was evident in the assays conducted with the asphalt paints. None of the asphalt paint samples were found to be mutagenic either in the absence or presence of S-9 activation.

Test substance : Four asphalt paint samples were used. They were composed of a bitumen cutback to which xylene was added in small quantities - see below.

The asphalt cutbacks were derived from petroleum asphalt cut back to 64% solid with mineral spirits

| <u>Sample</u> | <u>Component</u> | <u>% w/w</u> |
|---------------|------------------|--------------|
| Asphalt A | Asphalt cutback | 89 |
| | Xylene | 1 |
| | Mineral spirit | 10 |

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| | | |
|-----------|-----------------|----|
| Asphalt B | Asphalt cutback | 98 |
| | Xylene | 2 |
| Asphalt C | Asphalt cutback | 97 |
| | Xylene | 3 |
| Asphalt D | Asphalt cutback | 97 |
| | Xylene | 3 |

The PAH content of samples A and D were:

| PAH | Concentration of PAH (mg/g) | |
|--------------------------------|-----------------------------|----------|
| | Sample A | Sample D |
| Naphthalene | 0.2 | 0.3 |
| Biphenyl | <0.01 | >0.01 |
| Acenaphthalene | <0.01 | >0.01 |
| Acenaphthene | ND | ND |
| Fluorene | <0.01 | <0.01 |
| 9-H-Fluorene | <0.01 | ND |
| Phenanthrene | <0.01 | <0.01 |
| Anthracene | <0.01 | ND |
| Acridine | <0.01 | <0.01 |
| 2-Methylphenanthrene | <0.01 | <0.01 |
| 2-Methylanthracene | ND | |
| Fluoranthene | ND | ND |
| Pyrene | ND | ND |
| 1-Methylpyrene | ND | ND |
| Chrysene+ benzo(a)anthracene | ND | ND |
| Benzo(a)pyrene +benzo(e)pyrene | ND | ND |

Reliability : (2) valid with restrictions
It is doubtful that the study was conducted according to GLP. Nevertheless the study was reported fully, thus allowing a critical appraisal.

04.12.2003 (46)

Type : Ames test
System of testing : S. typhimurium TA98
Metabolic activation : With
Year : 1993
Test substance : Fume condensates of coal tar pitches, roofing asphalts and paving asphalts

Method : Fume generation
Fumes of the test material were generated in the laboratory.
Fumes of the roofing asphalts and coal tar pitches were generated by heating 10 kg samples to 232 or 316 °C for 6 hours. The samples were stirred at 200 rpm and air was passed over the materials at a rate of 10 liters per minute. Fumes and vapors were condensed in a series of traps. After each run, the condensates from all traps were combined and weighed. The material obtained consisted of oil and aqueous phases and the oil phase was separated and used in this study as fume condensate.

Paving asphalt fumes were generated in a similar manner except that air

was not passed over the material and the material was only heated to 163 °C (one sample was heated to 221 °C).

Preparation of DMSO extracts of condensates

DMSO extracts were prepared by heating a 200 mg/ml mixture of the condensate and DMSO at 60 °C for 1 hour with agitation at 150 rpm. After incubation, the samples were centrifuged at approximately 1000 rpm for 5 minutes at 22 °C. The DMSO layer was removed and used for testing.

Ames test

This was performed using Salmonella typhimurium TA98 using the Blackburn modification (Blackburn et al, 1984, 1986) of the Ames test (Ames et al, 1975).

The modified test system was used because asphalt fume condensates are very similar to water-insoluble petroleum distillates which exhibit low mutagenic activity in the standard Ames test.

The modified system used DMSO extracts of the test material.

All media and solutions were prepared according to the methods described originally by Ames et al. Metabolic activation was provided by Aroclor-induced hamster liver enzymes (S9). The final concentration in all assays was 400 µl/plate in order to optimize metabolic activation of PAHs in the samples. All concentrations were plated in triplicate. Testing was conducted using a pre-incubation assay in which the bacteria, test material and S9 were pre-incubated at 37 °C with shaking for 20 minutes before being plated. The plates were incubated for 48 hours at 37 °C and mutant colonies were counted.

The positive control was a commercial No 6 residual fuel oil containing vanadium and nickel. In each assay the positive control was tested at a concentration of 50 µl/plate.

If a dose-related doubling of the mean mutant count (relative to the mean solvent control) was reached, the material was considered to be mutagenic.

Non-linear regression was used to determine the slope of the initial linear portion of the dose-response curve. This value was used as an index of mutagenicity, or mutagenicity index (MI). When more than one experiment was conducted the MIs were pooled and an MI for the pooled data was calculated.

PAH analysis

Quantitative determination of the concentrations of 16-18 individual PAH was performed using EPA method 8310.

Result

: The results are shown in the following table.

Values shown are slope of dose response curve (± asymptotic standard error).

All positive control responses were stated to be within the expected range.

| Sample No. (description & generation temperature) | PAH* content ppm | Mutagenicity index Individual experiments | Pooled data |
|--|---------------------------------|--|------------------------|
| Coal tar pitch | | | |
| 1-a (232°C) | 4529 | 725(35) | |
| 1-b (316°C) | 12025 | 1555 (75) | |

Roofing asphalt

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| | | | | |
|---|-------|---------|---------|----------------|
| 2-a (232°C) | 34.1 | 12 (1) | | |
| 2-b (316°C) | 12.9 | 10 (1) | | |
| 3-a (232°C) | 34.2 | 12 (2) | | |
| 3-b (316°C) | 128.3 | 10 (1) | | |
| Paving asphalts (generated at 163°C- except as noted) | | | | |
| 4 | 16.34 | 24 (9) | 49 (10) | 16 (8) 29 (9) |
| 5-a | 16.37 | 22 (5) | 21 (6) | 22 (4) |
| 5-b (221°C) | 3.36 | 12 (10) | 30 (12) | 12 (5) 18 (13) |
| 6 | 10.76 | 18 (2) | 21 (3) | 20 (2) |
| 7 | 6.63 | 19 (3) | 21 (3) | 20 (2) |
| 8 | 8.76 | 14 (2) | 21 (2) | 18 (2) |
| 9 | 5.4 | 17 (3) | 15 (3) | 16 (2) |
| 10 | 7.62 | 11 (2) | 19 (3) | 15 (2) |
| 11 | 21.25 | 20 (4) | 9 (3) | 14 (3) |
| 12 | 12.15 | 12 (2) | 14 (2) | 13 (2) |
| 13 | 12.07 | 12 (6) | 16 (5) | 8 (5) 12 (3) |
| 14 | 7.15 | 10 (2) | 12 (2) | 11 (1) |
| 15 | 17.02 | 11 (3) | 12 (3) | 11 (2) |
| 16-a (6 hr.) | 7.18 | 13 (2) | 10 (1) | 11 (1) |
| 16-b (2 hr.) | | 7 (2) | 11 (2) | 9 (2) |
| 17-a (AC-10) | 8.92 | 7 (1) | | 7 (1) |
| 17-b (AC-20) | 18.66 | 7 (1) | 6 (1) | 7 (1) |
| 17-c (AC-30) | 10.76 | 9 (1) | 6 (2) | 7 (1) |
| 18 | 3.32 | 5 (2) | 8 (2) | 6 (2) |

* Value is the sum of 18 PAH

Test substance : The following materials were used:

Two coal tar pitches representing ASTM Type I specification for roofing products

Two asphalts conforming to ASTM Type III roofing specifications. These represented different crude oil sources. They were identified as:
Asphalt No. 2 which was air-blown without the use of catalyst

Asphalt No. 3 which was air-blown using ferric chloride as catalyst

Conclusion : 18 paving asphalts representing 14 different crude oil sources and various processing conditions.

The authors concluded that the asphalt fume condensates were weak to moderately mutagenic.

For the two roofing asphalts, mutagenic activity was unaffected by crude oil source, processing conditions or fume generation temperature.

For the paving asphalts derived from different crude oils, the mutagenicity indices differed over a five-fold range.

Reliability : (1) valid without restriction
04.12.2003

(37)

5. Toxicity

Id Asphalt

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Type : Modified Ames test
System of testing : S. typhimurium TA98
Metabolic activation : with
Year : 1990
GLP : no data
Test substance : Asphalts and their fumes

Result : Other bacterial mutagenicity studies (Ames test or modification of the Ames assay) have been conducted on asphalt fume condensates and all have shown similar supportive results and are not, therefore, described in detail here.

These studies have been reported by:

Reinke et al (2000)
De Meo et al (1998)
Blackburn and Kriech (1990)

A publication by Pasquini et al (1989) reports an Ames assay using S. typhimurium strains TA98 and TA100. The test was carried out on a DMSO extract of a whole asphalt and no mutagenic activity was found.

09.02.2006

(15) (29) (42) (45)

Type : Mouse lymphoma assay
System of testing : L5178Y TK+/- mouse lymphoma cell line
Test concentration : 0.061 to 1000 nI/ml
Metabolic activation : with and without
Year : 1984
GLP : yes
Test substance : Vacuum residue API sample 81-13 (See section 1.1.1.)

Method : Assays were carried out with and without metabolic activation. The activation system used was an S9 fraction of Araclor-induced male mouse liver homogenate.

Prior to the assay, doses were selected by exposing the cultures of mouse lymphoma cells to a series of concentrations of the test material to determine its cytotoxicity.

Non-activation assay

Cultures of mouse lymphoma cells were exposed to the test material for 4 hours at concentrations that had been preselected on the basis of the results of the preliminary cytotoxicity study. The cells were then washed and placed in growth medium for two to three days to allow recovery, growth and expression of the induced TK-/- phenotype.

At the end of the expression period, 3×10^6 cells for each selected dose were seeded onto soft agar plates with selection medium, and resistant (mutant) colonies were counted after 10 days incubation.

To determine the actual number of cells capable of forming colonies, a portion of the cell suspension was cloned in normal, nonselective, medium. The ratio of resistant colonies to total viable cell number is the mutant frequency.

Activation assay

The activation assay was run concurrently with the non-activation assay. The only difference was that the S9 fraction of mouse liver and the various

cofactors was added during the 4 hour incubation period.

The solvent control was acetone.

The positive control substance for the non-activation assay was ethyl methane sulfonate and for the activation assay was dimethyl nitrosamine.

The criteria used in assessing the results of the assay were:

the minimum condition necessary to demonstrate mutagenesis for any given treatment is a mutant frequency that exceeds 150% of the concurrent background frequency by at least 10×10^{-6} .

The background frequency is defined as the average mutant frequency of the solvent and untreated controls.

The observation of a mutant frequency that meets the minimum criteria for a single treated culture within a range of assayed concentrations is not sufficient evidence.

The following test results must also be obtained:

A dose-related or toxicity-related increase must be observed.
(Usually over three doses)

An increase in mutant frequency may be followed by only a small or no further increase at higher concentrations or toxicities.

If an increase of about two times the minimum criterion or greater is observed for a single dose near the highest testable concentration, the test material shall be considered mutagenic.

Result : Two trials were carried out, and the results for each trial are summarized in the following two tables.

| First trial | | | | |
|-----------------------|--|------------------------------|------------------------------|--|
| Test condition | Rel. susp. growth (% of controls) | Total mutant colonies | Total viable colonies | Mutant frequency ($10 E^{-6}$ units) |
| Non activation | | | | |
| solvent | 100 | 38 | 445 | 8.5 |
| solvent | 100 | 37 | 296 | 12.5 |
| untreated | 175.6 | 35 | 295 | 11.9 |
| EMS 0.25 μ l/ml | 47.6 | 572 | 36 | 1588.9 |
| test material | | | | |
| 62.5 nl/ml | 158.9 | 23 | 146 | 15.8 |
| 125 nl/ml | 131.8 | 34 | 171 | 19.9 |
| 250 nl/ml | 176.3 | 13 | 192 | 6.8 |
| 500 nl/ml | 152.9 | 39 | 247 | 15.8 |
| 1000 nl/ml | 123.4 | 44 | 187 | 23.5 |
| S9 activation | | | | |
| solvent | 100 | 97 | 307 | 31.6 |
| solvent | 100 | 122 | 319 | 38.2 |
| untreated | 88.1 | 60 | 213 | 28.2 |
| DMN 0.3 μ l/ml | 57.1 | 138 | 53 | 260.4 |
| test material | | | | |
| 62.5 nl/ml | 66.1 | 107 | 252 | 42.5 |

5. Toxicity

Id Asphalt
Date February 15, 2006

| | | | | |
|------------|-------|-----|-----|------|
| 125 nl/ml | 100.4 | 119 | 206 | 57.8 |
| 250 nl/ml | 97.3 | 105 | 152 | 69.1 |
| 500 nl/ml | 84.3 | 164 | 166 | 98.8 |
| 1000 nl/ml | 93.3 | 185 | 249 | 74.3 |

There was no evidence of mutagenic activity under non-activation conditions. However, with metabolic activation there was an indication of weak activity and a second trial using four doses in duplicate was carried out with activation only.

The results of the second trial confirmed those of the first and are summarized below.

| Test condition | Rel. susp. growth (% of controls) | Total mutant colonies | Total viable colonies | Mutant frequency (10 E-6 units) |
|-----------------------------|-----------------------------------|-----------------------|-----------------------|---------------------------------|
| solvent | 100 | 89 | 236 | 37.7 |
| solvent | 100 | 86 | 267 | 32.2 |
| untreated | 114.3 | 68 | 262 | 26.6 |
| DMN 0.3 µl/ml | 50.2 | 175 | 52 | 336.5 |
| DMN 0.3 µl/ml test material | 26 | 181 | 50 | 362 |
| 700 nl/ml | 47.6 | 214 | 214 | 100 |
| 700 nl/ml | 48 | 183 | 215 | 85.1 |
| 800 nl/ml | 40.1 | 162 | 208 | 77.9 |
| 800 nl/ml | 73.1 | 167 | 200 | 83.5 |
| 900 nl/ml | 58.4 | 175 | 149 | 117.4 |
| 900 nl/ml | 70.1 | 166 | 194 | 85.6 |
| 1000 nl/ml | 91.3 | 204 | 186 | 109.7 |
| 1000 nl/ml | 106.3 | 147 | 178 | 82.6 |

The mutant frequencies were all elevated over the negative controls and all exceeded the criterion of 58.0×10^{-6} used to indicate mutagenic activity. The increases varied between 2.4 - 3.7-fold.

Reliability : (1) valid without restriction (6)
04.03.2005

Type : Mouse lymphoma assay
System of testing : L5178Y TK+/- mouse lymphoma cell line
Test concentration : 0.061 to 1000 nl/ml
Metabolic activation : with and without
Year : 1984
GLP : yes
Test substance : Vacuum residue API sample 81-14 (See section 1.1.1.)

Result : The sample was weakly mutagenic with metabolic activation.
Reliability : (1) valid without restriction (7)
04.03.2005

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : 5 days
Doses : 0.3, 1.0 and 3.0 g/kg/day for five days
Year : 1984
GLP : yes
Test substance : Vaccum residue API sample 81-13 (See section 1.1.1.)

Method : Test material was administered once daily by gavage as solutions in corn oil to groups of ten male and ten female rats at doses of 0.3, 1.0 and 3.0 g/kg/day for five days.
 A negative control group of 10 rats of each sex received corn oil alone and the positive control group of ten rats of each sex received triethylenemelamine (TEM) as a single dose (1 mg/kg in 0.9% saline). All animals were killed 6 hours after the last exposure to either test material, vehicle control or TEM.
 Three hours prior to kill, all animals were given colchicines (4.0 mg/kg, intraperitoneally) to arrest cell division.
 Bone marrow was aspirated from the bone and transferred to Hank's balanced salt solution. The marrow button was collected by centrifugation and resuspended in 0.075M KCl.
 Cells were fixed in methanol:acetic acid and slides were prepared and stained with Giemsa.
 Slides were examined for chromosomal aberrations.
 Routinely, 50 spreads were read for each animal. A mitotic index based on at least 500 cells was recorded. It was calculated by scoring the number of cells in mitosis per 500 cells on each slide read.

Evaluation criteria and data interpretation

Gaps were not counted as significant aberrations.

Open breaks were considered as indicators of genetic damage, as were configurations resulting from the repair of breaks. Number of aberrations per cell was considered as significant. Cells with more than one aberration were considered to indicate more genetic damage than those containing evidence of single events. Consistent variations from the euploid number were also considered in the evaluation of the mutagenic potential.

In any event, the type of aberration, its frequency and its correlation to dose in a given time period were considered in evaluating a test article as being mutagenically positive or negative.

Result : Statistical analysis employed a Student t-test.
 Many animals representing all treatment groups showed bilateral puffiness of upper eyelids. Necropsy resulted in a diagnosis of probably SDAV infection (sialodacryoadenitis), a common viral infection in rats. The infection was not considered to have influenced the results of the assay.
 The pooled results for males and females is shown in the following table.

5. Toxicity

Id Asphalt

Date February 15, 2006

| | Neg control | Pos control | Test (g/kg/day) | | |
|---|------------------------|------------------------|------------------------|------------|------------|
| | | | 0.3 | 1.0 | 3.0 |
| No. animals | 16 | 16 | 18 | 19 | 18 |
| Total No. cells | 755 | 455 | 843 | 818 | 857 |
| No. structural aberrations | 2 | >956 | 3 | 4 | 3 |
| No. numerical aberrations | 8 | 16 | 4 | 8 | 8 |
| % cells with 1 or more structural aberrations | 0.3 | 47.5** | 0.4 | 0.5 | 0.4 |
| % cells with 2 or more structural aberrations | 0 | 29.9** | 0 | 0 | 0 |
| %Mitotic Index | 3.5 | 0.6 | 3.5 | 3.7 | 3.6 |

The authors concluded that the test material was negative in inducing chromosomal aberrations in rat bone marrow cells in this assay.

Reliability
21.06.2004

: (1) valid without restriction

(6)

Type : Cytogenetic assay
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : Once each day for 5 days
Doses : 0.4, 1.3 and 4 g/kg/day
Result : negative
Year : 1984
GLP : yes
Test substance : Vacuum residue API sample 81-14 (See section 1.1.1.)

