AECL--10245

CA9200396

ATOMIC ENERGY
OF CANADA LIMITED

ÉNERGIE ATOMIQUE
DU CANADA LIMITÉE

BIO-INDICATORS FOR RADIATION DOSE ASSESSMENT

UTILISATION D'INDICATEURS BIOLOGIQUES DANS L'ÉVALUATION DE LA DOSE D'IRRADIATION

A. TRIVEDI

Chalk River Laboratories

Laboratoires de Chalk River

Chalk River, Ontario K0J 1J0

December 1990 décembre

AECL Research

BIO-INDICATORS FOR RADIATION DOSE ASSESSMENT

by

A. Trivedi

Dosimetric Research Branch
Chalk River Laboratories
Chalk River, Ontario KOJ 1J0

1990 December

AECL-10245

EACL Recherche

UTILISATION D'INDICATEURS BIOLOGIQUES DANS L'ÉVALUATION DE LA DOSE D'IRRADIATION

par

A. Trivedi

Résumé

La dose reçue par les travailleurs sous rayonnements (TSR) (ARW) dans des installations nucléaires telles que les Laboratoires de Chalk River est évaluée, de façon courante, à l'aide de dosimètres physiques et d'essais biologiques, conformément aux recommandations réglementaires. Toutefois, ces procédés pourraient ne pas suffire dans certains cas : par exemple, lorsque la lecture du dosimètre est mise en question ou dans le cas d'accidents survenant alors que la(les) personne(s) touchée(s) ne portai(en)t pas de dosimètre ou bien en cas d'urgence radiologique pouvant donner lieu à une dose reçue qui dépasse la dose limite.

La possibilité d'évaluer la dose d'irradiation en se basant sur les effets radiobiologiques a conduit le Département de recherches dosimétriques à examiner la valeur des dispositifs biologiques et les techniques susceptibles d'être utilisés à cette fin. Les concepts de dosimétrie biologique actuels indiquent qu'il ne semble pas exister d'indicateur biologique donnant des résultats fiables dans le cas des doses très faibles qui sont mesurées de façon courante par les dispositifs physiques utilisés actuellement. Toutefois, les indicateurs biologiques pourraient apporter des informations précieuses dans les cas de radioexposition exceptionnelle, par exemple lorsqu'il est impossible de se fier aux doses corporelles estimatives en raison d'un manque de mesures physiques adéquates ou encore lorsque les résultats disponibles doivent être validés pour permettre la planification d'un traitement thérapeutique. Le présent rapport évalue l'état d'avancement actuel de la dosimétrie biologique, notamment l'efficacité et les limites d'indicateurs particuliers. Cette démarche a permis de recommander quelques domaines de recherche qui pourraient mener à la mise au point de dosimètres biologiques appropriés aux besoins de l'exploitation ou des situations d'urgence à Chalk River.

Recherche dosimétrique
Laboratoires de Chalk River
Chalk River (Ontario) K0J 1J0

Décembre 1990

AECL-10245

AECL Research

BIO-INDICATORS FOR RADIATION DOSE ASSESSMENT

by

A. Trivedi

Abstract

In nuclear facilities, such as Chalk River Laboratories, dose to the atomic radiation workers (ARWs) is assessed routinely by using physical dosimeters and bioassay procedures in accordance with regulatory recommendations. However, these procedures may be insufficient in some circumstances, e.g., in cases where the reading of the physical dosimeters is questioned, in cases of radiation accidents where the person(s) in question was not wearing a dosimeter, or in the event of a radiation emergency when an exposure above the dose limits is possible.

The desirability of being able to assess radiation dose on the basis of radio-biological effects has prompted the Dosimetric Research Branch to investigate the suitability of biological devices and techniques that could be used for this purpose. Current biological dosimetry concepts suggest that there does not appear to be any bio-indicator that could reliably measure the very low doses that are routinely measured by the physical devices presently in use. Nonetheless, bio-indicators may be useful in providing valuable supplementary information in cases of unusual radiation exposures, such as when the estimated body doses are doubtful because of lack of proper physical measurements, or in cases where available results need to be confirmed for medical treatment plannings. This report evaluates the present state of biological dosimetry and, in particular, assesses the efficiency and limits of individual indicators. This has led to the recommendation of a few promising research areas that may result in the development of appropriate biological dosimeters for operational and emergency needs at Chalk River.

