Urinary excretion as a function of uranium concentration in bladder cancer patients using kinetic phosphorimetry analyzer

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Abstract. Measurement of urinary excretion is most commonly used as a method of assessing internal contamination due to insoluble nuclides. The pulsed-laser Kinetic Phosphorimetry Analyzer (KPA-11 with analysis range 0.01µg/L up to 50mg/L for Uranium) has been used to determine the Uranium concentration (Uc) in urine samples of three groups of persons; Bladder cancer patients, Healthy and Infants (with age less than two month) and their mothers. The range of Uc excreted in all subjects have been found to be 0.735–3.876µg/L with an overall average of 2.00038 µg/L, 0.856–1.042µg/L with an overall average of 0.9464 µg/L and 0.505–0.979µg/L with an overall average of 0.7742 µg/L, respectively. The obtained results illustrated that there is statistically significant correlation between Uc and residential area. The obtained Mean values of Uc of the different groups were found to be approximately proportional to age up to 50 years. A noticeable drop is observed for subjects greater than 50 years old. A synthetic urine analysis was chosen in this study to proclaim any concern over biohazards, such as acidity of urine, increasing Amorphous urate, Uric acid, and decreasing Mucous.

Key words: Uranium concentration, Bladder cancer, KPA, Synthetic urine.

1. Introduction
Diagnosis of bladder cancer may occur by urine tests, cancer cells, and other signs of disease [1]. A risk factor of bladder cancer is anything that increasing the chance of getting a disease such as smoking, which can be changed by leaving this habit. Others, such as a person’s age or family history, can’t be changed [2]. The risk of bladder cancer increases with age, and about 9 out of 10 people with bladder cancer are older than 55. It is much more common in men than in women. Urinary infections, kidney and bladder stones, bladder catheters left in place a long time, and other causes of chronic bladder irritation have been linked with bladder cancer (especially squamous cell carcinoma of the bladder), but it’s not clear if they actually cause bladder cancer [3].

There are many studies indicated that radiation exposure is one of that carcinogenic reasons of bladder cancer or any type of cancer [4-6]. The radiation effect may be appear in the exposed person; directly or, take up along time [7]. Evidence of injury from low or moderate doses of radiation may not show up for months or even years. For leukemia, the minimum time period between the radiation exposure and the appearance of disease (latency period) is 2 years. For solid tumors, the latency period is more than 5 years [8].

Till now, the misuse of forbidden weapons in Gulf war I and II caused environmental pollution with uranium in wide areas of Iraq, which lead to different diseases, children malformations, leukemia,
and cancers [9]. Besides, Mc Diarmid et al. [10] found evidence of neurocognitive impairment in Gulf War veterans who had retained fragments of Depleted Uranium (DU) shrapnel, although these individuals showed little evidence of impaired kidney function. Hindin et al. [11] made a review about the teratogenic effects on animal and human. They concluded that the weight of evidence is consistent with an increased risk of birth defects in the progeny that exposed to DU. Accidental or chronic exposure to DU occur in military use of DU munitions in combat at the 1991 and 2003 Gulf Wars [12].

At the last decade in Iraq, the steady increasing in morbidity cases such as cancers, children with birth defects and abortion of pregnant women have been noted [13-16]. Uranium, principally, represents an internal radiation hazard. The risks associated with Uranium in the body are both chemical and radiological [6, 17]. The damage caused by ionizing radiation from radionuclide transformations is a result of the energy absorbed by body tissues [18]. Heavy metals compounds, like carbonate/bicarbonate compounds, e.g. [UO2(CO3)2]2−, may cause a number of cytotoxic effects. These compounds are stable at a neutral pH value (pH of blood) and in this form are not very reactive, but the highly reactive uranyl ion UO2+2 is released at low pH values (as urine) [19].

The effects of the radionuclides vary with many parameters, specifically its pathway, before reaching the target. Uranium can travel through the air, water (both groundwater and surface water), soil and through the food chain. The radionuclides may enter the human body by ingestion, inhalation and through the skin, and then played the internal exposure [20]. Moreover, the effect of radionuclides is depending on biokinetic of them inside the body. The behavior of a substance in the body, such as intake, uptake, distribution, excretion, and retention is called biokinetics [21]. These biokinetics are depending on the properties of Uranium compound such as bio-solubility. However, the Gastrointestinal (GI) tract absorption of Uranium at environmental levels is about (1%) [22]. The other property of Uranium is biological half-life (T1/2), which is the time was taken for the amounts of a particular element in the body to decrease to half its initial value due to elimination by biological processes alone, and hence some of radionuclides can be excrete by urine and/or feces. This means that a radioactive atom can be expelled before it has had the chance to decay. For a number of radioisotopes of particular medical interest, the rate of excretion has been specified in the form of an effective biological half-life. The overall elimination half-life of Uranium under conditions of normal daily intake has been estimated to be between 180 and 360 days in humans [23].

