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Testimony Regarding "Coal Combustion Waste Storage and Water Quality before the Subcommittee on Water Resources, Committee on Transportation and Infrastructure, U.S. House of Representatives

April 30, 2009
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My name is Dr. Conrad D. Volz, since 2004 I have been an Assistant Professor in the Department of Environmental and Occupational Health (EOH) at the Graduate School of Public Health (GSPH), University of Pittsburgh (UP) where I am also the Director of the Center for Healthy Environments and Communities (CHEC) and of the Environmental Health Risk Assessment Certificate Program. I also hold an appointment to the University of Pittsburgh, School of Law where I give technical and human and ecological toxicological guidance to the Environmental Law Clinic. I have over 30 years of experience in performing environmental health and human and aquatic risk assessment studies; working under contract or grants from the US Department of Defense and the Department of Energy, National Nuclear Security Agency (NNSA), in the USA and 24 countries, on 4 continents (See Attachment 1, Biography of Conrad Daniel Volz, DrPH, MPH and Attachment 2, CV of Conrad Daniel Volz, DrPH, MPH).

My current research is focused on using fish and other aquatic receptors as indicators of; industrial and municipal pollution sources; and as sentinels for human health effects from exposure to aquatic contaminants. This work is supported by the Centers for Disease Control and Prevention's (CDC), Environmental Public Health Tracking Network (EPHTN). At the GSPH I teach two (2) seminal courses related to; 1.) Transport processes of CCW and other toxic contaminants entry into surface water and groundwater and how these contaminants are cycled through other environmental media (air, soils and substrata, biota, sediments and foods); and 2.) The assessment and quantification of human and aquatic receptor exposure to environmental contaminants contained in these environmental media.

My testimony today before this Subcommittee on Water Resources and Environment concerns known and theoretical water quality impacts of coal combustion waste (CCW) storage including evidence; that CCW mixtures have direct ecotoxicological effects on aquatic animals and that these animals, once exposed to CCW can spread toxic trace elements to nearby uncontaminated terrestrial and aquatic environments; that trace toxic elements from CCW impoundments enter groundwater, especially during periods of low rainfall or draught, contaminating local drinking water wells, with a high probability of reentering surface water through freshwater seeps, springs, and movement of contaminated groundwater into surface water; that a toxic waste site with characteristics similar to unlined CCW impoundments, assessed by my group in June of 2008, is releasing significant levels of arsenic, lead and other metals and metalloids into groundwater and surface water and that this process is occurring under conditions of high alkalinity; that the predominant location of flyash piles and CCW surface impoundments near surface water-drinking water sources creates an unreasonable threat to public health and the environment because of rain water runoff and the demonstrated potential for catastrophic release of CCW into major river systems; and that placement of flyash piles and CCW impoundments constitutes a major environmental justice issue, in that these communities are generally located in areas with associated and other polluting sources, which are characterized by low socio-economic attainment, flight of residents that can afford to relocate, resulting in further erosion of municipal and school tax bases. Lastly, I will outline steps necessary to regulate and mitigate fly ash impoundments and storage facilities to protect human health and the environment.

Coal combustion waste (CCW) is a leachable mixture of carbon, sulfur compounds, nitrates/nitrites, toxic trace elements, radionuclides, and mutagenic polynuclear aromatic hydrocarbons. In 2005 coal-fired power plants (CFPP) produced 71.1 million tons of flyash, filling 44 million cubic yards of landfill space, in the forms of fly ash, bottom ash, boiler slag, and Flue Gas Desulfurization sludge. Studies show that masses of elements left in fly ash are much higher than in coal; Arsenic can have up to100 ppm in coal but 1,700 ppm in fly ash; Cadmium in coal isn't over .6 ppm but can reach 250 ppm in flyash; and alarmingly Manganese levels don't exceed 15.0 ppm in coal but can be as high as 4,400 ppm in fly ash. The Law of Conservation of Mass states that "matter cannot be created nor can it be destroyed." The burning of vast amounts of coal opens Pandora's Box and releases almost every element in the periodic table into the environment. This law also tells us that elements that don't go up the stack or into wastewater-stay in the fly ash. As air pollution control devices and wastewater treatment plant efficiency increases the amount of toxic elements in CCW. Overproduction of CCW has strained the holding capacity of many impoundment sites causing ecological and public health disasters, such as the recent Tennessee Valley Authority (TVA) spill, and the little known 2005 Forward

Township legacy flyash landslide. The staggering amounts of CCW sitting next to major source water bodies, dumped into landfills and pumped into impoundments creates a significant threat to environmental resources and a potential health hazard for communities, especially rural communities already impacted by coal mining and those already impacted by coal burning air pollution sources..

I. COAL COMBUSTION WASTE MIXTURES HAVE DIRECT ECOTOXICOLOGICAL EFFECTS ON AQUATIC ANIMALS AND THESE ANIMALS, ONCE EXPOSED TO CCW CAN SPREAD TOXIC TRACE ELEMENTS TO NEARBY UNCONTAMINATED TERRESTRIAL AND AQUATIC ENVIRONMENTS

Table 1, Studies Indicating Coal Combustion Waste (CCW) Effects on Animal Survival, Reproduction and Growth and Development (with an emphasis on aquatic organisms) lists 16 studies from the peer-reviewed academic literature that demonstrate that CCW has direct effects on aquatic animals and animals that spend part of their life-cycle in aquatic environments and species that feed on them. CCW effects in the southern toad (*Bufo terrestris*) have been extensively studied. CCW ash-exposed toads exhibited elevated levels of 11 of 18 metals measured. Increases ranged from 47.5% for lead to more than 5000% for arsenic (Ward et al., 2009). Toads exposed to CCW trace metal contamination gained significantly less mass (18.3 %) than control toads (31.3%) when food was limited and experienced significantly decreased Respiratory Quotient (RQ) after exercise (Ward et al., 2006). This study suggests that CCW trace metal exposure is associated with changes in the basal metabolic rate of these vertebrates and that decreased RQ after exercise suggests an inability to eliminate carbon dioxide and/or absorb oxygen due to trace metal exposure. Many of the metals in CCW are pulmonary toxicants even when the mode of exposure is through ingestion or skin absorption, just as in humans (Yoshida et al., 2004). In a study that assessed concentrations of As, Cd, Cu, Ni, Pb, Se, Sr, and Zn in whole bodies of larval, recently metamorphosed, and adult life stages in Bufo terrestris, after exposure to CCW, it was found that the elements As, Cd, Cu, Ni, Pb, Zn, concentrations were highest in larvae, but that Se and Sr concentrations remained elevated in later life stages (Roe et al, 2005). This study demonstrates that toads and frogs exposed to metals in CCW can transport trace elements from aquatic disposal basins to nearby uncontaminated terrestrial and aquatic habitats and additionally that, anurans utilizing naturally revegetated sites up to 30 years after CCW disposal ceases are exposed to elevated trace elements. A 1999 study of toads showed that initial circulating levels of corticosterone in toads captured at the CCW area were significantly higher than levels in toads from the reference site. Corticosterone levels in toads from the CCW site remained high even after 2 weeks of laboratory acclimation and injection with saline (Hopkins et al., 1999). This study demonstrates that CCW constituents display endocrine system disrupting effects that may be mediated through disruption of hepatic enzymes responsible for the metabolic clearance of steroid hormones.