Dosimetric Research Branch
Chalk River Laboratories
Chalk River, Ontario KOJ 1JO

1990 December

AECL-10245

Table of Contents

Page

1. INTRODUCTION 1

  1.1 Basic Requirements for Bio-indicators 2

2. BIOLOGICAL INDICATORS 3

  2.1 Haematological Indicators 3
  2.2 Spermatogenic Indicators 5
  2.3 Cytological Indicators 6
    2.3.1 Chromosomal aberration 6
    2.3.2 Micronuclei 7
    2.3.3 Sister chromatid exchanges 7
    2.3.4 Chromosome painting 7
  2.4 Genomic Indicators 8
    2.4.1 Mutation expression 8
      2.4.1.1 Hypoxanthine-guanine phosphoribosyltransferase 9
      2.4.1.2 β-globin 9
      2.4.1.3 Glycophorin A 10
      2.4.1.4 Human leukocyte antigen A (HLA-A) 10
    2.4.2 DNA base alteration 10
  2.5 Molecular Indicators 11
  2.6 Neurophysiological Indicators 11
  2.7 Immunological Indicators 11
    2.7.1 Cell surface markers 12
    2.7.2 Lymphocyte stimulation 12
    2.7.3 Cytotoxic functions 12

3. BIOCHEMICAL INDICATORS 13

  3.1 Blood Biochemical Indicators 13
  3.2 Urine Metabolite Indicators 13
  3.3 Other Biochemical Indicators 14

4. BIOPHYSICAL INDICATORS 14

  4.1 Electron Spin Resonance 14
  4.2 Optically Stimulated Luminescence 16

5. OTHER INDICATORS 16

6. CONCLUSION 17

  6.1 Using Cell Surface Markers on Blood Lymphocytes 18
  6.2 Using Bio-Organic Molecules for Free Radical Dosimetry 18

Page

7. SUMMARY 21

8. ACKNOWLEDGEMENTS 22

9. REFERENCES 22

1. INTRODUCTION

Many techniques and devices capable of measuring the dose to an individual in emergencies are in use or under development [Leake and Rawlings, 1988; Mendelsohn, 1990]. Numerous bio-techniques can be employed for early detection of radiation exposure, but the full spectrum of their application has not been assessed. The time appears appropriate to investigate the opportunities provided by these modern techniques in screening individuals in the event of a radiation emergency. The intention of this report is, therefore, to stimulate interest and discussion on potential applications of these biological techniques, and to assess the opportunities that are provided by them for improved monitoring of individuals in an occupational or emergency setting.

Physical procedures to estimate radiation dose are based on personal dosimeters worn on the body. These dosimeters can be a film, a quartz fiber electrometer, a thermoluminescence system, or specially designed devices for criticality dosimetry [Mann et al., 1988]. Such dosimeters have the capability to assess the amount of radiation exposure with adequate accuracy and over a sufficiently wide dose range. The standard thermoluminescent dosimeters worn by workers at Chalk River Laboratories can provide reliable dose measurements from 0.02 mGy to 10.0 Gy [Jones, 1989]. This means that, for occupationally exposed individuals, the data on the amount of radiation dose in the event of excessive exposure can be readily available within a short period of time. The personal dosimeters, however, measure the radiation dose to themselves only, and not to the worker. Under non-uniform fields, the personal dosimeters could record a very low absorbed dose to the body, while a substantial dose is received by some parts of the body; the reverse situation could also occur. The situation becomes complex when the value indicated by the personal dosimeter is questioned. Therefore, despite the convenience of physical dosimetry, such complications can make it difficult to establish an accurate assessment of radiation exposures in an unknown field. These limitations (Table 1), plus the fact that there is no absolute guarantee that those involved in a radiation accident will be wearing a personal dosimeter, have led to the search for suitable bio-indicators for dosimetry.

