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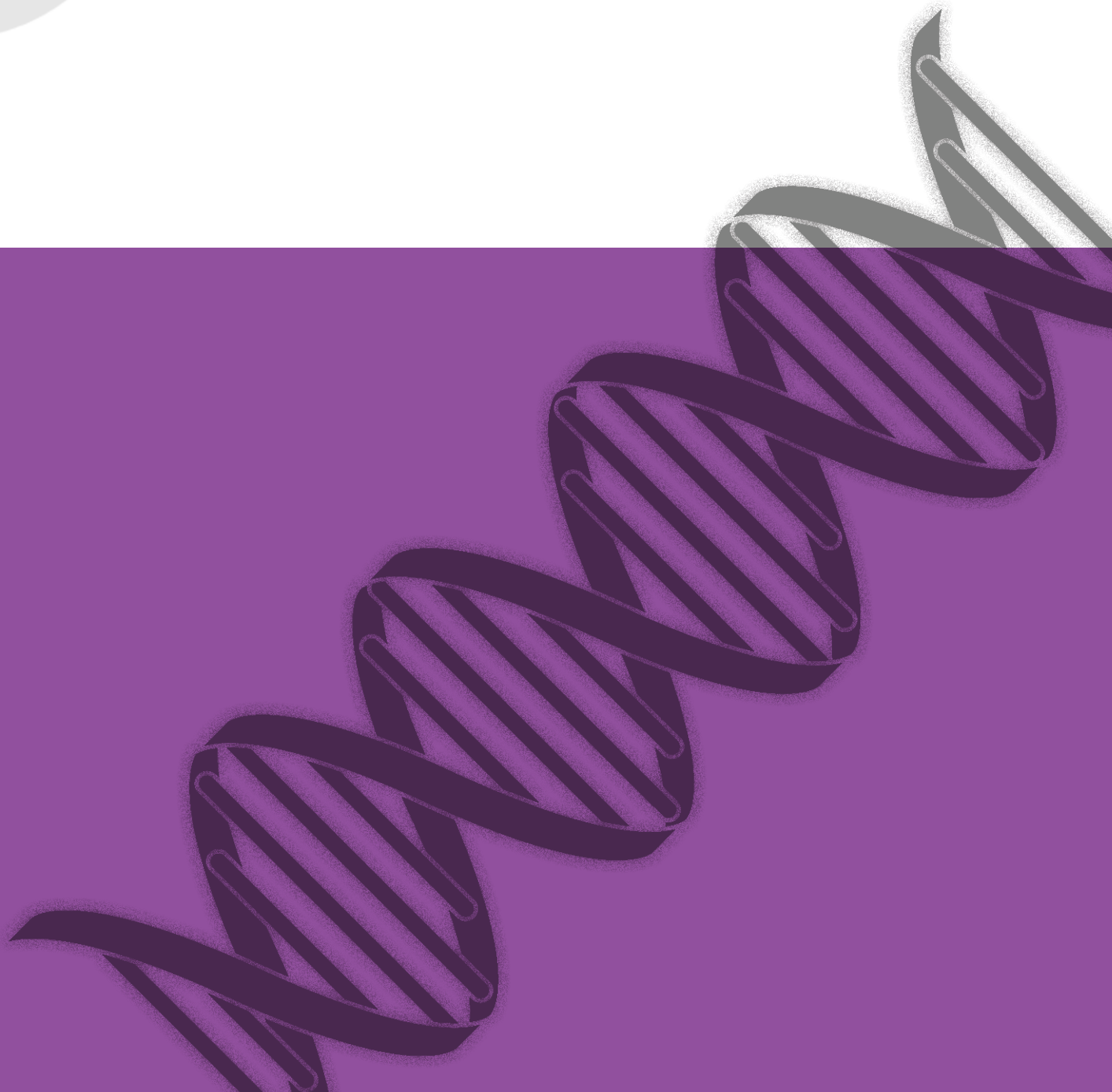
International Coalition to Ban Uranium Weapons

Malignant Effects:
depleted uranium as a genotoxin and carcinogen



Preface

Depleted uranium (DU) weapons have proved a controversial addition to the conventional arsenals of militaries since their first development in the Cold War. Opposition to their use has varied in pitch over the years but has tended to correlate closely with their deployment in conflict. Yet throughout this period, it has been clear from the column inches printed, the parliamentary debates and, more recently the bills, motions and resolutions passed, that the use of DU munitions appears to be intrinsically unacceptable to most people.

The stigmatisation of inhumane and unacceptable weapons has been crucial to extending the impact of the international treaties banning anti-personnel landmines and cluster bombs. But while DU has shown itself, to a degree, to be self-stigmatising – evidence for which is clearly demonstrated by the energetic public relations strategies of its proponents, the difficulty of establishing a causal link between its use and humanitarian impact requires a different approach to judging its acceptability to those that have historically been applied to explosive weapons.

Common sense lies at the heart of people's innate response to assessing the acceptability of DU's use in conventional weapons, thus it seemed only right for ICBUW to launch a discourse rooted in precaution. The Precautionary Principle provides a useful model for both health and environmental protection, particularly where scientific complexity and uncertainty meet.

The purpose of this report is to introduce the reader to the growing weight of evidence relating to how DU can damage DNA, interfere with cellular processes and contribute to the development of cancer. The report uses peer-reviewed studies, many of which have been published during the last decade and, wherever possible, has sought to simplify the scientific language to make it accessible to the lay reader. The executive summary simplifies the report's findings further. A glossary of key scientific terms is provided on each page to further aid understanding and a compendium of these terms and full bibliography are available at the end of the report.

A note on animal testing

Much of the information used in human toxicology comes from testing substances on experimental animals. For some people this is considered an unfortunate necessity, justified by the need to protect human health, but for others intentionally causing harm to animals can never be justified.

While a meaningful review of the literature on the toxicity of DU would be impossible without including the literature involving experiments using animals, we acknowledge that some readers do not regard these experiments as morally justifiable, and may find the description of these experiments upsetting. While we also acknowledge that some of this information could not have been generated by alternative methods, its inclusion should not be read as an endorsement of animal testing.

ICBUW does not have a single position on this issue; people involved in the campaign, like the wider public, have a range of views on the subject. However we do share a belief that the existing evidence is sufficient to justify a ban on the use of DU in conventional weapons, a view which if heeded would eliminate the need for further tests on animals, as well as reducing risks to human health and to the wider environment. We also wholly support moves within the discipline of toxicology to implement alternative methodologies which do not rely on animal testing.

This report has been a collaborative project, incorporating text written by Dr Katsumi Furitsu, Gretel Munroe and Dave Cullen, as well as edits and suggestions from Andy Garrity, Aneaka Kellay, Rae Street, Pat Sanchez and Rachel Thompson. Special thanks to Laurence Menhinick, Doug Weir, Dr Mohamed Ghalaieny and Dr Kat Arney.

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PDF available to download via: http://www.bandepleteduranium.org/malignant-effects

Contents

Acronyms

BAL Broncho-alveolar lavage
CHO Chinese hamster ovary
DU Depleted uranium
DNA Deoxyribonucleic acid
EU Enriched uranium
IARC International Agency for Research of Cancer (WHO)
ICBUW International Coalition to Ban Uranium Weapons
MOD Ministry of Defence (UK)
NU Natural uranium
ROS Reactive Oxygen Species
SCHER Scientific Committee on Health and Environmental Risks (EU)
UA Uranyl acetate
WHO World Health Organisation

Executive summary

What is depleted uranium?

Depleted uranium (DU) is a by-product of the uranium enrichment process. It is used by a number of states in armour-piercing tank shells and bullets.

The use of DU weapons is controversial because DU is radioactive and chemically toxic. Its use can generate particles that can be inhaled or ingested. DU creates large quantities of contaminated wreckage and hotspots of persistent contamination that present a hazard to civilians long after conflict ends.

What is DNA?

DNA is the chemical molecule which contains the inherited genetic information used by all known cell-based life-forms.

DNA is present in almost every cell in the body. DNA could be described as the instruction manual for the organism in which it is present. DNA has the ability to make copies of itself, this is done when cells divide and replicate.

What is genotoxicity?

Genotoxic substances can cause damage to DNA or change the way DNA functions within an organism.

In some cases, a damaged cell can become cancerous, or a mutation in the DNA can be passed on to daughter cells or even to the offspring of the organism.

How can DU damage DNA?

DU is known to damage DNA in several ways. DU emits ionizing radiation, which consists of subatomic particles travelling at high speed. If these particles hit DNA, the collision can cause damage to the DNA.

Experiments have also shown that DU can damage DNA, by joining to it in a chemical bond, forming what are called uranium-DNA adducts. Exposure to DU has also been shown to cause damage to DNA through a chemical process known as oxidation.

How do we know it can do this?

Scientists have shown that DU is genotoxic in a number of different ways.

As well as adducts, which have been observed in hamster cells, a number of experiments have shown that exposure to DU can cause breaks in the strands of DNA. Several studies have shown that DU exposure can cause mutations in rats and in cells in the laboratory. Exposure to DU has been shown to cause oxidative damage to DNA in rats and several kinds of small fish.

Experiments in human bone cells and in mice have shown that exposure to DU can cause genomic instability in immature human bone cells and in mice. Genomic instability means that cells are more likely to undergo changes. The offspring of the mice exposed to DU were more likely to have mutations in their DNA, meaning that genomic instability was passed from parent to child.

A number of studies show that DU exposure can cause different changes to chromosomes in human cells. Chromosomes are the structures formed by the coiled DNA within cells. The changes DU exposure causes in the chromosomes are often used by scientists to identify whether cells have been exposed to a genotoxic substance or effect. Biological indicators like this which are known to be associated with a given effect are called ‘biomarkers’.

What we don't know and why?

