Bitumen fumes: review of work on the potential risk to workers and the present knowledge on its origin

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Received 11 December 2001; accepted 26 June 2002

Abstract

Bitumens fumes contain polycyclic aromatic compounds (PAC). There is a possibility of long-term health effects following chronic exposure by inhalation or skin contamination in asphalt road pavers and highway maintenance workers. Epidemiological and experimental studies on this topic are reviewed and the possible causes of cancer discussed with a primary focus on heterocyclic polyaromatic compounds. In 2001, the results of the IARC epidemiological study confirmed an excess of lung cancer despite a lower cancer mortality. In vitro genotoxicity and mechanistic studies demonstrated a mutagenic effect of bitumen fume condensates (BFC) and some data suggested that the polycyclic aromatic hydrocarbons (PAH) analysed were not the major genotoxic compounds in bitumen fume condensates. Other compounds such as nitrogen-, sulfur- and/or oxygen-containing PAH or their alkyl substituted analogues, mutagenic in the Ames mutation assay, may be involved in the genotoxic effect of BFC. After skin painting with BFC, DNA adducts were found in skin, lung and lymphocytes of all the treated animals. Differences in the adduct patterns were also observed, but a more polar adduct was common to the three tissues and not observed in those from rats treated with coal-tar fume condensates (CTFC). Rat inhalation experiments with bitumen fumes confirmed the presence of a DNA-adduct in the lungs with the same Rf as the previous polar adduct. This adduct therefore merits further investigation as a potential biomarker in lymphocyte DNA to follow exposed workers. All the analytical data and the mechanistic data are complementary and suggest the potential role of thiophenes in the genotoxicity of bitumen fumes. Some thiophenes have lower mutagenic activity than their isosteric PAH, whereas others are very potent carcinogens. Generally, the sulfur analogues of PAH (SPAH) in bitumen fumes have a higher concentration than the PAH of similar molecular weight, whereas the SPAH in coal-tar fumes have a much lower concentration than the corresponding PAH. This may explain why the more polar adducts have been detected only in animals exposed to bitumen fume. In a skin carcinogenicity study of condensed asphalt roofing fumes, it has been demonstrated that the most active fractions were those containing a variety of aromatic SPAH. In conclusion to this review, there is an interest in determining the chemical identity of the major DNA adducts induced by BFC. This would allow experimental studies on the carcinogenic potency of these compounds and their validation as potential...
1. Introduction

Bitumens (asphalt in the USA) contain polycyclic aromatic compounds some of which are present in the fumes emitted when handling hot products containing bitumen during, for example, road paving or roofing. Although the exposure levels of workers are generally low, there is a possibility of long-term health effects following chronic exposure by inhalation or skin contamination. The relative risks of cancers in asphalt road pavers and highway maintenance workers is presented and the possible causes discussed.

2. Epidemiology

The data concerning the carcinogenic risk of coal-tar products were reviewed in 1984 by the IARC working group for the evaluation of carcinogenic risk of chemicals to humans (IARC, 1985). The group concluded: ‘Taken together, the data indicate that coal-tars and coal-tar pitches are causally associated with cancer in humans and that creosotes derived from coal-tars are probably carcinogenic in humans’.

The same working group also evaluated the data concerning bitumens and, on the basis of the information available on cancer in experimental animals, concluded that the evidence of carcinogenicity in experimental animals was: (a) sufficient for extracts of steam-refined and air-refined bitumens and mixtures of the two, (b) limited for undiluted steam-refined bitumens and for cracking residue bitumens; and, (c) inadequate for undiluted air-refined bitumens. No epidemiological studies of workers exposed solely to bitumens being available, the overall evaluation was: inadequate evidence that bitumens alone are carcinogenic to humans. No evaluation was made by the working group of the carcinogenic risk of exposure to bitumen fumes.

Coal-tar and coal-tar pitches were classified in group 1, human carcinogens in 1987 (IARC, 1987) and, in view of the paucity of new data, the working group classified bitumens in group 3 (compounds not classifiable as to their carcinogenicity to humans), and extracts of steam-refined and air-refined bitumens in group 2B (possible human carcinogens).

