



King's Research Portal

DOI:

[10.1021/acs.chemrestox.8b00250](https://doi.org/10.1021/acs.chemrestox.8b00250)

Document Version

Peer reviewed version

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Murray, J. R., Mesaros, C. A., Arlt, V. M., Seidel, A., Blair, I. A., & Penning, T. M. (2018). Role of human aldo-keto reductases in the metabolic activation of the carcinogenic air pollutant 3-nitrobenzanthrone. *Chemical Research in Toxicology*, 31(11), 1277-1288. <https://doi.org/10.1021/acs.chemrestox.8b00250>

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Role of Human Aldo-Keto Reductases in the Metabolic Activation of the Carcinogenic Air Pollutant 3-Nitrobenzanthrone

Jessica R. Murray¹, Clementina A. Mesaros^{1,2}, Volker M. Arlt^{3,4}, Albrecht Seidel⁵, Ian A. Blair^{1,2} and Trevor M. Penning^{*1,2}

¹Department of Systems Pharmacology and Translational Therapeutics, ²Center of Excellence in Environmental Toxicology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; ³Department of Analytical, Environmental and Forensic Sciences, MRC-PHE Centre for Environment and Health, King's College London, London, UK; ⁴NIHR Health Protection Research Unit in Health Impact of Environmental Hazards at King's College London in partnership with Public Health England and Imperial College London, London, UK; and ⁵Biochemical Institute for Environmental Carcinogens Prof. Dr. Gernot Grimmer-Foundation, Grosshansdorf, Germany

*Corresponding Author:

Trevor M. Penning, PhD

Department of Systems Pharmacology & Translational Therapeutics

Perelman School of Medicine

University of Pennsylvania,

1315 BRBII/III

421 Curie Blvd

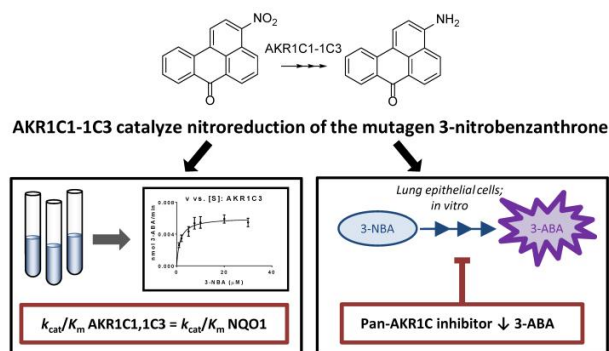
Philadelphia, PA 19104-6061

Email: penning@upenn.edu

Phone: 215-898-9445

Fax: 215-573-0200

Table of Contents Graphic.



Abstract

3-Nitrobenzanthrone (3-NBA) is a potent mutagen and suspected human carcinogen detected in diesel exhaust particulate and ambient air pollution. It requires metabolic activation via nitroreduction to promote DNA adduct formation and tumorigenesis. NAD(P)H:quinone oxidoreductase 1 (NQO1) has been previously implicated as the major nitroreductase responsible for 3-NBA activation, but it has recently been reported that human aldo-keto reductase 1C3 (AKR1C3) displays nitroreductase activity towards the chemotherapeutic agent PR-104A. We sought to determine whether AKR1C isozymes could display nitroreductase activity towards other nitrated compounds and bioactivate 3-NBA. Using discontinuous enzymatic assays monitored by UV-HPLC, we determined that AKR1C1-1C3 catalyze three successive 2-electron nitroreductions towards 3-NBA to form the reduced product, 3-aminobenzanthrone (3-ABA). Evidence of the nitroso- and hydroxylamino-intermediates were obtained by UPLC-HRMS. K_m , k_{cat} , and k_{cat}/K_m values were determined for recombinant AKR1C and NQO1 and compared. We found that AKR1C1, AKR1C3, and NQO1 have very similar apparent catalytic efficiencies ($8 \text{ min}^{-1} \text{ mM}^{-1}$ vs. $7 \text{ min}^{-1} \text{ mM}^{-1}$) despite the higher k_{cat} of NQO1 (0.058 min^{-1} vs. 0.012 min^{-1}). AKR1C1-1C3 possess a much lower K_m than NQO1 which suggests that they may be more important than NQO1

at the low concentrations of 3-NBA to which humans are exposed. Given that inhalation represents the primary source of 3-NBA exposure, we chose to evaluate the relative importance of AKR1C1-1C3 and NQO1 in human lung epithelial cell lines. Our data suggest that the combined activities of AKR1C1-1C3 and NQO1 contribute equally to the reduction of 3-NBA in A549 and HBEC3-KT cell lines and together represent approximately 50% of the intracellular nitroreductase activity towards 3-NBA. These findings have significant implications for the metabolism of nitrated polycyclic aromatic hydrocarbons and suggests that the hitherto unrecognized nitroreductase activity of AKR1C enzymes should be further investigated.

Keywords

NO₂-PAH

Nitroarene

Aldo-keto reductase

Metabolic activation

Nitroreduction

Chemical carcinogenesis

Air pollution

Introduction

Lung cancer is the leading cause of cancer death worldwide.¹ Although lung cancer is largely attributed to tobacco smoking,²⁻³ outdoor air pollution represents a growing threat to public health.⁴⁻⁶ The World Health Organization (WHO) estimated 223,000 lung cancer deaths in 2010 were due to air pollution which has since been designated as a Group 1 “known human carcinogen” by the International Agency for Research on Cancer (IARC).^{4, 7} A major source of air pollution includes diesel engine exhaust which has also been classified as a Group 1 “known human carcinogen” by IARC.⁸ Carcinogens found uniquely in diesel exhaust particulates include the nitrated polycyclic aromatic hydrocarbons (NO₂-PAHs) which may increase the risk of lung cancer in exposed individuals.⁹⁻¹⁴ NO₂-PAHs are adhered to superfine particulate matter (PM_{2.5}) in ambient air which can accumulate in the alveolar epithelia of the deep lung of exposed individuals¹⁵⁻¹⁶ and have been detected in lung tissue of non-smokers with lung cancer.^{12, 17}

However, not all individuals exposed to diesel exhaust develop lung cancer, indicating that gene-environment interactions may be important in determining individual risk for developing this disease.¹⁸⁻¹⁹ NO₂-PAHs require metabolic activation to exert their mutagenic and tumorigenic effects,²⁰ and identification of human enzymes that can metabolically activate these carcinogens will provide insight into identifying genetic variants and transcriptomic changes that may determine individual susceptibility. We chose to further characterize the metabolic activation of 3-nitrobenzanthrone (3-nitro-7H-benz[de]anthracen-7-one, 3-NBA)²¹ since it is the most mutagenic compound identified in the Ames test to date,²²⁻²³ and it is a potent mutagen and lung carcinogen in rodents.²⁴⁻²⁸ Metabolic activation of 3-NBA involves a 6-electron reduction of the nitro-group involving sequential formation of the nitroso-, hydroxylamino-, and amine products

catalyzed by cytosolic nitroreductases (**Figure 1**).²⁹⁻³¹ The hydroxylamino intermediate, *N*-hydroxy-3-aminobenzanthrone (*N*-OH-3-ABA) is intercepted by sulfonation or acetylation, creating a good leaving group for attack of the intermediate nitrenium or carbenium ion to DNA bases resulting in the formation of DNA adducts.³²⁻³⁶ Its final product, 3-aminobenzanthrone (3-ABA, **Figure 1**) can also be activated by peroxidases to yield either a nitrenium or carbenium ion that can contribute to DNA adduct formation.³⁷⁻³⁸ 3-NBA exposure leads to DNA adduct formation *in vitro* and in multiple organs *in vivo* in rodents^{34, 39-42} and in human cells.^{16, 43-46} 3-ABA has been found in the urine of salt-mine workers occupationally exposed to diesel exhaust which indicates human exposure to 3-NBA and their ability to metabolize it.⁴⁷

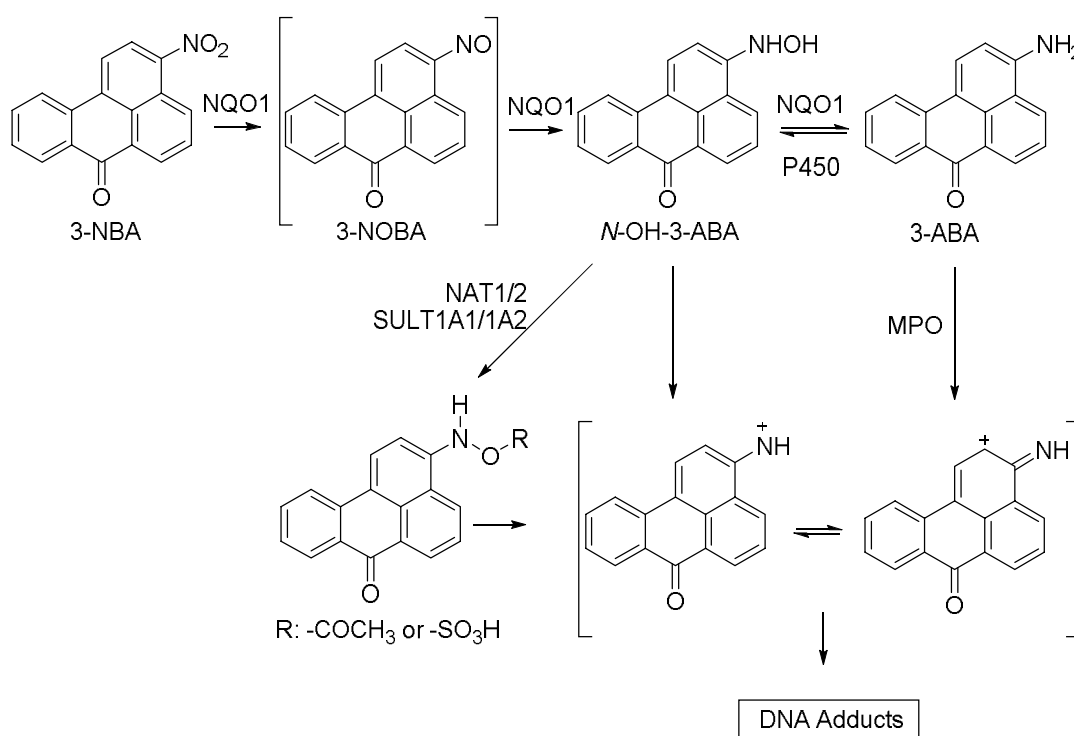


Figure 1. Proposed pathways of metabolic activation of 3-NBA and resulting DNA adduct formation. See text for details. Abbreviations used: NQO1, NAD(P)H:quinone oxidoreductase 1;

NAT1/2, *N,O*-acetyltransferase isozymes; SULT1A1/1A2, sulfotransferases 1A1 and 1A2; P450, cytochrome P450; MPO, myeloperoxidase. Modified from Arlt et al., 2006.³⁷

Identification of genes involved in the metabolic activation of representative NO₂-PAHs, such as 3-NBA, will be necessary to identify genotypes of sensitive individuals or predict phenotypic changes that may sensitize individuals to diesel exhaust exposures. 3-NBA has previously been shown to be metabolically activated by xanthine oxidase (XO), NADPH:cytochrome P450 oxidoreductase (POR), and NAD(P)H:quinone oxidoreductase 1 (NQO1) to yield *N*-OH-3-ABA or 3-ABA.^{33, 48-50} Inhibition studies have shown that NQO1 is the primary enzyme that catalyzes the nitroreduction of 3-NBA in liver *in vitro*.^{48, 51-52}

Since human aldo-keto reductase 1C3 (AKR1C3) displays greater nitroreductase activity than NQO1 towards the activation of the cancer chemotherapeutic agent PR-104A, we sought to characterize whether AKRs could contribute to the nitroreduction of 3-NBA in humans.⁵³ The ability of AKR1Cs to display nitroreductase activity against PR-104A was unexpected since these enzymes typically conduct a two electron reduction on carbonyl groups.⁵⁴ AKR1C1-1C3 are highly expressed in human lung tissue which is relevant as inhalation is the primary route of exposure to NO₂-PAHs.^{17, 55-56} We demonstrate here that human AKR1C1-1C3 are able to catalyze the nitroreduction of 3-NBA and therefore may be involved in toxification of NO₂-PAHs.

Materials and Methods

Caution: 3-NBA and its derivatives are potent mutagens and suspected carcinogens and should be handled in accordance with NIH Guidelines for the Use of Chemical Carcinogens.

