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Sensitivity of Salmonella YG5161 for Detecting PAH-Associated Mutagenicity in Air Particulate Matter

Running title: Sensitivity of YG5161 for detection of PAH-mutagenicity in air

Gisela A. Umbuzeiro[1]*, Fábio Kummrow[2], Daniel Alexandre Morales[1], Debora Kristina M. Alves[3], Hwanmi Lim[5], Ian W. H. Jarvis[4], Christoffer Bergvall[5], Roger Westerholm[5], Ulla Stenius[4], and Kristian Dreij[4]

[1]Faculty of Technology, State University of Campinas (Unicamp), Limeira, SP, Brazil.
[2]Institute of Environmental, Chemical and Pharmaceutical Sciences, Federal University of São Paulo (Unifesp), Diadema, SP, Brazil.
[3]Pharmaceutical Sciences Faculty, University of São Paulo (USP), São Paulo, SP, Brazil.
[4]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.
[5]Department of Analytical Chemistry, Stockholm University, Stockholm, Sweden.

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* Corresponding author: Tel: +55 19 21133419; E-mail: giselau@ft.unicamp.br

ABSTRACT

The Salmonella/microsome assay is the most used assay for the evaluation of air particulate matter (PM) mutagenicity and a positive correlation between strain TA98 responses and benzo[a]pyrene (B[a]P) levels in PM has been found. However, it seems that the major causes of PM mutagenicity in this assay are the nitro and oxy-PAHs. Salmonella YG5161, a 30-times more responsive strain to B[a]P has been developed. To verify if YG5161 strain was sufficiently sensitive to detect mutagenicity associated with B[a]P mutagenicity, PM samples were collected in Brazil and Sweden, extracted with toluene and tested in the Salmonella/microsome microsuspension assay. PAHs and B[a]P were determined and the extracts were tested with YG5161 and its parental strain TA1538. The extracts were also tested with YG1041 and its parental strain TA98. For sensitivity comparisons, we tested B[a]P and 1-nitropyrene (1-NP) using the same conditions. The minimal effective dose of B[a]P was 155 ng/plate for TA1538 and 7 ng/plate for YG5161. Although the maximum tested dose, 10 m3/plate containing 9 ng of B[a]P in the case of Brazilian sample, was sufficient to elicit a response in YG5161, mutagenicity was detected at a dose as low as 1 m3/plate (0.9 ng). This is probably caused by nitro-compounds that have been shown to be even more potent than B[a]P for YG5161. It seems that the mutagenicity of B[a]P present in PM is not detectable even with the use of YG5161 unless more efficient separation to remove the nitro-compounds from the PAH extract is performed.

INTRODUCTION

The mutagenicity of airborne particulate matter (PM) can be attributed to at least 500 identified compounds from different chemical classes [Claxton et al., 2004]. Among these, benzo[a]pyrene (B[a]P), along with other polycyclic aromatic hydrocarbons (PAHs), have received great attention because of their recognized carcinogenic and mutagenic potential [Srogi, 2007].

The Salmonella/microsome assay is the most widely used method for the evaluation of the mutagenic activity of pure compounds and environmental samples [Claxton et al., 2010], including atmospheric samples [Claxton and Woodall Jr, 2007]. The assay is sensitive to several PAHs, including B[a]P [DeMarini et al., 2011; Brito et al., 2013]. Some studies have correlated the mutagenic activity detected in the Salmonella/microsome assay with the levels of B[a]P and other non-substituted PAHs present in the samples [Viras et al., 1990; Nielsen et al., 1996; Claxton and Woodall Jr., 2007; Srogi, 2007], although it does not seem to demonstrate a direct relationship. The primary components responsible for the mutagenicity of air particulate matter in the Salmonella assay seem to be nitro and oxy-PAHs [Claxton et al., 2004; Sharma et al., 2007; Umbuzeiro et al., 2008 a, b; Walgraeve et al., 2010]. One explanation for this could be that the typically used strains (TA98 and TA100) are more sensitive to nitro- and oxy- PAHs than to non-substituted PAHs [Claxton et al., 2004; Enya et al., 1997; Enya et al., 1998; Kummrow et al., 2006; Franco et al., 2010]. To enhance the sensitivity of the Salmonella/microsome assay to non-substituted PAHs Matsui et al. [2006] developed the YG5161 strain which is more responsive to B[a]P and other non-substituted PAHs than its parental strain TA1538. The YG5161 strain overexpresses DNA polymerase IV, and has the *din*B gene of *Escherichia coli* encoded in the pYG768 plasmid, which also confers ampicillin resistance to the strain. DNA polymerase IV facilitates the error-prone bypass of the DNA guanine adducts formed by polycyclic aromatic compounds which, after