Other studies using a fish- Lake chubsuckers, again fellow vertebrates with hormonal systems much the same as humans, found that chubsuckers grazing CCW sediments had significantly elevated body burdens of Se, Sr, and V. Selenium levels were particularly elevated, reaching mean whole body concentrations of 5.6 micrograms/gram dry mass by the end of experimental manipulations. Twenty-five percent of fish exposed to pollutants died during the study. All surviving fish exposed to ash exhibited substantial decreases in growth and severe fin erosion

(Hopkins et al., 2000). This study indicates that fish exposed to ash utilized more energy for daily activities and/or were less efficient at converting available energy to tissues for growth and storage.

In a study of the bird-common grackle feeding in CCW basins, selenium was found in significantly higher concentrations in ash basin eggs (x = 5.88 +/- 0.44 microg/g DW) than in reference eggs (x = 2.69 +/- 0.13 microg/g DW). Selenium concentrations in eggs from the ash basins were above background levels (Bryan et al., 2003). This study shows maternal transfer of selenium to eggs in birds living near CCW settling ponds. Se was also found to be transferred maternally to turtle hatchlings at relatively high levels after exposure to CCW. Hatchlings from polluted-site females exhibited reduced O2 consumption rates compared to hatchlings from reference sites. Since Se was transferred to hatchlings at high levels it may be responsible for the observed physiological impairments.

There are several concepts concerning the ecotoxicology of Se that must be stated.

Selenium contamination represents one of the few clear cases where environmental pollution has led to devastation of wildlife populations, most notably in agricultural drainage evaporation and power plant coal-fly ash receiving ponds (Fan et al, 2002). Elevated concentrations have degraded many freshwater ecosystems throughout the United States, and additional systems are expected to be affected as anthropogenic activities, including runoff and leaching of selenium from CCW deposits and impoundments, increasingly mobilize Se into aquatic systems. Se is a very toxic essential trace element. Toxic threshold concentrations in water, dietary items, and tissues, for aquatic organisms are only 2-5 times normal background concentrations. Selenium toxicity in freshwater ecosystems is the result of a complex series of bioaccumulation and biotransformation mechanisms, and cycling of Se in aquatic food chains (Maier and Knight, 1994). Organic selenium bioaccumulation and toxicity patterns in the freshwater bivalve sentinel species *Corbicula fluminea* have recently been demonstrated. Waterborne selenomethionine (SeMet) exposure was used to mimic dietary organo-Se uptake. Results of this study demonstrate that SeMet is accumulated to a relatively high extent with a concentration factor of 770 (wet weight basis). The higher uptake than depuration rates suggest that bivalves deal with high Se amounts using a strategy of detoxification based on Se sequestration that could involve granules, as shown by a strong increase of Se in the particulate subcellular fraction. Selenium is persistent in the cytosol of bivalves exposed to SeMet where it is found in proteins of a wide range of molecular mass, indicating a possible replacement of methionine by selenomethionine. A subsequent alteration of protein function might be one of the mechanisms of Se toxicity that could explain the histopathological damage observed in gills by using transmission electronic microscopy. Those analyses showed changes in gill filament ultrastructure and suggested mitochondria as the first target for SeMet cytotoxicity, with alterations of the outer membrane and of cristae morphology. Organo-Se would thus not only be toxic via indirect mechanisms of maternal transfer as it is suggested for fish and turtles but also directly (Adam-Guillermin et al., 2009).

Table 1 describes CCW effects on shrimp, salamanders, water snakes, green frogs and leopard frogs. Many of these effects are related to perceived problems in hormonal regulatory processes. Larval leopard frogs exposed to CCW have high corticosterone levels that may be associated with jaw abnormalities and decreased survival rates. And the high mortality of green frog larvae exposed to CCW with raised concentrations of As, Se, Sr, and V occurred when control larvae were entering metamorphosis.

II. TRACE TOXIC ELEMENTS FROM CCW IMPOUNDMENTS ENTER GROUNDWATER, ESPECIALLY DURING PERIODS OF LOW RAINFALL OR DRAUGHT, CONTAMINATING LOCAL DRINKING WATER WELLS, WITH A HIGH PROBABILITY OF REENTERING SURFACE WATER THROUGH FRESHWATER SEEPS, SPRINGS, AND MOVEMENT OF CONTAMINATED GROUNDWATER INTO SURFACE WATER

Waste products from coal combustion have the highest potential human risk among the fossil fuel alternatives, even higher than wastes from the nuclear energy process. The highest risk is caused by metals, and the fly ash represents the effluent stream giving the largest contribution to the potential human health risk from trace metal exposure (Christensen et al, 1992). It has been observed that as much as 8% (approximately 10 microg g(-1) in fly ash) of total chromium is converted to the Cr(VI) species during oxidative combustion of coal and remains in the resulting ash as a stable species, however, it is significantly mobile in water based leaching (Kingston et al, 2005). Approximately 1.23 +/- 0.01 microg g(-1) of Cr(VI) was found in the landfill leachate from permanent deposits of aged fly ash. Thus Cr (VI), a known human carcinogen can enter groundwater and can runoff of CCW sites in tributary streams. Additionally it has been observed that fly ash and sludge mixing and transport to waste lagoons releases significant portions of zinc, nickel and chromium and that arsenic and manganese are released continuously during this transport process. Adsorbed portions of calcium, magnesium and potassium are also leached during coal ash transport (Popovic et al., 2001). These elements are then available to interact with unconfined aquifer water that is in hydrological connection with water in lagoon basins and can contaminate local well water and runoff through groundwater seeps and overflows to surface water. There is no known safe level of exposure to Cr (VI) any increase in its concentration in water carries with it an increased risk of the development of cancer.