Many of the experiments scientists use to assess whether a genotoxic effect has taken place look for the after-effects or biomarkers of the damage. These are often easier to locate and identify than the damage itself.

Because of this, it is not always possible to identify exactly how the DU has damaged DNA (this is called the mechanism), even though the biomarkers show that a genotoxic effect has occurred. Some studies suggest that radioactivity is the most significant mechanism in the genotoxicity of DU, others that a chemical reaction may play more of a role; it has not been possible however to reach a definite conclusion from the studies which have been carried out to date.

Most of what we know about whether DU is genotoxic comes from tests on cells and animals but it is important to work out whether DU is also genotoxic in humans. One of the major reasons for this is the lack of subjects for this kind of study. Very few studies have ever been carried out to identify civilians who have been exposed to DU. Identifying civilians at risk is difficult as militaries often do not say where they have fired DU weapons. Some studies have looked at soldiers from NATO countries but these have only found a few who had measurable levels of DU in their bodies. It is important for studies to look at as many people as possible.

The US Department of Veterans Affairs has funded some studies on a small group of veterans from the 1991 Gulf War, studies which have been running since 1994. The studies have some limitations in their design but some have investigated genotoxic effects. Most of these studies did not find any link between DU exposure and genotoxic effects but because of the small number of subjects it is difficult to draw meaningful conclusions. However, one experiment did show a significant increase of a type of mutation in the highest DU-exposed veterans.

Can DU cause cancer?

Cancer is usually the result of a number of independent DNA changes, which together promote cancerous cell growth and the development of a tumour.

Many studies have shown that exposure to DU can cause cells to transform to a malignant type, meaning that they have the characteristics of cancer cells. These changes have been shown in human lung and bone cells, as well as in rats and mice.

The World Health Organisation's (WHO) International Agency for Research on Cancer (IARC) has developed a framework to assess whether substances can cause cancer. Under the IARC framework DU inside the body is classified as carcinogenic in humans because of the type of radiation it emits. This is confirmed by the many studies on DU's genotoxic effects.

Because not enough studies have been done on civilians who have to live, work or play in or around sites contaminated by DU we cannot be certain about whether DU contamination in the environment is also a carcinogenic risk. As DU can get into people after it is used it seems clear that DU in the environment should be considered a probable, or at least a possible, carcinogen under IARC's framework.

Are civilians at risk from DU from weapons?

Without more information on how much DU might get into the bodies of civilians after DU weapons are used, it is very difficult to accurately quantify their risk of cancer or other health effects.

Most civilians in a country contaminated by DU weapons will not come into contact with contamination, and will face only minimal risks. However, those living or working near contaminated sites are more at risk of exposure, particularly if they are not aware of the contamination. DU weapons have been used in populated areas and against many different kinds of targets. This has made it more likely that people will come into contact with DU.

It is possible to use modelling to make an estimate of risk but until we have reliable data to assess how much DU can get into the bodies of civilians who have been exposed to DU, or what harm it would cause, there will be considerable uncertainties.

What does all this mean?

Peer-reviewed studies on the genotoxicity of DU show that DU has the potential to damage DNA or change how it works.

While the studies reviewed in this report mostly rely on data from laboratory and animal experiments, the range of studies, and the fact that these results have been observed in several different animal species amount to a strong body of evidence on the potential effects of DU on human health.

There have been several large-scale desk studies on the possible effects of DU weapons which assessed the risks to be relatively small. However, many were produced before most of the studies detailed in this report had been published, and others did not properly take this evidence into account.

There is an immediate and pressing need for more data on the exposure risk to civilians from DU in the environment; studies to quantify this risk should be carried out urgently. All sites where DU has been used must be identified and assessed.

Even without this work being done, it is clear that DU has the potential to cause cancer and other health problems through its genotoxic effects. DU is a dangerous substance, and should never be released into the environment in an uncontrolled fashion. Its use in weapons causes long-lived contamination and is wholly unacceptable. Enough is now known about the risks from their use to justify an immediate moratorium on the use of DU weapons, followed by a global ban.

While the evidence from laboratory studies supports this, many of these studies emerged a decade or more after the first battlefield use of DU weapons. The potential for harm to civilians during that time delay shows that in the future we cannot wait until after all the scientific evidence is assembled before acting.

A new review system is required in order to prevent toxic and environmentally damaging substances being used in weapons in the future. It must be rooted in precaution, open to external scrutiny and it must better balance military needs with the need to protect civilians.

Recommendations

1. Full disclosure of targeting data

Efforts to better understand the behaviour of DU in the environment, the risks residues may pose to civilians on a site-by-site basis and risk awareness and clearance work have all been hampered by the ongoing reluctance of users to release targeting data.

The issue of transparency has been raised by many, most notably the United Nations General Assembly, where a call for the transfer of quantitative and geographic data on DU use has featured in its biennial DU resolution since 2010. The UK Royal Society called for long-term environmental monitoring in 2003, while the WHO and UNEP have repeatedly stated that remedial work is necessary around target sites. Without detailed firing coordinates, this work cannot proceed in a meaningful way.

2. Determine the extent of civilian exposure

The models and projections intended to predict the extent of civilian exposure to DU particulate are imprecise and little real-world data is available to accurately determine the risks DU poses in the wide variety of settings that characterise its use in conflict.

The primary focus of research efforts to date has been on military personnel – and not communities or individuals living with DU contamination. Such data is skewed towards exposure scenarios specific to military settings and is unlikely to reflect the risks from DU exposure faced by vulnerable individuals, such as children or pregnant women. There is therefore a pressing need for the international community to assist with the commission and funding of civilian exposure studies. A desirable long-term outcome would be for all potentially exposed civilians to be offered tests analysing both DU excretion and biomarkers specific to DU-induced damage. The data from these studies would help inform efforts to reduce civilian harm by targeting remediation and management work and improving risk awareness projects.

3. Precaution must guide munitions development

The history of DU's development and use, which far outpaced the understanding of its risks, underscores the need for more stringent precautionary safeguards during the development of new weapons.

The rush to deploy DU weapons resulted in the dispersal of large quantities of contamination with little understanding of its potential health or environmental impact. Notably, most of the studies in this report post-date DU's first use by decades. Even today, significant knowledge gaps remain. The choice of toxic materials in munitions must carry with it a responsibility to understand their potential impact prior to deployment. No future weapons development should be undertaken without a risk assessment on their constituents, with a presumption against including those that behave in a similar way to known toxic, genotoxic or carcinogenic materials. Similar assessments on weapons currently in use would be desirable and problematic substances should be phased out.

4. Ban uranium weapons

This report has found that a growing body of research shows that DU is a carcinogen. Set against studies analysing its mode of use in conflict and in light of the lack of obligations to mitigate the risks it poses after conflict, it becomes clear that its use must stop.

The users of DU have shown themselves unwilling to be bound by the consequences of their actions. The failure to disclose targeting data or follow their own targeting guidelines has placed civilians at unacceptable risk. The recommendations of international and expert agencies have been adopted selectively or ignored. At times, users have actively opposed or blocked efforts to evaluate the risks associated with contamination. History suggests it is unlikely that DU use will be stopped voluntarily: therefore an international agreement banning the use of uranium in conventional weapons is required.

Introduction

What is depleted uranium?

Depleted uranium (DU) is a by-product of the uranium enrichment process, containing proportionally less of the fissionable uranium isotope U^{235}, and more of the isotope U^{238} than natural uranium.

As a material it is highly dense and pyrophoric, meaning that it has an incendiary effect upon impact. This effect can generate an aerosol of micron and sub-micron particles that can spread between tens and hundreds of metres from the target.

The use of DU creates hotspots of persistent contamination that present a hazard to civilians long after conflict ends, particularly when used in populated areas. Buildings and civilian infrastructure have been targeted with DU and its use can contaminate soils and groundwater and create vast quantities of contaminated military scrap[1]. Effectively managing DU's post-conflict legacy places a significant financial and technical burden on affected states.

DU is used by a number of states in armour-piercing-incendiary tank shells and aircraft and armoured vehicle ammunition. Six states are known to produce these weapons, and it is thought that around 20 currently possess them in their stockpiles[2].

Health concerns regarding the use of depleted uranium weapons

DU weapons have been controversial since their first significant use in the 1991 Gulf War. Following the use of DU weapons, DU contamination can find its way into the human body by inhalation, ingestion, or through wounds.

The radioactive and chemically toxic nature of DU weapons has meant that their use has been followed by claims that they are responsible for increased rates of cancer and birth defects in the areas where they have been used.

To date there have been no large-scale studies on the possible effects of DU weapons on civilians. Large-scale epidemiological studies on the health impact of environmental risk factors are challenging in peacetime and it is incredibly difficult to design and undertake them in insecure post-conflict settings. Studies have been further hampered by the reluctance of DU users to release targeting data.

In the absence of field data, a number of desk studies have examined the health impact of DU exposure, including studies by the United Kingdom Royal Society and World Health Organisation (WHO)[3]. These post-dated the first significant battlefield use of DU by a decade. In general these studies have divided their risk assessments, looking separately at the issues of radiological and chemical toxicological risk. Where the toxicological risk has been assessed, this has mainly been in terms of damage to the kidneys, which have historically been considered the organ most likely to suffer damage from an acute intake of uranium.

As these studies have assessed the risks from DU as not being very high, users of DU weapons have often cited them when defending their continued possession and use[4]. However, the state of knowledge regarding the genotoxic effects of DU has increased significantly since the use of these weapons first came to public prominence. Little research had been completed in this area when the earlier desk studies were undertaken in the early 2000s and genotoxicity remains under-represented in reports summarising the potential health risks from DU[5].