In 1989, Hansen published the results of two studies (Hansen, 1989a,b). The first, on cancer mortality in the asphalt industry, showed an increased incidence of cancer mortality in asphalt workers aged 45 or more and who had 5 years or more latency from enrolment. The second, a mortality study of Danish mastic asphalt workers, concluded that the incidence of cancer observed in the group significantly exceeded that of the total Danish male population (SMR 195 with an extremely large excess for the mouth, oesophagus, rectum and lung). In 1991, the same author also reported a significant increase for lung cancer, non-pulmonary cancer and liver cirrhosis among people between 40 to 89 years of age; bronchitis, emphysema and asthma also occurred in excess (Hansen, 1991). These two publications have been the subject of serious discussions in the scientific literature (Wong et al., 1992; Hansen, 1992). Partanen and Boffetta (1994) examined the 20 epidemiological studies describing cancer risks in asphalt workers and roofers in various countries. For roofers, they concluded that there were indications of an increased risk of lung, stomach and non-melanoma skin cancers, and of leukaemia, although some of the excess might be attributable to polycyclic aromatic hydrocarbons (PAH) from coal-tar products. The relative risks of the same cancers in road pavers and highway maintenance workers were lower than those in roofers.

The industry also criticised the results on the basis that the workers had also been exposed to
Table 1
Mutagenic capacity of coal-tar and bitumen fume condensates on *Salmonella typhimurium* strains (revertants/µl) (from De Méo et al., 1996)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>CTFC</th>
<th>BFC from bitumen 45/60 pen</th>
<th>BFC from bitumen 160/210 pen</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA98</td>
<td>3632</td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td>TA100</td>
<td>46450</td>
<td>7592</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>110</td>
<td>95</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>115</td>
<td>115</td>
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<tr>
<td></td>
<td>160</td>
<td>2296</td>
<td>2296</td>
</tr>
</tbody>
</table>

Coal-tar products and questioned the International Agency for Research on Cancer on the feasibility of an epidemiological study of workers exposed solely to asphalt fumes. Partanen et al. (1995) assessed the possibility of an European epidemiological study of cancer risk among asphalt workers. In 1997, Boffetta et al. stressed in an article on cancer risk from occupational exposure to polycyclic aromatic hydrocarbons that in this field ‘The most important current research question is the identification and the quantification of the risk—mainly lung cancer—from mixtures with relatively low PAH content, such as bitumen fumes and diesel engines. In these instances, the available epidemiological evidence is not able to identify with certainty, nor to quantify, a carcinogenic risk which in any case is expected to be rather low’. In its recent review of the subject, NIOSH (2001) concluded ‘Overall, the epidemiological evidence for an association between lung cancer and exposure to asphalt in paving is inconclusive at this time’ and ‘Although strong epidemiological evidence exists of an association between lung cancer and working as a roofer, it is uncertain whether exposure to asphalt is related to this association’.

The results of the most recent IARC epidemiological study have just been published (IARC, 2001). The authors’ evaluation confirms an excess of lung cancer despite a lower cancer mortality. Their statement is ‘Overall, mortality was decreased (SMR 0.91, 95% CI 0.86–0.96), as was cancer mortality (SMR 0.87, 95% CI 0.78–0.96). While there was no excess of mortality from oral and pharyngeal cancer, lung cancer mortality was significantly increased (SMR 1.23, 95% CI 1.02–1.48). The increased mortality from lung cancer was mainly apparent in the Netherlands, Germany and France, although in none of these countries the increase was statistically significant. The association between mortality from lung cancer and exposure to bitumen fumes was further assessed in the inception cohort, that is, among workers first employed in the companies included in the study after the beginning of the period of enumeration of the cohort’.

