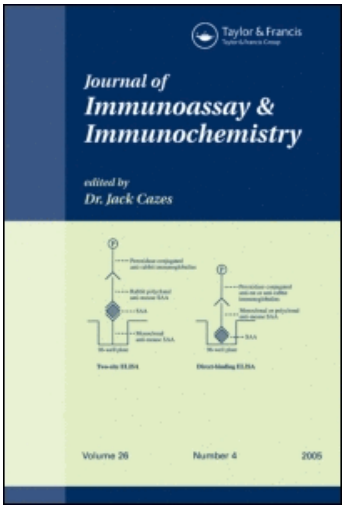

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QUANTIFICATION OF POLYNUCLEAR AROMATIC HYDROCARBONS IN TRANSFORMER OILS BY ENZYME IMMUNOASSAY

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ABSTRACT

Many polynuclear aromatic hydrocarbons (PAHs) are either known or suspected carcinogens and are a common constituent of mineral oils. Due to the large number of possible PAH structures, standard quantification methods fail since they either lack specificity or are too complex, requiring individual fractionation, identification, and quantification. A rapid, low-cost, novel analytical screening method, incorporating a silica-based solid-phase extraction (SPE) method linked to co-solvent dilution and quantification of total and carcinogenic PAH levels by immunoassay, is reported here. The method yielded high extraction efficiencies and minimal

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matrix effects. This novel approach yielded total and carcinogenic PAH levels $\times 5.7$ and $\times 126$, respectively, lower than that recorded by the industry-recognised BS2000 Pt. 346 (IP346) method which estimates the polyaromatic carbon (PAC) content of oils by gravimetry. The method is expected to be of benefit where an indication of PAH levels in oils is important for purchasing, management or disposal purposes and also for risk assessment and for appropriate labelling of oils in line with current legislation.

INTRODUCTION

Mineral oils have been used for over a century as an insulating medium and coolant in electrical transformer devices. Most of these transformer oils have a high naphthenic (cycloalkane) content, (oils containing less than 50% *n*-alkanes are typically considered naphthenic) since oils with a greater *n*-alkane content exhibit poorer oxidation product solubility and contain *n*-alkane waxes that precipitate at lower temperatures causing internal transformer problems.(1) All transformer oils also contain aromatic compounds that have a significant effect on transformer performance.

Monoaromatic compounds contain a single 6-carbon ringed aromatic structure and are alkylated, whilst polyaromatics contain 2 or more six-carbon ringed aromatic structures. The latter group of compounds are either naturally present in the oil or are generated during the hydrogenation processes commonly used to remove undesirable compounds such as polar and heteroatomic molecules from oils. Whilst polyaromatic compounds have the beneficial properties of oxidation inhibition and high gas absorption, their presence in oils can be detrimental due to undesirable electrical properties and the carcinogenic threat posed by many of the members of this family of compounds. PAHs are composed of two or more aromatic rings consisting, as the name suggests, solely of carbon and hydrogen. Many PAHs are known carcinogens or are suspected of being such and there is considerable epidemiological evidence to support a link between oil exposure and cancer.(2–4) A knowledge of the PAH composition of an electrical insulating oil prior to use would allow the selection of oils posing a low carcinogenic threat, but with sufficient levels of non-carcinogenic PAHs to provide adequate gas absorption and oxidation resistance. Furthermore, the analysis of oils currently in use would allow potential health risks to be identified and appropriate handling procedures to be implemented. Consequently, there is much interest in the development of methods capable of quantifying these compounds in transformer oils.

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The IP346 method(5) is used to determine which oils should be considered carcinogenic according to European Union regulations, with oils having an aromatic content of 3% or greater being labelled accordingly. IP346 assesses the levels of polar compounds in oils, a main constituent being PACs (polycyclic aromatic hydrocarbons) by measuring those compounds soluble in the polar organic solvent dimethylsulphoxide (DMSO). PACs are compounds that have several rings, some of which may be aromatic and therefore by definition includes all PAHs, many simple aromatics and naphthenes, especially those containing heteroatoms. Consequently, this method will significantly over-estimate the levels of PAH species in an oil, although there is a good correlation between IP346 data and the incidence of skin cancer in mice. One method(6) suggests a high-pressure liquid chromatographic (HPLC) based alternative to IP346, which avoids the need for handling DMSO. This method estimates the poly-aromatic content of an oil by quantifying the amount of compounds exhibiting greater polarity than a given marker compound, typically the di-aromatic ringed naphthalene or tri-aromatic ringed anthracene. Again this method will over-estimate the PAH content due to the relative non-specificity of the approach.