Chemicals and reagents

3-NBA, *N*-OH-3-ABA, and 3-ABA were synthesized as described previously.^{31, 57-58} The synthesis of 3-NOBA was performed from 3-ABA following the experimental protocol reported by Fifer et al. for the synthesis of similar nitroso-PAHs.⁵⁹ The purity and identity of these compounds were verified by UV spectroscopy, high resolution mass spectrometry, and high-field ¹H NMR spectroscopy. All other chemicals were of the highest grade available, and all solvents were of HPLC grade. (*S*)-(+)-1,2,3,4-tetrahydro-1-naphthol (*S*-tetralol), flavin adenine dinucleotide (FAD), dithiothreitol (DTT), 1-acenaphthenol, dicoumarol (Dic), allopurinol, rotenone, and D-glucose-6-phosphate (G6P) were purchased from Millipore-Sigma (St. Louis, MO). Nicotinamide adenine dinucleotide (NAD⁺) and nicotinamide adenine dinucleotide phosphate (NADP⁺) were purchased from Roche Diagnostics (Indianapolis, IN). Glucose-6-phosphate dehydrogenase (G6PD) was purchased from Worthington Biochemical Corporation (Lakewood, NJ). Androsterone was purchased from Steraloids (Wilton, NH); 2,6-dichloroindophenol (DCPIP) and salicylic acid were purchased from Acros Organics (Geel, Belgium). Flufenamic acid (FA), ursodeoxycholate, and indomethacin were purchased from ICN Biomedicals, Inc. (Aurora, Ohio).

Discontinuous enzymatic assays

Human recombinant enzymes AKR1C1-AKR1C3 were prepared and purified as previously described.⁶⁰ Homogenous human recombinant NQO1 was purchased from Millipore Sigma (St.

Louis, MO). Enzymes were shown to be homogenous by SDS-PAGE analysis prior to use and their specific activities were in accord with published values.⁶¹⁻⁶²

In order to identify whether AKR1C1-1C3 catalyzed the nitroreduction of 3-NBA, enzymatic assays were prepared in a cell-free system. Typical assays to determine catalytic efficiencies with AKR1C1-1C3 contained 1 – 30 μM 3-NBA, 4% DMSO (*v/v*), and a NADPH regeneration system to maintain 1 mM NADPH in 50 mM potassium phosphate buffer, pH 7.0. These reactions were maintained at 37 °C in the dark with gentle agitation. The NADPH regeneration system consisted of 1 mM G6P, 1 mM NADP⁺, and 2 units/mL G6PD. The reactions with AKR1C1-1C3 were initiated with 0.5 μM enzyme. Typical reaction mixtures with NQO1 contained 1 – 30 μM 3-NBA, 1 μM FAD, 1 mM DTT, and a NADPH-regeneration system in 50 mM potassium phosphate buffer, pH 7.0. Reactions were initiated with 0.25 μM enzyme and maintained in the dark at 37°C with gentle agitation. The amount of enzyme used was always in the linear range for each protein as determined by plots of initial velocity *versus* enzyme concentrations. In control reactions, AKR1C1-1C3 or NQO1 was omitted from the mixtures. Reactions for each substrate concentration were repeated 3 to 5 times.

To stop enzymatic reactions, aliquots were extracted twice with 1.5-fold volume of ice-cold ethyl acetate saturated with H₂O. Samples were vortexed vigorously for ³ 20 sec and centrifuged at 16,000 $\times g$ to help phase separation. The organic phase was then collected and dried under vacuum with a SpeedVac concentrator (Thermo Fisher Scientific, San Jose, CA). The residue was re-dissolved in methanol for analysis by UV-HPLC. 3-ABA was identified by its retention time and UV spectra which were compared to authentic standards and quantified using calibration curves.

High resolution mass spectrometry confirmed analyte identity. Amounts of 3-ABA (nmol) were plotted against time and the initial velocity was estimated by the slope of the linear portion of the progress curve in the first 5-10% of the reaction. Velocity vs. substrate concentration curves were analyzed using GraphPad Prism Version 7 (GraphPad Software, La Jolla, CA) to calculate V_{\max} , K_m , and k_{cat} .

UV-HPLC analysis

Data were acquired using a Waters Alliance 2695 HPLC system (Waters Corporation, Milford, MA) coupled to a Waters Alliance 2996 Photodiode Array Detector (Waters Corporation, Milford, MA). Separations were accomplished on a reverse-phase (RP) column (Zorbax-ODS C18, 5 μm , 4.6 mm \times 250 mm) (Dupont Co., Wilmington, DE) with a guard column at ambient temperature. The mobile phase consisted of 5 mM ammonium acetate and 0.1% trifluoroacetic acid (TFA) (v/v) in H_2O (solvent A) and 5 mM ammonium acetate and 0.1% TFA (v/v) in acetonitrile (solvent B) and was delivered at a flow rate of 0.5 mL/min. The linear gradient elution program was as follows: 5% to 95% B over 30 min, followed by an isocratic hold at 95% B for another 10 min. At 40 min, B was returned to 5% in 1 min and the column was equilibrated for 19 minutes before the next injection. The total run time for each analysis was 60 min.

Liquid chromatography high resolution mass spectrometry (LC-HRMS)

For LC-HRMS analysis, enzymatic reactions were prepared as above: aliquots were extracted twice with 1.5-fold volume of ice-cold ethyl acetate saturated with H_2O and centrifuged at 16,000 $\times g$ to help phase separation. The organic phase was dried under vacuum with a SpeedVac concentrator. The residue was re-dissolved in 50% methanol in water and cleaned up by passage

through a CoStar Spin-X HPLC 0.2 μm nylon filter (Corning Incorporated, Coning, NY). 5 μL aliquots were analyzed on a Waters nano-Acquity Ultra Performance Liquid Chromatography (UPLC) system (Waters Corporation, Milford, MA) coupled to a QE-HF mass spectrometer (Thermo Fisher Scientific, San Jose, CA). Separations were accomplished on an analytical column (C18, 1.7 μm BEH130, 150 $\mu\text{m} \times 100$ mm) (Waters Corporation, Milford, MA) at 55 $^{\circ}\text{C}$. The mobile phase consisted of 0.1% formic acid (v/v) in H_2O (solvent A) and 0.1% formic acid (v/v) in acetonitrile (solvent B) and was delivered at a flow rate of 1.6 $\mu\text{L}/\text{min}$. The linear gradient elution was as follows: an isocratic hold at 5% B for 5 min, 5% to 95 % B over 30 min, followed by an isocratic hold at 95% B for another 10 min. At 46 min, B was returned to 5% in 2 min and the column was equilibrated for 12 min before the next injection. The total run time for each analysis was 60 min. The mass spectrometer was operated in the positive ion mode, with a Microm source after system calibration with the manufacturer's calibration mixture. The ionization voltage was set to 1.5 kV and the capillary temperature was set to 200 $^{\circ}\text{C}$. Full scan spectra were acquired with a resolving power of 120,000 in a mass range from m/z 100 to 800. The Xcalibur software version 2.0 (Thermo Fisher Scientific, San Jose, CA) was used to control the UPLC-MS/MS system and to process data.

Cell Culture

The adenocarcinoma human alveolar basal epithelial cell line A549 was obtained from the American Type Culture Collection (ATCC, Manassas, VA; ATCC #CCL-185) and cultured in Kaighn's Modification of Ham's F-12 Medium (F-12K) supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin. A549 cells were passaged every 4 days at a dilution of 1:7. Normal human bronchial epithelial cells (HBEC3-KT) were a kind gift

from Dr. John Minna at the University of Texas Southwestern Medical Center and have been immortalized via the overexpression of cyclin-dependent kinase 4 (Cdk4) and human telomerase reverse transcriptase (hTERT). HBEC3-KT are considered the best model for normal HBEC cells as they do not form colonies in soft agar or tumors in nude mice.⁶³ HBEC3-KT cells were maintained in Keratinocyte-SFM supplemented with human recombinant Epidermal Growth Factor and Bovine Pituitary Extract. HBEC3-KT cells were passaged every 5 days at a dilution of 1:4. All cells were cultured in an atmosphere of 95% air and 5% CO₂ at 37°C in 100 mm culture plates. Cultured cells with low passage numbers of 10–20 were used in the experiments to prevent clonal selection of a cell strain that differed from the parental population. Cells were routinely authenticated by short-terminal repeat DNA analysis and were mycoplasma-free (DNA Diagnostics Center Medical, Fairfield, OH).

Detection of 3-ABA Formation in Cell Culture

The fluorescence of 3-ABA was used to measure the uptake and nitroreduction of non-fluorescent 3-NBA in cell culture. We adapted a 96-well fluorescence detection of 3-ABA reported by Pink and colleagues.⁶⁴ Metabolic assays with A549 cells were performed in 96-well plates 24 h (\pm 2 h) after seeding of 1×10^4 A549 cells per well. After the 24 h attachment period, cells were treated with 3-NBA in 0.1% DMSO (0.625 – 10 μ M 3-NBA) in phenol red-free DMEM/F-12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12). Excitation (λ_{ex}) and emission (λ_{em}) wavelengths for the Synergy 2 multi-detection microplate reader (Biotek Instruments Inc., Winooski, VT) were set at 520 and 650 nm, respectively, based on the spectral properties of 3-ABA. Fluorescence was monitored over a timespan from 1 h to 48 h. Cells treated with cell culture medium that contained 0.1% DMSO were used to correct for auto-fluorescence of the cells. Spontaneous formation of 3-

ABA was monitored in blank wells to determine background conversion rates of 3-NBA to 3-ABA in a cell-free environment but were found to be non-significant. Formation of 3-ABA (pmol) was quantified by using calibration curves constructed with authentic standards in phenol red-free DMEM/F-12 media.

Metabolic assays with HBEC3-KT cells were performed in 96-well plates as described above except HBEC3-KT cells rested for 48 h after seeding 2×10^4 cells per well before initiating treatment. After the 48 h attachment period, cells were treated with 3-NBA in 0.1% DMSO (0.625 – 10 μ M 3-NBA) in K-SFM. Fluorescence was monitored over a timespan from 1 h to 48 h. Formation of 3-ABA (pmol) was quantified by using calibration curves constructed with authentic standards in K-SFM.

To assess the contribution of individual enzymes, cells were treated with specific inhibitors prior to and throughout the duration of exposure to 3-NBA. The following inhibitors were selected: dicoumarol, NQO1 inhibitor;⁶⁵ flufenamic acid, pan-AKR1C inhibitor;⁶⁶ salicylic acid, AKR1C1 inhibitor;⁶⁷⁻⁶⁸ ursodeoxycholate, AKR1C2 inhibitor;⁶⁹ indomethacin, AKR1C3 inhibitor;⁷⁰ allopurinol, XO inhibitor;⁷¹ and rotenone, Complex I inhibitor⁷²⁻⁷³ to eliminate the contribution of mitochondrial nitroreductases. To determine the optimal treatment conditions, six concentrations between 0 – 100 μ M were tested for each inhibitor to determine the lowest concentration in which they produced maximal inhibition of 3-ABA formation. All assays were conducted in the 96-well assays as described above with 2.5 μ M 3-NBA. Cells were pretreated with inhibitors in 0.1% DMSO or vehicle control (0.1% DMSO) for 1 h prior to exposure to 3-NBA. After the 1 h incubation, cells were treated with 2.5 μ M 3-NBA + the inhibitor in 0.15% DMSO. Control cells

were exposed to inhibitor only (0.15% DMSO) and used as blanks to correct for cellular auto-fluorescence. We measured the effect of inhibitors on 3-ABA formation at 6 h for A549 cells and 24 h for HBEC3-KT cells. These time points were taken during a linear period of 3-ABA formation and within the linear range of the calibration curves for 3-ABA detection in the respective cell media.

Results

Activation of 3-nitrobenzanthrone by human aldo-keto reductases

Previous work by Guise et al. reported that AKR1C3 displayed nitroreductase activity towards the chemotherapeutic agent PR-104A.⁵³ To determine whether human AKR1C enzymes could display nitroreductase activity towards nitrated polycyclic aromatic hydrocarbons (NO₂-PAHs), we investigated whether AKR1C1-1C4 would be able to metabolically activate 3-NBA. Discontinuous enzymatic assays were initially performed with 1.68 μM enzyme (AKR1C1-1C4) and 8.2 μM 3-NBA in potassium phosphate buffer pH 7 at 37 °C in a 2 mL system to screen for nitroreductase activity. In order to characterize the reaction over time, 100 μL aliquots were sampled at 15 min, 30 min, 45 min and at 1, 3, 6, 9, 18, and 24 h. An NADPH-regeneration system consisting of glucose-6-phosphate (G6P) and glucose-6-phosphate dehydrogenase (G6PD) was necessary to drive the reaction to completion within 24 hours. The disappearance of 3-NBA and formation of the six-electron reduction product, 3-ABA, was monitored with reverse phase HPLC coupled to in-line photo-diode-array (PDA) detection.

