
Adducts, SCE's and Mutations following Benzo(a)pyrene Exposure: a Review of quantitative Data followed by some Considerations regarding Risk.

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105 REFERENCES
10 tables
4 figures

Abstract

Polycyclic aromatic hydrocarbons comprise important carcinogens and through their presence in ambient air, in tobacco smoke and in food, they contribute to the induction of cancer in the lung and in several other organs and to the induction of cardiovascular and airway disease. To investigate the dose-effect relationship following exposure to benzo(a)pyrene (BaP), the most extensively studied PAH, we reviewed the literature concerning quantitative in vitro and in vivo data on the formation of DNA adducts and the induction of sister chromatid exchanges (SCE's) and mutations. In vitro experiments on the formation of BaP-DNA-adducts and on the induction of SCE's and mutations show a trend towards relatively higher effects per dose unit at exposures below 5 μM compared to exposures above 5 μM. For instance, exposures below 5 μM induced, in human cells, a mean of 2.47 add./10^6 nucleotides per μM, while above 5 μM a mean of 0.104 add./10^6 nucleotides per μM were observed. BaP adducts were observed in human white blood cells in smokers (0.0177 add./10^6 nucl.), in occupationally exposed workers (0.0057-0.558 add./10^6 nucl.) and also in non-smoking controls (0.0054 add./10^6 nucl.). BaP adducts occur in much lower concentrations than several endogenous adducts like 8-hydroxyguanine and 7-methylguanine (1). The findings are discussed in terms of cancer risk potentially associated with the presence of a certain level of BaP adducts and with regard to a possible higher impact of low intensity exposures in terms of health effects per dose unit.

Introduction

Polycyclic hydrocarbons, a ubiquitous group of environmental pollutants produced during the incomplete combustion of organic material, exert their carcinogenic influence by covalent binding of highly reactive metabolic intermediates to DNA. Of this group of chemicals, the most extensively studied has been the potent carcinogen benzo(a)pyrene which is present in car exhaust fumes, coke oven emissions, cigarette smoke, environmental air and a variety of foods. Metabolic activation of BaP proceeds by two major mechanisms, one-electron oxidation with formation of radical cations and mono-oxygenation to yield bay-region diolepoxides (2). These pathways result respectively in depurinating adducts that are removed from DNA by cleavage of the glycosidic bond and in stable adducts that remain covalently bound to DNA . The major reactive metabolic intermediate of BaP obtained by monooxygenation is the diol epoxide derivative which is enzymatically formed in two stereoisomeric forms, 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (designated BPDE I or `anti`) and 7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (designated BPDE II or `syn`), each of which is formed as a (+) and (-), or as a (R) and (S) enantiomer. Of these four isomeric forms the anti BaP diol epoxide, in particular the (7R) or (+) enantiomer, is the most carcinogenic, and formation of stable adducts between this metabolite and deoxyguanosine (BPDE-10-N^2dG) of DNA predominates in cells. There can also be a significant reaction with the amino group of deoxyadenosine (with formation of BPDE-N6-dA) and, to a lesser extent, with the amino group of deoxycytidine (with formation of BPDE-N4-dC). Stable adducts can also be formed from o-quinones produced by dihydrodiol dehydrogenases and from the 4,5-epoxide (K region) of BaP (2). The major depurinating adducts following the one-electron pathway are BaP-6-N7-dG, BaP-6-C8dG and BaP-6-N7dA.

The induction of mutations by genotoxic carcinogens represents an important step in carcinogenesis. Mutations are often preceded by an interaction of the carcinogen with the DNA, resulting in the alteration of its chemical structure (adduct formation). Therefore, the analysis of DNA damage and resulting gene mutations in various organs and tissues is important for evaluating the mutagenic and/or carcinogenic potential of genotoxic agents. To investigate the dose-effect relationship following exposure to BaP, we conducted a literature study concerning quantitative aspects of the formation of DNA adducts, the induction of sister chromatid exchanges (SCE) and the formation of mutations.

Methodological Approach

We sought all potentially informative peer-reviewed publications reporting studies on DNA adducts, SCEs and mutations following BaP exposure. We included in vitro studies and in vivo human observations. We searched articles published until September 2004 using the following online electronic database: MEDILINE (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi). We excluded the following:

- Studies that used dose units not comparable and not transformable to the dose units we used in our analysis.
- Studies that measured SCEs or mutations following BPDE exposure.

For the sake of comparison, DNA adduct levels are expressed in a standardized way, including the number of adducts per 10^6 nucleotides. All relevant data are summarized in tables I to IX.

In order to study possible dose-response relationships we plotted the different adduct, SCE and mutation data against BaP exposure dose. We then divided the in vitro data sets into high

(≥ 5 μM) and low doses (< 5 μM) and calculated the mean adduct or SCE level and the mean mutation frequency per dose unit for high and for low doses and compared the two.

Possible confounding factors that complicate the comparison of different studies are the method of adduct-measurement, the investigated cell or tissue types, the use of external metabolic activation and the duration of exposure. To correct for these factors we performed an ANOVA analysis using the statistical programme Statview.

We also considered 5 studies that quantify BPDE-deoxyguanosine adducts or total BPDE adducts following BPDE exposure. In order to compare these studies to the former, we determined a conversion factor from BPDE exposure to BaP exposure and from BPDE-adducts to BaP-adducts. On the basis of all available studies on BPDE adduct formation after in vitro exposure to BPDE or BaP, we calculated, by dividing the number of adducts by the dose, that 18,58 μM BaP forms the same amount of BPDE-adducts as 1 μM of BPDE. Published data suggest that BPDE adducts constitute the vast majority of BaP adducts. Shugart (1985)(3) and Li et al. (1996)(4) measured the fraction of BPDE-dG adducts in total BPDE-DNA (80,0% for males and 60,2% for females, and 73%, respectively). We calculated the mean of these 2 studies to be 71,1%. Several other studies measured the fraction of BPDE-dG adducts in the total BaP-DNA population (5-15). We calculated a mean of 77,2% (77,5% for the in vivo studies and 76,8% for the in vitro studies). As the fraction of BPDE-dG adducts is about 71,1% of all BPDE adducts, and also about 77,2% of all BaP adducts, it appears that BPDE adducts comprise almost all BaP adducts. For further calculations, we assumed that BPDE adducts represent 100% of BaP adducts.