1. See: Zwijnenburg, W., *Laid to Waste: depleted uranium contaminated military scrap in Iraq*. PAX (2014), available via: http://www.bandepleteduranium.org/en/laid-to-waste

2. Further information on DU weapons can be found at www.icbuw.org

3. See: WHO, *Depleted uranium: Sources, Exposure and Health Effects*, [WHO/SDE/PHE/01.1] (2001); UK Royal Society, *The health hazards of depleted uranium munitions, Part I* (2001) *and II* (2003).

4. See: UK Ministry of Defence, *UK depleted uranium (DU) munitions policy and development* (2011), accessed via: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/210641/Depleted_Uranium.pdf

5. One more recent report on DU (SCHER 2010) referenced much of this research, but chose not to discuss it in detail or draw any conclusions from it, claiming that it was

What is genotoxicity?

Genotoxicity is the ability of an agent (usually a chemical or a type of radiation) to cause damage to DNA, or changes to DNA expression.

Genotoxic damage to cells may cause mutations which, if these occur in cells that are used in reproduction, can be passed on to offspring. These inherited mutations are known as trans-generational effects.

In some cases a damaged cell can become cancerous; this typically involves a series of mutations. An agent which has been shown to cause cancer is known as carcinogenic, or is said to possess the quality of carcinogenicity.

In 2009 the International Agency for Research on Cancer (IARC), a body of the WHO which classifies known and potential carcinogens, affirmed that all substances that emit alpha radiation are classified as Group 1 carcinogens when inside the body[6]. This includes DU, which primarily emits alpha radiation. Classification in Group 1 means that a causal relationship has been established between the agent and the development of cancer in humans.

The genotoxic and carcinogenic effects of DU are linked with the same health problems - cancers and congenital birth defects - that are being reported in areas where DU has been used. Because of this, studies on these effects are of particular interest in the debate about whether the military use of DU causes adverse health effects in civilians.

The purpose of this report is to bring together studies on the genotoxicity of DU and summarise the findings, in order to inform the debate about the use of DU in conventional weapons.

It is ICBUW's belief that the evidence more than justifies a precautionary approach to these weapons. In practice this would mean the discontinuation of their use.

How this report is organised

Each chapter of this report deals with a specific type of damage to DNA, or an expression or mechanism of DNA damage which has been documented in the literature.

These are:

1. **Induction of mutations**.

2. **Conversion of cells to a tumourigenic state,** i.e. a state where they are likely to form tumours.

3. **Generation of DNA strand breaks**, where the strands of the DNA double helix become broken.

4. **Induction of chromosome change**, changes to the structures of chromosomes – the collections of DNA that are visible at the cellular level.

5. **Oxidative damage**, a form of DNA damage caused by a class of molecules known as reactive oxygen species.

6. **Genomic instability**, where cells are likely to undergo changes in genetic material.

7. **The formation of uranium-DNA adducts**, where uranium chemically bonds to DNA.

The chapters are divided into sections detailing *in vitro* (Latin for 'in glass', the name used for scientific studies undertaken outside the body) and *in vivo* ('in the body') studies, using live organisms. Descriptions of studies are written with the aim of being understood by readers without a specialist scientific background. While specialists may also find the summaries useful, in many cases they will want to refer to the original studies for extra detail. The studies detailed in this report were published between 1990 and 2012.

not possible to use it for risk assessment, ICBUW's critique is available at: http://www.bandepleteduranium.org/en/critique-of-the-european-commissions-scher-risk-as

6. IARC, (2012) Monographs on the Evaluation of Carcinogenic Risks to Humans, Radiation Volume 100D: A Review of Human Carcinogens, WHO Press, Geneva, [online] available at http://monographs.iarc.fr/ENG/Monographs/vol100D/mono100D-1.pdf accessed 24 July 2014

1. Induction of mutations

A mutation is a change in the DNA sequence, which results from a failure of the cell's DNA-maintenance processes[7]. Mutations in the **somatic cells** of the human body can lead to cancers, as well as non-cancerous diseases. Usually, a single mutation is not enough to cause cancer. The development of cancer typically requires that a substantial number of independent, rare genetic accidents occur, either in the cell which becomes cancerous, or in one of its parent cells. Multiple genetic events in a single cell can then interact to promote cancerous cell growth and the development of a tumour[8].

Mutations in **germ-line cells** may cause trans-generational effects like congenital disorders, cancers and non-cancer diseases in the offspring of the individual in whom the original mutation occurred.

There are several studies in vitro, which indicate that DU-exposure can induce mutations in the DNA.

In vitro studies

A study by Miller and her group in 1998 was the first that showed that internalised DU can result in a significant enhancement of urinary mutagenicity[9].

Miller et al implanted DU pellets, composed of the same DU alloy used in US military munitions, within the muscles of experimental rats and collected urine samples after 6, 12 and 18 months. Enhancement of mutagenic activity in Salmonella bacteria (the induction of certain base substitution mutations) was observed after they were exposed to urine samples from the rats. In DU-implanted rats, urine mutagenicity increased with dose and the length of time elapsed, demonstrating a strong positive correlation with urinary uranium levels.

Stearns and her group published a paper in 2005, which indicated that uranium induced mutations at a marker locus in Chinese Hamster Ovary (CHO) cells. One of the doses of DU caused 5 times as many mutations in a line of cells that had a reduced level of DNA repair protein complex, compared to parent cells with a normal level of DNA repair protein complex. They also measured the formation of uranium-DNA adducts and the generation of DNA strand breaks by exposure to DU and discussed the mechanism behind the induction of mutations[10].

In a further study in 2006, Coryell and Stearns compared the mutations in CHO cells induced by DU, to mutations induced by hydrogen peroxide, as well as to spontaneous mutations in the same line[11]. The results showed that uranyl acetate (UA) induced significantly more major genomic rearrangements than were generated spontaneously. They also discussed the different ranges of mutations found in the three exposure groups and speculated that this might be indicative of different mechanisms at work.

In 2007, Miller and her group reported that DU-exposure caused a dose-dependent increase in mutagenic damage in cells. They also found that the frequency of mutations increased in proportion to the level of radioactive dose when cells were exposed to

Somatic cells: any cells in the body except germ cells

Germ cells and germ-line: germ cells are sperm cells and egg cells. A germ-line includes germ cells and those cells which give rise to them.

DU-uranyl nitrate: DU is primarily comprised of the uranium isotope U^{238} and the isotope U^{235}, though it contains proportionally less U^{235} than natural uranium and trace amounts of other isotopes. This experiment compared the effects of uranyl nitrate containing only U^{238} and uranyl nitrate containing DU. As the DU–uranyl nitrate will contain not only U^{238} but also a small amount of U^{235}, which has a greater specific activity (amount of radioactivity of a radionuclide per unit mass of the radionuclide), it is more radioactive than the U^{238}-uranyl nitrate.

7. Alberts, B. et al., (2008) *Molecular Biology of the Cell* 5th Edition, New York, Garland Science .

8. Ibid, p. 1208-1210.

9. Miller, AC., et al., *Urinary and serum mutagenicity studies with rats implanted with depleted uranium or tantalum pellets*, **Mutageneisis**, vol.13, No. 6, pp. 643-648, 1998.

10. Stearns, DM., et al., *Uranyl acetate induces hprt mutations and uranium-DNA adducts in Chinese hamster ovary EM9 cells*. **Mutagenesis** 2005;20:417–423.

11. Coryell, VH., and Stearns, DM., *Molecular Analysis of hprt Mutations Generated in Chinese Hamster Ovary EM 9 Cells by Uranyl Acetate, by Hydrogen Peroxide and Spontaneously*. **Molecular Carcinogenesis** 45, 2006.

U^{238}-uranyl nitrate and **DU-uranyl nitrate** at an equal uranium concentration. These results suggested that radiation can play a role in inducing mutations through DU-exposure[12]. As yet the mechanism for inducing mutations is not known.

Heintze and colleagues investigated in 2010 whether either uranyl acetate, or uranyl nitrate, could act as an activator of p53, a protein which is involved in suppressing tumours within the cell. The hypothesis was based on the evidence that heavy metals such as arsenic and chromium have been shown to induce p53-mediated responses, as has **ionising radiation**[13]. The results showed that the level of active p53 was not increased by either uranyl acetate or uranyl nitrate. They also showed that the toxicity of uranyl acetate did not change when p53 was absent. They concluded that these results supported the hypothesis that exposure to either uranyl acetate or uranyl nitrate does not activate a p53-mediated pathway, and that cellular response to uranium exposure occurs through a mechanism that is independent from p53.

Human studies

Few studies have sought to evaluate the presence of DNA mutations in human populations. This lack of studies means that currently, there is no clear scientific evidence of increased DNA mutations in humans exposed to DU.

The only study that has looked into this matter has been on a cohort of 74 Gulf War veterans with known exposure to DU, which resulted from their involvement in friendly-fire incidents with DU munitions. The study was undertaken by the U.S. Department of Veterans Affairs' surveillance programme for Gulf War veterans exposed to DU. Using uranium concentration in urine as the measure of the amount of uranium in their bodies, the cohort was divided into low-uranium and high-uranium groups. McDiarmid reported the results of a "comprehensive protocol" performed in a 2009 evaluation of a subgroup of this cohort (35 individuals). Four biomarkers of genotoxicity were examined.

There were no statistically significant differences in any outcome measure when results were compared between low-U and high-U groups. A significant increase of one type of mutation was found in the highest exposed veterans, and the authors of the paper consider this finding may indicate a possible threshold effect, meaning that the mutations may be induced only by exposure above a certain 'threshold' level[14].

