Additional Papers on Depleted Uranium from 2009-2011
ICBUW Science Team

Alexandra C. Miller et al., Preconceptional Paternal Exposure to Depleted Uranium: Transmission of Genetic Damage to Offspring, **Health Physics 99**(3), 371-379, 2010 (September)
Male transgenic mice with embedded DU pellets were exposed to low (2 DU pellets), medium (4 DU pellets) and high (6 DU pellets) doses of DU over a period of 7 months. They were mated to nontransgenic female mice at 7 months post implantation. The offspring of the mice where the male parent had been exposed to medium or high doses of DU had a significant increase in mutation frequency in the bone marrow as compared to control offspring.

Another experiment in which male transgenic mice were given acidified drinking water containing either DU or enriched uranium at a concentration of 50 mgUL-1. These male mice were mated at two months post-initial exposure. Offspring of the male mice given enriched uranium had a significantly higher bone marrow mutation frequency than the offspring of the male mice given DU.

In the 7 month experiments, litter size decreased with increasing dose in the mice with embedded DU pellets with the low dose mice having a mean litter size of approximately 5 pups per litter and the litter size from parental mice with the medium dose was approximately 4 pups per litter whereas the litter size of the male mice with high dose embedded DU was approximately 3.8 pups per litter. Mean litter size in the experiment using DU and enriched uranium in the male parents' drinking water were basically similar.

In both types of experiments, uranium content in the testes, kidneys and femur was measured and in all cases there was a statistically significant difference between experimental animals and controls with control levels in the testes being under 4 ngUg-1 tissue and ranging from approximately 456gUg-1 in the "low" DU group to approximately 618 ngUg-1 in the high DU group. Uranium concentrations in the kidney and femur were also significantly higher than controls. Similarly in the drinking water experiment the DU tissue uranium content was 539 ngUg-1 and 518 ngUg-1 in the mice given enriched uranium by mouth.

This is a solid experiment with sizeable sample sizes.

Special Report: Policy: A Review of human carcinogenesis – Part D: radiation. **The Lancet 10**(8), 751-752, August 2009.
The WHO International Agency for Research on Cancer Monograph Working Group categories all forms of ionizing radiation as "carcinogenic to humans". This includes alpha particles, beta particles and neutron radiation as well as gamma rays and x-rays. The paper lists anatomical sites in the human body where alpha and beta particle emissions have caused cancers. In addition neutrons have been shown to cause local tissue damage in laboratory animals to a greater extent than gamma-rays. The Working Group found that ionizing radiation caused various types of molecular lesions and "clustered, complex DNA damage".

Depleted uranium gives off alpha particles (and also beta particles to a lesser extent, and some gamma radiation) chiefly, alpha particles through the isotope Uranium^{238}.

Guoying Zhu et al. Accumulation and Distribution of Uranium in Rats After Implantation with Depleted Uranium Fragments. **J. Radiat. Res. 50**, 183-192, 2009.
This is a comprehensive study of DU distribution and accumulation in rats. DU fragments were inserted into the leg muscles of rats at three dosage levels – low, medium and high. Control rats had Tantalum fragments implanted in leg muscles. Uranium was distributed among the organs rapidly on the first day after implantation. The rats were sacrificed at 1, 7, 30, 90. 180, and 360 days. Uranium levels were significantly higher than in control rats at 7 and 30 days and thereafter, peaking at 90 days in the high-dose ratas and at 30 days in the other groups of experimental rats. At 360 days urinary uranium levels were still high. Throughout the study, uranium concentrations were significantly higher in kidney and bone at all dosage levels, than in controls. Uranium levels were initially higher in kidney than in bone but from 90 days on, uranium levels in the high-dose group in bone were higher than in the kidney (this was also true for bone in the medium-dose group and from 180 days.)

Uranium concentrations in other tissues were significantly less than in kidney and bone. However they were significantly higher in all dosage groups in the spleen, lung, and liver (with liver showing significant accumulation only in the medium- and high-dose groups at 180 days) as compared to controls. Data indicated that uranium levels in the high dose groups in muscle, thyroid and testes (in order of concentration) were significantly different from controls at 7 days and in the heart, the high-dose group had significantly greater levels of uranium at 90 and at 180 days.