3. Genotoxicity and carcinogenicity of bitumen fumes,

Few in vitro studies have been carried out concerning the evaluation of the genotoxic potency of bitumens or bitumen fumes. The study of the mutagenic potency of road tar extracts in DMSO after elimination of acids, alkali and phenols, and of solutions of volatiles generated in the laboratory by heating these compounds at three temperatures (290 °C, 180 °C, 120 °C) demonstrated a weak mutagenic effect in the presence of a metabolic activation system (Penalva et al., 1983). Blackburn et al. (1986) modified the Ames *Salmonella* microsome assay to improve its sensitivity and reproducibility with complex mixtures. On applying this test to DMSO extracts of whole asphalts (roofing and paving bitumen), marginally positive responses were measured (Blackburn and Kriech, 1990). However, in a similar study, Monarca et al. (1987) demonstrated that the samples contained low levels of PAH and were not mutagenic. Similar data had already been obtained by Tamakawa et al. (1983) when testing extracts of asphalt tar with a modified Ames test with pre-incubation, and by Robinson et al. (1984) who tested a series of petroleum asphalt paints. In a recent study (McGowan et al., 1992, cited in CONCAWE, 1992) tested penetration bitumens in a modified Ames
test and found them negative. No mutagenicity study has been carried out on bitumen fumes.

Using the $^{32}$P-postlabelling method for the detection of DNA adducts, Schoket et al. (1988a,b) reported an accumulation of DNA adducts in the skin and lungs of mice treated with solutions of bitumen in tetrahydrofuran and in mouse skin and human skin explants maintained in short-term culture, thus demonstrating the potential hazard to humans of epidermal contact with these compounds (Phillips et al., 1990). In a recent study by Qian et al. (1998), three rats received three intratracheal instillations of bitumen fume condensates which were mimics of those produced by Sivak et al. (1997) in a 24-h period with rather high cumulative doses which evoked lung DNA adducts only five to 10 times higher than the solvent controls, and no adducts in white blood cells.

In a comparative carcinogenicity study of petroleum roofing bitumen and coal-tar fumes Hueper and Payne (1960) obtained negative results with the bitumen fumes. However, positive results were obtained by Simmers (1964), one mouse in 20 exposed to bitumen fumes developing a lung adenoma.

When evaluating the mutagenicity data CON-CAWE (1992) concluded: ‘in most mutagenicity assays conducted, bitumens have given negative or marginally positive findings. Although there is some evidence that high-temperature bitumen fumes may have moderate mutagenic activity in the modified Ames test, it is questionable whether the fumes generated during normal operations have more than weak activity’. As to the data on adduct formation obtained, they stressed that ‘weak DNA binding has been seen with some bitumen... these studies involved the application of bitumen in a solvent vehicle and hence are subject to criticism... postlabelling studies have not been carried out on bitumen fumes and therefore the DNA adduct forming potential of this material is not known’. For the inhalation studies, CONCAWE concluded ‘Despite the shortcomings of the animal inhalation studies conducted... they do suggest that inhalation of such fumes is unlikely to result in cancer of the respiratory system’.

Recent data from Brandt et al. (1999) on the mutagenicity of bitumen fumes condensates should, however, allow, according to the criteria of Blackburn et al. (1986), their classification as potential carcinogens.

Therefore, a series of studies was recently initiated in which bitumen vapours/particulates were produced in such a way that they were as representative as possible of those produced in the field. Since coal-tar had been classified as a ‘human carcinogen’, coal-tar vapours/particulates were used as a positive control sample. To achieve this objective, fumes were produced according to the method of Brandt et al. (1985) and, Brandt and de Groot (1999). Not only the particulate phase was collected but also the vapour phase and the two were mixed in proportion. The mixtures were called bitumen fume condensates. The biological studies included testing of the mixed vapour/particulates with a modified Ames mutation assay (De Méo et al., 1988) and testing of their ability to form DNA adducts. DNA adduct formation was tested in vitro (De Méo et al., 1996), in vivo by skin application of the undiluted mixture (Genevois et al., 1996), and finally by nose only inhalation of the vapour/particulate phase produced under strictly controlled conditions (Genevois-Charmeau et al., 2001). The mechanism of DNA adducts formation was studied in a separate in vitro experiment with the condensates. (Genevois et al., 1998).

