



HAL
open science

Comparative genotoxic potential of 27 polycyclic aromatic hydrocarbons in three human cell lines

Florence Tomasetig, C. Tebby, Vanessa Graillot, F. Zeman, Alexandre R.R. Pery, Jean Pierre J. P. Cravedi, Marc Audebert

► To cite this version:

Florence Tomasetig, C. Tebby, Vanessa Graillot, F. Zeman, Alexandre R.R. Pery, et al.. Comparative genotoxic potential of 27 polycyclic aromatic hydrocarbons in three human cell lines. *Toxicology Letters*, 2020, 326, pp.99-105. 10.1016/j.toxlet.2020.03.007 . hal-02556779

HAL Id: hal-02556779

<https://hal.inrae.fr/hal-02556779>

Submitted on 20 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Comparative genotoxic potential of 27 polycyclic aromatic hydrocarbons in three human cell lines

Tomasetig, F.^{1, §}, Tebby, C.^{2, §}, Graillot, V.¹, Zeman, F.², Pery, A.³, Cravedi, J.P.¹ and Audebert, M.^{1,*}

§ These authors contributed equally to this work.

¹ Toxalim, Université de Toulouse, INRAE, INP-ENVT, INP-EI-Purpan, Université de Toulouse 3 Paul Sabatier, Toulouse, France

² Models for Ecotoxicology and Toxicology Unit (DRC/VIVA/METO), Institut National de l'Environnement Industriel et des Risques (INERIS), Verneuil en Halatte, France

³ UMR ECOSYS, INRA, AgroParisTech, Université Paris-Saclay, Versailles, France

Running title: PAHs genotoxicity screening

* Corresponding author:

Marc AUDEBERT, PhD

ORCID 0000-0001-7898-6912

INRAE-UMR1331, Toxalim, 180 chemin de Tournefeuille BP 93173

31027 Toulouse Cedex 3, France, Phone +33 582 066 311

E-mail address: marc.audebert@inrae.fr

21 **Abstract**

22 Polycyclic Aromatic Hydrocarbons (PAHs) form a family of compounds that are generally
23 found in complex mixtures. PAHs can lead to the development of carcinogenesis. The
24 Toxicity Equivalent Factor (TEF) approach has been suggested for estimating the toxicity of
25 PAHs, however, due to the relative weakness of available data, TEF have not been applied for
26 the risk characterization of PAHs as food contaminants in Europe. The determination of new
27 TEFs for a large number of PAHs could overcome some limitations of the current method and
28 improve cancer risk assessment. The present investigation aimed at deriving new TEFs for
29 PAHs, based on their genotoxic effect measured *in vitro* and analyzed with mathematical
30 models. For this purpose, we used a genotoxicity assay (γ H2AX) with three human cell lines
31 to analyze the genotoxic properties of 27 selected PAHs after 24 h treatment. For 11
32 compounds, we did not detect any genotoxic potential. For the remaining 16 PAHs, the
33 concentration-response for genotoxic effect was modelled with the Hill equation; equivalency
34 between PAHs at low dose was assessed by applying constraints to the model parameters. We
35 developed for each compound, in each cell line, Genotoxic Equivalent Factor (GEF).
36 Calculated GEF for the tested PAHs were similar in all cell lines and generally higher than the
37 TEF usually used. These new equivalent factors for PAHs should improve cancer risk
38 assessment.

39
40
41
42
43 **Keywords:** Polycyclic aromatic hydrocarbons; Modelization; Toxic Equivalent Factor;
44 genotoxicity; H2AX; *in vitro*.

46 Introduction

47 Polycyclic aromatic hydrocarbons (PAHs) are a family of substances containing two or more
48 aromatic rings that includes a few hundred compounds. They occur naturally in coal and
49 crude oils and are present in products made from fossil fuels (Harvey, 1991). They can also
50 result from the combustion or pyrolysis of various organic materials, such as coal, petroleum
51 and wood (Lima et al., 2005). Pyrogenic sources include among others forest fires, volcanic
52 eruptions, industrial facilities and power plants using fossil fuels, smelting, waste incinerators,
53 and combustion-powered vehicles. PAHs are widely distributed in the environment (air,
54 water, soil) and are also present in food (Purcaro et al., 2013). Smoking and grilling food,
55 particularly meat and fish, may contribute substantially to the formation and the intake of
56 PAHs. In the non-smoking general population, the main route of exposure to PAHs is *via* food
57 consumption (EFSA, 2008; IARC, 2010). Although processed fish, food supplement,
58 mollusks, barbequed and grilled meat were reported to be the most contaminated food
59 categories in the European Union, the highest contributors to the dietary exposure were
60 cereals and cereal products, due to the high consumption of these products (EFSA, 2008).
61 Sixteen priority PAHs were recommended by different international agencies for the analysis
62 of environmental and/or food samples (EFSA, 2008; EU, 2002; FAO/WHO, 2005; US-EPA,
63 1993).

64 A number of PAHs tested individually or in complex mixtures, have been shown to be
65 genotoxic *in vitro* and *in vivo* and to be carcinogens in laboratory animals. The International
66 Agency for Research on Cancer (IARC) evaluated the carcinogenicity to humans of 60 PAHs
67 (IARC, 2010). The agency classified benzo[a]pyrene as carcinogenic to humans (Group 1),
68 cyclopenta[c,d]pyrene, dibenz[a,h]anthracene and dibenzo[a,l]pyrene as probably
69 carcinogenic to humans (Group 2A) and benz[j]aceanthrylene, benz[a]anthracene,
70 benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[c]phenanthrene,

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

71 chrysene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, indeno[1,2,3-cd]pyrene and 5-
72 methylchrysene as possibly carcinogenic to humans (Group 2B). It has been clearly
73 demonstrated that tumorigenic PAHs require metabolic activation to electrophilic
74 intermediates and to ultimate carcinogens (Moorthy et al., 2015). As a first step, PAHs are
75 biotransformed by phase I enzymes and peroxidases, giving rise to DNA-reactive metabolites.
76 Then, phase I metabolites can be conjugated to polar metabolites by phase II enzymes.
77 Specific cytochrome P450 isozymes (mainly 1A1, 1A2 and 1B1) and epoxide hydrolase are
78 responsible for the oxidation of many PAHs to form phenols, diols and diol epoxides that are
79 ultimate carcinogenic metabolites. Other bioactivation pathways, such as the formation of
80 radical cations or quinones are known to lead ultimate carcinogenic metabolites.

81 For some chemical mixtures, the risk assessment can be based on an index compound, which
82 is often the compound for which the toxicological data are most robust (Committee et al.,
83 2019). The concept of Toxic Equivalency Factor (TEF), defined as an estimate of the toxicity
84 of a chemical related to that of a reference compound and also referred to as relative potency
85 factor (RPF) was proposed for PAHs by toxicologists and risk assessors (Bostrom et al., 2002;
86 Collins et al., 1998; Delistraty, 1997; Petry et al., 1996; US-EPA, 1993). These factors are
87 used to relate the carcinogenic potential of other PAHs to that of B(a)P. In some cases,
88 however, the concept of TEFs for PAHs is not accepted by risk assessors. In exploring
89 whether a TEF approach of the PAH mixtures in food was appropriate for risk
90 characterisation, the EFSA CONTAM Panel concluded that this approach was not valid
91 because of (1) the lack of data from oral carcinogenicity studies on individual PAHs, (2) their
92 different modes of action and (3) the evidence of poor predictivity of the carcinogenic
93 potency of PAH mixtures based on the currently proposed TEF values. The last two
94 objections lead us to consider the estimation of TEFs based on genotoxic events such as those
95 reflected by the induction of the phosphorylated histone H2AX (γ H2AX). Based on γ H2AX

96 genotoxicity testing, we compared recently the genotoxicity of 13 PAHs and derived
97 corresponding TEFs called genotoxic equivalent factors (GEFs) (Audebert et al., 2012).

