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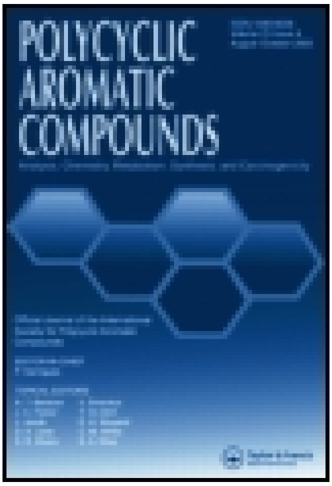

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Jan T. Andersson[a] & Christine Achten[b]

[a] Institute of Inorganic and Analytical Chemistry, University of Muenster, Muenster, Germany

[b] Institute of Geology and Palaeontology - Applied Geology, University of Muenster, Muenster, Germany

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Time to Say Goodbye to the 16 EPA PAHs? Toward an Up-to-Date Use of PACs for Environmental Purposes

Jan T. Andersson[1] and Christine Achten[2]

[1]Institute of Inorganic and Analytical Chemistry, University of Muenster, Muenster, Germany
[2]Institute of Geology and Palaeontology – Applied Geology, University of Muenster, Muenster, Germany

The 16 EPA PAHs have played an exceptionally large role above all in environmental and analytical sciences in the last 40 years, but now there are good reasons to question their utility in many circumstances even though their use is so established and comfortable. Here we review the reasons why the list has been so successful and why sometimes it is seen as less relevant. Three groups of polycyclic aromatic compounds (PAC) are missing: larger and highly relevant PAHs, alkylated PACs, and compounds containing heteroatoms. Attempts to improve the situation for certain matrixes are known and here: (1) an updated list of PAHs (including the 16 EPA PAHs) for the evaluation of the toxicity in the environment (40 EnvPAHs); (2) a list of 23 NSO-heterocyclic compounds and 6 heterocyclic metabolites; and (3) lists of 10 oxy-PAHs and 10 nitro-PAHs are proposed for practical use in the future. A discussion in the scientific community about these lists is invited. Although the state of knowledge has improved dramatically since the introduction of the 16 EPA PAHs in the 1970s, this summary also shows that more research is needed about the toxicity, occurrence in the environment and chemical analysis, particularly of alkylated PAHs, higher molecular weight PAHs and substituted PACs such as amino-PAHs, cyano-PAHs, etc.. We also suggest that a long overdue discussion of an update of regulatory environmental PAH analysis is initiated.

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Address correspondence to Jan T. Andersson, Institute of Inorganic and Analytical Chemistry, University of Muenster, Corrensstrasse 30, 48149 Muenster, Germany. E-mail: anderss@uni-muenster.de

THE 16 EPA PAHs

The list of 16 polycyclic aromatic hydrocarbons (PAHs), issued by the U.S. Environmental Protection Agency (EPA) in 1976 (1) with a view to use chemical analysis for assessing risks to human health from drinking water, has gained a tremendous role as a standardized set of compounds to be analyzed, quite especially in environmental studies. Although not mandated by law in most countries, it appears that the list has attained the authority of a legal document and that the 16 compounds ("priority PAHs") are routinely investigated in a large number of environmental situations. In this context, environmental is seen in a broader context and thus includes topics like food, too.

These 16 PAHs seem to have become accepted by many scientists as representatives for all PAHs, or even all polycyclic aromatic compounds (PAC), in a sample, despite the logical difficulty inherent in reducing a group of thousands of relevant compounds to only a handful of representatives. As described in detail by L. Keith (1), the list of PAHs was created in the 1970s under time pressure with respect to the following criteria:

(1) availability of analytical standards and, if available, whether they were measurable with current analytical methods (mainly GC-FID or GC-MS);

(2) whether they occurred in the environment; and

(3) whether they were known to be toxic.

The aim of selecting only a limited set of compounds was to cover the wide range of those PAHs that occur in samples as known at that time with a practical number of proxies. The aspect of having a manageable number of compounds—available in pure form—was important for the acceptance of the selection despite its limitations in accounting for the toxicity of contaminated sites. In the first approach, only parent compounds were selected. Without doubt, selecting the right compounds was a very difficult task within this complex working field and probably most scientists were—and continue to be—glad that an established and respected environmental institution implemented such a challenging list at all. That this selection was a big success and has advanced our understanding of environmental toxicity thoroughly is sufficiently indicated by the continued intense use of the EPA PAHs.

There are several advantages of the 16 EPA PAH list:

- only a limited number of PAHs are included, meaning a limited analytical complexity and acceptable costs, so that it can be widely used;
- only commercially available compounds are included on the list, and quantitative and qualitative standard solutions are obtainable from many purveyors;

- good comparability worldwide when most scientists analyze the same set of PAHs;
- it is often even more simple because in many cases only the sum of the 16 EPA PAHs are used for evaluation (practicability);
- consistency over time is assured because the same compounds have been analyzed for decades, meaning that trends in concentration changes over time can be compared;
- widespread and thorough experience in the evaluation of these priority PAHs; and
- experience with this list in lawsuits, making references to previous cases simpler.