3.1. In vitro genotoxicity studies

The US environmental protection agency (EPA) has published a list of 16 priority PAH to be analysed in water, which is currently used by the asphalt industry to assess the PAH content and the potential toxicity of bitumen fume condensates. The analysis of these PAH in bitumen and coal-tar fume condensates demonstrated that significantly lower levels of all the four- to six-ring (40–200-fold) and three- to seven-ring PAHs (120–550-fold) are found in bitumen fume condensates (BFC) than in coal-tar fume condensates (CTFC) (De Méo et al., 1996). The levels of UV absorbing materials are 3 to 10 times lower in BFC than in CTFC.
No mutagenic activity was detected in any of the samples without metabolic activation. The data from mutagenicity testing of the samples of condensates with metabolic activation are included in Table 1. These data demonstrate a mutagenic effect of all fume condensates prepared at all temperatures.

Typical autoradiograms of DNA adducts obtained by $^3$H-postlabelling demonstrate qualitative differences between adduct patterns obtained with the fume condensates generated from coal-tar and those generated from the two bitumens, thus implying qualitative differences in the nature of the compounds responsible for the formation of these adducts (Reddy and Randerath, 1986). Quantitatively, the adduct levels were four times lower for fumes from bitumen 45/60 pen as compared with those from coal-tar and 1.3 times lower for those from bitumen 160/210 pen.

When comparing the PAH contents data with those of the genotoxicity testing, it was obvious that four- to six-ring and three- to seven-ring PAHs analysed were not the major genotoxic compounds in BFC. It has already been shown that nitrogen, oxygen and sulfur derivatives of PAH as well as alkyl derivatives of PAH are mutagenic in the Ames mutation assay (reviewed in Kier et al., 1986; Jacob, 1990; IARC, 1983). As a somewhat better marker for total polycyclic aromatic compounds (PAC), the total amount of UV-absorbing species eluting up to coronene might be used. Using this marker a fairly linear relation was obtained with DNA adducts analysed for the BFC. It has recently been demonstrated that the total three- to six-ring PAC content, determined by DMSO extraction using flow injection analysis followed by coupled chromatographic analysis with flame ionisation injection detection (FIA-DMSO extraction), correlates well with mutagenicity (Brandt et al., 1999).

Differences in the adduct patterns induced in the various organs of rats treated with a particular bitumen fume condensate can be observed. For example, in the lungs one major adduct is formed (adduct A in Genevois et al., 1996). Although the same adduct is formed in the skin, it constitutes only part of a series of other adducts of comparable intensity in a diagonal zone. However, adduct A
Table 2
Quantitative relative adduct levels from the skin painting study (from Genevois et al., 1996)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>CTFC</th>
<th>BFC from bitumen 45/60 pen</th>
<th>BFC from bitumen 160/210 pen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes*</td>
<td>173.1 ± 36.0</td>
<td>5.5 ± 2.7</td>
<td>56.4 ± 11.6</td>
</tr>
<tr>
<td>Skin*</td>
<td>19.3 ± 10.7</td>
<td>17.2 ± 1.8</td>
<td>27.9 ± 2.8</td>
</tr>
<tr>
<td>Lung*</td>
<td>17.8 ± 6.4</td>
<td>5.5 ± 2.7</td>
<td>15.7 ± 3.3</td>
</tr>
</tbody>
</table>

* Mean ± S.D.

Note: For calculation of the mean adduct levels, the background adduct levels found in the corresponding organs of untreated animals have been subtracted.

is found in the skin, lungs and lymphocytes of rats treated with BFC and not of rats treated with CTFC, and therefore merit further investigation as a potential biomarker in lymphocyte DNA.

The differences in adduct patterns in the different organs can probably be explained by the fact that the genotoxic compounds from bitumen and coal-tar are quickly transferred through the skin and distributed in the various organs where they are metabolised. Each organ has an individual profile of, for example P450 enzymes (Gonzalez, 1992; Nelson et al., 1993), thus resulting in different adduct patterns as has been demonstrated by several groups (Grosse et al., 1995; Warshawsky et al., 1994).

The quantitative results are presented in Table 2 (from Genevois et al., 1996). Since some endogenous adducts were found in some control samples, the maximum RAL value of adducts present in the control animals in the areas where adducts were found after treatment in each organ has been subtracted from the corresponding average adduct level.