We provide quantitative in vivo data concerning BaP adducts in humans and compared these data to levels of endogenous DNA adducts (1).

Results

1/ Adducts

BaP exposure in vitro: dose-effect relationship for adduct-formation:

We considered studies that measured BaP adducts following BaP exposure in vitro. We found 6 studies on animal cells with a total of 11 measured adduct levels and 11 studies on human cells with a total of 23 measured adduct levels.

The entire set of adduct levels measured in human cells were plotted against the exposure dose (µM BaP) (see figure 1). Table I shows that exposures from 0,01 to 100 µM gave rise to adducts ranging from 0,004 to 11,8 adducts per 10^6 nucleotides. To get a clearer view over the dose-effect relationship, the data were divided into adducts following low dose exposure (< 5 µM BaP) (see figure 1 left) and adducts following exposure to BaP doses at least equal to 5 µM BaP (see figure 1 right). Doses from 0.01 to 4 µM gave rise to adduct levels ranging from 0.004 to 6.4 adducts per 10^6 nucleotides. Linear regression shows an R^2 of 0.579 at doses below 5 µM. Doses from 5 to 100 µM gave rise to adducts ranging from 0.14 to 11.8 adducts per 10^6 nucleotides and linear regression shows an R^2 of 0.719. In linear regression the slope of the curve for doses below 5 µM (1.676 with significance of the t-value of 0.002) is more than 10 times higher than the one for doses above 5 µM (0.105 with a significance of the t-value of 0.004). We also calculated the mean adduct level per dose unit to be higher for doses below 5 µM (2.47 add./10^6 nucl.) then for doses at least equal to 5 µM (0.104 add./10^6 nucl.). Possible confounding factors that could complicate the comparison of different studies are the method of adduct-measurement (HPLC or 32P), the investigated cell or tissue types (blood, lung, skin, bladder, mammary epithelial tissue), the use of external metabolic activation (S9) and the duration of exposure (2 to 48 hours). To correct for these factors we performed an ANOVA analysis using the statistical programme Statview. The ANOVA analysis requires a normal distribution so the adducts per dose values were first logarithmically transformed. Taking into account the tissue type, the use of external metabolic activation, the method of adduct measurement and the duration of exposure, the difference between adducts per dose between the high exposure group and the low exposure group was statistically significant ($p=0.0054$).

In vitro tests on animal cells showed that doses from 1 to 55 μM gave rise to adduct levels ranging from 0.1 to 11 adducts per 10^6 nucleotides (table II, figure 2). The mean adduct level per dose unit was higher for doses lower than 5 μM (1.445 add./10^6 nucl.) than for higher doses (0.106 add./10^6 nucl.) but there were too little data to test the significance of the difference in an ANOVA test taking into account the confounding factors.

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Adducts in vivo:

Data on adduct levels after in vivo treatment of animals with BaP are shown in table III Our search of the existing literature resulted in 8 animal in vivo studies concerning BaP or BPDE adducts following BaP exposure: 7 mouse studies (16 measured adduct levels) and 1 rat study. To eliminate ‘species’ as a confounding factor we excluded the rat study from our dose-response analysis. Assuming a mean mouse weight of 20 g, all the BaP doses were expressed in mg/kg. Exposed topically to 0.63-10 mg/kg BaP, mice show BaP adduct levels in skin of 0.13-3.56 add./10^6 nucleotides (3, 7, 8, 11, 28, 29). Exposure by intraperintoneal injection of 25 or 50 mg/kg BaP resulted in BaP adducts in the liver ranging from 0.043 to 0.86 add./10^6 nucleotides and from 0.079-1.38 add./10^6 nucleotides, respectively (using 4 different methods for adduct quantification) (30). Due to the limited data it was not possible to compare adducts per dose between high doses and low doses.

→ TABLE III NEAR HERE

BPDE adducts following BPDE exposure:

We also considered 5 studies that quantify BPDE-deoxyguanosine adducts or total BPDE adducts following BPDE exposure. Data on adduct formation are shown in table IV. After elimination of two outliers, doses from 0.01 to 10 µM gave rise to adduct levels ranging from 0.09 to 174.4 adducts per 10^6 nucleotides. Using the conversion factors calculated as described in the methodological approach section to transform BPDE exposure to BaP exposure and BPDE adducts to BaP adducts, it can be summarized that doses from 0.188 to 188.5 µM BaP gave rise to BaP adducts ranging from 0,117 to 225,91 adducts per 10^6 nucleotides. In contrast to the former data, the human data obtained after these conversions show that BaP doses below 5 µM induce a lower mean amount of adducts per µM of BaP than doses higher than 5 µM (1.011 vs. 4.136 add./10^6 nucleotides). This difference was not statistically significant taking the method of adduct measurement into account ($p=0.323$). Mouse data show a mean adduct level per dose unit of 0.955 for doses lower than 5 µM and of 1.518 for doses at least equal to 5 µM). Again, this difference was not statistically significant, when corrected for method of adduct measurement ($p=0.729$).

→ TABLE IV NEAR HERE

Human BaP adducts in vivo; Comparison with endogenous adducts:

Table V provides quantitative in vivo data concerning BaP adducts in humans. BaP and BPDE adducts (0.054-0.918 add./10^6 nucleotides and 0.013-0.787 add./10^6 nucleotides, respectively) occur in much lower concentrations than several endogenous adducts like 8-hydroxyguanine (0,24-59,70 adducts/10^6 nucleotides in white blood cells) and 7-methylguanine (0,10-2,36 adducts/10^6 nucleotides in white blood cells) (1). To get an idea of the total amount of endogenous DNA adducts in white blood cells we considered all quantitative data regarding endogenous DNA adducts in WBC as summarized by De Bont and van Larebeke (2004)(1). We included the following endogenous adducts: 5-hydroxy-2`-deoxycytidine, 5-hydroxy-2`-deoxyuridine, 5,6-dihydroxy-5,6-dihydro-2`-deoxyuridine, 8-oxo-7,8-dihydro-2`-deoxyguanosine, 7-methylguanine, 0^6-methylguanine, 7-(2-hydroxyethyl)guanine, 7-alkylguanine and 7-ethyl-dG. The total quantity of these endogenous adducts in human cells in vivo amounts to between 4,97 and 69,05 adducts/10^6 nucleotides. The minimum level (4.97) was reached by adding up the lowest level observed for each of the endogenous adduct types included, while the maximum level (69.05) was based on the highest observed level.