98 In the present study, we analysed the genotoxic potential of 27 PAHs comprising the 16
99 priority PAHs. First, the genotoxicity of the 27 selected compounds were tested in three
100 human cell lines representative of PAHs target: hepatoma cell line Hep3B, epithelial
101 colorectal adenocarcinoma cells LS-174T and in bronchioalveolar carcinoma NCI-H358 cell
102 line. The genotoxic potential of each PAHs was tested with the γ H2AX global genotoxic
103 endpoint (Kopp et al., 2019). This assay relies on the detection of the phosphorylation of the
104 histone H2AX that reflects a global genotoxic insult resulting from diverse type of DNA
105 damage, notably DNA adducts and oxidative lesions (Khoury et al., 2013, 2016a, b; Kopp et
106 al., 2019). Using this genotoxicity assay, we previously demonstrated that Hep3B liver cell
107 line was more sensitive than HepG2 to detect PAHs genotoxicity (Khoury et al., 2016b). In a
108 second step, the concentration-response relationship for genotoxic effects of each compound
109 in the three cell lines was modelled and analysed with the Hill equation. This equation is one
110 of the most often applied to dose-response relationships (Walker and Yang, 2005), because of
111 its ability to reflect the binding of ligands to target molecules. Based on this approach, and
112 using a complete dose-response curve covering low dose effects for each PAH, we derived
113 GEFs for PAHs. The GEF values we estimated for 16 PAHs would likely improve the
114 relevance of cancer risk assessment for humans exposed to PAHs.

116 **Material and Methods**

1
2
3 117 *Caution: PAHs are hazardous compounds and should be handled with care in accordance*
4
5 118 *with the NIH guidelines for the laboratory use of chemical carcinogens.*
6
7

8
9 119
10
11
12 120 *Chemicals and Reagents*
13

14
15 121 5-Methylchrysene (5MC), 7,12-Dimethyl-benz[*a*]anthracene (DMBA), acenaphthene (ACT),
16
17 122 acenaphthylene (ACTY), anthracene (ANT), benz[*a*]anthracene (BaA), benzo[*c*]fluorene
18
19 123 (BcF), benzo[*a*]pyrene (BaP), benzo[*b*]fluoranthene (BbF), benzo[*e*]pyrene (BeP),
20
21 124 benzo[*ghi*]perylene (BghiP), benzo[*j*]fluoranthene (BjF), benzo[*k*]fluoranthene (BkF),
22
23 125 chrysene (CHR), coronene (COR), cyclopenta[*c,d*]pyrene (CPP), dibenz[*a,h*]anthracene
24
25 126 (DBahA), dibenzo[*a,h*]pyrene (DBahP), dibenzo[*a,i*]pyrene (DBaiP), dibenzo[*a,l*]pyrene
26
27 127 (DBalP), dibenzo[*a,e*]pyrene (DBaeP), fluoranthene (FLA), fluorene (FLE), indeno[1,2,3-
28
29 128 *cd*]pyrene (IPY), naphthalene (NAP), phenanthrene (PHE), pyrene (PYR) (with chemical purity
30
31
32 129 > 97%) were obtained from AccuStandard (New Haven, USA) and Dr. Ehrenstorfer (CIL,
33
34 130 France) (Fig 1.). All compounds were diluted in dimethyl sulfoxide (DMSO) obtained from
35
36 131 Sigma-Aldrich (Saint Quentin Fallavier, France). Concentration of stock solutions was 5 mM.
37
38 132 Cells were exposed to 0.2% (v/v) DMSO in culture medium.
39
40
41
42
43
44
45 133

46
47
48 134 *Cell Lines and cultures*
49

50
51 135 Hep3B human hepatoblastoma cells (ATCC HB-8064) and LS-174T human epithelial
52
53 136 colorectal adenocarcinoma cells (ATCC CL-188) were cultured in α MEM medium, 10% fetal
54
55 137 calf serum v/v, penicillin (100 U ml⁻¹), streptomycin (100 μ g ml⁻¹), in a 5% CO₂ atmosphere
56
57
58 138 at 37°C. NCI-H358 human bronchioalveolar carcinoma cells (ATCC N° CRL-5807) were
59
60
61
62
63
64
65

139 cultured in RPMI-1640 medium, 10% fetal calf serum v/v, penicillin (100 U ml⁻¹),
140 streptomycin (100 µg ml⁻¹), in a 5% CO₂ atmosphere at 37°C.

141

142 *γH2AX In-Cell Western (ICW) assay*

143 The γH2AX In-Cell Western technique was performed as previously described (Audebert et
144 al., 2010; Audebert et al., 2012). This genotoxicity assay is based on histone H2AX
145 phosphorylation (named γH2AX) quantification. This biomarker has been linked to different
146 type of DNA damage including DNA bulk adducts as with PAHs exposure (Kopp et al.,
147 2019). To determine cytotoxicity, the DNA content (as a surrogate to the number of cells)
148 recorded in the different treated cells was compared to the DNA content in control cells and
149 was expressed as relative cell count (% RCC).

150

151 *Modelling genotoxicity of individual PAHs*

152 The toxic loads of chemicals with a similar mode of action can be expressed relative to one
153 reference compound by means of Toxic Units (Sprague, 1970) and Toxic Equivalency
154 Factors, often based on EC₅₀ ratios. This concept is only valid when all compounds can elicit
155 the same responses. An important feature of our data is that the maximum response levels
156 depend on the PAH studied; Toxic Units could therefore only be calculated for a limited range
157 of responses. Specific methods have been developed to deal with the analysis of partial
158 agonists, often encountered in the field of pharmacology in receptor-ligand relationships
159 (Dinse and Umbach, 2011; Dinse and Umbach, 2012; Gennings et al., 2004). In gene
160 expression data, supramaximal responses (also called superinduction) are often observed, for
161 example in oestrogenicity assays where the underlying mechanisms of superinduction are not

162 yet fully understood, and it is unclear whether this mechanism is relevant *in vivo* (Montano et
1
2 163 al., 2010; Sotoca et al., 2010). According to Dardenne et al. (2008), in gene induction data,
3
4 164 the maximum induction level for every gene reporter is not only determined by its intrinsic
5
6
7 165 regulatory mechanism but also is dependent on the nature of the inducing compound and on
8
9
10 166 the cytotoxicity. The EC_{50} therefore do not have any true biological meaning and toxic
11
12 167 equivalents cannot be based on the EC_{50} . This is a major impediment to the analysis of gene
13
14 168 expression data. We analyse genotoxicity using all dose-response data available, including
15
16
17 169 high response levels, and compare PAH genotoxicity based on the slope at low doses.