Improved specificity can be obtained using gas chromatography (GC) allied to a suitable detection technology such as mass-spectrometry and the United States Environmental Protection Agency (US EPA) provides detailed standard methods.(7) Since the sample is fractionated, individual PAH species must be identified and quantified. This would be an exacting process given that more than 345 polyaromatics have been identified in one highly characterised transformer oil.(6) It is usual to measure around 20 PAHs, including those 16 listed on the priority list of the EPA. Whilst such an approach clearly underestimates PAH levels, it is still used within the industry as an indicator of PAH levels.

In this study, we describe an alternative PAH measurement method based on antibody technology that circumvents many of the specificity issues described above. The method makes use of immunoglobulin G (IgG) antibodies, proteins secreted into the bloodstream as part of the vertebrate immune response to the presence of a foreign substance (termed the 'antigen'). In nature, a given IgG clone will recognise and bind to a particular structural entity on the invading species as a first step in the elimination process. This molecular recognition provides the analyst with a powerful tool with which to measure many different target compounds. Modern cell biology techniques allow the *in vitro* generation of homogeneous high affinity antibodies of almost any desired selectivity that can be simply isolated and incorporated into antibody-based test formats. Such an approach allows the simple, rapid, high-throughput,

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low-cost and highly sensitive screening of a given target analyte or group of structurally related analytes. The chosen approach is vindicated by the fact that the EPA have recently sanctioned the use of immunoassay procedures for the quantification of various organic pollutants, including PAHs in soil and water samples.(8–10)

This paper reports the performance of the antibody assay for the determination of PAHs in 11 transformer oils from two leading manufacturers and compares the resultant data with corresponding IP346 values. Since not all PAHs are known to be carcinogenic and the presence of non-carcinogenic PAHs in oil is beneficial, two immunoassay test kits were evaluated. The 'total PAH' test kit utilised an antibody preparation exhibiting a broad affinity for PAH species, whilst the 'carcinogenic PAH' test kit employed an antibody clone exhibiting an enhanced binding affinity towards the more carcinognic PAHs. Such data is useful in assessing the risk associated with an oil.

EXPERIMENTAL

Reagents

The 11 insulating oils (A–K) studied had previously been used within transformer plant and were donated by the National Grid Company (Leatherhead, UK). The standard mixture (QMX, Thaxted, UK) contained the 16 EPA priority PAHs and perylene (Sigma-Aldrich, Poole, UK), all at the $10\,\mu g\,mL^{-1}$ (total $170\,\mu g\,mL^{-1}$) level in methanol. All other reagents were of analytical grade and supplied by Sigma-Aldrich.

Sample Preparation

In nature, IgG antibodies occur in a predominantly hydrophilic matrix (blood) and selectively bind to the target compound via non-covalent interactions. Thus, in order to ensure high-affinity binding between the chosen anti-PAH antibody clone and target PAH analytes, the matrix in which the immuno-binding process occurs should also ideally be relatively hydrophilic. Thus transference of the target PAH compounds from the relatively non-polar oil matrix to a more assay-compatible solution was sought. Sample preparation was also required to ensure that the PAH concentrations of the oils fell within the dynamic range of the assay and also to remove or reduce interferent levels within the oil matrices. Samples were either diluted or pre-treated using silica gel as described below.

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Direct Dilution

Oils were sequentially diluted 10-fold (v/v), with vortexing, in propan-2-ol, methanol and aqueous assay diluent (the 2 mL spiked oil was further diluted 2-fold in assay diluent) and assayed. The poor reproducibility of the data was attributed to interference effects, despite the high dilution factor employed and consequently this approach was not further evaluated.