Interestingly, the implementation of the list that was accepted by the research community initiated to no small extent advancement of knowledge. Separation science benefitted immensely from it, as detailed by Wise et al. (2), and by striving for a complete resolution of all the 16 compounds, chromatographic phases both in liquid and in gas chromatography were developed and the separation mechanisms became better understood. When different laboratories use the same standardized set of analytes to investigate the toxic potential of an environmental sample, results are more easily comparable than if each laboratory use its own set of analytes.

It was also of benefit to the development of reference materials that feature certified concentrations of PAHs; these materials are important since they can be bought by any laboratory in the world and thus be used to validate their analytical methods using a common standard.

Hence, it is obvious that the implementation of the list of the 16 EPA PAHs constitutes a milestone in environmental chemistry.

16 EPA PAH LIST FOREVER?

So, why discuss modifying or replacing this list? Because the results of about 40 years of PAH research that show the shortcomings of the list simply cannot be ignored, otherwise a blind eye is turned to that scientific progress. Clearly, a re-evaluation of the list seems overdue. The toxicity of the PAHs presents one of the main aspects why we might need to consider saying good-bye to the traditional list of the EPA PAHs. There are PAHs of considerably higher toxicity than that of the priority PAHs in environmental samples but—from a regulatory perspective—many of these larger PACs may not have been studied in sufficient detail with respect to frequency of occurrence in the environment and to their toxicity to different organisms to justify their inclusion in routine measurements. Thus, it seems that the established list continues to be used although the state of knowledge (see Table 1 in (3) of this issue) and analytical potential have increased significantly since the 1970s.

Table 1: Proposed list of 40 PAHs in the environment (40 EnvPAHs) for expanding the 16 EPA PAH list; x = affirmative, x? = possibly affirmative but not certain, (?) = doubtful, ? = unknown

	Compound	Toxicity Equivalence Factor (Benzo[a]-pyrene = 1; max. value) [3]	Carcinogenicity (x = sufficient and limited evidence, 0 = inadequate evidence) [57]	C = carcinogenic, G = genotoxic, M = mutagenic, E = increased eco-toxicity [58]	At least one carcinogenic/ mutagenic/ celltoxic/ EROD proof available [3]	Commonly occurring in the environment? Authors' evaluation	Analytical separation possible? Authors' evaluation	16 EPA PAHs	Inclusion among the EU Food 15+1 PAHs [59]
40 EnvPAHs									
1	Naphthalene	0.001	0	C, (M), (G)	x	x	x	x	
2	2-Methylnaphthalene	?	?		?	x	x		
3	1,3-Dimethylnaphthalene	?	?		?	x	x		
4	Acenaphthylene		0	M, G	?	x	x	x	
5	Acenaphthene		0	E	?	x	x	x	
6	Fluorene		0	M, G	-	x	x	x	
7	Phenanthrene	0.001	0	M, G	-	x	x	x	
8	Anthracene	0	0	(M), (G)	?	x	x	x	
9	2-Methylphenanthrene	?	?		?	x	x		
10	9,10-Dimethylanthracene	?	?		?	x?	x		
11	Retene	?	?		?	x	x		
12	Fluoranthene	0.08	0	C, M, G, E	x	x	x	x	
13	Pyrene	0.001	0	(M), (G), E	x	x	x	x	
14	1-Methylpyrene	0.1	?		x	x	x?		
15	Benzo[c]fluorene	20	x		x	x	x?		x
16	Chrysene	0.1	x	C, M, G, E	x	x	x	x	x
17	5-Methylchrysene	?	x		x	x	x		x

18	6-Methylbenzo[a]anthracene	?	?		x	x?	(?)		x
19	Benzo[a]anthracene	0.2	x		x	x	x	x	
20	7,12-Dimethylbenzo[a]anthracene	20	?		x	x?	x?		
21	Benzo[b]fluoranthene	0.8	x		x	x	x	x	x
22	Benzo[j+k]fluoranthene	0.2	x		x	x	x	k: x	x
23	Benzo[j]aceanthrylene	60	x		x	x?	?		
24	Benzo[l]aceanthrylene	5	x		x	x?	?		
25	Cyclopenta[cd]pyrene	0.4	x		x	x	x		x
26	Cyclopenta[def]chrysene	0.3	0		x	x?	x?		
27	Benzo[a]pyrene	1	x	C, M, G, E	x	x	x	x	x
28	6-Methylbenzo[a]pyrene	?	?		?	x?	?		
29	3-Methylcholanthrene	5.7	?		x	x?	x?		
30	Indeno[1,2,3-cd]pyrene	0.1	x		x	x	x	x	x
31	Dibenzo[a,h]anthracene	10	x		x	x	x	x	x
32	Dibenzo[a,c]anthracene	0.04-50	x		x	x?	x?		
33	Benzo[ghi]perylene	0.009	0		?	x	x	x	
34	Anthanthrene	0.4	x		x	x	x		
35	Dibenzo[a,e]fluoranthene	0.9	x		x	x	?		
36	Dibenzo[a,l]pyrene	10	x		x	x	x		x
37	Dibenzo[a,e]pyrene	1	x		x	x	x		x
38	Dibenzo[a,i]pyrene	10	x		x	x	x		x
39	Dibenzo[a,h]pyrene	10	x		x	x	x		x
40	Naphtho[2,3-e]pyrene	0.3	0		x	x	?		