These data demonstrate that: (a) statistically higher adduct levels are present in the lymphocytes of rats treated with CTFC than in those treated with BFC, although there is a higher interindividual variability in the lymphocyte DNA adducts, which may reflect the short life of these adducts in the lymphocytes; (b) these differences are not found in the lungs and are preserved only marginally in the skin when comparing CTFC and bitumen 45/60 or 160/210 pen. There is no relationship between the quantitative data on DNA adduct formation in the various organs and the level of specific four- to six-ring or three- to seven-ring PAHs determined in the fume condensates. Overall, these results support the conclusions of the in vitro study on these fume condensates that the unsubstituted (three- to six-ring) PAHs from the EPA list are not the sole components responsible for adduct formation from BFC, but that an important contribution comes from other (hetero and/or alkylsubstituted) PAC. No relationship was found between the DNA adduct levels and the urinary excretion of 1-hydroxypyrene. There was, however, a linear relationship between the level of 1-hydroxypyrene in the urine and the pyrene content of the fume condensates.

3.3. Inhalation studies

A system comprising a fume generator and an inhalation chamber with plethysmographs for nose only inhalation was developed and tested in a series of interlaboratory experiments at different coal-tar and bitumen total particulate matter concentrations. The first series of experiments (Bonnet et al., 2000) on fumes generated from bitumen at approximately 5 mg/m³ total particulate matter (TPM) was devoted to the validation of the inhalation device. The results demonstrated that, at this level, the fume generator delivered a reproducible bitumen fume atmosphere to the inhalation chamber. The particle size distributions were somewhat different from those previously reported for roofing and indoor mastic laying (Brandt et al., 1985); more than 85% of the particles were <1 μm diameter whereas only 40% of the particles were reported to be below this size by Brandt et al. (1985). The PAH profile in the soluble matter of the fumes generated in the Shell small scale rig
ever, found for benzo a pyrene and, in general, the four- to six-ring PAC which constitute the biologically most active fraction (Brandt et al., 1985).

In the third series of experiments (Brandt et al., 2000), fumes were generated from coal-tar at the 5 mg/m³ target TPM (Brandt et al., 2000). One laboratory found much higher TPM and soluble matter levels than the others. This may have been due to the extreme volatility (90% boiling below 300 °C) of the fume components evaporated during transportation to the other laboratories. Close agreement was, however, found for benzo[a]pyrene and, in general, the four- to six-ring PAC which constitute the biologically most active fraction (Brandt et al., 1985).

In the third series of experiments (Brandt et al., 2000), fumes were generated from coal-tar at the 5 mg/m³ target TPM. The resulting BSM concentration was nine times that of the fumes at 5 mg/m³ TPM. However, the PAC profile of the 50 mg/m³ fume was very different from that of the 5 mg/m³ fume and also different from the fumes that workers are exposed to. Although the same molecules are found in both fumes, their proportions were dramatically different. The reason for these quantitative differences was not explained.

When rats were exposed to fumes from bitumen heated to 200 °C at a TPM concentration of 5.4±0.9 mg/m³, no DNA adducts were detected in any of the tissues analysed at the detection limit of =1 adduct per 10⁹ normal nucleotides. In addition, no adduct was detected in the lungs, liver, kidneys and lymphocytes of rats exposed to fumes from coal-tar heated to 110 °C at a TPM concentration of 4.0±1.3 mg/m³. Negative results were also obtained by Hueper and Payne (1960) in a comparative carcinogenicity study of petroleum roofing bitumen and coal-tar. Coal-tar is a known carcinogen by inhalation (Heinrich et al., 1994) which induces DNA adduct formation in rats (Lewtas et al., 1997). Therefore, the absence of adducts in both coal-tar and bitumen inhalation experiments might be due to doses of PAC insufficient to induce the formation of detectable DNA adducts.

