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Implications for environmental health of multiple stressors

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Abstract

Recent insights into the mechanisms underlying the biological effects of low dose effects of ionising radiation have revealed that similar mechanisms can be induced by chemical stressors in the environment. This means that interactions between radiation and chemicals are likely and that the outcomes following mixed exposures to radiation and chemicals may not be predictable for human health, by consideration of single agent effects. Our understanding of the biological effects of low dose exposure has undergone a major paradigm shift. We now possess technologies which can detect very subtle changes in cells due to small exposures to radiation or other pollutants. We also understand much more now about cell communication, systems biology and the need to consider effects of low dose exposure at different hierarchical levels of organisation from molecules up to and including ecosystems. Furthermore we understand, at least in part, some of the mechanisms which drive low dose effects and which perpetuate these not only in the exposed organism but also in its progeny and in certain cases, its kin. This means that previously held views about safe doses or lack of harmful effects cannot be sustained. The International Commission on Radiological Protection (ICRP) and all national radiation and environmental protection organisations have always accepted a theoretical risk and have applied the precautionary principle and the LNT (linear-non-threshold) model which basically says that there is no safe dose of radiation. Therefore even in the absence of visible effects, exposure of people to radiation is strictly limited. This review will consider the historical context and the new discoveries and will focus on evidence for emergent effects after mixed exposures to combined stressors which include ionising radiation. The implications for regulation of low dose exposures to protect human health and environmental security will be discussed.

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1. Biology of 'non-targeted' effects

Within conventional radiobiology as accepted in the 1950s continuing through to the 1990s there was little consideration of epigenetic effects, because the traditional concept of radiobiology was based on target theory [1, 2]. For an effect to occur, radiation had to hit a defined target within the cell, assumed to be DNA. Assumptions about the number of targets hit could then be made from measurements of dose and dose rate [3, 4]. The evolution of non-targeted radiobiology meant that certainly at low doses the previous assumptions needed to be reconsidered in the light of the existence of non-DNA mechanisms [5–7]. The mechanisms underlying radiation effects are not constant with respect to dose and it would now be generally accepted that low dose effects are mechanistically different to high doses effects. This is not to say the mechanisms are necessarily mutually exclusive but it does mean that non-targeted effects will contribute more to the overall outcome at low doses where targeted effects are small. Targeted effects will predominate at high doses and in situations where non-targeted effects have been inhibited or otherwise prevented. In terms of the progression of radiobiological thinking in this field, disease caused by radiation no longer had to be exclusively genetically based, but radiation could promote or exacerbate systemic disease. This disease could have been caused for example by a chemical mutagen [8–10]. Equally, the radiation could facilitate a non-mutation based inflammatory type disease [11–14]. These concepts, although largely accepted theoretically by the radiobiology community, have been difficult to prove epidemiologically because of what are generally called 'confounding variables' such as smoking, drinking, age, gender, or concurrent past or future exposures to the same or a different pollutant [15, 16]. Of course these actually reflect the futility of trying to assign causation, as defined in epidemiology, to one agent when the doses are low! It has also been argued that radiation and many chemical 'pollutants' might actually boost the immune system and be good [17–19]. The hormetic argument has many interesting applications but is unproven with regard to multiple pollutants. This further adds to the confusion and controversy surrounding low dose exposures. The essential point is that there will be huge individual variation due to involvement of epigenetic and non-targeted factors in the response [20–22]. At any one time we are as unique epigenetically as we are genetically. Epigenetic differences are linked to gender and lifestyle. In theory therefore a low dose of radiation could cause any number of effects ranging from beneficial to death-inducing disease depending on the context of the exposure and the interplay of factors such as cell communication, microenvironment, tissue infrastructure and a whole host of systemic variables which influence outcome from a cellular track of ionising radiation [23, 24].

2. Radiation as 'just another stressor'

Key developments leading to the current widespread acceptance of ionising radiation as 'just another stressor' include;

(1) The development of very sensitive techniques such as M-FISH, for detecting chromosomal abnormalities [25–28].
(2) The emergence of studies showing that delayed or persistent sub-optimal survival (reproductive death) could be seen in surviving progeny of irradiated cells [29–32].
(3) The gradual understanding of genomic instability as a mechanism by which low doses of radiation could cause delayed or persistent damage to chromosomes [33–37].
(4) The development of knowledge of 'bystander effects' whereby chromosome damage, death, DNA damage and various other consequences occur in cells receiving signals from cells irradiated with low doses of radiation [38–43].