Naturally, there are arguments in favor of continued use of the list. It has become established in different environmental fields and it seems difficult to make a change here. The question of continuity is also nontrivial. For instance, if trends have been studied according to this list over decades, it is not very likely that the set of analytes can be changed easily since there would be a break in the trends and the newer data would not be comparable with the older ones. Also, contaminated site owners may have cleaned up their sites to comply with analyses based on the EPA PAHs; a new set of compounds may invalidate this clean-up and demand new investments. This would surely meet with resistance.

LIMITATIONS OF THE 16 EPA PAH LIST

However, as useful as this list has been and continues to be, there is widespread agreement among PAC researchers that concentrating on only the 16 EPA PAHs may narrow our view of a sample considerably and, looking critically, it becomes evident that the information obtained from a sample studied only with respect to these compounds may be too limited to describe the toxic potential. Only a few examples will demonstrate this.

It is frequently found that the toxicity of a sample is not reflected by its PAH content as defined by the priority PAHs as proxies. In such cases, limiting the experimental work to these compounds leads the scientist to miss what is really important about the sample. This has been noted many times in the literature as indicated by the following short selection of works. Relying on this list in the analysis of petroleum contaminated sediments for a toxicity estimate was said to "grossly underestimate the load of PAH contamination" (4). Based on the comparison of chemical and bioanalytical characterization of PAH contaminated soils, it was concluded that "[t]he current risk assessment method for PAH-contaminated soil in use in Sweden along with other countries, based on chemical analysis of selected PAHs, is missing toxicologically relevant PAHs and other similar substances" (5). Another study reached the conclusion that "[r]isks to wildlife were underestimated by 40 to 70 times by only considering parent PAHs" (6). And in a series of coals, the EPA PAHs accounted for only 5–20% of the toxicity that was determined using an expanded set of 40 PAHs (predominantly toxic PAHs), mainly due to the presence of dibenzopyrenes and benzo[*c*]fluorene that are not included in the EPA list (7). Clearly, at least in environmental toxicity studies where fossil material is involved, adherence to the EPA list strongly underestimates the toxic potential of the analyzed material.

The 16 listed compounds only encompass parent PAHs. Much information is lost or the information obtained is distorted through the exclusion of other representatives of the class of polycyclic aromatic compounds (PAC) for purposes like estimating the toxicity of an environmental sample. It should be no wonder that the original list needs updating—the compounds originally included in it in 1976 were selected, i.e., on the basis of their commercial availability (1). Today, nearly 40 years later, many more PAHs are available in pure form than could be found at that time. As toxicology has increased our understanding of the adverse health influence of many more PACs, a wealth of new compounds have been added to the inventory of confirmed or suspect carcinogens. Not surprisingly, not all of the original PAHs are of much concern today in the field of toxicity assessment.

There are three main points that express the limitations of the priority PAHs:

(1) several commonly occurring PAHs possessing high toxicity are not included on the list;

(2) alkylated derivatives are not represented despite mounting evidence that their toxic potential may easily surpass that of the parent compounds; and, likewise,

(3) heterocyclic aromatic compounds of known toxicity and frequent occurrence in the environment are missing.

It should be noted that not all sources of PACs contain equally significant amounts of representatives of all these three classes. Petrogenic sources (mainly crude oil, coals, and similar materials) are rich in alkyl substituted PACs but pyrogenic (produced under the influence of high heat) are not and can be dominated by the parent PAHs and also contain more of the high molecular parent PAHs. Likewise, in coal derived materials, heteroatom containing compounds are more frequently encountered but in pyrogenic materials to a much lower degree. Also, patterns vary according to origin, e.g., either a rather pyrogenic (slope distribution of $C_0>C_1>C_2>C_3>C_4$ alkylated derivatives) or petrogenic pattern (bell-shape distribution).

Added to that is the selective change in the composition of the alkylation pattern of aromatic compounds through weathering in the environment, e.g., due to photodegradation (8,9) and biodegradation (10). Thus, not all compounds in a standard set of analytes may be equally relevant to each and every sample but a standardized set of compounds should be capable of dealing with all those situations.

(1) Point 1 above is well illustrated by the case of the dibenzopyrenes and benzo[*c*]fluorene as mentioned above. Dibenzo[*a,l*]pyrene, dibenzo[*a,i*]pyrene, and dibenzo[*a,h*]pyrene possess a carcinogenic potential about 10 times and benzo[*c*]fluorene about 20 times that of benzo[*a*]pyrene (11), meaning that also low concentrations of them will contribute significantly to the toxic effects of a sample. Moreover, these compounds occur in the environment, which is exemplarily shown by five

bituminous coals [7]: the content of benzo[*a*]pyrene, dibenzo[*a,l*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,h*]pyrene, and benzo[*c*]fluorene ranged from 1.68–3.96, 0.26–1.80, 0.54–2.06, 0.36–1.88, and 2.86–4.34 mg/kg. Here, the sum of the concentration of three dibenzopyrene isomers was in the same range as the content of benzo[*a*]pyrene alone but these compounds are about ten times more potent.

A similar case can be made for other (unsubstituted) PAHs, although it must be realized that many larger PAHs have not yet been studied conclusively (3) and therefore not enough generally accepted data on their adverse health properties are available.