A synthetic urine analysis has been chosen in this study to proclaim any concern over biohazards. Monitoring for occupational incorporation of Uranium and Thorium are usually carried out by urinary excretion analysis. Uranium is poorly absorbed by the digestive tract and most of the absorbed Uranium is eliminated in the urine. Thus, urine is considered to be the best sample for the detection of excessive intake of Uranium [24]. Measurement of urinary excretion is most commonly used as a method of assessing internal contamination due to insoluble nuclides. Analysis of human urine has revealed the presence of various nuclides such as Plutonium, Polonium, Uranium, Carbon-14, Strontium, Barium, Tritium, etc. [25].

The purpose of this research is to get answers of the questions: is the increasing of Uranium concentration (Uc) in urine represents a bio marker indication to get cancer or is a main cause of the cancer reasons?, is there any correlation relation between Uc in urine sample and its bio marker analyses?, and finally, is there any variance between patient and control groups due to their age and home location?.

2. Sample Collection
Urine samples were collected from different group of persons as illustrated in the table 1. The patient group participators were mostly from all districts of Baghdad governorate, from all age groups, and with both genders. The age of control group is asymptotic to that for patient, while the age of the infant is just a few days.
Table 1. Groups of persons from whom urine samples were taken.

| Group                        | Number of samples | Source                                                                 |
|------------------------------|-------------------|------------------------------------------------------------------------|
| Bladder cancer patients      | 60                | Al-Amal National Hospital for Cancer Management, Al-Imamein Al kadhimein city hospital / Al Jawad center for tumor treatment and Medical City / Oncology teaching hospital |
| Healthy (control)            | 30                | different position                                                     |
| Infants with their mother    | 10                | Al Khadraa Al Ahly hospital and Al Elwea teaching hospital for tocology |

2.1. Samples for biological test
The samples those taken for a biological test and from the entire groups that shown in table 1 were kept cool until the test was carried out. The sample was taken from the adult person with urine cup contains 60 ml, then was put in cooling box until reach the bio-laboratory, but in the case of the infant, the sample gathering by urine bag and also transfers in cooling box.

2.2. Samples for radiological test
The urine samples for a radiological test from the same participators in table 1 don't need stay cooling, and they were accumulated to be 500-1000 ml for almost 24 hour. Complete 24hour urine samples give better precision than spot samples in estimating Uc at these low levels, but presented more logistic difficulties in the collection [26]. In other wards these samples were taken in another day, because of all patient are visitors not residents. Total 24 hour urine samples were collected in polyethylene bottles, and immediately acidized by the addition of 1ml concentrated HCl for each sample to avoid precipitate formation.

3. Sample Analyses

3.1. Biological analyses
It could be done for all groups of participators in table 1 to study the relation between the cancer incidence probability and bio marker analysis of the urine. The biological tests are fixing in tables 2.

Table 2. The biological tests of urine samples.

| No. | Gender | pH | pus cell | Epithelial cell | Amorphous urate | Uric acid | Calcium Oxalate | Mucous | others | Bacteria |
|-----|--------|----|----------|-----------------|-----------------|-----------|-----------------|--------|--------|----------|

3.2. Radiological analyses
It could be done for all groups of participators in table 1 to study the relation between the cancer incidence probability and Uc measurements. In general, excretion of Uc in urine of healthy person was limited by several international agencies, for example World Health Organization (WHO), to about100 ng/L [27]. Therefore, Uc measurements in urine need a detecting system with low minimum detectable activity. Elements in environmental sample are presented in very small amount and their measurements required reliable and sensitive analytical technique. Environmental levels of Uranium in human excreta are highly variable, depending on Uc in air, food and water, and on the health of the individual [28]. Analytical techniques used as part of a radiation protection program should provide
limits of detection comparable to or below these levels, in order to differentiate between environmental and occupational Uranium exposures.