Todorov and colleagues at the Baltimore Veterans Affairs Medical Center studied the amount of uranium in semen of Gulf War veterans[15]. They analysed 6 samples from a cohort of veterans exposed to DU in 1991, with no knowledge of their exposure history. Some of the veterans had no detectable levels of uranium in their semen, but others had elevated levels[16]. This wide concentration range for uranium in semen was consistent with known differences in DU body burdens in these individuals, some of whom have retained embedded DU fragments. The evidence that some of the DU exposed veterans still have a detectable amount of DU in semen, almost 20 years after they were exposed, may pose concern for the possible induction of mutations of germ-cells, which could be transmissible to their offspring.

Ionising radiation: a type of radiation that has enough energy to liberate an electron from an atom or a molecule. Types of ionising radiation include alpha, beta and gamma radiation and X-rays.

12. Miller, AC., et al., *Observation of radiation-specific damage in cells exposed to depleted uranium: hprt gene mutation frequency*, **Radiation Measurement**, vol. 42, no. 6, pp. 1029-1032, 2007.

13. Heintze, E, *Toxicity of depleted uranium complexes is independent of p53 activity*. **Inorganic Biochemistry.** 105: 142-148, 2010.

14. McDiarmid, MA., et al., *Measures of genotoxicity in Gulf war I veterans exposed to depleted uranium*. **Environmental and Molecular Mutagenisis** 2011 Aug;52(7):569-81. doi: 10.1002/em.20658. Epub 2011 Jul 4.

15. Todorov, TI., et al., *Uranium quantification in semen by inductively coupled plasma mass spectrometry.* **Trace Elements in Medicine and Biology** 2012 Aug 31.

16. Uranium levels in the samples ranged from undetectable levels (<0.8pg/g) to 3350pg/g.

2. Conversion of cells to a tumourigenic state

A number of in vitro and in vivo studies indicate that DU can cause tumours or cancer and therefore has a potential role as a carcinogen. To date however, there have been no human studies investigating the development of cancerous cells inside the human body as a result of DU exposure.

In vitro studies

Research carried out by Miller and her team at the US Armed Forces Radiobiology Research Institute demonstrated that human cells exposed to DU transformed into a malignant type of cell. Another study, by Xie and colleagues, found that DU also transformed human bronchial cells to a malignant cell type.

The experiments undertaken by Miller and co-workers showed that exposing human bone cells to DU led to the cells becoming malignant. When injected into nude athymic mice (mice with an immune system that does not reject carcinogenic or human cells) these cells caused the formation of tumours. These findings resulted from the exposure of cells to a soluble DU compound[17]; similar results occurred when exposing the cells to an insoluble DU compound[18].

The human bone cells used in these experiments, known as **osteosarcoma** cells, have been employed to test the carcinogenicity of a range of different soluble metal salts such as nickel, chromium, lead and tungsten[19]. These research studies included a comparison with nickel, lead, and a tungsten alloy. With soluble DU, nickel or lead, a 24-hour exposure of human bone cells resulted in an increase in cells transforming to a **malignant phenotype**. In the case of DU, the number of cells undergoing this transformation was approximately 9 times greater, compared to untreated cells. When nickel was used, the increase in transformation frequency was only 7 times greater. With lead, the frequency increased only by a factor of 5.

With insoluble DU, the exposure led to an increase in transformation frequency that was 25 times greater, with an increase in frequency by a factor of 7 in the case of nickel, and by 9 in the case of tungsten alloy.

These experiments by Miller and co-workers indicate that DU is carcinogenic and can transform human bone cells to a malignant type, with the transformed cells subsequently causing tumours in mice. The rate of transformation in cells exposed to DU was greater than with nickel and tungsten alloy, both known carcinogens.

The 2010 paper by Xie and colleagues[20] also demonstrated that particulate or insoluble DU caused **neoplastic transformation** in human cells, in this case, human **bronchial epithelial cells**.

DU exposure at three different concentrations caused several pathological changes which are markers of neoplastic transformation in cells. Another finding consistent with the existence of malignant transformed cells, which have increased chromosome instability, was hypodiploidy (loss of chromosomes).

Tumourigenic: capable of producing tumours.

Osteosarcoma: an aggressive form of bone cancer (sarcomas are cancers that originate in bone or connective tissue.).

Malignant phenotype: the biological characteristics of cancer cells that divide without control, proliferate faster than normal cells and often invade surrounding tissues.

Neoplastic transformation: a change to the nature of cells which gives them the characteristics of cancer cells. For example, in the paper of Xie, lung epithelial cells lose "cell contact inhibition" when they are exposed to DU. The "cell contact inhibition" is considered one of the mechanisms controlling cell proliferation within a certain territory for normal cells.

Bronchial epithelial cells: cells that cover the inner wall of the bronchi, the branches of windpipe that lead to the lungs.

17. Miller, AC., et al., *Transformation of Human Osteoblast Cells to the Tumorigenic Phenotype by Depleted Uranium-Uranyl Chloride*. **Environmental Health Perspectives** 106(8), 465-471, 1998.

18. Miller, AC., et al., *Potential Late Health Effects of Depleted Uranium and Tungsten Used in Armor-Piercing Munitions: Comparison of Neoplastic Transformation and Genotoxicity with the Known Carcinogen Nickel*. **Military Medicine** 167, Supplement 1: 120-122, 2002.

19. *Ibid*, 120

20. Xie, H, et al., *Depleted Uranium Induces Neoplastic Transformation in Human Lung Epithelial Cells.* **Chemical Research in Toxicology**. 23, 373-378, 2010.

The researchers stated that: *"Fifty-three percent of the DU-induced transformed cell lines tested had a hypodiploid phenotype…which is consistent with a hypothesis that DU may be a human lung carcinogen."*[21]

All three of these studies showed that DU could transform human cells to a malignant type. However the mechanisms behind the malignant transformation of cells are not yet known.

In vivo studies

In the in vivo studies, papers by Hahn, Miller and their colleagues found that DU could cause tumours or leukaemia in rodents.

Hahn and his team studied the effect of DU implants in rat muscle tissue[22]. The study included three different sets of control rats because the researchers wanted to rule out the foreign body effect, where implanting a foreign object in an animal leads to tumours (known to be caused by tantalum) and to study the effect of pure radiation (radioactive Thorotrast injections were used for this). All rats, both experimental and control, were studied over their lifetimes.

The results of the experiments by the Hahn team indicated that DU exposure caused tumours, with the frequency of tumours increasing with the size of the DU fragments. Thorotrast-exposed rats had the most tumours, whereas tantalum implanted rats had practically no tumours, indicating that the results for DU-exposed rats were not due to the foreign body effect.

The in vivo study by Miller and co-workers[23], in which mice developed leukaemia, used male experimental mice with embedded DU fragments in a series of experiments. Two to three month old male mice had surgically implanted sterile pellets of DU and tantalum embedded in the lower leg. The ratio of DU to tantalum was varied across several groups.

Sixty days after implantation of the pellets, the mice were injected intravenously with murine **hematopoietic cells**, known as FDC-P1 cells. These cells are known to transform into leukaemia cells under certain conditions, including exposure to radiation.

The outcome of the experiments indicated that 76% of the mice with embedded DU pellets developed leukaemia. Mice with 6 or 8 DU pellets had more leukaemia cases than mice with only 2 DU pellets.

Miller and colleagues reported: *"This is the first report describing the consistent development of leukaemia transformation of FDC-P1 cells when injected intravenously into DU-implanted male mice…The results indicate that a uranium-altered environment plays an important role in the pathogenesis of DU-induced leukaemia."*[24]

Later in 2009, Miller and her group conducted a further study using this in vivo **leukaemogenesis** model and reported that **epigenetic** mechanisms are implicated in DU-associated leukaemia.

Hematopoietic cell: immature cells, usually situated in the bone marrow which become circulating blood cells, such as red blood cells, white blood cells and platelets.

Leukaemogenesis: the induction of leukaemia.

Epigenetics: epigenetic changes are heritable effects on gene expression which are not caused by a change in the DNA sequence. Gene expression refers to the way genetic information is passed on and used in the body to create products. DNA methylation is one of the important mechanisms of epigenetic regulation on gene expression. Epigenetic mechanisms also play a role in carcinogenesis.

21. *Ibid*, 377. The authors state that chromosome numbers *"ranged from 7 to 43."* They add that hypodiploidy is *"frequently"* found in several types of lung carcinomas *"characterized by manifold chromosomal deletions with losses of whole chromosome arms"*.

22. Hahn, FF., et al., *Implanted Depleted Uranium Fragments Cause Soft Tissue Sarcomas in the Muscles of Rats*. **Environmental Health Perspectives** 110(1), 51-59, 2002.

23. Miller, AC., et al., *Leukemic transformation of hematopoietic cells in mice internally exposed to depleted uranium.* **Molecular and Cellular Biochemistry** 279, 97-104, 2005.

24. *Ibid*, p. 102-103

3. Generation of DNA strand breaks

DNA is arranged in a structure known as a double helix. The double helix is comprised of two coiled strands of DNA, which hold the genetic information that makes up the genome. DU has been shown to cause breaks in one or both strands of DNA in both in vitro and in vivo studies. These are known as single and double strand breaks.

DU can cause single strand breaks in DNA due to both its chemical and radiological toxicity. Double strand breaks are also known to be induced by the radiological toxicity of uranium. DNA strand breaks may lead to mutations when they cannot be repaired properly. Double strand breaks can lead to serious damage at the scale of the chromosome.

In vitro studies

Yazzie and co-workers exposed **plasmid DNA** to DU in the form of uranyl acetate dehydrate[25]. They wanted to learn if **hexavalent** uranium in the form of the uranyl ion could be genotoxic in the same way that hexavalent chromium[26] (CrVI), a known carcinogen, is genotoxic.

To test whether DU could cause DNA damage in the same manner as CrVI, the researchers used ascorbate, a substance that has been shown to chemically reduce CrVI and increase the rate of DNA damage. The results showed that uranyl acetate dehydrate (UA) plus ascorbate, led to about 60% more **plasmid relaxation** than CrVI and ascorbate did. This suggests that uranium may cause strand breaks by a similar chemical mechanism to other metals.