A problem here would be that of the analysis of a large number of isomers, since the larger the molecular weight, the more isomers are possible and the more difficult is the correct analysis (2).

(2) Alkyl derivatives of PAHs can contribute a large part of the toxicity of a sample, point 2 in the list above, but none is included among the EPA priority PAHs. This is again an emerging field as by far not as many alkyl derivatives as parent compounds have undergone rigorous toxicity testing (3) but some data are available. For instance, 7,12-dimethylbenzo[*a*]anthracene has a 20 times higher toxic equivalence factor than its parent and twice that of benzo[*a*]pyrene (12). Structure-activity relationship calculations on methyl- and dimethyl PAHs have shown that they possess toxicities comparable with that of their parents and this of course makes it desirable to include them in any estimate of the toxicological properties of a sample (13) That altogether too little is known about the properties of alkylated derivatives is probably the reason why it may sound surprising that, in studies of sediments following the Exxon Valdez oil spill in Alaska in 1989, it was found that the "naphthalenes were the major contributors to any remaining sediment toxicity", not the higher PAHs that were found in the chrysene fraction (14).

(3) And what about the heterocyclic compounds as indicated in point 3, essentially those containing the elements nitrogen, sulfur, and/or oxygen? Today it is known that representatives of this group of compounds can exhibit just as strong adverse health effects as the corresponding hydrocarbons. Such knowledge has been tabulated in (3) that also shows that research efforts in this area seem to lag those in the PAH area and fewer compounds have been screened. However, in some materials, such as those deriving from coals, the NSO-heterocycles can be quite prominent and contribute to a nonnegligible extent to the toxicity. For example, the SARA fractions eluted with methanol (after removal of the nonpolar fractions with n-hexane and dichloromethane) contain more polar compounds, mostly NSO-heterocycles. This fraction from 5 bituminous coals

showed 100% mortality of fish embryos (*Danio rerio*), induced EROD activity (Bio-TEQs between 23 ± 16 and 52 ± 22 ng/g) and revealed mutagenic activity determined using the Ames Fluctuation test (7,15). Such contributions would of course not be possible to account for by measuring the priority PAHs.

PACs WITH HETEROATOMS—NOMENCLATURE

At this point, a brief explanation of the nomenclature used here for PACs containing heteroatoms is in order. "NSO-PAC" is the term used for PACs that somewhere in the molecule contain one (or several) nitrogen, sulfur, or oxygen atoms.

The term "NSO-heterocycles" is used to denote compounds with one (or several) nitrogen, sulfur or oxygen atoms in an aromatic ring. This term can be subdivided into several groups depending on the heteroatom:

- PANHs are polycyclic aromatic nitrogen heterocycles (e.g., indole, acridine; note that the H stands for "heterocycle" and not "hydrocarbon");
- PASHs are polycyclic aromatic sulfur heterocycles (e.g., dibenzothiophene); and
- PAOHs are polycyclic aromatic oxygen heterocycles (e.g., benzonaphthofuran).

It is necessary to distinguish these compounds from PACs with heteroatoms as substituents, for their origin and chemical and biological properties may be quite different. PAHs with a hydroxy group attached to the aromatic ring are termed hydroxy-PAHs, if keto groups are present we have oxy-PAHs (but that ring would not be aromatic anymore), nitro groups lead to nitro-PAHs, amino groups to amino-PAHs, etc.

NSO-HETEROCYCLES AND THE ANALYTICAL CHALLENGE FOR ALKYLATED PACs

Returning to the analysis of NSO-PACs in environmental materials and their prominence in such cases, we note as a case in point an investigation where heterocyclic PACs were identified in a contaminated sediment and, with respect to their induction of cytochrome P4501A as a measure of PAC carcinogenic potency, found to be "significantly more potent than the reference compound, benzo[*a*]pyrene, and among the most potent polycyclic inducers known" (16). An effect-directed fractionation and chemical analysis led to the identification of dinaphthofurans and 2-(2-naphthyl)benzothiophene as among the major cytochrome inducing compounds together with the nonpriority PAHs methylchrysenes and the priority PAH benzo[*a*]anthracene.

This may be considered a special case that cannot be covered by any list of defined compounds since the heterocyclic pollutants were generated as by-products in an industrial process involving a high-volume chemical, 2-naphthol, and entered the environment through uncleaned industrial effluents. No list can reasonably cover all industrial processes or all chemicals involved in them. However, the case illustrates the need to include in any set of compounds monitored a broad selection of not only parent compounds but also NSO-heterocycles as well as alkyl derivatives. Given the well-known toxic potential of heteroatom substituted PACs, such as those with nitro-, oxy-, amino-, and cyano- substituents, the question whether such representatives should also be taken into consideration must be raised. Support for this comes from investigations like those of a coastal sediment, where two mutagenicity maxima in the chromatographic separation of extracted compounds were found, one for 4- to 6-ring PACs and one for polar-substituted nitroarenes and azaarenes (17).