Yuhui HAO et al., A Study Assessing the Genotoxicity in Rats after Chronic Oral Exposure to a Low Dose of Depleted Uranium, **J. Radiat. Res. 50**, 521-528, 2009.
This paper investigated the genotoxic effect of long-term ingestion of DU (uranyl nitrate) in low-dose concentrations of 0, 4 and 40 mgKg-1 per day over four months in rats and over two generations. Although the most uranium went to the kidneys, uranium in blood and urine was higher in both the F0 and F1 generations than in control rats, these levels were not significantly different between treatment groups in the two generations of rats. For each treatment group, uranium levels were significantly higher in the F1 generation than in the F0 generation. Uranium found in the ovaries of the F1 generation was significantly greater than in the F0 generation. Sperm abnormality rates were significantly higher per treatment group as compared to controls. These rates which including different frequencies in headless sperm, were significantly greater in the F1 rats compared to the F0 rats. There was also significantly greater sperm DNA damage in the DU-treated rats, a dose-dependent phenomenon.
This study demonstrated that a chronic low dose of DU could cause genotoxic damage especially to sperm, over two generations.

Radjini Racine et al. Modifications of the Expression of Genes Involved in Cerebral Cholesterol Metabolism in the Rat Following Chronic Ingestion of Depleted Uranium. **J. Mol. Neurosci. 38**, 159-165, 2009.
The brain produces its own cholesterol although it accounts for 25 percent of the body pool. Racine and co-workers exposed rats to depleted uranium through their drinking water for 9

months. The DU concentration in the water was 40 mg/L. Focusing on changes in gene expression, the researchers investigated cholesterol biosynthesis, catabolism, transport, storage and regulatory pathways and the transcription factors controlling them.

Although chronic contamination by DU did not lead to pathological symptoms in the rats exposed to DU, all systems examined by the researchers except storage showed modifications at gene expression levels. In particular the m-RNA of the brain gene CYP46A1 showed a 39 percent increase. CYP46A1 has been linked with Alzheimer's Disease. The gene controlling the transport protein Apo E, increased by 75 percent and Apo E has been closely associated with Alzheimer's Disease. Another finding, an enzyme HMGCoA, the initiator of cholesterol biosynthesis, increased by as much as 91 percent.

Hong Yie et al., Depleted Uranium Induces Neoplastic Transformation in Human Lung Epithelial Cells, **Chem. Res. Toxicol. 23**: 373-378, 2010.
Using human bronchial epithelial cells, the researchers exposed the human cells to uranium trioxide in concentrations of 0, 0.25, and 25 ugcm2 in order to determine the potential carcinogenicity of DU. Cell lines derived from foci induced by DU exposure allowed the researchers to estimate plating efficiency (the measurement of the number of colonies generated from an individual cell, which indicates neoplastically transformed cells. The growth of these secondary cells were compared to nonexposed bronchial epithelial cells.
The research showed that the bronchial epithelial cell line was transformed through DU exposure, indicating various features such as contact inhibition and anchorage independent growth which occur in cancerous cells. Their chief finding was that 53 percent of the cell lines transformed by DU exposure showed loss of chromosomes (with chromosome numbers going from the least, 7 to 43).
The researchers state that this hyperdiploidy is found in several lung carcinomas. They add that "this article is the first to show that DU transformed human bronchial cells exhibited a hypodiploid phenotype". This chromosomal instability is an important aspect of lung tumors.

C. Darolles et al., Different genotoxic profiles between depleted and enriched uranium, **Toxicology Letters 192**:337-348, 2010.
This study contrasted the effects of depleted uranium and 12 percent enriched uranium (with a specific activity 20 times that of DU, specific activity being defined as the amount of radioactivity per unit mass) in mouse embryo fibroblasts as a way of estimating internal contamination. Using a CBNM assay to determine chromosome damage - loss or breakage of chromosomes (and the FISH), the research team found that DU was an aneugenic agent (leading to overall loss of chromosomes), like Vincristine sulphate, an established aneugenic agent used as a control. The 12 percent enriched uranium was found to be a weak aneugenic agent but a strong clastogenic agent (chromosome breakage). It was suggested that DU's ability to act as a aneugenic agent was likely mainly due to its chemical toxicity and partly due to its radioactivity. The aneuploidy induced by aneugenic agents "plays a significant role in cancer", according to the researchers. Their experiments showed that DU 's "significant aneugenic effect, even though DU was a weak clastogen, meant that the overall "global genotoxic effect was similar to that of the 12 percent enriched compound.