Bio-techniques have provided useful information and have been used at Chernobyl and in the Brazilian accident to estimate body dose [Cassel and Leanning, 1989; Cobb and Kasmauski, 1989]. In such accidents, the biological procedure involves serial determinations of the level of peripheral blood lymphocytes in the blood and analysis of dicentric chromosomes in spontaneously dividing or lectin-stimulated haematopoietic cells in blood and bone marrow [Stephan and Oesterlcher, 1989]. The calculation of dose is based on changes in these parameters and comparable effects in prior radiation accidents or in radiotherapy studies, as well as on effects observed in animals and *in vitro*. Clear estimates of doses of whole-body irradiation may remain questionable simply because of the unavailability of any direct physical estimate of doses in those who were most heavily irradiated in previous instances. However, the potential of the biological procedures in screening and monitoring the individuals under these situations is far greater. Many biological techniques may permit detection of radiation exposure when it is difficult to assess exposure by other means.

TABLE 1

CONDITIONS WHERE BIO-INDICATORS WOULD BE USEFUL

1. Exposures of personal dosimeters
   - Accidental contamination of personal dosimeter
2. Suspicion of receiving higher or lower dose than indicated by the dosimeter
   - Doubtful about wearing the personal dosimeter
3. Unusual radiation exposure of individuals not belonging to the atomic radiation worker group
4. Accidental or emergency exposure
5. Exposures of occupationally exposed persons under unusual circumstances
   - An exposure above an applicable dose limit
   - Medical assessment

1.1 Basic Requirement for Bio-indicators

The use of bio-indicators in radiation accidents support their applicability in the dose assessment and some of them hold promise for further development [Kaul et al., 1986]. Choice of a reliable bio-indicator includes a list of minimal requirements that must be considered in fulfilling the basic responsibility of a biological dosimeter. These requirements are listed in Table 2.

The standardization and evaluation of bio-indicator systems for a nuclear operating facility should be based on experimental work and practical experience. It should be able to detect biological response in a reproducible fashion. Biological variability and interference should be included in the measurement. The response of bio-indicators should have a good correlation to the radiation dose that produced the effect. Biological effects should be measurable over a range of doses. For example, the ability to measure dose well below 0.02 Gy is necessary for any operational dosimeter, while doses in the range 0.02 to 5.0 Gy should be measurable in a radiation emergency. The assay should be effective over a wide range of doses [Commission of the European Communities, 1988].

Irradiation will often not be uniform in an accident situation. The heterogeneity of irradiation is an important factor. The suitability of a bio-dosimeter depends upon monitoring the level of the absorbed dose to sensitive organs, the distribution of irradiation within the body, and its distribution in time. A bio-indicator should have a biological response that is detectable soon after exposure and persistent for some time,

TABLE 2

BASIC REQUIREMENTS FOR PRACTICAL APPLICABILITY OF BIO-INDICATORS

- Easy collection of test sample
  - Sample size
  - Minimal stress during sampling
- Reproducibility of observations
- Known dose effect relationship
- Persistence of effect
- Sensitivity
  - To detect the dose in the range of <0.05 Gy (for regulatory purposes)
  - Broad range capability to doses >0.50 Gy (for radiation emergency)
- Specificity to radiation
- Early availability of results

preferably several days at least. Any time-dependent changes should also be known and standardized. The bio-indicator has to be representative of the radiation exposure. It should preferably distinguish the response from other causative agents such as smoke, heat and chemicals. Most preferably, the biological sampling for the assay should be obtained in the least invasive or traumatic fashion.

2. BIOLOGICAL INDICATORS

The growing availability of biological markers has improved the effectiveness of radiation dose assessment. Much work has been devoted toward developing biological indicators that can meet most of the requirements discussed in section 1.1. The efficacy and limitations of bio-indicators are evaluated in the light of requirements outlined in Table 3.