repair, will lead to the deletion of two base pairs and consequently shifting of the reading frame [Matsui et al., 2006]. These authors suggested the possibility of using YG5161 as a major strain for the detection of the mutagenicity of non-substituted PAHs such as B[a]P. Because this compound needs to be metabolized to react with DNA [Uppstad et al., 2010], the addition of S9 mix is required for its detection in the Salmonella/mutagenicity assay.

Some strains have also been developed to be more sensitive to different compounds. For example YG1041 strain, a derivative of TA98, is more sensitive to nitroarenes and aromatic amines because it overproduces nitroreductase and *O*-acetyltransferases, both important enzymes in the activation of such compounds [Hagiwara et al., 1993]. Similarly, the strain YG7108 which is derived from TA1535 is more responsive to alkylating agents [Yamada et al., 1997]. Both strains have been used in the identification of the types of compounds predominantly responsible for the mutagenic activity of a test samples [Umbuzeiro et al., 2011]. Mutlu et al. [2013] demonstrated the applicability of strains with different sensitivities for analyzing environmental samples. In a hierarchical clustering analysis they showed that although PAHs, aromatic amines, and nitro-compounds were present in diesel exhaust extracts, oxy-PAHs were the cause of much of the mutagenicity.

When atmospheric particulate air samples are tested they usually demonstrate a clear increase with YG1041 in relation to TA98, indicating that the mutagenicity is related to nitro- and oxy- PAHs and not B[a]P, although PAHs are present in those samples when chemical analyses are performed [DeMarini et al., 2004; Umbuzeiro et al., 2008a,b].

The objective of this work was to verify if the YG5161 strain in the Salmonella/microsome microsuspension assay was sufficiently sensitive to B[a]P mutagenicity in air particulate matter collected in two different locations, Limeira, Brazil and Stockholm, Sweden. We determined the total PAH and B[a]P levels and tested the same extracts with YG5161 and its parental strain, TA1538. We also tested the same extracts with

YG1041 and its parental strain, TA98. For sensitivity comparisons we tested several concentrations of B[a]P and 1-nitropyrene (1-NP) using the same conditions.

MATERIAL AND METHODS

Sampling sites

Total atmospheric particulate matter (PM) was collected at two sites: the campus of the Faculty Technology at UNICAMP, Limeira, Brazil (LIMEIRA) and the campus of the Stockholm University, Stockholm, Sweden (STHOLM). The LIMEIRA site is impacted by heavy traffic including cars and trucks, industrial emissions, and sugar cane growing and harvesting activities, including biomass burning. The mutagenic potencies of previously evaluated extracts from this site using the Salmonella/microsome assay [Alves, 2011] and surrounding areas [Umbuzeiro et al., 2008a, b] were among the highest potencies found in the literature. The STHOLM site is also impacted by heavy traffic with emissions from cars and trucks as the main source of pollution. PM samples from this site have recently been used to investigate the toxicological impact of PAHs in air PM [Jarvis et al., 2013a,b].

