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Mutagenicity of selected polycyclic aromatic hydrocarbons (PAHs)

Awulu Emmanuel Aduojo[1,2]

[1]Department of Biological Sciences, Federal University Lokoja, Kogi State. Nigeria.
[2]School of Biological Sciences, University of Aberdeen. United Kingdom.

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants with a high octanol-water partition coefficient (*kow*) and are persistent in the environment. PAHs have various modes of action including mutagenicity, carcinogenicity, and teratogenicity. The mutagenic sensitivity to PAHs of single-cell gel electrophoresis (Comet assay) was compared with the Ames reversion *Salmonella* experiment (using *Salmonella typhimurium* TA98, TA100 and TA102) were with and without an exogenous metabolic activation system (S9 mix). *S. typhimurium* strains TA98, TA100 and TA102 treated with 4 and 40 µM of benzo[a]pyrene, 2-methylnaphthalene, and phenazine with and without S9 mix. Similarly, Caco-2 cells were treated with 5, 10, 20 and 40 µM of the chosen PAHs in the presence or absence of S9 mix. Even at the lowest treatment concentration (4 µM), benzo[a]pyrene, 2-methylnaphthalene and phenazine, significantly ($p < 0.05$) increased the number of revertant colonies of *S. typhimurium* TA98, TA100, and TA102 with S9 mix only. Similarly, the chosen PAHs significantly ($p < 0.05$) increased the tail moments of Caco-2 cells at the lowest treatment concentration (5 µM), resulting in decreased cell growth and viability as in the case of 2-methylnaphthalene. However, DNA damage to Caco-2 cells was not dependent on the S9 mix. The comet assay exhibits a comparable and more sensitive reaction to the tested PAHs than the Ames assay due to the inherent CYP450 metabolic pathway in mammalian cells.

Key words: Mutagenicity, Polycyclic aromatic hydrocarbons, benzo[a]pyrene, Ames assay, Comet assay.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants generated via pyrolytic (combustion of organic materials e.g., coal, oil, petrol and wood) and petrogenic processes (oil exploration and spillage) (Abdel-Shafy and Mansour, 2016). While most PAHs have anthropogenic sources, some derive from natural sources such as seepage, coal deposits and volcanic activities. PAH contamination have been reported in foods such as meat, vegetables, and fish via dry and wet deposition (Phillips, 1999; Zhao et al., 2008). Many PAH have toxic, mutagenic and/or carcinogenic effects post-metabolism (Abdel-Shafy and Mansour, 2016).

Short-term *in vitro* bioassays for genotoxic compounds include the Ames assay (Ames et al., 1973; Mortelmans and Zeigler, 2000) and single-cell gel electrophoresis (Comet assay) (Fairbairn et al., 1995; Collins, 2004), and sister chromatid exchange (SCE) (Tumini and Aguilera, 2021). These tests are based on the detection of DNA

E-mail: emmanuelawulu5@gmail.com

damage through various endpoints (gene mutations or DNA breaks or chromosomal abnormalities (Broerse et al., 1993; Yahagi et al., 1975).

The Ames assay has the highest predictivity (80-90%) of carcinogenesis among all the short-term bioassays (Benigni and Bossa, 2011). The Ames test is used to detect revertants in auxotrophic *Salmonella typhimurium* strains. It has been thoroughly validated and shown to be very sensitive for the identification of both DNA-reactive and non-DNA reactive genotoxins (Benigni and Bossa, 2011). While the Ames assay is extremely sensitive to mutagens, many chemicals, such as PAHs, are procarcinogens that display mutagenic activity only after metabolic products are released by microsomal cytochrome P450 enzyme complexes. As is the case with other bacterial reporter cells, the Ames *S. typhimurium* mutant strains lack the capacity to manufacture these enzyme complexes and so require external supply to detect procarcinogen mutagenic activity (Gelboin, 1980; Mortelmans and Zeigler, 2000).