2.1 Haematological Indicators

The radiosensitivity of blood-forming organs is well known [Duterix et al., 1987, Ogunranti, 1989]. Changes of cell concentrations in blood and bone marrow are considered to be a most useful biological indicator for radiation exposure. This system is more or less quantitative between 0.25 and 1.0 Gy whole-body dose. Abnormal features observed in the peripheral blood lymphocyte include nuclear changes, nuclear pycnosis and micronuclei

TABLE 3

CRITERIA FOR EVALUATING THE BIOLOGICAL INDICATOR

1. Dose Effect Relationship

   Experimental evidence *in vitro* and *in vivo*

   Transferability to man

   Dose range, type and energy of radiation

   Type of radiation: acute/fractionated/protracted

   Dose rate effects

   Biological variability, background corrections, interferences

   Time of sampling after exposure

   Reproducibility

2. Evaluation of Measured Values for Radiation Exposures

   Whole Body

   Partial Body

formation [Hall and Wells, 1988]. These are the result of chromosomal abnormalities, but they can be detected more easily and quickly than karyotype abnormalities. These changes can be observed at relatively low doses, typically 0.25 Gy *in vivo* and 0.02 Gy *in vitro* [Huber et al., 1983].

The earliest haematological indicator is a reduction in the concentration of blood lymphocytes. A falling rate of blood lymphocyte concentration estimated over the first three days is a potential biological indicator of the radiation dose [Baranov, 1981]. A fall in neutrophils and platelets can be monitored for large dose exposure. The observation between radiation dose and changes in blood cells count is summarized in Table 4.

At doses of more than 1.0 Gy, the uptake of thymidine is significantly reduced in lymphocytes, reflecting reduction in mitosis and cellular changes during irradiation [McFarland and Pearson, 1963]. The uptake of thymidine into lymphocytes is sometimes used in cases of accidental exposure to estimate the exposure. Radiation dose of 2 to 4 Gy also affects the electrophoretic mobility of lymphocytes and the size of cellular volumes, but these changes are transient and are undetectable a few hours after the accident. Also, the measurement is only useful if the pre-exposure value is known.

TABLE 4

CHANGES IN BLOOD CELL COUNTS FOLLOWING WHOLE BODY RADIATION

Cell Type	Normal levels in adults (per μL)	Dose range (Gy)			
		<1	1-2	2-5	5
Lymphocytes [days]	1000 to 4000	70% [3]	50% [2]	20% [1]	0% [0.5]
Neutrophils [days]	2000 to 7000	70% [40]	50% [40]	20% [25]	0% [10]
Platelets [days]	150 000 to 350 000	70% [30]	50% [30]	10% [25]	0% [15]

The mitotic index in bone-marrow cells is one of the most accurate biological indicators of cumulative dose, and also has a certain prognostic value in predicting death, recovery or acute radiation sickness [Potten, 1986]. Changes in the mitotic index are related to dose. Doses of 1.0 Gy or lower produce little change. Some progress has been made in extending the sensitivity of the mitotic indexing in bone marrow assays, but methods are still in the experimental stage and are far from practical application. Sampling of bone marrow is also much more stressful than sampling of peripheral blood.

It is evident that the effect on the haematopoietic system is transient due to recovery from radiation exposure followed by the repopulation of the bone marrow. Thus the usefulness of this system following sublethal doses is limited to days or a few weeks, depending upon dose and recovery of tissues, which is rather late to be useful in the early evaluation of radiation injury.

2.2 Spermatogenic Indicators

Spermatogenesis is very sensitive to irradiation and could be used as a biological indicator of dose in males [Ash, 1980]. The sperm count, motility, and morphology are changed by a dose of 0.15 Gy [Hacker et al., 1982]. Low doses can lead to severe oligospermia. The relevance of various parameters of morphology is under experimental study and needs further validation. This technique would be limited in use since it screens only a part of the population. Furthermore, the maturation of sperm takes almost seven weeks. The decrease in sperm count is thus an inappropriate indicator for early dosimetry and diagnosis of radiation injury. However, the technique is useful when extended long-term exposure is suspected. This technique could be potentially applied to monitor heritable sperm damage that may adversely affect the genetic characteristics of offspring. Questions of appropriate sampling methods

and control populations are important and further studies are need to establish the sensitivity of the assay.