Total PM samples from Limeira were collected at street level on a glass-fiber filter (254 × 233 mm; 0.33 mm pore size. Energética Ind. Com. LTDA, Rio de Janeiro, RJ, Brazil) using a high-volume sampler (Energética Ind. Com. LTDA, Rio de Janeiro, RJ, Brazil) operated at an average flow rate of 1,130 L/min for 24 h. The air total PM samples from Stockholm, Sweden were collected at roof-top level (22 m from the ground) on a fluorocarbon-coated glass fiber filter (235 mm of diameter; Fiberfilm Filters, Pallflex, Pall Corporation, Putnam, CT, USA) with an average flow rate of 1,209 L/min for 71 h. The total PM concentrations for Limeira were 95.8 ug/m^3 and for Stockholm, 7.16 ug/m^3.

Organic extraction and PAH analysis

Extraction was performed by pressurized fluid extraction using an ASE 200 accelerated solvent extraction system (Dionex Co., Sunnyvale, CA, USA) using toluene for 5 x 30 min. This extraction procedure was previously developed and validated for analysis of PAHs in air PM [Bergvall and Westerholm, 2008]. Aliquots of the extracts were evaporated gently under a nitrogen stream at 55°C until dryness and re-dissolved in dimethylsulfoxide (DMSO) for the biological assays. Portions of the extracts were analyzed for 42 PAHs (Table I) using silica solid phase extraction (SPE) cartridges and on-line liquid chromatography-gas chromatography/mass spectrometry as described in detail elsewhere [Sadiktsis et al., 2014]. A blank filter was also extracted and analyzed as an analytical control.

Salmonella/microsome microsuspension assay

The extracts were assayed in the Salmonella/microsome microsuspension assay [Kado et al., 1983; DeMarini et al., 1989] in dose-response experiments using the *Salmonella* strains TA1538, TA98, YG5161 and YG1041 with and without metabolic activation (S9). Strains were kindly provided by Dr. Takehiko Nohmi, except for TA98, which was kindly provided by Dr. Larry Claxton. Table II summarizes the genetic characteristics of the strains. B[a]P and 1-NP were also tested with the four strains in dose response experiments. Overnight cultures (around 10^9 cells/mL) were concentrated 5-fold by centrifugation (10,000xg at 4 ^{o}C for 10 min) and resuspended into 0.015 M sodium phosphate buffer. A volume of 50 µL of cell suspension, 50 µL of 0.015 M sodium phosphate buffer or S9 mix, and 5 µL of the sample were incubated at 37 ^{o}C for 90 min without shaking. To the mixture, 2 mL of molten agar was added and poured onto a minimal agar plate. Colonies were counted after 66 h of incubation at 37 ^{o}C by hand with the aid of a stereomicroscope. Toxicity was also carefully evaluated by observing the background of the agar plates. Metabolic activation was provided by Aroclor

1254-induced Sprague Dawley rat liver S9 mix (MolTox, Boone, NC) prepared at 4 % v/v and supplemented with the required co-factors [Mortelmans and Zeiger, 2000].

PM extracts were tested at 0.02, 0.2, 1, 2, 5 and 10 m^3 per plate. Positive controls without S9 were 4-nitroquinoline-oxide (4NQO) at 0.125 µg/plate for TA98 and YG5161 and 4-nitro-o-phenylenediamine (4NOP) at 2.5 µg/plate for YG1041. With S9, 2-aminoanthracene (2AA) at 0.625 µg/plate was used as a positive control for TA1538, TA98 and YG5161 and at 0.03125 µg/plate for YG1041. Duplicates of each concentration were tested, except for the negative control that was tested in triplicate. DMSO was used as negative control and to dilute the extracts and positive controls. The extract of a clean (blank) filter was also tested.

Data were analyzed with the Salanal computer program using the Bernstein model [Bernstein et al., 1982]. Samples were considered positive when a significant difference among the tested doses and the negative control (ANOVA) and a significant positive dose response were observed. Results were expressed as revertants per m^3 and per mg of PM equivalent. Also, the minimum effective dose (MED) to elicit a positive response was calculated for B[a]P and 1-NP. MED was defined as the dose that provided a doubling of revertants based on the calculated linear regression curve.