170 In the present paper, genotoxicity was represented as the fold-induction relative to the mean
21
22 171 induction in the control on the plate. Read-outs from duplicate wells were averaged. The log-
23
24
25 172 fold-induction was used to avoid heteroscedasticity due to larger variance of the higher
26
27 173 responses (Charles et al., 2002; Mumtaz et al., 2002) and has been used in oestrogen receptor
28
29
30 174 (ER) transcriptional activation assay result analysis (Gennings et al., 2003) and in micro-array
31
32 175 data analysis (Huber et al., 2002; McCarthy and Smyth, 2009).

33
34
35 176 The log- fold-induction dose-response relationship for individual chemicals was modelled by
36
37
38 177 least squares using a 3-parameter Hill (or log-logistic) model which is commonly used to
39
40 178 model dose-response relationships (Khinkis et al., 2003):

$$179 \quad \Phi(x) = \frac{Max}{1 + \left(\frac{x}{EC50}\right)^\beta}$$

41
42
43
44
45
46
47
48
49
50 180 where Max is the maximal response, $EC50$ is the concentration that induces a 50% effect, β is
51
52 181 the Hill slope, hereafter referred to as the Hill constant, and x is the PAH concentration.

53
54
55 182 At low doses, the Hill model simplifies into:

$$59 \quad 183 \quad \Phi(x) = ax^{-\beta} \text{ where } a \text{ is the slope at low doses, } a = \frac{Max}{EC^{-\beta}} \text{ (Audebert et al., 2012).}$$

184 Statistical tests were used to identify which PAHs were genotoxic and whether the model was
1
2 185 adequate. Genotoxic PAHs were identified as showing a significant difference in fit between
3
4 186 the 3-parameter Hill model and a Hill model with Max or β equal to 0, using an F-test. The
5
6
7 187 goodness of fit of the 3-parameter Hill model (full model) was assessed with a lack-of-fit test,
8
9
10 188 an approximate F-test which compares the dose-response model to the ANOVA model with a
11
12 189 separate level for each dose (Bates and Watts, 1988; Ritz and Martinussen, 2011). Adequacy
13
14 190 of a common Hill constant was checked by comparing the goodness of fit of the full model
15
16
17 191 with that of the reduced model with a common Hill constant β for all genotoxic PAHs. The
18
19 192 comparison was performed with an F-test analogous to the one used in nested models:

$$F = \frac{(SS_{M1} - SS_{M2}) / (p_{M2} - p_{M1})}{SS_{M2} / (n - 1 - p_{M2})}$$

194 where SS is the residual sum of squares, p is the number of model parameters, and n is the
27
28
29
30 195 number of observations. M1 refers to the reduced model and M2 refers to the full model.

31
32
33 196 Once genotoxicity of each compound had been assessed, genotoxicity equivalent factors
34
35 197 (GEF) of each PAH compared to BaP were then calculated as in (Audebert et al., 2012) by
36
37
38 198 first estimating a common value of the Hill constant β for all genotoxic PAHs and then
39
40 199 calculating the β -root of the ratios of a values. Finally, the differences in GEFs between the
41
42
43 200 three cell lines were analysed by calculating correlation coefficients between the logarithm of
44
45 201 the GEFs.

202 *Experimental designs*

50
51 203 Individual dose-responses were tested at a minimum of three geometrically-spaced
52
53
54 204 concentrations on independent plates, with at least three concentrations per plate. The
55
56 205 concentrations were selected based on previous range-finding experiments. With LS-174T
57
58
59 206 and NCI-H358 cell lines the highest concentration tested was at most 10 μ M. When high

207 cytotoxicity was observed (more than 50%), data was discarded from analysis. In LS-174T
208 cells, the number of independent experiments for each compound varied from 8 to 56, most
209 experiments being performed with 4 concentrations. With Hep3B and NCI-H358 cell lines,
210 the number of experiments varied from 3 to 7.

211

212 *Uncertainty estimation*

213 The uncertainty around the slopes at low doses was estimated by a 2-level bootstrap: at each
214 iteration, for both the genotoxic PAH and BaP, plates were sampled with replacement, and for
215 each of the sampled plates, at each concentration, the two wells were sampled with
216 replacement. The geometric mean of the two wells was modelled with a Hill model with a
217 fixed Hill constant: the final points could be either one of the two original wells or the mean
218 of the two. The GEF was calculated at each iteration based upon the slopes at low
219 concentrations for the genotoxic PAH and BaP. The 95% confidence interval was estimated
220 using the 2.5% and 97.5% quantiles.

221 Results

1
2
3 222 The sensitive genotoxic γ H2AX assay was used to determine the genotoxicity of 27 different
4
5 223 PAHs (Fig. 1) in three human cell lines (Hep3B, LS-174T and NCI-H358). All PAHs were
6
7
8 224 analyzed for cytotoxicity and genotoxicity after 24 h treatment at different concentrations
9
10 225 covering a four order of magnitude range. Only no and low-cytotoxic concentrations (cell
11
12 226 viability > 50%) were analyzed for genotoxicity. Eleven compounds (ACT, ACTY, ANT,
13
14 227 BghiP, COR, BeP, FLA, FLE, NAP, PHE and PYR) were found to be non-genotoxic,
15
16
17 228 whatever the cell line tested (F- test, $p > 0.01$) (Fig, S1-S3). In all cell lines, the most genotoxic
18
19 229 tested compound was DBaP, with a genotoxic potential at concentrations 10^{-9} M and
20
21 230 upwards in NCI-H358 and Hep3B cells and at concentrations 10^{-10} M and upwards in LS-
22
23 231 174T cell line (Table 1). A clear genotoxic dose-response effect was observed for 16 PAHs
24
25 232 tested in LS-174T cells (Table 1; Fig. S4). The estimated common Hill constant for the 16
26
27 233 genotoxic compounds was equal to -0.678; using this common slope resulted in a decrease in
28
29 234 goodness of fit only for DBaP and DBaP at a 1% significance threshold. The GEFs are
30
31 235 reported together with the dose-response parameters in Table 2.
32
33
34
35

36
37 236 Unlike in LS-174 cells, BcF did not demonstrate genotoxic potential in the two other cell
38
39 237 lines. CPP was not genotoxic in the NCI-H358 cell line and CPP and IPY were not genotoxic
40
41 238 in the Hep3B cell line (Table 1). With the Hep3B cell line, for 6 compounds (5MC, BaP,
42
43 239 DBaP, DBaP, DBaP, and DMBA), the 2-parameter exponential model did not fit as well as
44
45 240 the 3-parameter Hill model (Fig. S5). The estimated common Hill constant for the 13
46
47 241 genotoxic compounds in Hep3B was equal to -1.86 and was acceptable for all genotoxic
48
49 242 PAHs (F-test, $p > 1\%$) (Table 2).
50
51
52

53
54 243 With the NCI-H358 cell line, for 8 compounds 5MC, BaA, BbF, CHR, DBaP, DBaP,
55
56 244 DBaP, and IPY), the 3-parameter Hill model was selected (Fig. S6). The estimated common
57
58
59
60
61
62
63
64
65

245 Hill constant for the 15 genotoxic compounds was equal to -1.01 and was acceptable for all
1
2 246 genotoxic PAHs. The dose-response parameters and GEF are reported in Table 2.
3
4 247 We noted that for most compounds, GEFs calculated in LS-174T cells were lower than the
5
6
7 248 GEFs calculated for the two other cell lines (Table 2). The correlation coefficients between
8
9
10 249 the logarithms of the GEFs for genotoxic PAHs in all cell lines shows that Hep3B and NCI-
11
12 250 H358 cell lines produce very similar GEFs ($r=0.95$) (Table 3). The LS-174T cell line
13
14 251 produces slightly different GEFs (approximately $r=0.8$ on logGEFs of the two other cell lines)
15
16
17 252 still demonstrating a good agreement between the results from the three cell lines. This good
18
19 253 correlation between GEFs and between cell lines, demonstrated that despite different levels of
20
21
22 254 GEFs in each cell line, partly due to variation in potency of the reference compound, relative
23
24 255 genotoxicity of each tested PAH was conserved between cell lines. The greatest differences in
25
26 256 relative genotoxicity are observed for DBaiP and BkF.