A laboratory leaching test was employed to predict the potential mobility of As, and Se in landfilled fly ash produced by coal combustion. These waste residues also formed the basis of a speciation study in which the valency states of As and Se were determined. Selenium displayed the greatest leachability in CCW, despite being present at relatively low concentrations in CCW. A substantial amount As was also leached from coal ash. Water-soluble extracts of coal fly ash contained As exclusively as As(V). Selenium was present largely as Se(IV) in aqueous extracts of fly ash (Wadge et al., 1987). This is direct evidence that both As and Se are present in the water soluble fractions of CCW lagoons and can enter groundwater as well as surface waters. Distance of CCW particles from their injection points has also been shown to affect the metal characteristics of CCW impoundments. It has been found that the presence of fine particles (< 50 microns) increased with increasing distance from the ash slurry inlet zone an the ash pond. Wide variations in the bulk density (800-980 kg m(-3)), porosity (45-57%) and water-holding capacity (57.5-75.7%) of CCRs were recorded. With increasing distance the pH of the CCRs decreased (from 9.0 to 8.2) and electrical conductivity increased (from 0.25 to 0.65 dS m(-3)). The presence of almost all the heavy metals in CCRs exhibited an increase with distance from the ash slurry discharge zone due to the increase in surface area (from 0.1038 to 2.3076 m2 g(-1)) of CCRs particles (Askosan et al., 2004). These results suggest that CCW impoundments do not have monolithic physical-chemical properties and that the further away from the slurry inlet that CCW moves the greater its ability to become water soluble and move into groundwater and

surface waters. The increase in conductance indicates that species of chemicals are going into solution as they move from injection sites.
Finally, the coal fired power plant institutional control technique of purchasing residential and institutional properties as the levels of CCW raise in impoundments and surface water backs up into hollows indicates that environmental control personnel are aware of the intimate connection between standing water in settling CCW ponds and its connection to contamination of unconfined aquifers. At Little Blue CCW impoundment in Shippingsport PA the responsible company has purchased many properties bordering the impoundment and capped their wells (Site Survey, 2009). My group is in possession of 2 reports, one from the PA DEP of well water from a property in hydrogeological connection with this CCW lagoon that has arsenic levels above the drinking water standard for arsenic of 10 ppb. We would not expect such high background levels in groundwater in this area because it was not glaciated in the last ice age nor are their granite or other rock formations present that might leach arsenic into groundwater.

III. ASSESSMENTS OF A TOXIC WASTE SITE WITH CHARACTERISTICS SIMILAR TO UNLINED CCW IMPOUNDMENTS, SAMPLED BY THE CENTER FOR HEALTHY ENVIRONMENTS AND COMMUNITIES (CHEC) IN JUNE OF 2008, IS RELEASING SIGNIFICANT LEVELS OF ARSENIC, LEAD AND OTHER METALS AND METALLOIDS INTO GROUNDWATER AND SURFACE WATER AND THAT THIS PROCESS IS OCCURRING UNDER CONDITIONS OF HIGH ALKALINITY

It is commonly assumed that trace element migration from CCW lagoons into groundwater is minimal because the pH of the lagoon waste is extremely alkaline. It is believed that that high pH hinders the mobility of toxic elements through the CCW matrix itself and also through underlying soils. In the summer of 2008 the CHEC performed a site assessment of a highly alkaline waste glass dump with characteristics very similar to CCW ponds. Our results indicate that contrary to prevailing engineering opinions we found greatly increased levels of the following elements in different environmental media:

- Arsenic (As) concentrations in all waterfall effluents and 3 of 4 hole water samples exceed the US EPA Drinking Water Standard.; As in hole sediments range from .6-1X and 1.6 to 2.9X the Canadian PEL and ISQW respectively.
- Mercury (Hg) in all waterfall effluents exceeds the US EPA Drinking Water Standard (range 1.4-4.9X). Hg in waterfall effluent is approximately 7X the CMC.
- Cadmium (Cd) in hole sediments ranges from 1.6 to 2.8 times the Canadian ISQG.
- Lead (Pb) in waterfall effluent exceeds the EPA Drinking Water Standard in all samples (range 2.0 to 8.8X) and the CMC in one sample by a factor of 2. Pb in hole sediment exceeds the ISQG in 3 of 4 samples.
- Copper (Cu) exceeds the Freshwater CMC in all samples, in one by a factor of 8. Cu in hole water exceeds the CMC in 2 of 4 holes.
- Manganese (Mn) exceeds the NSDWR secondary water standards in 2 of 3 waterfall effluent samples and in all hole water samples. The Mn level in hole sediments exceeds the Missouri PEL in all samples (range, 1.9-2.7X).

This finding is important to this discussion because the prevailing pH was between 10.9 and 12.0. We propose as a result of this work that the environmental impact of metals is directly related to its bioavailability and that increasing pH can actually help mobilize and increase transport of toxic elements such as arsenic. This is because arsenic predominately exists as an oxyanion species in soils and freshwater. Arsenite [As(III)] species predominate in anoxic and reducing conditions while arsenate [As(V)] species are more so found in oxidizing solutions (T. W. Frankenberger, 2002). Sorption of arsenate/arsenite is highly dependent on pH and decreases greatly with increasing pH, as hydroxide competition is significant. High silica (SiO_2) levels have shown to interfere with arsenic sorption onto iron oxides and hydroxides (Cullen & Reimer, 1989; Ferguson & Gavis, 1972). High silica (SiO_2) levels, as seen in flyash, have been shown to interfere with arsenic sorption onto iron oxides and have been a concern of utilities striving to improve arsenic removal. It is suggested by Korte, Fernando and Moore that higher concentrations of silica in solution, coupled with higher pH, could cause mobilization of arsenic from sediments and soil (Korte & Fernando, 1991; Moore, 1991) into groundwater and breakout into surface water. Result of our survey of this site seems to confirm these observations.