Method : Test material was administered once daily by gavage as solutions in corn oil to groups of ten male and ten female adult Sprague Dawley rats at doses of 0.4, 1.3 and 4.0 g/kg/day for five days. A negative control group of 10 rats of each sex received corn oil alone and two positive control groups of ten rats of each sex received triethylenemelamine (TEM) as a single dose of either 0.75 or 1.0 mg/kg in 0.9% saline) . All animals were killed 6 hours after the last exposure to either test material, vehicle control or TEM. Three hours prior to kill, all animals were given colchicines (4.0 mg/kg, intraperitoneally) to arrest cell division. Bone marrow was aspirated from the bone and transferred to Hank's balanced salt solution. The marrow button was collected by centrifugation and resuspended in 0.075M KCl. Cells were fixed in methanol:acetic acid and slides were prepared and stained with Giemsa. Slides were examined for chromosomal aberrations. Routinely, 50 spreads were read for each animal. A mitotic index based on at least 500 cells was recorded. It was calculated by scoring the number of cells in mitosis per 500 cells on each slide read.

Evaluation criteria and data interpretation

Gaps were not counted as significant aberrations. Open breaks were considered as indicators of genetic damage, as were configurations resulting from the repair of breaks. Number of aberrations per cell was considered as significant. Cells with

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more than one aberration were considered to indicate more genetic damage than those containing evidence of single events. Consistent variations from the euploid number were also considered in the evaluation of the mutagenic potential.

In any event, the type of aberration, its frequency and its correlation to dose in a given time period were considered in evaluating a test article as being mutagenically positive or negative.

Result : Statistical analysis employed a Student t-test.
: No clinical signs of toxicity were reported following exposure to the test material.
The pooled results for males and females are shown in the following table.

| | Controls | | Test (g/kg/day) | | | |
|---|----------|------------------------|-----------------------|-----|-----|-----|
| | -ve | +ve 0.75 (mg/kg) | +ve 1.0 (mg/kg) | 0.4 | 1.3 | 4.0 |
| No. animals | 16 | 7 | 17 | 18 | 17 | 15 |
| Total No. cells | 759 | 182 | 350 | 792 | 820 | 750 |
| No. structural aberrations | 4 | >359** | >687** | 3 | 3 | 5 |
| No. numerical aberrations | 13 | 6 | 8 | 20 | 12 | 14 |
| % cells with 1 or more structural aberrations | 0.4 | 36.3** | 32.9** | 0.4 | 0.4 | 0.5 |
| % cells with 2 or more structural aberrations | 0.1 | 28.0** | 26.6** | 0 | 0 | 0.1 |
| %Mitotic Index | 5.9 | 0.6 | 0.3 | 6.3 | 6.0 | 6.4 |

The authors concluded that the test material was negative in inducing chromosomal aberrations in rat bone marrow cells in this assay.

Reliability : (1) valid without restriction (7)
21.06.2004

Type : Micronucleus assay
Species : rat
Sex : male
Strain : Sprague-Dawley
Route of admin. : Intratracheal
Doses : 0.45, 2.22 and 8.88 mg asphalt fume condensate/kg body weight
Result : positive
Year : 2002
GLP : no data
Test substance : Asphalt fume condensate

Method : Male Sprague-Dawley rats weighing approximately 150 g were used that had been acclimatized for one week before the study was begun. The animals were anesthetized with sodium methohexital and intratracheally instilled with saline or asphalt fume condensate at doses of 0.45, 2.22 or 8.888 mg AFC/kg (approximately 0.1, 0.5 or 2 mg AFC/0.25 ml sterile saline/rat for 3 consecutive days and were sacrificed the following day. These doses had been shown previously to cause no significant changes in pulmonary inflammation, lung damage or alveolar macrophage activity.

The nucleated cells in bone marrow (source unspecified) were isolated using a cellulose column. The erythrocytes were taken from the column, were pelleted by centrifugation and resuspended in fetal bovine serum. An aliquot of cell suspension was pelleted onto slides using a Shandon Cytospin II. The slides were allowed to dry, were then fixed in absolute methanol and stained with Giemsa-distilled water (1;6) for 10 minutes. All slides were scored.

The polychromatic erythrocytes (PCEs) showed a strong bluish tint. For each animal, the frequency of micronucleated PCEs (MNPCEs) in bone marrow was based on 10,000 PCEs scored.

The results were expressed as the mean number of cells with micronuclei per 1000 PCEs.

Statistical analysis

Results were expressed as means ± standard error from at least five different animals. The significance of the interaction among the different treatment groups was assessed using analysis of variance using the Tukey-Kramer post hoc test. Significance was set at p<0.05. The comparisons of MNPCEs between control and AFC-treated groups were carried out by the trend test at p<0.05. The significance between different dose groups of treated animals and the control was evaluated by chi square analysis. The group t-test for multiple samples was used to compare PCEs between treated and control animals.

Result : Results are given in the paper for only the controls and for the groups treated at 0.45 and 8.88 mg/kg 3 times.

These results are shown in the following table.

| Treatment | No. of MN/1000 PCEs | No. of PCEs in 1000 erythrocytes | PCE/NCE ratio |
|-----------------|---------------------|----------------------------------|---------------|
| Saline | 1.5 ± 0.4 | 499 ± 13 | 1.0 ± .05 |
| AFC, 0.45mg/kg | 2.0 ± 0.4 | 468 ± 22 | 0.90 ± .07 |
| AFC, 8.88 mg/kg | 2.9 ± 0.6* | 440 ± 8** | 0.79 ± .03 |

* significantly different from controls by chi-square test at p<0.05 and trend test valued z= 2.145, p<0.05

** significantly different from controls by group t-test for multiple samples, p<<0.05.

The authors concluded that there was a significantly lower number of PCEs in the high dose group animals compared to controls. They also demonstrated a significant increase in the frequency of micronuclei formation in the high dose group bone-marrow PCEs compared to controls whereas the low dose exposure did not significantly affect the number of micronuclei in PCEs.

Test substance : Asphalt fume condensate was collected at the top of a paving storage tank of asphalt materials, Indianapolis, IN. The condensate was collected using a cold trap.

The composition of the condensate was characterized by fluorescence analysis, gas chromatography with flame ionization detection (GC/FID) for simulated distillation, and gas chromatography/mass spectrometry (GC/MS).

The report does not include any results of the analyses but states that the condensate was found to be similar to the composition of condensate collected from road paving operations and cites Kriech et al, 1999.

Reliability : (2) valid with restrictions

The information was taken from a publication in the open scientific

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literature and consequently did not contain complete experimental details. For example, there was no indication of compliance with GLP.

Of particular concern - there was no indication why information from the mid-dose group animals was not presented. Also of concern was the fact that although analytical studies had been conducted, no results were presented.

Despite the above comments, a positive response was demonstrated in the assay.

05.03.2005

(36)

Type : DNA Adduct formation
Species : rat
Sex : male
Strain : CD
Route of admin. : Intratracheal instillation
Doses : 2250, 500 & 1000 mg/kg
Year : 1998
GLP : no data
Test substance : Condensed asphalt fumes

Method : Three male CD rats (4-6 weeks old) were instilled (tracheal) with solvent (negative control), benzo(a)pyrene (positive control) or test material at three dose levels three times every 8 hours for a total of 3 doses.

Six hours after the third dose, animals were anesthetized and lung tissues were harvested and were cut into small pieces for the isolation of DNA. Blood was also collected and after treatment with EDTA, the white blood cells were separated by density centrifugation.

DNA was isolated from rat lung cells using a standard procedure using a phenol/ethanol extraction and purification with RNase digestion.

The procedures for postlabeling DNA have been described elsewhere. ³²P-labelled adducts were separated by 2-dimensional chromatography and were visualized by autoradiography. The separated adducts were relatively quantified and adduct levels were calculated.

A 2-tailed Student's t-test was used to analyze the difference in the DNA adduct levels between the control and treated groups.

Remark : This study was carried out with a view to identifying a suitable biomarker for exposure to asphalt fumes.

Result : The number of adducts identified following the various treatments are shown in the following table.

| Dose | Adduct spot | Total adducts/10x ⁻⁸ nucleotides (mean±SD) | |
|-----------------------|-------------|---|------------------|
| | | Type I asphalt | Type III asphalt |
| Control(DMSO 3 ml/kg) | | 4.9 ± 4.0 | 5.8 ± 2.7 |
| 250 mg/kg | 1 | 25.8 ± 16.3 | 24.3 ± 4.5* |
| 500 mg/kg | 1 | 49.0 ± 4.9* | 33.7 ± 9.2* |
| 1000 mg/kg | 1 | 71.0 ± 3.5* | 67.8 ± 6.7* |
| B(a)P 10 mg/kg | 1 | 46.2 ± 1.9* | 44.1 ± 5.6* |

* P<0.01

Although clear adduct formation was detected in WBC of rats exposed to B(a)P, no adducts were found in the WBC of rats treated with fume condensate.

In conclusion, DNA adducts did occur in lung cells of rats that had been

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Test substance : instilled tracheally with fume condensates of either Type I or Type III roofing asphalt. In contrast, no adducts were found in the WBC of the same animals.
: Type I and Type III roofing asphalts were used in the study.
The fume condensates were prepared by heating small pieces of the asphalts to 316 ± 10 °C in round bottomed flasks.
The fumes that were generated were collected in glass impingers in cryotrap and organic solvents (50:50 mixture of cyclohexane/acetone). Collected materials from all impingers were combined and separated into water and organic phases. Water and solvents were removed and the condensates from both phases were combined.

09.02.2006 (44)

5.7 CARCINOGENICITY

Species : mouse
Route of admin. : Skin and inhalation
Test substance : Asphalts, various

Result : Many carcinogenicity studies have been reported for various types of asphalt.
The studies have included:
skin painting studies of whole asphalts and of extracts or solutions of whole asphalts

Skin painting studies of condensed asphalt fumes

Inhalation studies of asphalt fumes

The studies are presented in a summarized form in the attached table. The attachment also includes the references to the studies. These data have also been summarized previously by CONCAWE (CONCAWE 1992).

In general, whole asphalts have been shown to be non-carcinogenic when applied undiluted to the skin (but heated to assist application). When applied as solutions in organic solvents the asphalts have been shown to be weakly carcinogenic.

Inhalation of bitumen fumes has not demonstrated a carcinogenic effect.

Condensed fumes have been shown to cause skin tumors in mice. However, the use of organic solvent for skin application and higher than normal temperatures to generate the fumes casts some doubt on the validity or relevance of the results.

04.12.2003 : **See Appendix 1** (25)

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DERMAL CARCINOGENICITY STUDIES

| Material tested | Treatment | Duration | Results | Reference |
|---|---|----------------------------------|---|-----------------------|
| <u>Penetration asphalts</u> | | | | |
| Steam refined (1 sample) | Undiluted (heated) | 21 months | 5/63 mice with skin tumors 21/63 mice survived study | Simmers (1965) |
| Road bitumen (4 samples) | Diluted with acetone (concentration unspecified) Application twice/week | 2 years | 0/100, 2/50, 1/50 & 0/50 mice with skin tumors | Hueper & Payne (1960) |
| Penetration bitumens (4 samples) | 40% in benzene Application once/week | 19 months | 9/52, 4/47, 2/50 & 2/50 mice with skin tumors | Kireeva (1968) |
| Penetration bitumen (8 samples) | 10% in benzene Application twice/week | >81 weeks | Highest incidence 7% Lowest incidence 0% Overall incidence 2.7% | Walrave et al (1971) |
| Penetration bitumen (1 sample) | 30% in mineral oil Application twice/week | 24 months | 0/50 mice | McGowan et al (1992) |
| <u>Hard Asphalts</u> | | | | |
| Bitumen paint (1 sample) | 60% bitumen in mineral spirit Application once/week | 30 weeks | 1/40 mice with skin tumor | Robinson et al (1984) |
| <u>Oxidized bitumens</u> | | | | |
| Air blown bitumen (1 Sample) | Undiluted (heated) Application 1 to 3 times/week | 21 months | 1/50 mice with skin tumor 10 mice survived | |
| Air blown bitumen (1 Sample) | 90% in toluene Application three times/week | 2 Years | 9/20 mice with skin tumors | Simmers (1965) |
| Roofing bitumen (1 Sample) | Diluted in acetone, concentration unspecified Application twice/week | 2 Years | 1/50 mice with skin tumors | Hueper & Payne (1960) |
| Roofing bitumen (1 sample) | 50% in toluene Application twice/week | 80 weeks | 0/50 mice with skin tumors | Emmet et al (1981) |
| Roofing bitumen (1 sample) | 50% in acetone/cyclohexane Application twice/week | 2 Years | 3/30 mice with skin tumors | Sivak et al (1989) |
| <u>Mixed penetration & Oxidized bitumens</u> | | | | |
| Mixture of 6 air-blown and steam-refined bitumens | Diluted with benzene, concentration unspecified Application twice/week | Time unspecified, but > 54 weeks | 17/68 mice with skin tumors | Simmers et al (1959) |
| <u>Thermally cracked bitumens</u> | | | | |
| Oxidized residue bitumen (2 samples) | 40% in benzene Application once weekly | 19 months | 9/49 & 4/42 with skin tumors | Kireeva (1968) |
| <u>Vacuum residuum</u> | | | | |
| 2 samples API 81-13 & 81-14 | Diluted in toluene 50µl twice/week | 130 weeks | 5/50 & 2/50 mice with skin tumors Mean latency 113 & 120 weeks | API (1989) |

INHALATION CARCINOGENICITY STUDIES

| | | | | |
|---|---|-----------|--|-----------------------|
| Oxidized bitumen (1 sample) | Fumes generated at 250-275°F Exposure 5 hr/day, 4 days/week 65 Bethesda strrn rats 13 Guinea pigs used | 2 Years | No lung tumors, but extensive fibrosing pneumonitis was observed in rats | Hueper & Payne (1960) |
| Mixture of 6 penetration grades and oxidized bitumens | 20 C57 mice exposed 30 mins/day, five days/week Aerosol generated at 250°F | 17 months | 1 animal with lung adenoma | Simmers (1964) |
| Mixture of 6 penetration grades and oxidized bitumens | 30 C57 mice exposed 6-7½hrs/day five days/week Smoke generated at 250°F | 21 months | Bronchitis, loss of bronchial coilia, epithelial atrophy, necrosis, pneumonitis No lung tumors observed | Simmers (1964) |

SKIN APPLICATION OF CONDENSED FUMES

| | | | | |
|---------------------------|---|----------------|--|--------------------------|
| Type I & Type III asphalt | Fumes generated at 450 & 601°F Application twice/week as 50% solution in cyclohexane/acetone. Some animals also exposed to UV light CD 1 and C3H mice used | Up to 72 weeks | C3H more sensitive than CD-1. Greater tumor response from fume generated at the higher temperature. | Niemeier et al (1988) |
| Type III asphalt | Fumes generated same method as by Niemeier but at 601°F only C3H and Sencar mice used Sample applied twice weekly | 104 weeks | C3H mouse 20/30 mice with tumors Sencar : 14/30 mice with tumors | Sivak et al (1989, 1997) |

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High Production Volume Information System (HPVIS)

Genetic Toxicity *in vivo* – DNA Adducts

TEST SUBSTANCE

| Category Chemical: | <input type="text" value="Select a Chemical"/> Type in if not listed: 64742-93-4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---------------------------|----------------------|---------------------|----------------------|------------------------------|--|--|--|-------------|-----------|----------|------------|----------------|----|----|----|--------------|---------|----------|-----------|----------|---------|----------|-----------|--------------|----------|----------|-----------|------------|----|----|----|--------------|---------|----------|----------|--------|---------|----------|-----------|--------------------|--------|---------|---------|--------------|--------|---------|----------|----------|---------|---------|---------|----------------------|--------|---------|--------|----------------------|------|------|--------|----------------|--------|---------|---------|----------------|------|--------|--------|------------------------|------|------|--------|----------------------|------|------|---|--------------------|--------|--------|--------|
| Test Substance: | <input type="text" value="Select a Chemical"/> Type in if not listed: 64742-93-4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Test Substance Purity/Composition and Other Test Substance Comments: | <p>Fume vapor condensate from overhead space of a hot storage tank supplied with semi-blown paving asphalt [50/70 pen]</p> <p style="text-align: center;">Table 1. PAH Content of the Exposure Atmosphere</p> <table border="1" style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th>Dose group [Target conc.]</th> <th>4mg/m³</th> <th>20mg/m³</th> <th>100mg/m³</th> </tr> </thead> <tbody> <tr> <td>PAH [ng/m³ ± SD]</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Naphthalene</td> <td>1014±13.7</td> <td>3687±8.4</td> <td>19578±15.7</td> </tr> <tr> <td>Acenaphthylene</td> <td>SO</td> <td>SO</td> <td>SO</td> </tr> <tr> <td>Acenaphthene</td> <td>477±8.2</td> <td>1923±7.2</td> <td>10093±5.2</td> </tr> <tr> <td>Fluorene</td> <td>801±5.5</td> <td>3520±6.7</td> <td>21690±8.4</td> </tr> <tr> <td>Phenanthrene</td> <td>331±13.0</td> <td>1641±9.0</td> <td>11580±9.5</td> </tr> <tr> <td>Anthracene</td> <td>SO</td> <td>SO</td> <td>SO</td> </tr> <tr> <td>Fluoranthene</td> <td>43±11.6</td> <td>220±11.4</td> <td>1295±7.6</td> </tr> <tr> <td>Pyrene</td> <td>45±11.1</td> <td>219±13.7</td> <td>1139±17.8</td> </tr> <tr> <td>Benzo(a)anthracene</td> <td>7±14.3</td> <td>31±12.9</td> <td>169±8.9</td> </tr> <tr> <td>Triphenylene</td> <td>11±9.1</td> <td>47±12.8</td> <td>249±10.0</td> </tr> <tr> <td>Chrysene</td> <td>10±10.0</td> <td>45±13.3</td> <td>251±8.0</td> </tr> <tr> <td>Benzo(b)fluoranthene</td> <td>4±25.0</td> <td>15±13.3</td> <td>83±8.4</td> </tr> <tr> <td>Benzo(k)fluoranthene</td> <td><LOQ</td> <td><LOQ</td> <td>7±28.6</td> </tr> <tr> <td>Benzo(e)pyrene</td> <td>4±25.0</td> <td>19±10.5</td> <td>100±8.0</td> </tr> <tr> <td>Benzo(a)pyrene</td> <td><LOQ</td> <td>5±20.0</td> <td>30±6.7</td> </tr> <tr> <td>Indeno(1,2,3-cd)pyrene</td> <td><LOQ</td> <td><LOQ</td> <td>5±20.0</td> </tr> <tr> <td>Dibenz(ah)anthracene</td> <td><LOQ</td> <td><LOQ</td> <td>7</td> </tr> <tr> <td>Benzo(ghi)perylene</td> <td>3±33.3</td> <td>4±25.0</td> <td>24±8.3</td> </tr> </tbody> </table> <p>SO = Not quantitated due to signal overlap; LOQ = limit of quantitation</p> | Dose group [Target conc.] | 4mg/m ³ | 20mg/m ³ | 100mg/m ³ | PAH [ng/m ³ ± SD] | | | | Naphthalene | 1014±13.7 | 3687±8.4 | 19578±15.7 | Acenaphthylene | SO | SO | SO | Acenaphthene | 477±8.2 | 1923±7.2 | 10093±5.2 | Fluorene | 801±5.5 | 3520±6.7 | 21690±8.4 | Phenanthrene | 331±13.0 | 1641±9.0 | 11580±9.5 | Anthracene | SO | SO | SO | Fluoranthene | 43±11.6 | 220±11.4 | 1295±7.6 | Pyrene | 45±11.1 | 219±13.7 | 1139±17.8 | Benzo(a)anthracene | 7±14.3 | 31±12.9 | 169±8.9 | Triphenylene | 11±9.1 | 47±12.8 | 249±10.0 | Chrysene | 10±10.0 | 45±13.3 | 251±8.0 | Benzo(b)fluoranthene | 4±25.0 | 15±13.3 | 83±8.4 | Benzo(k)fluoranthene | <LOQ | <LOQ | 7±28.6 | Benzo(e)pyrene | 4±25.0 | 19±10.5 | 100±8.0 | Benzo(a)pyrene | <LOQ | 5±20.0 | 30±6.7 | Indeno(1,2,3-cd)pyrene | <LOQ | <LOQ | 5±20.0 | Dibenz(ah)anthracene | <LOQ | <LOQ | 7 | Benzo(ghi)perylene | 3±33.3 | 4±25.0 | 24±8.3 |
| Dose group [Target conc.] | 4mg/m ³ | 20mg/m ³ | 100mg/m ³ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PAH [ng/m ³ ± SD] | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Naphthalene | 1014±13.7 | 3687±8.4 | 19578±15.7 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Acenaphthylene | SO | SO | SO | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Acenaphthene | 477±8.2 | 1923±7.2 | 10093±5.2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluorene | 801±5.5 | 3520±6.7 | 21690±8.4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Phenanthrene | 331±13.0 | 1641±9.0 | 11580±9.5 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Anthracene | SO | SO | SO | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluoranthene | 43±11.6 | 220±11.4 | 1295±7.6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| Benzo(a)anthracene | 7±14.3 | 31±12.9 | 169±8.9 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Triphenylene | 11±9.1 | 47±12.8 | 249±10.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Chrysene | 10±10.0 | 45±13.3 | 251±8.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo(b)fluoranthene | 4±25.0 | 15±13.3 | 83±8.4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo(k)fluoranthene | <LOQ | <LOQ | 7±28.6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo(e)pyrene | 4±25.0 | 19±10.5 | 100±8.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo(a)pyrene | <LOQ | 5±20.0 | 30±6.7 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Indeno(1,2,3-cd)pyrene | <LOQ | <LOQ | 5±20.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Dibenz(ah)anthracene | <LOQ | <LOQ | 7 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo(ghi)perylene | 3±33.3 | 4±25.0 | 24±8.3 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