27
28
29
30 257
31
32 258
33
34
35 259
36
37 260
38
39
40 261
41
42 262
43
44
45 263
46
47 264
48
49
50 265
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

266 Discussion

1
2
3 267 In this study, we reported a detailed analysis of the genotoxicity of 27 different PAHs in three
4
5 268 human cell lines derived from target organs of PAHs. The LS-174T intestinal cell line was on
6
7 269 average more sensitive to the genotoxicity of PAHs than hepatoma Hep3B or lung NCI-H358
8
9
10 270 cell lines. This difference of PAHs genotoxicity between organs have also be observed *in vivo*
11
12 271 (Long et al., 2016). PAHs are genotoxic after metabolization by some cytochrome P450
13
14
15 272 isozymes or aldo-keto reductases (Xue and Warshawsky, 2005). The difference, in terms of
16
17 273 genotoxic sensitivity, between the three cell lines tested may be explained by the difference in
18
19
20 274 CYP expression and induction (Iwanari et al., 2002; Li et al., 1998). Iwanari and co-workers
21
22 275 found that in LS180 cells (parent cells from which LS-174T are derived), the constitutive
23
24 276 expression levels of CYP1A1, 1A2, and 1B1, the main CYPs involved in PAHs metabolism,
25
26
27 277 are expressed at higher levels than in other human-tissue derived cells lines such as MCF7,
28
29
30 278 HepG2, ACHN or Hela. In addition, the induction of CYP1 family by PAHs was found to
31
32 279 occur at a higher extent in LS180 cells compared to the other cell lines tested (Iwanari et al.,
33
34 280 2002).

35
36
37 281 In our study, no statistically significant difference to control was shown for 11 PAHs (ACT,
38
39 282 ACTY, ANT, BeP, BghiP, COR, FLA, FLE, NAP, PHE and PYR) on any of the three human
40
41
42 283 cell lines tested. These data are in accordance with other studies showing that most of them
43
44 284 were neither mutagenic nor genotoxic (Vaca et al., 1992, Durant et al. 1996; Sjogren et al.
45
46 285 1996; Long et al., 2016). Although the carcinogenic potential of some of them was considered
47
48
49 286 as equivocal (Stocker et al., 1996; Wang and Busby, 1993; Wang et al., 1995), most of the
50
51
52 287 published data do not support a carcinogenic effect of these PAHs (Table 4) (IARC, 2010).
53
54 288 ACTY was not classified by IARC. ACT, ANT, BeP, BghiP, COR, FLA, FLE, PHE and PYR
55
56 289 were classified 3 “not classifiable as to its carcinogenicity to humans” by IARC. Only NAP
57
58
59 290 was classified 2B “possibly carcinogenic to humans”. However, different reviews on the
60
61
62
63
64
65

291 genotoxicity of NAP have concluded that this PAH is not mutagenic in bacterial or
292 mammalian cell culture systems and is not genotoxic *in vivo* in either the liver or the bone
293 marrow (Recio et al., 2012). In contrast, most of the 16 PAHs showing a genotoxic potential
294 in our study were classified as 2A (CPP, DBahA and DBaP) or 2B (5MC, BaA, BbF, BkF,
295 BkF, CHR, DBahP, DBaP and IPY) by IARC (Table 4). The only exceptions were BcF and
296 DBaP that were classified in group 3. DMBA was not classified by IARC (IARC, 2010).

297 Formation of DNA adducts by genotoxic carcinogens and subsequent repair were expected to
298 be proportional to dose as long as the rates of the enzymatic and non-enzymatic activation and
299 inactivation reactions are all proportional to the substrate concentration. At low doses, this is
300 modelled with a Hill constant equal to 1 or -1. With LS-174T cells, the Hill constant equal to -
301 0.678 indicates supralinearity at low doses; with Hep3B cells, -1.86 indicates sublinearity at
302 low doses whereas with NCI-H358 cells, the constant is close to -1, indicating linearity
303 (Figure S7).

304 The GEFs calculated in this paper based on LS-174T cell line experimentations differ from
305 those published in 2012 by a factor 3.6 on average (Table 4). This difference is due to the fact
306 that here we modelled all concentration-responses with the same model whereas, in our
307 previous study (Audebert et al., 2012), BaP and DBaP were modelled directly with the 2-
308 parameter exponential model. With BaP, the reference for the calculation of the GEFs, the 2-
309 parameter exponential function overpredicted the response for the three lowest concentrations
310 thus yielding a lower estimate of the slope at low concentrations for BaP and consequently
311 higher GEFs for the other PAHs. The rather high within-treatment variability of the data had
312 been taken into account in the statistical test therefore leading to the conclusion that the
313 goodness of fit of the 3-parameter Hill model was not significantly different from that of the
314 2-parameter model. The choice of concentration-responses model is critical for the estimation
315 of the slope at low concentrations, far more than for the estimation of an EC_{50} in the case of

316 sigmoidal concentration-responses. With most PAHs a sigmoidal concentration-response
1
2 317 relationship with a plateau at high concentrations could not be observed due to cytotoxicity at
3
4
5 318 high concentrations. On the other hand, the inflexion point (*EC50*) was often readily
6
7 319 observed, with some uncertainty. We therefore settled on the use of a common model for all
8
9
10 320 PAHs with only the *Max* and *EC50* allowed varying from one to another. The slight
11
12 321 mispredictions that can be made with the 2-parameter exponential model on one compound
13
14 322 are likely to occur on other PAHs; the choice of a common model ensures that the same bias
15
16
17 323 is obtained on all concentration-responses if the actual concentration-responses are similar.
18
19 324 Another solution would consist in testing a large number of different models and selecting the
20
21
22 325 best fitting model (Scholze et al., 2001). In our application, this could impair the calculation
23
24 326 of the slope at low concentrations which, if estimable, would possibly not be valid for the
25
26
27 327 same range of response levels. The fact that we modelled log fold-inductions without
28
29 328 adjusting for heteroscedascity rather than fold-inductions taking heteroscedasticity into
30
31
32 329 account only affects the GEF to a small extent although the concentration-response parameter
33
34 330 estimates are different. The discrepancy for the BcF, pinpointed by our 2012 paper as being
35
36
37 331 more genotoxic than expected, was likely due to a lack of purity of the commercial solution in
38
39 332 this previous study: the present LS-174T GEFs are based on a larger amount of data.
40
41
42 333 The method we propose can be used to calculate Equivalency Factors even when the plateau
43
44 334 of the concentration-response relationships is not observed. TEFs are calculated as a ratio of
45
46
47 335 *EC50*. They are based on the assumption that the concentration-response curves are similar
48
49 336 (same *EC50*, same *Max*) and their values are independent of the response level chosen. GEFs
50
51
52 337 on the other are not equivalent to TEFs based on a low proportion (e.g. 1%) of maximal
53
54 338 response, as they are calculated as a ratio of slopes at low concentrations, the concentration-
55
56
57 339 response curves being modelled with a common Hill constant estimated separately for each
58
59 340 cell line. The GEFs are actually similar to TEFs based on a low response level (rather than a

341 proportion of the maximal response). The correlation coefficient between the logarithm of the
1
2 342 GEF and the log-concentration producing a 0.02 log-fold-induction (1.05 fold induction) is
3
4 343 0.9996. The advantage of our method is that there is no need for a user-defined response level
5
6
7 344 as we rely upon function approximation. Our method could be applied by only using data
8
9
10 345 from the low dose area of the concentration-response curve, with more closely spaced
11
12 346 dilutions. In this case, the 2-parameter function could be used directly. The experimental
13
14 347 design would however require a more precise design prior knowledge of the location of the
15
16
17 348 low concentration area or of the “point of departure” and may therefore require a higher
18
19 349 experimental effort.