(5) Criticism of the epidemiological research undertaken after the Hiroshima and Nagasaki bombs as ignoring the damage from residual radiation [44, 45].

The new paradigm emerged initially as a result of re examination of firmly held beliefs and some odd results in the laboratory which did not fit. Proof of the new hypotheses required the application of techniques such as molecular imaging, M-FISH, and SKY as well as the development of tissue culture techniques for human *normal* tissues which permitted functional studies to be performed [46]. Older studies tended to use high doses on a limited number of cell lines or highly inbred animal strains. These tended to thrive in the laboratory but were often unrepresentative of tissues in the outbred human or non-human [47–49].

The emerging non-targeted effects field has three important consequences for radiation protection and risk assessment:

2.1. The concept of hierarchical levels

Hierarchical levels stretch from the individual 'down' (organs–tissues–cells–organelles–genes) and 'up' to populations (multiples individuals/single species (multiples species—ecosystems)). Confusion in the low dose exposure field (radiation and chemical) arise from lack of consideration of this concept. Most of the arguments about whether radiation is 'good for you' or 'bad for you' fail due to lack of consideration of the hierarchical level at which the effect occurs and because most of the arguments are anthropocentric. For example cell death is seen as a 'bad' effect but if it removes a potentially carcinogenic cell from the population of cells in a tissue it could prevent cancer starting and could be seen as 'good'. Similarly in the non-human populations—death of radiosensitive individuals which cannot adapt to the changed (now radioactive/chemically polluted) environment, could be 'good' for the population in evolutionary terms depending on the life stage and reproductive status when the effects manifest, although death will always be 'bad' for the individual. It is only by considering responses in context, that any conclusions can be drawn about risk or harm.

2.2. Spatio-temporal concepts

There are two aspects to this—one is simply, the age of the irradiated unit and the spatial deposition pattern of the ionising energy. This concept is relevant across all hierarchical levels. Obvious considerations are the age or maturity of the unit (e.g. cell, organ, lifeform, ecosystem) receiving the track, the density of the energy deposition, the lifetime of the unit and its importance in the context of functionality of the higher hierarchical levels. Young units tend to be less stable and thus more vulnerable (or more adaptive?) than old or mature units because of their faster metabolic rate, higher rate of growth/cell division and at the ecosystem level, because of their less strongly developed interdependencies. There is also (usually), more redundancy in young units, for example there are more available individuals, better reproductive rates and better viability from young progenitors, whether cells or individuals. The other aspect is that the delayed effects of radiation and bystander effects mean that radiation effects are not fixed in time or space to the energy deposition along ionising track. The effects can persist and manifest at distant points in time and space. These concepts are also discussed elsewhere [8, 9].

2.3. The importance of mixed exposure analysis

Pollutants including radiation seldom occur in isolation. In fact most environmental radioactivity comes from radioisotopes which are chemical entities. This means that there

is always a mixed exposure and that both the chemical and radioactive aspects need to be considered. Additive damage used to be an acceptable way to deal with mixed exposures (if any way were used!). The new field of non-targeted effects with the consequent realisation that emergent properties can exist, which were not predictable from the individual agent dose response data, makes this no longer acceptable. Two simple examples of why new analytical thought is needed are presented below.

The following situations could occur and could be argued, but there is no consistently right or wrong answer. Without more information on the initial status of the irradiated unit;

(1) If a cell is irradiated it can be internally or externally induced to undergo apoptosis whether or not it is 'damaged', but if a chemical e.g. cadmium is present, it could interfere with apoptotic signalling so that the cell lives. If cell survival were measured, the assay would suggest cadmium were radioprotective but the surviving cells could carry mutations which might (or might not) be harmful.
(2) At the population level, the situation could occur where a population becomes adapted to a chronic radiation exposure. In this population, a further stress either radiation or chemical might have little effect due to cross resistance, but this same level of stressor might devastate a pristine, non-adapted population. Equally, withdrawal of the chronic stressor might damage the adapted population.

These complexities call for a re-think of fundamental approaches to both epidemiological causation after low dose exposures to anything. They also question the need regulators have to regulate to a number (dose unit/exposure unit). Some of the issues concerning the latter position include the following:

How to ensure compliance if there is no 'safe' or legal limit?
How to deal with multiple stressors especially if the interactions are not known?
How to correct for dose rate/time of exposure?
How to deal with mixed chronic and acute exposures?
How to factor in possible adaptive, hormetic or antagonistic effects?
How to regulate in pristine versus dirty environments?