| | |
|--|--|
| Category Chemical Result Type: | Measured |
| METHOD | |
| Type of Study: | DNA perturbation |
| Type of Test: | DNA adducts |
| Route of Administration: | Inhalation – Nose only |
| Species: | Rat |
| Strain: | SPF-Wistar |
| Gender: | Male and female |
| Dose: | Target: 0, 4, 20 and 100mg/m ³ . Actual: 0, 6.8, 34.4 and 172.5mg/m ³ [calculated by BIA method with a 1.66 conversion factor]. |
| Year Study Performed: | 2007 |
| Method/Guideline Followed: | DNA ³² P Postlabeling method of Gupta, et al., 1982 |
| GLP: | Yes |
| Duration of Treatment/Exposure Period and Units: | 5, 30 days or 12 months |
| Frequency of Treatment: | 6 hours/day, 5 days/week |
| Positive, Negative and Solvent Control Substance(s): | Clean air. No positive controls |
| Post-Exposure Period: | None |
| Number of Animals per Sex per Dose: | 8 rats/sex/group/exposure period |
| Method/Guideline and Test Condition Remarks: | Test material was collected and generated according to method reported in the parallel 2-year Carcinogenesis Nose-only Inhalation study at Fraunhofer ITEM described in a separate Carcinogenesis robust summary. <u>Animals:</u> SPF Wistar rats (8 weeks of age at study initiation) were exposed to bitumen fumes individually in acrylic glass tubes placed |

around the exposure cylinder, 6 hrs/day, 5 days/week. The whole exposure unit was placed under a laboratory hood. During non-exposure periods, rats were housed under barrier conditions in same sex pairs in polycarbonate type III cages with absorbent bedding and food and water *ad libitum*. Animal room temperatures was $22 \pm 2^{\circ}\text{C}$ and 40-70% relative humidity with a 12 hour light cycle and air flow rate of 12-15 changes/hour. Ninety-six male and 96 female rats were randomized by weight through a computer-generated randomization program into 4 groups consisting of 24 males and 24 females. Animals were sacrificed after 5 days, 30 days or 12 months. Alveolar epithelial cells were isolated from a pool of 3 lungs of male and female animals/group /exposure period. All other animals were anesthetized by an overdose of CO2 and necropsied. Lung and nasal epithelium and blood were collected for RNA and DNA preparation and tissue was frozen immediately at -80°C .

Endpoints evaluated in this extensive study included DNA adducts, determination of 8-oxo-dG and deoxyguanine, a micronucleus assay, biotransformation and urinary excretion of PAHs profiling of 16 genes with known function in inflammation, asthma and other pulmonary diseases, and Western blotting procedures to measure CYP1A1 activity stimulated by exposure to asphalt fume condensate. This robust summary addresses DNA adduct formation, and the micronucleus assay is presented in a separate robust summary. The other endpoints are not part of the HPV program and will not be discussed here.

DNA adducts:

Based on the method of Gupta, Reddy and Randerath, 1982, DNA collected from lung, nasal and alveolar epithelium and white blood cells [WBC] was hydrolyzed by endo- and exonucleases to monomeric nucleotides. Normal nucleotides were separated from modified nucleotides by butanol extraction before labelling the adduct-3'-phosphate in the presence of T4 polynucleotide kinase and the 5'-position with [γ - ^{32}P]-ATP of high specific activity. Labeled adducts were separated by thin layer chromatography on PEI-cellulose sheets, visualized by phosphoimaging and in the presence of definite spots quantitatively determined using calibration with the radiolabel. The intensity of adduct spots were calculated from the intensities from a calibration curve consisting of diluted ^{32}P labelled ATP of known concentrations. Because the intensities of the spots of the calibration curve correlate with a specific molar amount, the molar amount of the DNA adduct spots were calculated. The relative adduct level was calculated from the molar amount of the DNA-adduct and the total molar amount from the DNA ($5\mu\text{g}=0.15 \times 10^{-7} \text{ mol}$) used for the analysis. Adducts were quantitated but not analytically characterized.

Determination of 8-oxo-dG and deoxyguanine was performed by HPLC with electrochemical detection [pg amounts of 8-oxo-dG] and a UV detector connected downstream of the electrochemical detector for deoxyguanine.

Statistical Evaluation was performed via Kruskal Wallis Test (Chi Square approximation), $p < 0.05$.

TEST RESULTS

Systemic Toxicity:

No adverse effects were observed at any bitumen fume condensate exposure level but dose-dependent uptake of bitumen fumes was confirmed based on urinary excretion of PAHs and metabolites.

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|-------------------------------------|---|
| Genotoxic Effect: | <p>DNA adducts were identified in clean air exposed controls and all dose levels in male and female rats. Three stable adducts were seen in lung and nasal epithelium and 4 adducts were seen in alveolar epithelium. Statistically significant increases were seen in lung and nasal epithelium but no statistics were performed on alveolar epithelium data as only one measurement was made per group because tissue came from a pool of 3 lungs. In the lung, DNA adducts were identified in the high dose group as early as 5 days of exposure and in the mid-dose group after 4 weeks. After one year, adduct levels were dose dependently increased, however the increase seen in the low dose group after 1 year was not statistically significant. Similar results were seen with alveolar epithelium.</p> <p>In nasal epithelium, DNA adducts were detected in all dose groups at varying levels after 5 days of exposure. Females showed no statistically significant increase in adducts in the low and mid dose levels possibly due to an abnormally high adduct level in concurrent female controls. A decrease in all adducts levels in the medium group was seen after one year in both sexes but adduct levels steadily increased in the high dose group over time.</p> <p>In general, DNA adduct levels were highest in nasal epithelium followed by lung and alveolar epithelial cells. No adducts were identified in WBC that could be linked to exposure to bitumen fumes (data not shown).</p> <p>No 8-oxo-dG adducts, a marker of oxidative DNA damage was found in extracts from lung, alveolar or nasal epithelium at any dose levels, indicating that oxidative damage is not a major mechanism of DNA adduct formation from bitumen fume exposure.</p> |
| Results Remarks: | <p>None of the PAH metabolites identified in the urine [naphthols, phenanthrene, phenanthrene permercapturic acid, 1-OH-phenanthrene and phenanthrene 1,2 dihydrodiol were likely candidates for DNA adduct formation and the biological consequence of these DNA adducts are unknown. Moreover, no lung tumors were observed in rats exposed under the same dosage regimen after 2 years of treatment.</p> |
| Conclusion : | <p>Nose-only inhalation exposure to bitumen fume condensate over periods from 5 days to 1 year resulted observation of 3- 4 stable DNA adducts in lung, nasal and alveolar epithelium in excess of endogenous DNA adducts seen in clean air controls. which increased in a time and dose-dependent manner.</p> |
| RELIABILITY/DATA QUALITY | |
| Reliability: | 1. Reliable without restriction . |
| Reliability Remarks: | |
| Key Study Sponsor Indicator: | Not a key study – supplemental data |
| REFERENCE | |
| Reference: | <p>Halter, R., Hansen, T., Seidel, A, Ziemann, and Borlak, J. 2007. Importance of DNA-adduct formation and gene expression profiling of disease candidate genes in rats exposed to bitumen fumes. <i>J Occup Environ Hygiene</i> 4(S1): 44-64.</p> <p>Gupta, R.C. Reddy, M.V., and Randrath, K. 1982. ³²P postlabeling analysis of non-radioactive aromatic carcinogen-DNA adducts. <i>Carcinogenesis</i> 3: 1081-1092.</p> |



High Production Volume Information System (HPVIS)

Carcinogenicity

Test Substance

Category Chemical:

Select a Chemical

Type in CAS # if not listed: 64742-93-4

Test Substance:

Select a Chemical

Type in CAS # if not listed: 64742-93-4

Test Substance Purity/Composition and Other Test Substance Comments:

Fume vapor condensate from overhead space of a hot storage tank supplied with semi-blown paving asphalt [50/70 pen].

Table 1. PAH Content of the Exposure Atmosphere

| Dose group [Target conc.] | 4mg/m ³ | 20mg/m ³ | 100mg/m ³ |
|------------------------------|--------------------|---------------------|----------------------|
| PAH [ng/m ³ ± SD] | | | |
| Naphthalene | 1014±13.7 | 3687±8.4 | 19578±15.7 |
| Acenaphthylene | SO | SO | SO |
| Acenaphthene | 477±8.2 | 1923±7.2 | 10093±5.2 |
| Fluorene | 801±5.5 | 3520±6.7 | 21690±8.4 |
| Phenanthrene | 331±13.0 | 1641±9.0 | 11580±9.5 |
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| Pyrene | 45±11.1 | 219±13.7 | 1139±17.8 |
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| Chrysene | 10±10.0 | 45±13.3 | 251±8.0 |
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| Benzo(a)pyrene | <LOQ | 5±20.0 | 30±6.7 |
| Indeno(1,2,3-cd)pyrene | <LOQ | <LOQ | 5±20.0 |
| Dibenz(ah)anthracene | <LOQ | <LOQ | 7 |
| Benzo(ghi)perylene | 3±33.3 | 4±25.0 | 24±8.3 |

SO = Not quantitated due to signal overlap; LOQ = limit of quantitation

Category Chemical Result Type:

Measured

| | |
|---|---|
| Unable to Measure or Estimate Justification: | |
| METHOD | |
| Route of Administration: | Inhalation- nose-only |
| Other Route of Administration: | None |
| Type of Exposure: | 2 year inhalation |
| Species: | Rat |
| Other Species: | None |
| Mammalian Strain: | SPF-Wistar |
| Gender: | Male and Female |
| Number of Animals per Dose: | 50/sex/group; Additional high dose and control rats [36/sex/group] included for bronchio-alveolar lavage [BAL] at 7 and 90 days and 12 months. |
| Concentration: | |
| Dose: | Target: 0, 4, 20 and 100mg/m ³ . Actual: 0, 6.8, 34.4 and 172.5mg/m ³ [calculated by BIA method with a 1.66 conversion factor]. |
| Year Study Performed: | 2007 |
| Method/Guideline Followed: | OECD 454 |
| GLP: | Yes |
| Exposure Period: | 104 weeks |
| Frequency of Treatment: | 6 hrs/day, 5 days/week |
| Animals at Interim Sacrifice: | 6/sex/group in control and high dose for BAL |
| Interim Sacrifice Time: | 7 days, 90 days and 12 months |

| | |
|---|--|
| Animals at Final Sacrifice: | 50/sex/group |
| Final Sacrifice Time: | 104 weeks |
| Control Group Type: | Clean Air |
| Method/Guideline and Test Condition Remarks: | <p><u>Sample Collection:</u> Fume condensate was collected at an operating asphalt mixing plant supplied directly with bitumen from a nearby refinery. Fumes from the headspace of the storage tank maintained at approximately 175°C were representative of that to which workers are exposed during road paving. Collected fumes were passed through sampling units comprised of a cooling spiral and Peltier condenser running at approximately 5°C to cool and condense the fumes. Condensed fume and water were collected in 10L vacuum polyethylene bottles (pressure level 800mbar). Total of 16.2kg bitumen fume condensate was collected.</p> <p><u>Chamber fume generation and characterization:</u> Fumes for inhalation chambers were produced via an evaporation condensation generator. Condensate was fed by peristaltic pump to a nitrogen-operated dispersion nozzle, a droplet spray was generated and droplets evaporated in a heating tube. Hot vapor was cooled by a slow flowing cool air stream and directed into the inhalation units, diluted with clean air to achieve the final intended concentrations and fed into the nose-only tubes at a flow rate of 110L/min. Data on temperature and humidity in exposure cylinders were collected by computer and 10 minute averages stored. Fume concentration was determined every 2 weeks by sampling with glass fiber filter and XAD absorption tubes with sample flow rate of 2L/min, extracted and analyzed separately by IR spectroscopy according to BIA guideline #6305. Conversion factor of absolute bitumen fume concentration to BIA concentration was 1.66. Fumes were analyzed monthly for total polycyclic aromatic hydrocarbons (PAH) in exposure groups and every three months in controls. Continuous monitoring of total hydrocarbon concentration (THC) was performed with a flame ionization detector (FID). Particle size distribution was determined 24 times using a scanning mobility particle sizer.</p> <p><u>Animals:</u> SPF Wistar rats (5 weeks old at delivery) were exposed to bitumen fumes individually in acrylic glass tubes placed around the exposure cylinder, 6 hrs/day, 5 days/week. The whole exposure unit was placed under a laboratory hood. During non-exposure periods, rats were housed under barrier conditions in same sex pairs in polycarbonate type III cages with absorbent bedding and food and water <i>ad libitum</i>. Animal room temperatures was 22 ± 2°C and 30-70% relative humidity with a 12 hour light cycle and air flow rate of 12-15 changes/hour. Rats were observed daily for clinical symptoms. Individual body weights were recorded weekly for the first 13 weeks then monthly. Food consumption (10 rats/group- 5 cages/group) per cage by difference was recorded weekly during the first 13 weeks and every three months until study termination. Hematology was performed at final sacrifice with blood from the vena cava caudalis of carcinogenicity rats. Parameters were erythrocyte count, hemoglobin, hematocrit, mean erythrocyte volume, mean erythrocyte hemoglobin mass, mean erythrocyte hemoglobin concentration, total and differential leukocyte counts, platelet count and prothrombin time.</p> <p><u>Bronchio-alveolar Lavage (BAL):</u> Performed on 6 additional animals/sex/group in the control and high dose group following 7days, 90 days and 12 months exposure [total 36 rats/sex/group]. Lungs were lavaged twice and cell concentrations determined using a counting chamber. Slides were prepared for differential cell counts. Samples of lavage fluid supernatant were used for biochemical indicators. Endpoints were cell number, differential cell counts, total protein, lactic dehydrogenase (LDH), β-glucuronidase and gamma-glutamyl-transferase.</p> <p><u>Histopathology:</u> Forty-nine organs and tissues were collected and preserved from all animals at terminal sacrifice. Complete histopathologic examinations were conducted in all groups. Respiratory tract of animals used for cell proliferation evaluations after 7 days, 90 days of 12 months were also examined. Formalin-fixed epithelial cells of nasal cavity, terminal bronchioles and lung parenchyma were examined for cell proliferation using antibody to Ki-67. Lung sections were stained immunohistochemically using antibodies to Ki-67 and cytokeratin (labeled with Fastred) Sections of nasal cavities (labeled with chromagen DAB) were also stained. The numbers of positive cells were counted and the total length of analyzed bronchiolar or nasal epithelium were measured to calculate the unit length labeling index. Proliferative epithelial cells in pulmonary parenchyma were analyzed semi-automatically using image analysis software and the labeling index calculated as a percentage of positive cells.</p> <p><u>Statistics:</u> Body weight and food consumption data were analyzed for homogeneity by analysis of variance (ANOVA) and covariance (ANCOVA). Transformed data were analyzed for dose-related trend by the Williams test</p> |

(parametric data) and Shirley test (non-parametric data). If no trend was found but non-homogeneity of means was present, data were analyzed by stepwise Dunnett (parametric data) or modified Steel (non-parametric data) for differences from control (p=0.05). Hematological and biochemical indicators were analyzed by ANOVA followed by pair-wise comparisons with Dunnett's modification of the t-test (2-sided, p=0.05). Survival data was analyzed by Kaplan- Meier test. Tumor incidence data was evaluated by Peto's analysis of tumor incidence determined in fatal context [tumors directly or indirectly killed the host] or tumors determined in incidental context [death from non-tumor related causes or survival to terminal sacrifice]. In histopathology, the significance of differences of frequencies was analyzed by pair-wise comparison of controls and treatment groups using Fisher's exact test.