22 350 The LS-174T cell line was the most responsive one as 16 PAHs demonstrated genotoxicity,
23
24
25 351 compared to 14 in NCI-H358 and 13 in Hep3B cell lines. The LS-174T cells were also the
26
27 352 only ones to show supralinearity at low concentrations, thus making it a more suitable
28
29
30 353 biological model for studying low dose effects. The important cytotoxicity observed in Hep3B
31
32 354 at high concentrations makes it unsuitable for a reliable analysis of the concentration-response
33
34
35 355 of the least genotoxic compounds at high concentrations. On this basis, LS-174T cell line
36
37 356 appears as a good candidate for further study of the genotoxicity of PAH, notably in mixtures.
38
39 357 Table 4 shows the GEFs together with their confidence interval determined by bootstrap for
40
41
42 358 each compounds in each cell line. Calculated GEF for the tested PAHs were quite similar in
43
44
45 359 all cell lines with in most case an overlap of the confidence interval (Table 4). Generally,
46
47 360 GEFs were higher than the TEF usually used, notably for BaA, BbF and CHR (Nisbet and
48
49 361 LaGoy, 1992).

52 362 In a previous study, we started to model the GEFs for a limited number of PAHs, tested for
53
54
55 363 genotoxicity in two different cell lines, HepG2 and LS-174T (Audebert et al., 2012). In the
56
57 364 current article, the approach was expanded to a larger number of compounds and the
58
59
60 365 genotoxicity was tested on two different cell lines, in addition to LS-174T cells. As reported

366 by (Delistraty, 1997; Nisbet and LaGoy, 1992), our calculation is based on cancer-related
367 endpoint data, and our approach assumes that carcinogenic PAHs have a toxicological mode
368 of action similar to the model compound BaP. In addition to the fact that the data we have
369 produced are based on metabolically active human cell lines derived from the main PAH
370 target tissues and that γ H2AX assay allows genotoxicity testing at low concentrations, these
371 data offer the advantage of resulting from identical bioassay systems and from the same
372 mathematical treatments of the set of concentration-response curves, which makes the results
373 highly comparable. These methodological advances as well as the large number of PAHs
374 tested largely respond to the uncertainties highlighted by EFSA regarding the TEFs reported
375 so far in the literature (EFSA, 2008). The values we have obtained are all based on the
376 genotoxicity of PAHs and can be considered as predictive of the carcinogenic potential of
377 these substances. As recommended by the US EPA (1993), these values should therefore only
378 be used in relation to the carcinogenicity of PAHs, which is, however, the most sensitive
379 effect observed for these compounds. Conversely, the approach used by EFSA in its 2008 risk
380 assessment has significant uncertainties (EFSA, 2008). The EFSA CONTAM Panel based the
381 risk characterization on a two years experimental study carried out in rats orally exposed to a
382 complex PAH mixture (coal tar) or to B(a)P (Culp et al., 1998). In addition to the limitations
383 related to animal-human extrapolations, the CONTAM Panel assumed that the PAH mixture
384 tested by Culp and co-workers was representative of the dietary exposure of consumers to
385 PAHs, but it is likely that the data provided Culp et al. (1998) are specific to the mixtures
386 tested. In order to improve the consistency of GEF approach in risk assessment, it is obvious,
387 that toxicokinetic adjustments are needed to account for both routes of exposure and relative
388 concentrations in target tissues in the calculation of our TEFs.

389 In addition to the incorporation of toxicokinetic descriptors, the next step of this work will be
390 to investigate the genotoxicity of these PAHs as binary or complex mixtures, in order to

391 determine how interactions between different hydrocarbons can affect the cancer-related

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

392 endpoint responses.

393

394 **Funding**

1
2
3 395 This research was funded by the ANSES PNREST program and the “Institut National Centre
4
5 396 le cancer” (Contract N° 2010-371).

6
7
8 397

9
10
11 398

12
13 399 **Conflict of interest statement**

14
15
16 400 The authors are not aware of any conflicts of interest.

17
18 401

19
20
21 402

22
23
24 403 **Author contributions**

25
26
27 404 T.F. and G.V. performed all of the genotoxicity screening experiments. Data analysis and
28
29
30 405 mathematical modelling were performed by T.C. Z.F. and P.A. M.A. and C.JP. planned and
31
32
33 406 supervised the project. The manuscript was written by M.A. and C.JP. All the authors
34
35 407 discussed the results and contributed to editing of the manuscript.