The use mammalian reporter cells with the Comet assay for DNA damage offers an alternative approach to the Ames test and may more accurately model mutagenic effects of carcinogens in humans. Mammalian cells possess the microsomal enzyme systems to metabolize xenobiotics such as procarcinogens and therefore may not require exogenous supplementation (Gelboin, 1983). The comet assay involves suspending target cells in an agarose gel on a microscopic slide after exposure to the test compound. The immobilized cells are lysed by detergents and salts at high concentrations to release nuclear DNA, visualised with a fluorescent DNA binding dye (Singh et al., 1994). The shape of the "comet" depends on the number of single- and double-strand breaks in the nuclear DNA, and frequently reported parameters are the tail moment and tail intensity (Azqueta et al., 2011; Strubbia et al., 2019). The comet assay has been used to detect DNA damage in Annelids (Salagovic et al., 1996), mollusc (Wilson et al., 1998), fish (Nacci et al., 1996) and amphibians (Ralph et al., 1996). The assay can detect low level DNA damage (Chang et al., 2006; Morley et al., 2006; Olive and Banath, 2006). Because oral exposure to compounds such as PAHs and NPAHs is a significant exposure risk, this study used the human colon cancer cell line (Caco-2) (Hidalgo et al., 1989) as the target cell in the Comet Assay. Caco-2 cells are a model of human intestinal enterocytes that would be exposed to environmental contaminants shortly after oral ingestion and have been used to study the gut absorption and metabolism of exogenous chemicals such as PAHs (Ramesh et al., 2004).

This study assessed the compared mutagenic sensitivity of Ames *S. typhimurium* assay with single-cell gel electrophoresis (Comet assay) using benzo[a]pyrene, 2-methylnapthalene and phenazine as test models, due to their frequent detection in sediments (Dong et al., 2012), suspended particulate matter (Wang et al., 2013) and roasted food (Phillips, 1999). Benzo[a]pyrene is the most common PAH with established mutagenicity (Stiborova et al., 2016) while phenazine is a NPAH recently identified as a disinfectant-by-product (DBP) in drinking water (Zhao et al., 2008) and there is no information; its mutagenic activity is limited. Similarly, 2-methylnaphthalene has been detected in appreciable quantity in emissions from cigarettes, coal tar pitch, petroleum refineries, emission engines, and diesel exhaust fumes (HSDB, 2002; Warshawsky, 2001; ASTDR, 2005). 2-methylnaphthalene is absorbed rapidly post-ingestion, distributed and metabolised among tissues lungs and liver in less than 6 h (Melanon et al., 1982; Teshima et al., 1983; Ding et al., 2005; Fang et al., 2006).

MATERIALS AND METHODS

Chemicals and laboratory consumables

Oxoid Nutrient broth No. 2, Oxoid bacteriological agar, Benzo[a]Pyrene (CAS No. 50-32-8), 2Methylnaphhtalene (CAS No. 91-57-6), Phenazine (CAS No. 92-82-0), S9 fraction (Moltox), NADP (24292-60-2), Glucose-6-phosphate (5996-17-8), Sodium phosphate buffer recipes, Mitomycin C (5007-7), and Dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (Poole, UK) while S9 fraction was purchased from Moltox (Trinova, Giessen, Germany).

Metabolic activation system (S9 Mix)

S9 mix (pH 7.4) comprised 10% ($^{v}/_{v}$) mammalian liver homogenate S9 fraction from Aroclor 1254treated rats (Molecular Toxicology Inc. (Boone, NC)) in 4.2 mM NADP, 5.5 mM Glucose-6Phosphate, 32.6 mM potassium chloride, 17 mM Magnesium chloride, 21 mM Sodium Phosphate monobasic monohydrate, and 81.2 mM Sodium phosphate dibasic monohydrate (Mortelmans and Zeigler, 2000).

Preparation of test chemicals and controls

Stock solutions and of sodium azide (30 µg/mL), 4-nitro-0-phenylenediamine (400 µg/mL), and mitomycin-C (10 µg/mL) were prepared in Milli-Q water and applied as positive controls at concentrations (Mortelmans and Zeigler, 2000; Maron and Ames, 1983). Benzo[a]pyrene, 2methylnaphthalene and phenazine were dissolved in DMSO to a working concentration of 400, 500, 1000, 2000 and 4000 µM, respectively.

