A new model for toxicity of uranium dust

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ABSTRACT

There is growing evidence in scientific literature, resulting both from *in vitro* and *in vivo* analyses, that current models of the mechanisms of toxicity of uranium dust are not fully satisfactory. They should be refined in order to obtain more effective responses and predictions regarding health effects. A review of the most recent findings in the field of uranium toxicity is carried out, and a model based on the Toxicity Equivalent Factor approach is outlined.

Keywords *depleted uranium; dust; toxicity, radiotoxicity, synergistic effects*

1 INTRODUCTION

Depleted uranium (DU) is mostly composed of the natural radioactive isotope U^{238} and is a by-product of the enrichment process, which is part of the fuel cycle of nuclear power plants. DU characteristics are: low specific radioactivity (with emission of alpha particles), high specific weight, low cost, wide availability. Even if radioactivity of DU is weak compared to several other sources, its biological effects (chemical and radiological) cannot be neglected if its concentration is sufficiently high.

When DU bombs detonate, uranium oxide is formed in particulates of between 0.5 and 5 microns. These can be windborne several hundred miles or suspended in the atmosphere. The size of the particles varies greatly: larger fragments can be easily seen with bare eyes, while very fine particles are smaller than dust and can be inhaled and taken into the lungs. Whether large enough to be seen, or too small to be observed, DU particles and oxides contained in the body are all subject to various degrees of solubilisation — they dissolve in bodily fluids, which act as a solvent. Once dissolved in the blood, about 90% of the uranium will be excreted by the kidneys in urine within 24-48 hours. The remaining 10% of DU in blood is retained by the body. Insoluble uranium oxides can remain in the lungs for years.

Concerning chemical toxicity, uranium, being a heavy metal, is known to have toxic effects on specific organs in the body, the kidney in particular. Uranyl-carbonate complexes decompose in acidic urine in the kidney. This reaction forms the basis for the primary health effects of concern of uranium. The effects on the kidney of uranium resemble the toxic effects caused by other heavy metals, such as lead and cadmium. Concerning DU radiotoxicity, U-238 is a long-lived alpha-emitter, with a weak emission of beta and gamma rays. External exposure hazards mainly regard military personnel using tanks with DU shields, while it is negligible in other occasions. The most important pathways for DU exposure are therefore ingestion and inhalation.

There is growing evidence in scientific literature, resulting both from *in vitro* and *in vivo* analyses, that current models of the mechanisms of toxicity of uranium dust are not fully satisfactory. They should be refined in order to obtain more effective responses and predictions regarding health effects. The approach based solely on the determination of radiological toxicity must be disregarded. Chemical toxicity of uranium has to be reconsidered as a relevant mechanism through which uranium dust can cause health effects at long term. With regard to radiological toxicity, the ICRP (International Council for Radiation Protection) model has to be refined taking into account the peculiarity of uranium dust, α-emitting particles with a low concentration and fine particle size. The so-called "bystander effect" highlighted by recent literature should be included in a new model of toxicity for uranium, as well as the latest developments regarding the actual risk related to the so-called low doses. Moreover, emerging data on the different hazards of enriched uranium and depleted uranium indicate that the radiological toxicity cannot be neglected. Finally, the synergy between chemical and radiological toxicity must be taken into account in the new model.

2 RECENT FINDINGS ON DEPLETED URANIUM TOXICITY

2.1 Genotoxicity of uranium: in vivo and in vitro results

While depleted uranium is less radioactive than natural uranium, it still retains all the chemical toxicity associated with the original element. In large doses, the kidney is the target organ for the acute chemical toxicity of this metal, producing potentially lethal tubular necrosis. In contrast, chronic low-dose exposure to depleted uranium may not produce a clear and defined set of symptoms. Chronic low-dose, or subacute, exposure to depleted uranium alters the appearance of milestones in developing organisms. Adult animals that were exposed to depleted uranium during development display persistent alterations in behavior, even after cessation of depleted uranium exposure. Adult animals

exposed to depleted uranium demonstrate altered behaviors and a variety of alterations to brain chemistry. Despite its low level of radioactivity evidence continues to accumulate that depleted uranium, if ingested, may pose a radiologic hazard. [1]

