BENZO[a]PYRENE

This is a compilation of abstracts of articles identified during the preliminary toxicological evaluation of evidence on the developmental and reproductive toxicity of benzo[a]pyrene (BaP, CAS# 50-32-8). BaP is a polycyclic aromatic hydrocarbon and a component of air pollution. Polycyclic aromatic hydrocarbons are formed as a result of incomplete combustion of organic materials and are ubiquitous environmental contaminants. Exposures to the general population occur through mainstream and environmental tobacco smoke, grilled and smoked foods, foods grown in polluted areas, drinking water, air, and coal tar-based pharmaceutical products. For non-smokers and those not occupationally exposed, the major source of exposure is diet.

The abstracts compiled below are from animal toxicity and epidemiologic studies reporting on developmental and reproductive sequelae related to exposure to BaP, as well as other relevant investigations (e.g., *in vitro* studies or studies in non-mammalian animal species). This information was used to screen chemicals to propose for listing consideration by the Developmental and Reproductive Toxicant Identification Committee. The criteria for passing the current screen are the existence of the following number of reports of an increase in adverse developmental or reproductive toxicity outcomes in mammalian species

1) a total 15 or more reports across all of the endpoints (developmental toxicity, female reproductive toxicity, male reproductive); or
2) 10 or more reports for any one category of the following three categories: developmental toxicity; female reproductive toxicity, or male reproductive toxicity.

There were a total of 130 studies identified in the literature search on BaP (some studies may have reported more than one adverse effect). The Table below shows how BaP passed the screen.

Endpoints	Reports of adverse effects		Reports no adverse effects	
	Animal	Human	Animal	Human
Developmental	19	5	0	0
Female reproductive	11	2	0	0
Male reproductive	7	2	0	0
Total	37	9	0	0

In addition to the reports enumerated in the table, the search identified:

1) 71 other related studies or meeting presentations (titles of reports only provided below)
2) 23 publications with a relevant title but no abstract.

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I. Animal Developmental and Reproductive Toxicity Studies

A. Studies reporting developmental or reproductive toxicity

i. Developmental toxicity

a. *Studies identified in the open literature search*

Prenatal exposure to benzo(a)pyrene impairs later-life cortical neuronal function.
McCallister M. M., Maguire M., Ramesh A., Aimin Q., Liu S., Khoshbouei H., Aschner M., Ebner F. F. and Hood D. B.
Neurotoxicology. 2008;29(5):846-54.

Prenatal exposure to environmental contaminants, such as benzo(a)pyrene [B(a)P] has been shown to impair brain development. The overarching hypothesis of our work is that glutamate receptor subunit expression is crucial for cortical evoked responses and that prenatal B(a)P exposure modulates the temporal developmental expression of glutamatergic receptor subunits in the somatosensory cortex. To characterize prenatal B(a)P exposure on the development of cortical function, pregnant Long Evans rats were exposed to low-level B(a)P (300 microg/kg BW) by oral gavage on gestational days 14-17. At this exposure dose, there was no significant effect of B(a)P on (1) the number of pups born per litter, (2) the pre-weaning growth curves and (3) initial and final brain to body weight ratios. Control and B(a)P-exposed offspring were profiled for B(a)P metabolites in plasma and whole brain during the pre-weaning period. No detectable levels of metabolites were found in the control offspring. However, a time-dependent decrease in total metabolite concentration was observed in B(a)P-exposed offspring. On PND100-120, cerebrocortical mRNA expression was determined for the glutamatergic NMDA receptor subunit (NR2B) in control and B(a)P-exposed offspring. Neural activity was also recorded from neurons in primary somatic sensory (barrel) cortex. Semiquantitative PCR from B(a)P-exposed offspring revealed a significant 50% reduction in NR2B mRNA expression in B(a)P-exposed offspring relative to controls. Recordings from B(a)P-exposed offspring revealed that N-methyl-d-aspartate (NMDA) receptor-dependent neuronal activity in barrel cortex evoked by whisker stimulation was also significantly reduced (70%) as compared to controls. Analysis showed that the greatest deficit in cortical neuronal responses occurred in the shorter latency epochs from 5 to 20 ms post-stimulus. The results suggest that in utero exposure to benzo(a)pyrene results in diminished mRNA expression of the NMDA NR2B receptor subunit to result in late life deficits in cortical neuronal activity in the offspring. The findings from this study lead to a strong prediction that in utero exposure to benzo(a)pyrene at a time when synapses are first formed and adjusted in strength by activity in the sensory pathways will produce a strong negative effect on brain function in offspring progeny.

Down-regulation of early ionotrophic glutamate receptor subunit developmental expression as a mechanism for observed plasticity deficits following gestational exposure to benzo(a)pyrene.
Brown L. A., Khousbouei H., Goodwin J. S., Irvin-Wilson C. V., Ramesh A., Sheng L., McCallister M. M., Jiang G. C., Aschner M. and Hood D. B.
Neurotoxicology. 2007;28(5):965-78.

The focus of this study was to characterize the impact of gestational exposure to benzo(a)pyrene [B(a)P] on modulation of glutamate receptor subunit expression that is critical for the maintenance of synaptic plasticity mechanisms during hippocampal or cortical development in offspring. Previous studies have demonstrated that hippocampal and/or cortical synaptic plasticity (as measured by long-term potentiation and S1-cortex spontaneous/evoked neuronal activity) and learning behavior (as measured by fixed-ratio performance operant testing) is significantly impaired in polycyclic aromatic or halogenated aromatic hydrocarbon-exposed offspring as compared to controls. These previous studies have also revealed that brain to body weight ratios are greater in exposed offspring relative to controls indicative of intrauterine growth retardation which has been shown to manifest as low birth weight in offspring. Recent epidemiological studies have identified an effect of prenatal exposure to airborne polycyclic aromatic hydrocarbons on neurodevelopment in the first 3 years of life among inner-city children [Perera FP, Rauh V, Whyatt RM, Tsai WY, Tang D, Diaz D, et al. Effect of prenatal exposure to airborne polycyclic aromatic hydrocarbons on neurodevelopment in the first 3 years of life among inner-city children. Environ Health Perspect 2006;114:1287-92]. The present study utilizes a well-characterized animal model to test the hypothesis that gestational exposure to B(a)P causes dysregulation of developmental ionotropic glutamate receptor subunit expression, namely the N-methyl-d-aspartate receptor (NMDAR) and alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate receptor (AMPAR) both critical to the expression of synaptic plasticity mechanisms. To mechanistically ascertain the basis of B(a)P-induced plasticity perturbations, timed pregnant Long-Evans rats were exposed in an oral subacute exposure regimen to 0, 25 and 150mug/kg BW B(a)P on gestation days 14-17. The first sub-hypothesis tested whether gestational exposure to B(a)P would result in significant disposition in offspring. The second sub-hypothesis tested whether gestational exposure to B(a)P would result in down-regulation of early developmental expression of NMDA and AMPA receptor subunits in the hippocampus of offspring as well as in primary neuronal cultures. The results of these studies revealed significant: (1) disposition to the hippocampus and cortex, (2) down-regulation of developmental glutamate receptor mRNA and protein subunit expression and (3) voltage-dependent decreases in the amplitude of inward currents at negative potentials in B(a)P-treated cortical neuronal membranes. These results suggest that plasticity and behavioral deficits produced as a result of gestational B(a)P exposure are at least, in part, a result of down-regulation of early developmental glutamate receptor subunit expression and function at a time when excitatory synapses are being formed for the first time in the developing central nervous system. The results also predict that in B(a)P-exposed offspring with reduced early glutamate receptor subunit expression, a parallel deficit in behaviors that depend on normal hippocampal or

cortical functioning will be observed and that these deficits will be present throughout life.

Deleterious effects of polynuclear aromatic hydrocarbon on blood vascular system of the rat fetus.
Sanyal M. K. and Li Y. L.
Birth defects research Part B, Developmental and reproductive toxicology. 2007;80(5):367-73.

BACKGROUND: Polynuclear aromatic hydrocarbons (PAH), benzo[alpha]pyrene (B[alpha]P) and 7,12-dimethylbenz[alpha]anthracene (DMBA) are toxic environmental agents distributed widely. The relative deleterious effects of these agents on growth and blood vasculature of fetus and placental tissues of the rat were studied. METHODS: Pregnant rats (Day 1 sperm positive) with implantation sites confirmed by laparotomy were treated intraperitoneally (i.p.) on Pregnancy Days 10, 12, and 14 with these agents dissolved in corn oil at cumulated total doses 50, 100, and 200 mg/kg/rat, and control with corn oil only (3-20 dams/group). Fetal growth, tissue hemorrhage, and placental pathology were evaluated by different parameters on Pregnancy Day (PD) 20 in treated and control rats. RESULTS: DMBA was relatively more deleterious compared to B[alpha]P indicated by increased lethality and progressive reduction of body weight of the mother with increasing doses. At 200 mg/kg/rat doses of these agents, maternal survival was 45% and 100% and body weight reduced 24% and 52% of controls, respectively. The fetal survival rates in live mothers were similar to that of controls. They induced marked fetal growth retardation and necrosis of placental tissues. B[alpha]P and DMBA produced significant toxicity to differentiating fetal blood vascular system as exhibited by rupture of blood vessels and hemorrhage, especially in the skin, cranial, and brain tissues. CONCLUSIONS: Maternal PAH exposure induced placental toxicity and associated adverse fetal development and hemorrhage in different parts of the fetal body, in particular, marked intradermal and cranial hemorrhage, showing that developing fetal blood vasculature is a target of PAH toxicity.

Inhaled benzo(a)pyrene impairs long-term potentiation in the F1 generation rat dentate gyrus.
Wormley D. D., Chirwa S., Nayyar T., Wu J., Johnson S., Brown L. A., Harris E. and Hood D. B.
Cellular and molecular biology. 2004;50(6):715-21.

The purpose of this study is to provide a point of reference regarding the neurotoxic effects resulting from exposure to environmental contaminants. Benzo(a)pyrene is a member of the polycyclic aromatic hydrocarbon (PAH) family and it is a by-product of combustion processes. Thus, persons living near factories or hazardous waste sites face the danger of exposure through contact with contaminated air, water and soil. In an effort to understand the impact of environmental contaminants, we have investigated the effects of gestational B(a)P aerosol exposure on long-term potentiation (LTP), a cellular correlate of learning and memory in the F1 generation. Briefly, timed-pregnant

rats were exposed to B(a)P via nose-only inhalation on gestation days 11-21 for 4 hr per day. Dams were maintained to term and pups were weaned on postnatal day 30. Subsequent electrophysiological studies during postnatal days 60-70 revealed a diminution in LTP across the perforant path-granular cells synapses in the hippocampus of F1 generation animals that were transplacentally exposed to B(a)P aerosol relative to unexposed controls. Additionally, NMDA receptor subunit 1 (NR1) protein was found to be downregulated in the hippocampus of B(a)P exposed F1 generation animals. Taken together, our results suggest that gestational exposure to B(a)P aerosol attenuates the capacity for LTP in the F1 generation.

Embryonic prostaglandin H synthase-2 (PHS-2) expression and benzo[a]pyrene teratogenicity in PHS-2 knockout mice.
Parman T. and Wells P. G.
The FASEB journal. 2002;16(9):1001-9.