A major difficulty here lies in the fact that a very large number of alkyl derivatives and of NSO-heterocycles are conceivable and that the number rises quickly as the number of carbon atoms goes up. There is only one benzo[*a*]pyrene but it has 12 monomethyl derivatives and, for a modified list, a very strict selection which methylbenzopyrene isomer to include would need to be done. The reason is not whether they can be obtained as standards in pure form, because they can be synthesized once a need appears, but rather that the chosen compound must be of high toxicological relevance, chromatographically resolved, and of frequent occurrence in the environment. Chromatographic resolution may perhaps be achieved for the 12 compounds mentioned, but the issue is far more complex because there are a total of 12 isomers of the elemental composition $C_{20}H_{12}$, benzo[*a*]pyrene being one of them, and altogether there are 192 monomethyl derivatives $C_{21}H_{14}$ of them that would all need to be resolved. It is hardly imaginable that this can be achieved. The intricacies of this requirement for complete resolution are further discussed in (2).

It was said above that alkylaromatics may show similar adverse properties as the parent compounds do and that therefore any attempt at a reasonably correct representation of the toxicity of an environmental sample should include these or at least a more comprehensive selection of them than a few selected individual derivatives. Samples contaminated by petroleum spills would be expected to be particularly vulnerable to severe misjudgement if alkyl derivatives are not included in the analysis. However, no analytical technique is able to resolve all these compounds in a sample—for instance, the total number of isomers of C_1- to C_4 phenanthrenes is 588 compounds!—so that only a sum parameter that registers the sum of all isomers of each degree of alkylation for each PAH would be conceivable. This may be the more relevant concentration to measure rather than that of the parent compound alone, since at least for petroleum contaminated samples the alkylderivatives dominate the pattern. This is especially true in situations where a (partially) biodegraded fossil material constitutes the pollution. Microorganisms preferentially oxidize the parent and lower alkylated compounds so that their concentration does not reflect the toxicity of the whole sample anymore. On the other hand, this is not the only pathway of removal of aromatic compounds in the environment. Photodegradation can be as fast as biodegradation (18) and higher alkylated derivatives are photochemically removed considerably faster than the lower ones, thus changing the sample in the opposite direction to that of biodegradation (9).

The approach of including the lower alkylated PAHs was selected by EPA for the hydrocarbon narcosis risk model, where groups of C_1- to C_4-alkyl-PAHs of 16 PAHs (EPA-34) are included (19). In recent years the topic of the correct analysis of alkyl PACs has received increasing attention and in 2013 a standard reference material, SRM 1991, was issued by NIST to help in analyzing environmental samples (20). Detailed GC-MS studies on alkyl-PACs, including the sulfur aromatic compounds, show that a serious underestimation of the analyte concentration can result if the parent compounds are used as internal standards in GC-MS analysis (21). Very careful attention to retention windows and the use of several ions in the correct ratio are some of the necessary analytical virtues needed to obtain more reliable information (22–24). There are also known cases of dissimilar compounds of close but unresolved molecular weights in GC-MS using low-resolution mass selective detectors, e.g., between C_4-naphthalenes and dibenzothiophene that also have similar GC retention times using common stationary phases (25). Only recording the M^+ ion of C_4-naphthalenes would automatically include dibenzothiophene but these compounds should definitely be measured separately. The monitoring of several ions would be necessary to exclude artifacts that also have to fulfil the criterion of appearing in the correct ratios.

One reason for this uncertainty in GC-MS analysis is that for C_2- and higher alkylaromatics, not only methyl substituents are likely but also longer chains. With electron impact ionization and a monitoring of the M^+ ion, these longer-chain compounds show a lower response due to stronger fragmentation of that alkyl chain. For instance, the M^+ ion of 9-n-propylanthracene is only 25% of that of the base peak (which is formed through loss of CH_2CH_3) but for a trimethyl isomer, 2,3,5-trimethylphenanthrene, the M^+ signal is by far the largest one (26). Therefore, the concentration of a propyl aromatic compound would be severely underestimated as would that of any other aromatic compound with a side chain longer than a methyl group. Other examples for the phenomenon of very different fragmentation patterns for isomers are known, e.g. for dimethylbenzothiophenes (27).

The dearth of standard substances also hampers developments in this area. It seems advisable for future research to investigate if a somewhat more elaborate workup of the sample can reduce the matrix influence and thus improve the analysis, for instance through separation of the sulfur compounds (25) that frequently interfere with the PAH analysis (24). Following such a separation, both the PAHs and the PASHs can be analyzed without mutual interference. Even with these precautions, the correlation between the concentrations found and the toxicity of the sample to organisms is unclear since toxicity equivalence factors are unknown for most of these compounds. Lumping all isomers together and assigning an arbitrary TEF to the mixture leads to an unknown uncertainty since each sample may contain a unique mixture of isomers.

What we propose here is that these C_1-C_4-alkyl PACs are measured after taking all precautions into account with the goal to establish a data base that can be evaluated only after enough experience has been gained on a large variety of environmental samples. What is also urgently needed are more data on the physicochemical and toxicological properties of alkyl PACs. Without these, any toxicological assessment of a sample containing a prominent amount of alkyl-PACs cannot be based on analytical results and expected to lead to a reasonably accurate estimate. This would also mean a synthetic effort since many alkyl-PACs are not known in pure form or are not commercially available. A survey in CAS SciFinder of over 50 trimethylphenanthrenes revealed that only 8 can be purchased and none had been studied for its toxic properties.