Uranium is an alpha-particle-emitting heavy metal: its genotoxicity results both from its chemical and radiological properties, that vary with its isotopic composition. The influence of the isotopic composition of uranium on its genotoxic profile (clastogenic/aneugenic) has never been described. Recent studies [2,3] evaluated genotoxic profile of Uranium with the cytokinesis-block micronuclues centromere assay C3H10T1/2; mouse embryo fibroblasts were contaminated with either DU or EU (Enriched Uranium) at different concentrations. Cells received low doses ranging from 0.3 μGy to 760 μGy. The frequency of binucleated cells with one micronucleus increased with increasing concentrations of both DU and EU in the same way. EU induced more centromere-negative micronuclei and nucleoplasmic bridges than DU. A correlation between these two clastogenic markers and ionizing radiation doses was observed. Finally, the study showed that the genotoxic profile of uranium depends on its isotopic composition: DU and EU are low and high clastogens, respectively. However, DU aneugenic effects remain high: thus, there is a need to study the potential role of aneugenic effects of DU in carcinogenic risk assessment linked to uranium internal exposure.

A series of studies [4,5] have demonstrated that DU exposure *in vitro* to immortalized human osteoblast cells (HOS) is both neoplastically transforming and genotoxic. Recent animal studies have also shown that DU is leukemogenic and genotoxic. DU toxicity possesses both a radiological (alpha particle) and chemical (metal) component. DU has a lower specific activity in comparison to natural uranium, and it is not considered as a major cause of radiological hazard. The potential contribution of radiation to DU-induced biological effects is unknown, and the involvement of radiation in DU-induced biological effects could have significant implications for current risk estimates for internalized DU exposure. The purpose of those studies was to measure the induction of mutagenic damage in V79 cells and to determine if radiation plays a role in the induction of that damage. Mutagenicity at the hypoxanthine (guanine) phosphoribosyltransferase *(hprt)* locus was measured by selection with 6-thioguanine. There was a dose-dependent increase in mutagenic response following DU exposure. Using the same concentration of two uranyl nitrate compounds that have different uranium isotopic concentrations and, therefore, different specific activities, the effect on *hprt* mutant frequency *in vitro was examined*. Results showed that, at equal uranium concentration, a 1.33-fold increase in specific activity resulted in a 1.27±0.11-fold *(P* <0.05*)* increase in *hprt* mutant frequency. Taken together, these data support earlier results showing that radiation can play a role in DU-induced biological effects *in vitro*.

There is limited research information on the potential carcinogenicity of DU in human bronchial cells. Accordingly, a series of studies [6,7,8] determined the neoplastic transforming ability of particulate DU to human bronchial epithelial cells (BEP2D). Those studies observed the loss of contact inhibition and anchorage independent growth in cells exposed to DU after 24 h. They also characterized these DU-induced transformed cell lines and found that 40% of the cell lines exhibit alterations in plating efficiency and no significant changes in the cytotoxic response to DU. Cytogenetic analyses showed that 53% of the DU-transformed cell lines possess a hypodiploid phenotype. These data indicate that human bronchial cells are transformed by DU and exhibit significant chromosome instability consistent with a neoplastic phenotype.

2.2 The dose-enhancing effect

Ongoing controversy surrounds the adverse health effects of the use of depleted uranium (DU) munitions. The biological effects of gamma-radiation arise from the direct or indirect interaction between secondary electrons and the DNA of living cells. The probability of the absorption of X-rays and gamma-rays with energies below about 200 keV by particles of high atomic number is proportional to the third to fourth power of the atomic number. In such a case, the more heavily ionizing low-energy recoil electrons are preferentially produced; these cause dose enhancement in the immediate vicinity of the particles.

The dose-enhancing effect of high-atomic-number particles has been suggested as a mechanism by which low levels of radiation might lead to adverse health effects. As a mechanism of increasing the radiation dose in the vicinity of DU particles in the body, it has been suggested that the radiation dose to the tissue immediately surrounding DU particles arising from the inherent radioactivity of uranium is complemented by an enhancement in the radiation dose received from the natural background gamma-radiation [9]. It has been claimed that upon exposure to naturally occurring background gamma-radiation, particles of DU in the human body would produce dose enhancement by a factor of 500–1000, thereby contributing to a significant radiation dose in addition to the dose received from the inherent radioactivity of DU.