→ TABLE V NEAR HERE

2/ Sister Chromatid Exchanges

Literature research resulted in 8 studies investigating SCE`s following BaP exposure in vitro: 5 human studies and 3 animal studies (24 measurements) (table VI, figure 3). Doses from 0.8 to 500 µM gave rise to SCE amounts in human tissues ranging from 0.08 to 12.20 SCEs per cell and doses from 0.25 to 200 µM gave rise to 5 to 103.30 SCEs per animal cell. Seven of the eight studies determined more than 2 SCE levels and revealed a supralinear dose-response curve. Our analysis of the 8 studies showed that, for humans, BaP doses below 5 µM induce a higher mean amount of SCEs per µM of BaP than doses higher than 5 µM (1.48 vs. 0.18 SCEs per cell). The same can be concluded for animal studies (13.32 vs. 0.97 SCEs per cell). For the entire dataset this difference was statistically significant when corrected for species and the use of metabolic stimulation (after logarithmic transformation of adducts/dose) ($p = 0.046$).

→ TABLE VI NEAR HERE

→ FIGURE 3 NEAR HERE

3/ Mutations

Mutations in vivo following BaP exposure:

We found only 3 in vivo mutation studies following BaP exposure and comparison between high en low doses was not possible (58-60) (table VII). Several other in vivo studies used dose units not comparable to the dose unit we used in our analysis (µM) (61, 62). Brooks et al. (1999)(62) showed a clear linear relationship between the dose en the mutation frequency. Hakura et al. (1998)(58) measured the mutation frequency in 11 different tissues and showed a high variation in mutation frequencies (from 1.7×10^5 in brain to 267.6×10^5 in colon).

→ TABLE VII NEAR HERE

Mutations in vitro following BaP exposure:

We searched the literature looking for studies measuring the mutation frequency in human and animal cells following BaP exposure in vitro. This resulted in 3 human studies (7 measuring points) and 3 animal studies (20 measuring points) (table VIII, figure 4). In human tissues all experimental exposures were lower than 5 µM, demonstrating that mutations are already formed at low doses. The mutation frequency per dose unit showed a non-significant downward trend with increasing dose and the same trend was observed when only studies without metabolic activation were included. The animal studies showed mutation frequencies ranging from 0.30 to 312 per 10^5 following exposures from 0.1 to 31.68 µM. Doses lower than 5 µM BaP resulted in a higher mean mutation frequency than doses higher than 5 µM (27.84 vs. 0.88 per 10^5).

→ TABLE VIII NEAR HERE

→ FIGURE 4 NEAR HERE

An in vivo study pointed to the existence of a great variability in the induction of mutations by BaP in different tissues (58). Two out of three in vitro animal studies measured MF in ovarium cells of hamsters. One showed a supralinear dose-response curve (66), while the other showed a linear relationship (67). We analysed all the data from both studies and calculated a higher mean MF per dose unit BaP for doses lower than 5 µM than for doses higher or equal to 5 µM (4.30 vs. 0.88 per 10^5).

Three studies measuring MF's following BPDE exposure, all in the range from 0 to 1 μM, determined the dose-response curve: one gave a supralinear relationship (69), an other a linear relationship (70), and a third an infralinear relationship (71) (table IX).

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Discussion

Polycyclic aromatic hydrocarbons comprise important carcinogens and through their presence in ambient air, in tobacco smoke and in food, they contribute to the induction of cancer in the lung and in several other organs and to the induction of cardiovascular and airway disease (72, 73). There are many different PAH's and each parental PAH can, for example through atmospheric reactions, give rise to several different derivatives. Exposure to PAH's is generally assessed through measurement of a series of guide substances, of which benzo(a)pyrene is the most extensively studied. BaP DNA adducts have been found in human peripheral blood cells and in human tissues in most occasions when appropriate techniques have been applied. All humans are probably exposed to PAH’s.

Cancer risk associated with a certain level of BaP adducts

The data on the occurrence of BaP adducts and on the cancer incidence among workers in coke ovens and other industries with exposure to PAH’s can be used to estimate the cancer risk associated with a certain level of BaP adducts.

At least two considerations limit the meaning of such estimations. First, internal exposure can also originate from food sources and to a lesser extent from exposure through the skin. This might explain the fact that non-smoking controls, not exposed to coke oven emissions, show relatively high values of BaP adducts compared to coke oven workers or smokers (values for coke oven workers are maximum 10 times higher). Exposures through food or skin carry however a much lower risk on lung cancer than exposure through the airways (74) and although they might carry the same risk of cancers not related to the airways, total cancer risk will be substantially lower. So for persons exposed through food or skin, the level of BaP adducts will lead to an overestimation of cancer risk. Secondly, both experimental and epidemiological studies indicated that, for the induction of a certain lung tumour incidence, inhaled cigarette smoke contains about 100 times less BaP and inhaled diesel exhaust contains about 1000 times less BaP than the exhaust from coke ovens or heated tar pitch associated with the same risk of lung cancer (75). So for smokers or persons exposed to diesel exhaust the level of BaP adducts will lead to an underestimation of cancer risk. Notwithstanding these and also other considerations, risk estimations based on data from workers in coke ovens and other industries with exposure to PAH's can be relevant as many people are exposed to similar complex mixtures.

We calculated the mean BaP adduct level per dose unit (µg/m³) in vivo in coke oven and other factory workers to be able to estimate the cancer risk associated with the presence of BaP adducts based on the cancer risk experienced by these workers. Table X shows studies that measured BPDE or BaP adducts in white blood cells of coke oven or factory workers. We calculated the mean adduct level per dose unit to be 0.418 add./10^6 nucleotides per µg/m³.