IV. THE PREDOMINANT LOCATION OF FLYASH PILES AND CCW SURFACE IMPOUNDMENTS NEAR SURFACE WATER-DRINKING WATER SOURCES CREATES AN UNREASONABLE THREAT TO PUBLIC HEALTH AND THE ENVIRONMENT; AND PLACEMENT OF FLYASH PILES AND CCW IMPOUNDMENTS CONSTITUTES A MAJOR ENVIRONMENTAL JUSTICE ISSUE, IN THAT THESE COMMUNITIES ARE GENERALLY LOCATED IN AREAS WITH ASSOCIATED AND OTHER POLLUTING SOURCES, WHICH ARE CHARACTERIZED BY LOW SOCIO-ECONOMIC ATTAINMENT, FLIGHT OF RESIDENTS THAT CAN AFFORD TO RELOCATE, RESULTING IN FURTHER EROSION OF MUNICIPAL AND SCHOOL TAX BASES.

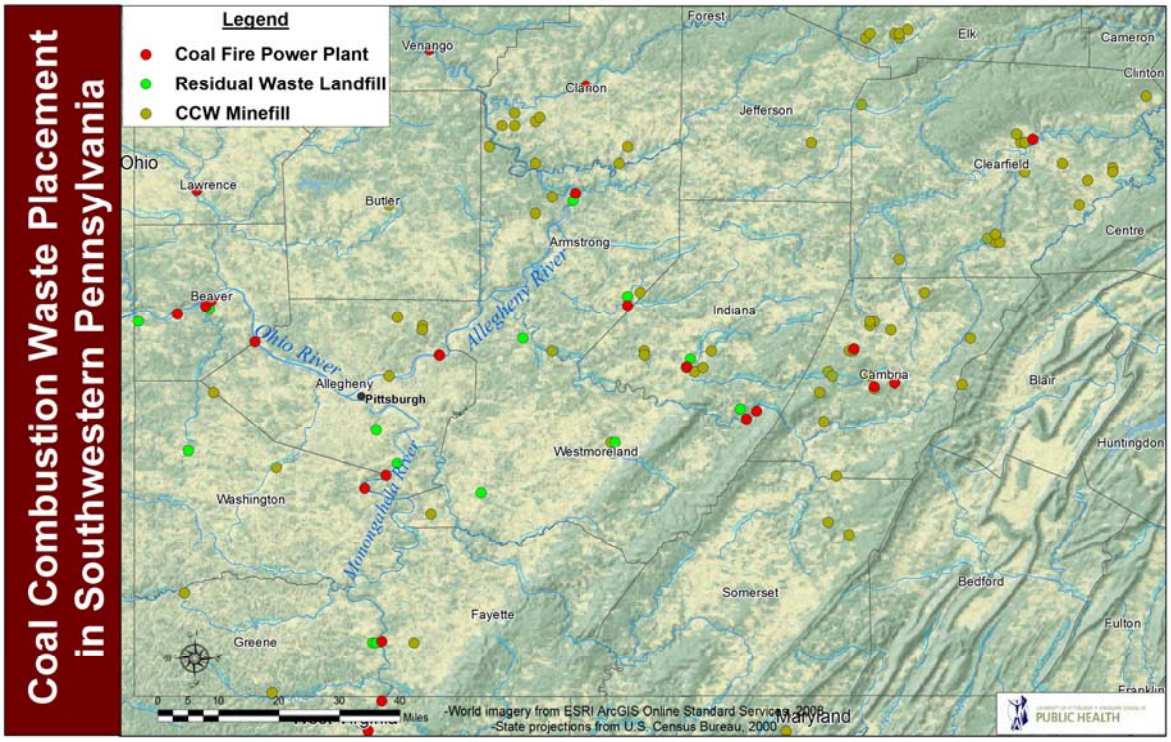
The CHEC used ArcView 9.3.1 to create interpolative geographical representations of CCW storage areas in Southwestern Pennsylvania. Coal fired power plants (CFPP), landfills, ash piles and impoundments were added as point sources by latitude and longitude or geocoding of addresses. Figure 1, Locations of Coal Powered Electrical Generation Stations in Southwestern PA and Associated Residual Waste Landfills presents the location of CCW sites in the region. While this map is representative only of the Southwestern PA region, CFPP locations across the country are located similarly because of their need for freshwater sources for proper operations, cooling and wastewater discharge.

This map revels that CCW sites are located principally along major river systems, which also serve as the source water for downstream communities. Leaching of toxic elements and potential catastrophic release of CCW impoundments are a direct threat to environmental resources and to public health. It is estimated by the PA DEP that failure of the Little Blue CCW impoundment would directly impact the lives of over 50,000 residents of the Upper Ohio Valley.

Additionally the communities that are affected by CCW impoundments and waste piles tend to be in areas already severely degraded environmentally from legacy industries (iron and steel, zinc smelting, foundry operations and coal mining) and active industries, including air and wastewater pollution from CFPP in close proximity to CCW storage areas. This constitutes a

major environmental justice issue because residents of these already environmentally degraded communities with sufficient economic resources simply move to areas more favorable to a healthy lifestyle and with better aesthetic value. As a result these communities have shown a shrinking municipal and school tax base, with resultant losses of municipal, educational, and social services. Federal and state tax dollars must therefore be directed to help stabilize these environmental justice areas; this necessary practice is an unrecognized subsidy to the coal fired electrical generation industry.

Figure 1, Locations of Coal Powered Electrical Generation Stations in Southwestern PA and Associated Residual Waste Landfills[1]



[1] Coal Fire Power Plants (CFPPs) are included in the list of impoundments because CFPPs have on-site temporary and permanent storage impoundments. This figure shows all forms of CCW at sites in the southwestern Pennsylvania region.

V. Problem Solutions

The Resource Conservation and Recovery Act (RCRA) of 1976, along with the Hazardous and Solid Wastes Amendments of 1984 are the principal Federal laws in the United States governing the disposal of solid waste and hazardous waste. These laws were intended to provide cradle to grave tracking of these wastes for the protection of public health and environmental resources. RCRA's intent was to maintain a system whereby legacy toxic waste dumps, covered under CERCLA (Superfund), would never occur again. CCW impoundments, flyash settling ponds,

flyash storage piles and flyash waste pits represent a clear hazard to humans and to the environment and should thus be covered under RCRA as hazardous waste. It is actually beyond my comprehension to think that the framers of this legislation did not have CCW in mind as a hazardous waste when this legislation was enacted. The US EPA should be tasked with the immediate review of all administrative decisions regarding the classification of the myriad forms of CCW and how to insure that disposal practices can insure proper protection of both human and ecological health. As a result of the failure of RCRA to stop CCW from being deposited into unlined pits and impoundments we are again confronted with massive potential Superfund sites that must be stabilized.