The developmental role of prostaglandin H synthase-2 (PHS-2), which converts xenobiotics such as benzo[a]pyrene (B[a]P) to toxic free radical intermediates, is poorly understood. In this study, we determined the embryonic expression and teratological relevance of PHS-2 in pregnant CD-1 and B6/129S7 PHS-2 knockout mice. Wild-type (+/+) B6/129S7 dams given B[a]P on gestational day (GD) 10 had three times more fetal malformations than did +/- PHS-2-deficient dams ($P < 0.05$). GD 10-13 CD-1 embryos had high PHS-2 protein expression, and both + /+ and +/- GD 19 B6/129S7 fetuses had more B[a]P-initiated malformations and postpartum lethality than did -/- littermates ($P < 0.05$). Thus, embryonic PHS-2 is expressed constitutively during organogenesis and contributes substantially to B[a]P teratogenicity.

Assessment of metabolites and AhR and CYP1A1 mRNA expression subsequent to prenatal exposure to inhaled benzo(a)pyrene.
Wu J., Ramesh A., Nayyar T. and Hood D. B.
International journal of developmental neuroscience. 2003;21(6):333-46.

Few studies have focused on environmental aerosol contaminant, mechanistically-based, dose-related neurotoxicity with respect to development of the central nervous system. To fill this important data gap and to highlight possible mechanistic pathways, a study was undertaken to determine metabolite concentrations associated with the transplacental disposition of inhaled benzo(a)pyrene (B(a)P) and the resulting effects on the status of aryl hydrocarbon receptor (AhR), and cytochrome P450 1A1 (CYP1A1) mRNA in preweaning F1 generation animals. In this study, laparotomy on GD 8 was performed on timed-pregnant rats followed by dosing via nose-only exposure for 4h a day for 10 days (GD 11-GD 20) to three concentrations of a B(a)P: carbon black aerosol (25, 75 and 100 microg/m^3). A dose-dependent decrease in birth index was observed in the B(a)P exposed group as compared to the controls ($P <0.05$). Analysis of cerebrocortical extracts from F1 generation pups revealed a dose-dependent ($P < 0.05$) increase in total B(a)P metabolites. Analysis of cerebrocortical and hippocampal mRNA developmental expression profiles for AhR and CYP1A1 using 18sRNA as the internal

standard, revealed that inhaled B(a)P upregulates AhR during the first postnatal month. The present study suggest that prenatal exposure to inhaled B(a)P upregulates hepatic aryl hydrocarbon receptor dependent mechanisms in the F1 generation. Hepatic upregulation of the aryl hydrocarbon receptor may modulate the potential for benzo(a)pyrene toxicity via the activation of cytochrome P450 and the subsequent deposition of lipophillic metabolites to developing central nervous system structures such as cerebral cortex and hippocampus.

Alteration of pregnancy related hormones and fetal survival in F-344 rats exposed by inhalation to benzo(a)pyrene.
Archibong A. E., Inyang F., Ramesh A., Greenwood M., Nayyar T., Kopsombut P., Hood D. B. and Nyanda A. M.
Reproductive toxicology. 2002;16(6):801-8.

The objective of this study was to evaluate the effect of subacute exposure to inhaled benzo(a)pyrene (BaP) on fetal survival and luteal maintenance using timed-pregnant Fisher 344 rats. Prior to assignment of pregnant rats to treatment and control groups, numbers of implantation sites were determined on gestation day (GD) 8 via midventral laparotomy. Subsequently, animals were assigned randomly to three treatment groups and two control groups. Treatment consisted of subacute exposure of rats via inhalation to BaP 25, 75, and 100 micro g/m^3, 4h daily for 10 days (GD-11-20). Control animals were either sham exposed to carbon black (CB) to control for inert BaP carrier or remained unexposed (UNC). Blood samples were collected on days 15 and 17 of gestation via sinus orbital veini-puncture for plasma. Number of pups per litter was determined postpartum and fetal survival rate was expressed as a percentage of the corresponding implantation sites. Radioimmunoassays were used to determine plasma progesterone, estrogen, and prolactin (indirect measurement of decidual luteotropin) concentrations. Fetal survival among BaP-treated rats declined in a dose-dependent manner (25 micro g/m^3, 78.3% per litter; 75 micro g/m^3, 38.0% per litter; 100 micro g/m^3, 33.8% per litter; $P < 0.05$) compared with CB (96.7% per litter) and UNC (98.9% per litter). Plasma progesterone, estrogen, and prolactin concentrations also declined as a result of subacute exposure of rats to BaP compared to controls. These data suggest that inhaled BaP compromised fetal survival and consequently luteotropic activity in the exposed animals.

Immunomodulation in progeny from thymectomized primiparous mice exposed to benzo(a)pyrene during mid-pregnancy.
Wolisi G. O., Majekodunmi J., Bailey G. B. and Urso P.
Immunopharmacology and immunotoxicology. 2001;23(2):267-80.

Previous studies have shown that Benzo(a)pyrene (B(a)P3) given to non-thymectomized (NTX) female mice alters expression of T cell subsets and suppresses cell mediated immunity (CMI) and humoral immunity (HI) in the progeny. Thus, maternal exposure to B(a)P may influence changes in progeny immune status. To understand how maternal cellular and humoral factors influence embryonic development of progeny

immunity, adult female mice were thymectomized (TX) at 6 weeks, mated and injected with 150 microg B(a)P)/g body weight at 12 days of pregnancy. After B(a)P exposure, the following studies were performed: (A) Maternal reproductive capacity and survival rate of progeny; (B) Detection of T cells in progeny thymus; (C) Functional characteristics of progeny thymus or spleen. Maternal thymectomy and B(a)P exposure reduced average litter size by 40%. Serological sensitivity of thymus cells with anti-Thyl + complement occurred at a higher dilution of mAb in progeny from TX mothers exposed to B(a)P, suggesting that B(a)P-thymectomy led to increased sensitivity of developing thymocytes to mAb plus complement. Progeny from TX mothers exposed to B(a)P showed enhanced thymic CMI, but suppressed splenic CMI and HI. Thus, thymectomy prevents CMI immunosuppression by B(a)P, while HI is still suppressed. These results indicate that the maternal thymus is necessary for incurring the effect of B(a)P on progeny CMI.

Modulation in the developmental expression profile of Sp1 subsequent to transplacental exposure of fetal rats to desorbed benzo[a]pyrene following maternal inhalation.
Hood D. B., Nayyar T., Ramesh A., Greenwood M. and Inyang F.
Inhalation toxicology. 2000;12(6):511-35.

Any alteration of the critical sequence of genes that are required to coordinate the differentiation of cells, the promotion of migration, dendritic arborization, synapse formation, and myelination in the developing nervous system would be expected to have deleterious consequences. The focus of this article is a molecular evaluation of the neurotoxicological effects that result subsequent to the transplacental exposure of fetal rats to desorbed benzo(a)pyrene (BaP) following maternal inhalation. A state-of-the-art, newly designed, fabricated, and tested model aerosol generation system was utilized in these studies. Timed-pregnant Sprague Dawley rats were exposed for 4 h on gestation day 15 of a 21-day gestation period to an acute dose of BaP:carbon black aerosol (100 microg/m^3). Controls received carbon black only. Nominal and chamber concentrations of the particulate aerosol were determined gravimetrically with a seven-stage cascade impactor. The aerosol exhibited a trimodal distribution with 95% cumulative mass less than 15.85 microm, 90% cumulative mass less than 10 microm, 67. 5% cumulative mass less than 2.5 microm and 66.2% cumulative mass less than 1.0 microm. Time-course bioavailability results indicated that greater than 95% of the parent compound is cleared from blood 240 min postexposure. An Sp1 transcription factor consensus sequence was examined by electrophoretic mobility shift analysis of nuclear extracts from various brain regions of resulting pups on postnatal days 3, 5, 7, 10, and 15. It revealed perturbations in the developmental expression profile of Sp1 abundance as a result of nose-only particulate aerosol exposure to the timed-pregnant dam. The data obtained on the temporal and spatial regulation of gene expression in the brain indicate that (1) Sp1 DNA-binding is developmentally regulated and expressed very highly in actively developing brain regions, and (2) a consequence of the transplacental deposition of desorbed BaP to the fetus is in utero neurotoxicity.

Fertility in mice after prenatal exposure to benzo[a]pyrene and inorganic lead.
Kristensen P., Eilertsen E., Einarsdóttir E., Haugen A., Skaug V. and Øvrebø S.
Environmental health perspectives. 1995;103(6):588-90.

Experimental evidence suggests that inorganic lead and benzo[a]pyrene (BaP) suppress the development of primordial oocytes during fetal life. We examined the single and combined effects of prenatal exposure to BaP and moderate doses of lead. The fertility and ovarian morphology of F1 female NMRI mice in four treatment groups (nine mice per group) were investigated: control; lead (F0 given 1 g PbCl2/L in drinking water until mating); BaP (10 mg/kg body weight daily by oral intubation on days 7-16 of F0 pregnancy); and combined lead and BaP. F1 groups exposed prenatally to BaP either alone or in combination with inorganic lead showed markedly reduced fertility with few ovarian follicles compared to controls, whereas the group exposed to lead only had measures comparable to the controls. Mice exposed to both lead and BaP had a significantly longer gestation period (days to litter) compared to mice exposed only to BaP, lead, or controls. There is a nonsignificant indication that the compounds together further reduce number of offspring, number of litters, and litter size. These results suggest that lead and BaP have synergistic effects on impairment of fertility. The possibility of synergism may be of human relevance as inorganic lead and BaP are ubiquitous environmental pollutants.

Fetal hematopoietic alterations after maternal exposure to benzo[a]pyrene: a cytometric evaluation.
Holladay S. D. and Smith B. J.
Journal of toxicology and environmental health. 1994;42(3):259-73.

In utero exposure to the environmental contaminant benzo[a]pyrene (BaP) was found to alter expression of murine thymocyte and liver fetal cell-surface markers. Pregnant mice were treated (via gavage) with 0, 50, 100, or 150 mg BaP/kg/d on gestational days (gd) 13-17, and offspring were examined on gd 18. Severe thymic atrophy and cellular depletion were found in BaP-exposed fetal mice. Flow cytometric analysis indicated that the BaP treatment resulted in a significant decrease in the percentage of CD4+8+ fetal thymocytes, as well as significantly increased CD4-8- and CD4-8+ thymocytes. Staining of thymocytes with anti-mouse heat-stable antigen (HSA) and CD8 monoclonal antibodies produced similar results. These data suggest that BaP, in addition to producing thymic hypocellularity, inhibits normal thymocyte maturation processes. The BaP treatment was also found to decrease total fetal liver cellularity including numbers of cells within resident hematopoietic subpopulations. In particular, prolymphocytic cells, identified by CD44 and CD45R antigen expression and by presence of nuclear terminal deoxynucleotidyl transferase (TdT), were significantly decreased in animals gestationally exposed to BaP. These data, taken together, indicate that postnatal suppression of cell and humoral-mediated immune function following in utero exposure to BaP may result from multiple targeting of immune cells at different hematopoietic levels. Furthermore, results of the present study identify both qualitative and quantitative changes in fetal immune cell antigen expression that correlate well with the postnatal

immunosuppression that occurs in experimental animals exposed to this carcinogenic polycyclic aromatic hydrocarbon.

Immunological consequences from exposure to benzo(a)pyrene during pregnancy.
Urso P., Zhang W. and Cobb J. R.
Scandinavian journal of immunology Supplement. 1992;11:203-6.

Progeny and maternal immune status after benzo(a)pyrene (BP) exposure of mothers at mid-pregnancy is disrupted in fetal liver (FL), in spleen and in thymus during pregnancy and postnatally. Mice suffer deficiencies in splenic and thymic mixed lymphocyte responses (MLR), and disorientations of T antigen expressing cells, punctuated by exorbitant increases in Lyt2, especially in FL. FL Lyt2 do not suppress an MLR, while Lyt1 mediate suppression. Isolated Thy1 show a weak response to Concanavalin A; FL Thy1 weakly express an MLR. Maternal macrophages and progeny B cells are also functionally abnormal. Thus, BP induces generalized immune deficiency that may affect ontogeny and which is potentially deleterious to health.