Thiebault and colleagues used normal rat kidney cells and exposed them to depleted uranyl nitrate[27]. They used the Comet Assay, a technique for the detection of single and double strand DNA breaks, in which, the damaged DNA appears to develop a tail like a comet when viewed under a microscope. This is due to the broken strands being pulled away from the cell when the DNA is placed in an electric field. Their results indicated that both sublethal and lethal exposures to depleted uranyl nitrate caused single and double DNA strand breaks, the frequency of which increased with increasing concentrations of DU.

In vivo studies

Monleau and colleagues published a paper dealing with inhalation exposure of DU in rats[28]. In their experiments, they used DU oxide powder, one an insoluble dust, uranium dioxide (UO2), and the other, a soluble dust, uranium peroxide (UO4). These powders had been collected from workplaces in uranium fuel cycle facilities. The dosages of the two uranium oxides used were similar to the estimated amounts of DU aerosol that might be inhaled on the battlefield[29]. In both series of experiments, rats were subjected to inhaling DU aerosols through their noses for varying amounts of time, with a range of doses. Some of the groups of rats were given repeated doses, and there was also a control group.

The Comet Assay was used to determine DNA damage. Lung cells known as Broncho-Alveolar Lavage (BAL) cells were taken from the rats. DNA damage was observed in BAL cells in

Plasmid DNA: a plasmid is a small circular double-strand DNA molecule which contains very few genes.

Hexavalent: valence is a measure of an atom or molecule's capacity to form bonds. It is based on the number of electrons in the outer (valence) shell of the atom. Hexavalent means having a valence of six; a hexavalent atom or molecule has a capacity to unite with six hydrogen atoms.

Plasmid relaxation: plasmids spontaneously form a twisted structure because of the tension in the helix-structure of a DNA strand. If either of the two DNA strands becomes broken through the action of agents such as radiation or chemicals, the tension is relieved because the free end can rotate. Then a plasmid becomes a relaxed form with fewer twists.

25. Yazzie, M, et al., *Uranyl Acetate Causes DNA Single Strand Breaks In Vitro in the Presence of Ascorbate (Vitamin C)*, **Chemical Research in Toxicology.** 16, 524-530, 2003

26. Hexavalent chromium was commonly used to prevent corrosion by the US military until 2009 when a memorandum requiring its aggressive phase-out was published, concurrent with a push to find effective alternatives.

27. Thiebault, C, et al., *Uranium Induces Apoptosis and Is Genotoxic to Normal Rat Kidney (NRK-58E) Proximal Cells*, **Toxicological Sciences** 98(2), 479-487k 2007.

28. Monleau, M, et al., *Genotoxic and Inflammatory Effects of Depleted Uranium Particles Inhaled by Rats*, **Toxicological Sciences** 89(1), 287-295, 2006.

29. *Ibid*, p. 288

the group of rats given repeated doses of UO2 over a three-week period, and a group given an **acute dose** of UO2.

DNA strand breaks in kidney cells occurred only in the rats subjected to repeated exposures. However, rats in the group given an acute dose of UO4 developed kidney failure, due to the solubility of UO4.

In conclusion, the researchers stated that: *"In BAL cells, DNA lesions were linked to the dose, independent of the solubility of uranium compounds"*, they were *"correlated with the type of inhalation and were composed partly of double-strand breaks, suggesting that radiation could contribute to DU genotoxic effects in vivo."*[30]

Monleau et al. also published an in vivo pilot study in 2006[31]. The protocol and uranium oxide powers used for this study were the same as for their earlier study. In this study, single and double DNA strand breaks were induced in nasal epithelial cells as well as in BAL and kidney cells. In this study, repeated UO2 exposures, coupled with exposure to soluble UO4 particles, caused DNA damage.

The researchers discussed the possibility that this response to the combined exposure could be the result of a synergistic effect, related to the differences in solubility between the different uranium oxides: *"as toxicity could depend on particle solubility"*.

A 2010 paper by Giovanetti and co-workers studied the accumulation of natural uranium (NU) and DU in the earthworm[32]. The earthworms had been living in soil with a range of concentrations of DU or NU. Exposure periods were 7 and 28 days. DNA strand breaks were determined through the Comet Assay. DNA damage was found at very low concentrations of DU[33].

After 7 days exposure, the percentage of DNA strand breaks was around 12 percent, with no statistically significant difference between the soil contaminated with NU and DU. After 28 days exposure, there were more DNA strand breaks in earthworms living in soil with higher concentrations of both DU and NU.

The relationship between dose and damage did not appear to be linear in earthworms examined after 7 days, but after 28 days, the increase in breaks did appear to be dose dependent. Overall, the increase in DNA strand breaks was greater for NU than for DU.

Acute dose: this term is used to describe a single large dose. In this experiment, the acute dose of UO4 given to a group of rats was a single dose of UO4 dust equal to the total dose of UO4 given to another group of rats over several doses. This allowed the researchers to assess the different effects of acute and chronic doses.

30. *Ibid*, p. 293

31. Monleau, M, et al., *Distribution and Genotoxic Effects After Successive Exposure to Different Uranium Oxide Particles Inhaled by Rats*, **Inhalation Toxicology** 18, 885-894, 2006 (herein referred to as "Monleau, D&G, 2006").

32. Giovanetti, A, et al., *Bioaccumulation and biological effects in the earthworm Eisenia fetida exposed to natural and depleted uranium*, **Environmental Radioactivity** 101,509-516, 2010.

33. *Ibid*, p. 515

4. Induction of chromosomal change

Chromosomal aberrations are important **biomarkers**, which can demonstrate the genotoxicity of substances or environmental factors. DNA double strand breaks induced by ionising radiation and certain chemicals may result in characteristic types of chromosomal aberrations such as 'ring formations' and 'dicentric chromosomes'.

These aberrations by themselves are unlikely to be linked to health problems, as the damaged chromosomes cannot replicate, meaning that the damage is not passed on to daughter cells. However, the aberrations are reliable markers of genotoxic damage, such as that caused by ionising radiation, which is known to cause health problems.

There are some other markers of genotoxic effects caused by chromosome change, which are not necessarily specific to radiation, such as 'sister-chromatid exchange' and '**micronuclei**'.

There have been many published studies of chromosomal changes induced by the exposure of cells to uranium or DU, both in experimental animals as well as in exposed groups of humans.

In vitro studies

Lin and his colleagues first reported in 1993 on the **cytotoxic** and genotoxic action of uranyl nitrate ($UO2^{2+}$) in Chinese hamster ovary (CHO) cells[34]. They examined the frequencies of micronuclei, chromosomal aberrations and sister-chromatid exchange in CHO cells, which were cultured in a medium with dissolved uranyl nitrate. Uranyl nitrates at certain concentrations increased the frequency of both micronuclei and chromosomal aberrations. Six types of chromosome aberrations: chromatid breaks, chromosome breaks, acentrics, dicentrics, interchange and ring, were observed in CHO cell cultures in the uranyl nitrate medium. The frequency of total aberrant cells was significantly increased by exposure to uranyl nitrate

As the exposure to uranyl nitrate in this study is short, only 2 hours, and the radiation exposure dose was very low, they assumed that the cytotoxic and genotoxic effects resulted from the chemical toxicity of uranium. They also suggested that this cytogenetic toxicity provides a biological basis for the potential **teratogenic** effect of uranium on developing foetal mice.

In the period since 2002, many papers on the genotoxicity of uranium have been published. Miller and her colleagues reported on the frequency of dicentric chromosomes in human osteoblast (HOS) cells that were exposed to soluble DU for a period of 24 hours[35]. The frequency of dicentric chromosomes was measured in HOS cells and was observed to increase with the concentration of DU. No significant increase in the number of dicentrics was observed in the cells that were exposed to nickel and tungsten controls, at any dose. According to the results, the researchers speculated that radiation contributes to DU-induced effects on chromosomes.

Wise and her colleagues showed that DU particles in the form of uranium trioxide (UO3) were **clastogenic** to human lung cells[36]. The frequency of

Biomarker: a measureable characteristic or substance that can be used as an indicator of a certain biological state.

Micronuclei: chromosomal fragments which are not incorporated into the nucleus at the time of the cell division. Testing for the formation of micronuclei is a reliable method for the evaluation of the genotoxicity of substances.

Cytotoxic: The property of being toxic to cells. Cytoxicity can cause inhibition of growth, functional disturbance or kill cells.

Teratogenic: capable of causing a malformation of a foetus during development.

Clastogenic: capable of causing breaks in chromosomes.

34. Lin, RH., et al., *Cytogenetic toxicity of uranyl nitrate in Chinese hamster ovary cells*. **Mutatation Research** 319: 197-203, 1993.

35. Miller, AC., et al., *Observation of radiation-specific damage in human cells exposed to depleted uranium: dicentric frequency and neoplastic transformation as endpoints.* **Radiation Protection Dosimetry** 99(1-4), 275-278. 2002.

36. Wise, SS., *Particulate Depleted Uranium is Cytotoxic and*

cells with damaged chromosomes increased in a time and concentration dependent manner. However, the soluble form of DU - as uranium acetate - was not found to be clastogenic in their experiment. It has been speculated that the human lung cells used in the study contributed to the difference between these results, and those in other studies that have shown a significant increase in chromosomal changes from exposure to a soluble form of DU.

Another research group, Darolles et al., studied a **mouse embryo fibroblast cell line** and examined the genotoxicity of DU and enriched uranium (EU) to the cells[37]. Their results showed that DU and EU are low and high clastogens respectively. Interestingly, DU showed a higher **aneugenic** effect compared to EU at the same concentration.