408 **References**

- 1
2 409 Audebert, M., Riu, A., Jacques, C., Hillenweck, A., Jamin, E.L., Zalko, D., Cravedi, J.P., 2010. Use of the
3
4 410 gammaH2AX assay for assessing the genotoxicity of polycyclic aromatic hydrocarbons in human cell
5
6 411 lines. *Toxicol Lett* 199, 182-192.
- 7
8 412 Audebert, M., Zeman, F., Beaudoin, R., Pery, A., Cravedi, J.P., 2012. Comparative potency approach
9
10 413 based on H2AX assay for estimating the genotoxicity of polycyclic aromatic hydrocarbons. *Toxicol*
11
12 414 *Appl Pharmacol* 260, 58-64.
- 13
14 415 Bates, D.M., Watts, D.G., 1988. *Nonlinear Regression Analysis and Its Applications*. Wiley Series.
- 15
16 416 Bostrom, C.E., Gerde, P., Hanberg, A., Jernstrom, B., Johansson, C., Kyrklund, T., Rannug, A.,
17
18 417 Tornqvist, M., Victorin, K., Westerholm, R., 2002. Cancer risk assessment, indicators, and guidelines
19
20 418 for polycyclic aromatic hydrocarbons in the ambient air. *Environ Health Perspect* 110 Suppl 3, 451-
21
22 419 488.
- 23 420 Charles, G.D., Gennings, C., Zacharewski, T.R., Gollapudi, B.B., Carney, E.W., 2002. An approach for
24
25 421 assessing estrogen receptor-mediated interactions in mixtures of three chemicals: a pilot study.
26
27 422 *Toxicological sciences : an official journal of the Society of Toxicology* 68, 349-360.
- 28
29 423 Collins, J.F., Brown, J.P., Alexeeff, G.V., Salmon, A.G., 1998. Potency equivalency factors for some
30
31 424 polycyclic aromatic hydrocarbons and polycyclic aromatic hydrocarbon derivatives. *Regul Toxicol*
32
33 425 *Pharmacol* 28, 45-54.
- 34 426 Committee, E.S., More, S.J., Bampidis, V., Benford, D., Bennekou, S.H., Bragard, C., Halldorsson, T.I.,
35
36 427 Hernández-Jerez, A.F., Koutsoumanis, K., Naegeli, H., Schlatter, J.R., Silano, V., Nielsen, S.S., Schrenk,
37
38 428 D., Turck, D., Younes, M., Benfenati, E., Castle, L., Cedergreen, N., Hardy, A., Laskowski, R., Leblanc,
39
40 429 J.C., Kortenkamp, A., Ragas, A., Posthuma, L., Svendsen, C., Solecki, R., Testai, E., Dujardin, B., Kass,
41
42 430 G.E., Manini, P., Jeddi, M.Z., Dorne, J.-L.C., Hogstrand, C., 2019. Guidance on harmonised
43
44 431 methodologies for human health, animal health and ecological risk assessment of combined
45
46 432 exposure to multiple chemicals. *EFSA Journal* 17, e05634.
- 47 433 Culp, S.J., Gaylor, D.W., Sheldon, W.G., Goldstein, L.S., Beland, F.A., 1998. A comparison of the
48
49 434 tumors induced by coal tar and benzo[a]pyrene in a 2-year bioassay. *Carcinogenesis* 19, 117-124.
- 50
51 435 Dardenne, F., Nobels, I., De Coen, W., Blust, R., 2008. Mixture toxicity and gene inductions: Can we
52
53 436 predict the outcome? *Environmental Toxicology and Chemistry* 27, 509-518.
- 54
55 437 Delistraty, D., 1997. Toxic equivalency factor approach for risk assessment of polycyclic aromatic
56
57 438 hydrocarbons. *Toxicological & Environmental Chemistry* 64, 81-108.
- 58 439 Dinse, G.E., Umbach, D.M., 2011. Characterizing non-constant relative potency. *Regul Toxicol*
59
60 440 *Pharmacol* 60, 342-353.

441 Dinse, G.E., Umbach, D.M., 2012. Parameterizing Dose-Response Models to Estimate Relative
1 442 Potency Functions Directly. *Toxicological Sciences* 129, 447-455.
2
3 443 EFSA, 2008. Polycyclic aromatic hydrocarbons in food. *The EFSA journal* 724, 1-114.
4
5 444 Gennings, C., Carter, W.H., Carney, E.W., Charles, G.D., Gollapudi, B.B., Carchman, R.A., 2004. A novel
6 445 flexible approach for evaluating fixed ratio mixtures of full and partial agonists. *Toxicological Sciences*
7 446 80, 134-150.
8
9 447 Gennings, C., Charles, G.D., Gollapudi, B.B., Zackarewski, T., Carney, E.W., 2003. Analysis of resulting
10 448 data from estrogen receptor reporter gene assays. *JABES* 8.
11
12 449 Harvey, R.G., 1991. Polycyclic aromatic hydrocarbons: Chemistry and carcinogenicity. Cambridge
13 450 monographs on cancer research V1.
14
15 451 Huber, W., von Heydebreck, A., Sultmann, H., Poustka, A., Vingron, M., 2002. Variance stabilization
16 452 applied to microarray data calibration and to the quantification of differential expression.
17 453 *Bioinformatics* 18 Suppl 1, S96-104.
18
19 454 IARC, 2010. Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures.
20 455 IARC Monographs on the Evaluation of Carcinogenic Risks to Humans V92.
21
22 456 Iwanari, M., Nakajima, M., Kizu, R., Hayakawa, K., Yokoi, T., 2002. Induction of CYP1A1, CYP1A2, and
23 457 CYP1B1 mRNAs by nitropolycyclic aromatic hydrocarbons in various human tissue-derived cells:
24 458 chemical-, cytochrome P450 isoform-, and cell-specific differences. *Arch Toxicol* 76, 287-298.
25
26 459 Khinkis, L.A., Levasseur, L., Faessel, H., Greco, W.R., 2003. Optimal design for estimating parameters
27 460 of the 4-parameter hill model. *Nonlinearity in biology, toxicology, medicine* 1, 363-377.
28
29 461 Kopp, B., Khoury, L., Audebert, M., 2019. Validation of the gammaH2AX biomarker for genotoxicity
30 462 assessment: a review. *Arch Toxicol*.
31
32 463 Li, W., Harper, P.A., Tang, B.K., Okey, A.B., 1998. Regulation of cytochrome P450 enzymes by aryl
33 464 hydrocarbon receptor in human cells: CYP1A2 expression in the LS180 colon carcinoma cell line after
34 465 treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin or 3-methylcholanthrene. *Biochem Pharmacol*
35 466 56, 599-612.
36
37 467 Lima, A.L.C., Farrington, J.W., Reddy, C.M., 2005. Combustion-Derived Polycyclic Aromatic
38 468 Hydrocarbons in the Environment—A Review. *Environmental Forensics* 6, 109-131.
39
40 469 McCarthy, D.J., Smyth, G.K., 2009. Testing significance relative to a fold-change threshold is a TREAT.
41 470 *Bioinformatics* 25, 765-771.
42
43 471 Montano, M., Bakker, E.J., Murk, A.J., 2010. Meta-analysis of Supramaximal Effects in In Vitro
44 472 Estrogenicity Assays. *Toxicological Sciences* 115, 462-474.
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

473 Mumtaz, M.M., Tully, D.B., El-Masri, H.A., De Rosa, C.T., 2002. Gene induction studies and toxicity of
1 474 chemical mixtures. *Environ Health Perspect* 110 Suppl 6, 947-956.
2
3 475 Nisbet, I.C., LaGoy, P.K., 1992. Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons
4 476 (PAHs). *Regul Toxicol Pharmacol* 16, 290-300.
5
6 477 Petry, T., Schmid, P., Schlatter, C., 1996. The use of toxic equivalency factors in assessing
7 478 occupational and environmental health risk associated with exposure to airborne mixtures of
8 479 polycyclic aromatic hydrocarbons (PAHs). *Chemosphere* 32, 639-648.
9
10 480 Purcaro, G., Moret, S., Conte, L.S., 2013. Overview on polycyclic aromatic hydrocarbons: occurrence,
11 481 legislation and innovative determination in foods. *Talanta* 105, 292-305.
12
13 482 Recio, L., Shepard, K.G., Hernandez, L.G., Kedderis, G.L., 2012. Dose-response assessment of
14 483 naphthalene-induced genotoxicity and glutathione detoxication in human TK6 lymphoblasts.
15 484 *Toxicological sciences : an official journal of the Society of Toxicology* 126, 405-412.
16
17 485 Ritz, C., Martinussen, T., 2011. Lack-of-fit tests for assessing mean structures for continuous dose-
18 486 response data. *Environ Ecol Stat* 18, 349-366.
19
20 487 Scholze, M., Boedeker, W., Faust, M., Backhaus, T., Altenburger, R., Grimme, L.H., 2001. A general
21 488 best-fit method for concentration-response curves and the estimation of low-effect concentrations.
22 489 *Environmental toxicology and chemistry* 20, 448-457.
23
24 490 Sotoca, A.M., Bovee, T.F.H., Brand, W., Velikova, N., Boeren, S., Murk, A.J., Vervoort, J., Rietjens,
25 491 I.M.C.M., 2010. Superinduction of estrogen receptor mediated gene expression in luciferase based
26 492 reporter gene assays is mediated by a post-transcriptional mechanism. *Journal of Steroid*
27 493 *Biochemistry and Molecular Biology* 122, 204-211.
28
29 494 Sprague, J.B., 1970. Measurement of Pollutant Toxicity to Fish II. Utilizing and Applying Bioassay
30 495 Results. *Water research* 4, 3-32.
31
32 496 Stocker, K.J., Howard, W.R., Statham, J., Proudlock, R.J., 1996. Assessment of the potential in vivo
33 497 genotoxicity of fluoranthene. *Mutagenesis* 11, 493-496.
34
35 498 US-EPA, 1988. COMPARATIVE POTENCY APPROACH FOR ESTIMATING THE CANCER RISK ASSOCIATED
36 499 WITH EXPOSURE TO MIXTURES OF POLYCYCLIC AROMATIC HYDROCARBONS. U.S. Environmental
37 500 Protection Agency, Washington, D.C., EPA/600/R-95/108.
38
39 501 US-EPA, 1993. Provisional guidance for quantitative risk assessment of polycyclic aromatic
40 502 hydrocarbons. EPA/600/R-93/089.
41
42 503 Wang, J.S., Busby, W.F., Jr., 1993. Induction of lung and liver tumors by fluoranthene in a
43 504 preweanling CD-1 mouse bioassay. *Carcinogenesis* 14, 1871-1874.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