Enhanced glutathione S-transferase (GST) activity in pregnant rats treated with benzo(a)pyrene.
Cervello I., Lafuente A., Giralt M. and Mallol J.
Placenta. 1992;13(3):273-80.

Administration of Benzo(a)pyrene (BP, 50 mg/kg/d) to pregnant rats significantly increased Glutathione S-transferase (GST) activity in placental tissue-extract (Vmax = 40 nmol/min/mg protein and 69 nmol/min/mg protein in controls versus treated animals respectively; P less than 0.01) and total fetal tissue-extract (Vmax = 51 nmol/min/mg protein and 82 nmol/min/mg protein in controls versus treated animals respectively; P less than 0.01) indicating an induction effect of BP on the GST system. An increase in the Km values was also observed: 1.61 x 10^{-3} M and 2.84 x 10^{-3} M in control versus treated placentae; 1.38 x 10^{-3} M and 2.05 x 10^{-3} M in control versus treated fetuses. A competitive effect on the enzyme by the BP present in the sample may also be involved. The glutathione content in both tissues did not show any changes after the treatment with BP. This increase in the GST system was not sufficient to protect the fetus. BP affected the reproductive performance of pregnant rats by significantly increasing the number of resorptions and fetal wastage, and, also, by decreasing the fetal weight.

Lasting impact of a single benzpyrene treatment in pre-natal and growing age on the thymic glucocorticoid receptors of rats.
Csaba G., Inczefi-Gonda A. and Szeberényi S.
General pharmacology. 1991;22(5):815-8.

1. Rats exposed to benzpyrene in utero at 19 days of pre-natal life showed a relative decrease in the number of thymic glucocorticoid receptors at 6 weeks of age. 2. Primary exposure to benzpyrene at 6 weeks of age had a similar effect on females 4 weeks

later, but did not change the glucocorticoid receptor number of males. 3. In utero exposure accounted for an increase in the fetal cytochrome P450 level within 1 day, whereas exposure at 6 weeks of age did not change it within 4 weeks. 4. It appears that exposure to benzpyrene gives rise to a faulty imprinting of the thymic glucocorticoid receptor in both fetal and growing age, to judge from a lasting change in the receptor number.

Early changes in T lymphocytes and subsets of mouse progeny defective as adults in controlling growth of a syngeneic tumor after in utero insult with benzo(a)pyrene.
Urso P. and Johnson R. A.
Immunopharmacology. 1987;14(1):1-10.

Benzo(a)pyrene, a potent carcinogen, severely suppresses the anti-SRBC plaque-forming cell response, the mixed lymphocyte response (afferent T cell function), and an in vivo graft-vs.-host response (efferent T cell function) of mouse progeny exposed to the carcinogen during gestation (11 to 13 days). Immunodeficiency occurs early after birth (1 week) and persists for 18 months. The abnormalities in the T cell-mediated responses led us to examine the quantitative profile of T cells and subsets (Lyt 1+, Lyt 2+) present in the lymphoid organs during fetogenesis (15 to 19 days) and postnatally. In addition, we examined the ability of 3- to 8-month-old progeny and their spleen cells to resist the in vivo growth of cells from a syngeneic fibrosarcoma (a tumor that had been induced by benzo(a)pyrene). Our observations included: (1) Depletion of T cells and subsets in the thymus late (19 days) in gestation and postnatally. (2) Depleted T and Lyt 1+ cells in the spleen during gestation, while postnatally the former were enhanced and the effect on the latter was variable (enhancement and reduction). (3) In the fetal liver, the T cells were reduced, but the Lyt 1+ cells were unchanged. (4) The Lyt 2+ cells were strikingly enhanced in the fetal liver and spleen, but most dramatically for the former. (5) The Lyt 1/Lyt 2 ratio was less than 1.00 or controls in the fetal liver and spleen, a condition which persisted for 30 days postnatally in the latter organ. (6) Benzo(a)pyrene-exposed progeny or their spleen cells were relatively ineffective in resisting in vivo growth of transferred tumor cells. These results show that this carcinogenic pollutant induces a marked disorientation of T cells and subsets which can persist for at least 4 weeks postnatally. This suggests disruption of T cell differentiation during ontogenesis which may have profound implications on the ability to resist induction and growth of neoplasias after in utero exposure to the carcinogen.

Embryotoxicity of benzo(a)pyrene and some of its synthetic derivatives in Swiss mice.
Barbieri O., Ognio E., Rossi O., Astigiano S. and Rossi L.
Cancer research. 1986;46(1):94-8.

We have studied the teratogenicity of benzo(a)pyrene (BP), benzo(a)pyrene-4,5-oxide, and a racemic mixture of 7 beta,8 alpha-dihydroxy-9 alpha,10 alpha-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene, a proximal metabolite and ultimate carcinogenic metabolite

of BP, respectively, and of 6-methylbenzo(a)pyrene after direct injection into embryonal Swiss mice. The compounds were dissolved in acetone and trioctanoin (1:1) and injected at doses ranging from 0.4 to 16.0 nmol/embryo on days 10, 12, and 14 of development. The transplacental effects of BP given at the same gestational days and at comparable dose levels were also evaluated. The control groups received 0.5, 1.0, or 2.0 microliter/embryo of vehicle on days 10, 12, or 14 of pregnancy, respectively. The fetuses were examined when they were 18 days old. On the basis of gross external and internal malformations, 7 beta,8 alpha-dihydroxy-9 alpha,10 alpha-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene appeared to be the most potent embryotoxic and teratogenic compound tested, causing 85% of embryolethality and 100% of malformed fetuses in the group treated on day 10 of intrauterine development. There were 61 and 27% of malformed fetuses following 7 beta,8 alpha-dihydroxy-9 alpha,10 alpha-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene treatment on days 12 and 14 of gestation, respectively. The effects of this BP metabolite were very specific and malformations such as exencephaly, thoraco- and gastroschisis, phocomelia, and edema were found. The administration of BP (both transplacental and direct intraembryonal injection) and benzo(a)pyrene-4,5-oxide caused no significant increase of malformed fetuses in any of the developmental stages considered. 6-Methylbenzo(a)pyrene induced multiple malformations (among these a high percentage of protruding tongue) in 50, 46 and 31% of the fetuses treated on days 10, 12, and 14 of gestational age, respectively. These results combined with previous data concerning the induction of lung tumors by the tested compounds in 15-day-old Swiss mouse embryos, emphasize the requirement of a common metabolic derivative of BP to induce both teratogenesis and carcinogenesis in mice. Furthermore present data show that midgestation Swiss embryos are also highly sensitive to the 6-methyl derivative of BP.

A comparative study of the reproductive effects of methadone and benzo[a]pyrene in the pregnant and pseudopregnant rat.
Bui Q. Q., Tran M. B. and West W. L.
Toxicology. 1986;42(2-3):195-204.

Benzo[a]pyrene (BP; 50 mg/kg) or methadone (5 mg/kg) was given subcutaneously to pregnant rats at different stages of gestation. Both BP and methadone affected the reproductive performance of pregnant rats by significantly increasing the number of resorptions and fetal wastage, and by decreasing the fetal weight. The same dosage levels of BP and methadone were also given to pseudopregnant rats (PSP) with an induced decidual cell reaction (DCR) in an attempt to distinguish whether adverse effects occur in the maternal or fetal compartment or both. Since the hormonal requirements for DCR and implantation are similar and the anatomical, histological, cytological, time sequential changes as well as appearance of the vasculature system for DCR and decidua are indistinguishable, PSP with DCR is similar to pregnancy except for the lack of a fetal compartment. BP, in this PSP model, significantly reduced the uterine wet weight and cyclic nucleotide (cAMP) and cGMP) levels whereas methadone was without a detectable effect. Our findings then suggest that BP may

exert its effects adversely on both the maternal and fetal compartments, whereas methadone may act primarily in the fetal compartment.

Importance of the route of administration for genetic differences in benzo[a]pyrene-induced in utero toxicity and teratogenicity.
Legraverend C., Guenthner T. M. and Nebert D. W.
Teratology. 1984;29(1):35-47.

C57BL/6N (Ahb/Ahb) mice have a high-affinity Ah receptor in tissues, whereas AKR/J and DBA/2N (Ahd/Ahd) mice have a poor-affinity Ah receptor. The cytochrome P1-450 induction response (enhanced benzo[a]pyrene metabolism) occurs much more readily in Ahb/Ahb and Ahb/Ahd than in Ahd/Ahd mice, at any given dose of the inducer benzo[a]pyrene. Embryos from the AKR/J X (C57BL/6N)(AKR/J)F1 and the reciprocal backcross were studied during benzo[a]pyrene feeding of the pregnant females. Oral benzo[a]pyrene (120 mg/kg/day) given to pregnant Ahd/Ahd mice between gestational day 2 and 10 produces more intrauterine toxicity and malformations in Ahd/Ahd than Ahb/Ahd embryos. This striking allelic difference is not seen in pregnant Ahb/Ahd mice receiving oral benzo[a]pyrene. Pharmacokinetics studies with [3H]benzo[a]pyrene in the diet and high-performance liquid chromatographic analysis of benzo[a]pyrene metabolism in vitro by the maternal intestine, liver, and ovary and the embryos of control and oral benzo[a]pyrene-treated pregnant females are consistent with "first-pass elimination" kinetics and differences in benzo[a]pyrene metabolism by the embryos and/or placentas versus maternal tissues. In the pregnant Ahd/Ahd mouse receiving oral benzo[a]pyrene, little induction of benzo[a]pyrene metabolism occurs in her intestine and liver; this leads to much larger amounts of benzo[a]pyrene reaching her embryos, and genetic differences in toxicity and teratogenesis are manifest. In the pregnant Ahb/Ahd mouse receiving oral benzo[a]pyrene, benzo[a]pyrene metabolism is greatly enhanced in her intestine and liver; this leads to less benzo[a]pyrene reaching her embryos, much less intrauterine toxicity and malformations, and no genetic differences are manifest. More toxic metabolites (especially benzo[a]pyrene 1,6- and 3,6-quinones) are shown to occur in Ahd/Ahd embryos than in Ahb/Ahd embryos. In additional studies, no prenatal or neonatal "imprinting" effect in C57BL/6N mice by 2,3,7,8-tetrachlorodibenzo-p-dioxin or Aroclor 1254 on benzo[a]pyrene metabolism later in life was detectable. These genetic differences in intrauterine toxicity and teratogenicity induced by oral benzo[a]pyrene are just opposite those induced by intraperitoneal benzo[a]pyrene [Shum et al., '79; Hoshino et al., '81). The data in the present report emphasize the importance of the route of administration when the teratogen induces its own metabolism.

Subnormal expression of cell-mediated and humoral immune responses in progeny disposed toward a high incidence of tumors after in utero exposure to benzo[a]pyrene.
Urso P. and Gengozian N.
Journal of toxicology and environmental health. 1984;14(4):569-84.