Salmonella mutagenicity test

S. typhimurium TA98, TA100 and TA102 were maintained according to standard protocols (Mortelsman and Zeiger, 2000). Each sample was tested in triplicate in the absence and presence of S9 Mix (2.5% $^{v}/_{v}$). *S. typhimurium* strains TA98, TA100 and TA102 were grown overnight in 250-ml Erlenmeyer flask containing 100 ml of Oxoid nutrient broth at 37°C, 200 rpm with appropriate antibiotics (25 µg/ml ampicillin for TA98 and TA100, and 2 µg/ml tetracycline for TA102). The cultures were incubated until they reached an absorbance of 1.0 at 600 nm (1-2 × 10^9 CFU/mL). For

the mutagenicity test conducted without S9 mix, 100 µL of each test chemical, 500 µL of 0.2 M phosphate buffer solution (pH7.4) and 100 µL of each *S. typhimurium* strain were transferred into a sterile glass tube and incubated for 25 min. Afterwards, 1.3 mL of melted top agar (0.6% agar and 0.6% NaCl) solution dissolved in distilled water, supplemented with histidine (0.5 mM) and biotin (0.5 mM) solution, and maintained at 45°C was added. The mixture was gently vortexed and poured onto the surface of minimal glucose agar plate. The plates were gently tilted and rotated to obtain an even distribution, placed onto a level surface to solidify, and incubated at 37°C for 48 h. Following the incubation, the revertant colonies were enumerated on a Gallenkamp colony counter. To conduct the assay with S9 mix, 500 µL of previously prepared S9 mix was added along with the 100 µL of tester strain and 100 µL of test chemical. Appropriate reagent and negative controls were included to quantify the spontaneous revertants. The two-fold threshold rule and ANOVA were used to determine mutagenicity and compare revertants colonies across the treatments.

Caco-2 cell culture

Caco-2 cells, a human colorectal adenocarcinoma cell line (ATCC, HTB-37), were maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml), streptomycin (100 µg/mL), glutamine (2 mM) and Sodium Pyruvate (0.1 mM) at 37^{O}C in a 5% CO_2 in a humidified atmosphere. For assay, sub-confluent cells were harvested with cell dissociation medium (TrypLE, Life Technologies, Paisley, UK) and diluted to the desired cell density.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) assay (Viability assay)

Caco-2 cells were seeded at a density of 2.0×10^5 cells/mL in DMEM in a 96-well plate. The cells were incubated for 24 h at 37°C, 5% CO_2. The old medium was replaced with a fresh medium and the cells were treated with each PAH and NPAHs to a final concentration of 10 and 40 µM in the presence and absence of S9 mix. The 96-well plate was incubated for another 24 h at 37°C, 5% CO_2. The medium from each well was replaced with 90 µl of fresh medium and 10 µL of MTT reagents. The 96-well plate was further incubated for 4 h. An aliquot of 100 µL of solubilization solution was then added to each well and incubated at room temperature in the dark for 2 h. The solution was mixed for complete solubilization. Absorbance was read at 570 nm using a microplate spectrophotometer (ABS plus, Molecular Devices, Wokingham, UK).

BrdU (5-Bromo-2-deoxyuridine) assay (Proliferation assay)

RAW and Caco-2 cells were seeded at a density of 2.0×10^5 cells/mL in DMEM in a 96-well plate. The cells were treated with each PAH and NPAHs to a final concentration of 10 and 40 µM in the presence and absence of S9 mix for 24 h. Cell proliferation was measured using the BrdU Cell Proliferation ELISA kit (Colourimetric, Abcam, AB126556, UK) according to the manufacturer instruction. Briefly, 20 µL of BrdU solution (10X) was added to each well containing 200 µL medium. After 24 h incubation the medium was removed and replaced with 100 µL of fixing/denaturing solution.