→ TABLE X NEAR HERE

The unit risk for lung cancer of BaP (after 70 years of exposure) is 1.1 to 3.3 x 10^{-3} per µg BaP/m³ (76) so 0.418 adducts/10^6 nucleotides could result in a unit lung cancer risk of 1.1-3.3 x 10^{-3} after exposure to pure BaP. The amount of endogenous DNA adducts in human

DNA, which is of the order of 4.97 to 69.05 adducts/10^6 nucleotides, is far greater than the amount of BaP adducts. This load of endogenous DNA adducts might underlie the spontaneous incidence of lung cancer, which is probably of the order of 6.6 x 10^{-3}, the cumulative incidence of lung cancer up to age 75 in Madras, India, in the period 1978-1982 (77). This suggests that the pathogenic potency of exogenous high molecular weight adducts such as those formed by BaP is higher than that of most endogenous adducts. That this might be the case is supported by the finding that, in male F344 rats, 0.85 aflatoxine adducts/10^6 nucleotides are already associated with a liver tumour incidence of 50% (78) at two years. The reported spontaneous liver tumour incidence in male F344 rats is only 1-2%. The level of endogenous DNA adducts as measured by Nath and Chung (1994)(79), Chung et al. (1999)(80), Wu et al. (1999)(81), Walker et al. (1993)(82), Föst et al. (1989)(83), Chung et al. (2000)(84), Nair et al. (1995)(85), Loureiro et al. (2002)(86), Schuler et al. (1997)(87), Fraga et al. (1990)(88), Biancini et al. (1993)(89), only including acrolein-dG, crotonaldehyde-dG, N7-(2-hydroxyethyl)guanine, HNE-dG, etheno-dA, etheno-dC, 1,N^2-etheno-2'-dG, 8-oxo-dG and 7-methyldeoxyguanosine, amounts to 2.18-137.3 adducts/10^6 nucleotides. The minimum level (2.18) was reached by adding up the lowest level observed for each of the endogenous adduct types considered, while the maximum level (137.3) was based on the highest observed level.

Occupational exposures involve complex mixtures of polycyclic aromatic hydrocarbons other than BaP. So BaP adducts can be considered as a measure of total PAH exposure, the impact of which on cancer incidence is much greater than that of the component BaP alone. We based the following calculations of the lung cancer risk associated with PAH exposure in industries measured as BaP, on the meta-analysis of Armstrong et al. (2004). Based on 39 studies they calculated the unit relative risk of industrial PAH exposures with BaP as a guide substance. The unit relative risk (URR) gives the relative risk at 100 microg/m³ years BaP. Based on all 39 studies the average estimated URR was 1.20. A calculation limited to the 22 studies on coke oven, gas works and aluminium production workers led to an average estimated URR of 1.17. As it is conceivable that extrapolation from high exposure studies to much lower exposures leads to an underestimation of risk (see below), it is of interest to make a risk assessment restricted to studies in which exposures were not above 20 microg BaP/m³ years. Based on 7 studies on coke oven, gas works and aluminium production workers in which exposures were not above 20 microg BaP/m³ years Armstrong et al. (2004)(90) found an average estimated URR of 1.87. Using the equation adapted from Armstrong et al. (2004)(90) we converted the unit relative risk factor into the lifetime risk ULR:

$$URR = 1 + \left[ULR * \left(\frac{1}{70} \right) * \left(\frac{10}{23} \right) * \left(\frac{230}{365} \right) * \left(\frac{1}{0.09} \right) \right]$$

where ULR is the lifetime risk per ng/m³ continuous (23 m³/dag vs. 10 occupational) exposure (365 days vs. 230 occupational) over 70 years, assuming, as did Armstrong et al. (2004)(90), a 9% baseline lifetime risk. Using a linear model we calculated that 0.418 BaP adducts/10^6 nucleotides as a marker of exposure to a mixture of PAH's such as coke oven emissions could be associated with a lung cancer risk of 39 to 200 x 10^{-3} (risks derived from URR's equal to 1.17 and 1.87 respectively). It should be noted that Armstrong et al. (2004)(90) find higher but less precisely estimated URR's if they include all 39 studies on cancer risk associated with PAH exposures in industry and do not restrict their calculations to coke oven, gas works and aluminium production workers. They find an estimated URR=1.20 based on all 39 studies and an estimated URR= 4.54 based on 21 studies concerning exposures not exceeding 20 microg/m³ years.

Besides lung cancer, exposure to coke oven emissions was also associated with kidney cancer, prostate cancer and genito-urinary cancer in male workers. Mandell et al. (1995)(91) found a relative risk of 1.7 for kidney cancer in male workers in the blast furnace or coke oven industry (95% CI: 1.1-2.7). Costantino et al. (1995)(92) reported significantly elevated relative risks for lung cancer and for prostate cancer and genito-urinary cancer in general (1.95, CI: 1.59-2.33; 1.57, CI: 1.09-2.30; 1.49, CI: 1.11-2.01, respectively). Based on the number of cancer deaths and relative risks reported by Costantino et al. it can be calculated that in a population of 5321 coke oven workers the induction of 124 excess cases of lung cancer by exposure to coke oven emissions was accompanied by the induction of 28 cases of genito-urinary cancers. So 0.418 BaP adducts/10^6 nucleotides as a marker of exposure to a mixture of PAH's such as coke oven emissions could be associated with a genito-urinary cancer risk of 9 to 45 x 10^{-3} and a cancer risk of 48-245 x 10^{-3} for lung and genito-urinary cancer combined.

Based on a linear model, DNA adduct levels in peripheral blood cells of the order of 0.05 BaP adducts/10^6 nucleotides observed in non-smokers (table V) could, if they resulted from exposure to air polluted by PAH's, be associated with cancer risks of the order of 5 to 25 x 10^{-3}. To the extent that BaP-DNA adduct levels are due to exposures through ingestion, lung cancer risk from PAH's will be lower. This will mainly be relevant for non-smokers.

Supralinear dose-effect relationship

The review by Armstrong et al. (2004)(90) shows that the calculation of the risk associated with exposure to PAH's not exceeding 20 microg BaP/m³ years (corresponding to an exposure to 1 µg/m3 BaP during 20 years) leads to a higher value than calculations also including more intensive exposures. This and some in vitro data (63; this review) and epidemiologic observations on humans (93) suggest a supralinear dose-effect relationship (low dose hypersensitivity). It is interesting to consider carcinogenic effects of environmental or other low intensity exposures to PAH’s in terms of existing knowledge on the induction of DNA repair by genotoxic agents such as ionising radiation. There is substantial evidence that exposure to a first dose (adaptive dose) of a few mGy up to a few hundred mGy (up to 500 mGy in some circumstances (94)) leads to a reduction in the mutagenic effects of a second higher dose (95, 96). This phenomenon called adaptation, which has also been described for genotoxic substances, is probably due to the induction of DNA repair mechanisms (95). The critical dose for induction of the adaptive response is probably of the order of 1 mGy with no induction of DNA double strand break repair nor adaptive response occurring below this dose (94, 97). The adaptive response takes one or more hours to be established and disappears after about 40 hours (95). Exposures to ionising radiation in doses below the threshold level for an adaptive response could be, per unit of dose, more efficient in the induction of mutations than higher doses (low dose hypersensitivity)(98).