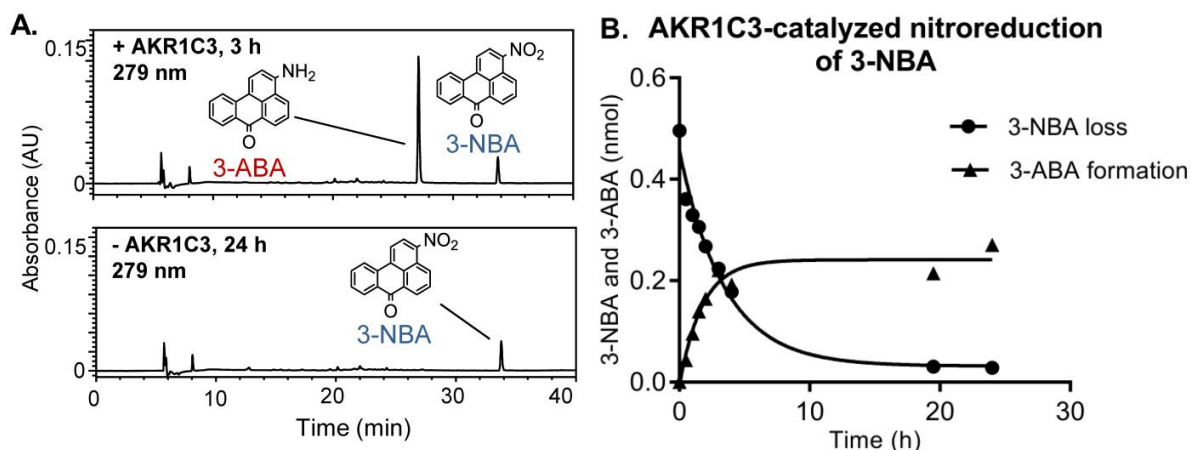


Figure 2. AKR1C3 catalyzes the nitroreduction of 3-NBA. 3-ABA was identified in discontinuous assays with AKR1C3 using UV-HPLC monitoring at 279 nm (A). 3-NBA and 3-ABA were identified based on retention times and UV spectra which were compared to standards. 3-NBA and 3-ABA levels were quantified over a 24 h time course (B). Results were similar for AKR1C1, AKR1C2, and NQO1.

Under these conditions, 3-ABA appeared within 15 minutes after initiation with AKR1C1-1C3 and continued to increase in a time-dependent manner (Figure 2). 3-ABA formation was quantified by integrating the peak that appeared at 27.2 minutes in the UV-chromatograms (Figure 2, A). The observed 3-ABA formation was due to enzyme activity as less than 2% of 3-NBA is converted to 3-ABA in control reactions that lacked enzyme. Human recombinant AKR1A1, AKR1B1, AKR1B10, AKR1D1, AKR7A2, and AKR7A3 were tested and were found to not contribute to the nitroreduction of 3-NBA (data not shown). AKR1C4 possessed less than 10% of the nitroreductase activity towards 3-NBA compared to AKR1C1-1C3 (data not shown).

UV-HPLC detection of 3-NBA and 3-ABA allowed us to determine that AKR1C enzymes displayed nitroreductase activity, but the nitroso- and hydroxylamino- intermediates were not identified in the UV-HPLC chromatograms. Evidence for the formation of the nitroso- and hydroxylamino-intermediates and 3-ABA were instead obtained using UPLC-HRMS/MS. These

analytes gave exact mass within 5 ppm for the parent ion and the corresponding fragment ions in the MS² spectrum.

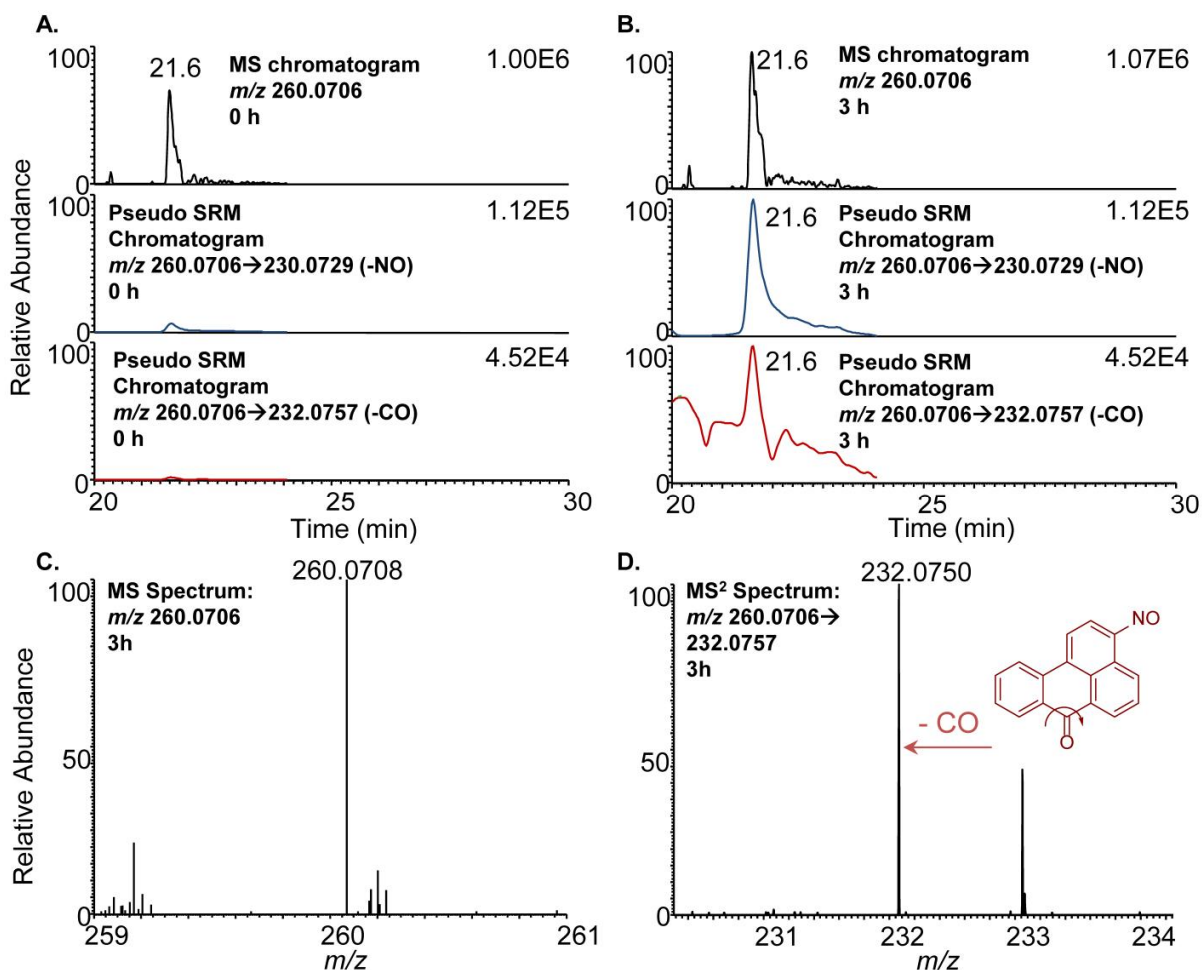


Figure 3. UPLC-HRMS/MS Detection of 3-NOBA. Aliquots of enzymatic reactions with AKR1C1-1C3 and NQO1 were sampled at 0 h (left panels A, C) and 3 h (right panels B, D). Data are shown for the reaction with AKR1C3 but are representative of each enzymatic reaction. 3-NOBA MH⁺ was detected at $m/z = 260.0706$, and two fragments were identified: MH⁺-CO $m/z = 232.0757$ and MH⁺-NO $m/z = 230.0729$. 3-NOBA and its fragments were detected within 5 ppm of their predicted m/z .

The two-electron reduction product, 3-NOBA, was identified in enzymatic reactions that contained AKR1C1-1C3 and NQO1 at 3 h with m/z of 260.0710 and an elution time of 21.6 min (**Figure 3**). Its fragment ion corresponding to the loss of the carbonyl ($MH^+ - CO$ $m/z = 232.0757$) and loss of the nitroso-group ($MH^+ - NO$ $m/z = 230.07290$) were detected within ± 5 ppm. The hydroxylamino intermediate *N*-OH-3-ABA (MH^+ $m/z = 262.0855$) was also identified in reactions with AKR1C1-1C3 and NQO1 at 3 h. It was identified in the pseudo-SRM chromatograms and eluted at 27.8-27.9 min with m/z of 262.0867 (**Figure 4**). Its fragments corresponded to the loss of the hydroxyl ($MH^+ - OH$ $m/z = 245.0835$) and carbonyl ($MH^+ - CO$ $m/z = 234.0913$) that were detected within the given parameters (± 5 ppm).

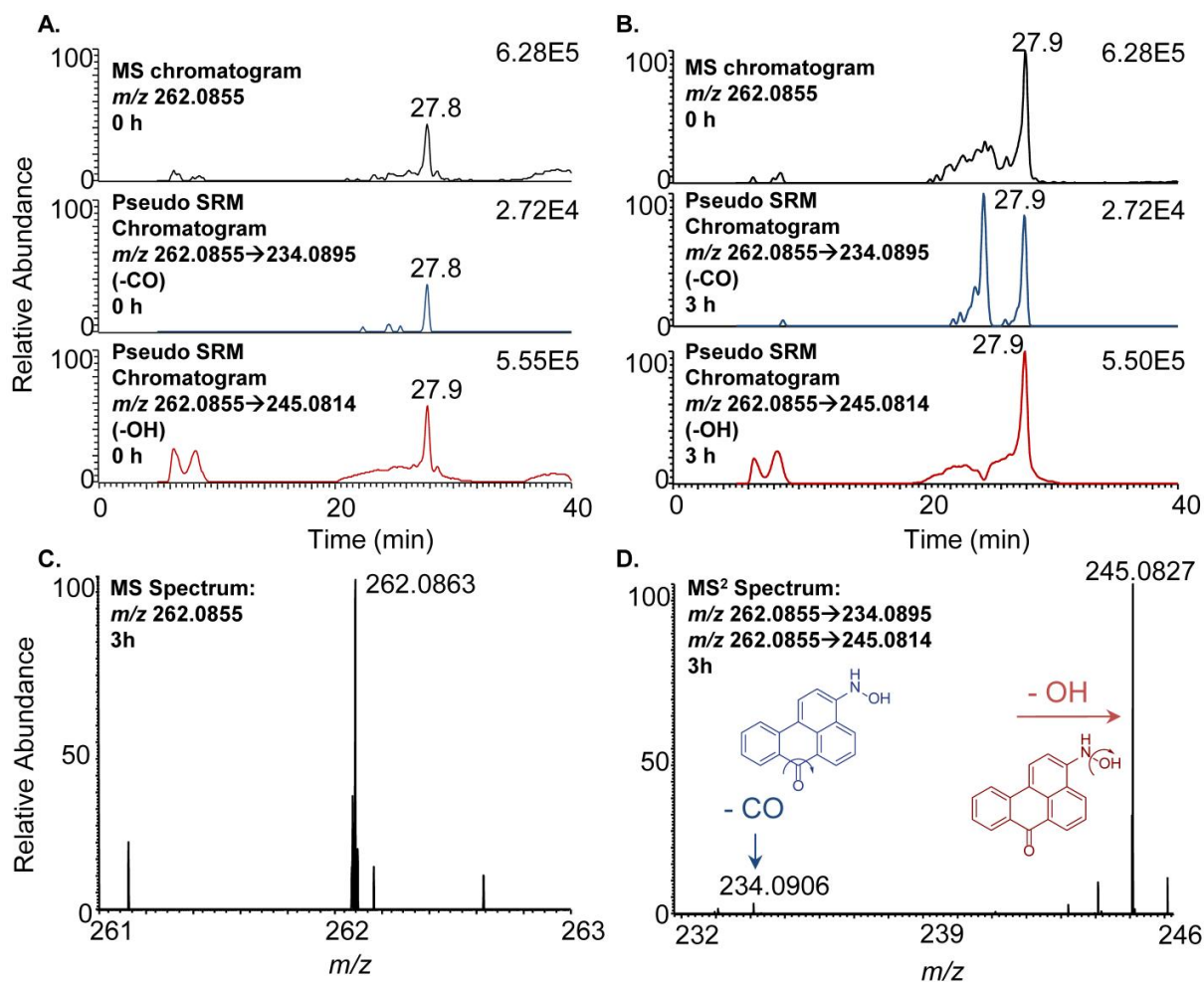


Figure 4. UPLC-HRMS/MS Detection of *N*-OH-3-ABA. Aliquots of enzymatic reactions with AKR1C1-1C3 and NQO1 were sampled at 0 h (left panels A, C) and 3 h (right panels B, D). Data are shown for the reaction with AKR1C3 but are representative of each enzymatic reaction. *N*-OH-3-ABA MH^+ was detected at $m/z = 262.0855$, and two fragments were identified: MH^+ -OH $m/z = 245.0814$ and MH^+ -CO $m/z = 234.0895$. *N*-OH-3-ABA and its fragments were detected within 5 ppm of their predicted m/z .