TEST RESULTS

MTD Indicator:

Decreased body weight at study termination was -3% males and -8% females in the 34.4mg/m³ group and -7% males and -8% females in the 172.5mg/m³ group compared to controls. Statistically significant decreases in body weight gain in the mid and high dose groups were seen at multiple but not all time points. Non-neoplastic toxic irritant effects in nasal cavity and lungs of very slight to slight degree showed dose related increases in all bitumen-treated groups up to >90% animals in the 172.5mg/m³ group. [See Remarks- Non-neoplastic Histopathology]

Neoplastic Effect:

Table 2. Summary of Tumor Incidence

| Doses mg/m ³ | Males | | | | Females | | | |
|---------------------------|---------|-----|------|-------|---------|-----|------|-------|
| | Control | 6.8 | 34.4 | 172.5 | Control | 6.8 | 34.4 | 172.5 |
| Number of Animals | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| with Tumors | 28 | 30 | 27 | 30 | 42 | 34 | 39 | 33 |
| with Single Tumor | 19 | 21 | 15 | 23 | 24 | 21 | 25 | 18 |
| with Multiple Tumors | 9 | 9 | 12 | 7 | 18 | 13 | 14 | 15 |
| with Benign Tumors | 25 | 27 | 27 | 25 | 39 | 32 | 36 | 32 |
| with Malignant Tumors | 7 | 5 | 5 | 6 | 6 | 8 | 5 | 4 |
| with Metastasizing Tumors | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 |

No substantial differences were observed in the number of tumor-bearing animals between the control and bitumen treated groups. The number of tumor bearing animals was slightly higher in females than males, irrespective of dose group. The number of benign tumors ranged from 31-37 /group in males and between 45 and 56/group in females. Of malignant tumors, 5 to 7 and 4 to 8 were observed in males and females respectively. There was no statistically significant increase in organ-related tumor types in bitumen treated groups compared to controls for males or females by Fisher test. No trends were found for males and females with the Peto's test, over controls and treated groups

Survival Rate prior to terminal sacrifice

| Male: | Female: | Total: |
|-------------------|--------------------|---------------------|
| Control: 5/50 10% | Control: 14/50 28% | Control: 19/100 19% |
| Low: 9/50 18% | Low: 6/50 12% | Low: 15/100 15% |
| Mid: 8/50 16% | Mid: 8/50 16% | Mid: 16/100 16% |
| High: 7/50 14% | High: 11/50 22% | High: 18/100 18% |

Mortality was comparable in all groups but slightly higher in females than males. Lifetime analysis over all groups via Kaplan Meier showed no significant differences between males and females.

Clinical Observations:

No clinical signs of intoxication were observed. No changes in food consumption for treatment groups compared to controls were reported.
Hematology: Effects observed were in the range expected for the species, strain, sex and age of rats and

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| | <p>represented minor changes (data not shown). <u>BAL</u>: collected at 7 days, 90 days and 12 months from additional 36 rats/sex from control and high dose groups [6/sex/sampling time/group]. Increased polymorphonuclear leucocyte levels in controls, primarily in females in this study after 12 months are known age-related effects. Statistically significant increase in lymphocytes in males after 12 months was considered an incidental finding expressed in 3/6 rats while the other 3/6 rats showed control level values. Increased lactic dehydrogenase (LDH) and gamma glutamyl-transferase (GGT) indicated very slight inflammatory effects in the bronchioalveolar region of the lung, indicating this region of the respiratory tract was not significantly impacted by exposure to bitumen fumes. Cell proliferation measurements: Increases in cell proliferation in terminal bronchiolar epithelium was detected after 7 days exposure in high dose females but not in males at any sampling time or in females at 90 days and 12 months. Cell proliferation in lung parenchymal cells was comparable between treated and control rats. In the nasal cavity, the only consistent increase was seen in the transitional zone of respiratory to olfactory epithelium in high dose males at all sampling times but not in females.</p> |
| <p>Carcinogenic Effect:</p> | <p>The number of animals with malignant tumors ranged between 5/50 (bitumen low and mid-dose groups) and 7/50 (clean air control group) in males and between 4/50 (Bitumen high dose group and 8/50 (Bitumen low dose group) in females. No significant differences were observed in the incidence of malignant tumors between clean air control and bitumen-treated rats. Metastasizing tumors (only distant metastases counted, not animals with systemic or localizing infiltrating tumors) were not observed in any male rats and in 1/50 and 2/50 females from clean air controls and bitumen high dose group, respectively. A poorly differentiated adenocarcinoma was observed in the nasal cavity of a single male rat from the Bitumen high dose group, probably derived from proliferated basal cells of the olfactory epithelium or of the underlying Bowman glands. The adenocarcinoma showed aggressive behavior for infiltration of surrounding bones and brain. An adenoma from the submucosal glands of the maxilloturbinate was observed in a single male of the clean air control group.</p> |
| <p>Results Remarks:</p> | <p><u>Exposure Atmosphere</u>: Mean actual concentration (aerosol + vapor phase) during a 6-hour exposure determined by IR spectroscopy according to the BIA guideline #6305 was 4.1, 20.7 and 103.9mg/m³ THC. Conversion of these data [factor 1.66 with absolute bitumen fume concentration] resulted in concentrations of 6.8, 34.4, and 172.5mg/m³ for low, mid and high dose groups respectively with a standard deviation in most cases below 10%. Exposure atmospheres comprised of mixed particulate/vapor were seen in the following ratios: 15%/85% [low dose], 27%/73% [mid dose] and 50%/50% [high dose]. Relative aerosol content decreased with increased dilution of fume due to mixture of volatile compounds with different vapor pressures. <u>Non-neoplastic Histopathology</u>: Bitumen-related toxic irritant effects were observed in the nasal cavity and lung. In the nasal cavity, dose-related degenerative (epithelial eosinophilic hyaline inclusions), inflammatory (mucosal mononuclear/inflammatory cell infiltration) and proliferative lesions (basal cell hyperplasia) of mainly very slight-to-slight degree were observed. In the lung, dose-dependent adaptive bronchio-alveolar hyperplasia was seen with slight dose dependent alveolar macrophage accumulation and slight mononuclear/inflammatory cell infiltrations. Slight alveolar bronchiolization occurred in 46/50 males and 44/50 females in the high dose group compared to 4/50, 1/50 and 22/50 males and 6/50, 7/50, and 21/50 females in control, low and mid dose groups. Incidence of mononuclear/inflammatory cell infiltrations was diagnosed in 37/50 males and 39/50 females in the high dose group compared to 7-9/50 males and 2-8/50 females in controls, low and mid dose groups.</p> |
| <p>Conclusion:</p> | <p>Exposure of Wistar rats to a paving Bitumen fume for 2 years did not result in any statistically significant increase in total or organ-specific tumor incidence between the clean air control and bitumen-exposed animals. Bitumen related irritant effects were observed in the nasal passages and the lungs. Bitumen fume is not considered tumorigenic in this assay system. NOAEL for carcinogenesis = 172.5mg/m³</p> |
| <p>RELIABILITY/DATA QUALITY</p> | |
| <p>Reliability:</p> | <p>1. Reliable without restrictions.</p> |

| | |
|-------------------------------------|--|
| Reliability Remarks: | |
| Key Study Sponsor Indicator: | Not a key study – Supplemental data |
| REFERENCE | |
| Reference: | <p>Furst, R., Creutzenberg, O., Heinrich, E., et al. 2007. 24 Months inhalation carcinogenicity study of bitumen fumes in Wistar (WU) rats. J Occupat Environ Hygiene 4(S1): 20-43.</p> <p>BIA Guideline #6305. Berufsgenossenschaftliches Institut für Arbeitssicherheit. 1989 <i>Messverfahren für Gefahrstoffe und Aerosole (Kennzahl 6305)</i>. BIA-Arbeitsmappe Messung von Gefahrstoffen. 19. Lfg. XI/97. Verlag Erich Schmid, Bielefeld, Germany.</p> |



High Production Volume Information System (HPVIS)

Genetic Toxicity *in vivo*

TEST SUBSTANCE

| Category Chemical: | <input type="text" value="Select a Chemical"/> Type in if not listed: 64742-93-4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|--|---------------------------|----------------------|---------------------|----------------------|------------------------------|--|--|--|-------------|-----------|----------|------------|----------------|----|----|----|--------------|---------|----------|-----------|----------|---------|----------|-----------|--------------|----------|----------|-----------|------------|----|----|----|--------------|---------|----------|----------|--------|---------|----------|-----------|-------------------|--------|---------|---------|--------------|--------|---------|----------|----------|---------|---------|---------|----------------------|--------|---------|--------|----------------------|------|------|--------|----------------|--------|---------|---------|----------------|------|--------|--------|------------------------|------|------|--------|----------------------|------|------|---|--------------------|--------|--------|--------|
| Test Substance: | <input type="text" value="Select a Chemical"/> Type in if not listed: 64742-93-4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Test Substance Purity/Composition and Other Test Substance Comments: | <p>Fume vapor condensate from overhead space of a hot storage tank supplied with semi-blown paving asphalt [50/70 pen]</p> <p style="text-align: center;">Table 1. PAH Content of the Exposure Atmosphere</p> <table border="1" style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th>Dose group [Target conc.]</th> <th>4mg/m³</th> <th>20mg/m³</th> <th>100mg/m³</th> </tr> </thead> <tbody> <tr> <td>PAH [ng/m³ ± SD]</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Naphthalene</td> <td>1014±13.7</td> <td>3687±8.4</td> <td>19578±15.7</td> </tr> <tr> <td>Acenaphthylene</td> <td>SO</td> <td>SO</td> <td>SO</td> </tr> <tr> <td>Acenaphthene</td> <td>477±8.2</td> <td>1923±7.2</td> <td>10093±5.2</td> </tr> <tr> <td>Fluorene</td> <td>801±5.5</td> <td>3520±6.7</td> <td>21690±8.4</td> </tr> <tr> <td>Phenanthrene</td> <td>331±13.0</td> <td>1641±9.0</td> <td>11580±9.5</td> </tr> <tr> <td>Anthracene</td> <td>SO</td> <td>SO</td> <td>SO</td> </tr> <tr> <td>Fluoranthene</td> <td>43±11.6</td> <td>220±11.4</td> <td>1295±7.6</td> </tr> <tr> <td>Pyrene</td> <td>45±11.1</td> <td>219±13.7</td> <td>1139±17.8</td> </tr> <tr> <td>Benz(a)anthracene</td> <td>7±14.3</td> <td>31±12.9</td> <td>169±8.9</td> </tr> <tr> <td>Triphenylene</td> <td>11±9.1</td> <td>47±12.8</td> <td>249±10.0</td> </tr> <tr> <td>Chrysene</td> <td>10±10.0</td> <td>45±13.3</td> <td>251±8.0</td> </tr> <tr> <td>Benzo(b)fluoranthene</td> <td>4±25.0</td> <td>15±13.3</td> <td>83±8.4</td> </tr> <tr> <td>Benzo(k)fluoranthene</td> <td><LOQ</td> <td><LOQ</td> <td>7±28.6</td> </tr> <tr> <td>Benzo(e)pyrene</td> <td>4±25.0</td> <td>19±10.5</td> <td>100±8.0</td> </tr> <tr> <td>Benzo(a)pyrene</td> <td><LOQ</td> <td>5±20.0</td> <td>30±6.7</td> </tr> <tr> <td>Indeno(1,2,3-cd)pyrene</td> <td><LOQ</td> <td><LOQ</td> <td>5±20.0</td> </tr> <tr> <td>Dibenz(ah)anthracene</td> <td><LOQ</td> <td><LOQ</td> <td>7</td> </tr> <tr> <td>Benzo(ghi)perylene</td> <td>3±33.3</td> <td>4±25.0</td> <td>24±8.3</td> </tr> </tbody> </table> <p>SO = Not quantitated due to signal overlap; LOQ = limit of quantitation</p> | Dose group [Target conc.] | 4mg/m ³ | 20mg/m ³ | 100mg/m ³ | PAH [ng/m ³ ± SD] | | | | Naphthalene | 1014±13.7 | 3687±8.4 | 19578±15.7 | Acenaphthylene | SO | SO | SO | Acenaphthene | 477±8.2 | 1923±7.2 | 10093±5.2 | Fluorene | 801±5.5 | 3520±6.7 | 21690±8.4 | Phenanthrene | 331±13.0 | 1641±9.0 | 11580±9.5 | Anthracene | SO | SO | SO | Fluoranthene | 43±11.6 | 220±11.4 | 1295±7.6 | Pyrene | 45±11.1 | 219±13.7 | 1139±17.8 | Benz(a)anthracene | 7±14.3 | 31±12.9 | 169±8.9 | Triphenylene | 11±9.1 | 47±12.8 | 249±10.0 | Chrysene | 10±10.0 | 45±13.3 | 251±8.0 | Benzo(b)fluoranthene | 4±25.0 | 15±13.3 | 83±8.4 | Benzo(k)fluoranthene | <LOQ | <LOQ | 7±28.6 | Benzo(e)pyrene | 4±25.0 | 19±10.5 | 100±8.0 | Benzo(a)pyrene | <LOQ | 5±20.0 | 30±6.7 | Indeno(1,2,3-cd)pyrene | <LOQ | <LOQ | 5±20.0 | Dibenz(ah)anthracene | <LOQ | <LOQ | 7 | Benzo(ghi)perylene | 3±33.3 | 4±25.0 | 24±8.3 |
| Dose group [Target conc.] | 4mg/m ³ | 20mg/m ³ | 100mg/m ³ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PAH [ng/m ³ ± SD] | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Naphthalene | 1014±13.7 | 3687±8.4 | 19578±15.7 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Acenaphthylene | SO | SO | SO | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Acenaphthene | 477±8.2 | 1923±7.2 | 10093±5.2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluorene | 801±5.5 | 3520±6.7 | 21690±8.4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Phenanthrene | 331±13.0 | 1641±9.0 | 11580±9.5 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Anthracene | SO | SO | SO | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluoranthene | 43±11.6 | 220±11.4 | 1295±7.6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pyrene | 45±11.1 | 219±13.7 | 1139±17.8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benz(a)anthracene | 7±14.3 | 31±12.9 | 169±8.9 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Triphenylene | 11±9.1 | 47±12.8 | 249±10.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Chrysene | 10±10.0 | 45±13.3 | 251±8.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo(b)fluoranthene | 4±25.0 | 15±13.3 | 83±8.4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo(k)fluoranthene | <LOQ | <LOQ | 7±28.6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo(e)pyrene | 4±25.0 | 19±10.5 | 100±8.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo(a)pyrene | <LOQ | 5±20.0 | 30±6.7 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Indeno(1,2,3-cd)pyrene | <LOQ | <LOQ | 5±20.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Dibenz(ah)anthracene | <LOQ | <LOQ | 7 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo(ghi)perylene | 3±33.3 | 4±25.0 | 24±8.3 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

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|---|--|
| Category Chemical Result Type: | Measured |
| METHOD | |
| Type of Study: | Cytogenetic |
| Type of Test: | Micronucleus |
| Route of Administration: | Inhalation – Nose only |
| Species: | Rat |
| Strain: | SPF-Wistar |
| Gender: | Male and female |
| Dose: | Target: 0, 4, 20 and 100mg/m ³ . Actual: 0, 6.8, 34.4 and 172.5mg/m ³ [calculated by BIA method with a 1.66 conversion factor]. |
| Year Study Performed: | 2007 |
| Method/Guideline Followed: | Other – Heddle et al., 1984 for bone marrow and standard peripheral blood staining technique; similar to OECD 474 |
| GLP: | Yes |
| Duration of Treatment/Exposure Period and Units: | 5, 30 days or 12 months |
| Frequency of Treatment: | 6 hours/day, 5 days/week |
| Positive, Negative and Solvent Control Substance(s): | Clean air. No positive controls |
| Post-Exposure Period: | None |
| Number of Animals per Sex per Dose: | 6 rats/sex/group/exposure period |
| Method/Guideline and Test Condition Remarks: | Test material was collected and generated according to method reported in the parallel 2-year Carcinogenesis Nose-only Inhalation study at Fraunhofer ITEM described in a separate Carcinogenesis robust summary. <u>Animals:</u> SPF Wistar rats (8 weeks of age at study initiation) were exposed to bitumen fumes individually in acrylic glass tubes placed |

| | |
|----------------------------------|--|
| | <p>around the exposure cylinder, 6 hrs/day, 5 days/week. The whole exposure unit was placed under a laboratory hood. During non-exposure periods, rats were housed under barrier conditions in same sex pairs in polycarbonate type III cages with absorbent bedding and food and water <i>ad libitum</i>. Animal room temperatures was 22 ± 2°C and 40-70% relative humidity with a 12 hour light cycle and air flow rate of 12-15 changes/hour.</p> <p>Endpoints evaluated in this extensive study included DNA adducts, determination of 8-oxo-dG and deoxyguanine, a micronucleus assay, biotransformation and urinary excretion of PAHs profiling of 16 genes with known function in inflammation, asthma and other pulmonary diseases, and Western blotting procedures to measure CYP1A1 activity stimulated by exposure to asphalt fume condensate. This robust summary addresses the micronucleus assay and the DNA adduct formation study is presented in a separate robust summary. The other endpoints are not part of the HPV program and will not be discussed here.</p> <p>Peripheral blood from each animal was collected from the tail vein at each exposure interval and treated with Li-heparin as anticoagulant. Two smears were prepared on defatted clean slides. The smears were air-dried for 24 hours and stained according to Pappenheim with May-Gruenwald and Giemsa-solution. Slides were coded prior to microscopic analysis.</p> <p>After 12 months exposure, bone marrow from 6 rats/sex/group was isolated from the femur. Ends of the femur were cut off and bone marrow pushed out with a fine wire and transferred to a tube containing fetal calf serum. The raw bone marrow suspension was gently pulled up and down in the tube to obtain a fine cell suspension, centrifuged for 5 minutes at 1000RPM and most of the supernatant discarded. Cell pellet was carefully resuspended in a small volume of fetal calf serum, resulting in approximately 2 drops of bone marrow cell suspension per animal. Two smears were prepared on defatted clean slides, air-dried for 24 hours and stained with May-Gruenwald and Giemsa-solution.</p> <p>Slides were analyzed microscopically under 630-1000x magnification. For each animal, the incidence of micronucleated cells per 2000 total erythrocytes of the peripheral blood was determined. For bone marrow after 12 months exposure, the incidence of micronucleated cells per 2000 polychromatic erythrocytes was determined and the number of polychromatic erythrocytes per 200 red blood wells was scored. The ratio of polychromatic to normochromatic erythrocytes was calculated to evaluate cell toxicity.</p> |
| <p>TEST RESULTS</p> | |
| <p>Systemic Toxicity:</p> | <p>No adverse effects were observed at any bitumen fume condensate exposure level but dose-dependent uptake of bitumen fumes was confirmed based on urinary excretion of PAHs and metabolites. Also after 12 months exposure at the highest dose, 4/6 male rats displayed slight repression in bone marrow erythrocyte count compared to controls suggesting that bitumen fume components reached to bone marrow without causing cytogenetic damage. Two other male rats of the high dose group exhibited a slight increase in red blood cells when compared to controls.</p> |