Lifelong exposure of a human population to ionising radiation at a level corresponding to the threshold dose per time period during which the adaptive response persists, that is 1 mGy per 40 hours or 15.3 Gy per 70 years can be estimated to lead to a risk of death from cancer of 100% before age 70. Indeed, according to UNSCEAR (2000)(96) the risk of death from all solid cancers combined amounts to 11% following an acute dose of 1 Sv, with total solid cancer incident risks roughly twice those for mortality while chronic exposure might lead to only 50% of the above mentioned risk. That exposures to very low doses of ionising radiation do lead to the induction of cancer in humans is demonstrated by observations concerning radon in houses (99), by observations made after the Chernobyl disaster (100) and by the fact that low dose gamma radiation induced cytogenetic effects in residents of buildings (101). Risks associated with environmental and most occupational exposures to BaP and complex PAH mixtures are far lower than the risk associated with exposure to ionising radiation at the threshold dose per time period during which the adaptive response persists. It is likely that these exposures to PAH's do not reach a threshold level for induction of DNA repair and that consequently a higher efficiency in the induction of biological and health effects (compared to high experimental or occupational doses) might be expected. This might explain supralinear effects suggested by some epidemiological data.

PAH's such as BaP play an important role in the induction of lung cancer by tobacco smoke and by particulate matter in ambient air (102, 103). A recent study from Norway (104) found that smoking 1 to 4 cigarettes a day is already associated with an almost three-fold increase in relative risk of dying from cardiovascular disease or from lung cancer, whereas Vineis et al. (2000)(105) found that the increase of lung cancer risk in heavy smokers levelled of above a relative risk of 20. According to the WHO, the response per dose unit in the induction of lung cancer and of cardiopulmonary disease by particulate matter in ambient air is higher for low exposures than for more intensive exposures (93). The existence of a supralinear dose response relationship in the induction of DNA-adducts and biological effects following BaP exposure, as suggested through our analysis of the published literature, is consistent with the findings concerning smoking and with the recommendations of the WHO concerning particulate matter (93).

Tables

Table I: Human BaP adducts following BaP exposure in vitro.

Method	**Cell- or tissue type**	**Dose (µM)**	**Quantity**		**Reference**
				Adducts/10^6 nucleotides	
HPLC	monocytes	0.30	2.07 fmol/µg DNA	0.680	(16)
32P	Peripheral lymphocytes	8.00	14.40 add./10^8 nucl.	0.140	(17)
		20.00	19.93 add./10^8 nucl.	0.200	
Technique based on radioactivity	Skin fibroblasts	0.25	6.89 fmol/µg DNA	2.260	(18)
32P	Lung fibroblasts	0.01	1.8 add./10^8 nucl.	0.018	(19)
		1.00	237 add./10^8 nucl.	2.370	
HPLC	Mammary epithelial cells	2.00	16.6 pmol/mg DNA	5.440	(20)
32P	White blood cells	10.00	2.2 add./10^6 nucl.	2.200	(13)
		25.00	4.2 add./10^6 nucl.	4.200	
		50.00	9.3 add./10^6 nucl.	9.300	
		100.00	11.8 add./10^6 nucl.	11.800	
HPLC	Epidermal cells	0.40	1.5 µmol/mol DNA	1.500	(21)
		4.00	6.0 µmol/mol DNA	6.000	
HPLC	Bladder	1.00	6.4 µmol/mol DNA	6.400	(22)
	bronchus	1.00	3.1 µmol/mol DNA	3.100	

32P	White blood cells	100.00	929.9 add./10^8 nucl.	9.300	(23)
32P	lymphocytes	0.01	0.035 add./10^7 nucl.	0.004	(24)
		0.05	0.13 add./10^7 nucl.	0.013	
		0.10	0.27 add./10^7 nucl.	0.027	
		0.50	1.99 add./10^7 nucl.	0.199	
		1.00	3.17 add./10^7 nucl.	0.317	
		5.00	5.56 add./10^7 nucl.	0.556	
32P	fibroblasts	50.00	65-70 add./10^8 nucl.	0.675	(25)

HPLC: high performance liquid chromatography, 32P: 32P-postlabeling

Table II: Animal BaP adducts following BaP exposure in vitro.

Method	**Cell- or tissue type**	**Dose (µM)**	**Quantity**		**Reference**
				Adducts/ 10^6 nucleotides	
32P	Mouse Fœtal liver	8.0	69.81-217.72 add./10^8 nucl.	1.44	(17)
		20.0	103.24-376.42 add./10^8 nucl.	2.40	
		40.0	268.81-573.73 add./10^8 nucl.	4.22	
HPLC	Rat dermal fibroblasts	1.2	9.62 pmol/mg DNA	3.15	(26)
	Rabbit dermal fibroblasts	1.2	5.12 pmol/mg DNA	1.68	
HPLC	Rat Mammary epithelial cells	2.0	10.1 pmol/mg DNA	3.31	(20)
HPLC	Rat hepatocytes	55.0	3.1 pmol/mg DNA	1.02	(5)
		55.0	5.2 pmol/mg DNA	1.71	
Sephadex LH 20 chromatography	Rat lung	1.0	0.2-0.4 pmol/mg DNA	0.10	(27)
32P	Rat trachea	19.8	3.7 add./10^6 nucl.	3.70	(14)

	Hamster trachea	19.8	11.0 add./10^6 nucl.	11.0

HPLC: high performance liquid chromatography, 32P: 32P-postlabeling

Table III: Animal BaP adducts following BaP exposure in vivo.