Both the nitroso- and hydroxylamino-intermediates were unstable, and were subsequently detected in low amounts, with intensities of 1.07E6 and 6.28E5, respectively. Much higher levels of the product 3-ABA were detected with an intensity of 1.80E6 in the peak eluting at 18.03 min with m/z of 246.0917 (**Figure 5**). 3-ABA was not detected by HRMS/MS in any enzymatic reactions at the 0 h time point. Small amounts of both intermediates were detected at the 0 h time point however, indicating either minimal contamination of 3-NBA or rapid conversion of 3-NBA to these intermediates prior to organic extraction.

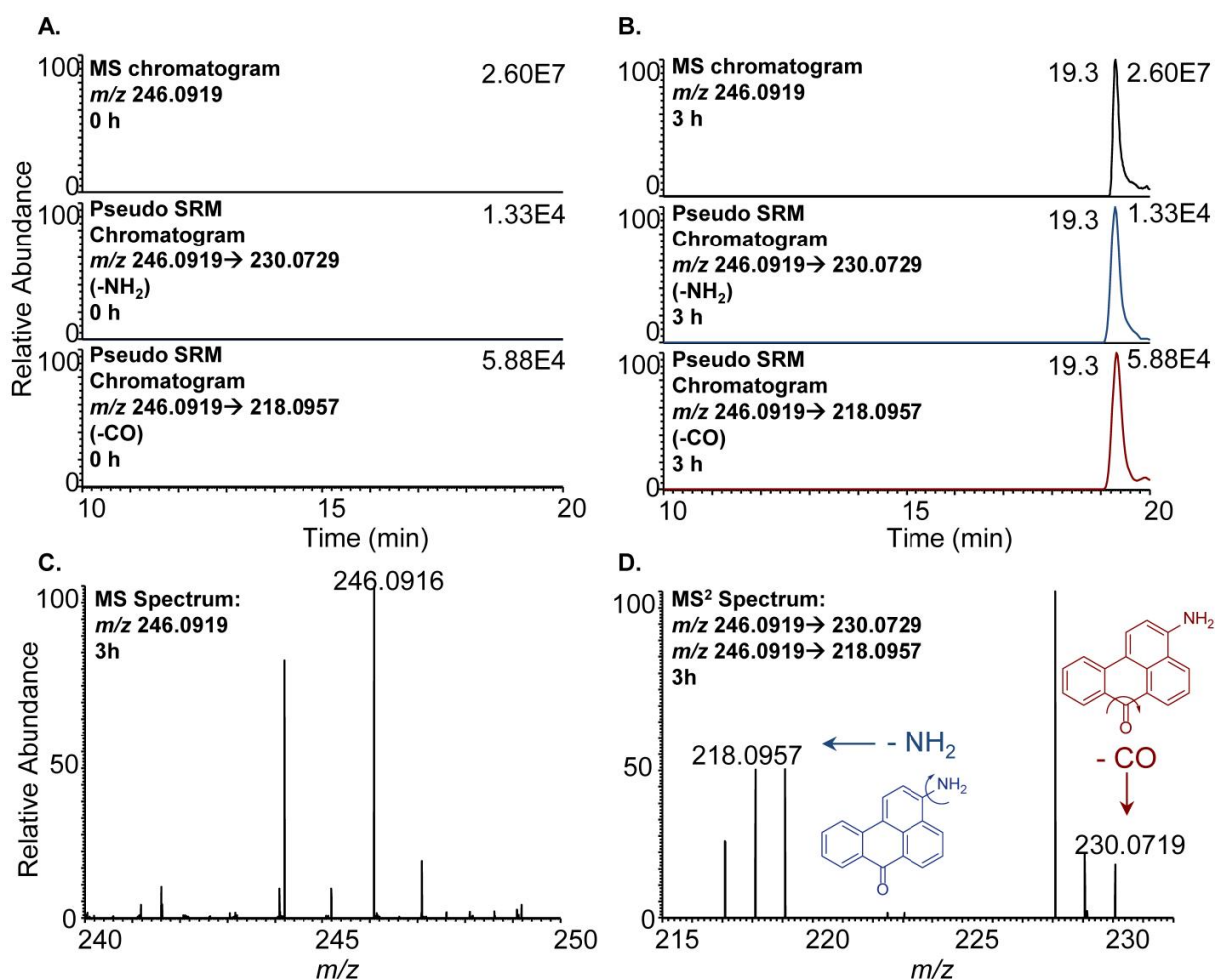


Figure 5. UPLC-HRMS/MS Detection of 3-ABA. Aliquots of enzymatic reactions with AKR1C1-1C3 and NQO1 were sampled at 0 h (left panels A, C) and 3 h (right panels B, D). Data are shown for the reaction with AKR1C3 but are representative of each enzymatic reaction. 3-ABA MH^+ was detected at $m/z = 246.0919$, and two fragments were identified: MH^+-NH_2 $m/z = 230.0729$ and MH^+-CO $m/z = 218.0957$. 3-ABA and its fragments were detected within 5 ppm of their predicted m/z .

Comparison of AKR1C1-1C3 to NQO1

Since NQO1 had been previously identified as the major nitroreductase in the metabolic activation of 3-NBA *in vitro*, we calculated K_m , k_{cat} , and catalytic efficiencies (k_{cat}/K_m) of AKR1C1-1C3 and NQO1 to compare these enzymes for their ability to reduce 3-NBA and form 3-ABA. These assays were optimized so that the amount of enzyme used was in the linear range for each protein as determined by plots of initial velocity *versus* enzyme concentrations. Discontinuous enzymatic assays with AKR1Cs were performed in 50 mM potassium phosphate buffer pH 7 at 37 °C in a 1 mL system using 0.5 μ M AKR1C1-1C3 with 0 – 30 μ M 3-NBA to construct v vs. $[S]$ curves. To capture the initial velocity during the first 5-10% of the reaction, multiple aliquots were taken in the first 30 min. The formation of the six-electron reduction product, 3-ABA, was monitored with reverse phase HPLC coupled to in-line PDA detection at 279 nm. Discontinuous enzymatic assays with NQO1 were performed as described above with 0.25 μ M NQO1. Initial velocity (v) vs. $[S]$ curves were fit with the Michaelis-Menten equation using GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA) to calculate K_m and k_{cat} , and k_{cat}/K_m (**Figure 6**). k_{cat}/K_m values were termed “apparent catalytic efficiencies” since substrate concentrations were not significantly lower than K_m values due to the detection limits of this assay.

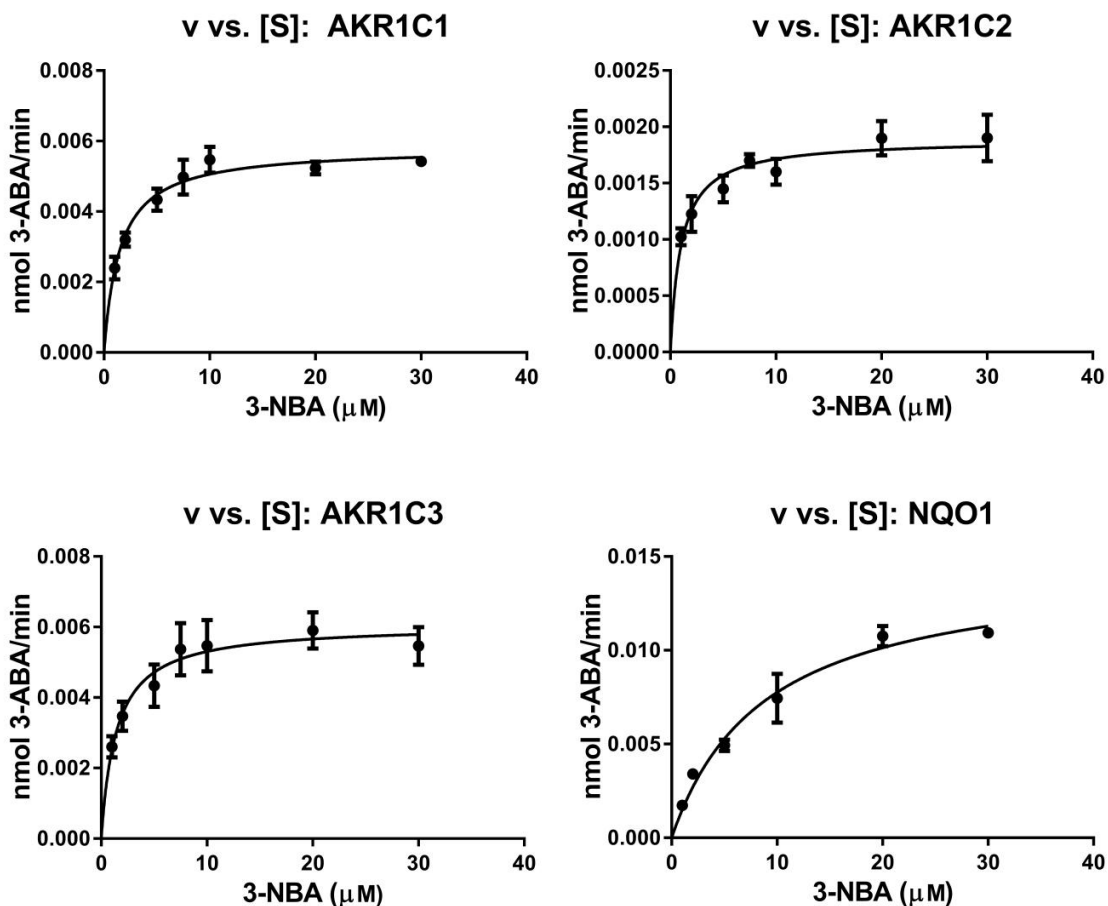


Figure 6. v vs. $[S]$ curves for AKR1C1-1C3 and NQO1. Discontinuous enzymatic assays were performed in 50 mM potassium phosphate buffer pH 7 at 37 °C in a 1 mL system using 0.5 μ M AKR1C1-1C3 or 0.25 μ M NQO1 with 0 – 30 μ M 3-NBA to construct v vs. $[S]$ curves. Initial velocity (v) vs. $[S]$ curves were fit with the Michaelis-Menten equation using GraphPad Prism 7.0 to calculate V_{\max} , K_m , and k_{cat} .

We found that AKR1C1, AKR1C3, and NQO1 had very similar apparent catalytic efficiencies ($\sim 8 \text{ min}^{-1} \text{ mM}^{-1}$ vs. $\sim 7 \text{ min}^{-1} \text{ mM}^{-1}$) despite the much higher k_{cat} of NQO1 (0.058 min^{-1} vs. 0.012 min^{-1})

¹). AKR1C1-1C3 possessed a much lower K_m than NQO1: AKR1C1 and AKR1C3 had K_m values 1.43 – 1.5 μM while the K_m for NQO1 was 8.8 μM . AKR1C2 had a similar K_m when compared to AKR1C1 and AKR1C3 but had a lower k_{cat} , and thus its apparent catalytic efficiency was 50% less than AKR1C1 and AKR1C3. AKR1C4 was a very poor nitroreductase for 3-NBA and so it was difficult to obtain steady state kinetic parameters for AKR1C4. AKR1C4 is a liver-specific AKR1C isozyme and we expect that it will not contribute to 3-ABA formation in the lung.