However, the challenging task of designing a more adequate list of compounds should not be dismissed or postponed with the argument that the problem is too complex and not enough is known about other compounds that may come into question. Such reticence in tackling a difficult issue may be one of the reasons for the long-lasting tradition of the 16 EPA PAH list.

This state of affairs applies not only to toxicity studies but, as illustrated by Stout et al. (28), also to environmental forensics. Indeed, the EPA PAHs were never meant to be used for such purposes but they are still often included in an investigation. Again, such studies frequently involve petroleum related materials and thus components of petroleum are in the focus of the analysis. A fairly wide-ranging selection of alkyl derivatives and some heterocyclic compounds, for instance sulfur heterocycles of the dibenzothiophene kind, together with carefully selected PAHs and nonaromatic compounds provide considerably more relevant information than the priority PAHs.

OTHER PAH LISTS AND RELEVANCE OF THE DIBENZOPYRENES

In studies of food safety, the 16 EPA PAHs were considered not to be the best choice of relevant compounds. In 2005, the European Commission responded to this by introducing a modified list for food analysis that contains 15+1 PAHs, eight of which are also included among the priority PAHs. The added ones possess four to six aromatic rings and represent known carcinogens, such as several isomers of dibenzopyrene. In this way a more relevant selection of PAHs for the particular purpose of estimating the mutagenic/carcinogenic potential of food contaminants was created. This topic is treated in detail by Zelinkova and Wenzl (29).

A certain urgency to look beyond the priority PAHs, at least in some areas, is already noticeable. For example, the EPA narcosis model for benthic (invertebrate) organisms requires 18 PAHs (16 EPA PAHs + benzo[*e*]pyrene + perylene) and 16 groups of prominent C_1- to C_4-alkyl PAH derivatives, the so-called EPA-34 for contaminated sediments (30).

This development leads to the question if now, about ten years after the re-evaluation of PAH toxicity in food safety, would not be the time, to re-evaluate them in the environmental field, too. If this is attempted, one of the key questions, apart from the toxicity of single PAC compounds and their reliable analytical measurements, clearly is the occurrence in the environment. Are they frequently recorded or only rarely, and are they very (site-)specific compounds? Above, the dibenzopyrene isomers were mentioned as highly toxic PAHs that may be relevant for inclusion on any list of environmentally significant PAHs, but up to today no more than some 40 studies report the detection of these compounds in environmental samples: their presence in the environment has been shown in vehicle exhaust (31–37), air particulate matter (36,38–42), house dust (43), coal tar (31,40,44), coal extracts (7), automobile tires (45), particulates from coal combustion (46, 47), fly ash (48), sediment and soil (31,40,49–55), cigarette smoke (34), and exhaust gases from sewage sludge incineration (56).

This look into the occurrence of only one set of isomers shows that the dibenzopyrenes occur not only in scattered samples but in a wide variety of environments, but of course this needs to be verified through further work that deliberately incorporates these compounds in the analytical approach. On the other hand, their size leads to a poor water solubility—or, more appropriately, a high partition coefficient as described by K_{oc} and K_{ow}—that may reduce their uptake by organisms via the aqueous phase and thus lessen their impact in exposure scenarios where only derivatives with more pronounced water solubility are taken up (pure aqueous phase, neither considering nonaqueous phase liquids, NAPLs, nor particles, including nanoparticles) in an environmental situation. However, this is already the case with benzo[*a*]pyrene (log K_{ow} of 6.1), which is practically insoluble in water, and it may be questioned if the difference in K_{ow} values (vs., e.g., log K_{ow} of 7.7 for dibenzo[*a,l*]pyrene) have meaningful effects in the environment. Here is much room for more detailed research, but at the present time it seems advisable to discuss the inclusion of the dibenzopyrenes in an updated priority PAH list.

THE FUTURE OF PAC PRIORITIZATION

As there are good arguments for modifying or replacing this set of PAHs, especially for environmental work, it may now be the time to make a first concrete proposal of compounds that should be included in such a list. Given the more advanced scientific stand today, it would surely be a more comprehensive list than the EPA PAH one. We agree that no matter how many compounds are included in such a list, it will always be no more than a substitute for a complete analysis of the sample. The criterion of its being manageable in daily practice is of highest concern.

In giving our opinion here, we would like to initiate a discussion on how such a list (lists) should be composed: which compounds are particularly relevant because of their frequent occurrence in the environment, which compounds because of their toxicity, which compounds are needed for particular analytical tasks, etc. Indeed, it may be argued that several lists would be more appropriate, depending on the purpose of the analysis: a set of compounds that is relevant for estimating the toxicity of a sample may not be relevant for the purpose of distinguishing between different sources of the PACs. On the other hand, as few sets of compounds as possible should be in common use to allow for useful comparisons with other laboratories or with different sample types. The EPA list is so entrenched that a proposal to replace it after these 40 years by a list that includes other PAHs that are now known to be the most toxic ones that commonly occur in the environment and are analyzable may not appear to be the first choice. The described advantages of the long-term use of the list in the past would be lost. That is the reason why we propose amending the existing list and add a series of PAHs that appear to be particularly relevant. Thus, an analysis based on this new set of compounds would automatically include the traditional 16 PAHs to allow for an uninterrupted study of time trends. To keep the number of compounds manageable, we limit it to 40 representatives, i.e., the 16 original ones and 24 new ones; see Table 1. Due to their frequent occurrence and toxicity, inevitably alkylated PAHs need to be included. We suggest the inclusion of no more than C_2-alkylated derivatives because of the large possible number of isomers at $>C_3$-alkylated compounds and thus decreasing analytical reliability of them. The choice of the alkylated indicator compounds should again be dictated by their toxicity, the frequency of occurrence and ease of analysis. A suite of higher molecular weight PAHs is also included in this proposed list because, to our current knowledge, they are the most toxic PAHs and it is known that many of them occur in environmental samples. In this area, we see research needed to develop reliable separation and analysis methods of such compounds which – fortunately for the environment but unfortunately for the analytical chemists – often occur at fairly low concentrations. Doubtlessly, toxicity and occurrence of these PAHs and alkylated PAHs need to be better defined and studied.