Pregnant mice were exposed to 150 micrograms benzo[a]pyrene (BaP) per gram of body weight during fetogenesis (d 11-17 of gestation) and the progeny were assayed for humoral and cell mediated immune responses at different time intervals after birth. Immature offspring (1-4 wk) were severely suppressed in their ability to produce antibody-(plaque-) forming cells (PFC) against sheep red blood cells (SRBC) and in the ability of their lymphocytes to undergo a mixed lymphocyte response (MLR). Lymphocytes from these progeny showed a moderate to weak capacity to inhabit production of colony-forming units (CFU) in host spleens following transfer with semiallogeneic bone marrow (BM) cells into lethally X-irradiated recipients syngeneic to the BM (in vivo graft-versus-host response, GVHR). A severe and sustained suppression in the MLR and the PFC response occurred from the fifth month up to 18 mo. The in vivo GVHR, also subnormal later in life, was not as severely suppressed as the other two parameters. Tumor incidence in the BP-exposed progeny was 8- to 10-fold higher than in those encountering corn oil alone from 18 to 24 mo of age. These data show that in utero exposure to the chemical carcinogen BaP alters development of components needed for establishing competent humoral and cell-mediated functions of the immune apparatus and leads to severe and sustained postnatal suppression of the defense mechanism. The immunodeficiency exhibited, particularly in the T-cell compartment (MLR, GVHR), before and during the increase in tumor frequency, may provide a favorable environment for the growth of nascent neoplasms induced by BaP.

ii. Female reproductive toxicity

a. *Studies identified in the open literature search*

Deleterious effects of polynuclear aromatic hydrocarbon on blood vascular system of the rat fetus.
Sanyal M. K. and Li Y. L.
Birth defects research Part B, Developmental and reproductive toxicology. 2007;80(5):367-73.

BACKGROUND: Polynuclear aromatic hydrocarbons (PAH), benzo[alpha]pyrene (B[alpha]P) and 7,12-dimethylbenz[alpha]anthracene (DMBA) are toxic environmental agents distributed widely. The relative deleterious effects of these agents on growth and blood vasculature of fetus and placental tissues of the rat were studied. METHODS: Pregnant rats (Day 1 sperm positive) with implantation sites confirmed by laparotomy were treated intraperitoneally (i.p.) on Pregnancy Days 10, 12, and 14 with these agents dissolved in corn oil at cumulated total doses 50, 100, and 200 mg/kg/rat, and control

with corn oil only (3-20 dams/group). Fetal growth, tissue hemorrhage, and placental pathology were evaluated by different parameters on Pregnancy Day (PD) 20 in treated and control rats. RESULTS: DMBA was relatively more deleterious compared to B[alpha]P indicated by increased lethality and progressive reduction of body weight of the mother with increasing doses. At 200 mg/kg/rat doses of these agents, maternal survival was 45% and 100% and body weight reduced 24% and 52% of controls, respectively. The fetal survival rates in live mothers were similar to that of controls. They induced marked fetal growth retardation and necrosis of placental tissues. B[alpha]P and DMBA produced significant toxicity to differentiating fetal blood vascular system as exhibited by rupture of blood vessels and hemorrhage, especially in the skin, cranial, and brain tissues. CONCLUSIONS: Maternal PAH exposure induced placental toxicity and associated adverse fetal development and hemorrhage in different parts of the fetal body, in particular, marked intradermal and cranial hemorrhage, showing that developing fetal blood vasculature is a target of PAH toxicity.

Alteration of pregnancy related hormones and fetal survival in F-344 rats exposed by inhalation to benzo(a)pyrene.
Archibong A. E., Inyang F., Ramesh A., Greenwood M., Nayyar T., Kopsombut P., Hood D. B. and Nyanda A. M.
Reproductive toxicology. 2002;16(6):801-8.

The objective of this study was to evaluate the effect of subacute exposure to inhaled benzo(a)pyrene (BaP) on fetal survival and luteal maintenance using timed-pregnant Fisher 344 rats. Prior to assignment of pregnant rats to treatment and control groups, numbers of implantation sites were determined on gestation day (GD) 8 via midventral laparotomy. Subsequently, animals were assigned randomly to three treatment groups and two control groups. Treatment consisted of subacute exposure of rats via inhalation to BaP 25, 75, and 100 micro g/m^3, 4h daily for 10 days (GD-11-20). Control animals were either sham exposed to carbon black (CB) to control for inert BaP carrier or remained unexposed (UNC). Blood samples were collected on days 15 and 17 of gestation via sinus orbital veini-puncture for plasma. Number of pups per litter was determined postpartum and fetal survival rate was expressed as a percentage of the corresponding implantation sites. Radioimmunoassays were used to determine plasma progesterone, estrogen, and prolactin (indirect measurement of decidual luteotropin) concentrations. Fetal survival among BaP-treated rats declined in a dose-dependent manner (25 micro g/m^3, 78.3% per litter; 75 micro g/m^3, 38.0% per litter; 100 micro g/m^3, 33.8% per litter; $P < 0.05$) compared with CB (96.7% per litter) and UNC (98.9% per litter). Plasma progesterone, estrogen, and prolactin concentrations also declined as a result of subacute exposure of rats to BaP compared to controls. These data suggest that inhaled BaP compromised fetal survival and consequently luteotropic activity in the exposed animals.

Ovotoxicity in female Fischer rats and B6 mice induced by low-dose exposure to three polycyclic aromatic hydrocarbons: comparison through calculation of an ovotoxic index.
Borman S. M., Christian P. J., Sipes I. G. and Hoyer P. B.
Toxicology and applied pharmacology. 2000;167(3):191-8.

Extensive destruction of primordial follicles by exposure to ovarian toxicants can cause early menopause in women. Primordial follicle destruction is known to result from dosing of mice and rats with three polycyclic aromatic hydrocarbons (PAHs), contaminants commonly found in cigarette smoke. Therefore, the purpose of this study was to compare relative ovotoxicity in mice and rats using the PAHs, 9, 10-dimethylbenzanthracene (DMBA), 3-methylcholanthrene (3-MC), and benzo[a]pyrene (BaP). Female B6C3F(1) mice and Fischer 344 rats (age 28 days) were dosed daily (ip) with vehicle control or a range of doses of the PAHs. Two groups were dosed with the occupational chemicals 4-vinylcyclohexene (VCH; 500 mg/kg ip) or its diepoxide metabolite (VCD; 80 mg/kg ip), other known ovotoxicants. After 15 days, ovaries were collected, histologically prepared, and follicles were microscopically classified (primordial, primary, or secondary) and counted. The dose of each chemical that produced 50% loss of primordial follicles ($p < 0.05$) was determined (ED50) and used to calculate an ovotoxic index (OI) in mice and rats (ED50 x 15 days). Thus, a chemical with a lower OI is more toxic. Primordial follicles in mice displayed a lower OI than rats to all chemicals tested (mouse: DMBA, 0.0012; 3-MC, 0.003; BaP, 0.18; VCD, 6.8; VCH, 69; rat: DMBA, 0.45; 3-MC, >3.4; BaP, >3.6; VCD, 8.6; VCH, >69). In mice, DMBA targeted primordial follicles at a 10-fold lower concentration than primary and secondary follicles, whereas 3-MC exposure targeted primordial and primary follicles to a similar degree. BaP exposure targeted primordial and primary follicles at a 100-fold higher concentration than DMBA or 3-MC. Although BaP and 3-MC did not target secondary follicles in mice, secondary follicles in rats were most susceptible to 3-MC. Furthermore, all three PAHs were more ovotoxic (lower OI) with repeated low-dose exposure compared with OIs calculated from other studies using single high-dose exposures. The earliest day of impending primordial follicle loss (increase in percentage of unhealthy follicles, $p < 0.05$) in mice was factored into the OI (ED50 x first day of damage, $p < 0.05$ x % healthy follicles remaining, relative to control). The revised OI became DMBA d15, 0.0006; 3-MC d12, 0.0008; BaP d15, 0.132; and VCD d8, 2.96. These results predict that DMBA is the most potent ovarian toxicant (lower OI) in both species but VCD damages primordial follicles after shorter exposures. Calculation of the OI in mice and rats represents a method for comparing the relative potential risk of a variety of chemicals that produce ovarian damage at low levels following repeated exposures. The results also demonstrate that low-dose repeated exposures are substantially more toxic to the ovary than a single high-dose exposure. This finding is particularly important in view of the implications for chronic low-dose exposures of women to environmental chemicals.

Fertility in mice after prenatal exposure to benzo[a]pyrene and inorganic lead.
Kristensen P., Eilertsen E., Einarsdóttir E., Haugen A., Skaug V. and Øvrebø S.
Environmental health perspectives. 1995;103(6):588-90.

Experimental evidence suggests that inorganic lead and benzo[a]pyrene (BaP) suppress the development of primordial oocytes during fetal life. We examined the single and combined effects of prenatal exposure to BaP and moderate doses of lead. The fertility and ovarian morphology of F1 female NMRI mice in four treatment groups (nine mice per group) were investigated: control; lead (F0 given 1 g PbCl2/L in drinking water until mating); BaP (10 mg/kg body weight daily by oral intubation on days 7-16 of F0 pregnancy); and combined lead and BaP. F1 groups exposed prenatally to BaP either alone or in combination with inorganic lead showed markedly reduced fertility with few ovarian follicles compared to controls, whereas the group exposed to lead only had measures comparable to the controls. Mice exposed to both lead and BaP had a significantly longer gestation period (days to litter) compared to mice exposed only to BaP, lead, or controls. There is a nonsignificant indication that the compounds together further reduce number of offspring, number of litters, and litter size. These results suggest that lead and BaP have synergistic effects on impairment of fertility. The possibility of synergism may be of human relevance as inorganic lead and BaP are ubiquitous environmental pollutants.

Enhanced glutathione S-transferase (GST) activity in pregnant rats treated with benzo(a)pyrene.
Cervello I., Lafuente A., Giralt M. and Mallol J.
Placenta. 1992;13(3):273-80.

Administration of Benzo(a)pyrene (BP, 50 mg/kg/d) to pregnant rats significantly increased Glutathione S-transferase (GST) activity in placental tissue-extract (Vmax = 40 nmol/min/mg protein and 69 nmol/min/mg protein in controls versus treated animals respectively; P less than 0.01) and total fetal tissue-extract (Vmax = 51 nmol/min/mg protein and 82 nmol/min/mg protein in controls versus treated animals respectively; P less than 0.01) indicating an induction effect of BP on the GST system. An increase in the Km values was also observed: 1.61 x 10^{-3} M and 2.84 x 10^{-3} M in control versus treated placentae; 1.38 x 10^{-3} M and 2.05 x 10^{-3} M in control versus treated fetuses. A competitive effect on the enzyme by the BP present in the sample may also be involved. The glutathione content in both tissues did not show any changes after the treatment with BP. This increase in the GST system was not sufficient to protect the fetus. BP affected the reproductive performance of pregnant rats by significantly increasing the number of resorptions and fetal wastage, and, also, by decreasing the fetal weight.

The effect of benzo(a)pyrene on murine ovarian and corpora lutea volumes.
Miller M. M., Plowchalk D. R., Weitzman G. A., London S. N. and Mattison D. R.
American journal of obstetrics and gynecology. 1992;166(5):1535-41.

OBJECTIVE: Women who smoke have impaired fertility and experience menopause at an earlier age. This experiment determined the effect of benzo(a)pyrene, a polycyclic aromatic hydrocarbon contained in cigarette smoke, on murine ovarian volume, total corpora lutea volume, individual corpora lutea volumes, and corpora lutea numbers. STUDY DESIGN: C57BL/6N mice were treated with intraperitoneal injections of 0 to 500 mg/kg benzo(a)pyrene in corn oil. The 20 mice at each dose were divided into four groups of five each and were killed at 1, 2, 3, or 4 weeks after treatment. Ovaries were serially sectioned and analyzed morphometrically. RESULTS: Benzo(a)pyrene produced a dose- and time-dependent decrease in ovarian volume, total corpora lutea volume, and number of corpora lutea per ovary. This effect was transitory at low doses with complete recovery of corpora lutea by 4 weeks. Compensatory hypertrophy of the individual corpora lutea occurred during the recovery phase. Ovarian function did not return in animals treated with the two highest doses. CONCLUSION: Benzo(a)pyrene is a murine ovarian toxicant that inhibits corpus luteum formation in a dose- and time-dependent fashion.