LaCerte and her colleagues observed the clastogenic effects of DU on human bronchial epithelial cells[38]. The cells were examined for chromosome damage in the form of breaks, **gaps**, dicentrics and acentric fragments. Cells treated with DU, in the form of UO3 for 24 hours, did not show a significant increase in chromosome damage, while cells treated with DU for 48 hours showed a uranium-dose-dependent increase in chromosome damage. They concluded that DU may be a human bronchial carcinogen through a mechanism that involves DNA breakage after longer exposure.

In vivo studies

As early as 1990, Hu and Zhu published a study on uranium causing chromosomal aberrations in male mouse germ cells[39]. The background to this study was a discussion of the toxicity of EU, which is one of the main fuels for nuclear power stations. They injected uranyl fluoride into the testes of mice; the mice were killed and samples of **spermatogonia and spermatocytes** were taken at 1, 13, 36 and 60 days after the injection.

Significant increases in the frequency of gap damage to the chromosomes of spermatogonia were observed at 1 and 13 days after injection, compared to the control. The general tendency was for the production of breaks to increase with the dose of uranyl fluoride. The incidence of aberrant cells in spermatocytes was largely dependent on the administered dose.

Human studies

A study on the rate of chromosome aberrations in uranium miners in Namibia was reported in 1997[40]. A representative cohort of 75 non-smoking, HIV negative miners was compared to 31 non-smoking control individuals with no occupational history of mining and who lived in the same country more than 12 miles from the mine.

The average background radiation dose of the miners was lower than the recommended annual radiation dose for workers but higher than the recommendation for the general public[41]. The most likely route of uranium exposure for miners was through inhalation. In the miners, the excretion in urine of the isotope U^{238} was 6 times higher than in the control group, which suggested significantly higher internalised uranium contamination in the miners.

The chromosome aberrations were analysed in 32,177 cultured blood **lymphocytes** collected from 16 miners, and 9,376 from 4 control individuals; an

Mouse embryo fibroblast cell line: a type of cell used in experiments, which can be cultured for many generations.

Aneugenic: capable of causing a condition in which a cell has an abnormal number of chromosomes.

Gap: gaps are a kind of chromosome damage which appear similar to a break. Gaps are a localised area of thinning in a chromatid - one copy of the duplicated chromosome. Gaps have the potential to become a break.

Spermatogonia and spermatocytes: both spermatogonia and spermatocytes are immature male germ cells. Spermatogonia differentiate into spermatocytes after a type of cell division that is specific to germ cells.

Lymphocyte (and peripheral lymphocytes): a lymphocyte is a type of white blood cell that works in the immune system. Peripheral lymphocytes are lymphocytes which circulate in the body's blood flow.

Clastogenic to Human Lung Cells. **Chemical Research in Toxicology** 20(5): 815-820, May 2007.

37. Darolles, C, et al., *Different genotoxic profiles between depleted uranium and enriched uranium.* **Toxicolology Letters** 192, 337-348, 2010.

38. LaCerte, C, et al., *Particulate depleted uranium is cytotoxic and clastogenic to human lung epithelial cells.* **Mutation Research** 697, 31-37, 2010.

39. Hu, QY., and Zhu, SP., *Induction of chromosomal aberrations in male mouse germ cells by uranyl fluoride containing enriched uranium.* **Mutation Research** 244: 209-214, 1990.

40. Zaire, R., *Unexpected Rates of Chromosomal Instabilities and Alterations of Hormonal Levels in Namibian Uranium Mines.* **Radiation Research** 147, 579-584. 1997.

41. The average dose in the group was 1.8 mSv/year.

average of 2,000 cells per individual for both groups. The rate of lymphocytes with chromosomal aberrations was 0.82% in miners and 0.245% in the control group.

Various categories of chromosomal aberrations were observed in the miners, such as translocation, dicentrics, chromosome breaks, chromatid breaks, centric rings, inversions and trisomies. Cells with multiple chromosome aberrations were also detected in the miners. A similar type of aberrant cells has been observed in the survivors of the Hiroshima and Nagasaki atomic bombs, as well as in children exposed to radiation as a result of the Chernobyl accident.

The exposure conditions faced by uranium miners are different from the exposure to the fine DU particles which result from DU weapons use in military settings. However, the data from uranium miners who have significant internalised uranium contamination may be helpful in considering the effect of **chronic low dose** radiation from internalised alpha particles, such as DU.

Several studies, from 2003 onwards, have examined chromosome aberrations in the peripheral lymphocytes of DU-exposed populations. These examined military veterans and residents living near sites with DU contamination.

Schroeder and her colleagues reported the results of chromosome analysis of peripheral lymphocytes in a group of British veterans; 13 of whom served in the 1991 Gulf War, two in the Balkans and one who had served in both[42]. All sixteen veterans had experienced a situation involving exposure to DU through inhalation. The study excluded veterans who had undergone previous radiation or chemical therapy, had greater than average exposure to medical diagnostic radiation, were heavy smokers or who had previously worked in the nuclear industry.

The results were compared to a laboratory control group of 40 healthy volunteers in Germany, and about 1,000 lymphocytes were analysed for each veteran. The frequency of dicentric and ring chromosomes, both of which can be indicative of radiation effects, was more than 5 times greater in the veterans group than in the control group.

This was a pilot study with some limitations, such as the small sample size, not excluding all possible sources of radiation, and the possibility that the exposed and non-exposed laboratory controls had different backgrounds.

The authors did not expect the rate of aberrations in veterans to be so high, as many of the dicentric-chromosomes would have been expected to die within ten years of a single radiation exposure event. Possible mechanisms behind the observed cytogenetic effects were discussed, such as synergetic effects with other toxic factors on the battlefield, continuous environmental contamination with DU, and chronic **alpha-irradiation** from internalised DU particles to the local tissue.

In a study of US veterans of the 1991 Gulf War, researchers did not find a significant increase in the frequency of micronuclei in peripheral lymphocytes of veterans with DU embedded fragments in their bodies, compared to the frequency in veterans without DU fragments[43].

There have been several studies from the Balkan countries where DU weapons have been used. In 2005, the research team of Krunić, Ibrulj and Haverić in Bosnia and Herzegovina reported a significant increase in micronuclei frequency in the peripheral blood

Chronic dose: small doses of a toxic substance or radiation over a long period of time that result in a cumulative negative effect, in contrast to a single acute dose.

Alpha particles, alpha radiation: alpha radiation is an emission of alpha particles. Alpha particles consist of two protons and two neutrons. This type of radiation can cause serious damage to local tissue or cells when the alpha emitting substance is situated inside the body.

42. Schroeder, H., *Chromosome aberration analysis in peripheral lymphocytes of Gulf War and Balkans War veterans.* **Radiation Protection Dosimetry** 103(3), 211-219. 2003.

43. Bakhmutsky, MV., *Long term depleted uranium exposure in Gulf War I veterans does not cause elevated numbers of micronuclei in peripheral blood lymphocytes.* **Mutatation Research** 2011 Feb 28;720(1-2):53-7. Epub 2010 Dec 15.

lymphocytes of a DU exposed group, in comparison to a control group. Their exposed group included 30 employees of a tank-repair facility and ammunition storage depot (which had both been hit by DU ammunition from US A10 aircraft) at Hadžići, near Sarajevo, who were environmentally exposed to DU. The control group consisted of 30 inhabitants from West Herzegovina, which was considered environmentally clean[44].

The same research group also reported the results of chromosome aberrations of the peripheral lymphocytes of 3 population groups: 26 employees of the contaminated tank-repair facility in Hadžići, 30 inhabitants of Sarajevo (possibly exposed to war-related activities) and 28 inhabitants in Posušje (not exposed to war-related activities). They found a higher frequency of chromosome aberrations in the sample of the employees of the facility in Hadžići (3.2%) compared to the inhabitants of Sarajevo (1.6%) and Posušje (1.5%)[45].

In 2004, Milačić and her colleagues reported a higher incidence of chromosome aberrations in the peripheral lymphocytes of residents of Vranje and Bujanovac, DU-contaminated areas in southern Serbia. They also reported that the increased incidence was not higher than the incidence of chromosome aberrations in workers who lived in an uncontaminated area in central Serbia and who had been occupationally exposed to radiation[46].

Another similar study by Milačić and colleagues reported in 2009 on four groups: two test groups and two reference groups. One test group was composed of media workers from the Radio Television Station who had been working at locations that were attacked with DU ammunition during the war, the other group included occupationally exposed radiation workers from Vranje, an area contaminated with DU. They were compared to two test groups: one group that had been occupationally exposed to ionising radiation, and another that had not.

The media workers thought to have been exposed to DU were twice as likely to have chromosome aberrations when compared to workers who were not occupationally exposed to radiation. The occupationally exposed radiation workers from the DU contaminated area were found to have a higher risk of chromosome aberrations compared to occupationally exposed radiation workers in clean areas[47].

44. Krunić, A., *Micronuclei frequencies in peripheral blood lymphocytes of individuals exposed to depleted uranium.* **Arhiv za higijenu rada i toksikologiju** 56(3), 227-32, Sept. 2005.

45. Ibrulj, S., *Chromosome aberrations as bioindicators of environmental genotoxicity*, **Bosnian J Basic Medical Science**, 2007, 7 (4), 311-316.

46. Milačić, S., *Examination of the health status of populations from depleted uranium contaminated regions.* **Environmental Research** 75(2-10) 2004.

47. Milačić, S., *Identification of Health Risks in Workers Staying and Working on the Terrains Contaminated with Depleted Uranium.* **Radiation Research** 50: 213-222. 2009.

5. Generation of oxidative damage to DNA

The energy from ionizing radiation can break down the water molecules that are ubiquitous in living things, giving off hydroxyl radicals, one of the most reactive **free radicals** found in the body. Hydroxyl radicals, together with other molecules such as superoxide and hydrogen peroxide, are called Reactive Oxygen Species (ROS). ROS are unstable and easily react with other molecules in a cell.