Solid Phase Extraction Using Silica

Silica SepPak solid phase extraction (SPE) cartridges (Waters) containing 0.5 μg silica sorbent were preconditioned with 4 mL hexane and loaded with 100 μg oil. Hexane (2 mL) and benzene (10 mL) were passed through the cartridges and the resultant eluent collected, mixed and a 100 μL fraction removed and evaporated at room temperature. Fractions were then reconstituted in 100 μL propan-2-ol and diluted in methanol (v/v) to a final dilution of 10^5 (total PAH kit) or 10^6 (carcinogenic PAH kit) and assayed.

Acid Degradation of Residual Aliphatic Compounds

It was reasoned that the presence of residual aliphatic hydrocarbons in the SPE column extracts was a potential source of undesirable matrix effects within the immunoassay. Correspondingly, the use of an acid treatment step to degrade these compounds was considered. Large silica gel columns were prepared by mixing 15 mL silica gel (Sigma-Aldrich) with 30 mL cyclohexane and slowly decanting into a glass column. Columns were preconditioned under positive pressure using 6 × 5 mL hexane aliquots. 0.3 mL volumes of oil E, either unspiked or spiked with PAH as described below, were applied to the columns and the main fluorescent band of material (measured with a 366 nm UV source) displaced to the base of the column with 44 mL hexane and eluted with 5 mL benzene. The residual, hence more polar PAHs, were eluted with 50 mL propan-2-ol. Each fraction was independently evaporated to dryness and reconstituted in 5 mL benzene with vigorous vortexing. Next, 1 mL of this material was mixed 1 : 1 with conc. sulphuric acid and vortexed in an Eppendorf tube for 1 min. on a bench vortexer. The benzene fraction, which contained the purified PAHs was recovered after phase separation and the process repeated. The resultant extracts were serially diluted in propan-2-ol and methanol as described for the commercial silica column extracts and assayed.

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Spiking of Transformer Oil

Transformer oil E was used for spiking experiments since it was known to have a low aromatic content by virtue of its IP346 value. First, 1 or 2 mL of the standard PAH mixture was dispensed into glass test tubes and placed in a waterbath at 60°C to evaporate the methanol solvent. Next, 1 mL of the oil was added to each tube followed by sonication to ensure complete PAH resuspension. The spiked oils were extracted using the large silica column then sequentially diluted 10-fold (v/v), with vortexing, in propan-2-ol, methanol and assay diluent (the 2 mL spiked oil was diluted a further 2-fold in assay diluent) and assayed.

PAH Immunoassay Test Kits

PAH levels in the extracted oils were quantified using either a total PAH RaPID assay kit or a carcinogenic PAH RaPID assay kit, supplied by Strategic Diagnostics Inc. (Newark, DE, USA) and used according to the manufacturer's instructions. Operation of the two assay kits was essentially identical. The assay reagents consisted of anti-PAH antibodies bound to small paramagnetic beads and a PAH analogue (phenanthrene and benzo-[a]anthracene for the total and carcinogenic test kits respectively) linked to an enzyme (horseradish peroxidase, termed HRP) label. Each oil sample was prepared and diluted as previously described and 250 µL incubated with 250 µL of PAH-HRP conjugate and 500 µL of the antibody-magnetic bead solution in a glass tube for 30 min. at room temperature (Figure 1a).

During incubation, the sample PAH and PAH-HRP compete for available antibody binding sites (Figure 1b). This *competitive assay* format results in a inverse proportionality in signal in that the greater the quantity of PAH in the sample, the smaller the proportion of sites that will become occupied by the labelled analogue and *vice-versa*. Next a magnetic field is applied to capture the magnetic beads which will contain antibody-analyte and antibody-conjugate complexes, allowing excess unbound sample and conjugate to be removed by washing (Figure 1c). The beads are then resuspended in buffered HRP enzyme substrate (hydrogen peroxide) and chromogen (3,3′,5,5′-tetramethylbenzidine). The concentration of labelled PAH analogue, and hence PAH present in the test sample is determined colourimetrically at 450 nm after addition of 0.5 mL 2 M sulphuric acid stop solution (Figure 1d).