2.3 Cytological Indicators

Cytogenetic dosimetry allows an estimate of the mean dose in the body. The analysis of chromosome aberrations in the circulating lymphocytes is widely used to assess dose [Eisert and Mendelsohn, 1984]. Even in cases of partial-body exposure, chromosome changes are indicators of the average absorbed dose to the whole body [Lloyd, 1987]. The evidence to justify the technique is well-founded and covers various irradiated populations in nuclear medicine, radiotherapy and radiation accidents [International Atomic Energy Agency Technical Report #260, 1986; Ramalho et al., 1988].

Cytogenetic monitoring techniques in blood lymphocytes permit the detection of chromosomal mutations microscopically visible as numerical or structural alterations affecting one or more genes. The technique provides a reliable indication of acute dose since lymphocytes are widely dispersed in the various tissues and organs. Lymphocytes have a long life and circulate rapidly in the body. The number of aberrations in lymphocytes is dose-dependent down to doses of 0.15 Gy for low LET and to 0.05 Gy for high LET (neutron) radiation [Workshop on low dose radiation, 1988]. However, the reproducibility of lymphocyte sensitivity to radiation is questionable as some individuals have exhibited far more radiosensitivity than others [Gentner et al., 1988]. It is possible that different sensitivities may be shown by the same individual at different times. If this is so, this might be a distinct advantage of such assays in that it might estimate damage rather than simply the physical dose [Myers, personal communication].

The common cytogenetically observed biological markers are chromosomal aberration, micronuclei and sister chromatid exchange. Other approaches for estimating dose to humans by using other cytogenetic techniques are discussed in detail elsewhere [Bender et al., 1988].

2.3.1 Chromosomal aberration

Many types of radiation-induced chromosomal aberrations appear in irradiated lymphocytes [Martin et al., 1989], but the dicentric aberration is currently taken as providing the most valuable information on dose [Savage, 1989]. Dose-response for dicentric aberrations in the irradiated lymphocytes of normal individuals is little affected by factors such as the age or sex of the individuals [Anderson and Standefer, 1983]. So far, the dicentric aberrations have been used for recent exposures, because irradiated lymphocytes with dicentric aberrations self-destruct over months to years due to unequal positioning of their chromosomes in mitosis. Irradiated lymphocytes with reciprocal translocation aberrations, which theoretically occur at the same frequencies as dicentrics, do not self-destruct and generally persist indefinitely. However, the reciprocal translocation aberrations are much more difficult to detect. Thus, to use chromosomal aberrations for detecting long-term exposures in nuclear workers, rapid and reliable methods would be needed to detect translocation numbers in irradiated chromosomes.

The use of chromosomal aberration as a biological dosimeter is continually being challenged [Twan and Binks, 1989]. Gerber [1989] has questioned the

validity of this assay by pointing out that more chromosomal aberrations are observed for workers in the conventional coal-fired power industry than in the nuclear industry, and that the assay is not always reproducible. Chromosomal aberrations are produced by a wide variety of genotoxic chemicals, as well as by ionizing radiation. The second difficulty in using aberrations is the great labor needed to get reasonable confidence limits on the results. The same difficulties apply to the other cytological indicators discussed below.

2.3.2 Micronuclei

Counting micronuclei has been suggested as a dosimetric method for situations that include the evaluation of damage of chemical origin and the identification of particularly sensitive individuals with higher than average potential risks of developing cancer or genetic disorders [Bender et al., 1988]. In principal, counting micronuclei appears easier, faster and less expensive than scoring chromosome aberrations. However, due to a higher background incidence of micronuclei than of dicentric aberrations, the lower limit of dose detection by micronuclei is restricted to 0.25 Gy.

Currently, the micro nuclei assay is being improved by the use of cytochalasin-B [Bender et al., 1988]. Cytochalasin-B inhibits microtubule assembly in cell division. This technique ensures that micronuclei are scored only in those cells that have just completed their first post-irradiation mitosis. This technique is amenable to automation using pattern recognition systems, e.g., in polychromatic erythrocytes. The effect of factors such as dose protraction, fractionation and partial body exposure have yet to be investigated. The test can be envisaged as being particularly useful after a serious accident, when many people may need to be tested quickly.