Concentrations of various elements in different samples were determined using many analytical techniques, whereas, Uranium content as been measured in various foods and drink samples using Gamma-Ray Spectrometry, but this technique needs to use of special food and liquid calibration standards [29]. Neutron Activation Analysis and radiochemical separation can use to estimate Uc in some biological samples [30]. Photometric techniques such as fluorometry and phosphorometry can use to measure Uc with very good resolution and sensitivity. KPA-11 spectrometer, which is one of the updated phosphorometry techniques, has been used to evaluate Uc in urine samples of the present work. KPA-11 provides highly precise and accurate measurements that eliminate the need for internal standards. The advantages of this technique are the extremely low detection limit (10 ng/L), and only required pretreatment of samples. KPA-11 is a bench-top instrument that rapidly performs single-measurement, manufactured by Chemchek Instruments (Richland, USA). This model is equipped with a pulsed nitrogen/dye laser to supply monochromatic ultraviolet light to excite Uranium atoms in the sample solution. It is a fully integrated computerized system for data collection and analysis. Chemchek's KPA Win software controls the KPA-11 along with storing and interpreting the analytical data returned from the KPA [31].

The urine samples were prepared and Uc measured in μg/l in Radiation Protection Center of Iraqi Environmental Ministry.

Uranium octoxide U₂O₃ used to preparing Uranium standard solutions. Firstly, a stock standard solution of 1000 mg/l (1000 ppm) was prepared by dissolving 117.9 mg of U₂O₃ in 100 ml of 0.82 M nitric acid (HNO₃) in volumetric flask. A calibration curve for KPA at the low Uc is obtained from 0.05, 0.1, 0.5, 1, 5 and 10 μg/l concentrations. Secondly, the desired Uc for the standard sample was prepared by further diluting the stock solution in 0.82M HNO₃ utilizing the dilution formula:

\[1^{st \text{ volume}} \times 1^{st \text{ concentration}} = 2^{nd \text{ volume}} \times 2^{nd \text{ concentration}} \ldots (1)\]

4. Data Processing

Questionnaire data, related to all participators included in table 1, had been done as fixed in table 3. They were taken from participators and checked for validity and consistency with hospital computer files of them. Identities were stored separately from questionnaire data, which were indexed by an anonymous unique participant number. In order to stand about all the effected parameters on the bladder cancer incidence probability, Uc and biological measurements have been gathered with this questionnaire and studied against each other.

The Statistical Analysis System- SAS (2012) program was used to study the effect of factors differences in studied parameters [32]. Chi-square test was used to significant compare between percentage and T-test instead of Least Significant Difference –LSD test (because of the comparison is between two groups) was used to significant compare between Mean of the studied parameters.

| Table3. The questionnaire of all participators of study. |
|---|---|---|---|---|---|---|---|---|---|
| No | Gender | age | Home location | study type | Degree of | The work | Are you marrying? | Are you having children? | Are you smoking? | Are you drinking water |
|---|---|---|---|---|---|---|---|---|---|---|
|---|---|---|---|---|---|---|---|---|---|---|
5. Results and Discussion

5.1. Results of biological analyses

T-test was used to significant compare between the Mean of each bio marker of urine, related to the patient, healthy (control) and infants with their mothers, who participated in this study. The comparison showed that there are high significant differences between patients and controls in pH, Amorphous urate, Mucous, Uric acid, Epithelial and Pus cell. Also a significant difference appeared in other bacteria, as shown in table 4. The comparison between control participants and infants with their mothers showed no significant differences as illustrated in table 5.

| Parameters          | Patients Mean ± SE | Control Mean ± SE | T-Test (P-value) |
|---------------------|--------------------|-------------------|------------------|
| pH                  | 6.56 ± 0.08        | 7.17 ± 0.06       | 0.2457 ** (0.0001) |
| Pus cell            | 1.541 ± 0.14       | 0.766 ± 0.08      | 0.426 ** (0.0005) |
| Epithelial          | 0.233 ± 0.04       | 0.766 ± 0.07      | 0.1542 ** (0.0001) |
| Amorphous rate      | 2.14 ± 0.16        | 0.917 ± 0.09      | 0.4833 ** (0.0001) |
| Uric acid           | 0.316 ± 0.07       | 0.00 ± 0.00       | 0.217 ** (0.00047) |
| Calcium oxalate     | 0.950 ± 0.25       | 0.716 ± 0.12      | 0.5026 NS (0.3532) |
| Mucous              | 0.00 ± 0.00        | 0.216 ± 0.04      | 0.0643 ** (0.0001) |
| Other bacteria      | 1.10 ± 0.16        | 0.467 ± 0.11      | 0.4931 * (0.0120) |