The plate was incubated at room temperature for 30 min. The solution was removed and replaced with 100 µL of detection antibody solution. The plate was kept at room temperature for 1 h. The solution was removed. Each well was washed three (3) times with wash buffer (1X). An aliquot of 100 µL of HRP-conjugated secondary antibody solution was aliquoted into each well. The plate was kept at room temperature for 30 min. The antibody solution was aspirated, and the wells washed three times with the wash buffer. 100 µL TMB substrate was added to each well and incubated at room temperature for 30 min. Finally, an aliquot of 100 µL of the stop solution was added and the absorbance was read using a 96-well microplate reader set at 450 nm wavelength.

COMET assay

Caco-2 cell cultures seeded in aliquot of 2 mL in a 24 well plate (2.0×10^5 cell/well) were treated with benzo[a]pyrene, 2-methylnaphtalene, fluorene, acridine and phenazine (the concentration of DMSO was maintained at 1% $^{v}/_{v}$) to a final concentration of 0, 5, 10, 20 and 40 µM for 24 h, respectively in the absence and presence of S9 mix (2.5% $^{v}/_{v}$). Hydrogen peroxide (20 µM) was used as a positive control. The comet assay followed the procedure of Singh et al. (1988).

After the duration of treatment, the medium from each well was replaced with 200 µL of cell dissociation medium (TrypLE, Life Technologies) and incubated for 15 min to allow detachment of the adherent cells. 1800 µL of medium was added to each well and the cells were dispersed in the medium using 1ml-micropipette. An aliquot of 50 µL of the Caco-2 cell was mixed with 450 µL of 0.5% ($^{v}/_{v}$) low melting point agarose dissolved in PBS and held at 37°C. From this mixture, 50 µL aliquot was taken and placed onto appropriate wells of a pre-treated and pre-warmed 20-well Trevigen microscope slide (Trevigen, #4250-050-03, Gaithersburg, MD). This was repeated for each treatment. The slide was cooled at 4°C for 15 min and then submerged in a freshly prepared lysis solution (2.5 M NaCl, 100 mM Na_2 EDTA, 10 mM Tris, pH 10, 1% sodium sarcosinate, 1% Triton X-100) and incubated at 4°C for 4 h. Afterwards, the slides were transferred into a horizontal gel electrophoresis tank filled with freshly prepared electrophoresis solution (1 mM Na_2EDTA, 300 mM NaOH, pH 13) maintained at 4°C for 30 mins followed by voltage application for 30 mins (0.74 V/cm, 300 mA). After the electrophoresis, the slides were rinsed with Tris buffer (0.4 M Tris, pH 7.5) for 10 min and rinsed with distilled water for 5 min. The slides were then transferred into ethanol solution (80% $^{v}/_{v}$ in water) for 5 min to remove excess water. The slides are then incubated at 37°C for drying. Before the slide image analysis, the slides were stained with Gelred dissolved in water (Sigma-Aldrich, #9Q05FE, (10000x)) for 30 min, rinsed with water and dried at 37°C. Slides were examined at x200 magnification using an epifluorescence microscope (LEICA, DMLB) equipped with excitation filter of 515-560 nm, connected through a gated CCD camera to Comet IV image analysis software (Instem, Stone, UK). Images were analysed and tail moment expressed in arbitrary units generated autonomously. The advantage of tail moment as an index of DNA damage is that both the amount of damage DNA and the distance of migration of the genetic material in the tail are represented by a single number (Ashby et al., 1995). Data are presented as median ± IQR. The Kruskal Wallis test with Dunn's post-hoc test was used to compare the medians of each treatment using GraphPad Prism statistical software.

RESULTS

The Ames *Salmonella* reversion assay

All the tested strains of *S. typhimurium* tested positive to

the positive control. However, in contrast, benzo [a]pyrene, 2-methylnaphthalene and phenazine caused no changes in the revertant frequency of the *Salmonella* strains tested at 4 and 40 µM in the absence of S9 mix. In the presence of S9 mix, benzo[a]pyrene, phenazine and acridine caused a fold-increase above two (2) in *S. typhimurium* TA98. Benzo[a]pyrene and 2-methylnaphthalene produced a fold-increase above (2) in *S. typhimurium* TA100. Similarly, a fold-increase of above two was observed in *S. typhimurium* TA102 treated with 2-methylnaphthalene and acridine at 4 and 40 µM, respectively (Table 1).