In recent studies [10], the Monte Carlo code EGSnrc was used to accurately estimate the likely maximum dose enhancement arising from the presence of micrometre-sized uranium particles in the body. It was found that although the dose enhancement is significant, of the order of 1–10, it is considerably smaller than that suggested previously.

2.3 The Bystander effect

The low radioactivity level of depleted uranium could cause damage to cells adjacent to those directly irradiated, a phenomenon known as "bystander effect" [11]. This phenomenon undermines the stability of the genetic system

of the human body, and many scientists consider it hypothetically linked to cancer and other diseases. The effect is typical of alpha emitters, as uranium-238, the main component of depleted uranium.

The bystander effect is predominant in the case of tissues that receive low doses, where only a few cells interact with alpha particles. At higher doses, in fact, the higher number of cells directly interacting with alpha particles increases the number of killed cells and the magnitude of other effects on nearby cells, placing the bystander effect in the background.

For this reason, low-dose irradiation drom uranium particles seems a perfect candidate to highlight the importance of the bystander effect. We must take also into account that focused sources of alpha radiation (such as uranium particles) can induce different effects than a uniform irradiation.

In the particular case of uranium, in proximity to individual particles or aggregates, there might be the highest concentrations of uranium in soluble form: this might cause synergistic effect with irradiation of localized tissue.

In considering these effects, it must be remembered that uranium particles could accumulate or aggregate in interstitial tissues of the lungs, lymph nodes and in reticuloendothelial tissues. This type of behavior and exposure has not been studied in any previous situation of exposure to alpha emitters in the lungs, found in the civil sphere. The exposure is very different from those under which they are derived equivalences dose-damage by ICRP.

The fact that irradiation with small doses could also affect cells not directly affected by the radiation had already been reported in the 50s. Recently, it has been shown that in cells not directly affected by radiation, but close to the irradiated cells, could occur mutations, micronucleus formation, chromosomal structural damage [12]. Soon it was realized that these were not isolated and random events, but complex effects on tissue reactions [13]. Among the molecular mediators involved in the process, were found not only the oxygen radicals [14] (directly produced by radiation in radiolysis of 'water), but also the radical citochine [15]. The new situation indicates clastogenic molecules as the mediators of inflammation-oxidative stress implicated in the bystander effect.

The bystander effect contradicts the basis of the classical model (the direct action of radiation on DNA, the earliness of genetic damage, the linearity Dose-effect damage, etc...).

3 PROPOSAL FOR A NEW MODEL FOR DU TOXICITY

Alpha particle radiation is known to be a potent cause of unusual effects, as seen above, particularly in the form of genomic instability and, since heavy metals can also cause instability, there is a strong case that the mixed radio-chemical exposure may be acting in this context.

The implication of the combined chemical and radiological transforming capability of uranium, the dose-enhancing effect and the bystander effect, means that, in estimating its significance in causing cancer, the simple assumptions, based on committed effective dose, ie (committed absorbed dose to the lung, modified by a radiation weighting factor for the fact that the radiation arises from alpha particles) would probably underestimate risks.

When several toxic compounds share a mechanism of action, their toxic effects are similar, and, to understand and measure their toxicity, it is common practice to employ a value that shows the relative potency of each compound compared to the toxicity of a reference compound in that group [16]. The term potency refers to the dose needed to attain a certain effect, which is usually the LD50 (the dose that kills half the animals tested). The ratio of the toxic potency of each compound compared to the potency of the reference compound is referred to as the toxic equivalency factor (TEF). These values are usually applied with regard to control or surveillance systems, where the presence of groups of toxins in food needs to be defined by means of one single parameter that gives a reliable measure of the total toxicity present in a sample. As many of the biological effects elicited by these compounds are receptor mediated, such differences can be attributed to different receptor binding affinities. Therefore, an evaluation of the environmental risk from such complex mixtures requires detailed information on each substance.