Carolyne LaCerte et. al., Particulate depleted uranium is cytotoxic and clastogenic to human lung

epithelial cells, **Mutation Research 697**, 31-37, 2010.
These researchers showed that particulate depleted uranium (DU) in the form of slightly soluble uranium trioxide ($UO^{3)}$ is cytotoxic and clastogenic to human lung bronchial epithelial cells. The clastogenicity (chromosomal damage) was noted after 48 hours exposure. Concentrations of UO^{3} used was 0.25, 2.5 25 and 50 ug/cm^{2} (with a hexavalent chromium that served as a positive control).
After 24 hours, the above concentrations of UO^{3} resulted in 79, 73, 36 and 8 percent relative survival whereas after 48 ours, these concentrations led to 89, 73, 46 and 23 percent relative survival.
Total chromosomal damage caused by 0, 0.25, 2.5, 5, 10 and 25 ug/cm^{2} of UO^{3} led to 6, 6, 11, 15, 13 and 13 aberrations per 100 metaphases (metaphase is a stage in mitosis where chromosomes line up along a spindle). Fifty microns/centimeter squared caused up to 100 percent cell death.
Concentrations of 0, 0.25, 2.5 and 25 ug/cm^{2} UO^{3} caused damage, leading to 6, 11, 31 and 59 aberrations per 100 metaphases.
Overall findings were that UO^{3} led to concentration-dependent cytotoxicity as well as clastogenic changes at 48 hours exposure.

Mufen Yan et al., Effects of uranium depletion on 1-alpha-hydroxylase in kidneys of rats, **Hum. Exp. Toxicol.**, 2010 July 29, E-pub.
This study demonstrated the ability of depleted uranium (DU) to reduce levels of 1-alpha hydroxylase, which is involved in vitamin D metabolism in the kidney. Four groups of rats had DU pellets implanted in the gastrocnemius (leg) muscle. Dosage ran from 0.1g DU to 0.2g DU and 0.3g DU. The control group had no implanted DU. The rats were studied for 3, 6 and 12 months. Rats with 0.2g DU and 0.3g DU showed significant reduction in 1-alpha hydroxylase at 3 months. That this finding was not evident at 6 and 12 months may be related to the aging of the rats and increased kidney dysfunction. Uranium renal levels peaked at 3 months in the three DU groups. This chronic exposure to DU in the kidney and the consequent reduction in 1-alpha hydroxylase concentrations adversely affected the anabolism of vitamin D and may have also negatively influenced bone metabolism resulting from injury to the kidneys consequent to exposure to DU.

In a neurotoxicity experiment, there was approximately a 30 percent decrease in acetylcholinesterase (AChE) activity with a significant decrease occurring between 36 hours and 3 days of DU exposure followed by a transient over-activation of AChE which was dose-dependent, existing in fish exposed to 20 and 100 ug/L of DU. In fish exposed to 100g/L of DU AChE activity was reduced after 10 days as compared to its activity at 5 days. AChE allows for "the fast clearance of the released neurotransmitter acetylcholine (AcH) within the synaptic cleft" which may increase electrical activity in the brain. In the experiment with AChE, "the combined effects of these modulations aimed at reducing the bioavailability of AcH within the synaptic cleft". The researchers stated that Uranium has an impact on the brain cholinergic system in zebrafish".

Sabrina Barillet et al., Ultrastructural effects on gill, muscle and gonadal tissues induced in zebrafish (Danio rerio) by a waterborne uranium exposure, **Aquatic Toxicology 100**, 295-302, 2010.

These researchers showed that uranium in water has a deleterious effect on zebrafish. The uranium concentration was 100 ug DU/L of water, a concentration similar to the concentration of uranium found near uranium mines.
Another group of zebrafish received 93.35 ug DU/L water plus 6.65 ug U233/L water which had a much higher radioactivity than the water with DU alone. There was also a control group of fish.
The fish were exposed to uranium over a period of 20 days. Total Uranium mass concentration the two uranium groups were similar.