Peripheral blood: After 5 days, 20 days and 1 year exposure, only a few micronucleated erythrocytes were observed and there was no evidence of a significant increase in micronucleated erythrocytes with exposure to bitumen fumes [Table 1]

Table 1. Micronuclei Formation in Peripheral Blood

| Treatment [target] | Sex | Micronuclei/2000 Erythrocytes [Mean of 6 animals ± SD] | | |
|-----------------------|--------|--|------------|------------|
| | | 5 days | 30 days | 12 months |
| 0 mg/m ³ | Male | 1.0 ± 1.09 | 0.8 ± 0.75 | 0.5 ± 1.23 |
| | Female | 0.3 ± 0.52 | 1.7 ± 1.21 | 0.5 ± 0.84 |
| 4 mg/m ³ | Male | 0.7 ± 0.52 | 1.5 ± 1.05 | 0.2 ± 0.41 |
| | Female | 0.2 ± 0.41 | 0.5 ± 0.55 | 0.2 ± 0.45 |
| 20 mg/m ³ | Male | 0.8 ± 0.98 | 0.7 ± 0.82 | 0.7 ± 0.82 |
| | Female | 0.5 ± 0.55 | 1.0 ± 0.63 | 0.3 ± 0.52 |
| 100 mg/m ³ | Male | 0.0 ± 0.55 | 0.7 ± 0.82 | 0.3 ± 0.52 |
| | Female | 0.5 ± 0.84 | 0.8 ± 1.17 | 0.2 ± 0.41 |

After 12 months inhalation exposure to bitumen fume condensate, there was no evidence of an increase in micronucleated polychromatic erythrocytes in bone marrow cells [Table 2]

Table 2. Micronuclei Formation in Bone Marrow of Rats after 12 months of Bitumen fume exposure

| Sex | Micronuclei/2000 Polychromatic Erythrocytes [Mean of 6 Animals ± SD] | | | |
|---------|--|---------------------|----------------------|------------------------|
| | 0 mg/m ³ | 4 mg/m ³ | 20 mg/m ³ | 1000 mg/m ³ |
| Males | 41.3 ± 16.80 | 39.2 ± 14.39 | 32.3 ± 15.54 | 29.3 ± 18.50 |
| Females | 35.2 ± 13.35 | 25.8 ± 6.53 | 36.5 ± 14.21 | 31.5 ± 10.13 |

Genotoxic Effect:

Results Remarks:

No concurrent positive control was used in these assays

Conclusion:

Nose-only inhalation exposure to bitumen fume condensate did not induced an increase in micronucleated red blood cells in peripheral blood over periods from 5 days to 1 year. No increase in micronucleated polychromatic erythrocytes was seen in bone marrow cells of rats exposed to bitumen fume condensate for 1 year. Paving bitumen fume condensate is not a clastogen under these assay conditions.

RELIABILITY/DATA QUALITY

Reliability:

2. Reliable with restriction . No concurrent positive control was used .

Reliability Remarks:

Key Study Sponsor Indicator:

Key study. This study was performed in conjunction with the DNA adduct study reported in a separate robust summary.

REFERENCE

Reference:

Halter, R., Hansen, T., Seidel, A, Ziemann, and Borlak, J. 2007. Importance of DNA-adduct formation and gene expression profiling of disease candidate genes in rats exposed to bitumen fumes. *J Occup Environ Hygiene* 4(S1): 44-64.
Heddle, J.A., Stuart, E., and Salamone, M.F. 1984. The bone marrow micronucleus test. In: Kilbey, B.J. et al. (eds) *Handbook of Mutagenicity Test Procedures*. 2nd ed. Pp. 441-457. Elsevier Press Amsterdam.



High Production Volume Information System (HPVIS)

| Developmental Toxicity/Teratogenicity | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|--|--|----------------------|----------------------|----------------------|-------------|-------|-------|----------------|----|----|--------------|------|------|----------|-------|-------|--------------|-------|-------|------------|------|------|--------------|------|------|--------|------|------|---------------------|------|------|--------------|------|------|----------|------|------|------------------------|------|------|------------------------|------|------|------------------|------|------|------------------|------|------|--------------------------|------|------|------------------------|------|------|----------------------|------|------|
| Test Substance | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Category Chemical: (CAS #) | 64742-93-4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Test Substance: (CAS #) | 64742-93-4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Test Substance Purity/Composition and Other Test Substance Comments: | Roofing asphalt fume condensate (RAFC) prepared by Heritage Research Group. D = 0.8745g/ml, Refractive Index 1.4831 at 25 ^o C, Kinematic viscosity 8.3616 cst at 100 ^o F. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Concentration of PAH in RAFC | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | <table border="1"> <thead> <tr> <th>Compound</th> <th>Analysis A [µg/g]</th> <th>Analysis B [µg/g]</th> </tr> </thead> <tbody> <tr> <td>Naphthalene</td> <td>164.0</td> <td>165.0</td> </tr> <tr> <td>Acenaphthylene</td> <td>*)</td> <td>*)</td> </tr> <tr> <td>Acenaphthene</td> <td>29.2</td> <td>28.8</td> </tr> <tr> <td>Fluorene</td> <td>254.0</td> <td>253.0</td> </tr> <tr> <td>Phenanthrene</td> <td>248.0</td> <td>250.0</td> </tr> <tr> <td>Anthracene</td> <td>31.9</td> <td>32.0</td> </tr> <tr> <td>Fluoranthene</td> <td>10.5</td> <td>9.97</td> </tr> <tr> <td>Pyrene</td> <td>48.8</td> <td>47.7</td> </tr> <tr> <td>Benz (a) anthracene</td> <td>7.81</td> <td>7.99</td> </tr> <tr> <td>Triphenylene</td> <td>20.7</td> <td>21.6</td> </tr> <tr> <td>Chrysene</td> <td>19.8</td> <td>19.8</td> </tr> <tr> <td>Benzo (b) fluoranthene</td> <td>4.80</td> <td>4.70</td> </tr> <tr> <td>Benzo (k) fluoranthene</td> <td>1.72</td> <td>1.15</td> </tr> <tr> <td>Benzo (e) pyrene</td> <td>7.72</td> <td>7.73</td> </tr> <tr> <td>Benzo (a) pyrene</td> <td>4.09</td> <td>4.10</td> </tr> <tr> <td>Indeno (1,2,3-cd) pyrene</td> <td>0.70</td> <td>0.60</td> </tr> <tr> <td>Dibenz (ah) anthracene</td> <td>1.41</td> <td>1.37</td> </tr> <tr> <td>Benzo (ghi) perylene</td> <td>2.93</td> <td>2.76</td> </tr> </tbody> </table> | Compound | Analysis A [µg/g] | Analysis B [µg/g] | Naphthalene | 164.0 | 165.0 | Acenaphthylene | *) | *) | Acenaphthene | 29.2 | 28.8 | Fluorene | 254.0 | 253.0 | Phenanthrene | 248.0 | 250.0 | Anthracene | 31.9 | 32.0 | Fluoranthene | 10.5 | 9.97 | Pyrene | 48.8 | 47.7 | Benz (a) anthracene | 7.81 | 7.99 | Triphenylene | 20.7 | 21.6 | Chrysene | 19.8 | 19.8 | Benzo (b) fluoranthene | 4.80 | 4.70 | Benzo (k) fluoranthene | 1.72 | 1.15 | Benzo (e) pyrene | 7.72 | 7.73 | Benzo (a) pyrene | 4.09 | 4.10 | Indeno (1,2,3-cd) pyrene | 0.70 | 0.60 | Dibenz (ah) anthracene | 1.41 | 1.37 | Benzo (ghi) perylene | 2.93 | 2.76 |
| | Compound | Analysis A [µg/g] | Analysis B [µg/g] | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Naphthalene | 164.0 | 165.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Acenaphthylene | *) | *) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Acenaphthene | 29.2 | 28.8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Fluorene | 254.0 | 253.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Phenanthrene | 248.0 | 250.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Anthracene | 31.9 | 32.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Fluoranthene | 10.5 | 9.97 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Pyrene | 48.8 | 47.7 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Benz (a) anthracene | 7.81 | 7.99 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Triphenylene | 20.7 | 21.6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Chrysene | 19.8 | 19.8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (b) fluoranthene | 4.80 | 4.70 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (k) fluoranthene | 1.72 | 1.15 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (e) pyrene | 7.72 | 7.73 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (a) pyrene | 4.09 | 4.10 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Indeno (1,2,3-cd) pyrene | 0.70 | 0.60 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Dibenz (ah) anthracene | 1.41 | 1.37 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (ghi) perylene | 2.93 | 2.76 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *) Not determined due to overlapping signals. From Fraunhofer ITEM Rpt 02N07532 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Category Chemical Result Type: | Measured | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Method | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Route of Administration: | Inhalation | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Type of Exposure: | Nose only | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Species: | Rat | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

| | |
|---|--|
| Mammalian Strain: | Wistar (Cr1:WU) |
| Gender: | Male and female |
| Number of Animals per Dose: | 12 males/12 satellite breeding females/group |
| Concentration: | Target: 0, 30, 100, 300mg/m ³ total hydrocarbons (THC), Actual: 0, 30.0, 100.1, 297.3mg/m ³ THC measured continuously during the entire exposure period by aerosol photometers |
| Year Study Performed: | 2008 |
| Method/Guideline Followed: | OECD 422 [Developmental Toxicity Screening segment of study described in the Repeated Dose Toxicity section] |
| GLP: | Yes |
| Exposure Period: | 28 days for subchronic males; approximately 35-48 days for pregnant satellite females based on 14 days pre-mating, up to 14 days mating and Gestation days 0-20;and 54 days [26 days after end of cohabitation] for females with no evidence of copulation |
| Frequency of Treatment: | 6 hours/day, 7 days/week |
| Post-Exposure Period: | none |
| Method/Guideline and Test Condition Remarks: | <p>The selection of dose levels of 30, 100 and 300mg/m³ THC for this study was based on results from a range finding study [See robust summary for Repeated dose toxicity for details]. Body weights and weight gain and food consumption were reduced at 300 and 1000mg/m³. A decrease in fetal body weight was seen at 1000mg/m³.</p> <p><u>Exposure:</u> The rats were exposed to asphalt fumes in a direct flow nose only inhalation exposure system. In this system the fume was supplied to each animal individually, and exhaled air was exhausted immediately. The rats were placed around the exposure cylinder in tapered acrylic glass tubes with adjustable backstops. The different exposure units were placed under separate laboratory hoods to avoid cross-contamination. The roofing asphalt fume condensate was pumped via a stainless steel tube directly into the pneumatic dispersion nozzle. This nozzle was operated with heated nitrogen (160°C) at a flow rate of 5 l/min and generated droplets with a mean diameter of about 6µm. The droplets were fed directly into a tube heated at approximately 220 °C, where they evaporated. The vapor was then issued through the nozzle and recondensed by mixing with cool air. From the generator, the fume was directed through stainless steel tubes to the inhalation units. Flow resistors controlled the flow of asphalt fume to each inhalation unit. The flow rate through these resistors was maintained by keeping a constant pressure difference between the inhalation units and the generator controlling the flow rate of the cooling air in the generator. The final concentrations were achieved by mixing the asphalt fume with dilution air, regulated by mass flow controllers. The whole inhalation system was microprocessor controlled and supervised by a computer. Fume concentration was determined by sampling from each nose-only unit (2</p> |

samples/week) using a combination of glass fiber filter and XAD absorption tube. Samples were extracted and analyzed separately by IR spectroscopy and reported in mg Total Hydrocarbons (THC/m³) aerosol and vapor phase. UV fluorescence determined as diphenylanthracene equivalents (mg DPA/kg) was measured in all exposure groups once a week to verify uniformity of atmosphere in all chambers. Particle size distribution was also determined using a scanning mobility particle sizer.

Animals: Male and virgin female rats received at 7 weeks of age were acclimated for 3 weeks. During the first week, training began to acclimatize the animals to the exposure tubes for increasing periods of time. Clinical observations were made once daily. Rats were randomized by weight using a computer generated program (PROVANTIS) into each of 4 groups. Male groups were evaluated for homogeneity of mean body weights and variances and did not exceed $\pm 20\%$ within or between groups. However, due to an error in the randomization program, females were assigned to groups based on their body weights instead of by randomising them resulting in statistically significant differences in absolute body weights between controls and treated groups at study start. Animals were identified by ear tattoos and cage cards. Animals were housed individually in Makrolon® Type III cages with softwood bedding with food and water *ad libitum* except during exposure. Room temperature was $22 \pm 2^{\circ}\text{C}$ and relative humidity at $55 \pm 15\%$ with 12 hour light-dark cycle and 15 air changes/hour.

Groups of 12 young, adult, male Crl:WU rats were exposed to atmospheres containing 0, 30, 100, or 300mg/m³ of RAFC for 28 days. Satellite groups of 12 young, nulliparous, non-pregnant female rats were exposed to 0, 30, 100, or 300mg/m³ during a pre-mating period of approximately 2 weeks, a cohabitation period of up to 2 weeks, and a gestation period of approximately 3 weeks. Following the 2 week pre-mating period, each satellite female was paired with a male of the same respective dosage group during an approximately 2 week cohabitation period. Presumed pregnant females were exposed from gestation day [GD] 0-20 but were not exposed after gestation day 20, or during the approximately 4-day postpartum lactation period [LD]. Females without evidence of mating continued to be exposed for 26 days after the end of the cohabitation period.

Body weights, clinical signs, and food consumption were recorded throughout the study. Presumed pregnant females were weighed weekly during pre-mating and cohabitation, on GD0, 7, 14, 21 and on LD0 and 4. Food consumption data were collected at the same intervals. On postpartum day 4, lactating females and offspring were sacrificed. Organs (liver, kidneys, lungs, adrenal glands, thymus brain, spleen, heart, ovaries and uterus) were weighed. In addition to standard histopathological evaluations, the number of implantation sites [using ammonium sulphide staining] and corpora lutea were counted. Offspring were sexed, weighted and evaluated for external abnormalities.

No soft tissue or skeletal evaluations were performed on offspring.

Statistical analysis: Body weight, food and water consumption, organ weight data analyzed by Analysis of Variance (ANOVA) followed by Dunnett's modification if necessary. Kruskal Wallis ANOVA and Mann Whitney U-test were used for non-homogeneous data. Qualitative data was analyzed by 2-tailed Fisher test with Bonferroni correction or Chi square test.

Test Results

Concentration (LOEL/LOAEC/NOEL/NOAEC)

| Type | Population | Value Description | Value/Lower Concentration | Upper Concentration | Units |
|-------|------------------|-------------------|---------------------------|---------------------|-------------------|
| NOAEL | Develop | ≥ | 297.3 | | mg/m ³ |
| LOAEL | Parental females | = | 100.1 | | mg/m ³ |
| NOAEL | Parental females | = | 30.0 | | mg/m ³ |
| | | | | | |
| | | | | | |

Results Remarks:

Mortality did not occur at any exposure concentration. No RAFC-related effects were seen in body weight gain or food consumption in pregnant females. Higher body weights of RAFC exposed females compared to controls throughout gestation resulted from randomization error at initiation of study. Overall gestation body weight gain and food consumption were slightly but not statistically decreased in the high dose group. Absolute lung weights were statistically significantly increased at mid and high doses but relative weights were only statistically significantly increased at 297.3mg/m³. Minimal histopathological effects of the 297.3mg/m³ THC exposure occurred in the lungs, where a slight increase of alveolar macrophage accumulation was observed in combination with minimal mononuclear/inflammatory cell infiltration and minimal to slight (adaptive) alveolar hyperplasia of the bronchiolar type (alveolar bronchiolization). This type of hyperplasia is considered to be non-preneoplastic. In the nasal cavity, a single control animal but 7/12 pregnant rats of the high dose group showed (multi)focal very slight to slight mucosal mononuclear/inflammatory cell infiltration. This effect was not seen in the nasal cavity of subchronic female rats.