505 Wang, J.S., Busby, W.F., Jr., Wogan, G.N., 1995. Formation and persistence of DNA adducts in organs
1 of CD-1 mice treated with a tumorigenic dose of fluoranthene. *Carcinogenesis* 16, 2609-2616.
2
3
4 507 Xue, W., Warshawsky, D., 2005. Metabolic activation of polycyclic and heterocyclic aromatic
5 508 hydrocarbons and DNA damage: a review. *Toxicol Appl Pharmacol* 206, 73-93.
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

509 Table 1: Lowest effective concentration (in μM) for γH2AX induction for each genotoxic
 510 PAH in the three cell lines tested.

511

Compound	CAS number	NCI-H358	Hep3B	LS-174T
5-Methylchrysene (5MC)	3697-24-3	10^{-7}	10^{-7}	10^{-7}
7,12-Dimethylbenz(a)anthracene (DMBA)	57-97-6	10^{-7}	10^{-7}	10^{-8}
Benz(a)anthracene (BaA)	56-55-3	10^{-6}	10^{-6}	10^{-7}
Benzo(a)pyrene (BaP)	50-32-8	10^{-7}	10^{-7}	3.10^{-8}
Benzo(b)fluoranthene (BbF)	205-99-2	10^{-7}	10^{-7}	10^{-7}
Benzo(c)fluorene (BcF)	205-12-9	nd	nd	10^{-6}
Benzo(j)fluoranthene (BjF)	205-82-3	10^{-6}	10^{-6}	10^{-6}
Benzo(k)fluoranthene (BkF)	207-08-9	10^{-6}	10^{-6}	10^{-6}
Chrysene (CHR)	218-01-9	10^{-6}	10^{-6}	10^{-7}
Cyclopenta(c,d)pyrene (CPP)	27208-37-3	nd	nd	10^{-6}
Dibenz(a,h)anthracene (DBahA)	53-70-3	10^{-7}	10^{-7}	10^{-7}
Dibenzo(a,e)pyrene (DBaeP)	192-65-4	10^{-7}	10^{-7}	10^{-8}
Dibenzo(a,h)pyrene (DBahP)	189-64-0	10^{-8}	10^{-7}	10^{-8}
Dibenzo(a,i)pyrene (DBaiP)	189-55-9	10^{-8}	10^{-7}	10^{-6}
Dibenzo(a,l)pyrene (DBalP)	191-30-0	10^{-9}	10^{-9}	10^{-10}
Indeno(1,2,3-c,d)pyrene (IPY)	193-39-5	10^{-6}	nd	10^{-6}

512

513 Table 2: Parameters of individual PAH dose-responses with a common Hill constant.

514

	LS-174T				Hep3B				NCI-H358			
PAH	Max	EC50	slope at low dose	GEF	Max	EC50	slope at low dose	GEF	Max	EC50	slope at low dose	GEF
5MC	0.726	0.635	0.988	0.48	0.748	0.129	33.6	1.83	0.38	0.116	3.33	1.95
BaA	0.495	0.394	0.930	0.44	0.339	0.197	6.93	0.78	0.259	0.187	1.41	0.83
BaP	0.511	0.180	1.63	1	0.846	0.253	10.9	1	0.632	0.376	1.69	1
BbF	0.647	0.371	1.27	0.69	0.496	0.13	22.2	1.46	0.366	0.171	2.17	1.28
BcF	0.572	2.36	0.320	0.09	0	Inf	0	0	0	Inf	0	0
BjF	0.474	0.628	0.649	0.25	44.1	14.6	0.3	0.15	0.181	0.367	0.496	0.30
BkF	0.351	1.37	0.284	0.07	0.237	0.211	4.29	0.61	0.208	0.328	0.638	0.38
CHR	0.530	0.646	0.712	0.29	2.38	2.98	0.31	0.15	0.257	0.429	0.605	0.36
CPP	0.135	0.293	0.311	0.08	0	Inf	0	0.93	0	Inf	0	0
DBaeP	0.678	0.090	3.47	3.04	0.543	0.069	78.2	2.89	0.242	0.052	4.73	2.77
DBahA	0.555	0.403	1.03	0.50	0.37	0.086	35	1.87	0.329	0.071	4.72	2.77
DBahP	0.335	0.025	4.1	3.89	0.362	0.026	325	6.21	0.385	0.010	39.5	22.74
DBaiP	0.182	0.113	0.799	0.35	0.245	0.032	147	4.05	0.515	0.026	20.1	11.64
DBalP	1.18	0.027	13.6	22.9	0.83	0.008	6220	30.35	0.708	0.003	244	139
DMBA	0.71	0.101	3.36	2.90	0.59	0.085	57.1	2.44	0.36	0.057	6.41	3.75
IPY	0.303	0.362	0.602	0.23	0	Inf	0	0	0.198	0.33	0.606	0.36

515 Inf: Infinite

516 Table 3: Pearson correlation coefficients between the logarithms of the GEFs calculated on
1
2 517 the three cell lines.
3
4