RESULTS AND DISCUSSION

In this study, we evaluated the mutagenicity of PM organic extracts from two cities: Limera, Brazil and Stockholm, Sweden. The concentration of PM collected in LIMEIRA was 95.8 µg/m^3 and in STHOLM was 7.16 µg/m^3. The total PAH content in the LIMEIRA sample was 10.66 ng/m^3 with 0.9 ng/m^3 of B[a]P, and the STHOLM sample contained 4.43 ng/m^3 with 0.29 ng/m^3 of B[a]P (Table I). The mean of the number of revertants per plate obtained for the four strains including negative, blank, positive controls and calculated potencies are presented in Table III for LIMEIRA and Table IV for STHOLM. In LIMEIRA,

the potency for TA98 without S9 was 100 revertants/m^3, similar what had been reported for other cities in Brazil [Sato et al., 1995; Ducatti and Vargas, 2003; Umbuzeiro et al., 2008 a, b]. The potency of the STHOLM sample was 4 times lower (25 revertants/m^3). The potency values decreased for both locations when S9 was used (Tables III and IV). According to Claxton et al. [2004] this is a first indication of a response of nitroaromatics. In general the samples from LIMEIRA were more mutagenic than the STHOLM in all strains/conditions tested, especially with TA98 with S9 (13-fold difference). However, because only one sample from each site was analyzed it was not possible to compare the mutagenicity of the two sites with a high level of confidence. A comprehensive study, which includes additional sampling at the same season of the year, is being conducted to provide additional information on the potencies at both sites.

To establish how sensitive each strain was to B[a]P, the prototypical non-substituted PAH, and 1-NP, a typical nitroaromatic, their mutagenicities were assessed using the same protocol used to evaluate the PM samples.

YG5161 was the most sensitive strain for B[a]P showing a MED of 7 ng/plate in contrast to 77.5 ng/plate for TA1538, 265 ng/plate for YG1041, and 1,400 ng/plate for TA98 (Table V). Although several non-substituted PAHs were detected in both PM samples (Table II), the potency of the YG5161 response did not provide a typical B[a]P response, which would be an increase of 30-fold in relation to TA1538 (Table V). This is most likely due to the fact that the amount of B[a]P present in the 10 m^3 of PM (the maximum dose tested) was 9 ng/plate and the MED of B[a]P for YG5161 is 7 ng/plate (Table V). All the comparisons were made with S9 because non-substituted PAHs require S9 to be activated.

YG1041, as expected, demonstrated much higher sensitivity to 1-NP than any other strain, with a MED of 0.03 ng/plate. MEDs for TA98, TA1538 and YG5161 were 0.45, 1.8 and 1.8 ng/plate, respectively (Table VI). 1-NP was chosen as an example for testing purpose,

but other nitrocompounds (e.g. dinitropyrenes, nitrobenzanthrones) are more mutagenic and are likely to be present in PM samples. For both PM samples we observed a typical response of nitroaromatics because the potency of YG1041 was 35 times higher than TA98. This comparison was made without S9 because all the potencies decreased with S9. This behavior is consistent with the hypothesis that nitroaromatics are the major cause of the mutagenic effect.

The data suggest that B[a]P is not the compound predominantly causing the mutagenic responses of the PM analyzed in this study, but that nitroaromatics such as 1-NP seems to be causing the observed effect. Chemical analysis of nitroaromatics could be performed in the same extracts in order to determine their contribution to the mutagenicity.

Other authors also used YG5161 to test different samples. Sharma et al. [2007] used TA98, YG1041, and YG5161 strains, in the Salmonella/microsome microsuspension assay to evaluate the genotoxicity of organic fractions of PM collected from an incineration energy plant and urban air. The fraction containing moderately polar PAHs, alkyl-PAHs and *O*- and *S*-heterocyclics was mutagenic only in the presence of S9 mix, indicating that compounds such as B[a]P could be responsible for the observed effect. Because the mutagenic activity with the YG5161 strain was lower in comparison to TA98 it is plausible to suspect that non-substituted PAHs are not predominantly contributing to the observed mutagenic activity. Conversely, the fraction containing N-heterocyclics, nitro-, amino- and oxy-PAHs was more mutagenic without S9 and higher in YG1041 than TA98, suggesting that nitroarenes and aromatic amines are likely to be the major cause of the mutagenic activity.