Proliferation and viability bioassay

The tolerance of Caco-2 cells to the solvent, DMSO, was assessed using two independent measures of cell viability, measuring cell proliferation (BrdU incorporation) assay and metabolic activity (MTT reduction). While DMSO concentrations at 2% ($^{v}/_{v}$) or more caused a significant reduction in both measures of cell viability (Figure 1a and b), lower (1% or less) did not have any significant effect on viabilityThe effect of benzo[a]pyrene, 2-methylnaphthalene, phenazine treatment on the proliferation and metabolic activity of Caco-2 cells in the presence and absence of S9 mix are presented in Table 2. In the presence of S9 mix, none of the PAHs and NPAHs caused a significant inhibition of cell proliferation. Benzo[a]pyrene and 2-methylaphthalene caused significant ($p < 0.05$) decrease in Caco-2 cell proliferation in the absence of S9 mix (Table 2). Metabolic activity was not significantly affected by any of the treatments, with the exception of 2-methylnaphthalene that caused a small but significant reduction in the viability of Caco-2 cells significantly ($p < 0.05$) in the presence (at 40 µM) and absence (at 4 and 40 µM) of S9 mix (Table 2). Table 3 expresses the median and inter-quartile range of the tail moments for Caco-2 cells exposed to different concentrations of benzo[a]pyrene, 2-methylnapthalene and phenazine. Hydrogen peroxide (20 µM) (positive control) caused a significant ($p < 0.001$) increase in the tail moment in Caco-2 cells (Table 3). Similarly, benzo[a]pyrene, 2-methylnaphthalene and phenazine caused a comparable dose dependent damage to DNA. However, these damages are not dependent on the S9 mix (Table 3). DNA damage was observed at the least tested concentration (5 µM) in benzo[a]pyrene, 2-methylnaphthalene and phenazine in the absence of S9 mix.

DISCUSSION

Benzo[a]pyrene, 2-methylnaphthalene and phenazine exerted different levels of biological effect on the modelled specimen. An indication of a possible exposure danger. benzo[a]pyrene, 2-methylnaphthalene and phenazine caused different mutations only in the presence of the S9 mix on the test models (Table 1). Ames *Salmonella* test models have the *rfa* mutation, which alters the bacterial cell membrane to enhance chemical uptake (Mortelmans and Zeigler, 2000), and coupled with the lack of CYP450 metabolic enzymes, makes sensitivity to PAHs impossible. Therefore, the metabolites of PAHs are released in response to the exogenous supply of metabolic activation systems, causing the observed mutation. These observations corroborate the procarcinogen status of PAHs, whose metabolites through the CYP450 metabolic pathway produce diols and epoxides that form DNA adducts leading to mutations (Shimada et al., 2001; Rubin et al., 2005; Locasale and Cantley, 2011; Chapman et al., 2020). Similarly, the findings are consistent with the report that implicates the metabolites of benzo[a]pyrene and 2-methylnaphthalene in causing base-pair mutation, acre mutation, sister chromatid exchange, chromatid breaks, and frameshift mutation (Madill et al., 1999; Kulka et al., 1988; EPA, 2016). No information is available on the mutagenicity of phenazine via the Ames assay, and the available data was on the comet assay by McGuigan and Li (2014). However, our data showed that phenazine caused a frameshift mutation (Table 1).