Method	Cell- or tissue type	Dose (mg/kg)	Quantity		Reference
				Add./10^{6} nucleotides	
32P	Rat lung	10	3.1 add./10^{8} nucl.	0.031	(31)
	Rat liver		2.9 add./10^{8} nucl.	0.029	
	Rat hart		6.3 add./10^{8} nucl.	0.063	
32P	Mouse skin	0.63[a]	125 add./10^{9} nucl.	0.125	(28)
ELISA	Mouse skin	1.6[a]	0.7 fmol/µg DNA	0.230	(29)
		3.2[a]	1.2 fmol/µg DNA	0.393	
		6.4[a]	1.6 fmol/µg DNA	0.525	
(1,3-^{3}H)BPDE-radiolabelling	Mouse liver	25[a]	86.4 add./10^{8} nucl.	0.864	(30)
		50[a]	137.6 add./10^{8} nucl.	1.376	
			11.4 add./10^{8} nucl.		
32P		25[a]	22.1 add./10^{8} nucl.	0.114	
		50[a]	4.3 add./10^{8} nucl.	0.221	
DELFIA		25[a]	7.9 add./10^{8} nucl.	0.043	
		50[a]	11.0 add./10^{8} nucl.	0.079	
CIA		25[a]	17.0 add./10^{8} nucl.	0.110	
		50[a]		0.170	
HPLC	Mouse skin	10[a]	15-20 add./10^{7} nucl.	1.75	(32)
32P	Mouse skin	2.52[a]	1.07 add./10^{6}nucl.	1.07	(8)
32P	Mouse skin	2.52[a]	3.56 µmol/mol DNA	3.56	(11)
HPLC	Mouse skin	2.52[a]	9.6 pmol/mg DNA	3.15	(7)

[a] Dose units transformed into mg/kg using: mean weight of mice: 20g.

HPLC: high performance liquid chromatography, 32P: 32P-postlabeling, ELISA: enzyme-linked immunosorbent assay, DELFIA: dissociation-enhanced lanthanide fluoroimmunoassay, CIA: Chemiluminescence immunoassay

Table IV: BPDE adducts following BPDE exposure in vitro.

Method	**Cell or tissue type**	**Dose (µM)**	**Quantity**		**Reference**
				Add./10^6 nucleotides	
	Epidermal mouse cells		BPDE-dG (fmol/µg DNA):		(33)
ELISA		10	422	138	
		1	47	15.4	
		0.1	5	1.64	
USERIA		10	479	156	
		1	39	12.8	
		0.1	4	1.31	
HPLC	Epidermal mouse cells		BPDE-dG (fmol/µg DNA):		(34)
		33	335	109.8	
		3.3	532	174.4	
ELISA	Human white blood cells		BPDE-dG (add./10^7 nucl.):		(10)
		0	0	0	
		0.01	0.9	0.09	
		0.1	20.4	2.04	
		1	373.5	37.35	

		10	5400	540	
32P	Human peripheral blood lymphocytes	1	BPDE (add./10^7 nucl.): 63.7	6.37	(35)
	Human mammary epithelial cells	1	BPDE (add./cell): 180000	28	(36)

HPLC: high performance liquid chromatography, 32P: 32P-postlabeling, ELISA: enzyme-linked immunosorbent assay, USERIA: ultrasensitive enzymatic radioimmunoassay

Table V: Human BaP adducts in vivo

Method	**Cell or tissue type**	**Exposure type**	**Quantity**		**Reference**
				Add./10^6 nucleotides	
BaP adducts					
HPLC	monocytes	Cancer Patients	2.8 fmol/µg DNA	0.918	(37)
		Controls	2.1 fmol/µg DNA	0.698	
32P	Leukocytes	Controls:			(38)
		Non-smokers	0.83 fmol/50 µg DNA	0.0054	
		Smokers	2.7 fmol/50 µg DNA	0.0177	
		Factory workers	0.87-59.29 fmol/50 µg DNA	0.0057-0.389	
BPDE adducts					
IAC	Placenta		1 add./10^7 nucl.	0.100	(39)

USERIA	lymphocytes	Coke oven workers	1.7 fmol/µg DNA	0.558	(40)
USERIA	white blood cells	factory workers	2-120 fmol/50 µg/DNA	0.013-0.787	(41)
ELISA	white blood cells	factory workers	0.155 fmol/µg DNA	0.051	(42)
		controls	0.083 fmol/µg DNA	0.027	
ELISA	placenta	Smokers	10-60 fmol/50 µg DNA	0.066-0.393	(43)
		non-smokers	10-13.25 fmol/50 µg DNA	0.066-0.087	
	umbilical cord blood	smokers	10-22.15 fmol/50 µg DNA	0.066-0.145	
		non-smokers	10 fmol/50 µg DNA	0.066	
HPLC	Lung tissue		1-40 add./10^8 nucl.	0.010-0.400	(44)
HPLC	lymphocytes	Coke oven workers	1.54 fmol/µg DNA	0.505	(45)
32P	white blood cells	Controls	9.8-30 add./10^8 nucl.	0.098-0.300	(46)

HPLC	lympho-monocyte fraction	Coke oven workers	4.37 add./10^8 nucl.	0.044	(47)
HPLC	Lung tissue	cancer patients	0.6-9.9 add./10^8 nucl.	0.006-0.099	(48)
HPLC	lympho-monocyte fraction	Controls	3.9 add./10^8 nucl.	0.039	(49)
		anode aluminium workers	11.5 add./10^8 nucl.	0.115	
		chimney sweeps	5.5 add./10^8 nucl.	0.055	
		coke oven workers	19.5 add./10^8 nucl.	0.195	
		psoriatic patients	2.3 add./10^8 nucl.	0.023	

IAC : immunoaffinity chromatography, HPLC: high performance liquid chromatography, 32P: 32P-postlabeling, ELISA: enzyme-linked immunosorbent assay, USERIA: ultrasensitive enzymatic radioimmunoassay

Table VI: SCE's following BaP exposure in human and animal tissues.

Cell or tissue type	**Dose (µM)**	**SCE's/cell**	**Reference**
Humans			
Lymphocytes	50	3.40	(50)
	100	4.90	
	500	6.30	
Lymphocytes	1.00	0.08	(51)
	10.00	2.32	
	100.00	5.92	
Lymphocytes	39.60	3.44	(52)
	198.00	3.94	
Lymphocytes	0.80	1.60	(53)
	20.00	8.80	
	40.00	12.20	
Lymphocytes	1.00	3.23	(54)
	10.00	5.97	
	100.00	9.50	
Animals			
Rat liver epithelial cells	50	8.00	(55)
	200	23.50	
	400	27.70	
	2000	35.60	

Chinese hamster ovary cells	0.25	7.37		(56)
	2.50	13.67		
	25.00	14.07		
Rat liver epithelial cells	1.00	5.00		(57)
	10.00	48.60		
	100.00	103.30		

Table VII: Mutation frequency following BaP exposure in vivo.