Maertens et al. [2009] used the Salmonella/microsome assay with several strains/conditions, including TA98, YG1041 and YG5161 to, evaluate the mutagenicity of tobacco and marijuana smoke condensates. All of the tested smoke condensates presented positive responses. The mutagenic potencies obtained with YG1041 and with YG5161 were

higher than the ones with TA98 indicating that aromatic amines and non-substituted PAHs were contributing to the mutagenicity. Although the authors identified B[a]P and benzo[a]anthracene in the samples, they contributed to less than 0.1% of the total mutagenicity indicating that there are other major mutagens in the mixture.

Yauk et al. [2012] observed mutagenic activity in cigarette smoke condensate extracts using the Salmonella/microsome assay with the TA98, YG1041, and YG5161 strains in the presence of S9 mix. The authors observed that the potencies with YG1041 were greater than those for TA98 but the increase of YG5161 potencies in relation to TA98 was not significant, indicating that non-substituted PAHs could not fully explain the mutagenicity.

Therefore we conclude that even the highly sensitive strain to B[a]P, YG5161, does not seem to be able to distinguish the response of B[a]P in samples containing nitroaromatics. We observe that only 1 m^3 is sufficient to elicit a positive response in the Salmonella/microsuspension assay for the majority of the strains/conditions. Both for the LIMEIRA and STHOLM air samples.

Therefore we can suggest that although associations were observed between B[a]P content and mutagenicity, this compound does not totally explain the mutagenicity of PM air samples tested in the Salmonella/microsome assay unless amounts of B[a]P are higher than 7 ng/plate and nitrocompounds are eliminated by the fractionation procedure.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Contribution of the Authors

Gisela A. Umbuzeiro: designed experiments, performed the Salmonella/microsome assay, analyzed data and drafted the manuscript

Fábio Kummrow: designed experiments, performed the Salmonella/microsome assay, analyzed data and drafted the manuscript

Daniel Alexandre Morales: designed experiments, performed the Salmonella/microsome assay and analyzed data

Debora Kristina M. Alves: designed experiments, performed the Salmonella/microsome assay and analyzed data

Hwanmi Lim: performed the chemical analysis and analyzed data

Ian W. H. Jarvis: designed experiments, analyzed data and drafted the manuscript

Christoffer Bergvall: designed experiments, analyzed data and drafted the manuscript

Roger Westerholm: designed experiments, analyzed data and drafted the manuscript

Ulla Stenius: designed experiments, analyzed data and drafted the manuscript

Kristian Dreij: designed experiments, analyzed data and drafted the manuscript

TABLE I. PAHs content (pg/m^3) in extracts of the PM samples collected in Limeira, Brazil and Stockholm, Sweden.