A new inhalation study was performed with the same bitumen batch heated to 200 °C, aimed at a TPM concentration in the inhalation chamber approximately 10 times higher (i.e. 45.9±11.8 mg/m³). A DNA-adduct was found by postlabelling analysis (Genevois-Charmeau et al., 2001) in the lungs of the three rats exposed to bitumen fumes at this level. This adduct had the same Rf as the major adduct detected in the lungs during the skin-painting studies with BD 4 and BD 6 rats. The relative adduct labelling (RAL) was 4.3±0.4 adducts per 10⁹ normal nucleotides. When co-chromatographed with the lung DNA adducts from the skin painting study, only one spot was observed with a RAL of 7.9±0.8 adducts per 10⁹ normal nucleotides while the expected calculated value was 8.5±0.5 adducts per 10⁹ normal nucleotides. It is therefore probable that this DNA adduct spot was due to the same compound(s).

Levels of 1-hydroxypyrene ranging from 1.1 to 9.8 μmol/mol of creatinine were detected in the urine of the rats exposed to 50 mg/m³ of bitumen fumes, and were similar to those in the urine of animals exposed to coal-tar fumes (5 mg/m³). Together with the absence of any adduct in the lungs of the rats exposed to coal-tar fumes (5 mg/m³) these results confirmed the conclusion of the skin-painting experiment (Genevois et al., 1996) that 1-hydroxypyrene cannot be used as a biomarker of bitumen fume exposure.

4. In vitro mechanistic study, possible implication of sulfur heterocyclic compounds

The DNA adducts formed by incubation of CTFC or BFC have been investigated with liver microsomes from several type of mice and with yeast microsomes expressing individual human CYP enzymes (Genevois et al., 1998). The overall results demonstrated that: (1) the aryl hydrocarbon receptor (AHR) plays an important role in the biotransformation of BFC and, to a lesser extent, of CTFC; (2) for CTFC, both cytochrome P450 (CYP) 1A isoforms are involved in the formation of genotoxic compounds, and the reactive metabolites formed via CYP 1A1, are substrates for
epoxide hydrolase (mEH); (3) for BFC, the genotoxicity is partially dependent upon CYP 1A1 and the reactive metabolites are not substrates for mEH; (4) CYP 1A isoforms are not exclusively responsible for the genotoxicity of the CTFC and BFC as other CYPs and also enzymes of the [Ah] gene battery may play an important role.

Implication of CYP 3A4 and 2C9 was clearly demonstrated using incubation of BFC with human CYP 3A4 or 2C9, both of which induced a very high level of DNA adducts. The nitrogen-containing dibenzacridine is metabolised to 3,4-dihydrodiol-dibenzacridine by CYP 3A4 in addition to CYP 1A2 and 1A1 (Roberts-Thomson et al., 1995; Warshawsky et al., 1996). It has also been reported that BFCs contain SPAH (Table 3). Little is known about the oxidative metabolism of SPAH compounds. Benzo[b]naphtho[2,1]thiophene, a sulfur analogue of chrysene also known to be present in several coal products, is metabolised primarily to a sulfoxide and sulfone (Jacob et al., 1986). Moreover, it can be transformed into diol-epoxides, which are highly mutagenic (Misra and Amin, 1990). Chryseno[4,5-bcd]thiophene is one of the SPAH which are potential environmental contaminants found in fossil fuels. This SPAH is less mutagenic in the Ames test than benzo[a]pyrene (B[a]P), but exhibits chromosomal aberration activity in vivo in bone marrow cells of mice equivalent to that of B[a]P. Its genotoxicity increases after conversion into diol-epoxide (Sinsheimer et al., 1992). The predominant route of biotransformation is metabolic oxidation of the sulfur atom leading to a sulfone, which is less mutagenic. However, this involves an initial activation step into a reactive sulfoxide (Valadon et al., 1996). According to Dansette et al. (1991) and Lopez-Garcia et al. (1993), human CYP 2C9 catalyses the hydroxylation of tienilic acid to thiophene sulfoxide, an electrophilic metabolite responsible for covalent binding with proteins (Lopez-Garcia et al., 1994). Some SPAH can be bioactivated to DNA adduct-forming species (Devanaboyina et al., 1993; King et al., 2001) and, for example, Ashby et al. (1993) have demonstrated that 6,11-dimethylbenzo[b]naphtho[2,3-d]thiophene (S-DMBA) binds covalently to mouse skin DNA. The adducts formed by S-DMBA may be more mutagenic than those from the mouse-skin carcinogen 7,12-dimethylbenz[a]anthracene (DMBA), as on an equivalent dose basis S-DMBA produces approximately 1/60th the level of DNA adducts and approximately 1/10th the level of DNA mutations in mouse skin than does DMBA (Ashby et al., 1993). In the case of BFC, the B[a]P induction of CYP 2C9, independently of AHR, could increase the formation of thiophene sulfoxides which may bind to DNA.