At minimum actions should be immediately taken to stop the flow of CCW into unlined impoundments. CCW should be stored only in areas of low hydrogeological significance-away from source drinking water supplies. These disposal pits should be lined with clay and appropriate liners that can withstand the harsh physical-chemical demands of these mixtures and geologic weathering. All CCW disposal areas should be ringed with monitoring wells that are at a depth well below the level of the waste so that monitoring can be done at various intervals between the surface and any confining unit; monitoring wells should be monitored in perpetuity, at least once monthly and after significant rain/wet weather events or during low flow periods such as droughts. CCW disposal areas should also be covered with structurally sound materials so that wind erosion cannot aerosolize dried materials, especially those that can be inhaled and retained in the deep lung.

Unlined CCW impoundments should slowly be drained, especially if they are in critical watersheds and moved to less hydrogeologically sensitive areas. This will not be easy and it will be expensive. There may be technologies that can be used to encapsulate these existing lagoons in place using newly discovered flexible pipes used in the gas and oil shale industries. Focused research will be needed to find ways to stop groundwater intrusion by CCW if removal to proper storage areas proves to be cost ineffective. Minimally no fly ash pile temporary, semi-permanent or permanent should be placed on bare ground. All such piles should be within sealed brims preferably made of concrete and underlayed with both clay and a non-permeable fabric. All fly ash piles must be covered with durable fabric to stop entrainment of particles into the air from the ash pile surface.

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Table 1, Studies Indicating Coal Combustion Waste (CCW) Effects on Animal Survival, Reproduction and Growth and Development (Emphasis on Aquatic Organisms)

Study Number	Species Name	Study Type	Results	Significance	Authors Affiliations	Citation
i	Southern toads (*Bufo terrestris*)	In situ exposure of toads to ash containing wastes over a 5 month period with sacrifice of animals throughout the study period.	Ash-exposed toads exhibited elevated levels of 11 of 18 metals measured. Increases ranged from 47.5% for lead to more than 5000% for arsenic. Ten of eighteen metals decreased in toads removed from ash, ranging from -25% for cobalt to -96% for thallium.	First report of field studies that examine the uptake of metals over time from vertebrates exposed to CCW. Additionally, this study showed the extent of recovery from long term exposure and excretion of trace metals.	C. Ward and M. Mendonca, at Department of Biological Sciences, Auburn University, Auburn, AL 36866, USA S. Hassan at Forest Utilization Laboratory, University of Georgia, Athens, GA 30602, USA	Ward C, Hassan S, Mendonca M., (2009). Accumulation and depuration of trace metals in southern toads, *Bufo terrestris*, exposed to coal combustion waste. Archives of Environmental Contamination and Toxicology 56:268–275.
ii	Southern toads (*Bufo terrestris*)	Experimental exposure of southern toads to metal-contaminated sediment and food; measured changes in standard and exercise metabolic rates as well as changes in body, liver and muscle mass, blood glucose, and corticosterone.	Toads exposed to trace metal contamination gained significantly less mass (18.3 %) than control toads (31.3%) when food was limited and experienced significantly decreased Respiratory Quotient after exercise.	Trace metal exposure is associated with changes in the basal metabolic rate of these vertebrates. Decreased RQ after exercise suggests an inability to eliminate carbon dioxide and/or absorb oxygen due to trace metal exposure.	Department of Biology, Auburn University, Montgomery, P.O. Box 244023, Montgomery, AL 36124-4023, USA. cward3@mail.a	Ward CK, Appel AG, and Mendonca MT (2006). Metabolic measures of male southern toads (*Bufo terrestris*) exposed to coal combustion waste. Comp Biochem Physiol A Mol Integr Physiol. ;143(3):353-

Study Number	Species Name	Study Type	Results	Significance	Authors Affiliations	Citation
					um.edu	60. Epub 2006 Jan 31.
iii	Southern toads (*Bufo terrestris*) and southern leopard frogs (*Rana sphenocephala*)	Assessed concentrations of As, Cd, Cu, Ni, Pb, Se, Sr, and Zn in whole bodies of larval, recently metamorphosed, and adult life stages in Bufo terrestris and *Rana sphenocephala* from a site that currently receives coal combustion waste (CCW) discharge, a site where CCW was formerly discharged that has undergone natural attenuation for 30 years, and a nearby reference site.	For elements As, Cd, Cu, Ni, Pb, Zn, concentrations were highest in larvae, but Se and Sr concentrations remained elevated in later life stages. Element concentrations were generally higher in B. terrestris than in R. sphenocephala. Concentrations of As, Se, and Sr were up to 11-35 times higher in metamorphs emigrating from CCW-polluted wetlands compared to unpolluted wetlands	The study suggests that toads and frogs exposed to metals in CCW can transport trace elements from aquatic disposal basins to nearby uncontaminated terrestrial and aquatic habitats. In addition, anurans utilizing naturally revegetated sites up to 30 years after CCW disposal ceases are exposed to elevated trace elements.	University of Georgia, Savannah River Ecology Laboratory, P.O. Drawer E, Aiken, SC 29802, USA.	Roe JH, Hopkins WA, Jackson BP. (2005). Species- and stage-specific differences in trace element tissue concentrations in amphibians: implications for the disposal of coal-combustion wastes. Environ Pollut.;136(2):353-63.
iv	Southern toads (*Bufo terrestris*)	In vivo study to assess the responsiveness of the interrenal axis to stress from adrenocorticotropic hormone (ACTH), as well as the vehicle alone (saline) in CCW exposed toads against those from control sites.	Initial circulating levels of corticosterone in toads captured at the CCW area were significantly higher than levels in toads from the reference site. Corticosterone levels in toads from the CCW site remained high even after 2 weeks of laboratory acclimation and injection with saline. Injection of toads from the	CCW constituents display endocrine system disrupting effects. This may be through disruption of hepatic enzymes responsible for the metabolic clearance of steroid hormones. Toads exposed to CCW wastes may be less efficient at responding to additional environmental stressors.	Hopkins WA, Mendonça MT, Congdon JD. Department of Zoology and Wildlife, Auburn University, AL 36849, USA. hopkins@srel.edu	Hopkins WA, Mendonça MT, Congdon JD (1999). Responsiveness of the hypothalamo-pituitary-interrenal axis in an amphibian (*Bufo terrestris*) exposed to coal combustion wastes. Comp Biochem Physiol C Pharmacol Toxicol