No heterocyclic compounds are included in this PAH list (Table 1); they would expand the list beyond a reasonable number despite their recorded relevance in many situations. Therefore, we propose establishing a second set of compounds, namely NSO-heterocycles that are likely to be of major concern in similar areas as the compounds on the PAH list. Such lists have already been proposed by experts (58,60) and selected compounds of the combined list are suggested here (Table 2). A further reason for a separate list of heterocycles is that they have other properties than PAHs, for instance with respect to polarity, and may therefore need a different sample preparation to allow for a correct analysis. This arrangement would also fulfil the requirement of the Federal Soil Protection Act in Germany and similar regulations in other countries that mandate the analysis of PAHs and recommend the additional analysis of heterocycles.

Metabolites are listed separately because they may not always be in the focus of an investigation in the environmental sector. Here, too, a discussion about the analysis of alkylated derivatives is desirable because so far they are listed as group parameters (e.g., dimethylbenzofurans that include several isomers).

The concept of toxicity equivalence factors (TEFs, in relation to benzo[*a*]pyrene) has proven to be a helpful tool for evaluating risks and in Table 2, it is obvious that more TEFs for NSO-heterocycles would be beneficial.

Compared with the 16 EPA PAHs, the substituted PACs which include oxy-PAHs and nitro-PAHs are often neglected although their occurrence has often been documented and their toxicity is comparable with that of the PAHs or heterocyles (e.g., TEFs of 10 for 6-nitrochrysene and 1,6-dinitropyrene) (Table 3). The oxy-PAHs are emitted from the same sources as PAHs, because they are both products of incomplete combustion, however, they may additionally be formed through chemical, photo- or biological oxidation of PAHs in the environment. They have been described to be persistent in soils and aquifers (63,64). Particularly in cases where PAH degradation is enhanced, e.g., in remediation of PAH polluted soil and groundwater, oxy-PAHs, which have been proposed to be "dead-end products", can be formed (64). These compounds are expected to be more mobile than the parent PAHs and in the worst case new, even more toxic, contaminants in the remediated remains are formed during these treatments. Oxy-PAHs occur in diesel and gasoline exhaust, flue gases from various combustion processes, fly ash, urban aerosols, sediments, river and coastal waters, sewage sludge, industrial waste, and soil (reviewed in 64).

Due to the significance of these compounds in the environment, attempts are currently underway to include the oxy- and nitro-PAHs in regulative standards (65).

Table 2: Proposed list of 23 NSO-heterocycles in the environment (23 NSO-Heterocycles) and additional metabolites; x = affirmative, x? = possibly affirmative but not certain, (?) = doubtful, ? = unknown

	Compound	Toxicity Equivalence Factor (Benzo[a]-pyrene = 1; max. value) [3]	Carcinogenicity (x = sufficient and limited evidence, 0 = inadequate evidence) [61, 62]	C = carcinogenic, G = genotoxic, M = mutagenic, E = increased eco-toxicity L = effect in luminescence test [58, 60]	At least one carcinogenic/ mutagenic/ celltoxic/ EROD proof available [3]	Commonly occurring in the environment? Authors' evaluation	Analytical separation possible? Authors' evaluation	Modified KORA NSO-PAC list for contaminated sites [58, 60]
23 NSO-Heterocycles								
	PAOH							
1	Benzofuran		x	C, M, G		x	x	x
2	Methylbenzofurans				?	x	?	x
3	Dimethylbenzofurans				?	x	?	x
4	Dibenzofuran			M, G		x	x	x
5	Methyldibenzofuran			E	?	x	?	x
6	Xanthene			L		x	x	x
	PANH							
7	Indole			L		x	x	x
8	Quinoline			C, M, G, L		x	x	x
9	Methylquinolines			C, M, G, E	x	?	?	x
10	Dimethylquinolines				?	x	?	x
11	Isoquinoline			M, G	x	x	x	x
12	Methylisochinolines			M, G	x	?	?	x
13	Carbazole		x	(C), (M), (G)		x	x	x