Calibration of the total PAH kit was performed by assaying a phenanthrene standard in duplicate at three separate concentrations (2, 10 and 50 ng mL^{-1}). The carcinogenic kit was similarly calibrated using 0.1, 1,

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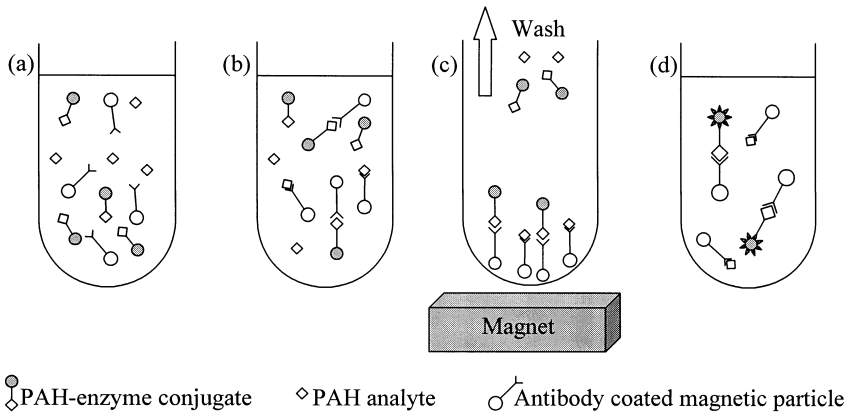

Figure 1. Magnetic particle competitive immunoassay format. Full details in text.

and 5 ng mL^{-1} benzo[a]anthracene. A phenanthrene/benzo[a]anthracene standard of known concentration was also included in each run to allow the accuracy of a given assay to be calculated. All values were within 10% of those expected.

Total vs. Carcinogenic PAH Test Kit

The 'Total PAH' test kit utilised an anti-PAH antibody clone exhibiting a broad binding affinity for a wide range of PAHs whilst the 'Carcinogenic PAH' test kit used a clone with a greater affinity towards those PAHs known to be, or suspected of being, carcinogenic. In effect the Total PAH antibody clone binds to a structural entity common to the wider PAH family whilst the carcinogenic PAH clone recognised a functionality more prevalent within the carcinogenic PAH group. It is a standard procedure, when developing an immunoassay from first principles, to isolate and examine a wide range of target specific antibodies then propagate those immunoglobulins exhibiting the desired specificity, a time-consuming and expensive empirical process. However, the reward is ideally an effectively inexhaustible supply of high specificity, high affinity antibody.

Since the antibodies recognise structural features and bind via non-covalent interactions, such as electrostatic attraction, hydrophobic interaction and hydrogen bonding, a given antibody clone can bind to structurally similar entities with varying degrees of affinity. A measure of the binding affinity between antibody and antigen can be obtained by

Table 1. Least Detectable Dose (LDD), Affinity (50%B/B_0), and Percent Cross Reactivity (%CR) Data for the Total PAH and Carcinogenic PAH RaPID Assay Test Kits

	Total PAH Kit			Carcinogenic PAH Kit		
Compound	LDD[a] (ng mL^{-1})	50% B/Bo[a] (ng mL^{-1})	% CR	LDD[a] (ng mL^{-1})	50% B/Bo[a] (ng mL^{-1})	%CR
Anthracene[b]	0.54	11.0	150.0	0.22	20.50	2.34
Phenanthrene[b,c]	0.70	16.5	100.0	1.35	67.20	0.71
Benzo[a]anthracene[d]	0.77	28.4	58.1	0.01	0.48	100.0
Benzo[b]fluoranthene[b]	0.91	54.2	30.4	0.02	1.30	36.92
Benzo[k]fluoranthene[b]	0.77	524	3.1	0.01	0.63	76.19
Acenapthalene	12.9	688	2.4	539	> 10 000	< 0.005

[a]Data supplied with test kit.
[b]Previously identified in naphthenic oil.
[c]Total PAH test kit calibrator.
[d]Carcinogenic PAH test kit calibrator.

determining the concentration of antigen required to reduce the maximum assay signal (B_0) by 50%, expressed as 50%B/B_0. An inverse relationship is apparent, since the lower the50%B/B_0 value, the greater the binding affinity. The relative binding affinity of an antibody towards different target compounds, such as the various PAH congeners, is termed cross-reactivity (CR) and is expressed as a percentage ratio of the affinity of a given compound against a pre-selected standard compound. Affinity and CR data for 4 PAHs previously identified in transformer oil and the test kit calibrators are provided in Table 1. The enhanced binding affinity of the Carcinogenic PAH antibody clone to the more carcinogenic benzo-PAHs is evident. The least detectable dose (LLD) of analyte necessary to elicit a positive response from the kits was defined as the concentration of analyte required to reduce B_0 by 90% and was generally in the sub-ng mL^{-1} range for the total PAH kit and around 10–20 pg mL^{-1} for the benzo-PAHs using the requisite test kit.