Study Number	Species Name	Study Type	Results	Significance	Authors Affiliations	Citation
			CCW site with ACTH had no effect on plasma corticosterone levels, whereas a similar treatment of toads from the reference site stimulated a marked increase in corticosterone.			Endocrinol.;122(2):191-6.
v	Southern toads (*Bufo terrestris*)	In situ experiment, which compared in phase 1, the circulating levels of corticosterone (B) and testosterone in male toads captured at CCW sites versus reference sites. In phase 2 of this study, male toads from reference sites were transplanted to enclosures at the polluted site or an uncontaminated site and B levels were checked up to 12 weeks following exposure in each group.	Free-ranging male toads captured at the CCW site exhibited significantly higher circulating levels of corticosterone (B) in both June/July and August than conspecifics captured at uncontaminated sites. Additionally, both calling and noncalling males from the polluted site had higher B levels than conspecifics engaged in the same behaviors at reference sites. Testosterone levels were elevated in toads from the polluted site, regardless of capture month or behavioral state. In phase 2 of this study toads held at the polluted site exhibited significant increases in B after 10 days of exposure compared to toads held at the reference site. B levels remained significantly elevated in	This study describes an interrenal stress response in adult toads after exposure to coal combustion waste (CCW). CCW exhibited endocrine-disrupting capabilities by increasing testosterone levels, which suggests altered androgen production, utilization, and/or clearance. CCW exposure also increased circulating levels of corticosterone.	Department of Zoology and Wildlife Science, Auburn University, 331 Funchess Hall, Auburn, Alabama, 36849, USA.	Hopkins WA, Mendonça MT, Congdon JD. (1997). Increased circulating levels of testosterone and corticosterone in southern toads, *Bufo terrestris*, exposed to coal combustion waste. Gen Comp Endocrinol.;108(2):237-46.

Study Number	Species Name	Study Type	Results	Significance	Authors Affiliations	Citation
			toads transplanted to the polluted site after 12 weeks.			
vi	Larval southern leopard frogs, (*Rana sphenocephala*)	CCW are documented to negatively impact oral morphology, growth, and development in larval amphibians. It is currently unclear what physiological mechanisms may mediate these effects. Corticosterone, a glucocorticoid hormone, is a likely mediator because when administered exogenously it, like CCRs, also negatively influences oral morphology, growth, and development in larval amphibians. In an attempt to identify if corticosterone mediates these effects, the authors raised larval Southern Leopard Frogs, *Rana sphenocephala*, on either sand or CCR substrate and documented effects of sediment type on whole body corticosterone,	CCW treated tadpoles contained significantly more corticosterone than controls throughout metamorphosis. However, significantly more oral abnormalities occurred early in metamorphosis when differences in corticosterone levels between treatments were minimal. Overall, CCR-treated tadpoles took significantly more time to transition between key stages and gained less mass between stages than controls, but these differences between treatments decreased during later stages when corticosterone differences between treatments were greatest.	CCW treatment has effects on corticosterone levels in tadpoles and exposed tadpoles took significantly more time between stages than controls and gained less mass between stages than controls. The mechanism for these effects may be more complex than once thought.	Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA. peterj1@auburn.edu	Peterson JD, Peterson VA, and Mendonça MT. (2009). Exposure to coal combustion residues during metamorphosis elevates corticosterone content and adversely affects oral morphology, growth, and development in *Rana sphenocephala*. Comp Biochem Physiol C Toxicol Pharmacol.;149(1):36-9. Epub 2008 Jun 25.

Study Number	Species Name	Study Type	Results	Significance	Authors Affiliations	Citation
		oral morphology, and time to and mass at key metamorphic stages.				
vii	Green frogs (*Rana clamitans*)	In contaminated aquatic environments, a prolonged larval phase means prolonged exposure to pollutants and, potentially, more severe toxic effects. In the laboratory, the authors tested this hypothesis by exposing green frog larvae (*Rana clamitans*) to commercial clean sand (control), sediment from an abandoned surface mine (mine), or sediment contaminated with coal combustion waste (CCW). By collecting eggs late in the breeding season, they obligated larvae to overwinter and spend a protracted amount of time exposed to contaminated sediments. The experiment was continued until all larvae either successfully	Larvae exposed to CCW-contaminated sediment accumulated significant levels of As, Se, Sr, and V. Larvae exposed to CCW-contaminated sediment suffered greatly reduced survival (13%) compared to both control and mine treatments. Moreover, among larvae in the CCW treatment, the majority of mortality occurred during the latter part the overwintering period (after day 205), corresponding to the onset of metamorphosis in the controls.	Mortality in CCW exposed larvae corresponding with metamorphosis in control larvae suggests possible disruption of hormone signaling from exposure to CCW and/or constituent elements and/or chemicals.	Department of Biological Sciences, Towson University, Towson, Maryland 21252, USA. jsnodgrass@towson.edu	Snodgrass JW, Hopkins WA, Jackson BP, Baionno JA, and Broughton J. (2005). Influence of larval period on responses of overwintering green frog (*Rana clamitans*) larvae exposed to contaminated sediments. Environ Toxicol Chem. ;24(6):1508-14.