14	Phenanthridine				x	x	x	x
15	Acridine				x	x	x	x
16	Benzo[h]quinoline				x	?	?	
17	Dibenzo[c,g]carbazole	1	x			x	?	
18	Dibenzo[a,h]acridine	0.1	x			x	?	
19	Dibenzo[a,j]acridine	0.1	x			x	?	
	PASH							
20	Benzothiophene			(G), E	?	x	x	x
21	Methylbenzothiophene			E	?	x	?	x
22	Dibenzothiophene		0					x
23	Methyldibenzothiophene							x
6 NSO-Heterocycles Metabolites								
1	Quinolinone			M, G				x
2	Isoquinolinone							x
3	Methylquinolinones			G				x
4	Acridone			M, G	x	x	?	x
5	Phenanthridone			M, G	x	x	?	x
6	Xanthenone							x

Table 3: Proposed lists of oxy- and nitro-PAHs in the environment (10 Oxy-PAHs, 10 Nitro-PAHs) and additionally amino-PAH; x = affirmative, x? = possibly affirmative but not certain, (?) = doubtful, ? = unknown

	Compound	Toxicity Equivalence Factor (Benzo(a)-pyrene = 1; max. value) (3)	Carcinogenicity (x = sufficient and limited evidence, 0 = inadequate evidence) (3)	Commonly occurring in the environment? Authors' evaluation	Analytical separation possible? Authors' evaluation
10 Oxy-PAHs					
1	1-Indanone			x	?
2	Fluoren-9-one		x	x	?
3	Phenanthrene-9,10-dione		x	x	?
4	2-Methylanthracenedione			x	?
5	Cyclopenta(def)phenanthrenone			x	?
6	Benzo(a)fluorenone			x	?
7	Benzo(de)anthracene-7-one			x	?
8	Benzo(a)anthracene-7,12-dione			x	?
9	Naphthacene-5,12-dione			x	?
10	Benzo(cd)pyren-6-one			x	?
10 Nitro-PAHs					
1	2-Nitronaphthalene		x	x	?
2	3,7-Dinitrofluoranthene		x	x	?
3	3,9-Dinitrofluoranthene		x	x	?
4	1-Nitropyrene	0.1	x	x	?
5	4-Nitropyrene	0.1	x	x	?
6	1,6-Dinitropyrene	10	x	x	?
7	1,8-Dinitropyrene	1	x	x	?
8	6-Nitrochrysene	10	x	x	?
9	7-Nitrobenzo(a)anthracene		x	x	?
10	6-Nitrobenzo(a)pyrene		x	x	?
6 Amino-PAHs					
1	1-Aminonaphthalene		x	x	?
2	2-Aminonaphthalene		x	x	?
3	Aminophenanthrenes		x	x	?
4	2-Aminoanthracene		x	x	?
5	Aminofluoranthenes		x	x	?
6	Aminopyrenes		x	x	?

Less is known about amino- and cyano-PAHs but that does not mean that they may be less toxic than other PACs. Hydroxy-PAHs such as hydroxynaphthalenes, -phenanthrenes, -pyrenes, -benzo[*a*]pyrene or tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenes are compounds of interest in the field of human or animal PAH exposure, metabolism and biomonitoring and should not be included here.

It must be stressed that if the aim of the PAC analysis is source identification, for instance in environmental forensics studies, it is neither recommended to use the 16 nor the 40 PAH list as proposed here, but a specific and fairly wide-ranging selection of alkyl derivatives and selected heterocyclic compounds, for instance sulfur heterocycles of the dibenzothiophene kind, together with carefully selected PAHs and nonaromatic compounds (e.g., alkanes, isoprenoids, steranes, hopanes, levoglucosan, etc.).

CHALLENGES AND FUTURE NEEDS

As was the case when the 16 EPA PAHs were introduced, these new lists of analytes may be a challenge to analytical chemistry. To be useful, it must be ensured that all the compounds are chromatographically resolved from each other and also from other compounds of common appearance in environmental samples, or that a selective detection is available to avoid erroneous results. This puts new demands on analytical chemists and on companies offering stationary phases for this kind of analysis. This is certainly a nontrivial task also with modern developments like GCxGC. For instance, the best separation that could be obtained for 23 isomeric methyl-PAHs with a molecular weight of 242 (e.g., methylbenzophenanthrenes, methylchrysenes, methylbenzoanthracenes), showed that 11 compounds were completely and two were partially separated, whereas the rest still coeluted (66).

We will probably always have the EPA list of the 16 priority PAHs with us, for better or for worse, because for some purposes it is a convenient set of compounds that lets us compare new results with past ones. On the other hand, we imagine that updated lists, which account for progress done in the last several decades, may better reflect the needs of scientists in various areas. It would probably be wrong to imagine that one list, no matter how many compounds are included in it, can be the answer to requirements from all the different areas in which PACs are studied and therefore we feel that the proposed set of PAHs, that combines the long-established PAHs with several representatives of particularly toxic compounds including alkylated derivatives, may more adequately reflect current and evolving needs.

In the future, information about the toxicity of C_1-C_4-alkylated derivatives and other PACs is particularly necessary. Also, the occurrence of known toxic compounds such as, e.g., 3-methylcholanthrene and 7,12-dimethylbenzo[*a*]anthracene needs to be investigated. A promising approach, which has been shown to produce invaluable information on the toxic compounds of a sample, including those that do not appear on any lists, should increasingly be applied, namely the combination of effect-directed and chemical analysis of environmental samples. This is a non target analysis strategy that can be used to identify the most toxic PACs even if they have not been described before.

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