RESULTS AND DISCUSSION

Spiked Oil Analysis

A key aspect of the study centred on the development of a suitable PAH extraction process that delivered a sample with suitably reduced levels

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of potential interferent species, compatibility with the subsequent immunoassay step and exhibiting a minimum loss of the target analytes during the treatment process. Since the study focussed on developing a PAH screening process for naphthenic oils, it was considered pertinent that the combined sample preparation-assay procedure be investigated with such oils, which by definition would be expected to contain significant amounts of naturally occurring PAH species. Therefore it was considered that the efficiency of the extraction procedure and quantitative nature of the immunoassay process be examined with PAH-spiked naphthenic oils. Immunoassay data for the silica column extracted oils, both spiked and unspiked, and also uncleaned, and acid cleaned, is given in Table 2.

Interpretation of Table 2 is as follows. Acid cleaned oil E, spiked with 2 mL of the 17 PAH standard mixture (i.e., total PAH loading of 0.34 mg in 2 mL of spiking solution) yielded a total PAH concentration by immunoassay of 92 ng mL^{-1}, compared with 40 ng mL^{-1} for the unspiked oil. The difference of 52 ng mL^{-1} was multiplied by 5 to give the total amount of PAHs recovered in the 5 mL benzene eluent collected after passage through the silica column. This amount (0.26 mg mL^{-1}) was recovered from a 0.3 mL

Table 2. Data Treatment for Transformer Oil 4, Unspiked, or Spiked with 1 mL and 2 mL Standard PAH Mixture (Both the Acid Washed (Cleaned) and Non-Acid Washed (Uncleaned) Extracted Sample Data Is Shown)

	Unspiked Oil	1 mL Spiked Oil	2 mL Spiked Oil
Cleaned benzene fraction			
Immunoassay measured PAH conc. (ng mL^{-1})	40.00	54.00	92.00
Difference, spiked − unspiked oil (ng)		14.00	52.00
Mass PAHs in 5 mL benzene eluent (ng)		70.00	260.00
Mass PAHs in 0.3 mL loaded sample oil (mg)		0.07	0.26
Mass PAHs in 1 mL oil (mg)		0.23	0.87
Mass of added spiked standard (mg)		0.17	0.34
% Difference (spiked value – measured value)		137.25	255.00
Uncleaned benzene fraction			
Immunoassay measured PAH conc. (ng mL^{-1})	30.00	60.00	74.00
Difference, spiked − unspiked oil (ng)		30.00	44.00
Mass PAHs in 5 mL benzene eluent (ng)		150.00	220.00
Mass PAHs in 0.3 mL loaded sample oil (mg)		0.15	0.22
Mass PAHs in 1 mL oil (mg)		0.50	0.73
Mass of added spiked standard (mg)		0.17	0.34
% Difference (spiked value − measured value)		294.12	215.69

oil loading, therefore the amount of PAHs recovered in 1 mL of oil would be 0.87 mg. Comparing this value with the actual amount of spiked PAH standard (0.35 mg) yields an 'over-calculation' of 255%.

Overall, the assay over-calculated the amount of standard PAH mixture spiked into oil E by 137% and 255% for the 1 mL and 2 mL spiked, acid cleaned oil and 294% and 216% for the 1 mL and 2 mL spiked uncleaned oil. It was postulated that this over-calculation was due to CR effects, whereby the PAH species in the standard mixture had, overall, a higher binding affinity for the antibody and hence yielded a greater reduction in assay signal when compared against the phenanthrene assay calibrant. This postulation was given credence when over-calculation factors of 247–279% were recorded for buffer solutions spiked with PAH standard mixtures and tested by immunoassay both with and without the SPE extraction method. Three main points were therefore concluded: the extracted oil matrix had no apparent detrimental effect on immunoassay performance; the SPE method yielded high extraction efficiencies; and acid treatment had no measurable effect on the immunoassay method.