The issues of legal causation are highly relevant to the former point but outside the scope of this review. Discussion of these issues can be found elsewhere [50–52]. Ultimately, in order to resolve these issues, more data are needed for mixed exposure scenarios using relevant species. Systems biology approaches involving close interaction between experimental biologists and modellers are also required.

3. Data concerning low dose effects of mixed exposures

There are very little data where low dose exposures to multiple stressors/mixed contaminants involving radiation and a chemical are investigated. The field was reviewed by Mothersill *et al* [53] in 2006. Recent interest in non-targeted effects probably means more attention will be paid to this area in future. Gowans *et al* [54] have data showing chemical induction of genomic instability. Data from the authors' own and other laboratories shows that heavy metals singly or in combination can cause genomic instability [55–67]. Delayed death and chromosome aberrations in human cells following nickel, titanium or cadmium exposure have been reported [64–67]. Similar effects have been reported in fish cell lines [58–63], and more recently in live fish exposed to very low doses of gamma radiation 4–75 mGy over 48 h in the presence of heavy metals at levels just above background [68, 69].

Organic pesticides and detergents such as prochloras, nonoylphenol, nonoxynol and dichloroaniline have also been found to cause delayed lethal mutations in fish cells [57, 58, 61].

Chromium and vanadium used in implants and dentures lead to a variety of genetic and reproductive delayed effects *in vivo* and to multiple endpoints associated with non-targeted effects *in vitro* [64–67].

4. Implications for environmental protection and human health

While many of the studies cited above are concerned with fish rather than humans, the data show that non-targeted effects can be induced by low dose exposures to a number of environmental chemicals as well as ionising radiation. This means that combined exposures to low doses of these agents cannot be regulated in isolation and that studies of potential mechanistic interactions are important. Radiation protection of humans could find use from the approaches which are being taken by the task groups within ICRP, IAEA and the US-DoE (see for example [70, 71]) who have to formulate policy to regulate exposure of non-human biota. Many of the issues involved such as dealing with non-cancer endpoints, mixed contaminants or chronic low dose exposure are real issues in human radiation protection.

5. Summary thoughts and recommendations

The challenge in the low dose exposure field is to tease out the 'noise'. Noise is the euphemistic term we use when the level of the disease which is un attributable to our favoured causative agent, is too high to prove causation formally in any strict scientific or legal sense. Perhaps we should accept that we cannot assign causation and instead view ionising radiation as one among many agents which *together* contribute to cause disease. Before we can do this it is vital to understand the key mechanisms and in particular to find areas of mechanistic commonality suggesting common causation. Biomarkers may be useful to identify possible common mechanisms and to validate their relevance across different hierarchical levels. If this is achieved it should be possible to model links between *effects* at one level e.g. cellular or individual leading to *harm and risk* at higher levels—in this example the individual or the population. Biomarker studies do need to be interpreted cautiously however because they are often used as surrogates for risk when in fact they may merely be pointing to change in the system. Without the back-up modelling and multi-level analysis of their relevance they may lead to false conclusions and confusion about the true risk of an inducing agent.

The problem of establishing causation following mixed exposures remains along with the issue of what constitutes 'harm'. In the non-human biota field, there is great concern about doing more harm than good, if action levels are enforced which might require 'remediation' of a habitat—i.e. removal of contaminated vegetation and soil. This could cause much more harm to the ecosystem than the original stressor. In the realm of human protection against low dose stressors, issues might include the ethics of genetic screening to identify sensitive sub-populations. If a sensitivity marker were available, who should be tested and when? Should diagnostic screening be forbidden to these individuals because of their possible sensitivity to low doses of radiation? There are also issues regarding lifestyle choices and risk benefit analysis at the biological level. Evolutionary adaptation leads to a fitter population (of cells, individuals) by eliminating the weak units but how is that population changed? In dealing with concepts of adaptation to environmental stressors where 'nature' sorts things out in the optimal way, is 'nature's way' to Nietzian for Man?

It would be nice to conclude this reflection with a 'way forward' but as we are still in the very early stages of accepting that radiation doses effects at low doses are non-linear, that multiple stressors impact the final outcome, and that what appears to be bad (or good) may

be good (or bad)—it is perhaps best to recommend caution and consideration of these points rather than a great new regulatory framework!

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