Ovarian toxicity of benzo(a)pyrene and metabolites in mice.
Mattison D. R., Singh H., Takizawa K. and Thomford P. J.
Reproductive toxicology. 1989;3(2):115-25.

The effect of intraovarian injection of benzo(a)pyrene (BP) or one of three metabolites: +7,8-oxide (7,8-O), (-)-dihydrodiol (DHD), and (+)-diol-epoxide-2 (DE2) on ovarian volume, weight, and follicle number was investigated in DBA/2N (D2), C57BL/6N (B6), and (DBA/2N x C57BL/6N)F1 (F1) mice. Female mice, 6 to 8 weeks old, were treated by injection into the right ovary with the indicated compound (10 micrograms in 1 microL DMSO). The left ovary was untreated. Two weeks following treatment both ovaries were removed, fixed in Bouin's medium, serially sectioned, and stained with hematoxylin and eosin. Right ovarian weight was decreased in D2 mice treated with BP (P less than 0.01 and DHD (P less than 0.01). Left ovarian weight was increased in D2 mice treated with DE2 (P less than 0.05). BP decreased right ovarian volume in D2 (P less than 0.01) and F1 (P less than 0.01) mice. 7,8-O decreased right ovarian volume in D2 mice (P less than 0.05). DHD decreased right ovarian volume in D2 (P less than 0.01) and F1 (P less than 0.05) mice. DE2 decreased right ovarian volume in D2 (P less than 0.01) and F1 (P less than 0.01) mice. Left ovarian volume was increased in B6 (P less than 0.01) and D2 (P less than 0.05) mice treated with DE2. The number of small follicles was decreased in D2, B6, and F1 mice treated with DE2 (P less than 0.01). BP and DHD also decreased small follicle number in D2 and F1 mice (P less than 0.01). The number of growing follicles was decreased in B6, D2, and F1 mice treated with DE2 (P less than 0.01). Treatment with DHD decreased the number of growing follicles in D2 mice (P less than 0.05). The number of antral follicles was reduced in F1 mice treated with BP (P less than 0.05), DHD (P less than 0.01), and DE2 (P less than 0.01). The number of

antral follicles was also reduced in B6 mice treated with DE2 (P less than 0.01) and in D2 mice treated with DHD (P less than 0.05) and D2 mice treated with DE2 (P less than 0.01). These experiments suggest that toxic effects to one ovary may result in compensatory hypertrophy of the contralateral ovary. Morphometric analysis of the ovary, including ovarian volume, represents a useful objective measure of ovarian toxicity.

A comparative study of the reproductive effects of methadone and benzo[a]pyrene in the pregnant and pseudopregnant rat.
Bui Q. Q., Tran M. B. and West W. L.
Toxicology. 1986;42(2-3):195-204.

Benzo[a]pyrene (BP; 50 mg/kg) or methadone (5 mg/kg) was given subcutaneously to pregnant rats at different stages of gestation. Both BP and methadone affected the reproductive performance of pregnant rats by significantly increasing the number of resorptions and fetal wastage, and by decreasing the fetal weight. The same dosage levels of BP and methadone were also given to pseudopregnant rats (PSP) with an induced decidual cell reaction (DCR) in an attempt to distinguish whether adverse effects occur in the maternal or fetal compartment or both. Since the hormonal requirements for DCR and implantation are similar and the anatomical, histological, cytological, time sequential changes as well as appearance of the vasculature system for DCR and decidua are indistinguishable, PSP with DCR is similar to pregnancy except for the lack of a fetal compartment. BP, in this PSP model, significantly reduced the uterine wet weight and cyclic nucleotide (cAMP) and cGMP) levels whereas methadone was without a detectable effect. Our findings then suggest that BP may exert its effects adversely on both the maternal and fetal compartments, whereas methadone may act primarily in the fetal compartment.

Benzo(a)pyrene inhibits ovulation in C57BL/6N mice.
Swartz W. J. and Mattison D. R.
The Anatomical Record. 1985;212(3):268-76.

Successful female reproductive function requires follicle growth, ovulation, and formation of the corpus luteum. Treatment of C57BL/6N mice with a single intraperitoneal injection of benzo(a)pyrene in doses ranging from 1 to 500 mg/kg produced a dose- and time-dependent decrease in the number of corpora lutea. This effect on the number of corpora lutea is most pronounced at 1 week after treatment, with a threshold of about 1 mg/kg, and an ED50 of 1.6 mg/kg. By 2 weeks after treatment partial recovery of follicle growth and ovulation occurred, as indicated by an increase in the ED50 to 20 mg/kg. Complete recovery of normal corpora lutea number occurs in mice treated with less than 100 mg/kg by 3 weeks after treatment, with little change in the ED50 noted between 3 and 4 weeks post-treatment, 78 mg/kg at both times. Mice treated with 100 or 500 mg/kg did not recover normal corpora lutea number over the course of this experiment. These data indicate that acute exposure to benzo(a)pyrene, and perhaps other polycyclic aromatic hydrocarbons, may have a

transient adverse effect on follicle growth, ovulation, or formation of corpora lutea. A consequence of this effect, transient infertility, has been observed previously when exploring the effect of polycyclic aromatic hydrocarbons on murine reproduction.

The effect of intraovarian injection of benzo(a)pyrene on primordial oocyte number and ovarian aryl hydrocarbon [benzo(a)pyrene] hydroxylase activity.
Shiromizu K. and Mattison D. R.
Toxicology and applied pharmacology. 1984;76(1):18-25.

The effect of intraovarian (io) injection of benzo(a)pyrene (BP) on primordial oocyte number, ovarian aryl hydrocarbon hydroxylase (AHH, EC 1.14.14.1) activity, and hepatic AHH activity and P-450 content was investigated in C57BL/6N (B6) and DBA/2N (D2) mice. Ovaries were exteriorized through dorsolumbar incisions under ether anesthesia, and 1.0 microliters of corn oil or corn oil containing BP was injected directly into the ovary. Bilateral io injection of BP was ovotoxic in a time and dose dependent manner. Primordial oocyte destruction was maximal 8 days after injection in both B6 and D2 mice. Unilateral io injection of BP destroyed oocytes only in the treated ovary. The threshold dose for primordial oocyte destruction was similar in both strains, approximately 50 ng/ovary. However, ED50's were different; 1.1 micrograms/ovary in B6 and 8.9 micrograms/ovary in D2 mice. Intraperitoneal treatment with alpha-naphthoflavone (80 mg/kg) inhibited the oocyte destruction by io treatment with BP (10 micrograms/ovary) in both B6 and D2 mice. In B6 mice, ovarian AHH activity was induced by BP in the treated ovary after either bilateral, or unilateral io treatment. However, io BP treatment did not change ovarian AHH activity in D2 mice. Hepatic AHH activity and P-450 content was not altered by io BP treatment in either mouse strain. These results strengthen the hypothesis that the ovary has the capability of metabolizing BP to ovotoxic products.

Murine strain differences in ovotoxicity following intraovarian injection with benzo(a)pyrene, (+)-(7R,8S)-oxide, (-)-(7R,8R)-dihydrodiol, or (+)-(7R,8S)-diol-(9S,10R)-epoxide-2.
Takizawa K., Yagi H., Jerina D. M. and Mattison D. R.
Cancer research. 1984;44(6):2571-6.

Murine ovarian tumors produced by polycyclic aromatic hydrocarbons like benzo(a)pyrene (BP) require small oocyte destruction. Small oocyte destruction was evaluated in C57BL/6N (B6), DBA/2N (D2), and C57BL/6J X DBA/ 2JF1 (B6D2F1) mice following intraovarian injection with BP, (+)-(7R ,8S)-oxide, (-)-(7R , 8R)-dihydrodiol [(-)-DHD], or (+)-(7R ,8S)-diol-(9S, 10R)-epoxide-2 [(+)- DE2] at doses ranging from 0.01 to 30 micrograms/ovary. BP, (-)-DHD, and (+)- DE2 produced small oocyte destruction in a dose-dependent fashion. The (+)-(7R ,8S)-oxide did not destroy small oocytes at the highest dose tested (10 micrograms/ovary). The rank orders of the calculated doses which resulted in the destruction of 50% of the small oocytes (ED50S) for small oocyte destruction were BP approximately equal to (-)-DHD greater than (+)-DE2 in all three groups of mice. However, the ED50S for BP and (-)-DHD differed

considerably among B6, D2, B6D2F1 mice; ED50S were smallest in B6 mice and largest in D2 mice. The ED50S for oocyte destruction in B6D2F1 mice were intermediate or similar to ED50S for B6 mice, depending on the method used for calculation. In spite of large strain differences in ED50S for BP and (-)-DHD, the ED50S for (+)- DE2 were similar in B6, D2, and B6D2F1 mice. The similar ED50 for (+)- DE2 suggests that it is an ultimate ovotoxin and ovarian carcinogen and that the target molecule(s) and mechanism(s) of detoxification are similar in B6, D2, and B6D2F1 mice.

iii. Male reproductive toxicity

a. *Studies identified in the open literature search*

Benzo(a)pyrene induces similar gene expression changes in testis of DNA repair proficient and deficient mice.
Verhofstad N., Pennings J. L., van O. C. T., van B. J., van S. F. J., van S. H. and Godschalk R. W.
BMC genomics. 2010;11:333.

BACKGROUND: Benzo [a]pyrene (B[a]P) exposure induces DNA adducts at all stages of spermatogenesis and in testis, and removal of these lesions is less efficient in nucleotide excision repair deficient Xpc-/- mice than in wild type mice. In this study, we investigated by using microarray technology whether compromised DNA repair in Xpc-/- mice may lead to a transcriptional reaction of the testis to cope with increased levels of B[a]P induced DNA damage. RESULTS: Two-Way ANOVA revealed only 4 genes differentially expressed between wild type and Xpc-/- mice, and 984 genes between testes of B[a]P treated and untreated mice irrespective of the mouse genotype. However, the level in which these B[a]P regulated genes are expressed differs between Wt and Xpc-/- mice ($p = 0.000000141$), and were predominantly involved in the regulation of cell cycle, translation, chromatin structure and spermatogenesis, indicating a general stress response. In addition, analysis of cell cycle phase dependent gene expression revealed that expression of genes involved in G1-S and G2-M phase arrest was increased after B[a]P exposure in both genotypes. A slightly higher induction of average gene expression was observed at the G2-M checkpoint in Xpc-/- mice, but this did not reach statistical significance ($P = 0.086$). Other processes that were expected to have changed by exposure, like apoptosis and DNA repair, were not found to be modulated at the level of gene expression. CONCLUSION: Gene expression in testis of untreated Xpc-/- and wild type mice were very similar, with only 4 genes differentially expressed. Exposure to benzo(a)pyrene affected the expression of genes that are involved in cell cycle regulation in both genotypes, indicating that the presence of unrepaired DNA damage in testis blocks cell proliferation to protect DNA integrity in both DNA repair proficient and deficient animals.

DNA adduct kinetics in reproductive tissues of DNA repair proficient and deficient male mice after oral exposure to benzo(a)pyrene.
Verhofstad N., van O. C. T., van B. J., van S. F. J., van S. H. and Godschalk R. W.
Environmental and molecular mutagenesis. 2010;51(2):123-9.