PAH	LIMEIRA (pg/m^3)		STHOLM (pg/m^3)	
	Mean	**SD**	**Mean**	**SD**
Phenanthrene	798	27	250	2
Anthracene	180	14	33.6	0.7
3-Methylphenanthrene	47.0	2.5	23.8	0.7
2-Methylphenanthrene	65.4	2.1	32.0	1.3
2-Methylanthracene	14.8	0.7	5.75	0.52
9-Methylphenanthrene	34.5	1.5	18.0	1.4
1-Methylphenanthrene	43.3	4.2	37.2	0.8
4H-Cyclopenta[def]phenanthrene	58.9	2.0	47.8	0.1
2-Phenylnaphthalene	35.0	2.9	26.9	2.6
3,6-Dimethylphenanthrene	5.03	0.20	1.70	0.11
3,9-Dimethylphenanthrene	22.9	1.5	8.31	0.75
Fluoranthene	671	6	368	6
Pyrene	895	25	439	8
1-Methylfluoranthene	40.9	3.5	46.1	3.7
Benzo[a]fluorene	28.7	1.2	27.0	2.4
Benzo[b]fluorene	16.2	1.5	13.0	0.8
2-Methylpyrene	30.8	2.9	18.4	1.4
4-Methylpyrene	52.3	4.7	30.7	2.9
1-Methylpyrene	47.7	3.9	32.1	3.2
Benzo[ghi]fluoranthene	346	15	207	8
Benzo[c]phenanthrene	48.5	3.4	77.6	2.1
Benzo[b]naphto(1,2-d)thiophene	9.95	0.31	2.16	0.71
Benz[a]anthracene	332	3	306	10
3-Methylchrysene	39.1	2.3	21.2	2.0
2-Methylchrysene	61.0	3.3	47.9	3.5
6-Methylchrysene	40.2	2.5	32.6	2.5
1-Methylchrysene	47.1	2.6	51.5	3.3
Benzo[b]fluoranthene	974	19	465	8
Benzo[k]fluoranthene	440	5	180	4
Benzo[e]pyrene	1050	31	352	13
Benzo[a]pyrene	**899**	**17**	**289**	**4**
Perylene	164	6	45.1	0.2
Indeno[1,2,3-cd]fluoranthene	65.4	1.5	28.2	0.3
Indeno[1,2,3-cd]pyrene	908	28	230	12
Dibenz[a,h]anthracene	55.9	2.7	35.6	3.3
Picene	76.2	2.2	30.9	1.7
Benzo[ghi]perylene	1620	35	351	7
Dibenzo[a,l]pyrene	4.79	0.37	5.14	0.28
Dibenzo[a,e]pyrene	15.7	0.6	32.4	2.0
Coronene	366	8	171	10
Dibenzo[a,i]pyrene	9.23	0.28	3.66	0.17
Dibenzo[a,h]pyrene	3.03	0.11	2.53	0.19
Total	**10,663**		**4,427**	

TABLE II. Summary of the main genetic characteristics of *Salmonella typhimurium* specific strains.

Strain	Description	Reversion event	Plasmids	Reference
TA1538	*his*D3052, Δ(*uvr*B, *bio*), *rfa*	Frameshift	No plasmids	Maron and Ames, [1983]
TA98	*his*D3052, Δ(*uvr*B, *bio*), *rfa*, Ap^r	Frameshift	pKM101	Maron and Ames, [1983]
YG1041	*his*D3052, Δ (*uvr*B, *bio*), *rfa*, Ap^r and Km^r, NR and O-AT overproducing strain	Frameshift	pKM101, pYG233	Hagiwara et al. [1993]
YG5161	*his*D3052, Δ(*uvr*B, *bio*), *rfa*, Ap^r, DNA Pol IV overproducing	Frameshift	pYG768	Matsui et al. [2006]

his – mutation in the histidine operon
Δ*uvrB* – deletion of *uvr*B gene
Δ*bio* – deletion of biotin gene
rfa – mutation cause partial loss of the lipopolysaccharide barrier
Ap^r – resistant to ampicillin
NR – nitroreductase
O-AT – O-acetyltransferase
DNA Pol. IV – DNA Polymerase IV
Km^r – resistant to Kanamycin

TABLE III. Mutagenicity for LIMEIRA PM extracts in the Salmonella/microsome microsuspension assay with TA98, YG1041, TA1538 and YG5161 without and with S9.