Radiation, including DU's alpha radiation, can therefore damage DNA indirectly through the generation of ROS. Studies have found that ROS can cause **oxidative** damage in living cells, and they play an important role in the development of various diseases, including cancer.

ROS are produced through normal cell metabolism and are countered by natural defence mechanisms that protect the cell by keeping a balance between ROS and antioxidants.

DU can also act as a **catalyst**, speeding up the chemical reaction of ROS, which can damage DNA even without significant irradiation.

A number of articles indicate that the presence of low and high concentrations of DU can cause oxidative damage to DNA.

In vitro studies

In 2002, Miller and co-workers showed that DU could catalyse the oxidation of ascorbic acid 6 times more effectively than the known carcinogen nickel, under the same experimental conditions[48]. The researchers wrote that: *"DU complexes might contribute to a gradual accumulation of oxidative damage that is important in tumour production".*[49] The researchers determined that this was done by chemical means.

This observation was further supported by a 2006 study by Pourahmad and colleagues. Their study found that ROS were developed in rat liver cells that were exposed to a uranium-based compound[50].

Periyakaruppan and colleagues looked at oxidative stress in rat lung epithelial cells exposed to uranium acetate for 3 hours, and found there was a significant generation of ROS at higher concentrations of uranium acetate[51].

Orona and Tasat also investigated the effect of DU exposure on rat lung cells (alveolar **macrophages**)[52]. DU exposure led to the generation of strong ROS at very low DU concentrations. The researchers hypothesised that the conditions created by DU exposure in the macrophages could lead to: *"the development of lung pathologies associated with uranium exposures".*[53]

In vivo studies

A number of papers have been published that have sought to measure the levels of oxidative damage caused by DU exposure in vivo. These studies have primarily studied the effects of exposure on rats and fish.

Free radicals: atoms or molecules that have unpaired electrons and are highly reactive with surrounding molecules. The hydroxyl radical is an important free radical that can cause damage to all types of macromolecules in the body, including DNA.

Oxidation: refers to the interaction between oxygen molecules and other molecules. It is more precisely defined as the loss of electrons when molecules interact chemically. Oxidative stress in a body caused by free radicals can lead to various diseases including cancer.

Catalyst: a substance that speeds up a chemical reaction.

Macrophage: a large white blood cell that ingests foreign particles and infectious microorganisms. Macrophages are key players in the immune response to foreign invaders of the body.

48. Miller, AC., et al., *DU-catalyzed oxidative DNA damage: absence of significant alpha particle decay.* **Inorganic Blochemistry**. 91(1):246-252, 2002.

49. *Ibid*, p. 251.

50. Pourahmad, J, et al., *A search for cellular and molecular mechanisms involved in depleted uranium (DU) toxicity.* **Environmental Toxicology**, 21: 349–354. doi: 10.1002/tox.20196, 2006.

51. Periyakaruppan, A, et al., *Uranium induces oxidative stress in lung epithelial cells.* **Archives of Toxicology**. 8(16):389-395, 2007.

52. Orona, NS., and Tasat, DR., *Uranyl nitrate-exposed rat alveolar macrophages cell death: Influence of superoxide anion and TNF alpha mediators.* **Toxicology and Applied Pharmacology.** 261(3): 309-316, 2012.

53. *Ibid*, p. 315.

The exposure levels used in the studies differed but they generally sought to replicate low or moderate exposures. Some researchers used chronic exposure to uranium while others utilised short-term exposures. The studies used significant changes in **antioxidant** defence systems as biomarkers to measure oxidative stress. These were compared to antioxidant activity in control groups.

Another biomarker for the presence of oxidative stress was the hydroperoxidation of the lipids found in cell membranes. Researchers found that a secondary product from the **lipid peroxicidation** by ROS forms DNA adducts, which may lead to mutations[54].

DU exposure has been found to cause oxidative stress in vivo in rats[55,56,57,58] and different small fish[59,60,61,62,63,64], often at low or moderate waterborne DU exposure. Oxidative stress was observed in a variety of tissues: lung (and gills), kidney, testes, brain, muscle and liver. Several researchers pointed out that ROS attack followed by DNA damage was intrinsic to heavy metal toxicity.

Antioxidant: a molecule that inhibits the oxidation of other molecules. Endogenous anti-oxidant defence systems are the internal systems responsible for controlling oxidation in a body. The system is composed of an enzyme called superoxide dismutase (SOD) and other molecules such as glutathione and Co-enzyme Q 10.

Lipid peroxidation: lipids are hydrophobic molecules such as cholesterol, fatty acid, phospho-lipid and others. Lipid peroxidation is oxidative degradation of lipids.

54. Marnett, LJ., *Lipid peroxidation – DNA damage by malondialdehyde*. **Mutation Research** 424, 83-95, 1999 and L.J. Marnett. *Oxy radicals, lipid peroxidation and DNA damage.* **Toxicology** 181-182, 219-222, 2002.

55. Monleau, M, et al., *Genotoxic and Inflammatory Effects of Depleted Uranium Particles Inhaled by Rats.* **Toxicological Sciences** 89(1): 287-295, 2006c.

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6. Genomic instability caused by DU

Genomic instability is a state where a cell is prone to genomic changes or has an increased propensity for genomic alterations[65].

Genomic instability is a characteristic of almost all cancer cells and can take many forms, ranging from subtle changes of DNA sequences, to alterations of chromosome numbers and/or the structure of chromosomes.

Genomic instability is a major driving force of **tumourigenesis**. Additional genetic alterations among the offspring of cancer cells can result in sub-populations of cells with even more aggressive cancerous properties.

In vitro studies

Miller and her research group published a paper that showed that DU can cause genomic instability in human **osteoblast cells** (HOS)[66].

They measured cell lethality and micronuclei formation, at various times after exposure to DU, nickel or gamma radiation, and at various doses. They found that DU can induce delayed cell death and micronuclei formation. Compared to gamma radiation or nickel, DU exposure resulted in a greater occurrence of genomic instability in their in vitro experiments.

In vivo studies

If a germ-line cell (i.e. an egg or sperm) of an organism has a genomic instability that can be transmitted as the cell divides, some trans-generational changes might occur in the offspring of the organism.

There are many studies on the impact of DU exposure on reproduction[67]. However, there are very few studies that specifically focus on transmissible genetic damage to offspring.

Another study by Miller and her team indicated that male mice with DU pellets implanted in their muscles could pass on genomic instability to the somatic cells of unexposed offspring[68].

They used a transgenic mouse system employing a **vector** that carries a marker gene. The researchers looked at the mutation frequencies of the marker gene in a vector, which was recovered from the bone marrow cells of the first generation offspring of exposed male parents and then examined in vitro.

The frequency of mutations was compared to the offspring of mice that had been exposed to tantalum, nickel and gamma radiation, and to a control group. The results demonstrated that as paternal DU dose increased, there was a trend towards higher mutation frequency in the DNA obtained from the offspring.

Genomic: of or relating to the genome. A genome is an organism's complete set of DNA, including all of its genes.

Tumourigenesis: the process involved in the creation of tumours.

Osteoblast cells: juvenile bone cells.

Vector: a nucleic acid that is able to maintain itself within a host cell. Vectors are used by researchers to transfer genetic material into cells.

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7. Formation of uranium-DNA adducts

Uranium-DNA adducts are formed when uranium chemically binds to molecules on DNA strands.

Stearns and her colleagues found that DU in the form of **uranyl acetate** led to the formation of uranium-DNA adducts in Chinese hamster ovary (CHO) cells[69].

Uranyl acetate: is an acetate salt of uranium that is water soluble so its aqueous solution is often used in animal and cellular experiments to investigate the toxicity of uranium. Uranyl acetate is used as a stain for DNA during electron microscopy because of its propensity to bind to phosphorous.

In vitro study

The only piece of research done on this subject to date was an in vitro study by Stearns and her team. The researchers used two CHO lines, the parental CHO cells which had the usual component of DNA repair proteins and the daughter cells, which were deficient in a repair protein, and so were presumably more sensitive to any DNA damage.

Mutagen: a physical or chemical agent that causes changes to genetic materials such as DNA, resulting in mutations. A mutation is a change of the nucleotide sequence of genetic materials.

The experiment used a range of concentrations of uranyl acetate, and grew the CHO lines for either 48 hours or 24 hours before they were exposed.

After various procedures, diluted samples were tested for uranium and phosphorus. This allowed the researchers to calculate the ratios of uranium to DNA-Phosphorus and to evaluate the number of uranium-DNA adducts. The results showed that DU can cause the formation of uranium-DNA adducts[70].

Other findings of the experiments were that the uranyl acetate was weakly **mutagenic**. It also caused DNA strand breaks in CHO cells.

69. Stearns, DM., et al., *Uranyl acetate induces hprt mutations and uranium-DNA adducts in Chinese hamster ovary EM9 cells.* **Mutagenesis** 20(6), 417-423, 2005

70. *Ibid*, p.420.

Is DU a carcinogen?

As mentioned in the introduction, the International Agency for Research on Cancer (IARC) has classified all substances that emit alpha radiation as Group 1 carcinogens when inside the body. This means that DU inside the body is a known carcinogen.

Even if internalised radioactive substances that emit alpha radiation were not already categorised in this way, the studies listed in this report together make a compelling case for DU *inside the body* to be categorised as Group 1.

This is because there is *sufficient* evidence of carcinogenicity in animals, as DU has been shown to cause tumours in at least two different species of animal. Although the direct evidence for carcinogenicity in humans would currently be considered *inadequate* according to the IARC framework[71], due to the lack of studies on humans known to have been exposed, the weight of other types of evidence is sufficient for a Group 1 classification.