Benzo(a)pyrene (B[a]P) can induce somatic mutations, whereas its potential to induce germ cell mutations is unclear. There is circumstantial evidence that paternal exposure to B[a]P can result in germ cell mutations. Since DNA adducts are thought to be a prerequisite for B[a]P induced mutations, we studied DNA adduct kinetics by (32)P-postlabeling in sperm, testes and lung tissues of male mice after a single exposure to B[a]P (13 mg/kg bw, by gavage). To investigate DNA adduct formation at different stages of spermatogenesis, mice were sacrificed at Day 1, 4, 7, 10, 14, 21, 32, and 42 after exposure. In addition, DNA repair deficient (Xpc(-/-)) mice were used to study the contribution of nucleotide excision repair in DNA damage removal. DNA adducts were detectable with highest levels in lung followed by sperm and testis. Maximum adduct levels in the lung and testis were observed at Day 1 after exposure, while adduct levels in sperm reached maximum levels at approximately 1 week after exposure. Lung tissue and testis of Xpc(-/-) mice contained significantly higher DNA adduct levels compared to wild type (Wt) mice over the entire 42 day observation period ($P < 0.05$). Differences in adduct half-life between Xpc(-/-) and Wt mice were only observed in testis. In sperm, DNA adduct levels were significantly higher in Xpc(-/-) mice than in Wt mice only at Day 42 after exposure ($P = 0.01$). These results indicate that spermatogonia and testes are susceptible for the induction of DNA damage and rely on nucleotide excision repair for maintaining their genetic integrity.

Exposure to di(n-butyl)phthalate and benzo(a)pyrene alters IL-1β secretion and subset expression of testicular macrophages, resulting in decreased testosterone production in rats.
Zheng S. J., Tian H. J., Cao J. and Gao Y. Q.
Toxicology and applied pharmacology. 2010;248(1):28-37.

Di(n-butyl)phthalate (DBP) and benzo(a)pyrene (BaP) are environmental endocrine disruptors that are potentially hazardous to humans. These chemicals affect testicular macrophage immuno-endocrine function and testosterone production. However, the underlying mechanisms for these effects are not fully understood. It is well known that interleukin-1 beta (IL-1β), which is secreted by testicular macrophages, plays a trigger role in regulating Leydig cell steroidogenesis. The purpose of this study was to reveal the effects of co-exposure to DBP and BaP on testicular macrophage subset expression, IL-1β secretion and testosterone production. Adult male Sprague-Dawley rats were randomly divided into seven groups; two groups received DBP plus BaP (DBP+BaP: 50+1 or 250+5mg/kg/day) four groups received DBP or BaP alone (DBP: 50 or 250 mg/kg/day; BaP: 1 or 5mg/kg/day), and one group received vehicle alone (control). After co-exposure for 90 days, the relative expression of macrophage subsets and their functions changed. ED2(+) testicular macrophages (reactive with a differentiation-related antigen present on the resident macrophages) were activated and

IL-1β secretion was enhanced. DBP and BaP acted additively, as demonstrated by greater IL-1β secretion relative to each compound alone. These observations suggest that exposure to DBP plus BaP exerted greater suppression on testosterone production compared with each compound alone. The altered balance in the subsets of testicular macrophages and the enhanced ability of resident testicular macrophages to secrete IL-1β, resulted in enhanced production of IL-1β as a potent steroidogenesis repressor. This may represent an important mechanism by which DBP and BaP repress steroidogenesis.

Hesperidin attenuates benzo[alpha] pyrene-induced testicular toxicity in rats via regulation of oxidant/antioxidant balance.
Arafa H. M., Aly H. A., Abd-Ellah M. F. and El-Refaey H. M.
Toxicology and industrial health. 2009;25(6):417-27.

Benzo[alpha]pyrene (BaP) is one of the polycyclic aromatic hydrocarbons, which has shown carcinogenic, teratogenic, and mutagenic potentials. The reproductive toxicity of BaP in male was not well investigated. Thereby, we have addressed in the current study the testicular toxicity of BaP and the postulate whether or not the citrus flavonoid, hesperidin (HDN), could ameliorate such toxicity in male Swiss albino rats. In this sense, animals were challenged with BaP (50 mg/kg/day, orally) for 10 consecutive days. HDN (200 mg/kg/day, orally) was administered ahead of BaP challenge for 10 consecutive days. BaP induced testicular toxicity that was well characterized histologically and biochemically. It decreased the relative testis weight and induced pyknosis and necrobiotic changes as well as chromatolysis in the nuclei of the spermatocytes in the seminiferous tubules. It also markedly deteriorated epididymal function as shown by decreased sperm count, motility, and daily sperm production. The polyaromatic hydrocarbon also reduced the testicular activities of lactate dehydrogenase (LDH-X), superoxide dismutase (SOD), and glutathione-S-transferase (GST). Besides, it decreased the testicular reduced glutathione (GSH) but increased malondialdehyde (MDA) contents. Prior administration of HDN ahead of BaP challenge ameliorated all the histological and biochemical alterations induced by BaP. It improved the epididymal function and mitigated the injurious effects of BaP on the seminiferous tubules. In conclusion, HDN has proven protective effects in BaP-induced testicular toxicity paradigm, and this protection resides, at least in part, on its antioxidant properties.

Effects of benzo(a)pyrene on intra-testicular function in F-344 rats.
Archibong A. E., Ramesh A., Niaz M. S., Brooks C. M., Roberson S. I. and Lunstra D. D.
International journal of environmental research and public health. 2008;5(1):32-40.

The objective of this study was to evaluate the reproductive risk associated with exposure of adult male Fisher-344 (F-344) rats to inhaled benzo(a)pyrene (BaP), a ubiquitous environmental toxicant present in cigarette smoke, automobile exhaust fumes and industrial emissions. Rats were assigned randomly to a treatment or control

group. Treatment consisted of exposure of rats via nose-only inhalation to 75 microg BaP/m3, 4 hours daily for 60 days, while control animals were unexposed (UNC). Blood samples were collected immediately on day 60 of exposures (time 0) and subsequently at 24, 48, and 72 hours, to assess the effect of exposures to BaP on plasma testosterone and luteinizing hormone (LH) concentrations. Mean testis weight, total weight of tubules and total tubular length per paired testes were reduced 33% (P < 0.025), 27% (P with UNC rats. The number of homogenization -resistant spermatids was significantly reduced in BaP-exposed versus UNC rats. Plasma testosterone and intra-testicular testosterone (ITT) concentrations were significantly decreased by BaP compared with those of UNC rats. The decreases in circulating plasma testosterone were accompanied by concomitant increases in plasma LH concentrations in BaP-exposed versus control rats (P < 0.05). These data suggest that 60 days exposure to inhaled BaP contribute to reduced testicular endocrine and spermatogenic functions in exposed rats.

Alteration of fertility endpoints in adult male F-344 rats by subchronic exposure to inhaled benzo(a)pyrene.
Ramesh A., Inyang F., Lunstra D. D., Niaz M. S., Kopsombut P., Jones K. M., Hood D. B., Hills E. R. and Archibong A. E.
Experimental and toxicologic pathology. 2008;60(4-5):269-80.

The objective of this study was to evaluate the reproductive risk associated with exposure of adult male Fisher-344 rats to inhaled benzo(a)pyrene (BaP). Rats were assigned randomly to a treatment or control group. Treatment consisted of sub-chronic exposure of rats via inhalation to 75microgBaP/m^3, 4h daily for 60 days, while control animals were unexposed (UNC). Blood samples were collected immediately after the cessation of exposures (time 0) and subsequently at 24, 48, and 72h, to assess the effect of bioavailable BaP on plasma testosterone and luteinizing hormone (LH) concentrations. Rats were sacrificed after the last blood collection. Testes were harvested, weighed and prepared for histology and morphometric analysis, and cauda epididymides were isolated for the determination of progressive motility and density of stored spermatozoa. BaP exposure reduced testis weight compared with UNC (mean+/-SE; 2.01+/-0.11 versus 3.04+/-0.16g; P <0.025), and caused significant reductions in the components of the steroidogenic and spermatogenic compartments of the testis. Progressive motility and mean density of stored spermatozoa were reduced (P < 0.05). Plasma testosterone concentrations were decreased by two-thirds in BaP-exposed rats throughout the time periods studied compared with those of their UNC counterparts (P < 0.05), concomitant with increased concentrations of LH in BaP-exposed rats (P < 0.05). These data suggest that sub-chronic exposure to inhaled BaP contribute to reduced testicular and epididymal function in exposed rats.

Inhibition of meiotic divisions of rat spermatocytes in vitro by polycyclic aromatic hydrocarbons.
Georgellis A., Toppari J., Veromaa T., Rydström J. and Parvinen M.
Mutation research. 1990;231(2):125-35.

The toxic effects of polycyclic aromatic hydrocarbons (PAH) on spermatogenic cells undergoing meiotic division were investigated in vitro. Toxicity was assayed as alterations in cell nucleus morphology and cell survival and by DNA flow cytometry. Benzo[a]pyrene (BP) and 7,12-dimethylbenz[a]anthracene (DMBA) inhibited the progression of spermatocytes through meiotic division and were highly cytotoxic at concentrations higher than 1 microM. These results were obtained upon addition of a drug-metabolizing system, indicating that the seminiferous tubules lack the enzymes required for the initiation of PAH metabolism. The spindle poisons, e.g., vincristine and Colcemid, a group of direct-acting agents, affected spermatogenesis during meiotic division in a manner similar to that observed with PAH. In contrast, adriamycin did not inhibit meiotic division, although it did induce the formation of meiotic micronuclei as a result of chromosome breakage. It is concluded that low concentrations, i.e., 0.1 microM of PAH, strongly inhibit meiotic division, presumably after metabolic activation to reactive molecules functionally resembling direct-acting alkylating agents. High concentrations of PAH are cytotoxic.

B. Studies reporting no developmental or reproductive toxicity

There were no reports identified for this category.

II. Epidemiologic Developmental and Reproductive Toxicity Studies

A. Studies reporting increased risk of adverse developmental or reproductive outcomes

i. Developmental toxicity

Prenatal polycyclic aromatic hydrocarbon exposure leads to behavioral deficits and downregulation of receptor tyrosine kinase, MET.
Sheng L., Ding X., Ferguson M., McCallister M., Rhoades R., Maguire M., Ramesh A., Aschner M., Campbell D., Levitt P. and Hood D. B.
Toxicological sciences. 2010;118(2):625-34.

Gene by environment interactions (G × E) are thought to underlie neurodevelopmental disorder, etiology, neurodegenerative disorders, including the multiple forms of autism spectrum disorder. However, there is limited biological information, indicating an interaction between specific genes and environmental components. The present study focuses on a major component of airborne pollutants, polycyclic aromatic hydrocarbons

(PAHs), such as benzo(a)pyrene [B(a)P], which negatively impacts cognitive development in children who have been exposed in utero. In our study, prenatal exposure of Cpr(lox/lox) timed-pregnant dams to B(a)P (0, 150, 300, and 600 µg/kg body weight via oral gavage) on embryonic day (E14-E17) consistent with our susceptibility-exposure paradigm was combined with the analysis of a replicated autism risk gene, the receptor tyrosine kinase, Met. The results demonstrate a dose-dependent increase in B(a)P metabolite generation in B(a)P-exposed Cpr(lox/lox) offspring. Additionally, a sustained persistence of hydroxy metabolites during the onset of synapse formation was noted, corresponding to the peak of Met expression. Prenatal B(a)P exposure also downregulated Met RNA and protein levels and dysregulated normal temporal patterns of expression during synaptogenesis. Consistent with these data, transcriptional cell-based assays demonstrated that B(a)P exposure directly reduces human MET promoter activity. Furthermore, a functional readout of in utero B(a)P exposure showed a robust reduction in novel object discrimination in B(a)P-exposed Cpr(lox/lox) offspring. These results confirm the notion that common pollutants, such as the PAH B(a)P, can have a direct negative impact on the regulated developmental expression of an autism risk gene with associated negative behavioral learning and memory outcomes.