Doses	Mean of Number of Revertants/plate and *Standard Deviation (SD*)							
m^3/plate	TA98				YG1041			
	-S9		+S9		-S9		+S9	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Negative control	52.7	*4.51*	53.3	*14.4*	157.7	*17.1*	145.7	*9.1*
0.02	53.5	*2.12*	58.0	*7.1*	262.0	*24.1*	151.5	*17.7*
0.2	68.5	*6.4*	59.5	*0.7*	849.5	*7.8*	329.5	*16.3*
1	144.0*	*22.6*	69.0	*8.5*	1,922.5	*160.5*	*1,344.0*	*73.5*
2	207.5	*45.9*	59.0	*2.8*	3,583.5	*231.2*	3,312.5	*50.2*
5	595.5	*37.5*	76.0	*9.9*	4,145.5	*381.1*	5,308.0	*151.3*
10	1,110.0	*70.7*	133.0	*18.4*	3,861.5	*362.7*	6,355.5	*0.7*
Blank Filter	60.0	*1.4*	47.0	*2.8*	146.0	*30.4*	137.0	*11.3*
Positive control	927.0	*101.1*	1,518.0	*352.8*	908.0	*173.9*	2,344.0	*7*
Potency revertants/m^3	**100**		**6.3**		**3,500**		**1,100**	
Doses	Mean of Number of Revertants/plate and *Standard Deviation (SD*)							
m^3/plate	TA1538				YG5161			
	-S9		+S9		-S9		+S9	
	Mean	*SD*	Mean	SD	Mean	*SD*	Mean	SD
Negative control	15.0	*2.0*	12.7	*2.5*	26.3	*4.0*	28.0	*2.65*
0.02	16.5	*0.7*	14.5	*2.1*	23.5	*0.7*	29.5	*7.8*
0.2	28.0	*2.8*	18.5	*2.1*	43.0	*7.1*	34.0	*7.1*
1	89.5	*0.7*	52.0	*9.9*	99.0	*2.8*	57.5	*2.1*
2	146.0	*18.3*	69.0	*14.1*	136.0	*4.2*	85.5	*3.5*
5	306.5	*24.7*	147.5	*13.4*	272.0	*4.2*	162.0	*7.1*
10	429.0	*38.2*	209.0	*1.4*	395.0	*8.5*	179.0	*28.3*
Blank Filter	20.0	*1.4*	21.0	*9.2*	18.0	*0.7*	29.0	*6.4*
Positive control	392.0	*50.2*	2,451.0	*746.0*	677.0	*111.0*	3,114.0	*514.1*
Potency revertants/m^3	**62**		**28**		**61**		**27**	

*Shaded values represent the number of revertants that equal or more than twice the negative controls

TABLE IV. Mutagenicity data for STHOLM PM extracts of the Salmonella/microsome microsuspension assay with TA98, YG1041, TA1538 and YG5161 without and with S9.

Doses	Mean of Number of Revertants/plate and *Standard Deviation (SD*)							
m^3/plate	TA98				YG1041			
	-S9		+S9		-S9		+S9	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Negative control	52.7	*4.5*	53.3	*14.4*	157.7	*17.1*	145.7	*9.1*
0.02	58.5	*6.4*	51.5	*3.5*	173.5	*6.4*	157.0	*5.7*
0.2	61.0	*12.7*	43.0	*1.4*	364.5	*2.1*	173.5	*16.3*
1	73.0	*1.4*	50.0	*5.7*	936.0	*135.7*	418.5	*4.9*
2	93.0	*14.1*	58.0	*8.5*	2,108.0	*159.8*	683.5	*37.5*
5	195.0*	*59.4*	70.5	*3.5*	3,790.0	*181.0*	1,059.5	*33.2*
10	728.0	*31.1*	98.5	*0.7*	4,695.0	*29.7*	2,885.5	*191.6*
Blank filter	50.0	*11.3*	57.0	*14.8*	178.0	*24.0*	153.0	*3.5*
Positive control	927.0	*101.1*	1,518.0	*352.8*	908.0	*173.9*	2,344.0	*171.1*
Potency revertants/m^3	**25**		**4.8**		**890**		**270**	

Doses	Mean of Number of Revertants/plate and *Standard Deviation (SD*)							
m^3/plate	TA1538				YG5161			
	-S9		+S9		-S9		+S9	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Negative control	15.0	*2.0*	12.7	*2.5*	26.3	*4.0*	28.0	*2.6*
0.02	15.0	*1.4*	11.5	*3.5*	26.5	*2.1*	20.0	*0.0*
0.2	24.0	*2.8*	20.5	*3.5*	27.5	*9.2*	28.5	*2.1*
1	38.0	*0.0*	30.0	*4.2*	40.0	*0.0*	36.0	*2.8*
2	66.0	*8.5*	47.0	*1.4*	55.5	*0.7*	58.0	*2.8*
5	162.5	*9.2*	93.5	*19.1*	129.0	*5.6*	101.5	*16.3*
10	264.5	*41.7*	117.0	*1.4*	213.5	9.2	143.0	5.7
Blank filter	14.0	4.2	18.0	7.1	26.0	2.8	25.0	7.8
Positive control	392.0	50.2	2,451.0	746.0	677.0	111.0	3,114.0	514.1
Potency revertants/m^3	**26**		**12**		**18**		**15**	