Table 1. Catalytic Efficiencies for 3-NBA Turnover by NQO1 and AKR1C1-1C3			
Recombinant Enzyme	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m ($\text{min}^{-1} \text{mM}^{-1}$)
NQO1	8.8 ± 2.5	0.058 ± 0.0066	6.59
AKR1C1	1.5 ± 0.27	0.012 ± 0.0004	8.00
AKR1C2	1.1 ± 0.29	0.004 ± 0.0002	3.63
AKR1C3	1.4 ± 0.43	0.012 ± 0.0007	8.39

Relative contributions of AKR1C1-1C3 and NQO1 *in vitro*

Apparent catalytic efficiencies suggest that AKR1C1-1C3 may compete with NQO1 and contribute to the nitroreduction of 3-NBA. To determine the relative contributions of these enzymes in human lung epithelial cells, we modified a 96-well plate assay to monitor the formation of 3-ABA in A549 cells.⁶⁴ First we established that human lung cells metabolically activate 3-NBA in submerged cell culture. A549 cells exposed to five different concentrations of 3-NBA ranging from 0.625 – 10 nM revealed time- and dose-dependent formation of 3-ABA measured by an increase of cellular fluorescence intensity compared to non-exposed cells. Elevated fluorescence was observed by 60 min following exposure to 0.625 – 10 nM 3-NBA and increased throughout the 48 h time course.

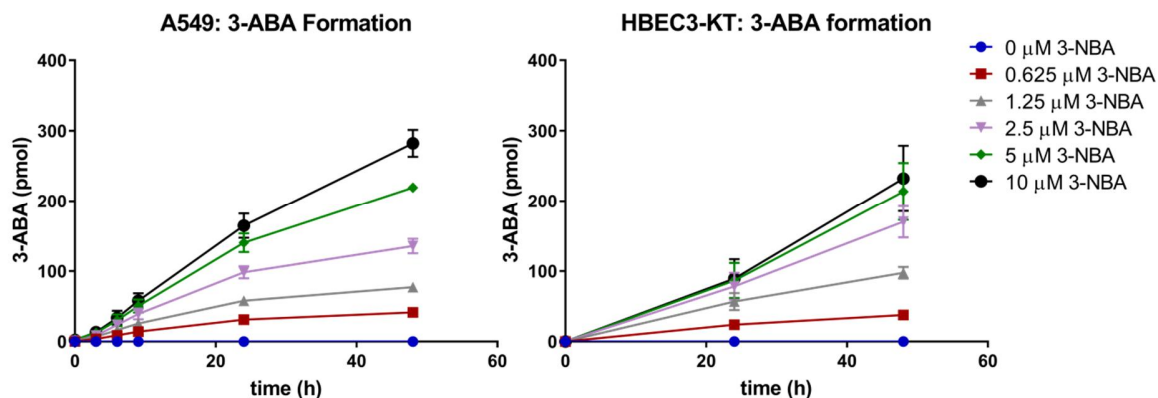


Figure 7. 3-ABA formation in A549 and HBEC3-KT cells. Determination of the metabolic activation of 3-NBA to 3-ABA in A549 (A) and HBEC3-KT (B) cells. The intrinsic fluorescence of 3-ABA (λ_{ex} 520 nm, λ_{em} 650 nm) was used to detect the final reduction product, 3-ABA. Fluorescence was monitored over a timespan from 1 h to 48 h in A549 (n=3) and 1 h to 48 h in HBEC3-KT (n=5). Elevated fluorescence levels were observed at 0.625 μM by 1 h in A549 cells and by 6 h in HBEC3-KT.

HBEC3-KTs also metabolically activate 3-NBA and form 3-ABA in a time- and dose-dependent manner. Due to the lower metabolic activity of this cell line and reduced sensitivity of 3-ABA detection in K-SFM, the time to first detect an increase in fluorescence was 6 h, but this was below the linear range of 3-ABA detection (Figure 7, B). Reliable measurements for formation of 3-ABA after exposure to 0.625 – 10 μM 3-NBA concentrations could be obtained after 24 h and continued to increase over 48 h.

To assess the contribution of NQO1 vs AKR1C1-1C3 in each cell line, we monitored the formation of 3-ABA in the presence and absence of dicoumarol (a NQO1 inhibitor) and flufenamic acid (a pan-AKR1C inhibitor). Together, these enzymes account for approximately 50% of the

nitroreduction of 3-NBA, and AKR1C enzymes make a significant contribution that is similar to that of NQO1 in both cell lines, Fig. 8. Flufenamic acid reduced total 3-ABA formation by $34 \pm 5\%$ in A549 and $33 \pm 7\%$ in HBEC3-KT while dicoumarol reduced total 3-ABA formation by $23 \pm 6\%$ in A549 and $40 \pm 6\%$ in HBEC3-KT. The combination treatment (10 μM dicoumarol + 50 μM flufenamic acid, abbreviated DF) led to $45 \pm 8\%$ reduction of 3-ABA formation in A549 and $53 \pm 8\%$ in HBEC3-KT.

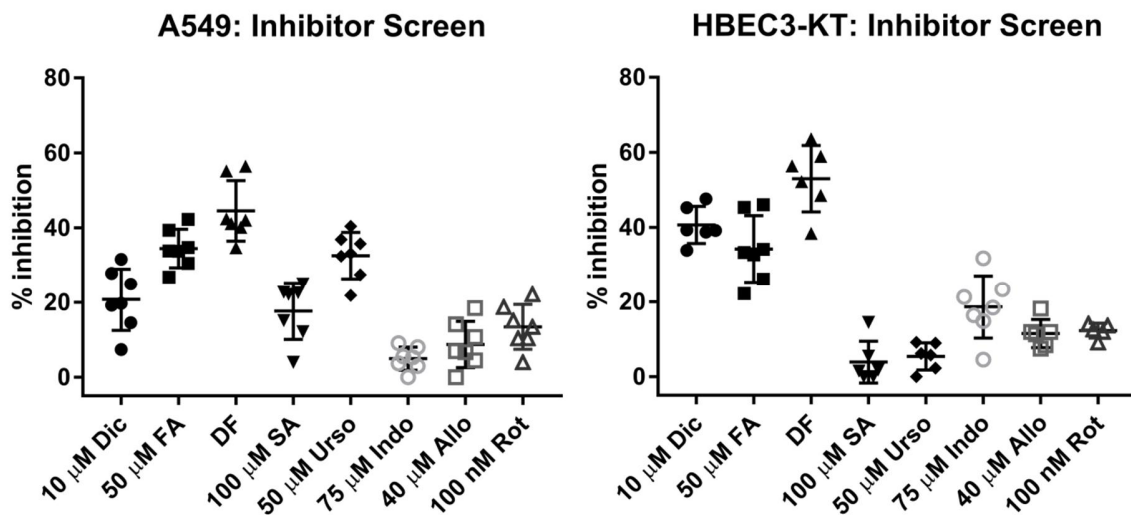


Figure 8. Inhibition of 3-ABA formation by AKR1C and NQO1 inhibitors. Specific inhibitors were used to assess individual contributions of AKR1C1-1C3, NQO1, XO, and complex 1 in catalyzing nitroreduction of 3-NBA in A549 and HBEC3-KT cell lines exposed to 2.5 μM 3-NBA. Abbreviations used: Dic, Dicoumarol; FA, Flufenamic Acid; DF, combination treatment with Dicoumarol and Flufenamic Acid; SA, Salicylic Acid; Urso, Ursodeoxycholate; Indo, Indomethacin; Allo, Allopurinol; and Rot, Rotenone. Experiments were repeated at least 5 independent times ($n = 5-7$).

To further assess the role of individual AKR1C enzymes, salicylic acid was selected as a specific inhibitor against AKR1C1 (IC₅₀: 5.8 μM), ursodeoxycholate is a specific inhibitor against AKR1C2 (IC₅₀: 236 nM), and indomethacin is a specific inhibitor against AKR1C3 (IC₅₀: 108 nM). Several concentrations between 0 – 100 μM were tested for each inhibitor to determine the lowest concentration in which they produced maximal inhibition of 3-ABA formation. Salicylic acid inhibited 3-ABA formation by approximately 18% in A549 cells and 4% in HBEC3-KT, ursodeoxycholate inhibited 3-ABA formation by approximately 33% in A549 cells and 5% in HBEC3-KT, and indomethacin inhibited 3-ABA formation by approximately 5% in A549 cells and 19% in HBEC3-KT.

Allopurinol (XO inhibitor) was included in the inhibitor screen as XO has been previously implicated in the reduction of 3-NBA.³⁷ XO plays a minor role in the metabolic activation of 3-NBA and contributes approximately 9-11% of 3-ABA formation in A549 and HBEC3-KT cell lines. Rotenone was included to rule out contribution of mitochondrial nitroreductases as it is a Complex I inhibitor. Rotenone inhibits approximately 12% of total 3-ABA formation in A549 and HBEC3-KT cells, but with high variability. It is likely that mitochondrial nitroreductases play a minor role in the formation of 3-ABA.

Discussion

3-NBA is a potent mutagen and suspected human carcinogen present in diesel engine exhaust and ambient air particulate matter which requires metabolic activation in order to promote its mutagenic and tumorigenic effects.^{8-14, 22, 74} Previous research has suggested that NQO1 is the major nitroreductase responsible for 3-NBA activation, but this work has largely been conducted

in human hepatic cytosols⁵¹ and primary human hepatocytes⁴⁹ which may not be reflective of 3-NBA bioactivation following inhalation exposures. To further characterize the metabolic activation of 3-NBA, we examined the role of human AKR enzymes due to reports that AKR1C3 nitroreductase activity superseded that of NQO1 towards the cancer therapeutic PR-104A.⁵³ *AKR1C* genes are also among the most upregulated genes in non-small cell lung cancer and in bronchial epithelial cells exposed to environmental carcinogens.^{53, 79-83}

We established the ability of AKR1C1-1C3 to act as nitroreductases via detection of the final reduction product 3-ABA in discontinuous enzymatic assays using HPLC-UV chromatography, and a product-precursor relationship was established. We found evidence for the formation of the nitroso- and hydroxylamino-intermediates via UPLC-HRMS, but these intermediates were not produced in significant quantities which suggests that these intermediates are unstable or are quickly metabolized to 3-ABA, the final reduction product. The ability of AKR1C enzymes to catalyze the reduction of 3-NBA and sequentially generate the nitroso- and hydroxylamino-intermediates is unusual since the AKRs conduct a single 2-electron reduction of carbonyl groups followed by the ordered release of reduced product and NADP⁺.⁸⁴ This is the first time to our knowledge that AKR1C enzymes have been implicated in the metabolic activation of NO₂-PAHs by acting as nitroreductases.

The present study examined AKR1C1-1C3 and NQO1 in cell-free systems to compare their kinetic constants (K_m , k_{cat} , k_{cat}/K_m). We found that the apparent catalytic efficiencies (k_{cat}/K_m) for 3-ABA formation were nearly equivalent for AKR1C1, AKR1C3, and NQO1. Other AKR1C isozymes have variable nitroreductase activity towards 3-NBA: AKR1C2 had a lower k_{cat}/K_m than AKR1C1 and AKR1C3 while liver-specific AKR1C4 had negligible nitroreductase activity. k_{cat}/K_m is best applied to measure catalytic efficiency when the substrate concentrations cover the range 0.2-5.0

K_m , but this was not achieved due our limitations in detecting 3-ABA in the low substrate range. Our lower limit of detection of 3-ABA was 0.005 nmol, which required higher substrate concentrations (1 – 30 μ M) in the reaction system to form sufficient quantities of 3-ABA within the period that 3-ABA formation was linear with time. Despite these limitations, these apparent catalytic efficiencies are a useful comparator of the abilities of AKR1C1-1C3 and NQO1 to reduce 3-NBA. Although NQO1 has a higher turnover rate, AKR1Cs possess much lower K_m values than NQO1. The low K_m observed with AKR1C enzymes suggests that that they may be more important than NQO1 at the low concentrations of 3-NBA to which humans are exposed by inhalation.

We chose to evaluate the relative importance of AKR1C1-1C3 and NQO1 in human lung epithelial cell lines as the respiratory tract is the primary site of exposure to 3-NBA. 3-NBA-induced DNA adduct formation (i.e. 3-NBA activation) has also been observed in cultured human A549^{46, 75-76}, TT1¹⁶ and BEAS-2B⁷⁷⁻⁷⁸ lung cell lines. Our data suggest that the combined activities of AKR1C1-1C3 and NQO1 contribute equally in the reduction of 3-NBA in A549 and HBEC3-KT cell lines. Flufenamic acid, a pan-AKR1C inhibitor, and dicoumarol, a NQO1 inhibitor, reduced 3-ABA formation in cell culture to a similar extent in both cell lines. Although the contribution of NQO1 to the total nitroreductase activity is greater than any single AKR1C enzyme, the combined activities of AKR1C1-1C3 are roughly equivalent to NQO1 in these lung cell lines. However, the contribution of individual AKR1C isozymes may differ between cell lines. The use of specific inhibitors against individual AKR1C enzymes (salicylic acid, ursodeoxycholate, and indomethacin) suggests that AKR1C2 contributes to a much greater percentage of 3-NBA reduction than AKR1C1 and AKR1C3 in A549 cells while AKR1C3 appears to be the dominant isozyme in HBEC3-KT cells. This may be due to variability in relative expression of *AKR1C1-1C3* between cell lines.