Furthermore, the use of mammalian cells to screen for mutagenicity was inherent in their ability to initiate the CYP450 metabolic pathway in the degradation of xenobiotics (Buesen et al., 2003). Interestingly, the data showed that only 2-methylnaphthalene significantly reduced the proliferation and viability of Caco-2 cells by ~16% and ~22% respectively, at the minimal tested dose (Table 2). In contrast to our findings, benzo[a]pyrene has been shown to reduce cell proliferation and mitochondrial activity in A549 cells (Wang et al., 2015) while increasing cell proliferation in hepatoma cells (Chen et al., 2014). There is no previously published data on the effects of 2-methylnaphthalene on the proliferation and viability of Caco-2 cells or other mammalian cell lines. Phenazine has been shown to reduce mitochondrial activity in T24 cells at concentrations of 61.5 and 123 µM, as well as HepG2 cells at 7.7 µM (McGuigan and Li, 2014), however, in our study, except for 2-methylnaphthalene, none of the PAHs or NPAHs tested changed the mitochondrial activity of Caco-2 cells (Table 2).

In addition, our data showed the tested PAHs caused DNA damage in Caco-2 cells without obvious implication for proliferation or viability, except for 2-methyl-naphthalene. Benzo[a]pyrene, 2-methylnaphthalene, and phenazine caused DNA damage in Caco-2 cells (Table 3), even at the least tested concentration (5 µM). These damages occurred independent of the exogenous S9 mix. Benzo[a]pyrene caused significant DNA damage at all concentrations, caused significant DNA damage at all

Table 1. Induction of reverse his- mutation in Ames *Salmonella typhimurium* TA98, TA100 and TA102 strains.

Compound[d]	Dose (µM/plate) [b]	Number of revertants/plate (Mean ± SD)[a]					
		Without S9 mix			With S9 mix		
		TA98	TA100	TA102	TA98	TA100	TA102
Benzo[a]pyrene	0	17 ± 2	151 ± 59	323 ± 10	13 ± 7	155 ± 18	345 ± 33
	4	10 ± 2	189 ± 90	317 ± 4	599 ± 7*	960 ± 180*	461 ± 53*
	40	17 ± 1	169 ± 9	375 ± 35	629 ± 22*	1137 ± 94*	597 ± 36*
Positive control[c]		1645 ± 97*	1441 ± 42*	1620 ± 53*	224 ± 28*	535 ± 74*	770 ± 89*
2-Methylnaphthalene	0	24 ± 4	160 ± 71	302 ± 26	26 ± 11	160 ± 21	287 ± 21[d]
	4	25 ± 4	144 ± 15	164 ± 46	29 ± 1	837 ± 237*	632 ± 116*
	40	17 ± 1	156 ± 27	170 ± 55	25 ± 5	1025 ± 253*	837 ± 60*
Positive control[c]		1128 ± 120*	686 ± 57*	1656 ± 140*	1367 ± 135*	1190 ± 125*	997 ± 10*
Phenazine	0	10 ± 1[d]	158 ± 40	326 ± 23	16 ± 6	155 ± 19	345 ± 33
	4	13 ± 2	130 ± 70	400 ± 23	599 ± 7*	231 ± 31*	565 ± 66*
	40	14 ± 2	168 ± 31	470 ± 44	629 ± 22*	278 ± 22*	598 ± 43*
Positive control[c]		1235 ± 31*	695 ± 23*	1655 ± 134*	224 ± 28*	1060 ± 17*	639 ± 54*

[a]The mean number of histidine revertant colonies per plate of three replicates ± standard deviation b: Concentration is based on 10 × 15 mm Petri dish containing 20-25 cm^3 of minimal glucose agar. The recommended positive controls for *S. typhimurium* strains in the absence of S9 mix. TA98 (4-Nitro-0-diphynylenediamine (20 µg/plate)). TA100 (Sodium azide (2.0 µg/plate)) and Mitomycin-C (0.5 µg/plate). With S9 mix, 2-aminoanthracene (2 µg/plate) was used as the positive control for all *S. typhimurium* strains. [d]Test PAHs (Benzo[a]pyrene, 2-Methylnaphthalene and phenazine). *Significant ($p < 0.05$) increase in revertant colonies over the solvent control (underlined and italicised).
Source: Author

Table 2. Measurement of DNA synthesis (proliferation via BrdU assay) and mitochondrial activity (viability via MTT assay) of Caco-2 cells supplemented with concentrations of selected PAHs and NPAHs in the presence and absence of S9 mix.