Under the globally accepted IARC system of evaluation, an agent can still be placed in Group 1 if there is *inadequate* evidence of carcinogenicity in humans - due for example to a lack of research, but there is *sufficient* evidence of carcinogenicity in animals and the agent is known to act through a relevant mechanism of carcinogenicity. We can therfore say that, *inside the body*, DU is a Group 1 carcinogen.

This raises the question of whether DU as an environmental contaminant is also a carcinogen. In other words, can we say that DU *in the environment*, such as the contamination left by DU weapons, is also carcinogenic?

It is known that DU in the environment can enter the body through a number of different pathways but only a handful of studies have been done on persons known to be exposed to DU. Very few of the subjects in those studies were exposed to DU as an environmental hazard.

Without reliable experimental data on the quantities of DU which might get into the body following exposure to DU in the environment, it is difficult to make an exact judgement. Studies on high risk civilian groups living or working in proximity to DU contamination are therefore urgently required.

In the interim, DU *in the environment* should be considered a probable, or at the least a possible, carcinogen.

71. Preamble to the IARC Monogrpahs, Scientific Review and Evaluation: http://monographs.iarc.fr/ENG/Preamble/currentb6evalrationale0706.php

Conclusion

The studies reviewed in this report demonstrate without a doubt, that DU is a genotoxic agent. We reviewed around 50 peer-reviewed studies on the induction of mutations, the conversion of cells to a tumourigenic state, DNA strand breaks, chromosome change, oxidative damage to DNA, genomic instability and uranium-DNA adducts; the results showing that DU is implicated in numerous expressions and mechanisms of DNA damage.

While the studies reviewed mostly rely on data from laboratory and animal experiments, the range of studies, and the fact that these results have been observed in several different animal species amount to a strong body of evidence on the potential effects of DU on human health. The potential for DU to cause genotoxic effects in human populations seems unarguable, given the weight of the evidence.

Genotoxic effects are known to be involved in the development of cancer and other diseases, and there is also the potential for genetic damage to be passed on to children, creating health problems for future generations. It should also be noted that the genotoxic effects of DU will not be limited to humans, and are likely to be hazardous to other organisms in the environment.

The Precautionary Principle is a central pillar of environmental protection and holds that preventative measures should be undertaken if there is potential for harm, even if there are still some knowledge gaps[72].

Applying precautionary values to DU weapons provides a robust framework for assessing their acceptability and for informing a global response[73]. The use of DU weapons in conflict is uncontrolled and unpredictable, targeting guidelines intended to reduce harm are commonly disregarded and there are no legal obligations to address contamination nor any associated civilian health impact.

ICBUW believes that the evidence for the potential harm from DU has passed the 'threshold of plausibility', i.e. even though there are ongoing uncertainties, the available information is sufficient to justify action. This calculation is not only based on DU's potential health effects but also those factors relating to its use in conflict and its lack of effective post-conflict management, factors that strongly influence the likelihood of civilian exposure.

This compelling evidence on the genotoxic effects of DU only strengthens the case for an immediate moratorium on their use, followed by their prohibition in international law.

72. See: Principle 15 of the Rio Declaration on Environment and Development at the 1992 Conference on Environment and Development. http://www.un.org/documents/ga/conf151/aconf15126-1annex1.htm

73. See: Weir, D, *Precaution in Practice: challenging the acceptability of depleted uranium weapons*. ICBUW, 2012. Available via: http://www.bandepleteduranium.org/en/precaution-in-practice

Glossary

Acute dose A single large dose over a short period of time. (p15)

Alpha particles, alpha radiation Alpha radiation is an emission of alpha particles. This radiation can cause serious damage to local tissue or cells when the alpha emitting substance is situated inside the body. (p18)

Aneugenic Capable of causing a condition in which a cell has an abnormal number of chromosomes. (p17)

Antioxidant A molecule that inhibits the oxidation of other molecules. (p21)

Biomarker A measureable characteristic or substance that can be used as an indicator of a certain biological state. (p16)

Bronchial epithelial cells Cells that cover the inner wall of the bronchi, the branches of windpipe that lead to the lungs. (p12)

Catalyst A substance that speeds up a chemical reaction. (p20)

Chronic dose Small doses of a toxic substance or radiation over a long period of time that result in a cumulative negative effect, in contrast to a single acute dose. (p18)

Clastogenic Capable of causing breaks in chromosomes. (p16)

Cytotoxic Property of being toxic to cells. The toxicity can cause inhibition of growth, functional disturbance or the death of the cells. (p16)

Epigenetics Epigenetic changes are heritable effects on gene expression which are not caused by a change in the DNA sequence. Gene expression refers to the way genetic information is passed on and used in the body to create products. (p13)

Free radicals Atoms or molecules that have unpaired electrons and are highly reactive with surrounding molecules and which can therefore cause damage to all types of macromolecules in the body, including DNA. (p20)

Gap A form of chromosome damage which appears similar to a break. Gaps are actually a localised area of thinning in a chromatid, which is one copy of duplicated chromosome. (p17)

Genome A genome is an organism's complete set of DNA, including all of its genes. (p22)

Genotoxicity The propensity to cause damage to DNA or to change DNA expression. (p9)

Germ cells and germ-line Germ cells are sperm cells and egg cells. A germ-line, includes germ cells and those cells that give rise to them. (p10)

Hematopoietic cell Immature cells, usually situated in the bone marrow and which become circulating blood cells, such as red blood cells, white blood cells and platelets. (p13)

Hexavalent Valence is a measure of an atom or molecule's capacity to form bonds. It is based on the number of electrons in the outer (valence) shell of the atom. Hexavalent means having a valence of six. (p14)

Ionising radiation A type of radiation that has enough energy to liberate an electron from an atom or a molecule; types of ionising radiation include alpha, beta and gamma radiation and X-rays. (p11)

Leukaemogenesis Induction of leukaemia. (p13)

Lipid peroxidation Lipids are hydrophobic molecules such as cholesterol, fatty acid, phospho-lipid and others. Lipid peroxidation is the oxidative degradation of lipids. (p21)

Lymphocyte A lymphocyte is a type of white blood cell that works in the immune system. (p17)

Macrophage A large white blood cell that ingests foreign particles and infectious microorganisms. Macrophages are key a player in the immune response to foreign invaders of the body. (p20)

Malignant phenotype The biological characteristics of cancer cells that divide without control, proliferate faster than normal cells and often invade surrounding tissues. (p12)

Micronuclei Chromosomal fragments that are not incorporated into the nucleus at the time of the cell division. Testing the formation of micronuclei is a reliable method for the evaluation of the genotoxicity of substances. (p16)

Mouse embryo fibroblast cell line A type of experimental cell, which can be cultured for many generations. (p17)

Mutagen Physical or chemical agent that causes changes to genetic materials such as DNA, resulting in mutations. A mutation is a change of the nucleotide sequence of genetic materials. (p23)

Neoplastic transformation A change to the nature of cells that gives them the characteristics of cancer cells. (p12)

Osteoblast cells Juvenile bone cells. (p22)

Osteosarcoma An aggressive form of bone cancer (sarcomas are cancers that originate in bone or connective tissue). (p12)

Oxidation Oxidation refers to the interaction between oxygen molecules and different molecules. It is more precisely defined as the loss of electrons when molecules interact chemically. Oxidative stress in a body caused by free radicals can lead to various diseases including cancer. (p20)

Plasmid DNA A plasmid is a small circular double-strand DNA molecule that contains very few genes. (p14)

Plasmid relaxation Plasmids spontaneously form a twisted structure because of the tension in the helix-structure of a DNA strand. If either of the two DNA strands becomes broken through the action of agents such as radiation or chemicals, the tension is relieved because the free end can rotate. Then a plasmid becomes a relaxed form with fewer twists. (p14)

Somatic cells Any cells in the body except germ cells. (p10)

Spermatogonia and spermatocytes Both are immature male germ cells. Spermatogonia differentiate into spermatocytes after a type of cell division which is specific to germ cells. (p17)

Teratogenic Capable of causing a malformation of a foetus during the development. (p16)

Tumourigenic Capable of producing tumours. (p12)

Tumourigenesis The process involved in the creation of tumours. (p22)

Uranyl acetate Acetate salt of uranium that is water soluble so its aqueous solution is often used in animal and cellular experiments to investigate the toxicity of uranium. (p23)

Vector A nucleic acid that is able to maintain itself within a host cell. Vectors are used by researchers to transfer genetic material into cells. (p22)

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About ICBUW

The International Coalition to Ban Uranium Weapons (ICBUW) was launched in 2003 to take an evidence-based approach to examining the acceptability of the use of depleted uranium weapons. ICBUW is based in Manchester, UK and represents more than 160 civil society organisations worldwide.

ICBUW campaigns for a ban on the use of uranium in all conventional weapons and weapon systems and for monitoring, health care, compensation and environmental remediation for communities affected by their use.

The main focus of ICBUW's work has been to inform and advise policy makers and governments on the threat to human health and the environment from uranium weapons. ICBUW also researches, produces and disseminates information, offers advice to its member groups and encourages domestic and regional coalition building and skills sharing. ICBUW is grateful to the Polden-Puckham Charitable Foundation for supporting its educational outreach work and to the Norwegian Ministry of Foreign Affairs for its support for our core and project costs.

In 2012, and together with PAX, we launched the Toxic Remnants of War Project, which aims to take a broader view of the humanitarian and environmental impact of the toxic legacy of military activities. To learn more about the project, please visit: **www.toxicremnantsofwar.info** or follow **@detoxconflict**

For more information on ICBUW's work, please visit **www.icbuw.org** or follow **@ICBUW**
Other publications on DU from ICBUW and our members are available online via: **http://www.icbuw.org/publications**

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