A549 cells displayed greater nitroreductase activity towards 3-NBA than HBEC3-KT cells which is to be expected since A549 cells have higher levels of AKR1C1-1C3 and NQO1 due to constitutively active nuclear factor (erythroid-derived 2)-like 2 (Nrf2).⁸⁵⁻⁸⁶ *AKR1C* and *NQO1* genes are induced by the Nrf2-Keap1 pathway and possess antioxidant response elements (ARE) in their promoters. *AKR1C1-AKR1C3* genes contain multiple AREs (6-16 based on a positional matrix search) in their promoters and are among the most highly induced genes by the Nrf2-Keap1 system in humans yet the least studied.^{84, 89-92} *NQO1* is a prototypic Nrf2-regulated gene which is induced by exposures to NO₂-PAHs, and so it is thought that NO₂-PAHs may induce their own genotoxicity by increasing transcription of *NQO1*.^{52, 87-88} A similar phenomena is observed in the metabolic activation of PAH by AKRs as their reactive metabolites (PAH *ortho*-quinones, ROS) upregulate Nrf2 and further upregulate *AKR1C* genes, thus enhancing PAH bioactivation and genotoxicity.^{89, 93} *AKR1C* genes are also part of the “smoking gene battery” – genes that are upregulated in bronchial epithelial cells of smokers and are downregulated in smokers who have quit.⁸⁰⁻⁸³ Interestingly, Nrf2-targeted chemopreventatives such as *R*-sulforaphane (*R*-SFN) induce *AKR1C* and *NQO1* genes.⁸⁹ Given the similarities in the induction of *AKR1C1-1C3* and *NQO1*, both gene families will likely be over-expressed during exposures to NO₂-PAHs which will exacerbate metabolic activation of these compounds. This raises the question about the safety of indiscriminate use of Nrf2-targeted chemoprevention therapies as there may be certain environmental exposures that are toxified by Nrf2 activity.

Our data from this study suggest that monitoring expression of both *NQO1* and *AKR1C1-1C3* may provide insight into ability of cells and tissues to bioactivate 3-NBA. Human *AKR1C* isozymes have been previously implicated in the metabolic activation of PAHs via their dihydrodiol dehydrogenase activity to produce electrophilic and redox-active *ortho*-quinones.⁹⁴⁻⁹⁸

However, our work suggests for the first time that they may also be important for the metabolic activation of NO₂-PAHs via nitroreductase activity.

Funding Sources

This publication was made possible by the Pharmaceutical Research and Manufacturers of America (PhRMA) Foundation Pharmacology/Toxicology Pre-Doctoral Fellowship (J.R.M.) and P30 ES013508 (T.M.P.) and T32019851 (J.R.M.) from the National Institute of Environmental Health Sciences (NIEHS), NIH, DHHS. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS or NIH. Work at King's College London is supported by the National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in Health Impact of Environmental Hazards at King's College London in partnership with Public Health England (PHE) and Imperial College London. The views expressed are those of the authors and not necessarily those of the UK National Health Service, the NIHR, the UK Department of Health & Social Care or PHE.

Notes

The authors declare no competing financial interest.

Abbreviations

NO₂-PAHs, nitrated polycyclic aromatic hydrocarbons; PAHs, polycyclic aromatic hydrocarbons; 3-NBA, 3-nitrobenzanthrone; 3-NOBA, 3-nitrosobenzanthrone; *N*-OH-3-ABA, *N*-hydroxy-3-aminobenzanthrone; 3-ABA, 3-aminobenzanthrone; Dic, dicoumarol; FA, flufenamic acid; AKR,

aldo-keto reductase; NQO1, NAD(P)H:quinone oxidoreductase 1; XO, xanthine oxidase; nuclear factor (erythroid-derived 2)-like 2, Nrf2

References

1. Esposito, L.; Conti, D.; Ailavajhala, R.; Khalil, N.; Giordano, A., Lung Cancer: Are we up to the Challenge? *Current Genomics* **2010**, *11* (7), 513-8.
2. Fontham, E. T.; Correa, P.; Reynolds, P.; Wu-Williams, A.; Buffler, P. A.; Greenberg, R. S.; Chen, V. W.; Alterman, T.; Boyd, P.; Austin, D. F.; et al., Environmental tobacco smoke and lung cancer in nonsmoking women. A multicenter study. *Jama* **1994**, *271* (22), 1752-9.
3. Doll, R.; Hill, A. B., Lung Cancer and Other Causes of Death in Relation to Smoking. *Br Med J* **1956**, *2* (5001), 1071-81.
4. Loomis, D.; Grosse, Y.; Lauby-Secretan, B.; Ghissassi, F. E.; Bouvard, V.; Benbrahim-Tallaa, L.; Guha, N.; Baan, R.; Mattock, H.; Straif, K., The carcinogenicity of outdoor air pollution. *The Lancet Oncology* **2013**, *14* (13), 1262-1263.
5. Brauer, M.; Amann, M.; Burnett, R. T.; Cohen, A.; Dentener, F.; Ezzati, M.; Henderson, S. B.; Krzyzanowski, M.; Martin, R. V.; Van Dingenen, R.; van Donkelaar, A.; Thurston, G. D., Exposure assessment for estimation of the global burden of disease attributable to outdoor air pollution. *Environ Sci Technol* **2012**, *46* (2), 652-60.
6. Vineis, P.; Forastiere, F.; Hoek, G.; Lipsett, M., Outdoor air pollution and lung cancer: recent epidemiologic evidence. *Int J Cancer* **2004**, *111* (5), 647-52.
7. Straif, K.; Cohen, A.; Samet, J., *IARC Scientific Publications. Air Pollution and Cancer*. 2013; Vol. 161.
8. IARC, Diesel and gasoline engine exhausts and some nitroarenes. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* **2012**, *105*.
9. Attfield, M. D.; Schleiff, P. L.; Lubin, J. H.; Blair, A.; Stewart, P. A.; Vermeulen, R.; Coble, J. B.; Silverman, D. T., The Diesel Exhaust in Miners study: a cohort mortality study with emphasis on lung cancer. *J Natl Cancer Inst* **2012**, *104* (11).
10. Garshick, E.; Schenker, M. B.; Munoz, A.; Segal, M.; Smith, T. J.; Woskie, S. R.; Hammond, S. K.; Speizer, F. E., A case-control study of lung cancer and diesel exhaust exposure in railroad workers. *Am Rev Respir Dis* **1987**, *135* (6), 1242-8.
11. Garshick, E.; Laden, F.; Hart, J. E.; Rosner, B.; Smith, T. J.; Dockery, D. W.; Speizer, F. E., Lung cancer in railroad workers exposed to diesel exhaust. *Environ Health Perspect* **2004**, *112* (15), 1539-1543.
12. Tokiwa, H.; Sera, N.; Horikawa, K.; Nakanishi, Y.; Shigematu, N., The presence of mutagens/carcinogens in the excised lung and analysis of lung cancer induction. *Carcinogenesis* **1993**, *14* (9), 1933-8.
13. Vermeulen, R.; Silverman, D. T.; Garshick, E.; Vlaanderen, J.; Portengen, L.; Steenland, K., Exposure-response estimates for diesel engine exhaust and lung cancer mortality based on data from three occupational cohorts. *Environ Health Perspect* **2014**, *122* (2), 172-7.
14. Silverman, D. T.; Samanic, C. M.; Lubin, J. H.; Blair, A. E.; Stewart, P. A.; Vermeulen, R.; Coble, J. B.; Rothman, N.; Schleiff, P. L.; Travis, W. D.; Ziegler, R. G.; Wacholder, S.; Attfield, M. D., The Diesel Exhaust in Miners study: a nested case-control study of lung cancer and diesel exhaust. *J Natl Cancer Inst* **2012**, *104* (11), 855-68.
15. Boström, C.-E. E.; Gerde, P.; Hanberg, A.; Jernström, B.; Johansson, C.; Kyrklund, T.; Rannug, A.; Törnqvist, M.; Victorin, K.; Westerholm, R., Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. *Environ Health Perspect* **2002**, *110 Suppl 3*, 451-488.

16. Jarvis, I. W. H.; Enlo-Scott, Z.; Nagy, E.; Mudway, I. S.; Tetley, T. D.; Arlt, V. M.; Phillips, D. H., Genotoxicity of fine and coarse fraction ambient particulate matter in immortalised normal (TT1) and cancer-derived (A549) alveolar epithelial cells. *Environ Mol Mutagen* **2018**, *59* (4), 290-301.
17. Tokiwa, H.; Ohnishi, Y.; Rosenkranz, H. S., Mutagenicity and carcinogenicity of nitroarenes and their sources in the environment. *Crit Rev Toxicol* **1986**, *17* (1), 23-60.
18. Samet, J. M.; Avila-Tang, E.; Boffetta, P.; Hannan, L. M.; Olivo-Marston, S.; Thun, M. J.; Rudin, C. M., Lung cancer in never smokers: clinical epidemiology and environmental risk factors. *Clin Cancer Res* **2009**, *15* (18), 5626-45.
19. Boffetta, P.; Nyberg, F., Contribution of environmental factors to cancer risk. *British medical bulletin* **2003**, *68*, 71-94.
20. Benbrahim-Tallaa, L.; Baan, R. A.; Grosse, Y.; Lauby-Secretan, B.; El Ghissassi, F.; Bouvard, V.; Guha, N.; Loomis, D.; Straif, K., Carcinogenicity of diesel-engine and gasoline-engine exhausts and some nitroarenes. *The Lancet. Oncology* **2012**, *13* (7), 663-4.
21. Arlt, V. M., 3-Nitrobenzanthrone, a potential human cancer hazard in diesel exhaust and urban air pollution: a review of the evidence. *Mutagenesis* **2005**, *20* (6), 399-410.
22. Enya, T.; Suzuki, H.; Watanabe, T.; Hirayama, T.; Hisamatsu, Y., 3-Nitrobenzanthrone, a Powerful Bacterial Mutagen and Suspected Human Carcinogen Found in Diesel Exhaust and Airborne Particulates. *Environ Sci Technol* **1997**, *31* (10), 2772-2776.
23. Takamura-Enya, T.; Suzuki, H.; Hisamatsu, Y., Mutagenic activities and physicochemical properties of selected nitrobenzanthrones. *Mutagenesis* **2006**, *21* (6), 399-404.
24. Nagy, E.; Zeisig, M.; Kawamura, K.; Hisamatsu, Y.; Sugeta, A.; Adachi, S.; Moller, L., DNA adduct and tumor formations in rats after intratracheal administration of the urban air pollutant 3-nitrobenzanthrone. *Carcinogenesis* **2005**, *26* (10), 1821-8.
25. Arlt, V. M.; Zhan, L.; Schmeiser, H. H.; Honma, M.; Hayashi, M.; Phillips, D. H.; Suzuki, T., DNA adducts and mutagenic specificity of the ubiquitous environmental pollutant 3-nitrobenzanthrone in Muta Mouse. *Environ Mol Mutagen* **2004**, *43* (3), 186-95.
26. Arlt, V. M.; Gingerich, J.; Schmeiser, H. H.; Phillips, D. H.; Douglas, G. R.; White, P. A., Genotoxicity of 3-nitrobenzanthrone and 3-aminobenzanthrone in MutaMouse and lung epithelial cells derived from MutaMouse. *Mutagenesis* **2008**, *23* (6), 483-490.
27. White, P. A.; Douglas, G. R.; Phillips, D. H.; Arlt, V. M., Quantitative relationships between lacZ mutant frequency and DNA adduct frequency in MutaMouse tissues and cultured cells exposed to 3-nitrobenzanthrone. *Mutagenesis* **2017**, *32* (2), 299-312.
28. Kucab, J. E.; Zwart, E. P.; van Steeg, H.; Luijten, M.; Schmeiser, H. H.; Phillips, D. H.; Arlt, V. M., TP53 and lacZ mutagenesis induced by 3-nitrobenzanthrone in Xpa-deficient human TP53 knock-in mouse embryo fibroblasts. *DNA Repair (Amst)* **2015**.
29. Fu, P. P., Metabolism of nitro-polycyclic aromatic hydrocarbons. *Drug Metab Rev* **1990**, *22* (2-3), 209-68.
30. IARC, Diesel and gasoline engine exhausts and some nitroarenes. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* **1989**, *46*, 1-458.
31. Arlt, V. M.; Glatt, H.; Muckel, E.; Pabel, U.; Sorg, B. L.; Seidel, A.; Frank, H.; Schmeiser, H. H.; Phillips, D. H., Activation of 3-nitrobenzanthrone and its metabolites by human acetyltransferases, sulfotransferases and cytochrome P450 expressed in Chinese hamster V79 cells. *Int J Cancer* **2003**, *105* (5), 583-92.