PAH Analysis of the 11 Oil Samples

IP346, total PAH and carcinogenic PAH data for the 11 oils tested are shown in Table 3. The IP346 data yielded significantly higher concentration values than those recorded by the immunoassay methods. This is exemplified in the fact that the mean IP346 concentration value for the 11 oils was $\times$ 5.71 and $\times$ 125.5 times higher than the mean values recorded by the total and carcinogenic immunoassays respectively. The relative magnitude of the IP346 values can be attributed to the inherent non-specificity of the gravimetric method in which the overall level of polar in the oil is measured. Both kits yielded lower concentration values, due to the inherent PAH-specificity of the antibodies.

IP346 vs. Total PAH Immunoassay

A comparison of the concentration values obtained for the oils by IP346 and the total PAH immunoassay is given in Figure 2. In general, it can be observed that a higher polar compound content corresponds (by IP346) to a higher PAH level in a given oil. The trend is not exact, since the immunoassay specifically targets PAH species, whilst IP346 measures PACs. It was further noted that since IP346 yielded, on average, concentration values 5.7 $\times$ those recorded by the total PAH immunoassay,

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Table 3. IP346 and Immunoassay Data for the 11 Transformer Oils Studied

Oil No.	Manufacturer	IP346 (mg mL^{-1})	PAH Content by Total PAH Assay (mg mL^{-1})	PAH Content by Carcinogenic PAH Assay (mg mL^{-1})
A	1	10	2.8	< 0.1
B	1	10	3.8	0.14
C	1	18	4.4	< 0.1
D	1	33	2.8	< 0.1
E	2	10	0.6	< 0.1
F	2	18	4.9	0.32
G	2	21	5.8	0.58
H	2	22	7.2	0.16
I	2	26	9.0	0.48
J	2	45	8.0	0.24
K	2	89	> 10*	0.70

The IP346 measures the levels of polar compounds in the oils, whilst the total and carcinogenic immunoassay kits provide, an indication of general PAHs and carcinogenic PAH concentrations in the oils respectively.
*Off scale.

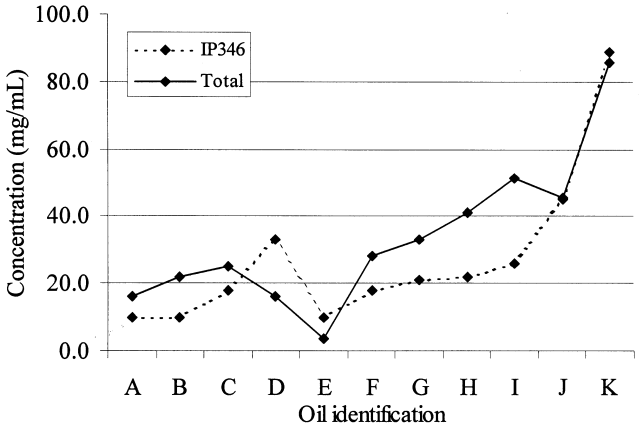

Figure 2. Comparison of IP346 and total PAH immunoassay data. To allow trend comparison, the immunoassay data was multiplied by 5.71, the mean difference between the immunoassay data and PAC concentration as determined by IP346.

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then only around one sixth of the polar compounds recovered by IP346 are in fact PAHs. Those oils exhibiting increased immunoassay responses relative to their IP346 values (oils A–C and F–I) could be considered as having a polar compound content richer in PAH species. No obvious difference in the immunoassay/IP346 responses was evident between the oils of manufacturers 1 (oils A–D) and 2 (oils E–K).

IP346 vs. Carcinogenic Immunoassay

A comparison of the carcinogenic PAH immunoassay and IP346 data is provided in Figure 3. As with the total PAH assay data, the carcinogenic immunoassay yielded concentration values significantly lower ($\times$ 125.5) than IP346. Again, this difference can be ascribed to the high specificity of the antibody-based assay. A less obvious correlation between the IP346 and carcinogenic immunoassay data (compared with the total immunoassay data) is apparent. It is evident from the relative response values that the polar extracts of oils F, G and I are proportionally richer in carcinogenic PAHs, with the inverse being true in oil D.