Compound	Dose (µM)	% Proliferation (Mean ± SD)[a]		% Viability (Mean ± SD)[a]	
		-S9 mix	+S9 mix	-S9 mix	+S9 mix
Benzo[a]pyrene	10	73.15 ± 6.69*	119.2 ± 14.54	87.63 ± 10.14	89.36 ± 11.42
	40	71.60 ± 6.70*	110.90 ± 14.52	89.58 ± 20.12	85.51 ± 20.12
2-Methylnaphthalene	10	84.81 ± 3.24*	116.6 ± 9.18	77.34 ± 8.81*	79.75 ± 22.75
	40	81.86 ± 3.23*	106.6 ± 9.20	69.81 ± 8.91*	**78**.41 ± 5.71*
Phenazine	10	84.40 ± 8.90	105.3 ± 8.84	86.08 ± 19.25	89.31 ± 8.83
	40	107.9 ± 8.84	92.17 ± 8.83	90.70 ± 13.53	88.78 ± 2.43

*Significant ($p < 0.05$) decrease compared to the control (the control is designated a 100% response). [a]Data point represent mean ± standard deviation of quadruplicate treatments expressed as a percentage of the solvent control. DNA Damage in Caco-2 cells.
Source: Author

concentrations, with a clear concentration dependent increase, consistent with previous studies showing dose-dependent DNA fragmentation in sperm (Gelboin, 1980; Sipinen et al., 2010). The levels of DNA damage in benzo[a]pyrene in S9 treated Caco-2 cells were at least 2-fold lower than in untreated cells. As well as demonstrating the endogenous metabolic activation available in Caco-2 cells. However, 2-methylnaphthalene

Table 3. Comet assay tail moment in CaCo-2 cells after treatment with control mutagens and study PAH.

Compound	Tail moment median (IQR)					
	Concentrations					
	0 µM	5 µM	10 µM	20 µM	40 µM	H_20_2 20 µM
B[a]P	1.75 (0.11 - 4.48)	17.75 (8.66 - 29.78) ***	29.02 (14.69 - 42.35) ***	ND	48.60(38.53 - 83.47)***	10.37 (5.12 - 38.18)***
B[a]P + S9	5.25 (0.59 - 10.64)	12.13 (6.82 - 20.71) ***	17.78 (12.09 - 25.34) ***	17.65 (8.68 - 30.28) ***	18.46 (9.87 - 28.30)***	18.22 (10.80 - 25.25)***
2-MN	5.10 (1.76 - 11.74)	ND	2.81 (1.85 - 12.55)	6.63 (1.85 - 12.55)	3.00 (0.42 - 16.36)	
2-MN +S9	13.27(6.15 - 21.52)	32.80 (25.34 -47.21) ***	33.36 (21.83 - 40.10) ***	18.28 (11.57 - 46.39) *	19.92 (8.35 - 50.80)**	
Phenazine	0.46 (0.00 - 4.28)	ND	2.20 (0.02 - 9.58)	13.04 (9.02 - 16.31) ***	13.53 (9.88 - 21.94)***	
Phenazine +S9	0.42 (0.00 - 4.29)	5.70 (1.94 - 9.16) *	10.07 (3.79 - 21.12) ***	17.72 (7.13 - 19.60) ***	13.42 (7.13 - 19.60)***	

ND: No data. *Significantly increased over control (0 µM) p<0.01. **Significantly increased over control (0 µM) p<0.001. ***Significantly increased over control (0 µM) p<0.0001.
Source: Author

also exerted antiproliferative effects on Caco-2 cells in the presence of S9 mix, and yet in this case, DNA damage is only caused by S9 mix in the comet assay. Possibly, this reflects differences in the activation pathways for each of these PAHs and bioaccessibility as limited by the partition coefficient (Xue and Warshawsky, 2005; Penning, 2021). The variation in the structure of benzene, 2-methylnapthalene and phenazine may account for the different metabolic pathways and oxidative stress induced in the test bacterial and mammalian models. These likely account for the observed differential mutagenicity.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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