32. Arlt, V. M.; Glatt, H.; Muckel, E.; Pabel, U.; Sorg, B. L.; Schmeiser, H. H.; Phillips, D. H., Metabolic activation of the environmental contaminant 3-nitrobenzanthrone by human acetyltransferases and sulfotransferase. *Carcinogenesis* **2002**, *23* (11), 1937-1945.
33. Arlt, V. M.; Stiborova, M.; Hewer, A.; Schmeiser, H. H.; Phillips, D. H., Human enzymes involved in the metabolic activation of the environmental contaminant 3-nitrobenzanthrone: evidence for reductive activation by human NADPH:cytochrome p450 reductase. *Cancer Res* **2003**, *63* (11), 2752-2761.
34. Arlt, V. M.; Sorg, B. L.; Osborne, M.; Hewer, A.; Seidel, A.; Schmeiser, H. H.; Phillips, D. H., DNA adduct formation by the ubiquitous environmental pollutant 3-nitrobenzanthrone and its metabolites in rats. *Biochem Biophys Res Commun* **2003**, *300* (1), 107-114.
35. Bieler, C. A.; Wiessler, M.; Erdinger, L.; Suzuki, H.; Enya, T.; Schmeiser, H. H., DNA adduct formation from the mutagenic air pollutant 3-nitrobenzanthrone. *Mutat Res* **1999**, *439* (2), 307-11.
36. Stiborova, M.; Frei, E.; Schmeiser, H. H.; Arlt, V. M.; Martinek, V., Mechanisms of enzyme-catalyzed reduction of two carcinogenic nitro-aromatics, 3-nitrobenzanthrone and aristolochic acid I: Experimental and theoretical approaches. *Int J Mol Sci* **2014**, *15* (6), 10271-95.
37. Arlt, V. M.; Henderson, C. J.; Wolf, C. R.; Schmeiser, H. H.; Phillips, D. H.; Stiborova, M., Bioactivation of 3-aminobenzanthrone, a human metabolite of the environmental pollutant 3-nitrobenzanthrone: evidence for DNA adduct formation mediated by cytochrome P450 enzymes and peroxidases. *Cancer letters* **2006**, *234* (2), 220-231.
38. Arlt, V. M.; Hewer, A.; Sorg, B. L.; Schmeiser, H. H.; Phillips, D. H.; Stiborova, M., 3-aminobenzanthrone, a human metabolite of the environmental pollutant 3-nitrobenzanthrone, forms DNA adducts after metabolic activation by human and rat liver microsomes: evidence for activation by cytochrome P450 1A1 and P450 1A2. *Chemical research in toxicology* **2004**, *17* (8), 1092-1101.
39. Arlt, V. M.; Bieler, C. A.; Mier, W.; Wiessler, M.; Schmeiser, H. H., DNA adduct formation by the ubiquitous environmental contaminant 3-nitrobenzanthrone in rats determined by ³²P-postlabeling. *Int J Cancer* **2001**, *93* (3), 450-454.
40. Bieler, C. A.; Cornelius, M. G.; Klein, R.; Arlt, V. M.; Wiessler, M.; Phillips, D. H.; Schmeiser, H. H., DNA adduct formation by the environmental contaminant 3-nitrobenzanthrone after intratracheal instillation in rats. *Int J Cancer* **2005**, *116* (6), 833-8.
41. Arlt, V. M.; Schmeiser, H. H.; Osborne, M. R.; Kawanishi, M.; Kanno, T.; Yagi, T.; Phillips, D. H.; Takamura-Enya, T., Identification of three major DNA adducts formed by the carcinogenic air pollutant 3-nitrobenzanthrone in rat lung at the C8 and N2 position of guanine and at the N6 position of adenine. *International journal of cancer*. **2006**, *118* (9), 2139-2146.
42. Arlt, V. M.; Meinel, W.; Florian, S.; Nagy, E.; Barta, F.; Thomann, M.; Mrizova, I.; Kraus, A. M.; Liu, M.; Richards, M.; Mirza, A.; Kopka, K.; Phillips, D. H.; Glatt, H.; Stiborova, M.; Schmeiser, H. H., Impact of genetic modulation of SULT1A enzymes on DNA adduct formation by aristolochic acids and 3-nitrobenzanthrone. *Arch Toxicol* **2017**, *91* (4), 1957-1975.
43. Kanno, T.; Kawanishi, M.; Takamura-Enya, T.; Arlt, V. M.; Phillips, D. H.; Yagi, T., DNA adduct formation in human hepatoma cells treated with 3-nitrobenzanthrone: analysis by the (³²)P-postlabeling method. *Mutation research* **2007**, *634* (1-2), 184-191.
44. Lamy, E.; Kassie, F.; Gminski, R.; Schmeiser, H. H.; Mersch-Sundermann, V., 3-Nitrobenzanthrone (3-NBA) induced micronucleus formation and DNA damage in human hepatoma (HepG2) cells. *Toxicol Lett* **2004**, *146* (2), 103-9.

45. Phousongphouang, P. T.; Grosovsky, A. J.; Eastmond, D. A.; Covarrubias, M.; Arey, J., The genotoxicity of 3-nitrobenzanthrone and the nitropyrene lactones in human lymphoblasts. *Mutat Res* **2000**, *472* (1-2), 93-103.
46. Arlt, V. M.; Glatt, H.; Gamboa da Costa, G.; Reynisson, J.; Takamura-Enya, T.; Phillips, D. H., Mutagenicity and DNA adduct formation by the urban air pollutant 2-nitrobenzanthrone. *Toxicol Sci* **2007**, *98* (2), 445-57.
47. Seidel, A.; Dahmann, D.; Krekeler, H.; Jacob, J., Biomonitoring of polycyclic aromatic compounds in the urine of mining workers occupationally exposed to diesel exhaust. *Int J Hyg Environ Health* **2002**, *204* (5-6), 333-8.
48. Stiborova, M.; Arlt, V. M.; Henderson, C. J.; Wolf, C. R.; Frei, E.; Schmeiser, H. H.; Phillips, D. H., Molecular mechanism of genotoxicity of the environmental pollutant 3-nitrobenzanthrone. *Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia* **2005**, *149* (2), 191-7.
49. Stiborová, M.; Martínek, V.; Svobodová, M.; Sístková, J.; Dvorák, Z.; Ulrichová, J.; Simánek, V.; Frei, E.; Schmeiser, H. H.; Phillips, D. H.; Arlt, V. M., Mechanisms of the different DNA adduct forming potentials of the urban air pollutants 2-nitrobenzanthrone and carcinogenic 3-nitrobenzanthrone. *Chem Res Toxicol* **2010**, *23* (7), 1192-1201.
50. Arlt, V. M.; Stiborova, M.; Henderson, C. J.; Osborne, M. R.; Bieler, C. A.; Frei, E.; Martinek, V.; Sopko, B.; Wolf, C. R.; Schmeiser, H. H.; Phillips, D. H., Environmental pollutant and potent mutagen 3-nitrobenzanthrone forms DNA adducts after reduction by NAD(P)H:quinone oxidoreductase and conjugation by acetyltransferases and sulfotransferases in human hepatic cytosols. *Cancer Res* **2005**, *65* (7), 2644-52.
51. Arlt, V. M.; Stiborova, M.; Henderson, C. J.; Osborne, M. R.; Bieler, C. A.; Frei, E.; Martinek, V.; Sopko, B.; Wolf, R. C.; Schmeiser, H. H.; Phillips, D. H., Environmental Pollutant and Potent Mutagen 3-Nitrobenzanthrone Forms DNA Adducts after Reduction by NAD(P)H:Quinone Oxidoreductase and Conjugation by Acetyltransferases and Sulfotransferases in Human Hepatic Cytosols. *Cancer Research* **2005**, *65* (7), 2644-2652.
52. Stiborová, M.; Dracínská, H.; Hájková, J.; Kaderábková, P.; Frei, E.; Schmeiser, H. H.; Soucek, P.; Phillips, D. H.; Arlt, V. M., The environmental pollutant and carcinogen 3-nitrobenzanthrone and its human metabolite 3-aminobenzanthrone are potent inducers of rat hepatic cytochromes P450 1A1 and -1A2 and NAD(P)H:quinone oxidoreductase. *Drug Metab Dispos* **2006**, *34* (8), 1398-1405.
53. Guise, C. P.; Abbattista, M. R.; Singleton, R. S.; Holford, S. D.; Connolly, J.; Dachs, G. U.; Fox, S. B.; Pollock, R.; Harvey, J.; Guilford, P.; Donate, F.; Wilson, W. R.; Patterson, A. V., The bioreductive prodrug PR-104A is activated under aerobic conditions by human aldo-keto reductase 1C3. *Cancer Res* **2010**, *70* (4), 1573-84.
54. Penning, T. M., Aldo-Keto Reductases. In *Comprehensive Toxicology*, McQueen, C. A., Ed. Academic Press: Oxford, 2010; Vol. 4, pp 149-167.
55. Penning, T. M.; Drury, J. E., Human aldo-keto reductases: Function, gene regulation, and single nucleotide polymorphisms. *Arch Biochem Biophys* **2007**, *464* (2), 241-250.
56. Hsu, N. Y.; Ho, H. C.; Chow, K. C.; Lin, T. Y.; Shih, C. S.; Wang, L. S.; Tsai, C. M., Overexpression of dihydrodiol dehydrogenase as a prognostic marker of non-small cell lung cancer. *Cancer Res* **2001**, *61* (6), 2727-31.
57. Arlt, V. M.; Glatt, H.; Muckel, E.; Pabel, U.; Sorg, B. L.; Schmeiser, H. H.; Phillips, D. H., Metabolic activation of the environmental contaminant 3-nitrobenzanthrone by human acetyltransferases and sulfotransferase. *Carcinogenesis* **2002**, *23* (11), 1937-45.