** (P<0.01), NS: Non-significant.

| Parameters          | Mean ± SE | T-Test (P-value) |
|---------------------|-----------|------------------|
| pH                  | 7.17 ± 0.06 | 0.3714 NS (0.3742) |
| Pus cell            | 0.766 ± 0.08 | 0.3002 NS (0.3239) |
| Epithelial          | 0.766 ± 0.07 | 0.3193 NS (0.0501) |
| Amorphous rate      | 0.917 ± 0.09 | 0.50907 NS (0.9097) |
| Uric acid           | 0.00 ± 0.00  | 0.00 NS (1.000)   |
| Calcium oxalate     | 0.716 ± 0.12 | 0.3725 NS (0.2509) |
| Mucous              | 0.216 ± 0.04 | 0.1874 NS (0.8581) |
| Other bacteria      | 0.467 ± 0.11 | 0.4417 NS (0.4496) |

** (P<0.01), NS: Non-significant.

However, the bio markers of urine are really considered as indicators on healthiness states, and the infants with their mothers considered as a reference to healthiness states. A comparison among all groups of participants was showed in figure 1. It's clear that the uric salts (Amorphous urate, Calcium oxalate and Uric acid) increase in urine as much as the pH decreasing for all groups, and the infants with their mothers had minimum amount of these salts and highest pH. Furthermore, other bacteria had the same behavior, while Epithelial and Pus cell in the second and third groups had disconcerted behavior due to sample related to women in all most cases.
5.2. Results of Radiological Analyses

The Mean of the measured Uc in urine samples of patient and control participants had been compared significantly using T-test. The comparison showed there is high significant difference between participants as in table 6. Additionally, the comparison between control and infants with their mothers showed a high significant difference as in table 7.

Table 6. The Mean of Uc (µg/l) in the urine samples of the bladder cancer patients and control participants.

| The group | No  | Mean ± SE of Uc |
|-----------|-----|-----------------|
| Patients  | 60  | 2.0003 ± 0.112  |
| Control   | 30  | 0.946 ± 0.009   |
| T-Test    | --- | 0.3165 **       |
| P-value   | --- | (0.0001)        |
| ** (P<0.01)**                             |

Table 7. The Mean of Uc (µg/l) in the urine samples of the control and Infants with their mothers.

| The group                        | No  | Mean ± SE of Uc |
|----------------------------------|-----|-----------------|
| Control                          | 30  | 0.946 ± 0.009   |
| Infants with their mother        | 10  | 0.774 ± 0.06    |
| T-Test                           | --- | 0.0732 **       |
| P-value                          | --- | (0.0001)        |
| ** (P<0.01)**                    |

Figure 2 illustrates a comprehensive comparison between the studied groups and the international organizations; World Health Organization (WHO) and International Commission on Radiological Protection (ICRP). Publication 23 of ICRP listed the values 0.04-0.4 µg/L for urinary Uc excretion [28]. WHO reviewed data from the early 1990s suggesting that urinary Uc excretion in the general population ranged from about 0.04 to 0.57 µg/L [27]. However, comparison shows that Mean of Uc of patient has the highest value against all other groups, while Mean Uc of infants with their mothers is the lowest value, but it is higher than the permissible recommended values of WHO and ICRP.
Figure 2. Comparison of Uc of the studied groups and international organizations.

The correlation coefficients between measured Uc and the biological parameters were shown in table 8. The results showed a reverse correlation coefficient with high significant between Uc and pH, Mucous and Epithelial, while a positively proportional correlation coefficient with high significant with Amorphous urate, and a non-significant correlation coefficient with respect to the other parameters.