Exposure to polycyclic aromatic hydrocarbons and missed abortion in early pregnancy in a Chinese population.
Wu J., Hou H., Ritz B. and Chen Y.
The Science of the total environment. 2010;408(11):2312-8.

BACKGROUND: Polycyclic aromatic hydrocarbons (PAHs) are formed during incomplete burning of fossil fuels, wood, and tobacco products. High PAH exposure has been associated with low birth weight, intrauterine growth restriction, and preterm birth, but little is known about its impact on adverse outcomes in early pregnancy such as in-utero fetal death. OBJECTIVES: To examine associations between exposure to PAHs and missed abortion in which the embryo has died but a miscarriage has not yet occurred during early pregnancy in a Chinese population in Tianjin. METHODS: A case-control study was conducted from April to November, 2007 in Tianjin, China. Cases experienced a missed abortion while controls underwent elective abortions before 14weeks of pregnancy. Eighty-one cases were recruited from four hospitals, with the same number of controls matched on hospital, maternal age (+/-8years), gravidity (1 or > 1), and gestational age (+/-30days). Two maternal measures of PAH exposures were obtained based on benzo[a]pyrene (BaP) DNA adducts in 1) aborted tissues and 2) maternal blood (for a subset of subjects). In addition, proxy measures for PAH exposures from different sources were derived from maternal interviews. RESULTS: In conditional logistic regression analyses, we estimated more than 4-fold increase in risk of having experienced a missed abortion in women with above the median levels of blood BaP-DNA adducts (adjusted OR=4.27; 95% CI, 1.41-12.99); but no increase with adduct levels in aborted tissues (adjusted OR=0.76; 95% CI, 0.37-1.54). BaP-DNA adduct levels in maternal blood and aborted tissues were poorly correlated (r=-0.12; n=102). Missed abortion risk also was higher among women reporting traffic congestion

near the residence, commuting by walking, and performing regular cooking activities during pregnancy. CONCLUSION: High levels of maternal PAH exposures may contribute to an increased risk of experiencing a missed abortion during early pregnancy.

Benefits of reducing prenatal exposure to coal-burning pollutants to children's neurodevelopment in China.
Perera F., Li T. Y., Zhou Z. J., Yuan T., Chen Y. H., Qu L., Rauh V. A., Zhang Y. and Tang D.
Environmental health perspectives. 2008;116(10):1396-400.

BACKGROUND: Coal burning provides 70% of the energy for China's industry and power, but releases large quantities of polycyclic aromatic hydrocarbons (PAHs) and other pollutants. PAHs are reproductive and developmental toxicants, mutagens, and carcinogens. OBJECTIVE: We evaluated the benefit to neurobehavioral development from the closure of a coal-fired power plant that was the major local source of ambient PAHs. METHODS: The research was conducted in Tongliang, Chongqing, China, where a coal-fired power plant operated seasonally before it was shut down in May 2004. Two identical prospective cohort studies enrolled nonsmoking women and their newborns in 2002 (before shutdown) and 2005 (after shutdown). Prenatal PAH exposure was measured by PAH-DNA adducts (benzo[a]pyrene-DNA) in umbilical cord blood. Child development was assessed by the Gesell Developmental Schedules at 2 years of age. Prenatal exposure to other neurotoxicants and potential confounders (including lead, mercury, and environmental tobacco smoke) was measured. We compared the cohorts regarding the association between PAH-DNA adduct levels and neurodevelopmental outcomes. RESULTS: Significant associations previously seen in 2002 between elevated adducts and decreased motor area developmental quotient (DQ) ($p = 0.043$) and average DQ ($p = 0.047$) were not observed in the 2005 cohort ($p = 0.546$ and $p = 0.146$). However, the direction of the relationship did not change. CONCLUSION: The findings indicate that neurobehavioral development in Tongliang children benefited by elimination of PAH exposure from the coal-burning plant, consistent with the significant reduction in PAH-DNA adducts in cord blood of children in the 2005 cohort. The results have implications for children's environmental health in China and elsewhere.

PAH-DNA adducts in cord blood and fetal and child development in a Chinese cohort.
Tang D., Li T. Y., Liu J. J., Chen Y. H., Qu L. and Perera F.
Environmental health perspectives. 2006;114(8):1297-300.

Polycyclic aromatic hydrocarbons (PAHs) are an important class of toxic pollutants released by fossil fuel combustion. Other pollutants include metals and particulate matter. PAH-DNA adducts, or benzo[a]pyrene (BaP) adducts as their proxy, provide a chemical-specific measure of individual biologically effective doses that have been associated with increased risk of cancer and adverse birth outcomes. In the present

study we examined the relationship between prenatal PAH exposure and fetal and child growth and development in Tongliang, China, where a seasonally operated coal-fired power plant was the major pollution source. In a cohort of 150 nonsmoking women and their newborns enrolled between 4 March 2002 and 19 June 2002, BaP-DNA adducts were measured in maternal and umbilical cord blood obtained at delivery. The number of gestational months occurring during the period of power plant operation provided a second, more general measure of exposure to plant emissions, in terms of duration. High PAH-DNA adduct levels (above the median of detectable adduct level) were associated with decreased birth head circumference ($p=0.057$) and reduced children's weight at 18 months, 24 months, and 30 months of age ($p < 0.05$), after controlling for potential confounders. In addition, in separate models, longer duration of prenatal exposure was associated with reduced birth length ($p=0.033$) and reduced children's height at 18 ($p=0.001$), 24 ($p < 0.001$), and 30 months of age ($p < 0.001$). The findings suggest that exposure to elevated levels of PAHs, with the Tongliang power plant being a significant source, is associated with reduced fetal and child growth in this population.

Molecular evidence of an interaction between prenatal environmental exposures and birth outcomes in a multiethnic population.
Perera F. P., Rauh V., Whyatt R. M., Tsai W. Y., Bernert J. T., Tu Y. H., Andrews H., Ramirez J., Qu L. and Tang D.
Environmental health perspectives. 2004;112(5):626-30.

Inner-city, minority populations are high-risk groups for adverse birth outcomes and also are more likely to be exposed to environmental contaminants, including environmental tobacco smoke (ETS), benzo[a]pyrene (BaP), and other polycyclic aromatic hydrocarbons (PAHs) found in urban air. In a sample of nonsmoking African-American and Dominican women, we evaluated the effects on birth outcomes of prenatal exposure to ETS, using questionnaire data and plasma cotinine as a biomarker of exposure, and environmental PAHs using BaP-DNA adducts as a molecular dosimeter. We previously reported that among African Americans, high prenatal exposure to PAHs estimated by prenatal personal air monitoring was associated with lower birth weight ($p = 0.003$) and smaller head circumference ($p = 0.01$) after adjusting for potential confounders. In the present analysis, self-reported ETS was associated with decreased head circumference ($p = 0.04$). BaP-DNA adducts were not correlated with ETS or dietary PAHs. There was no main effect of BaP-DNA adducts on birth outcomes. However, there was a significant interaction between the two pollutants such that the combined exposure to high ETS and high adducts had a significant multiplicative effect on birth weight ($p = 0.04$) and head circumference ($p = 0.01$) after adjusting for ethnicity, sex of newborns, maternal body mass index, dietary PAHs, and gestational age. This study provides evidence that combined exposure to environmental pollutants at levels currently encountered in New York City adversely affects fetal development.

ii. Female reproductive toxicity

Exposure to polycyclic aromatic hydrocarbons and missed abortion in early pregnancy in a Chinese population.
Wu J., Hou H., Ritz B. and Chen Y.
The Science of the total environment. 2010;408(11):2312-8.

BACKGROUND: Polycyclic aromatic hydrocarbons (PAHs) are formed during incomplete burning of fossil fuels, wood, and tobacco products. High PAH exposure has been associated with low birth weight, intrauterine growth restriction, and preterm birth, but little is known about its impact on adverse outcomes in early pregnancy such as in-utero fetal death. OBJECTIVES: To examine associations between exposure to PAHs and missed abortion in which the embryo has died but a miscarriage has not yet occurred during early pregnancy in a Chinese population in Tianjin. METHODS: A case-control study was conducted from April to November, 2007 in Tianjin, China. Cases experienced a missed abortion while controls underwent elective abortions before 14weeks of pregnancy. Eighty-one cases were recruited from four hospitals, with the same number of controls matched on hospital, maternal age (+/-8years), gravidity (1 or > 1), and gestational age (+/-30days). Two maternal measures of PAH exposures were obtained based on benzo[a]pyrene (BaP) DNA adducts in 1) aborted tissues and 2) maternal blood (for a subset of subjects). In addition, proxy measures for PAH exposures from different sources were derived from maternal interviews. RESULTS: In conditional logistic regression analyses, we estimated more than 4-fold increase in risk of having experienced a missed abortion in women with above the median levels of blood BaP-DNA adducts (adjusted OR=4.27; 95% CI, 1.41-12.99); but no increase with adduct levels in aborted tissues (adjusted OR=0.76; 95% CI, 0.37-1.54). BaP-DNA adduct levels in maternal blood and aborted tissues were poorly correlated (r=-0.12; n=102). Missed abortion risk also was higher among women reporting traffic congestion near the residence, commuting by walking, and performing regular cooking activities during pregnancy. CONCLUSION: High levels of maternal PAH exposures may contribute to an increased risk of experiencing a missed abortion during early pregnancy.

Quantification of benzo[a]pyrene and other PAHs in the serum and follicular fluid of smokers versus non-smokers.
Neal M. S., Zhu J. and Foster W. G.
Reproductive toxicology. 2008;25(1):100-6.

Cigarette smoking is a well-established reproductive hazard that has been linked with decreased fertility in both smokers and those exposed to second hand smoke. The chemical components responsible for the reproductive toxic effects of cigarette smoke are unknown. Moreover, exposure of reproductive tissues to the chemical constituents of cigarette smoke is largely unknown. Therefore, we measured the levels of benzo[a]pyrene (B[a]P), and other polycyclic aromatic hydrocarbons (PAH) present in

cigarette smoke, in the serum and follicular fluid of women exposed to mainstream (n=19) and side stream smoke (n=7) compared to non-smokers (n=10). Women exposed to mainstream smoke had significantly higher levels of B[a]P (1.32+/-0.68ng/ml) in their follicular fluid compared to side stream exposed (0.05+/-0.01ng/ml) or their non-smoking (0.03+/-0.01ng/ml) counterparts. More importantly we found significantly higher ($p < 0.001$) levels of B[a]P in the follicular fluid of women who did not conceive (1.79+/-0.03ng/ml) compared to those that achieved a pregnancy (0.08+/-0.03ng/ml). Other PAHs known to be present in cigarette smoke were also detectable in both serum and follicular fluid of study subjects studied but with lower frequency compared to B[a]P and no differences in serum or follicular fluid levels between the groups could be demonstrated. The important finding that B[a]P reaches the follicular fluid and the fact that it is found at much higher levels in women who smoke provides further evidence that of the many toxicants present in cigarette smoke, B[a]P may be a key compound that is central to the documented adverse effects of cigarette smoke on follicular development and subsequent fertility.

iii. Male reproductive toxicity

In vitro evaluation of baseline and induced DNA damage in human sperm exposed to benzo[a]pyrene or its metabolite benzo[a]pyrene-7,8-diol-9,10-epoxide, using the comet assay.
Sipinen V., Laubenthal J., Baumgartner A., Cemeli E., Linschooten J. O., Godschalk R. W., Van S. F. J., Anderson D. and Brunborg G.
Mutagenesis. 2010;25(4):417-25.