A key reason for the use of IP346 by naphthenic users is that an approximation regarding the carcinogenic threat posed by the oil can

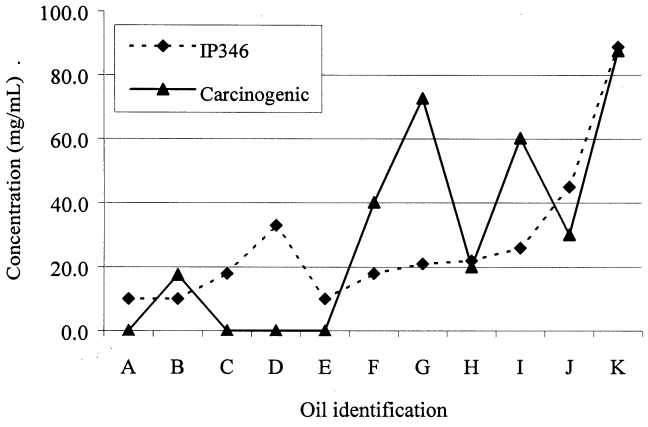

Figure 3. Comparison of IP346 and carcinogenic PAH immunoassay data. To allow trend comparison, the immunoassay data was multiplied by 125.5, the mean difference between the immunoassay data and polar compound concentration as determined by IP346. Oils containing -1 carcinogenic PAH by immunoassay are shown as having zero analyte levels.

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be made. IP346, whilst showing a degree of correlation with mice skin tests, may still lead to incorrect classification of oils since many of the polar compounds extracted by the DMSO are not mutagenic or carcinogenic. The preliminary data in Figure 3 suggests that, owing to the specificity of the carcinogenic immunoassay towards known or suspected carcinogenic PAHs, a more accurate diagnosis and labelling of a naphthenic oil can be made. For example, IP346 yields concentration values of 10–33 mg mL^{-1} for oils A, C, D and E, yet the carcinogenic PAH levels in these oils is −1 by the carcinogenic immunoassay method. Oils F, G and I have an intermediate polar compound content by IP346, but a proportionally high carcinogenic PAH load. This data suggests that, for the 11 oils studied, the antibody-based approach shows promise as a rapid, simple and reliable diagnostic tool for more accurate oil carcinogenicity measurement.

Total vs. Carcinogenic Immunoassay

Data from the two immunoassays is compared in Figure 4. The total PAH kit yielded far higher PAH concentration values than the carcinogenic kit (mean difference of × 26.9 between the total and carcinogenic PAH kits. This decrease in assay response was expected. For example, a relatively

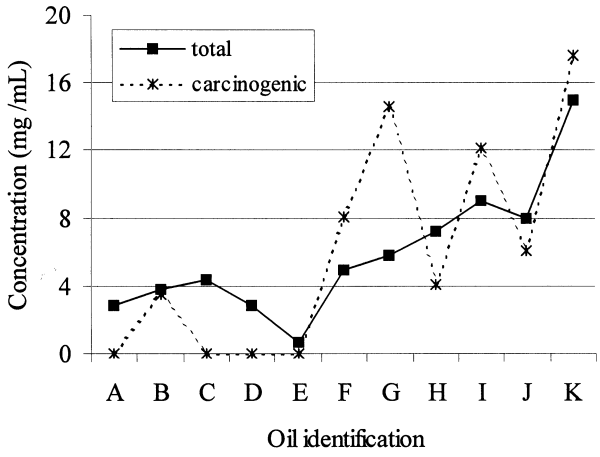

Figure 4. Comparison of data from the two immunoassay methods. On average, the total immunoassay test kit yielded total oil PAH loadings × 25.2 times greater than the carcinogenic assay. Correspondingly, data from the latter assay was multiplied by this factor to allow data trend comparison.