*Shaded values represent the number of revertants that equal or more than twice the negative controls

TABLE V. Mutagenic responses in the *Salmonella*/microsome microsuspension assay for benzo[a]pyrene with the strains, TA98, YG1041, TA1538 and YG5161 in the presence of S9.

Doses	Mean of Number of Revertants/plate and *Standard Deviation (SD)*							
ng/plate	TA98		YG1041		TA1538		YG5161	
	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
Negative control	28.0	*7.1*	159.0	*21.2*	15.5	*0.7*	21.0	*5.7*
0.5							32.0	*7.1*
1.0							28.5	*0.7*
2.0							34.0	*2.8*
4.0							48.5	*3.5*
7.8	32.5	*3.5*	164.5	*29.0*	14.5	*0.7*	47.5	*2.1*
15.6	31.0	*1.7*	183.0	*35.4*	25.0	*5.7*	71.5	*6.4*
31.2	37.5	*3.5*	210.5	*10.6*	30.5	*4.9*	114.0	*1.4*
62.5	30.5	*7.8*	214.0	*21.2*	44.0	*17.0*	110.5	*12.0*
125	37.5	*9.2*	306.0	*31.1*	49.5	*12.0*	155.0	*8.5*
250	42.5	*6.4*	339.0	*38.2*	68.0	*4.2*	203.5	*19.1*
500	39.0	*5.7*	462.5	*17.7*	98.5	*10.6*	230.0	*5.7*
1,000	60.0*	*14.1*	471.0	*9.9*	125.5	*27.6*	285.0	*1.4*
2,000	63.0	*11.3*	470.5	*0.7*	106.0	*5.7*	271.5	*2.1*
Potency revertants/ng	**0.02**		**0.6**		**0.2**		**3.0**	
MED**	**1,400**		**265**		**77.5**		**7**	

*Shaded values represent the number of revertants that equal or more than twice the negative controls

**MED = Minimal Effective Dose in ng/plate

TABELA VI. Mutagenic responses in the *Salmonella*/microsome microsuspension assay for 1-nitropyrene with the strains, TA98, YG1041, TA1538 and YG5161 in the absence of S9.

Doses	Mean of Number of Revertants/plate and *Standard Deviation (SD)*							
ng/plate	TA98		YG1041		TA1538		YG5161	
	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
Negative control	42.5	*4.9*	201.0	*21.2*	18.0	*0*	24.0	*4.2*
0.01			257.5	*10.6*				
0.02			310.5	*23.3*				
0.03			383.0	*35.4*				
0.04			410.5	*2.1*				
0.05			506.5	*24.7*				
0.1	48.0	*2.8*	791.5	*3.5*	18.5	*0.7*	33.5	*10.6*
0.3	58.0	*15.6*	3,063.5	*101.1*	17.5	*7.8*	29.0	*5.7*
0.7	101.0*	*7.1*	4,353.0	*110.3*	28.5	*6.4*	45.5	*0.7*
1.0	129.5	*0.7*			19.0	*5.7*	36.0	*5.7*
2.5	266.0	*41.0*			36.5	*3.5*	68.0	*11.3*
5.0	536.5	*140.7*			63.5	*0.7*	76.5	*13.4*
10	1,001.0	*75.0*			139.5	*46.0*	135.0	*33.9*
20	1,883.5	*82.7*			595.0	*110.3*	334.0	*19.8*
Potency revertants/ng	**94**		**5,900**		**10**		**13**	
MED**	**0.45**		**0.03**		**1.8**		**1.8**	

*Shaded values represent the number of revertants that equal or more than twice the negative controls

**MED - Minimal Effective Dose in ng/plate