Study Number	Species Name	Study Type	Results	Significance	Authors Affiliations	Citation
		completed metamorphosis or died (301 d).				
viii	Lake chubsuckers (*Erimyzon sucetta*) FISH	Lake chubsuckers (*Erimyzon sucetta*) were exposed to coal ash-polluted sediments under conservative experimental conditions (filtered artificial soft water and abundant uncontaminated food). Four months after exposure fish were analyzed for incorporation of toxic elements, growth-survival and physical deformities, and total non-polar lipids.	Fish grazing the CCW sediments had significantly elevated body burdens of Se, Sr, and V. Selenium levels were particularly elevated, reaching mean whole body concentrations of 5.6 microg/g dry mass by the end of experimental manipulations. Twenty-five percent of fish exposed to pollutants died during the study. All surviving fish exposed to ash exhibited substantial decreases in growth and severe fin erosion. Total nonpolar lipids were two times higher in fish from the control treatment.	Fish exposed to ash utilized more energy for daily activities and/or were less efficient at converting available energy to tissues for growth and storage. Study implicates CCW and/or constituents as possible endocrine-disrupting agents.	Savannah River Ecology Laboratory, Aiken, South Carolina 29802, USA.	Hopkins WA, Snodgrass JW, Roe JH, Jackson BP, Gariboldi JC, and Congdon JD. (2000). Detrimental effects associated with trace element uptake in lake chubsuckers (*Erimyzon sucetta*) exposed to polluted sediments.Arch Environ Contam Toxicol. ;39(2):193-9.
ix	Juvenile lake chubsuckers (*Erimyzon sucetta*)	A controlled laboratory study evaluating the responses of individual fish to ash exposure and its effect on swimming performance. To test this hypothesis, the authors measured sprint speed and critical swimming speed (U(crit)) of juvenile lake	Fish exposed to ash for 90-100 days accumulated significant concentrations of As, Se, Sr, and V; exhibited severe fin erosion; and had reduced sprint speed and U(crit). Compared to controls, sprint speed of ash-exposed fish was reduced by 30% at 5 cm and the percent reduction was further	Ash exposed fish have changes in their ability to move effectively in their environment, making them less able to avoid predators and procure foods.	Savannah River Ecology Laboratory, Aiken, South Carolina 29802, USA.	Hopkins WA, Snodgrass JW, Staub BP, Jackson BP, and Congdon JD (2003). Altered swimming performance of a benthic fish (*Erimyzon sucetta*) exposed to contaminated sediments. Arch

Study Number	Species Name	Study Type	Results	Significance	Authors Affiliations	Citation
		chubsuckers (*Erimyzon sucetta*) exposed to ash in the laboratory versus control fish.	reduced to 104% at 20 cm. Critical swimming speed was approximately 50% lower in fish exposed to ash compared to controls. Additionally, the typical positive relationship between standard length and U(crit) was absent in fish exposed to ash.			Environ Contam Toxicol. ;44(3):383-9.
x	Lake chubsuckers (*Erimyzon sucetta*) FISH and benthic invertebrates	Because aquatic disposal of ash can also adversely affect food resources for benthic fish, the authors hypothesized that changes in resources might exacerbate the effects of ash on fish observed in laboratory studies. They exposed juvenile *E. sucetta* in outdoor microcosms to water, sediment, and benthic resources from an ash-contaminated site or a reference site for 45 days and compared findings to previous laboratory studies.	Benthic invertebrate biomass was nearly three times greater in controls compared to ash microcosms. Total organic content of control sediment (41%) was also greater than in ash sediments (17%), suggesting that additional benthic resources may have also been limited in ash microcosms. Benthic invertebrates isolated from the ash microcosms had trace element concentrations (As, Cd, Co, Cr, Cs, Se, Sr, and V) up to 18 times higher than in weathered ash used in laboratory studies. The concentrations of trace elements accumulated by fish reflected the high	This study combined with the results of the previous study suggest that ash discharge into aquatic systems is a more serious threat to the health of benthic fish than previously predicted based upon laboratory toxicity tests.	Savannah River Ecology Laboratory, University of Georgia, Drawer E, Aiken, SC 29802, USA. hopkins@srel.edu	Hopkins WA, Staub BP, Snodgrass JW, Taylor BE, DeBiase AE, Roe JH, Jackson BP, and Congdon JD. (2004).Responses of benthic fish exposed to contaminants in outdoor microcosms--examining the ecological relevance of previous laboratory toxicity tests. Aquatic Toxicology (Amsterdam, Netherlands),;v. 68 no. 1, pp. 1-12.

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			dietary concentrations encountered in the ash microcosms and were associated with reduced growth (final mass = 0.07 g) and survival (25%) compared to controls (0.37 g and 67%, respectively).			
xi	Common grackles (*Quiscalus quiscala*) BIRD	Tested hypothesis concerning aquatic disposal of CCW and effects in avian fauna. Individual eggs were collected from common grackles (*Quiscalus quiscala*) nesting in association with coal fly ash settling basins and a reference site to determine if females from the contaminated site transferred trace elements to their eggs. Whole clutches were also collected from both sites to examine inter- and intra-clutch variability of maternally transferred contaminants.	Selenium was found in significantly higher concentrations in ash basin eggs (x = 5.88 +/- 0.44 microg/g DW) than in reference eggs (x = 2.69 +/- 0.13 microg/g DW). Selenium concentrations in eggs from the ash basins were above background levels. Inter- and intra-clutch variation was higher for ash basin clutches than reference clutches.	This study shows maternal transfer of selenium to eggs in birds living near CCW settling ponds.	Savannah River Ecology Laboratory, University of Georgia, P. O. Drawer E, Aiken, South Carolina 29802, USA. bryan@srel.edu	Bryan AL Jr, Hopkins WA, Baionno JA, and Jackson BP. (2003) Maternal transfer of contaminants to eggs in common grackles (*Quiscalus quiscala*) nesting on coal fly ash basins. Arch Environ Contam Toxicol.;45(2):273-7.
xii	Slider turtles (*Trachemys scripta*)	The authors examined two potential pathways by which female *T.*	Incubation in contaminated soil was associated with reduced embryo	CCW contaminant constituents accumulated in female turtles. Se was	University of Georgia, Savannah River	Nagle RD, Rowe CL, and Congdon JD. (2001).