Hence, there is some possibility that the bitumen adducts, more polar than those from coal-tar, are derived from oxidation of SPAH compounds (Genevois et al., 1998). In their skin carcinogenicity study of condensed asphalt roofing fumes, Sivak et al. (1997) demonstrated that the most active fractions were those containing a variety of aromatic SPAH. Carmichael et al. (1992) suggested that benzo[a]naphtho[2,1-d]thiophene was a possible source of an adduct.

Since the review by Jacob (1990) on the biological activity of SPAH very little has been published on these compounds. Several of these compounds are mutagenic in Salmonella typhimurium strains TA 98 and TA 100. In this review, it is stated that ‘the carcinogenic potency of crude oil fractions may depend on their sulphur content. Desulfuration of such fractions decreased their biological activity and resulfuration could partially or completely restore it’. Some SPAH have lower activity than their isosteric PAH, whereas others are very potent carcinogens. There is, however, no simple correlation between the carcinogenic potential of carboxylic systems and that of their isosteric SPAH.

5. Exposure to SPAH vs. PAH

Generally, in bitumen fumes the SPAH have a higher concentration than the average PAH of similar molecular weight, whereas in coal-tar fumes SPAH have a much lower concentration than the corresponding PAH (Table 3). For example, more than five times higher concentrations of benzonaphtothiophene than those of B[a]P have been reported in bitumen fumes while, in coal-tar fumes, they were two to four times lower (Grimmer et al., 1987; Heinrich et al., 1994; Lewtas et al., 1996).
Table 3
Levels of SPAH and PAH of corresponding molecular weights in various type of fumes

<table>
<thead>
<tr>
<th>Mol. wt.</th>
<th>CTFC&lt;sup&gt;a&lt;/sup&gt; (μg/m&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>Coke-oven emissions CTFC&lt;sup&gt;b&lt;/sup&gt; (μg/m&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>Indoor Mastic laying with R80/25 bitumen&lt;sup&gt;b&lt;/sup&gt; 280–300 °C (ng/m&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>Laboratory rate from bitumen BFC&lt;sup&gt;c&lt;/sup&gt; 180 °C (μg/h)</th>
<th>PAH emission B45 250 °C (μg/h)</th>
<th>Laboratory animal exposure study: fume from 45/60 bitumen concentration in exposure chamber&lt;sup&gt;d&lt;/sup&gt; 200 °C (ng/m&lt;sup&gt;3&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>128</td>
<td>1.18</td>
<td>5.58</td>
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<tr>
<td>Benzo[b]thiophene</td>
<td>134</td>
<td>1.46</td>
<td>6.72</td>
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<td></td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>152</td>
<td>0.15</td>
<td>1.02</td>
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<tr>
<td>Acenaphthene</td>
<td>154</td>
<td>0.67</td>
<td>4.69</td>
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<tr>
<td>Phenanthrene</td>
<td>178</td>
<td>7.79</td>
<td>48.91</td>
<td>125</td>
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<td>Anthracene</td>
<td>178</td>
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<td>8.99</td>
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<tr>
<td>Dibenzothiophene</td>
<td>184</td>
<td>4.86</td>
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<td>87</td>
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<td></td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>202</td>
<td>0.44</td>
<td>4.42</td>
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<tr>
<td>Pyrene</td>
<td>202</td>
<td>0.93</td>
<td>9.71</td>
<td>65</td>
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<tr>
<td>Benz[a]anthracene</td>
<td>228</td>
<td>58</td>
<td>20.3</td>
<td>123</td>
<td>0.25</td>
<td>11.30</td>
</tr>
<tr>
<td>Chrysene</td>
<td>228</td>
<td>59</td>
<td>27.9</td>
<td>279</td>
<td>0.50</td>
<td>7.39</td>
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<tr>
<td>Benzo[b]naphtho[2.1-d]thiophene</td>
<td>234</td>
<td>12</td>
<td>9.9</td>
<td>189</td>
<td>0.61</td>
<td>11.11</td>
</tr>
<tr>
<td>Benzo[fluorantheno][b+j+k]</td>
<td>252</td>
<td>93</td>
<td>32.8</td>
<td>94</td>
<td>0.12</td>
<td>2.99</td>
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<tr>
<td>Benzo[a]pyrene</td>
<td>252</td>
<td>46</td>
<td>19</td>
<td>37</td>
<td>0.09</td>
<td>2.02</td>
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<tr>
<td>SPAH134/weighed ave PAH128&amp;152</td>
<td></td>
<td></td>
<td>1.8</td>
<td>0.4</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>SPAH184/weighed ave PAH178&amp;202</td>
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<td></td>
<td>0.1</td>
<td>1.9</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>SPAH234/weighed ave PAH228&amp;252</td>
<td></td>
<td></td>
<td>0.2</td>
<td>0.5</td>
<td>1.6</td>
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<tr>
<td>Benzo[b]naphtho[2.1-d]thiophene/BaP</td>
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<td></td>
<td>0.3</td>
<td>0.5</td>
<td>5.1</td>
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<sup>a</sup> From Heinrich et al. (1994).
<sup>b</sup> From Grimmer et al. (1987).
<sup>c</sup> From Woitowitz et al. (1997).
<sup>d</sup> From Brandt et al. (2000).
al., 1997; Woitowitz et al., 1997). This may explain why more polar adducts were detected only in animals exposed to bitumen fumes. A recent manuscript by Jaycox and Olsen (2000) has also described the presence of large amounts of benzothiazole, especially in the vapours of crumb-rubber modified asphalt. Little is known about the toxicity of this class of compounds.