2.3.3 Sister chromatid exchanges

This method has been used successfully in experimental and operational programs as an indicator of exposure. Sister chromatid exchanges (SCE) are observed when DNA damage to complementary strands of sister chromatid leads to intrachromosomal exchanges during DNA replication. Exchanges are efficiently induced by ionizing radiation that forms single and double strand breaks or DNA adducts that interfere with DNA synthesis/repair. Until now, effective use of this technique in monitoring occupational and accidental exposures has not been fully exploited, since other compounding factors can influence the observations [DeMarini et al., 1989].

2.3.4 Chromosome painting

Chromosomal painting uses fluorescent *in situ* hybridization (FISH) with chromosome-specific DNA probes to stain selected chromosome pairs. The FISH technique has been applied successfully to the detection of structurally aberrant chromosomes, especially with reciprocal translocations [Pinkel et al., 1986].

The chromosomal painting is achieved by using DNA probes that are chemically modified and then hybridized with the chromosomal DNA in conventional cytogenetic preparations. The modified DNA probe is rendered visible (green) by using fluorescently labelled aridin to the biotin. A

fluorescent counterstain, such as propidium iodide, renders all of the DNA visible (red). Thus, the regions of a chromosome or nucleus to which the probes have bound appear green, while the remaining DNA is red. Under circumstances where chromosomal aberrations occurred, exchanges between painted and unpainted chromosomes have distinct appearances: green on one side of the breakpoint and red on the other. Such aberrant chromosomes are clearly and easily recognizable against a backdrop of chromosomes of one color or the other. Several different chromosomes can be painted in the same sample.

Two classes of DNA probes have proven useful in chromosomal painting studies. The first contains probes specific to DNA sequences that are repeated hundreds or thousands of times in a small portion of one chromosome type. The second class is made up of collections of cloned sequences specific for a whole chromosome or chromosomal part.

Lucas et al. (1989) have used the first class of DNA probes to measure translocation and dicentric frequencies in human lymphocytes irradiated *in vitro*. They obtained tight-fitting linear quadratic response for each aberration type in the range of biologically relevant doses (0.1 Gy). The principal limitations of this approach are the relatively small chromosomal target being used and the present lack of suitable pairs of repetitive probes for other chromosomes.

Composites of probes for entire chromosomes, the second class of DNA probes, already exist in the form of human chromosome-specific recombinant DNA libraries [Van Dilla et al., 1986]. Studies in progress using these probes clearly show two things: an increase of perhaps two orders of magnitude in the rate of scoring for translocations, and the ready recapture of biological dose information from decades-old radiation exposures using translocations as an end point [Mendelsohn, 1990]. Full validation for fractionated and small exposures is difficult, but may best be done in the context of nuclear workers.

2.4 Genomic Indicators

Ionizing radiation may induce a small proportion of non-lethal DNA damage in a cellular genome, which can be manifested in permanent heritable alterations in the genomes of afflicted cells and their descendants. Though most such damage is repaired shortly after exposure, it is possible to detect DNA damage caused by irradiation over hours and days, such as measurement of the incidence of DNA strand breaks, or the incorporation of new bases in the repair of DNA, or the use of specific antibodies to detect specific base alterations in DNA [Springer, 1989]. It is clear that to quantify an exposure a method should detect more or less permanent genomic damage. This essentially implies the detection of permanent changes in DNA composition or structure that may or may not be expressed phenotypically as mutational changes.

2.4.1 Mutation expression

The ability to detect even the smallest mutations caused by radiation in human genes is currently being developed. These techniques may be capable of picking up changes in a single base pair, and thus promise a far more accurate assessment of the biological consequences of exposure to radiation

than is currently available. Eventually, the methods may be able to quantify the dose of radiation that has produced excess gene mutations. The specificity of this method for radiation, as distinct from other mutagenic agents is, however, questionable.

2.4.1.1 Hypoxanthine-guanine phosphoribosyltransferase (HGPRT)

A T-cell assay is being developed to detect human gene mutation. The procedure uses the HGPRT (hypoxanthine-guanine phosphoribosyltransferase) gene as a site-specific mutation indicator, because there is a simple selection method that can distinguish cells in which mutations have inactivated the HGPRT gene from cells in which the gene is functional [Morley et al., 1983]. The cells themselves can be readily obtained from a sample of an individual's blood.