Reproductive/Developmental Toxicology: There were no significant RAFC related differences in mean number of pregnant animals, number of animals delivering, mating index, fertility index, gestation length, number of corpora lutea, number of implantation sites or percent of post implantation loss for any exposure group. All animals mated within the first 8 days of cohabitation yielding 10, 10, 11 and 12 sperm positive females and 11, 9, 11, and 11 pregnancies in control, 30, 100.1, and 297.3mg/m³ groups,

| | |
|--|--|
| | <p>respectively. Mating Indices were 100, 91.7, 100, and 100% and the Female Fertility Indices were 91.7, 81.8, 91.7 and 91.7 in controls, 30, 100.1 and 297.3mg/m³ groups, respectively. The Fertility Index included 2 control, 1 low dose, 1 mid-dose, and 2 high dose females who were pregnant although no sperm was detected in vaginal smears. One control, 2 low dose, 1 mid-dose and 1 high dose female had sperm in vaginal smears but did not become pregnant. One high dose female (#4207) was killed as non-pregnant but was found to have 12 implantation sites at necropsy. One low dose female did not mate. The number of females completing delivery was 11, 9, 11, and 10 and the Live birth Indices were 99.1, 97.9, 100 and 100% in control, 30, 100.1 and 297.3mg/m³ group respectively. Duration of gestation was approximately 22 days in all groups. Stillborn pups were seen in one control litter [1 pup] and in one low dose litter [2 pups]. One control litter (1204, 7 pups) was terminated on postpartum day 0 due to lack of maternal care and one pup died between days 1-4. In the 100.1mg/m³ group 3 pups died between postpartum days 1-4. Overall Viability Indices were 92.5, 100. 97.3 and 100% in control, 30, 100.1 and 297.3mg/m³ group respectively. Pup sex ratio, body weights and weight gain from treated litters over postpartum day 0-4 were comparable to controls [Additional details in robust summary for Reproductive Toxicity.] No apparent teratogenic effects were seen in gross morphological examination of pups. At necropsy, average implantation sites per litter were 10.7, 11.1, 11.5 and 9.8 and the average corpora lutea were 11.7, 12.0, 11.5 and 10.9 in control, 30, 100.1 and 297.3mg/m³ group respectively. Values for RAFC groups were comparable to controls for these parameters.</p> |
| <p>Conclusion:</p> | <p>Exposure to RAFC did not cause significant adverse in any reproductive parameters [mean number of pregnant animals, number of animals delivering, mating index, fertility index, gestation length, number of corpora lutea, number of implantation sites or percent of post implantation loss] for any exposure group. No gross morphological malformations were seen in offspring from postpartum day 0-4. Systemic effects in maternal animals demonstrated that treatment at concentrations up to 297.3mg/m³ were sufficient to induce some level of toxicity, as specified in OECD 422.</p> |
| <p>Reliability/Data Quality</p> | |
| <p>Reliability:</p> | <p>2.- Reliable with restrictions</p> |
| <p>Reliability Remarks:</p> | <p>Error in randomization of females by group at study onset OECD 422 Developmental screening protocol, not a complete developmental study</p> |
| <p>Key Study Sponsor Indicator:</p> | <p>Key study This is the developmental toxicity screen segment of OECD 422 described in the Repeated Dose Toxicity Section and in the Reproductive Toxicity Section. No soft tissue or skeletal evaluations of offspring were performed.</p> |

| Reference | |
|-------------------|---|
| Reference: | <p>Fraunhofer Institute of Toxicology and Experimental Medicine [ITEM]. 2009. Combined Repeated Dose Toxicity Study with Reproductive/Developmental Screening Test and Mammalian Erythrocyte Micronucleus Test via Inhalation with Roofing Asphalt Fume Condensate. Fraunhofer ITEM Study # 02G08016. Hannover, Germany Sponsored by Petroleum HPV Testing Group, API, Washington, DC.</p> <p>Fraunhofer ITEM. 2008. Chamber Trials for the Combined Repeated Dose Toxicity Study with Reproductive/ Developmental Screening Test and Mammalian Erythrocyte Micronucleus Test via Inhalation with Roofing Asphalt Fume Condensate. Fraunhofer ITEM Study # 02N07532 Hannover, Germany. Sponsored by Petroleum HPV Testing Group, API, Washington, DC.</p> |



High Production Volume Information System (HPVIS)

Genetic Toxicity *in vivo*

TEST SUBSTANCE

| Category Chemical: | <input type="text" value="Select a Chemical"/> Type in if not listed: 64742-93-4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|--|----------------------|----------------------|----------------------|-------------|-------|-------|----------------|----|----|--------------|------|------|----------|-------|-------|--------------|-------|-------|------------|------|------|--------------|------|------|--------|------|------|---------------------|------|------|--------------|------|------|----------|------|------|------------------------|------|------|------------------------|------|------|------------------|------|------|------------------|------|------|
| Test Substance: | <input type="text" value="Select a Chemical"/> Type in if not listed: 64742-93-4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Test Substance Purity/Composition and Other Test Substance Comments: | <p>Roofing asphalt fume condensate (RAFC) prepared by Heritage Research Group. d=0.8745g/ml, Refractive Index 1.4831 at 25°C, Kinematic viscosity 8.3616 cst at 100°F.</p> <p>Concentration of PAH in RAFC</p> <table border="1" data-bbox="562 873 1325 1414"> <thead> <tr> <th>Compound</th> <th>Analysis A [µg/g]</th> <th>Analysis B [µg/g]</th> </tr> </thead> <tbody> <tr><td>Naphthalene</td><td>164.0</td><td>165.0</td></tr> <tr><td>Acenaphthylene</td><td>*)</td><td>*)</td></tr> <tr><td>Acenaphthene</td><td>29.2</td><td>28.8</td></tr> <tr><td>Fluorene</td><td>254.0</td><td>253.0</td></tr> <tr><td>Phenanthrene</td><td>248.0</td><td>250.0</td></tr> <tr><td>Anthracene</td><td>31.9</td><td>32.0</td></tr> <tr><td>Fluoranthene</td><td>10.5</td><td>9.97</td></tr> <tr><td>Pyrene</td><td>48.8</td><td>47.7</td></tr> <tr><td>Benz (a) anthracene</td><td>7.81</td><td>7.99</td></tr> <tr><td>Triphenylene</td><td>20.7</td><td>21.6</td></tr> <tr><td>Chrysene</td><td>19.8</td><td>19.8</td></tr> <tr><td>Benzo (b) fluoranthene</td><td>4.80</td><td>4.70</td></tr> <tr><td>Benzo (k) fluoranthene</td><td>1.72</td><td>1.15</td></tr> <tr><td>Benzo (e) pyrene</td><td>7.72</td><td>7.73</td></tr> <tr><td>Benzo (a) pyrene</td><td>4.09</td><td>4.10</td></tr> </tbody> </table> | Compound | Analysis A [µg/g] | Analysis B [µg/g] | Naphthalene | 164.0 | 165.0 | Acenaphthylene | *) | *) | Acenaphthene | 29.2 | 28.8 | Fluorene | 254.0 | 253.0 | Phenanthrene | 248.0 | 250.0 | Anthracene | 31.9 | 32.0 | Fluoranthene | 10.5 | 9.97 | Pyrene | 48.8 | 47.7 | Benz (a) anthracene | 7.81 | 7.99 | Triphenylene | 20.7 | 21.6 | Chrysene | 19.8 | 19.8 | Benzo (b) fluoranthene | 4.80 | 4.70 | Benzo (k) fluoranthene | 1.72 | 1.15 | Benzo (e) pyrene | 7.72 | 7.73 | Benzo (a) pyrene | 4.09 | 4.10 |
| Compound | Analysis A [µg/g] | Analysis B [µg/g] | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Naphthalene | 164.0 | 165.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Acenaphthylene | *) | *) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Acenaphthene | 29.2 | 28.8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluorene | 254.0 | 253.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Phenanthrene | 248.0 | 250.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Anthracene | 31.9 | 32.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluoranthene | 10.5 | 9.97 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pyrene | 48.8 | 47.7 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benz (a) anthracene | 7.81 | 7.99 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Triphenylene | 20.7 | 21.6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Chrysene | 19.8 | 19.8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (b) fluoranthene | 4.80 | 4.70 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (k) fluoranthene | 1.72 | 1.15 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (e) pyrene | 7.72 | 7.73 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (a) pyrene | 4.09 | 4.10 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

| | | | | | | | | | | |
|---|---|--------------------------|------|------|------------------------|------|------|----------------------|------|------|
| | <table border="1"> <tr> <td>Indeno (1,2,3-cd) pyrene</td> <td>0.70</td> <td>0.60</td> </tr> <tr> <td>Dibenz (ah) anthracene</td> <td>1.41</td> <td>1.37</td> </tr> <tr> <td>Benzo (ghi) perylene</td> <td>2.93</td> <td>2.76</td> </tr> </table> <p>*) Not determined due to overlapping signals. From Fraunhofer ITEM Rpt 02N07532</p> | Indeno (1,2,3-cd) pyrene | 0.70 | 0.60 | Dibenz (ah) anthracene | 1.41 | 1.37 | Benzo (ghi) perylene | 2.93 | 2.76 |
| Indeno (1,2,3-cd) pyrene | 0.70 | 0.60 | | | | | | | | |
| Dibenz (ah) anthracene | 1.41 | 1.37 | | | | | | | | |
| Benzo (ghi) perylene | 2.93 | 2.76 | | | | | | | | |
| Category Chemical Result Type: | Measured | | | | | | | | | |
| METHOD | | | | | | | | | | |
| Type of Study: | Cytogenetic | | | | | | | | | |
| Type of Test: | Micronucleus | | | | | | | | | |
| Route of Administration: | Inhalation – Nose only | | | | | | | | | |
| Species: | Rat | | | | | | | | | |
| Strain: | Wistar (CrI:WU) | | | | | | | | | |
| Gender: | Male and female animals selected from Fraunhofer OECD 422 subchronic animals [Details of Subchronic segment presented in Repeated Dose robust summary]. Additional Positive control rats [5/sex/group] | | | | | | | | | |
| Dose: | Target: 0, 30, 100, 300mg/m ³ total hydrocarbons (THC), Actual: 0, 30.0, 100.1, 297.3mg/m ³ THC measured continuously during the entire exposure period by aerosol photometers. | | | | | | | | | |
| Year Study Performed: | 2008 | | | | | | | | | |
| Method/Guideline Followed: | OECD 474, OPPTS 870.5395 | | | | | | | | | |
| GLP: | Yes | | | | | | | | | |
| Duration of Treatment/Exposure Period and Units: | 28 days | | | | | | | | | |
| Frequency of Treatment: | 6 hours/day, 7 days/week | | | | | | | | | |
| Positive, Negative and Solvent Control Substance(s): | Positive control: Cyclophosphamide, monohydrate, 60mg/kg in water; Nose-only Inhalation negative control: clean room air | | | | | | | | | |

| | |
|---|--|
| Post-Exposure Period: | None |
| Number of Animals per Sex per Dose: | 5 |
| Method/Guideline and Test Condition Remarks: | <p>The selection of dose levels of 30, 100 and 300mg/m³ THC for this study was based on results from a range finding study [See robust summary for Repeated dose toxicity for details]. Body weights and weight gain and food consumption were reduced at 300 and 1000mg/m³. Impairment of blood formation was seen bone marrow of female rats at 1000mg/m³.</p> <p><u>Exposure:</u> The rats were exposed to asphalt fumes in a direct flow nose only inhalation exposure system. In this system the fume was supplied to each animal individually, and exhaled air was exhausted immediately. The rats were placed around the exposure cylinder in tapered acrylic glass tubes with adjustable backstops. The different exposure units were placed under separate laboratory hoods to avoid cross-contamination. The roofing asphalt fume condensate [RAFC] was pumped via a stainless steel tube directly into the pneumatic dispersion nozzle. This nozzle was operated with heated nitrogen (160°C) at a flow rate of 5 l/min and generated droplets with a mean diameter of about 6µm. The droplets were fed directly into a tube heated at approximately 220°C, where they evaporated. The vapor was then issued through the nozzle and recondensed by mixing with cool air. From the generator, the fume was directed through stainless steel tubes to the inhalation units. Flow resistors controlled the flow of asphalt fume to each inhalation unit. The flow rate through these resistors was maintained by keeping a constant pressure difference between the inhalation units and the generator controlling the flow rate of the cooling air in the generator. The final concentrations were achieved by mixing the asphalt fume with dilution air, regulated by mass flow controllers. The whole inhalation system was microprocessor controlled and supervised by a computer. Fume concentration was determined by sampling from each nose-only unit (2 samples/week) using a combination of glass fiber filter and XAD absorption tube. Samples were extracted and analyzed separately by IR spectroscopy and reported in mg Total Hydrocarbons (THC/m³) aerosol and vapor phase. UV fluorescence determined as diphenylanthracene equivalents (mg DPA/kg) was measured in all exposure groups once a week to verify uniformity of atmosphere in all chambers. Particle size distribution was also determined using a scanning mobility particle sizer.</p> <p><u>Animals:</u> Male and virgin female rats received at 7 weeks of age were acclimated for 3 weeks. During the first week, training began to acclimatize the animals to the exposure tubes for increasing periods of time. Clinical observations were made once daily. Rats were randomized by weight using a computer generated program (PROVANTIS) into each of 4 groups. Male groups were evaluated for homogeneity of mean body weights and variances and did not exceed ± 20% within or between groups. However, due to an error in the randomization program, females were assigned to groups based on their body weights instead of by randomising them resulting in statistically significant differences in absolute body weights between controls and treated groups at study start. Animals were identified by ear tattoos and cage cards. Animals were housed individually in Makrolon® Type III cages with softwood bedding with food and water <i>ad libitum</i> except during exposure. Room temperature</p> |

was 22 ± 2°C and relative humidity at 55 ± 15% with 12 hour light-dark cycle and 15 air changes/hour.

Subchronic animals were exposed by nose-only inhalation to target concentrations of 0, 30, 100, 300mg/m³ total hydrocarbons (THC) of roofing asphalt fume condensate (RAFC) for 28 days. The positive control, cyclophosphamide 60mg/kg in water was administered by oral gavage to 10 additional rats [5/sex/group] 24 hours prior to sacrifice. Five rats/sex/group were selected at random from each RAFC group and sacrificed 24 hours after the start of the final exposure. One femur was collected from each rat, marrow was flushed out with 5 ml fetal calf serum and a fine cell suspension prepared. The bone marrow suspension was cleaned of nucleated cells by passage through a cellulose column to facilitate erythrocyte counts and eliminate granulocytes. Bone marrow suspensions were prefiltered through microscope cleaning paper, loaded onto cellulose columns and allowed to drain into 15ml centrifuge tubes. Cleansed bone marrow was centrifuged and most of the supernatant discarded. The cell pellet was resuspended in a small additional amount of fetal calf serum and 2 smears were prepared, fixed and stained with May Gruenwald-Giemsa stain. Slides were coded prior to analysis and evaluated microscopically under 630 x 1000x magnification. Incidence of micronucleated cells per 2000 polychromatic erythrocytes (PCE) was determined. Ratio of PCE (immature erythrocytes) to normochromatic (NCE) erythrocytes(mature erythrocytes) was calculated by counting the number of PCE in 500 total red blood cells to determine toxic effects if any. The PCE ratio should not fall below 20% of clean air control values to avoid unspecific effects due to excessive cytotoxicity in bone marrow.

Statistics: Mann Whitney U test for evaluation of treated groups and positive control, and the H test of Kruskal Wallis for treated groups.

TEST RESULTS

Systemic Toxicity: RAFC did not repress formation of red blood cells in animals of any group at target concentrations of 30, 100 or 300mg/m³ compared to clean air treated controls. However in the earlier range finding study, exposure to RAFC at 1000mg/m³ demonstrated significant depression of red blood cell formation in females confirming that the test material was able to reach bone marrow as a target organ for this assay. Other effects in all subchronic rats are described in the Repeated Dose Toxicity robust summary for the Fraunhofer OECD 422 study.

No significantly enhanced mean frequency of micronucleated polychromatic erythrocytes (MN per 2000 PCE) was seen in any dose group [see Table below]. The ratios of PCE to NCE were comparable to controls indicating the absence of RAFC induced bone marrow toxicity.

Micronucleus induction in bone marrow of rats exposed to RAFC for 28 days

| RAFC Group | PCE per 500 RBC | PCE/NCE | MN per 2000 PCE | % MN in PCE |
|------------------------------|-----------------|------------------|------------------------|------------------|
| 0 Negative control | M 250 F 247 | M 1.00 F 0.98 | M 1.0 F 1.0 | M 0.05 F 0.05 |
| 30mg/m ³ | M 252 F 247 | M 1.02 F 0.98 | M 0.6 F 1.8 | M 0.03 F 0.09 |
| 100mg/m ³ | M 250 F 247 | M 1.00 F 0.98 | M 1.4 F 0.6 | M 0.07 F 0.03 |
| 300mg/m ³ | M 253 F 252 | M 1.03 F 1.01 | M 1.4 F 1.4 | M 0.07 F 0.07 |
| CP (60mg/kg) Pos. control | M 248 F 252 | M 0.99 F 1.01 | M 15.0*** F 12.8*** | M 0.75 F 0.64 |

*** P < 0.001

Genotoxic Effect:

Results Remarks:

The positive control induced the expected statistically significant increase in micronucleated polychromatic erythrocytes without causing bone marrow toxicity as demonstrated in the PCE/NCE ratio.

Conclusion:

Roofing asphalt fume condensate is not a clastogenic in this test system. NOAEL = 300mg/m³ target concentration [297.3 mg/m³ actual concentration].

RELIABILITY/DATA QUALITY

Reliability:

2. Reliable with restriction.

Reliability Remarks:

Error in randomization of all females by group at study onset.

Key Study Sponsor Indicator:

Key study
The OECD 422 subchronic and reproductive/developmental toxicity segments of this study are described in separate Robust Summaries.

REFERENCE

Reference:

Fraunhofer Institute of Toxicology and Experimental Medicine [ITEM]. 2009. Combined

Repeated Dose Toxicity Study with Reproductive/Developmental Screening Test and Mammalian Erythrocyte Micronucleus Test via Inhalation with Roofing Asphalt Fume Condensate. Fraunhofer ITEM Study # 02G08016. Hannover, Germany Sponsored by Petroleum HPV Testing Group, API, Washington, DC.

Fraunhofer ITEM. 2008. Chamber Trials for the Combined Repeated Dose Toxicity Study with Reproductive/Developmental Screening Test and Mammalian Erythrocyte Micronucleus Test via Inhalation with Roofing Asphalt Fume Condensate. Fraunhofer ITEM Study # 02N07532 Hannover, Germany. Sponsored by Petroleum HPV Testing Group, API, Washington, DC.