Table 8. Correlation coefficients between Uc and studied biological parameters.

| biological Parameters   | Correlation coefficient-r | Level of sig. |
|-------------------------|----------------------------|---------------|
| pH                      | -0.40                      | **            |
| Pus cell                | 0.45                       | **            |
| Epithelial              | -0.30                      | **            |
| Amorphous rate          | 0.57                       | **            |
| Uric acid               | 0.11                       | NS            |
| Calcium oxalate         | 0.09                       | NS            |
| Mucous                  | -0.34                      | **            |
| Other bacteria          | 0.10                       | NS            |

** (P<0.01), NS: Non-significant.

5.3. Results of Questionnaire Data

Chi-square test was used to significant comparison between percentages of distribution of sample which related to patient character according to Questionnaire that fixed in table 3. The results showed that the bladder cancer patients had high percentage in some factors as shown in table 9, such as gender and period of illness of 1 year.
Table 9. Percentage distribution of bladder cancer patient according to their character.

| Factor : character | No.of cases | Percentage (%) | Chi-Square |
|--------------------|------------|----------------|------------|
| Male               | 50         | 83.33          | 13.409 **  |
| Primary study      | 18         | 30.00          | 7.271 **   |
| Farmer or Gainer   | 23         | 38.33          | 8.341 **   |
| Marred             | 58         | 96.67          | 14.594 **  |
| 4-7 (no. of child ) | 31        | 51.67          | 9.025 **   |
| Smoker             | 38         | 63.33          | 9.017 **   |
| Pipe /Filter       | 29/31      | 48.33/ 51.677  | 1.082 NS   |
| 1 year / (Period of illness:1-5year) | 26 | 43.33 | 10.671 ** |
| Family history(No) | 42         | 70.00          | 10.750 **  |

** (P<0.01).

Bladder cancer patients’ distribution with their Mean of Uc according to home location is shown in table 10. The home location with symbols 2, 8 and 9 registered highest numbers of cases with highest Mean of Uc, with taking into consideration that almost the patients living in home location since 10-15 years or from the born. In spite of that City center (symbolized with 1) registered 7cases, but their Mean of Uc is lower although the high population density of this location (Mansour, Ghazaliya, Al-Shurtah, Juafir, Muasalat, America and Hafa Street) against others. However, the archives of the patients in the Al Jwad center for tumor treatment demonstrated that the registered cases, for all types of cancer, got from home location with symbol 2 represent 30-40% of all other home locations. Also Al-Amal National Hospital for Cancer Management registered cancer cases with 30-40% from home location with symbols 8 and 9.

Table 10. Distribution of bladder cancer patients classified according to home locations and their Mean of Uc (µg/l-1)

| Home location with symbol | No.of cases | Mean ± SE of Uc | Percentage (%) |
|---------------------------|------------|----------------|----------------|
| City center (1):{Mansour, Ghazaliya, Al-Shurtah, Juafir, Muasalat, Ameria, Hafa Street, Bayaa, Turath, Jihad, Harthiya, Attaifiya, Dora, Karada, Amil District} | 7 | 1.75 ± 0.12 | 11.67 |
| Shuala, Kadhimiya, Hurriya, Dabass, Chekok (2) | 11 | 2.27 ± 0.10 | 18.33 |
| Hosrinia, Taji (3) | 4 | 1.87 ± 0.10 | 6.67 |
| Abu Ghraib, Sabaa AL-Bour, Khan Dhari (4) | 2 | 1.56 ± 0.08 | 3.33 |
| Rasheed, Yusufiya, Mahmutiyah (5) | 3 | 2.09 ± 0.11 | 5.00 |
| Shaab, Hay Ure (6) | 3 | 2.22 ± 0.14 | 5.00 |
| Mustansiriya, Falastin street (7) | 2 | 1.58 ± 0.11 | 3.33 |
| Sadr city, Habibiya (8) | 9 | 2.35 ± 0.11 | 15.00 |
| Baghdad AL Jadeeda , AL Baladiyat (9) | 10 | 2.05 ± 0.08 | 16.67 |
| Nahrawan (10) | 3 | 2.80 ± 0.16 | 5.00 |
| AL Zaafrianiya, JistDIyala , Madain (11) | 2 | 2.01 ± 0.09 | 3.33 |
| AL Fallujah, Ramadi, Baqubah, Amerli, Kut (12) | 4 | 1.69 ± 0.11 | 6.67 |
| Chi-Square | --- | | 7.271 ** |

** (P<0.01).
To emphasize the results that obtained from table 10, a comprehensive comparison had been made among all participants of studied groups, where control group (included healthy and infant and their mothers), with respect to home location as shown in figure 3-a. The Uc of participants of control group in home location (1) is lower than in other home locations (2, 8 and 9).