Exposure to genotoxins may compromise DNA integrity in male reproductive cells, putting future progeny at risk for developmental defects and diseases. To study the usefulness of sperm DNA damage as a biomarker for genotoxic exposure, we have investigated cellular and molecular changes induced by benzo[a]pyrene (B[a]P) in human sperm in vitro, and results have been compared for smokers and non-smokers. Sperm DNA obtained from five smokers was indeed more fragmented than sperm of six non-smokers (mean % Tail DNA 26.5 and 48.8, respectively), as assessed by the alkaline comet assay ($P < 0.05$). B[a]P-related DNA adducts were detected at increased levels in smokers as determined by immunostaining. Direct exposure of mature sperm cells to B[a]P (10 or 25 microM) caused moderate increases in DNA fragmentation which was independent of addition of human liver S9 mix for enzymatic activation of B[a]P, suggesting some unknown metabolism of B[a]P in ejaculates. In vitro exposure of samples to various doses of B[a]P (with or without S9) did not reveal any significant differences in sensitivity to DNA fragmentation between smokers and non-smokers. Incubations with the proximate metabolite benzo[a]pyrene-r-7,t-8-dihydrodiol-t9,10-epoxide (BPDE) produced DNA fragmentation in a dose-dependent manner (20 or 50 microM), but only when formamidopyrimidine DNA glycosylase treatment was included in the comet assay. These levels of DNA fragmentation were, however, low in relation to very high amounts of BPDE-DNA adducts as measured with (32)P postlabelling. We

conclude that sperm DNA damage may be useful as a biomarker of direct exposure of sperm using the comet assay adapted to sperm, and as such the method may be applicable to cohort studies. Although the sensitivity is relatively low, DNA damage induced in earlier stages of spermatogenesis may be detected with higher efficiencies.

The in vitro effect of benzo[a]pyrene on human sperm hyperactivation and acrosome reaction.
Mukhopadhyay D., Nandi P., Varghese A. C., Gutgutia R., Banerjee S. and Bhattacharyya A. K.
Fertility and sterility. 2009;94(2):595-8.

OBJECTIVE: To evaluate the in vitro effect of benzo[a]pyrene on sperm hyperactivation and acrosome status in normozoospermic semen samples of nonsmokers analyzed by computer-assisted semen analysis (CASA). DESIGN: Experimental in vitro study. SETTING: Andrology laboratory. PATIENT(S): Thirteen proven fertile, normozoospermic, and nonsmoking men. INTERVENTION(S): Spermatozoa were washed free of seminal plasma and were treated with different concentrations of benzo[a]pyrene and compared with controls treated with medium alone. The benzo[a]pyrene concentrations were: 100, 50, 25, and 12.5 microg/mL. MAIN OUTCOME MEASURE(S): Effect of varying concentrations of benzo[a]pyrene on sperm hyperactivation and acrosomal reaction. RESULT(S): A statistically significant increase in sperm hyperactivation was observed in presence of benzo[a]pyrene at concentrations of > or=50 microg/mL. The result of the acrosome halo test showed that concentrations of benzo[a]pyrene > or=25 microg/mL statistically significantly decreased the percentage of halo formation, indicating an inappropriate (false) acrosome reaction. CONCLUSION(S): Benzo[a]pyrene statistically significantly affected sperm functional competence as evidenced by increased hyperactivation as well as premature acrosomal reaction.

B. Studies reporting no increased risk of adverse developmental or reproductive outcomes

There were no reports identified for this category.

III. Other Relevant Information

A. Meeting Abstracts

A mutant allele of the aryl hydrocarbon receptor protects the developing kidney from hydrocarbon-induced deficits in cellular differentiation.
Nanez A. and Ramos K. S. Birth Defects Res A Clin Mol Teratol. 2007;79(5):372.

Disruption of ovarian function by subchronic inhaled benzo(a)pyrene.
Archibong A. and Ramesh A. Biol Reprod. 2005:141.

Chronic administration of the environmental pollutant benzo[a]pyrene disrupts the reproductive system in adult male rats.
Raychoudhury S. S. and Blake C. A. Biol Reprod. 2005:135.

Effects of polycyclic aromatic hydrocarbons on murine embryonic weight and placental morphology.
Detmar J., Taniuchi Y., Shang X., Casper R. and Jurisicova A. J Soc Gynecol Investig. 2004;11(2 Suppl).

DNA repair in testicular cells-consequences of exposure to environmental genotoxicants.
Lindeman B., Brunborg G. and Olsen A. K. Toxicol Appl Pharmacol. 2004;197(3):173-4.

Perturbation of testicular and epididymal function of adult f-344 rats by subchronic inhaled benzo(a)pyrene exposure.
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Metabolism of inhaled benzo(a)pyrene in the developing central nervous system and partial ablation of long-term potentiation in F1 generation animals.
Hood D. B., Wu J., Ramesh A., Wormley D., Nayyar T. and Greenwood M. Toxicologist. 2003;72(S-1):125.

Prenatal exposure to dioxin and inhaled benzo(a)pyrene: reduced capacity for long-term potentiation in the F1 generation.
Wormley D., Chirwa S., Zhang W., Nayyar T., Greenwood M., Ebner F. F. and Hood D. B. Toxicologist. 2003;72(S-1):125.

Benzo(a)pyrene and dioxin induced down-regulation of hippocampal NMDAR1 expression and deficits in fixed-ratio performance in F1 generation rats.
Wu J., Nayyar T., Tu T., Johnson S., Greenwood M. and Hood D. B. Toxicologist. 2003;72(S-1):125.

Midgestational, subacute, mixed TCDD/B(a)P exposure: effects on F1 generation long-term potentiation and temporal expression of developmental CNS neonatal markers I.
Hood D. B., Wu J., Nayyar T., Wormley D., Greenwood M. and Chirwa S. Toxicologist. 2002;66(1-S):335.

Role of benzo(a)pyrene (BaP) metabolism in luteotropic activity and fetal survival of timed-pregnant rats exposed to BaP : carbon black aerosol.
Ramesh A., Inyang F., Archibong A. E., Greenwood M., Nayyar T., Hood D. B., Kopsombut P. and Nyanda A. M. Toxicologist. 2002;66(1-S):369.

Effect of benzo(a)pyrene on placentation.
Tomikawa J., Yan J., Ohgane J., Hattori N., Makino T., Tanaka S. and Shiota K. Biol Reprod. 2002;66(Suppl 1):199.

Midgestational, subacute, mixed TCDD/B(a)P exposure: effects on F1 generation pups in radial arm maze performance and temporal expression of developmental CNS neonatal markers II.
Zhang W., Nayyar T., Wu J., Johnson S., Greenwood M. and Hood D. B. Toxicologist. 2002;66(1-S):335.

Reduction in luteotropic activity and embryonic survival in pregnant rats by inhaled benzo(a)pyrene.
Archibong A. E., Inyang F. E., Ramesh A., Hood D. B., Greenwood M., Nunes M. G., Nayyar T., Kopsumbut P. and Nyanda A. M. Toxicologist. 2001;60(1):274.

Subacute exposure of timed-pregnant rats to a benzo(a)pyrene : carbon black aerosol; effects on development in the F1 generation-I.
Hood D. B., Nayyar T., Greenwood M. M., Aramandla R., Nunes M., Inyang F., Nyanda A. and Archibong A. Toxicologist. 2001;60(1):275.

Neurobiological effects of mid-gestational transplacental exposures to dioxin and a benzo(a)pyrene: carbon black aerosol.
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Hood D. B., Greenwood M. M., Nayyar T., Ramesh A., Inyang F. and Knuckles M. E. Toxicologist. 1999;48(1-S):10.

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Wells P. G., Nicol C. J. and Wiley M. J. Toxicologist. 1999;48(1-S):18-9.

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Parman T., Kim D. and Wells P. G. Toxicologist. 1998;42(1-S):121.

Deleterious influence of maternal polynuclear aromatic hydrocarbon treatment on developing blood vascular system of fetuses in the rat.
Li Y. L., Biggers W. J. and Sanyal M. K. Teratology. 1997;55(1):43.

Effects of benzo(a)pyrene on preimplantation mouse embryos in vitro.
Park K. L., Kim J. I., Kim P. G., Lee Y. M., Shin J. H., Kang T. S., Kang T. H., Park K. S. and Jang S. J. Teratology. 1995;52(4).

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Schalue T. K. and Kipersztok S. Biol Reprod. 1995;52(Suppl 1):172.

Benzo(a)pyrene (BaP) adversely affects conception and pregnancy outcome in mice.
Schalue T. K. and Kipersztok S. American Society for Reproductive Medicine. 1995;51(Suppl).

Altered immune development following gestational exposure to environmental pollutants.
Holladay S. D. Neurotoxicol Teratol. 1994;16(3):330.

Immunomodulation in progeny from thymectomized primiparous mice exposed to benzo(a)pyrene during mid-pregnancy.
Wolisi G., Majekodunmi J. and Urso P. Faseb J. 1994;8(4).

The effect of intratracheal instillation of benzo(a)pyrene during pregnancy.
Thomas K. L., Cannon V., Cobb J. R. and Urso P. Faseb J. 1991;5(6).

B. Related articles

Benzo(a)pyrene causes PRKAA1/2-dependent ID2 loss in trophoblast stem cells.
Xie Y., Abdallah M. E., Awonuga A. O., Slater J. A., Puscheck E. E. and Rappolee D. A. Molecular reproduction and development. 2010;77(6):533-9.

Placental transfer and DNA binding of benzo(a)pyrene in human placental perfusion.
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Slotkin T. A. and Seidler F. J. Brain research bulletin. 2009;80(1-2):17-21.

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Bouayed J., Desor F., Rammal H., Kiemer A. K., Tybl E., Schroeder H., Rychen G. and Soulimani R. Toxicology. 2009;259(3):97-106.

Urinary 1-hydroxypyrene, air pollution exposure and associated life style factors in pregnant women.
Llop S., Ballester F., Estarlich M., Ibarluzea J., Manrique A., Rebagliato M., Esplugues A.,Iñiguez C.. The Science of the total environment. 2008;407(1):97-104.

Estrogenic activity of environmental polycyclic aromatic hydrocarbons in uterus of immature Wistar rats.
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Effects of prenatal exposure to coal-burning pollutants on children's development in China.
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Benzo(a)pyrene inhibits growth and functional differentiation of mouse bone marrow-derived dendritic cells. Downregulation of RelB and eIF3 p170 by benzo(a)pyrene.
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Follicle growth is inhibited by benzo-[a]-pyrene, at concentrations representative of human exposure, in an isolated rat follicle culture assay.
Neal M. S., Zhu J., Holloway A. C. and Foster W. G. Human reproduction (Oxford, England). 2007;22(4):961-7.

Species-specific testicular and hepatic microsomal metabolism of benzo(a)pyrene, an ubiquitous toxicant and endocrine disruptor.
Smith T. L., Merry S. T., Harris D. L., Joe F. J., Ike J., Archibong A. E. and Ramesh A. Toxicology in vitro :. 2007;21(4):753-8.

Relationship between polycyclic aromatic hydrocarbon-DNA adducts, environmental tobacco smoke, and child development in the World Trade Center cohort.
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