High Production Volume Information System (HPVIS)

| Repeated-Dose Toxicity | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|--|--|-------------------|-------------------|-------------------|-------------|-------|-------|----------------|----|----|--------------|------|------|----------|-------|-------|--------------|-------|-------|------------|------|------|--------------|------|------|--------|------|------|---------------------|------|------|--------------|------|------|----------|------|------|------------------------|------|------|------------------------|------|------|------------------|------|------|------------------|------|------|--------------------------|------|------|------------------------|------|------|----------------------|------|------|
| Test Substance – Repeated-Dose Toxicity | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Category Chemical: | 64742-93-4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| | Concentration of PAH in RAFC | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| | Compound | Analysis A [µg/g] | Analysis B [µg/g] | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Naphthalene | 164.0 | 165.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Acenaphthylene | *) | *) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Acenaphthene | 29.2 | 28.8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Fluorene | 254.0 | 253.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Phenanthrene | 248.0 | 250.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Anthracene | 31.9 | 32.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Fluoranthene | 10.5 | 9.97 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Pyrene | 48.8 | 47.7 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Benz (a) anthracene | 7.81 | 7.99 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Triphenylene | 20.7 | 21.6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Chrysene | 19.8 | 19.8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (b) fluoranthene | 4.80 | 4.70 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (k) fluoranthene | 1.72 | 1.15 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (e) pyrene | 7.72 | 7.73 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (a) pyrene | 4.09 | 4.10 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Indeno (1,2,3-cd) pyrene | 0.70 | 0.60 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Dibenz (ah) anthracene | 1.41 | 1.37 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (ghi) perylene | 2.93 | 2.76 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | *) Not determined due to overlapping signals. From Fraunhofer ITEM Rpt 02N07532 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Category Chemical Result Type: | Measured | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Method – Repeated-Dose Toxicity | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Route of Administration: | Inhalation | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Type of Exposure: | Nose only | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Species: | Rat | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

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| Mammalian Strain: | Wistar (Cr1:WU) |
| Gender: | Male and female |
| Number of Animals per Dose: | 12 males/12 subchronic females/12 satellite breeding females/group |
| Dose: | Target: 0, 30, 100, 300mg/m ³ total hydrocarbons (THC), Actual: 0, 30.0, 100.1, 297.3mg/m ³ THC measured continuously during the entire exposure period by aerosol photometers. |
| Year Study Performed: | 2008 |
| Method/Guideline Followed: | OECD 422; OPPTS 870.3650 [Details of Reproductive/ Developmental Screen and Micronucleus assay presented in separate robust summaries] |
| GLP: | Yes |
| Exposure Period: | 28 days for subchronic males and females |
| Frequency of Treatment: | 6 hours/day, 7 days/week |
| Post-Exposure Period: | None |
| Method/Guideline and Test Condition Remarks: | <p>The selection of dose levels of 30, 100 and 300mg/m³ THC for this study was based on results from a range finding study.</p> <p><u>Range finding study:</u> 5 male and 5 timed-pregnant female Wistar rats/group) were exposed to roofing asphalt fume condensate (RAFC) 6hr/day, 7 days/week over 21 days at target concentrations of 100, 300 or 1000mg/m³. Body weights and weight gain and food consumption were reduced at 300 and 1000mg/m³. Impairment of blood formation was seen bone marrow of animals at 1000mg/m³. A decrease in fetal body weight was seen at 1000mg/m³.</p> <p><u>Exposure:</u> The rats were exposed to asphalt fumes in a direct flow nose only inhalation exposure system. In this system the fume was supplied to each animal individually, and exhaled air was exhausted immediately. The rats were placed around the exposure cylinder in tapered acrylic glass tubes with adjustable backstops. The different exposure units were placed under separate laboratory hoods to avoid cross-contamination. The roofing asphalt fume condensate was pumped via a stainless steel tube directly into the pneumatic dispersion nozzle. This nozzle was operated with heated nitrogen (160°C) at a flow rate of 5 l/min and generated droplets with a mean diameter of about 6µm. The droplets were fed directly into a tube heated at approximately 220 °C, where they evaporated. The vapor was then issued through the nozzle and recondensed by mixing with cool air. From the generator, the fume was directed through stainless steel tubes to the inhalation units. Flow resistors controlled the flow of asphalt fume to each inhalation unit. The flow rate through these resistors was maintained by keeping a constant pressure difference between the inhalation units and the generator controlling the flow rate of the cooling air in the generator. The final concentrations were achieved by mixing the asphalt</p> |

fume with dilution air, regulated by mass flow controllers. The whole inhalation system was microprocessor controlled and supervised by a computer. Fume concentration was determined by sampling from each nose-only unit (2 samples/week) using a combination of glass fiber filter and XAD absorption tube. Samples were extracted and analyzed separately by IR spectroscopy and reported in mg Total Hydrocarbons (THC/m³) aerosol and vapor phase. UV fluorescence determined as diphenylanthracene equivalents (mg DPA/kg) was measured in all exposure groups once a week to verify uniformity of atmosphere in all chambers. Particle size distribution was also determined using a scanning mobility particle sizer.

Animals: Male and virgin female rats received at 7 weeks of age were acclimated for 3 weeks. During the first week, training began to acclimatize the animals to the exposure tubes for increasing periods of time. Clinical observations were made once daily. Rats were randomized by weight using a computer generated program (PROVANTIS) into each of 4 groups. Male groups were evaluated for homogeneity of mean body weights and variances and did not exceed $\pm 20\%$ within or between groups. However, due to an error in the randomization program, females were assigned to groups based on their body weights instead of by randomising them resulting in statistically significant differences in absolute body weights between controls and treated groups at study start. Animals were identified by ear tattoos and cage cards. Animals were housed individually in Makrolon® Type III cages with softwood bedding with food and water *ad libitum* except during exposure. Room temperature was $22 \pm 2^{\circ}\text{C}$ and relative humidity at $55 \pm 15\%$ with 12 hour light-dark cycle and 15 air changes/hour.

Observations: Animals were inspected at least once a day and once a week outside the home cage. Body weights were recorded weekly. Food and water consumption was measured weekly as the difference between the initial and remaining food or water weight. Hematology and clinical chemistry analyses were performed on all rats fasted overnight immediately prior to necropsy after 28 days exposure. Hematology parameters were erythrocyte count, hemoglobin, hematocrit, mean erythrocyte volume, mean erythrocyte hemoglobin mass, mean erythrocyte hemoglobin concentration, total and differential leukocyte count, platelet count, prothrombin time, and thromboplastin time. Clinical chemical parameters were aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase, alkaline phosphatase, sorbitol dehydrogenase, cholinesterase, creatinase, total bilirubin, urea, creatinine, total protein, albumin, cholesterol, glucose, sodium, potassium, calcium, chloride, and inorganic phosphate and triglyceride. Globulin and albumin/globulin ratio were calculated from the albumin and total protein data.

Neurobehavioral Evaluations:

Spontaneous locomotor activity over 90 minutes using the "Actimot" computerized light-beam system (TSE, Homburg/Ts., Germany) was determined around day 28 in all subchronic animals. Data were analyzed in 15 minutes intervals. In addition, the total values for distance, time in rest, time in movement, rearing time, and number of rearings were determined.

A functional observational battery (FOB) was performed around day 28 in all subchronic animals. Endpoints were righting reflex, body temperature, salivation, startle response, respiration, urination, mouth breathing, convulsions, pineal response, piloerection, diarrhea, pupil response, lacrimation, impaired gait, stereotypy, toe pinch, tail pinch, wire maneuver, hind leg splay, tremors, extensor thrust, positive geotropism, activity and limb rotation, and determination of forelimb grip strength.

Necropsy and Pathology: Animals were sacrificed by CO₂ overdose and exsanguinations. Animals were examined macroscopically and the following organs were weighed: lung (including 2/3 of trachea, heart, liver, kidney, brain, spleen, adrenals, testes, epididymides, thymus, ovaries and uterus. Forty-nine tissues [brain, pituitary, tongue, eyes, lacrimal glands, nasal and paranasal cavities, larynx, pharynx, trachea, thyroid, parathyroids, lungs, thymus, heart, aorta, lung associated lymph nodes, salivary glands, mandibular lymph nodes, liver, pancreas, spleen, kidneys, adrenals, esophagus, forestomach, glandular stomach, duodenum, jejunum, ileum, caecum, colon, rectum, mesenterium and lymph nodes, urinary bladder, testes (fixation in modified Davidson's fluid), one epididymis (fixation in modified Davidson's fluid), prostate, seminal vesicles, ovaries with oviduct (fixation in modified Davidson's fluid), uterus, vagina, mammary glands, skeletal muscle, one femur including joint, vertebrae with spinal cord, skin, peripheral nerve, sternum with bone marrow] and gross lesions were fixed in formalin. Tissues from control and high dose rats were processed for microscopic examination and staging of seminal epithelium was performed in testes. Respiratory tract of mid and low dose groups were also evaluated.

Spermatology: One epididymis /male was used for sperm collection. The number of cauda epididymal sperm reserves was counted and morphological evaluation of sperm performed. Epididymal sperm motility was determined immediately after sacrifice.

Statistical analysis: Body weight, food and water consumption, organ weight data analyzed by Analysis of Variance (ANOVA) followed by Dunnett's modification if necessary. Kruskal Wallis ANOVA and Mann Whitney U-test were used for non-homogeneous data. Qualitative data was analyzed by 2-tailed Fisher test with Bonferroni correction or Chi square test.

Test Results – Repeated-Dose Toxicity

| Concentration (LOAEL/LOAEC/NOAEL/NOAEC): | LOAEL/LOAEC/NOAEL/NOAEC | Population | Value Description | Value/Lower Concentration | Upper Concentration | Units |
|--|-------------------------|------------|-------------------|---------------------------|---------------------|-------------------|
| | LOAEL | males | = | 297.3 | | mg/m ³ |
| | NOAEL | males | = | 100.1 | | mg/m ³ |
| | LOAEL | females | = | 100.1 | | mg/m ³ |
| | NOAEL | females | = | 30.0 | | mg/m ³ |

Results Remarks:

Average chamber concentrations measured continuously by aerosol photometers were 30.0, 100.1 and 297.3mg/m³ THC. UV fluorescence as DPA equivalent was 251, 260 and 252mg/kg for low, mid and high dose groups respectively, indicating uniform composition in all exposure chambers. All animals survived exposure. No adverse clinical signs were observed. Body weight gain and food consumption were significantly reduced in 297.3mg/m³ males. No test material-related effects were seen in body weight gain or food consumption in females. Statistically significant differences from controls in absolute body weight of females were due to an error in the randomization program. Water consumption was unaffected in males and females of all groups compared to controls. No treatment-related adverse effects were reported for haematology or clinical chemistry endpoints. Sporadic statistically significant differences were incidental and not considered toxicologically relevant.

Necropsy/Organ weights: No adverse effects were seen in gross pathology. Absolute and relative lung weights were increased in 297.3mg/m³ males. Absolute lung weights were statistically significantly increased in all subchronic females (p 0.05 at low dose, p 0.01 at mid and high doses) and mid and high dose breeding females [see reproductive and developmental robust summaries]. Relative lung weights were statistically significantly increased in mid and high dose subchronic females (p 0.01) and in high dose breeding females. Absolute liver weights demonstrated dose-dependent increases in all subchronic females with statistical significance at 297.3mg/m³ but no statistically significant relative liver weight increases were seen.

Pathology: In the nasal cavity, a statistically significant decrease of inflammatory cell infiltration was observed in the 297.3mg/m³ THC dose group as compared to the control group. This observation was unexpected and is also in contrast to results of the dose range finding study with RAFC where inflammatory changes were observed in all groups but more frequently in the 300 and 1000 mg/m³ THC dose groups than in the respective control groups. It is also in contrast to the breeding females of the present study (nasal cavities of groups 1 and 4 examined), where a single control animal but 7/12 rats of the 297.3mg/m³ THC high dose group showed (multi)focal very slight to slight mucosal mononuclear/inflammatory

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| | <p>cell infiltration of the nasal cavity. The reason for this marked discrepancy between both subsets of females is presently unknown. Minimal adverse effects of the 297.3mg/m³ THC exposure occurred in the lungs, where a slight increase of alveolar macrophage accumulation was observed in combination with minimal mononuclear/ inflammatory cell infiltration and minimal to slight (adaptive) alveolar hyperplasia of the bronchiolar type (alveolar bronchiolization). This type of hyperplasia is considered to be non-preneoplastic and is interpreted as an attempt to facilitate a more efficient removal of inhaled materials via the mucociliary escalator by extension of bronchiolar epithelium into the alveolar ducts.</p> <p>In all other organs, including larynx and trachea, no effects of RAFC exposure were observed.</p> <p><u>Spermatology:</u> No statistically significant effect was observed on sperm count, sperm motility and percent abnormal sperm, although there is a statistically non-significant but dose dependent trend towards a decrease in sperm number in all exposed groups: control 38.125, low 32.250, mid 27.125, high 24.542 million sperm/ epididymis. However all values are within historical control data of this laboratory with a minimum of 16.4 million sperm per epididymis.</p> <p><u>Neurobehavioral Toxicology:</u> No adverse effects were seen on locomotor activity or any parameter in the functional observational battery.</p> |
| <p>Conclusion:</p> | <p>Male LOAEL=297.3mg/m³ is based on decreased body weight gain, food consumption and increased absolute and relative lung weight correlated with slight respiratory system changes primarily adaptive in nature. Male NOAEL=100.1mg/m³.</p> <p>Female LOAEL=100.1mg/m³ is based on statistically significant increase in relative lung weight at 100 and 300mg/m³ correlated with slight histopathologic effects in the lungs at 297.3mg/m³ primarily adaptive in nature. Errors in the randomization program for female initial body weight resulting in statistically significant increases in absolute body weight and some organ weights make it unreliable to use absolute weights to determine LOAEL/NOAEL values. Changes in body weight gain and organ weights relative to body weight were used for this purpose. Female NOAEL = 30.0 mg/m³</p> |
| <p>Reliability/Data Quality – Repeated-Dose Toxicity</p> | |
| <p>Reliability:</p> | <p>2. Reliable with restriction.</p> |
| <p>Reliability Remarks:</p> | <p>Error in randomization of females by group at study onset.</p> |
| <p>Key Study Sponsor Indicator:</p> | <p>Key study The reproductive/developmental toxicity segment of this study and the Micronucleus segment (OECD 474) are described in separate Robust Summaries.</p> |

Reference – Repeated-Dose Toxicity

Reference:

Fraunhofer Institute of Toxicology and Experimental Medicine [ITEM]. 2009. Combined Repeated Dose Toxicity Study with Reproductive/Developmental Screening Test and Mammalian Erythrocyte Micronucleus Test via Inhalation with Roofing Asphalt Fume Condensate. Fraunhofer ITEM Study # 02G08016. Hannover, Germany Sponsored by Petroleum HPV Testing Group, API, Washington, DC.

Fraunhofer ITEM. 2008. Chamber Trials for the Combined Repeated Dose Toxicity Study with Reproductive/Developmental Screening Test and Mammalian Erythrocyte Micronucleus Test via Inhalation with Roofing Asphalt Fume Condensate. Fraunhofer ITEM Study # 02N07532 Hannover, Germany. Sponsored by Petroleum HPV Testing Group, API, Washington, DC.



High Production Volume Information System (HPVIS)

| Reproductive Toxicity | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|--|--|-------------------|-------------------|-------------------|-------------|-------|-------|----------------|----|----|--------------|------|------|----------|-------|-------|--------------|-------|-------|------------|------|------|--------------|------|------|--------|------|------|---------------------|------|------|--------------|------|------|----------|------|------|------------------------|------|------|------------------------|------|------|------------------|------|------|------------------|------|------|--------------------------|------|------|------------------------|------|------|----------------------|------|------|
| Test Substance | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Category Chemical: (CAS#) | 64742-93-4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Test Substance: (CAS#) | 64742-93-4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Test Substance Purity/Composition and Other Test Substance Comments: | Roofing asphalt fume condensate (RAFC) prepared by Heritage Research Group. d=0.8745g/ml, Refractive Index 1.4831 at 25°C, Kinematic viscosity 8.3616 cst at 100°F. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Concentration of PAH in RAFC | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | <table border="1"> <thead> <tr> <th>Compound</th> <th>Analysis A [µg/g]</th> <th>Analysis B [µg/g]</th> </tr> </thead> <tbody> <tr> <td>Naphthalene</td> <td>164.0</td> <td>165.0</td> </tr> <tr> <td>Acenaphthylene</td> <td>*)</td> <td>*)</td> </tr> <tr> <td>Acenaphthene</td> <td>29.2</td> <td>28.8</td> </tr> <tr> <td>Fluorene</td> <td>254.0</td> <td>253.0</td> </tr> <tr> <td>Phenanthrene</td> <td>248.0</td> <td>250.0</td> </tr> <tr> <td>Anthracene</td> <td>31.9</td> <td>32.0</td> </tr> <tr> <td>Fluoranthene</td> <td>10.5</td> <td>9.97</td> </tr> <tr> <td>Pyrene</td> <td>48.8</td> <td>47.7</td> </tr> <tr> <td>Benz (a) anthracene</td> <td>7.81</td> <td>7.99</td> </tr> <tr> <td>Triphenylene</td> <td>20.7</td> <td>21.6</td> </tr> <tr> <td>Chrysene</td> <td>19.8</td> <td>19.8</td> </tr> <tr> <td>Benzo (b) fluoranthene</td> <td>4.80</td> <td>4.70</td> </tr> <tr> <td>Benzo (k) fluoranthene</td> <td>1.72</td> <td>1.15</td> </tr> <tr> <td>Benzo (e) pyrene</td> <td>7.72</td> <td>7.73</td> </tr> <tr> <td>Benzo (a) pyrene</td> <td>4.09</td> <td>4.10</td> </tr> <tr> <td>Indeno (1,2,3-cd) pyrene</td> <td>0.70</td> <td>0.60</td> </tr> <tr> <td>Dibenz (ah) anthracene</td> <td>1.41</td> <td>1.37</td> </tr> <tr> <td>Benzo (ghi) perylene</td> <td>2.93</td> <td>2.76</td> </tr> </tbody> </table> | Compound | Analysis A [µg/g] | Analysis B [µg/g] | Naphthalene | 164.0 | 165.0 | Acenaphthylene | *) | *) | Acenaphthene | 29.2 | 28.8 | Fluorene | 254.0 | 253.0 | Phenanthrene | 248.0 | 250.0 | Anthracene | 31.9 | 32.0 | Fluoranthene | 10.5 | 9.97 | Pyrene | 48.8 | 47.7 | Benz (a) anthracene | 7.81 | 7.99 | Triphenylene | 20.7 | 21.6 | Chrysene | 19.8 | 19.8 | Benzo (b) fluoranthene | 4.80 | 4.70 | Benzo (k) fluoranthene | 1.72 | 1.15 | Benzo (e) pyrene | 7.72 | 7.73 | Benzo (a) pyrene | 4.09 | 4.10 | Indeno (1,2,3-cd) pyrene | 0.70 | 0.60 | Dibenz (ah) anthracene | 1.41 | 1.37 | Benzo (ghi) perylene | 2.93 | 2.76 |
| | Compound | Analysis A [µg/g] | Analysis B [µg/g] | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Naphthalene | 164.0 | 165.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Acenaphthylene | *) | *) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Acenaphthene | 29.2 | 28.8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Fluorene | 254.0 | 253.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Phenanthrene | 248.0 | 250.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Anthracene | 31.9 | 32.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Fluoranthene | 10.5 | 9.97 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Pyrene | 48.8 | 47.7 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Benz (a) anthracene | 7.81 | 7.99 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Triphenylene | 20.7 | 21.6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Chrysene | 19.8 | 19.8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (b) fluoranthene | 4.80 | 4.70 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (k) fluoranthene | 1.72 | 1.15 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (e) pyrene | 7.72 | 7.73 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (a) pyrene | 4.09 | 4.10 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Indeno (1,2,3-cd) pyrene | 0.70 | 0.60 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Dibenz (ah) anthracene | 1.41 | 1.37 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Benzo (ghi) perylene | 2.93 | 2.76 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | *) Not determined due to overlapping signals. From Fraunhofer ITEM Rpt 02N07532 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Category Chemical Result Type: | Measured | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Method | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Route of Administration: | Inhalation | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Type of Exposure: | Nose only | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Species: | Rat | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