Moreover, bladder cancer patients' distribution with their Mean of Uc according to age groups is shown in table 11. Age group larger than 50 years registered highest number of cases but with lower Mean ± SE of Uc, while the other age groups registered lower number of cases but with highest Mean ± SE of Uc. This result can be explained as follow: the archives of the patients in the hospital that registered cases with bladder cancer refer to largest number of cases in age 25-50, but they couldn’t survive firstly; because of highest Uc contaminated in their body that attributed for many reasons like type of working, food consumed, etc. . Secondly, from table 9, where the period of illness was (1-5) years and as the period increase the probability of death increase, where cases with period of 1 year represent highest percentage in distribution of studied samples. To emphasize the results that deduced from table 11 a comprehensive comparison according to age had been made among all participants of study, where control group (included healthy and infant and their mothers), as shown in figure 3-b.

Table 11. Distribution of bladder cancer patients classified according to age groups and their Mean of Uc (µgL⁻¹)

| Age groups (year) | No. of cases | Mean ± SE of Uc | Percentage (%) |
|-------------------|--------------|-----------------|----------------|
| ≤ 30              | 3            | 2.24 ± 0.05     | 5.00           |
| 31-50             | 12           | 2.19 ± 0.07     | 20.00          |
| >50               | 45           | 1.93 ± 0.07     | 75.00          |
| Chi-Square        | ---          |                 | 12.632 **      |

** (P<0.01).
6. Conclusions
Through the results of this work, one can strongly conclude that the increasing of Uc in bladder cancer patients represents one of the main reasons of the increasing bladder cancer incidence probability in the Iraqi populations. The second important conclusion is the Uc increment in the urine samples of control and infants and their mothers compared with international agencies such as WHO and ICRP, which indicates on the Uc increment in the Iraqi environment and, therefore, the increasing health hazards in populations. However, there are other remarkable conclusions one can be deduced from this work. The decreasing pH of urine caused increasing uric salts especially amorphous urate and this considered as strong indicator to renal failure in particular and generally in urinary system. Additionally, increasing of acidity of urine, which accompanying with activity of Uc, leads to increase the incidence rate of a bladder cancer. Finally, the home location 2, 8 and 9 suffer from highest Uc pollution.

References
[1] Carmack A J, Soloway M S 2006 The diagnosis and staging of bladder cancer from RBCs to TURs. Urology 67 (3(suppl 1) pp 3-10.https://doi.org/10.1016/j.urology.2006.01.026
[2] Smith A, Balar AV, Milowsky MI, Chen RC, Chapter 83: Bladder Cancer. In: Niederhuber JE, Armitage JO, Dorshow JH, Kastan MB, Tepper JE, eds 2014.. Abeloff’s Clinical Oncology. 5th ed. (Philadelphia: Elsevier)
[3] American Cancer Society 2016 Cancer Facts and Figures 2016 (250Williams street, NW, Atlanta, Ga: American Cancer Society Inc.) GA 30303-1002.
[4] United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) 2000 The Sources and Effects of Ionization Radiations vol.1 (United Nation, New York: Report to the general assembly with scientific annexes) pp126-127.
[5] International Atomic Energy Agency 2006 environmental consequences of the Chernobyl accident and their remediation: twenty years of experience report of the Chernobyl forum expert group ‘environment’ Radiological assessment reports series (Austria : IAEA STI/PUB/ 1239) ISSN1020- 6566
[6] Durakovi A 1999 Medical Effects of Internal Contamination with Uranium (Department of nuclear medicine, Georgetown University School of Medicine, Washington D.C., USA) 40 No.1.
[7] Cameron J R and Skofronick J G 1992 Medical Physics (New York, United State of American: John Wiley and sons Inc.)
[8] U.S. Environmental Protection Agency 2005, "Radiation Information: Fact sheets series on Ionizing Radiation 2
[9] Annual report to Congress Federally Sponsored Research on Gulf War Veterans’ Illnesses for 2007. Department of veterans' affairs.
[10] Diarmid Mc M A, Keogh J P , Hooper F J , Mc Phaul K, Squib K, Kane R, Di Pino R, Kabat M, Kaup B, Anderson L, Hoover D, Brown L, Hamilton M, Jacobson-Kraum D, Burrows B and Walsh M 2000 Health effects of depleted uranium on exposed gulf war veterans Environ. Res. 82 pp 168–180
[11] Hindin R, Brugge D and Panikkar B 2005, Teratogenicity of depleted uranium aerosols: a review from an epidemiological perspective Environmental Health: a global access science source, http://www.ehjournal.net/content/4/1/17.
[12] Xiongxin Dai 2011 Isotopic uranium analysis in urine samples by alpha spectrometry J RadioanalNucl Chem.289:595–600
[13] Haider Aabood Neaamah M 2010 Statistical study of depleted uranium effectiveness in increasing cancer disease in Iraq Journal of Baghdad college of economic sciences university no.24 327-340
[14] Huda Saleh Mahdy Aamash and Baha Al-deen Maarouf March 2002 Baghdad, Iraq Using depleted uranium weaponry against Iraq and legal confronting it at international level (Baghdad: decisions of scientific conference about effectiveness of using depleted uranium in human and environment in Iraq) part 1 p 32