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		scripta may influence the survivorship and quality of their offspring in a contaminated habitat: (1) nesting in contaminated soil and (2) maternal transfer of pollutants. Eggs were collected from turtles captured in coal ash-polluted or unpolluted sites; individual clutches were incubated in both ash-contaminated and uncontaminated soil in outdoor, artificial nests.	survivorship. Adult females from the polluted site accumulated high levels of As, Cd, Cr, and Se in their tissues, yet Se was the only element transferred maternally to hatchlings at relatively high levels. Hatchlings from polluted-site females exhibited reduced O2 consumption rates compared to hatchlings from reference sites.	transferred to hatchlings at high levels and may be responsible for observed physiological impairments.	Ecology Laboratory, Aiken, South Carolina 29802, USA	Accumulation and selective maternal transfer of contaminants in the turtle *Trachemys scripta* associated with coal ash deposition. Arch Environ Contam Toxicol.;40(4):531-6.
xiii	Banded water snakes, (*Nerodia fasciata*) and prey	Trace element concentrations in banded water snakes, *Nerodia fasciata,* and representative prey items from a site polluted by coal combustion wastes were compared with concentrations in conspecifics from a nearby reference site.	Water snakes accumulated high concentrations of trace elements, especially arsenic (As) and selenium (Se), in the polluted habitat. In addition to being exposed to contaminants in water and sediments, snakes in the polluted site are exposed to contaminants by ingesting prey items that have elevated whole-body concentrations of trace elements, including As, cadmium (Cd), and Se. Snakes from the polluted site exhibited mean standard	CCW exposed snakes appear to have elevated allocation of energy to maintenance and theoretically should have less energy available for growth, reproduction, and storage. These findings are consistent with physiological responses recently documented in other organisms from the polluted site. The authors hypothesize that long-term exposure to coal ash–derived trace elements and the resultant	William A. Hopkins[1, 2], Christopher L. Rowe[1, 3], and Justin D. Congdon[1] 1. Savannah River Ecology Laboratory, Aiken, South Carolina 29802, USA, 2. Department of Zoology and Wildlife, Auburn University,	William A. Hopkins, Christopher L. Rowe, and Justin D. Congdon (1999). Elevated trace element concentrations and standard metabolic rate in banded water snakes (*nerodia fasciata*) exposed to coal combustion wastes. Environmental Toxicology and Chemistry . Volume 18, Issue 6

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			metabolic rates (SMR) 32% higher than snakes from the reference site.	accumulation of some elements are responsible for observed increases in SMR.	Auburn, Alabama 36849, USA, 3. Department of Biology, University of Puerto Rico, PO Box 23360, San Juan, Puerto Rico, 00931	Article: pp. 1258–1263.
xiv	Salamander (*Ambystoma talpoideum*)	The authors exposed the salamander *Ambystoma talpoideum* to coal-combustion wastes at low and high larval density throughout aquatic development in mesocosms simulating temporary wetlands.	CCW and high density reduced survival to metamorphosis by 57-77% and 85-92%, respectively, and the effects of these two factors together were additive. Reduced metamorphosis was due in part to larval mortality prior to initiation of pond drying, but CCW and high density also extended the larval period, causing mortality of larvae that were not ready to metamorphose before the pond dried. A. talpoideum metamorphs accumulated high concentrations of a suite of trace elements (As, Se, Sr, and V).	This was the first demonstration of a CCW pollutant-induced extension of larval period leading to reduction in amphibian recruitment.	Savannah River Ecology Laboratory, University of Georgia, Aiken, SC 29802, USA. roe@aerg.canberra.edu.au	Roe JH, Hopkins WA, Durant SE, and, Unrine JM. (2006). Effects of competition and coal-combustion wastes on recruitment and life history characteristics of salamanders in temporary wetlands. Aquat Toxicol.;79(2):176-84. Epub 2006 Jul 13.
xv	Grass shrimp	Grass shrimp were	Survival to metamorphosis	These findings suggest that	University of	Kuzmick DM,

Study Number	Species Name	Study Type	Results	Significance	Authors Affiliations	Citation
	(*Palaemonetes pugio Holthius*)	exposed in the laboratory to CCW-enriched sediments and food over a full life cycle. Survival to metamorphosis was monitored. The COMET assay, a general but sensitive assay for genotoxicity, was used to quantify DNA single strand breaks (SSB) in adults. Total antioxidant potential was examined to assess the overall antioxidant scavenging capacity of CCW-exposed and non-exposed adult grass shrimp.	was significantly reduced in CCR-exposed larvae (17+/-4 versus 70+/-13% in the controls) but not in the juveniles or adults. Grass shrimp exposed to CCR significantly accumulated selenium and cadmium compared to unexposed shrimp. Chronic CCR exposure caused DNA SSB in hepatopancreas cells, as evidenced by the significantly increased percent tail DNA, tail moment, and tail length as compared to reference shrimp.	genotoxicity may be an important mode of toxicity of CCR, and that DNA SSB may serve as a useful biomarker of exposure and effect of this very common, complex waste stream. This study also suggests that CCW exposure during a critical window of development may predispose larvae to not survive to metamorphosis.	Maryland Center for Environmental Science, Chesapeake Biological Laboratory, 1, Williams Street, PO Box 38, Solomons, MD 20688, USA.	Mitchelmore CL, Hopkins WA, and, Rowe CL. (2007). Effects of coal combustion residues on survival, antioxidant potential, and genotoxicity resulting from full-lifecycle exposure of grass shrimp (*Palaemonetes pugio Holthius*). Sci Total Environ. ;373(1):420-30. Epub 2006 Dec 19.
xvi	Paramecium protozoan (Mutagenic Assay)	The use of the established mutagenesis assay in Paramecium as a prescreen for hazardous environmental particles is described. Since these protozoans ingest particles of the size respired by animals and man, the biological effects of the respirable fraction of fly ash	Fly ash from coal combustion was utilized for these studies and was found to be mutagenic. The effects of physical and chemical treatment of the particle mutagenicity provided evidence for heat-stable, heat-labile and acid extractable mutagenic agents.	Flyash and/or specific constituents of fly ash are mutagenic in the Paramecium-protozoan assay. This finding implies genotoxic effects to both humans and animals from inhalation of repairable flyash particles. Genotoxic effects are associated with increased risk of cancer development and	Department of Biological and Exercise Sciences, Northeastern Illinois University, Chicago 60625-4699, USA.	Smith-Sonneborn J, Fisher GL, Palizzi RA, and Herr C. (1981). Mutagenicity of coal fly ash: a new bioassay for mutagenic potential in a particle feeding ciliate. Environ Mutagen.;3(3):239-52.

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Study Number	Species Name	Study Type	Results	Significance	Authors Affiliations	Citation
		particles were monitored in particle-feeding eukaryotic cells. Fly ash from coal combustion was utilized for these studies.		teratogenic effects.		

Table compiled by Conrad Daniel Volz, DrPH, MPH Assistant Professor **http://www.pitt.edu/~cdv5/**
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