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|---|--|
| Mammalian Strain: | Wistar (Cr1:WU) |
| Gender: | Male and female |
| Number of Animals per Dose: | 12 males/ 12 satellite breeding females/group |
| Dose: | Target: 0, 30, 100, 300mg/m ³ total hydrocarbons (THC), Actual: 0, 30.0, 100.1, 297.3mg/m ³ THC measured continuously during the entire exposure period by aerosol photometers. |
| Year Study Performed: | 2008 |
| Method/Guideline Followed: | OECD 422 [Reproductive Toxicity Screening segment of study described in the Repeated Dose Toxicity section] |
| GLP: | Yes |
| Exposure Period: | 28 days for subchronic males; approximately 35-48 days for pregnant satellite females based on 14 days pre-mating, up to 14 days mating and Gestation days 0-20;and 54 days [26 days after end of cohabitation] for females with no evidence of copulation |
| Frequency of Treatment: | 6 hours/day. 7 days/week |
| Post-Exposure Period: | Lactation days 0-4 for dams with litters only. |
| Method/Guideline and Test Condition Remarks: | <p>The selection of dose levels of 30, 100 and 300mg/m³ THC for this study was based on results from a range finding study [See robust summary for Repeated dose toxicity for details]. Body weights and weight gain and food consumption were reduced at 300 and 1000mg/m³. A decrease in fetal body weight was seen at 1000mg/m³.</p> <p><u>Exposure:</u> The rats were exposed to asphalt fumes in a direct flow nose only inhalation exposure system. In this system the fume was supplied to each animal individually, and exhaled air was exhausted immediately. The rats were placed around the exposure cylinder in tapered acrylic glass tubes with adjustable backstops. The different exposure units were placed under separate laboratory hoods to avoid cross-contamination. The roofing asphalt fume condensate was pumped via a stainless steel tube directly into the pneumatic dispersion nozzle. This nozzle was operated with heated nitrogen (160°C) at a flow rate of 5 l/min and generated droplets with a mean diameter of about 6µm. The droplets were fed directly into a tube heated at approximately 220 °C, where they evaporated. The vapor was then issued through the nozzle and recondensed by mixing with cool air. From the generator, the fume was directed through stainless steel tubes to the inhalation units. Flow resistors controlled the flow of asphalt fume to each inhalation unit. The flow rate through these resistors was maintained by keeping a constant pressure difference between the inhalation units and the generator controlling the flow rate of the cooling air in the generator. The final concentrations were achieved by mixing the asphalt fume with dilution air, regulated by mass flow controllers. The whole inhalation system was microprocessor controlled and supervised by a computer. Fume concentration was determined by sampling from each</p> |

nose-only unit (2 samples/week) using a combination of glass fiber filter and XAD absorption tube. Samples were extracted and analyzed separately by IR spectroscopy and reported in mg Total Hydrocarbons (THC/m³) aerosol and vapor phase. UV fluorescence determined as diphenylanthracene equivalents (mg DPA/kg) was measured in all exposure groups once a week to verify uniformity of atmosphere in all chambers. Particle size distribution was also determined using a scanning mobility particle sizer.

Animals: Male and virgin female rats received at 7 weeks of age were acclimated for 3 weeks. During the first week, training began to acclimatize the animals to the exposure tubes for increasing periods of time. Clinical observations were made once daily. Rats were randomized by weight using a computer generated program (PROVANTIS) into each of 4 groups. Male groups were evaluated for homogeneity of mean body weights and variances and did not exceed $\pm 20\%$ within or between groups. However, due to an error in the randomization program, females were assigned to groups based on their body weights instead of by randomising them resulting in statistically significant differences in absolute body weights between controls and treated groups at study start. Animals were identified by ear tattoos and cage cards. Animals were housed individually in Makrolon® Type III cages with softwood bedding with food and water *ad libitum* except during exposure. Room temperature was $22 \pm 2^{\circ}\text{C}$ and relative humidity at $55 \pm 15\%$ with 12 hour light-dark cycle and 15 air changes/hour.

Groups of 12 young, adult, male Crl:WU rats were exposed to atmospheres containing 0, 30, 100, or 300mg/m³ of RAFC for 28 days. Satellite groups of 12 young, nulliparous, non-pregnant female rats were exposed to 0, 30, 100, or 300mg/m³ during a pre-mating period of approximately 2 weeks, a cohabitation period of up to 2 weeks, and a gestation period of approximately 3 weeks. Following the 2 week pre-mating period, each satellite female was paired with a male of the same respective dosage group during an approximately 2 week cohabitation period. Presumed pregnant females were exposed from gestation day [GD] 0-20 but were not exposed after gestation day 20, or during the approximately 4-day lactation period [LD]. Females without evidence of mating continued to be exposed for 26 days after the end of the cohabitation period.

Body weights, clinical signs, and food consumption were recorded throughout the study. Body weight data were collected weekly for males, and satellite females without evidence of copulation. Satellite females were weighed weekly during pre-mating and cohabitation, on GD0, 7, 14, 21 and on LD0 and 4. Food consumption data were collected at the same intervals for presumed pregnant females. Males were sacrificed after 28 days of exposure, organs (liver, kidneys, lungs, adrenal glands, thymus, brain, spleen, heart, testes with epididymides, were weighed, and 49 tissues were fixed in formalin. Histopathology was performed on tissues of all control and high dose animals

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| | <p>and on respiratory tissues of mid and low dose rats. On postpartum day 4, lactating females and offspring were sacrificed. Organs (liver, kidneys, lungs, adrenal glands, thymus brain, spleen, heart, ovaries and uterus) were weighed. In addition to standard histopathological evaluations, the number of implantation sites [using ammonium sulphide staining] and corpora lutea were counted. Offspring were sexed, weighted and evaluated for external abnormalities.</p> <p><u>Spermatology</u>: One epididymis /male was used for sperm collection. The number of cauda epididymal sperm reserves was counted and morphological evaluation of sperm performed. Epididymal sperm motility was determined immediately after sacrifice.</p> <p><u>Statistical analysis</u>: Body weight, food and water consumption, organ weight data analyzed by Analysis of Variance (ANOVA) followed by Dunnett's modification if necessary. Kruskal Wallis ANOVA and Mann Whitney U-test were used for non-homogeneous data. Qualitative data was analyzed by 2-tailed Fisher test with Bonferroni correction or Chi square test.</p> |
| Pre-Mating Exposure / Males: | 14 days |
| Pre-Mating Exposure / Females: | 14 days |

Test Results

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

| Type | Population | Value Description | Value/Lower Concentration | Upper Concentration | Units |
|-------|----------------------|-------------------|---------------------------|---------------------|-------------------|
| NOAEL | Reproduction | ≥ | 297.3 | | mg/m ³ |
| LOAEL | Systemic maternal | = | 100.1 | | mg/m ³ |
| NOAEL | Parental maternal | = | 30.0 | | mg/m ³ |
| NOAEL | Parental male-Repro | = | 297.3 | | mg/m ³ |
| NOAEL | Systemic Parent male | = | 100.1 | | mg/m ³ |

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| Results and Remarks: | <p>Details of systemic effects in males are presented in detail in the Repeated Dose Subchronic Toxicity Robust Summary for this OECD 422 study. Body weight gain and food consumption were significantly reduced in 297.3mg/m³ males. Reproductive performance of RAFC exposed males were comparable to controls yielding 10, 10, 11 and 12 sperm positive females and 11, 9, 11, and 11 pregnancies in control, 30, 100.1, and 297.3mg/m³ groups, respectively. All animals mated within the first 8 days. No adverse effects were seen in histopathological evaluation of reproductive organs. No statistically significant effect was observed on sperm count, sperm motility and percent abnormal sperm, although there is a statistically non-significant but dose dependent trend towards a decrease in sperm number in all exposed groups: control 38.125, low 32.250, mid 27.125, high 24.542 million sperm per epididymis. However all values are within historical</p> |
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control data of this laboratory with a minimum of 16.4 million sperm per epididymis. A NOAEL for reproductive performance in males was 297.3mg/m³. The systemic NOAEL of 100.1mg/m³ reported in the Subchronic Toxicity robust summary for males were based on decreased body weight gain, food consumption and increased absolute and relative lung weight correlated with slight respiratory system changes primarily adaptive in nature at 297.3mg/m³.

Mortality did not occur at any exposure concentration. No RAFC-related effects were seen in body weight gain or food consumption in females. Higher body weights of RAFC exposed females compared to controls throughout gestation resulted from a randomization error at initiation of study. Overall gestation body weight gain and food consumption were slightly but not statistically decreased in the high dose group. Absolute lung weights were statistically significantly increased at mid and high doses but relative weights were only statistically significantly increased at 297.3mg/m³. Minimal histopathological effects of the 297.3mg/m³ THC exposure occurred in the lungs, where a slight increase of alveolar macrophage accumulation was observed in combination with minimal mononuclear/ inflammatory cell infiltration and minimal to slight (adaptive) alveolar hyperplasia of the bronchiolar type (alveolar bronchiolization). This type of hyperplasia is considered to be non-preneoplastic. In the nasal cavity, a single control animal but 7/12 rats of the high dose group showed (multi)focal very slight to slight mucosal mononuclear/inflammatory cell infiltration. This effect was not seen in the nasal cavity of subchronic rats.

Reproductive Toxicology: There were no significant RAFC related differences in mean number of pregnant animals, number of animals delivering, mating index, fertility index, gestation length, number of corpora lutea, number of implantation sites or percent of post implantation loss for any exposure group. Mating Indices were 100, 91.7, 100, and 100% and the Female Fertility Indices were 91.7 (11/12 females), 81.8 (9/11 females), 91.7 (11/12 females) and 91.7 (11/12 females) in controls, 30, 100.1 and 297.3mg/m³ groups, respectively. The Fertility Index included 2 control, 1 low dose, 1 mid-dose, and 2 high dose females who were pregnant although no sperm was detected in vaginal smears. One control, 2 low dose, 1 mid-dose and 1 high dose female had sperm in vaginal smears but did not become pregnant. One high dose female (#4207) was killed as non-pregnant but was found to have 12 implantation sites at necropsy. One low dose female did not mate. The number of females completing delivery was 11, 9, 11, and 10 and the Live birth Indices were 99.1, 97.9, 100 and 100% in control, 30, 100.1 and 297.3mg/m³ group respectively. Duration of gestation was approximately 22 days in all groups. Stillborn pups were seen in one control litter [1 pup] and in one low dose litter [2 pups]. One control litter (#1204, 7 pups) was terminated on postpartum day 0 due to lack of maternal

| | <p>care and one pup died between days 1-4. In the 100.1mg/m³ group 3 pups died between postpartum days 1-4. Overall Pup Viability Indices were 92.5, 100. 97.3 and 100% in control, 30, 100.1 and 297.3mg/m³ group respectively. Pup sex ratio, body weights and weight gain from treated litters over postpartum day 0-4 were comparable to controls.</p> <p style="text-align: center;">Summary of Pup data from Postpartum days 0-4</p> <table border="1" data-bbox="529 449 1455 978"> <thead> <tr> <th colspan="2"></th> <th>Control</th> <th>30mg/m³</th> <th>100mg/m³</th> <th>300mg/m³</th> </tr> </thead> <tbody> <tr> <td colspan="2">Pups Day 0 [Liveborn]</td> <td>107</td> <td>93</td> <td>113</td> <td>90</td> </tr> <tr> <td colspan="2">Pups Day 4</td> <td>99</td> <td>93</td> <td>110</td> <td>90</td> </tr> <tr> <td colspan="6" style="text-align: center;">Sex Ratio: Male pups/Total pups</td> </tr> <tr> <td rowspan="2">Day 0</td> <td>N</td> <td>59</td> <td>52</td> <td>49</td> <td>47</td> </tr> <tr> <td>%</td> <td>55.1</td> <td>55.9</td> <td>43.4</td> <td>52.2</td> </tr> <tr> <td rowspan="2">Day 4</td> <td>N</td> <td>54</td> <td>52</td> <td>46</td> <td>47</td> </tr> <tr> <td>%</td> <td>54.5</td> <td>55.9</td> <td>41.8</td> <td>52.2</td> </tr> <tr> <td colspan="6" style="text-align: center;">Pup Weight/Litter [grams]</td> </tr> <tr> <td rowspan="3">Day 0</td> <td>Mean</td> <td>5.8</td> <td>5.8</td> <td>5.6</td> <td>5.7</td> </tr> <tr> <td>S.D.</td> <td>0.67</td> <td>0.45</td> <td>0.37</td> <td>0.52</td> </tr> <tr> <td>N</td> <td>11</td> <td>9</td> <td>11</td> <td>10</td> </tr> <tr> <td rowspan="3">Day 4</td> <td>Mean</td> <td>9.8</td> <td>9.5</td> <td>9.3</td> <td>9.9</td> </tr> <tr> <td>S.D.</td> <td>1.21</td> <td>0.80</td> <td>0.77</td> <td>0.85</td> </tr> <tr> <td>N</td> <td>10</td> <td>9</td> <td>11</td> <td>10</td> </tr> </tbody> </table> <p>At necropsy, average implantation sites per litter were 10.7, 11.1, 11.5 and 9.8 and the average corpora lutea were 11.7, 12.0, 11.5 and 10.9 in control, 30, 100.1 and 297.3mg/m³ group respectively. Values for RAFC groups were comparable to controls for these parameters.</p> | | | Control | 30mg/m ³ | 100mg/m ³ | 300mg/m ³ | Pups Day 0 [Liveborn] | | 107 | 93 | 113 | 90 | Pups Day 4 | | 99 | 93 | 110 | 90 | Sex Ratio: Male pups/Total pups | | | | | | Day 0 | N | 59 | 52 | 49 | 47 | % | 55.1 | 55.9 | 43.4 | 52.2 | Day 4 | N | 54 | 52 | 46 | 47 | % | 54.5 | 55.9 | 41.8 | 52.2 | Pup Weight/Litter [grams] | | | | | | Day 0 | Mean | 5.8 | 5.8 | 5.6 | 5.7 | S.D. | 0.67 | 0.45 | 0.37 | 0.52 | N | 11 | 9 | 11 | 10 | Day 4 | Mean | 9.8 | 9.5 | 9.3 | 9.9 | S.D. | 1.21 | 0.80 | 0.77 | 0.85 | N | 10 | 9 | 11 | 10 |
|--|--|---------|---------------------|----------------------|----------------------|----------------------|----------------------|-----------------------|--|-----|----|-----|----|------------|--|----|----|-----|----|--|--|--|--|--|--|-------|---|----|----|----|----|---|------|------|------|------|-------|---|----|----|----|----|---|------|------|------|------|----------------------------------|--|--|--|--|--|-------|------|-----|-----|-----|-----|------|------|------|------|------|---|----|---|----|----|-------|------|-----|-----|-----|-----|------|------|------|------|------|---|----|---|----|----|
| | | Control | 30mg/m ³ | 100mg/m ³ | 300mg/m ³ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pups Day 0 [Liveborn] | | 107 | 93 | 113 | 90 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pups Day 4 | | 99 | 93 | 110 | 90 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Sex Ratio: Male pups/Total pups | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Day 0 | N | 59 | 52 | 49 | 47 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | % | 55.1 | 55.9 | 43.4 | 52.2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Day 4 | N | 54 | 52 | 46 | 47 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | % | 54.5 | 55.9 | 41.8 | 52.2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pup Weight/Litter [grams] | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Day 0 | Mean | 5.8 | 5.8 | 5.6 | 5.7 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | S.D. | 0.67 | 0.45 | 0.37 | 0.52 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | N | 11 | 9 | 11 | 10 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Day 4 | Mean | 9.8 | 9.5 | 9.3 | 9.9 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | S.D. | 1.21 | 0.80 | 0.77 | 0.85 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | N | 10 | 9 | 11 | 10 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Conclusion: | <p>Exposure to RAFC did not cause significant adverse effects in any reproductive parameters [mean number of pregnant animals, number of animals delivering, mating index, fertility index, gestation length, number of corpora lutea, number of implantation sites or percent of post implantation loss] for any exposure group. Pup viability indices, sex ratio, body weight and body weight gain over lactation days 0-4 were comparable to controls. Systemic effects in maternal animals demonstrated that treatment at concentrations up to 297.3mg/m³ were sufficient to induce some level of toxicity, as specified in OECD 422.</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Reliability/Data Quality | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Reliability: | 2. Reliable with restriction | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Reliability Remarks: | Error in randomization of females by group at study onset | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Key Study Sponsor Indicator: | Key study. This is the reproductive toxicity screen segment of OECD 422 described in the Repeated Dose Toxicity Section. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Reference | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Reference: | Fraunhofer Institute of Toxicology and Experimental Medicine [ITEM]. 2009. Combined Repeated Dose Toxicity | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

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| | <p>Study with Reproductive/Developmental Screening Test and Mammalian Erythrocyte Micronucleus Test via Inhalation with Roofing Asphalt Fume Condensate. Fraunhofer ITEM Study # 02G08016. Hannover, Germany Sponsored by Petroleum HPV Testing Group, API, Washington, DC.</p> <p>Fraunhofer ITEM. 2008. Chamber Trials for the Combined Repeated Dose Toxicity Study with Reproductive/Developmental Screening Test and Mammalian Erythrocyte Micronucleus Test via Inhalation with Roofing Asphalt Fume Condensate. Fraunhofer ITEM Study # 02N07532 Hannover, Germany. Sponsored by Petroleum HPV Testing Group, API, Washington, DC.</p> |
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