6. Conclusions and perspectives

The main conclusions which can be drawn from this review are as follows.

a. Recent epidemiological studies indicate that bitumen fumes display a carcinogenic effect in humans, the lung being the target organ.

b. Dermal transfer of the bitumen fume condensates results in a distribution of their components and/or their metabolites in the lymphocytes and the lungs where adduct formation also occurs because the compounds are transferred directly into the blood and hence escape liver detoxification. They are thus delivered to the lung where they are metabolised into reactive derivatives.

c. Bitumen fume condensates display a mutagenic activity and can induce the formation of DNA adducts both in vitro and in vivo.

d. In the skin painting study, skin penetration of the fume condensates is extremely rapid, possibly due to a lowering of the viscosity by the mixing of the vapour and particulate phase. Skin can thus be a major route of penetration in humans.

e. Some DNA adducts are present in both the peripheral blood lymphocytes and the internal organs of rats exposed to BFC, and are therefore of potential use as biomarkers of exposure to bitumen fumes. It is, however, necessary to determine their persistence in the lymphocytes and to compare this to their persistence in the various organs.

f. In the skin painting study, there was a linear relation between 1-hydroxypyrene excretion and the pyrene content of the fume condensates, but no relation between either of these parameters and adduct formation was found.

g. The 16 individual PAH (from the EPA list) analysed are not good markers of the genotoxic activity of bitumen fumes. SPAH might be major genotoxic compounds in bitumen fumes.

A number of areas of further investigations regarding the mutagenic and carcinogenic role of the DNA damage reported above are indicated below.

a. First, it could be advantageous to determine the chemical identity of the major DNA adducts induced by BFC as this would allow the development of more specific assays for individual DNA adduct. All the analytical data and the mechanistic data are complementary and suggest a potential role of SPAH in the genotoxicity of bitumen fumes.

b. Methods should then be developed to determine the exposure to SPAH.

c. The relationship between DNA adducts resulting from specific compounds in BFC and the carcinogenic potency of these compounds could be examined in rodent studies (i.e. see Heinrich et al., 1994, for coal-tar).

d. For the DNA adduct(s) earmarked as potential biomarkers of exposure to bitumen fumes more information on the kinetics of adduct(s) formation and removal is required as well as pilot epidemiological studies in exposed populations to investigate the occurrence of the same adduct(s) in man.

References