Cell or tissue type	Gene or locus	Dose (mg/kg)	Mutation frequency (MFx10^5)	Reference
Mouse:Colon	Lac Z	625	267.60	(58)
Ilium		625	176.40	
Forestomach		625	137.00	
Bonemarrow		625	87.00	
Spleen		625	80.70	
Glandular stomach		625	49.90	
Liver		625	17.90	
Lung		625	16.60	
Kidney		625	11.70	
Heart		625	8.90	
Brain		625	1.70	
Mouse liver	Lac I	120	6.40	(59)
Mouse splenic T cells	Hprt	50	0.09	(60)
		150	0.91	
	Lac I	50	4.40	
		150	13.80	
	CII	150	1.90	
	CI	50	1.70	
		150	0.50	

Table VIII: Mutation frequency following BaP exposure in vitro.

Cell or tissue type	**Gene or locus**	**Dose (µM)**	**Mutation frequency ($MFx10^5$)**	**Reference**
Humans				
Lymphoblastoid cells	HPGRT	0.020	0.08	(63)
		0.100	0.21	
		0.500	0.28	
		1.000	0.38	
Fibroblast without BER for BaP adducts	6-thioguanine resistance	0.100	6.50	(64)
		0.200	9.90	
Epidermal keratinocytes	HPRT	3.960	3.08	(65)
Animals				
Chinese hamster ovary cells	HGPRT	0.396	1.45	(66)
		0.990	7.83	
		1.980	12.99	
		3.960	12.15	
		7.920	13.44	
		15.840	7.08	
Chinese hamster ovary cells	XPRT	0.396	2.80	(67)
		1.980	2.80	
		3.960	2.10	
		7.920	6.20	

		15.840	13.50	
		31.680	18.90	
Chinese hamster V79 cells	Azaguanine resistance	0.100	5.00	(68)
		0.400	45.00	
		1.200	70.00	
		4.000	312.00	
	Ouabian resistance	0.200	0.30	
		0.400	6.80	
		1.200	18.80	
		4.00	106.80	

Table IX: Mutation frequency following BPDE exposure in vitro.

Cell or tissue type	Gene or locus	Dose (µM)	Mutation frequency (MFx10^5)	Reference
Human bronchial epithelial cells	CGG^{arg} to CTG^{leu} transversion at p53 codon 248	0	0.27	(69)
		0.5	2.6	
		1.0	4.3	
	Codon 249 AGG^{arg} to ATG^{met}	0	0.6	
		0.5	3.0	
		1.0	3.2	
	Codon 249 AGG^{arg} to AAG^{lys} transition	0	0.24	
		0.5	2.3	
		1.0	1.56	
	Codon 250 CCC^{pro} to TCC^{ser} transition	0	0.7	
		0.5	3.5	
		1.0	3.2	
V79 chinese hamster cells	Ouabain-resistant mutations	BPDE I:		(70)
		0	0.2	
		0.16	2.5	
		0.33	6.3	
		0.66	10.3	
		BPDE II:		
		0	0.1	
		0.16	0.2	

		0.33	0.4	
		0.66	0.9	
V79 chinese hamster cells		Syn-BPDE:		(71)
	8-azaguanine	0.1	11.1	
		0.2	6.3	
		0.5	42.4	
		1.0	242.5	
	Ouabain-resistant mutations	0.1	4.3	
		0.2	6.5	
		0.5	168	
		1.0	68.8	
		Trans-BPDE :		
	8-azaguanine	0.1	12.6	
		0.2	47.6	
		0.5	827.6	
	Ouabain-resistant mutations	0.1	15.7	
		0.2	23.7	
		0.5	111.0	

Table X: BPDE or BaP adducts in white blood cells of coke oven or factory workers.

Reference	**Dose (µg/m³)**	**Adduct level (add./10^6 nucleotides)**	**Adducts per dose unit**
(40)	2.5	0.210 BPDE add.[b]	0.084
	8	0.255 BPDE add.	0.032
(38)	0.64	0.034 BaP add.	0.053
(49)	0.0289[a]	0.039 BPDE add.	1.349
	0.0867[a]	0.115 BPDE add.	1.326
	0.202[a]	0.055 BPDE add.	0.272
	0.347[a]	0.195 BPDE add.	0.562
(47)	2.69[a]	0.044 BPDE add.	0.016
(42)	0.8	0.051 BPDE add.	0.064
			Mean: 0.418

[a] Exposure doses were originally expressed in µmol 1-pyrenol/mol creatine. 1 µmol 1-pyrenol/mol creatine = 0.289 µg BaP/m³ (38).

[b] Excluding the only women in the study which presented a very large number of adducts.

Figures

Figure 1: BaP adducts in human tissues following BaP exposure in vitro: doses < 5µM (left), doses ≥ 5 µM (right)

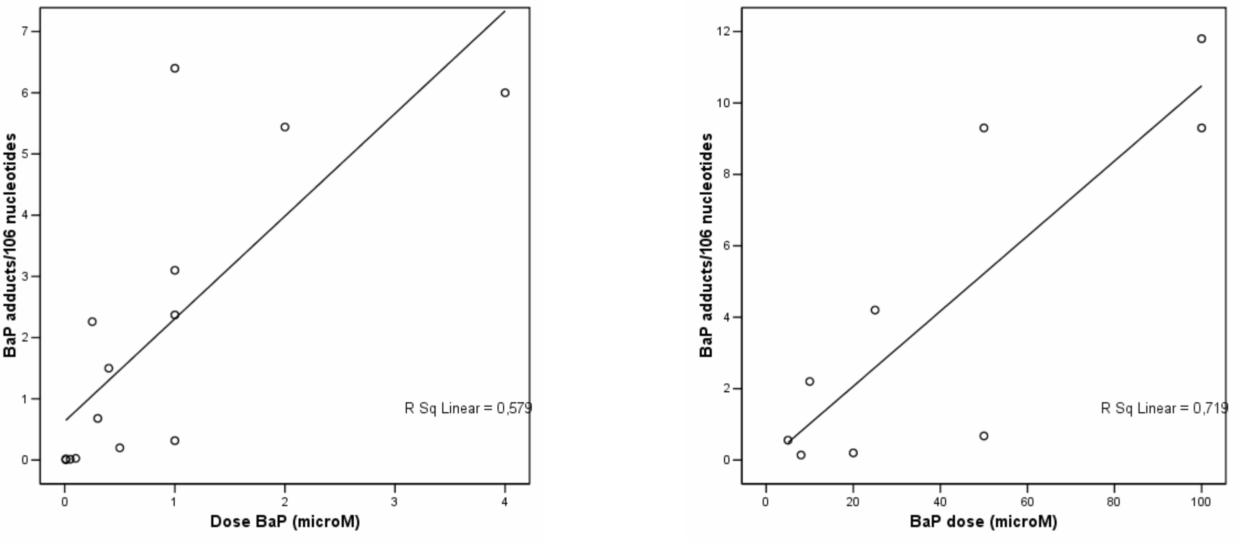


Figure 2: BaP adducts in animal tissues following BaP exposure in vitro.