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carcinogenic PAH such as benzo[k]fluoranthene has a %B/Bo value of 524 ng mL^{-1} (Table 1) using the total kit, compared with 0.63 ng mL^{-1} using the carcinogenic kit. In other words, 524 ng mL^{-1} of benzo[k]fluoranthene is required to reduce the assay signal by 50% using the total kit, compared with only 0.63 ng mL^{-1} for the carcinogenic kit. This data indicates the usefulness of the carcinogenic kit in providing a far truer carcinogenicity index when assessing a naphthenic or other mineral oil. Less carcinogenic PAHs such as acenapthylene must be present in much larger concentrations in a carcinogenic test kit assay to register the same signal decrease as in a total PAH test kit assay (5770 ng mL^{-1} compared with 688 ng mL^{-1} respectively).

DISCUSSION

Currently, there is no true means of determining PAH concentrations in oil rapidly, efficiently and cost-effectively, primarily because a typical naphthenic oil contains such a wide array of PAH species. Thus standard analytical techniques, such as chromatography require each PAH species to be independently identified and quantified – prohibitively expensive and time-consuming for routine analysis. Consequently, the recognised 'standard' for assessing the PAH levels in oils relies on IP346.

The immunoassay method circumvents this problem by employing PAH-specific antibodies. It is recognised that there is a consequent issue with regard to CR, in that some PAHs (the more carcinogenic in the case of the carcinogenic test kit) have greater binding specificities to the antibody. Therefore, to safely assess oil carcinogenicity, it is important to select a predefined PAH level, based on the more carcinogenic PAHs to prevent the mis-diagnosis of an oil sample. In other words, it is more acceptable to over-estimate the carcinogenic load of an oil than to generate a false negative result. An attractive alternative approach would be to calibrate the total PAH kit using a lower affinity less carcinogenic PAH to prevent under-estimation of carcinogenic PAH levels, or to calibrate the assay against a recognised carcinogenicity test, such as the Ames test.

The immunoassays appear highly specific towards PAH species. For example, the carcinogenic PAH immunoassay exhibited high binding specificity towards the higher PAHs (e.g. benzo[a]anthracene (100%), benzo[k]fluoranthene (76%), chrysene (70%)) with significantly lower cross-reactivities for the lower PAHs (naphthalene (0.01%), acenapthylene (0.008%), acenaphthene (< 0.005%)). Thus the antibody has a demonstrably high affinity for multi-ringed fused benzenoid structures and little or no-activity for the lower aromatic species.

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Current knowledge concerning the chemical content of naphthenic oils is incomplete. However, it would be unlikely that there are non-PAH species in oil extracts that have a more-PAH-like structure than those PAHs with low antibody binding affinities (for example, acenaphthene a simple 2-fused benzene ring structure with %CR values of 2.4% and < 0.005% for the total and carcinogenic antibodies respectively). US EPA method 4035 [US EPA method 4035, 1996] lists 'compounds that might be expected to be found *(in soil and water)* in conjunction with PAH contamination', presumably from oil-based pollution, as: benzene, toluene, CCA, phenol, creosote, 2,4,6-trichlorobenzene, 2,3,5,6-tetrachlorobenzene, pentachlorobenzene, bis(2-ethylhexyl)pthalate and Aroclors 1254 and 1260. All had %CRs of < 0.5% using a commercially available immunoassay test kit to screen soil samples. It would be pertinent to analyse a range of naphthenic oils to establish the presence of such compounds, and if identified, to determine their %CR values using the immunoassay test kits.

CONCLUSIONS

In this study, we present, for the first time, a specific analytical method for the routine screening of PAH levels in mineral oils. It was shown that extraction by silica SPE columns, linked to eluent evaporation, then reconstitution and dilution in solvents compatible with the subsequent immunoassay process, allowed meaningful analytical data to be obtained with respect to overall levels of PAHs and carcinogenic PAHs in oils. Extraction efficiencies were high and quantitative data obtained with no apparent matrix effects. A trend between data obtained by the highly PAH-specific immunoassay approach and less-specific industry recognised IP346 method was evident. It is recognised that the broad cross-reactivity of the antibody towards various PAH species will necessitate the judicious selection of threshold values on a case-by-case basis, in order to minimise the risk of generating false-negative data.

The simplicity and specificity of the approach represents an improvement over current methods of rapid PAH quantification in oils. It is expected to be of benefit to oil manufacturers, distributors and end-users and to be of use in risk assessment procedures and allow more accurate labelling of oils according to current or impending legislation.

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