Radiation increases the frequency of T-cells with HGPRT mutations, and its effect can be very long-lived. Researchers of the Radiation Effects Research Foundation in Hiroshima, Japan, have shown that survivors of the 1945 atom blasts in Hiroshima still have higher than normal HGPRT mutant frequencies more than 40 years after their radiation exposure [Kondo, 1988; Chiyoko, 1988]. However, recent studies detecting the effect on the HGPRT locus in T-cell show that it is not significant [Kodama et al., 1989]. The disappearance of mutant T-cells from the bomb survivors seems to be attributable to the *in vivo* kinetics of T-cells rather than to the presumable selection against HGPRT mutant cells.

The effects of radiation on the HGPRT locus is also a continuing research interest of a Canadian team, where an increased rate of mutation frequency has been observed even with very low dose exposures. Dr. Karen Messing and her colleagues at the University of Quebec in Montreal have looked at T-cell mutant frequencies in nuclear medicine, and x-ray technicians who appeared to have a dose-related increase in HGPRT mutant levels at very low doses of radiation exposures [Seifert et al., 1987]. However, recent data from the same laboratory have not confirmed some of the initial results [Bradely and Messing, 1989]. One important consequence of these observations is that the hemizygosity of the HGPRT locus may not permit detection of all the large deletion events which affect this gene. Other studies also point out the pitfall of this technique, as the incidence of such mutational frequency is not specific to irradiation, but may be influenced by a variety of mutagens, including cigarette smoke and heat. It may, therefore, be appropriate to develop a mutational assay system employing an autosomal marker specific to radiation. Further evaluation of such techniques is in progress.

2.4.1.2 β-globin

Not all the mutation detection methods now being explored use the HGPRT gene as an indicator for biological dosimetry. An alternative approach involves the use of fluorescence-tagged monospecific antibodies to detect the presence of mutant proteins in red blood cells (RBC) to quantify the previous exposures [Klasen et al., 1982]. Antibodies to various abnormal haemoglobin (e.g., sickle cell haemoglobin [HbS or β-globin]) have been used to detect the presence of presumed cells containing mutant proteins in blood samples from normal individuals. Mutant cells can be detected by flow cytometry, which enables the analysis of some million cells per

second. Although such mutations are probably induced by ionizing radiation, they may be very infrequent events relative to those radiation-induced mutations that involve the loss of a gene for its functional inactivation. The draw-back with this technique is that it is limited in its use because of the time dependency of the assay.

2.4.1.3 Glycophorin A

Another approach, which eventually will detect loss mutations in RBC, is currently being developed at the Lawrence Livermore National Laboratory. It involves the use of fluorescence-labelled monoclonal antibodies to human glycophorin A, the RBC protein responsible for the M and N blood serotypes [Langlois et al., 1988]. In findings similar to those obtained with the HGPRT gene, the Livermore group has shown that the frequency of mutant RBCs is increased in atom bomb survivors [Langlois et al., 1987]. This technique was also employed in screening the people who received substantial radiation exposure (>0.05 Gy) at Chernobyl [Edwards and Raymer, 1987]. However, the RBC method lacks the ready ability to characterize the mutations underlying the protein defect, because as red blood cells mature they lose their genetic material. This approach is promising, but far from being a proven technique to detect early radiation exposure.

2.4.1.4 Human leukocyte antigen A (HLA-A)

A mutation assay using the human leukocyte antigen A (HLA-A) locus on chromosome 6 has recently been reported [Janatipour et al., 1988]. The mutant cells are identified or selected using antibodies to $HLA-A_2$ and $HLA-A_3$, two commonly occurring alleles. Radiation-induced (1.0 Gy) mutants show a large component of deletions and mitotic recombination. The method has the potential to be used as a biological indicator, but will first require more uses and further validation.

Of the four approaches, the glycophorin method seems best suited for studies on nuclear workers. The human leukocyte antigen and haemoglobin methods are not yet suitably validated or available. The HGPRT method would be preferred if the exposures were in a short period of time. But the worker situation is one of cumulative exposure over a working lifetime, and at the moment none of these methods is a candidate to deal with this at a practical level. There is no direct evidence that any of these methods will respond to small, essentially whole-body exposures fractionated over years.