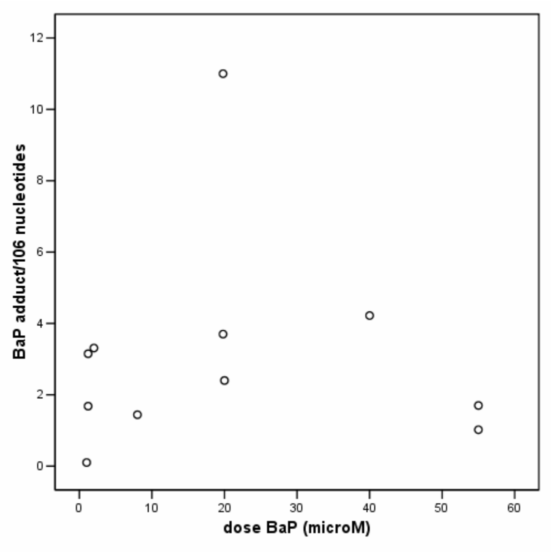


Figure 3: SCEs following BaP exposure in vitro: humans (left), animals (right).

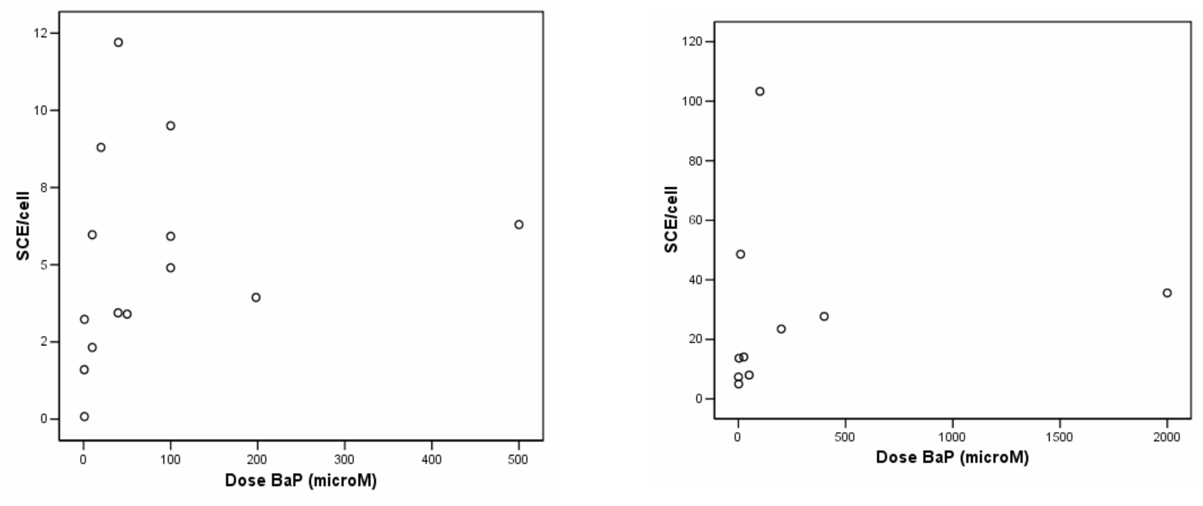
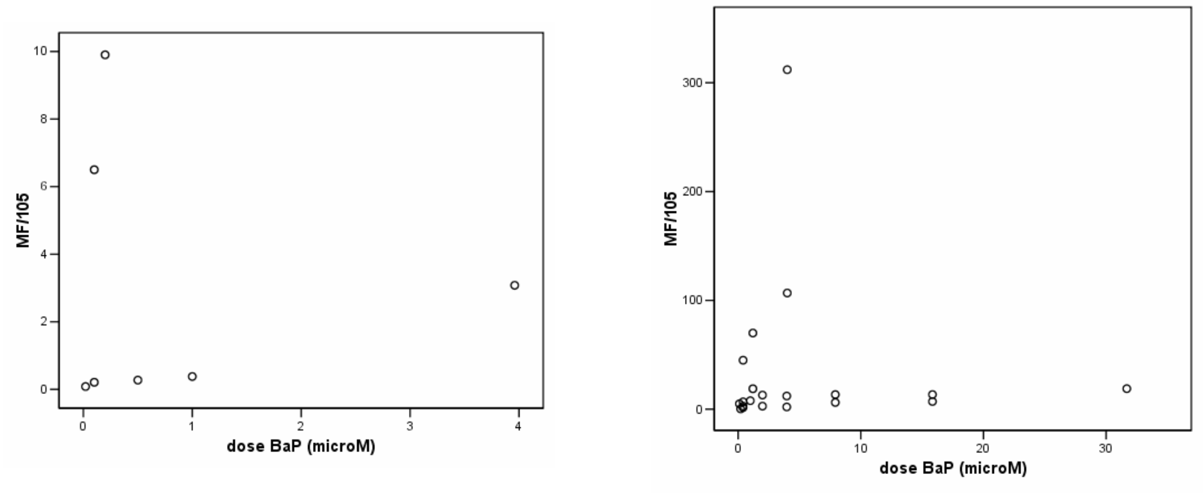


Figure 4: Mutation frequency in vitro in human (left) and animal (right) cells following BaP exposure.



Legend to Figures

Figure 1: BaP adducts in human tissues following BaP exposure in vitro: doses < 5µM (left), doses ≥ 5 µM (right)

Figure 2: BaP adducts in animal tissues following BaP exposure in vitro.

Figure 3: SCEs following BaP exposure in vitro: humans (left), animals (right).

Figure 4: Mutation frequency in vitro in human (left) and animal (right) cells following BaP exposure.

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