The combination of different toxicities (chemical, radiological) of the same material to compute a Toxicity Equivalent Factor (TEF) has never been attempted for several reasons, such as the difficulty to combine different actions and different mechanisms. We will restrict our approach to long-term effects of Depleted Uranium, and in particular the carcinogenetic effects.

The sketch of ideas brought forward in the scientific analysis of combined effects, in experimental sciences and in epidemiology, shows that very different lines were followed with little cross-referencing and so far resulted in very different approvals of the state of art. Experimental sciences claim their potential in disclosing principles of combined effects in a degree of confidence that is sufficient to encourage regulations. In contrast, from an epidemiologic point of view the scientific means are considered rather modest.

The description and prediction of combined effects is primarily not interested in how combined agents act but whether the combined effects are more/less significant than expected from the effects of the single components. The focus is on optimizing wanted effects or safeguarding against unwanted effects. Describing combined effects is often done on a case-by-case basis. For example, in Germany, lung cancer from the combined exposure against asbestos and polycyclical aromatic hydrocarbons is now legally considered an occupational disease when individual exposures exceed limits derived from an additive model. In

epidemiology, this approach might also be useful for scoring risk profiles. The prediction of combined effects focuses on the expectation rather than on the deviations from the expectation. Prediction typically calls for simple instruments to enable prospective risk management and regulations taking combined effects into account.

Plackett and Hewlett [17] introduced a generalized model of correlated independent action. For a combination of two agents with concentrations c_1, c_2 the response surface $P_{1,2}$ was modeled by the bivariate normal distribution f. The correlation coefficient U was used to differentiate 3 subtypes of independent action:

$$P_{1,2} = 1 - \int_{y_2}^{\infty} \int_{y_1}^{\infty} f(c_1, c_2, \phi) dc_1 dc_2$$

Where:

$\varphi = 0$	$P_{1,2} = P_1 + P_2 - P_1P_2$	independent action	(1)
$\varphi = 1$	$P_{1,2} = \max(P_1,P_2)$	"no addition"	(2)
$\varphi = -1$	$P_{1,2} = P_1 + P_2$	effect summation	(3)

Within this model uncorrelated susceptibility of organisms leads to the (simple) independent action , whereas in case of a total correlation of susceptibilities the effect of the combination equals the effect of most potent agent. The latter case has also been termed ''no addition'' and bridges as part of the mixture toxicity index to the concept of concentration additivity.

The same model could be used for DU combined toxicity evaluation, given that a new Eq. (1) is introduced:

$\varphi = 0$ $P_{1,2} = P_1 + P_2 + P_1P_2$ enhancement, synergy (1')

That is the case, in fact, that better applies to our problem, where enhancement effects are part of the experimental evidence.

If we now determine:

P_1 = long-term teratogenic effect due to chemical toxicity

P_2 = long-term teratogenic effect due to radioactive toxicity

We should be able to give a first estimate of the enhancement effects.

As far as P_1 is concerned, reference can be made to [18] and other similar assessments, however a relatively scarse database is available on teratogenic (long-term) effects. Some authors [19] hypothesize that hexavalent uranium, as uranyl ion, may have a chemical genotoxicity similar to that of hexavalent chromium (a known human carcinogen), since there are some parallels between their chemistry. They concluded that there are two possible molecular mechanisms that could result in a uranium chemically induced strand breaks: indirectly by free radical generation (Fenton type chemistry) or through direct interactions.

As far as P_2 is concerned, the National Research Council Committee on the Biological Effects of Ionizing Radiation BEIR IV report calculated that the ingestion of additional 1 pCi/day (0.0015 mg/day) of soluble natural uranium would lead to a fractional increase in the incidence rate of osteogenic sarcoma (bone cancer) of 0.0019. The above listed references concerning radioatoxicity show that the enhancement factor between 1 and 10 can be further enlarged by the bystander effect. We should therefore choose a factor close to 10 for a new estimate of the long-term radiotoxicity effects of DU.

However no final conclusion can be said about the results of the model, we think we posed a good basis for further assessment, that will be subject of the future work of the authors.

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