58. Schmeiser, H. H.; Furstenberger, G.; Takamura-Enya, T.; Phillips, D. H.; Arlt, V. M., The genotoxic air pollutant 3-nitrobenzanthrone and its reactive metabolite N-hydroxy-3-aminobenzanthrone lack initiating and complete carcinogenic activity in NMRI mouse skin. *Cancer Lett* **2009**, *284* (1), 21-9.
59. Fifer, E. K.; Heflich, R. H.; Djurić, Z.; Howard, P. C.; Beland, F. A., Synthesis and mutagenicity of 1-nitro-6-nitrosopyrene and 1-nitro-8-nitrosopyrene, potential intermediates in the metabolic activation of 1,6- and 1,8-dinitropyrene. *Carcinogenesis* **1986**, *7* (1), 65-70.
60. Penning, T. M.; Burczynski, M. E.; Jez, J. M.; Hung, C. F.; Lin, H. K.; Ma, H.; Moore, M.; Palackal, N.; Ratnam, K., Human 3 α -hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. *The Biochemical journal* **2000**, *351* (Pt 1), 67-77.
61. Adeniji, A.; Uddin, M. J.; Zang, T.; Tamae, D.; Wangtrakuldee, P.; Marnett, L. J.; Penning, T. M., Discovery of (R)-2-(6-Methoxynaphthalen-2-yl)butanoic Acid as a Potent and Selective Aldo-keto Reductase 1C3 Inhibitor. *Journal of Medicinal Chemistry* **2016**, *59* (16), 7431-7444.
62. Burczynski, M. E.; Harvey, R. G.; Penning, T. M., Expression and characterization of four recombinant human dihydrodiol dehydrogenase isoforms: oxidation of trans-7, 8-dihydroxy-7,8-dihydrobenzo[a]pyrene to the activated o-quinone metabolite benzo[a]pyrene-7,8-dione. *Biochemistry* **1998**, *37* (19), 6781-6790.
63. Ramirez, R. D.; Sheridan, S.; Girard, L.; Sato, M.; Kim, Y.; Pollack, J.; Peyton, M.; Zou, Y.; Kurie, J. M.; Dimairo, J. M.; Milchgrub, S.; Smith, A. L.; Souza, R. F.; Gilbey, L.; Zhang, X.; Gandia, K.; Vaughan, M. B.; Wright, W. E.; Gazdar, A. F.; Shay, J. W.; Minna, J. D., Immortalization of human bronchial epithelial cells in the absence of viral oncoproteins. *Cancer res* **2004**, *64* (24), 9027-9034.
64. Pink, M.; Verma, N.; Zerries, A.; Schmitz-Spanke, S., Dose-Dependent Response to 3-Nitrobenzanthrone Exposure in Human Urothelial Cancer Cells. *Chem Res Toxicol* **2017**, *30* (10), 1855-1864.
65. Ernster, L.; Ljunggren, M.; Danielson, L., Purification and some properties of a highly dicumarol-sensitive liver diaphorase. *Biochemical and Biophysical Research Communications* **1960**, *2* (2), 88-92.
66. Steckelbroeck, S.; Jin, Y.; Gopishetty, S.; Oyesanmi, B.; Penning, T. M., Human Cytosolic 3 α -Hydroxysteroid Dehydrogenases of the Aldo-keto Reductase Superfamily Display Significant 3 β -Hydroxysteroid Dehydrogenase Activity: IMPLICATIONS FOR STEROID HORMONE METABOLISM AND ACTION. *Journal of Biological Chemistry* **2004**, *279* (11), 10784-10795.
67. Byrns, M. C.; Penning, T. M., Type 5 17 β -Hydroxysteroid Dehydrogenase/Prostaglandin F Synthase (AKR1C3): Role In Breast Cancer and Inhibition by Nonsteroidal Antiinflammatory Drug Analogs. *Chem Biol Interact* **2009**, *178* (1-3), 221-7.
68. Dhagat, U.; Carbone, V.; Chung, R. P.; Matsunaga, T.; Endo, S.; Hara, A.; El-Kabbani, O., A salicylic acid-based analogue discovered from virtual screening as a potent inhibitor of human 20 α -hydroxysteroid dehydrogenase. *Medicinal chemistry (Sharjah (United Arab Emirates))* **2007**, *3* (6), 546-50.
69. Hara, A.; Matsuura, K.; Tamada, Y.; Sato, K.; Miyabe, Y.; Deyashiki, Y.; Ishida, N., Relationship of human liver dihydrodiol dehydrogenases to hepatic bile-acid-binding protein and an oxidoreductase of human colon cells. *Biochemical Journal* **1996**, *313* (Pt 2), 373-6.
70. Byrns, M. C.; Steckelbroeck, S.; Penning, T. M., An indomethacin analogue, N-(4-chlorobenzoyl)-melatonin, is a selective inhibitor of aldo-keto reductase 1C3 (type 2 3 α -HSD,

type 5 17beta-HSD, and prostaglandin F synthase), a potential target for the treatment of hormone dependent and hormone independent malignancies. *Biochem Pharmacol* **2008**, 75 (2), 484-93.

71. Watts, R. W. E.; Watts, J. E. M.; Seegmiller, J. E., Xanthine oxidase activity in human tissues and its inhibition by allopurinol (4-hydroxypyrazolo[3,4-d] pyrimidine). *The Journal of Laboratory and Clinical Medicine* **1965**, 66 (4), 688-697.

72. Oberg, K. E., The site of the action of rotenone in the respiratory chain. *Exp Cell Res* **1961**, 24, 163-4.

73. Palmer, G.; Horgan, D. J.; Tisdale, H.; Singer, T. P.; Beinert, H., Studies on the respiratory chain-linked reduced nicotinamide adenine dinucleotide dehydrogenase. XIV. Location of the sites of inhibition of rotenone, barbiturates, and piericidin by means of electron paramagnetic resonance spectroscopy. *J Biol Chem* **1968**, 243 (4), 844-7.

74. Arlt, V. M.; Stiborova, M.; Henderson, C. J.; Osborne, M. R.; Bieler, C. A.; Frei, E.; Martinek, V.; Sopko, B.; Wolf, R. C.; Schmeiser, H. H.; Phillips, D. H., Environmental Pollutant and Potent Mutagen 3-Nitrobenzanthrone Forms DNA Adducts after Reduction by NAD(P)H:Quinone Oxidoreductase and Conjugation by Acetyltransferases and Sulfotransferases in Human Hepatic Cytosols. *Cancer Res* **2005**, 65 (7), 2644-2652.

75. Nagy, E.; Adachi, S.; Takamura-Enya, T.; Zeisig, M.; Moller, L., DNA adduct formation and oxidative stress from the carcinogenic urban air pollutant 3-nitrobenzanthrone and its isomer 2-nitrobenzanthrone, in vitro and in vivo. *Mutagenesis* **2007**, 22 (2), 135-45.

76. Rossner, P.; Strapacova, S.; Stolcpartova, J.; Schmuczerova, J.; Milcova, A.; Neca, J.; Vlkova, V.; Brzicova, T.; Machala, M.; Topinka, J., Toxic effects of the major components of diesel exhaust in human alveolar basal epithelial cells (A549). *Int J Mol Sci* **2016**, 17 (9).

77. Oya, E.; Ovrevik, J.; Arlt, V. M.; Nagy, E.; Phillips, D. H.; Holme, J. A., DNA damage and DNA damage response in human bronchial epithelial BEAS-2B cells following exposure to 2-nitrobenzanthrone and 3-nitrobenzanthrone: role in apoptosis. *Mutagenesis* **2011**, 26 (6), 697-708.

78. Ovrevik, J.; Arlt, V. M.; Oya, E.; Nagy, E.; Mollerup, S.; Phillips, D. H.; Lag, M.; Holme, J. A., Differential effects of nitro-PAHs and amino-PAHs on cytokine and chemokine responses in human bronchial epithelial BEAS-2B cells. *Toxicol Appl Pharmacol* **2010**, 242 (3), 270-80.

79. MacLeod, A. K.; Acosta-Jimenez, L.; Coates, P. J.; McMahon, M.; Carey, F. A.; Honda, T.; Henderson, C. J.; Wolf, C. R., Aldo-keto reductases are biomarkers of NRF2 activity and are co-ordinately overexpressed in non-small cell lung cancer. *Br J Cancer* **2016**, 115 (12), 1530-9.

80. Woenckhaus, M.; Klein-Hitpass, L.; Grepmeier, U.; Merk, J.; Pfeifer, M.; Wild, P.; Bettstetter, M.; Wuensch, P.; Blaszyk, H.; Hartmann, A.; Hofstaedter, F.; Dietmaier, W., Smoking and cancer-related gene expression in bronchial epithelium and non-small-cell lung cancers. *J Pathol* **2006**, 210 (2), 192-204.

81. Gümüş, Z. H.; Du, B.; Kacker, A.; Boyle, J. O.; Bocker, J. M.; Mukherjee, P.; Subbaramaiah, K.; Dannenberg, A. J.; Weinstein, H., Effects of tobacco smoke on gene expression and cellular pathways in a cellular model of oral leukoplakia. *Cancer Prev Res* **2008**, 1 (2), 100-11.

82. Penning, T. M.; Lerman, C., Genomics of smoking exposure and cessation: lessons for cancer prevention and treatment. *Cancer Prev Res* **2008**, 1 (2).

83. Nagaraj, N. S.; Beckers, S.; Mensah, J. K.; Waigel, S.; Vigneswaran, N.; Zacharias, W., Cigarette smoke condensate induces cytochromes P450 and aldo-keto reductases in oral cancer cells. *Toxicology letters* **2006**, 165 (2), 182-194.

84. Jin, Y.; Penning, T. M., Aldo-keto reductases and bioactivation/detoxication. *Annu Rev Pharmacol Toxicol* **2007**, *47*, 263-292.
85. Ohta, T.; Iijima, K.; Miyamoto, M.; Nakahara, I.; Tanaka, H.; Ohtsuji, M.; Suzuki, T.; Kobayashi, A.; Yokota, J.; Sakiyama, T.; Shibata, T.; Yamamoto, M.; Hirohashi, S., Loss of Keap1 Function Activates Nrf2 and Provides Advantages for Lung Cancer Cell Growth. *Cancer Research* **2008**, *68* (5), 1303-1309.
86. Singh, A.; Misra, V.; Thimmulappa, R. K.; Lee, H.; Ames, S.; Hoque, M. O.; Herman, J. G.; Baylin, S. B.; Sidransky, D.; Gabrielson, E.; Brock, M. V.; Biswal, S., Dysfunctional KEAP1–NRF2 Interaction in Non-Small-Cell Lung Cancer. *PLoS Medicine* **2006**, *3* (10), e420.
87. Stiborová, M.; Dracínská, H.; Mizerovská, J.; Frei, E.; Schmeiser, H. H.; Hudecek, J.; Hodek, P.; Phillips, D. H.; Arlt, V. M., The environmental pollutant and carcinogen 3-nitrobenzanthrone induces cytochrome P450 1A1 and NAD(P)H:quinone oxidoreductase in rat lung and kidney, thereby enhancing its own genotoxicity. *Toxicology* **2008**, *247* (1), 11-22.
88. Hajos, A. K.; Winston, G. W., Dinitropyrene nitroreductase activity of purified NAD(P)H-quinone oxidoreductase: role in rat liver cytosol and induction by Aroclor-1254 pretreatment. *Carcinogenesis* **1991**, *12* (4), 697-702.
89. Agyeman, A. S.; Chaerkady, R.; Shaw, P. G.; Davidson, N. E.; Visvanathan, K.; Pandey, A.; Kensler, T. W., Transcriptomic and proteomic profiling of KEAP1 disrupted and sulforaphane-treated human breast epithelial cells reveals common expression profiles. *Breast Cancer Res Treat* **2012**, *132* (1), 175-87.
90. Ciaccio, P. J.; Shen, H.; Jaiswal, A. K.; Lyttle, M. H.; Tew, K. D., Modulation of detoxification gene expression in human colon HT29 cells by glutathione-S-transferase inhibitors. *Mol Pharmacol* **1995**, *48* (4), 639-47.
91. Ciaccio, P. J.; Jaiswal, A. K.; Tew, K. D., Regulation of human dihydrodiol dehydrogenase by Michael acceptor xenobiotics. *J Biol Chem* **1994**, *269* (22), 15558-62.
92. Lou, H.; Du, S.; Ji, Q.; Stolz, A., Induction of AKR1C2 by phase II inducers: identification of a distal consensus antioxidant response element regulated by NRF2. *Mol Pharmacol* **2006**, *69* (5), 1662-72.
93. Burczynski, M. E.; Lin, H. K.; Penning, T. M., Isoform-specific induction of a human aldo-keto reductase by polycyclic aromatic hydrocarbons (PAHs), electrophiles, and oxidative stress: implications for the alternative pathway of PAH activation catalyzed by human dihydrodiol dehydrogenase. *Cancer Res* **1999**, *59* (3), 607-614.
94. Burczynski, M. E., Harvey, R.G., and Penning, T.M., Expression and characterization of four recombinant human dihydrodiol dehydrogenase isoforms: Oxidation of *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene to the activated *o*-quinone metabolite benzo[*a*]pyrene-7,8-dione. *Biochemistry* **1998**, *37*, 6781-6790.
95. Palackal, N. T., Burczynski, M.E., Harvey, R.G. and Penning, T.M., The ubiquitous aldehyde reductase (AKR1A1) oxidizes proximate carcinogen *trans*-dihydrodiols to *o*-quinones: Potential role in polycyclic aromatic hydrocarbon activation. *Biochemistry* **2001**, *40*, 10901-10910.
96. Palackal NT, L. S., Harvey RG, Blair IA, Penning TM., Activation of polycyclic aromatic hydrocarbon *trans*-dihydrodiol proximate carcinogens by human aldo-keto reductase (AKR1C) enzymes and their functional overexpression in human lung carcinoma (A549) cells. *J. Biol. Chem.* **2002**, *24*, 24799-808.

97. Smithgall, T. E., Harvey, R.G. and Penning, T.M., Regio- and stereospecificity of homogeneous 3 α -hydroxysteroid-dihydrodiol dehydrogenase for *trans*-dihydrodiol metabolites of polycyclic aromatic hydrocarbons. *J. Biol. Chem.* **1986**, **261**, 6184-6191.
98. Smithgall, T. E., Harvey, R.G. and Penning, T.M., Spectroscopic identification of *ortho*-quinones as the products of polycyclic aromatic *trans*-dihydrodiol oxidation catalyzed by dihydrodiol dehydrogenase. A potential route of proximate carcinogen metabolism. *J. Biol. Chem.* **1988**, **263**, 1814-1820.