Gill structures in the fish were badly damaged by both the DU and the U233 treatments, with cellular collapse with the latter treatment. DU-exposed gill tissues had "extensive edema of epithelial cells and hyperplasia of chloride cells at the base of the gill lamellae". Uranium was found in the nuclei of of pillar cells (sites of gas exchange) within gill tissues of experimental fish exposed to 80 ug/L DU with 20ug/L^{233}. (This amount of radioactivity is equivalent to 7150 Bq/L in water (p. 298). The gill is a delicate complex vital organ that has many functions. The researchers noted, "Uranium exposure…produces profound effects on gill structure and might also have deep impacts on gill function".

In muscle, the sarcomere, a basic unit of the myofibril, was found to be twisted or split by DU exposure. Genes in muscle were also altered. The concentration of uranium in muscle tissue was related to the level of radioactivity.
While spermatogenesis in the DU-exposed zebrafish was not altered, vacuoles appeared in their nuclei of spermatozoa possibly due to changes in genes. Little is known about this phenomenon.

The authors conclude: "Ultrastructural responses in gill, muscle and gonadal tissues were indeed proven to be sensitive enough to indicate early toxic effects of environmentally relevant uranium concentrations." (p. 302).

Sabrina Barillet et al., Uranium bioaccumulation and biological disorders induced in zebrafish (Danio rerio) after a depleted uranium waterborne exposure, **Environmental Pollution** 159(2) 495-502, Feb. 2011.
This paper found that DU in the form of uranyl nitrate was highly bioconcentrated in zebrafish, with from 30 ug/gram to 50 ug/gram of fresh tissue after 20 days of exposure to DU. The bioconcentration factor (BCF) was higher when the uranium concentration in the water was lower. There was also considerable inter-individual variability so that a large number of fish had low uranium concentrations whereas only a few fish had higher concentrations. DU concentrations in water ranged from 20, to 100 to 500 ug/L, similar to the uranium concentrations found near uranium mines. The fish were exposed for 12 and 36 hours and also for 3, 10 and 20 days.

DU was found to cause oxidative stress in liver tissue due to the fact that it lowered several endogenous anti-oxidant defense systems in liver tissue. These changes were significant but transient. The researchers surmised that the oxidative response or formation of free radicals might affect proteins or DNA or lipids in the zebrafish. DU exposure also increased DNA

damage in fish red blood cells, with a dose-dependent reaction, resulting from oxidative stress or possibly the creation of DNA adducts.

Caroline Rouas et al. Distribution of Soluble Uranium in the Nuclear Cell Compartment at Subtoxic Concentrations. **Chem. Res. Toxicol. 23**, 1883-1889, 2010.

Rouas and colleagues studied the effects of subtoxic concentrations of soluble DU on human kidney (HEK-293) cells, liver (HepG2) cells and neuronal (IMR-32) cells as well as the localization of DU in these different human cells. Uranyl nitrate hexahydrate represented DU and was used in 9 different concentrations, from 1uM to 1000uM with incubation times of either 24 or 48 hours. SIMMS technology was used to determine the presence of DU in the cytoplasm and the nucleus of the different cell types.

Brain cells were more sensitive to DU exposure than kidney or liver cells.

For kidney cells, with a basal level of less than 10 percent mortality after 24 hours, mortality did not increase until 700uM (a 12 percent increase) and had only increased by 26 percent at 1000 uM DU. The same basal level was observed after 48 hours. At 300 uM DU, mortality was up by 18 percent and attained 59 percent mortality by 1000 uM.

For liver cells, a basal level of less than 20 percent mortality was true for both exposures, 24 hours and 48 hours. After 24 hours exposure, cell mortality had increased by 37 percent, plateauing at about 40 percent from 500 uM DU. After 48 hours exposure, cell mortality began at 300 uM (50 percent) with a 90 percent mortality at 500 uM, remaining at this level at higher concentrations.

With regard to neuronal cells, after 24 hours exposure, cell mortality was at a basal level of under 30 percent. It stayed there even at 300 uM but by 500 uM the mortality rate increased, plateauing at about total cell death. After a 48 hour exposure, brain cells suffered a basal mortality of greater than 50 percent. Total cell death occurred at 500 uM.

Results from the localization of DU (Uranium $^{238)}$) in kidney, liver and brain cells found DU in cytoplasm and nucleus of each type of cell, including precipitates. In kidney cells, after 24 hour incubation, no uranium was present in either control cells or cells incubated in 10 uM DU. However, at 50 and 100uM, DU was found “mainly in the nucleus”.