5 518
6

	Hep3B	NCI-H358	LS-174T
Hep3B	1		
NCI-H358	0.95	1	
LS-174T	0.78	0.81	1

12 519
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

520 Table 4: Classification and toxicity relative to benzo(a)pyrene of the tested PAHs.

1
2 521
3
4
5

Compound	CAS number	IARC	TEF ^a	TEF ^b	GEF in LS-174T ^c	GEF in Hep3B ^c	GEF in NCI-H358 ^c
6 Methylchrysene	3697-24-3	2B	nd	nd	0.48 [0.14; 0.56]	1.83 [1.4 ; 2]	1.95 [0.56; 2.9]
7 1,2-Dimethylbenz(a)anthracene	57-97-6	nd	nd	nd	2.9 [1.8; 3.8]	2.44 [1.9; 2.6]	3.75 [0.95; 6.0]
8 Benaphthene	83-32-9	3	0.001	nd	0	0	0
9 Acenaphthylene	208-96-8	nd	0.001	nd	0	0	0
10 Anthracene	120-12-7	3	0.01	nd	0	0	0
11 Benz(a)anthracene	56-55-3	2B	0.1	0.145	0.44 [0.3; 0.71]	0.78 [0.27; 1.1]	0.83 [0.26; 1.2]
12 Benzo(a)pyrene	50-32-8	1	1	1	1	1	1
13 Benzo(b)fluoranthene	205-99-2	2B	0.1	0.140	0.69 [0.31; 0.74]	1.46 [0.17; 2.2]	1.28 [0.37; 2.1]
14 Benzo(c)fluorene	205-12-9	3	nd	nd	0.09 [0.06 ; 0.18]	0	0
15 Benzo(e)pyrene	192-97-2	3	nd	0.004	0	0	0
16 Benzo(ghi)perylene	191-24-2	3	0.01	0.022	0	0	0
17 Benzo(j)fluoranthene	205-82-3	2B	nd	0.061	0.26 [0.15; 0.53]	0.15 [0.12; 0.22]	0.30 [0.068; 0.42]
18 Benzo(k)fluoranthene	207-08-9	2B	0.1	0.066	0.076 [0.058; 0.24]	0.61 [0.10; 1.1]	0.38 [0.089; 0.74]
19 Chrysene	218-01-9	2B	0.01	0.0044	0.29 [0.12; 0.45]	0.15 [0.11; 1.2]	0.36 [0.10; 0.52]
20 Coronene	191-07-1	3	0.001	nd	0	0	0
21 Cyclopenta(c,d)pyrene	27208-37-3	2A	0.1	0.023	0.086 [0.024; 0.13]	0	0
22 Dibenz(a,h)anthracene	53-70-3	2A	5	1.11	0.50 [0.29; 0.74]	1.87 [1.1; 3.4]	2.77 [0.65; 4.4]
23 Benzo(a,e)pyrene	192-65-4	3	nd	nd	3.0 [2.6; 6.6]	2.89 [2.2; 3.3]	2.77 [0.96; 3.8]
24 Dibenzo(a,h)pyrene	189-64-0	2B	nd	nd	3.9 [2.3; 7.5]	6.21 [2.9; 6.2]	22.7 [7.2; 31]
25 Benzo(a,i)pyrene	189-55-9	2B	nd	nd	0.35 [0.057; 2.8]	4.05 [1.1; 5]	11.6 [2.6; 18]
26 Dibenzo(a,l)pyrene	191-30-0	2A	nd	nd	23 [5.5; 73]	30.3 [22; 69]	139 [36; 200]
27 Fluoranthene	206-44-0	3	0.001	nd	0	0	0
28 Fluorene	86-73-7	3	0.001	nd	0	0	0
29 Indeno(1,2,3-c,d)pyrene	193-39-5	2B	0.1	nd	0.23 [0.11; 0.47]	0	0.36 [0.070; 0.55]
30 Naphthalene	91-20-3	2B	0.001	nd	0	0	0
31 Phenanthrene	85-01-8	3	0.001	nd	0	0	0
32 Pyrene	129-00-0	3	0.001	nd	0	0	0

33 522 nd: Not determined

34 523 ^a from (Nisbet and LaGoy, 1992)

35 524 ^b from (US-EPA, 1988)

36 525 ^c from this study

526 **Figure Legends**

1 527

2
3 528 **Figure 1.** Structures of the different PAHs tested in this study.

4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Figure

[Click here to download Figure: Figures criblage v5.pptx](#)

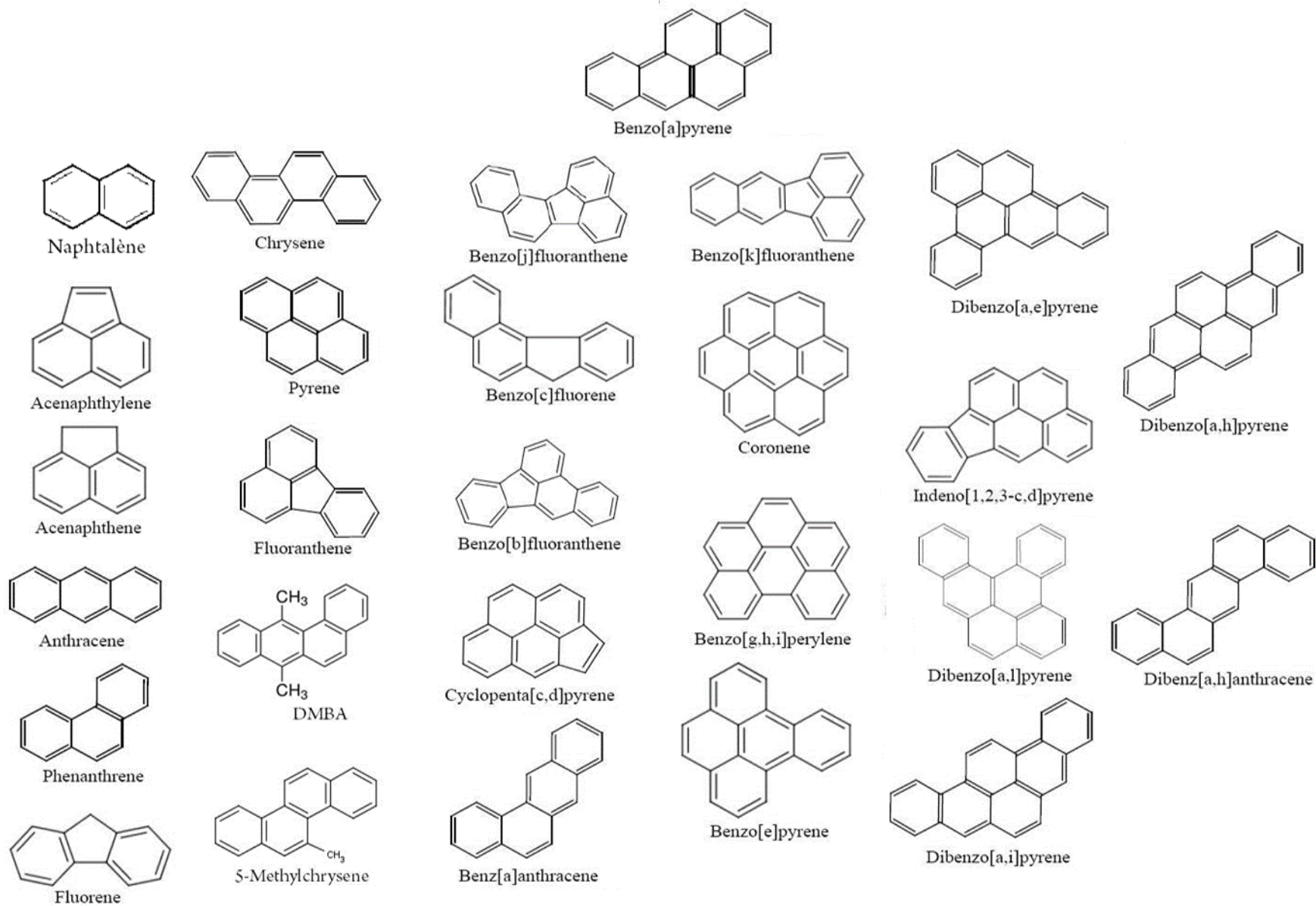


Fig 1.Tomasetig et Al.