[15] Awqati Naira A, Ali Mohamed M, Al-Ward Nada J, Majeed Faiza A, Khawla Salman, Mahdi Al-Alak and Naeema Al-Gasseer 2009 Causes and differentials of childhood mortality in Iraq. *Bio Med Central BMC Pediatrics* 9:40. doi: 10.1186/1471-2431-9-40

[16] Samira Alaani, Mohammed Tafash, Christopher Busby, Malak Hamdan and Eleonore Blaurock-Busch 2011 Uranium and other contaminants in hair from the parents of children with congenital anomalies in Fallujah Iraq. *Conflict and Health* 5 pp 1-15

[17] Diehle P, Fahey D, Bertell R, Robicheau D, Bristow R, Arbuthnot F and Van der kreu H 1999 Depleted uranium: A post-war disaster for environmental and health (Laka Foundation, Amsterdam, Netherland: IAEA international nuclear information system) INIS vol. 30 INIS issue 46

[18] Zarkadas Ch, Karydas A G and Paradellis T 2001 Determination of uranium in human urine by total reflection X-ray fluorescence *Spectrochim. Acta* Part B 56 p 2505-11

[19] Benton E V and Makeover S W S 2001 Proc. of the 20th Int. Conf. Radiation Measurement on Nucl. Tracks in Solids vol 34 pp 1-6

[20] Murray L 1994 *Understanding Radioactive Waste* 4th Edition (Columbus, Ohio: Battelle Press)

[21] Fisenne I M, Perry P M and Harley N H 1988 Uranium in humans *Radiation Protection Dosimetry* 24 pp127-131

[22] Wrenn M, Durbin P, Howard B, Lipzstein J, Rundo J, Sill E and Willis D 1985 Metabolism of ingested U and Ra. *Health Physics* vol. 48, p 601-603

[23] Berlin M and Rudell B 1986 Uranium. In: *Handbook on the Toxicology of Metals*, (Amsterdam: Elsevier Science Publishers) pp 623-637

[24] AllainP, Berre S, Preamble-Cabic A and Maura Y 1991 Investigation of the direct determination of uranium in plasma and urine by inductively coupled plasma mass spectrometry *Analytica Chimica Acta* 251 pp 183–185

[25] Chakarvarti S K, NandLal and Nagpaul K K 1980 Content of uranium trace in urine determined from fission track etch technique *international Journal of Applied Radiation & Isotopes* vol.31 pp 793 – 795

[26] Jones A D, Miller B G, Walker S, Anderson J, Colvin A P, Hutchison P A and Soutar C A2007 A normative value pilot study: Levels of uranium in urine samples from UK civilians *Environmental Research* 104 pp 216–223.

[27] WHO Department of Protection of the Human Environment 2001 Depleted Uranium: Sources, Exposure and Health Effects (Geneva, Switzerland: World Health Organization)

[28] Snyder W S and Cook M J 1975 Report of Task Group on Reference Man (Oxford: International Commission on Radiological Protection) ICRP Publication 23

[29] Yu K N and Mao S Y 1999 *Health Phys.* 77 p 686

[30] Dang H S, Pullat V R and Pullai K C 1992 *J. Radioanal. Nucl. Chem.* 162 p 163

[31] Chemchek Instruments inc.2006 Operation Manual of Manual and automatic Kinetic phosphorescence analyzer KPA-11 richland, W A 99354 U.S.A.

[32] Statistical Analysis System SAS. 2012 *User’s Guide. Statistical*. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.