In liver cells, there was no DU in control cells at 24 hours, but DU was present in both nucleus and cytoplasm in the cells exposed to 10 uM DU. At 50 uM, DU was observed “mainly in the cell nucleus” and in the 100 uM exposed cells, most of the DU had precipitated and was observed in and outside of the nucleus. (100 uM is a subtoxic concentration.)

In brain cells, no DU was seen in control cells or in brain cells incubated in 10 uM DU for 24 hours. Cells exposed to concentrations of 50 uM and 100 uM DU, were found to have DU in the cytoplasm and “especially” in the nucleus. Also at 100 uM, a significant amount of DU had precipitated.

Cell viability was not affected by the number of uranium precipitates at the 100 uM DU concentration.

The mechanism by which the U^{238} enters the cell or the nucleus is not known at present.

In their conclusion the researchers state, "A new and interesting result was that at 50-100 uM soluble uranium is localized in the cytoplasmic area but also and mainly in the nucleus.

Also a paper from 2003:

A.C. Miller et al. Genomic instability in human osteoblast cells after exposure to depleted uranium: delayed lethality and micronuclei formation. **J. Environ. Radiat. 64:** 247-259, 2003.

Miller and her team investigated the ability of DU to cause genomic instability in human osteosarcoma cells (HOS). Genomic instability is found in cancer cells.
In the Miller experiments on HOS cells, the results of soluble DU exposure were compared with the results of exposure from gamma radiation and from soluble Nickel (Ni). Nickel, a heavy metal, is a known carcinogen.
The endpoints of genomic instability chosen by the researchers were delayed reproductive death (a lethal event) and micronuclei formation.
In the experiments with the end results of delayed reproductive death and delayed micronuclei expression, HOS cells were exposed to differing concentrations (0, 25, 50 and 100uM) of soluble DU, or soluble Ni and compared with 0, 2, 4, 6, and 8 Gy of gamma radiation. (p. 251).
Following incubation with soluble DU, soluble Ni or gamma radiation, cell survival was noted 3, 12, 24 or 36 days after exposure. Survival levels of all three experimental substances were universally lower than that of untreated controls.

Results for DU were similar to those for Ni and gamma radiation in the experiments on delayed reproductive death.

With delayed micronuclei expression, levels of micronuclei were for the most part higher in the DU experiment than in the Ni or gamma radiation experiments. Even at 12, 24, and 36 days post-exposure, levels in the DU experiment were significantly higher than in controls. (Micronuclei expression in the Ni and gamma radiation experiments, were not significantly higher than control levels at 24 and 36 days after exposure).

In micronuclei frequency experiments, soluble DU and soluble Ni exposure concentrations were 0, 10 and 25uM as compared to 0, 2, and 3 Gy in the gamma radiation experiments. (p. 251).
In the experiments on micronuclei frequency in individual clones from cells exposed to DU, Ni or gamma radiation, three to five individual clones from the original cells exposed to the different concentrations as per above, were analyzed and compared to clones from untreated control cells. Control clones had a frequency of micronuclei below 1.8 percent. By contrast, in the words of the researchers, "For DU exposure, a persistent increase in the frequency of micronuclei was observed in all clones examined. All clones generated by DU-exposed

proliferating cells exhibited a frequency ranging from 2.2 to 4.5." (p. 255). Other researchers had found a similar range in micronuclei frequency in experiments with alpha radiation. In the Miller study, micronuclei frequency in the gamma radiation experiment ranged from 2.2 to 3.6, showing a persistent frequency as in the DU experiments. In the Ni experiments, there was only a very small increase in frequency of micronuclei.

The researchers stated, "Our data indicate that delayed lethality is associated with a significant increase in micronuclei frequency after exposure to DU. ….The DU-associated delayed increase in micronuclei frequency exhibited a clear concentration-dependent response in the low-dose range".

The researchers concluded: "In summary, we have presented data showing the production of genomic instability in the progeny of human cells exposed to DU. The findings demonstrate that DU can induce delayed cell death and genetic alterations in the form of micronuclei. Compared to gamma radiation or Ni, DU exposure resulted in a greater manifestation of genomic instability." (p. 257)

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