2.4.2 DNA base alteration

In any ionizing radiation event, a wide range of changes in the DNAs of exposed cells occurs. Many are short-lived and none have been shown to yield useful signals that can be used to monitor or detect radiation damage in the DNA of exposed individuals [Watt, 1989]. Comparison of unique sequence reference DNAs with homologous DNAs extracted from cells of irradiated individuals by hybridization properties or direct sequencing may be theoretically possible, but not immediately practical for such heterogeneous DNA samples. It is possible that detection of changes at sites of DNA cleavage by restriction enzymes can provide a workable approach. This would involve using certain repetitive sequence DNAs in the

human genome to detect radiation doses <0.05 Gy [Unrau, personal communication].

The development of modern molecular tools to reveal changes in DNA bases provides a promising avenue that may ultimately lead to a powerful method to detect and quantify radiation-induced DNA damage. Recent technical progress in molecular biology may aid in detecting early changes in a genome before being clinically observable. Techniques like restriction-fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) could be useful in identifying DNA base alterations and mismatches.

2.5 Molecular Indicators

Changes in other biomolecules hold great potential for ascertaining *in vivo* exposures and may also facilitate identification of groups or individuals who are at increased risk of genetic damage due to irradiation [Ashby, 1988]. This approach would be of particular interest when the intake of chemically active radionuclide is suspected [Wogan and Gorelick, 1985]. Post-labelling techniques can be employed as they recognize a spectrum of molecular adducts that can provide a direct measure of target tissue dose. While still under development, these techniques are sensitive, especially for detecting adducts corresponding to large chemical electrophiles.

2.6 Neurophysiological Indicators

Doses of radiation (1.5-6.0 Gy) can affect the function of peripheral receptors, nerve conduction, and synaptic transmission, and they can change the acetylcholine-cholinesterase balance [Court et al., 1984]. As such, neurophysiological changes are important indicators of the direct effect of radiation on the optic nerve. However, examinations of synaptic transmission produces results that are harder to interpret. Disturbances in the neurophysiological equilibrium are usually indicated by changes in excitability, consisting of successive phases of inhibition and excitation. On the electro-encephalogram (EEG), changes in the excitation waves are noted that are characteristic of radiation-induced effects. The dose-effect relationship observed in animals shows a response above 0.25 Gy and appears at 15 minutes after exposure [Court et al., 1984]. The persistence of the effect observed during the hours following irradiation tend to indicate changes in protein synthesis and the coding of information of the neurons. This could be a valuable tool in supplying supplementary dose information. This is especially true if the assessment is done soon after irradiation and in cases where it has not been possible to undertake chromosome analysis immediately after transfusion of blood components. Currently, the use of this technique is limited and restricted to those radiotherapy patients who receive high doses of radiation for medical treatments.

2.7 Immunological Indicators

Immunological tests have been evaluated as potential biological indicators for routine monitoring of radiation exposure [Anderson and Standefer, 1983; Conklin and Monroy, 1987]. Information on cellular mechanisms in immune defence is constantly evolving. Many of the immunological techniques that have been applied for the purpose of estimating radiation dose are in use

for other purposes in clinical immunology. Attempts to relate these tests to radiation exposure include tests on humans receiving partial-body exposure in radiotherapy [Lloyd, 1987]. The screening of immunological tests depends on the duration of the effect, a demonstrable dose response and individual variability. Other confounding factors, such as stress, intercurrent infections, and medication, can interfere with immunological response as well. These factors complicate the use of immunological indicators in measuring radiation exposure. However, an expert working group at Oak Ridge National Laboratory concerned with the determination of radiation dose has recently reviewed immunological approaches that would be more appropriate for biological dosimetry [Bender et al., 1988].

2.7.1 Cell surface markers

Lymphocytes do not constitute a homogeneous population, but belong to two classes with distinctive functional capabilities. The T-cells express cellular immunity, while the B cells are responsible for antibody production. The composition of different lymphocyte subpopulations is examined by means of phenotypic markers and various *in vitro* functions [Mazurick et al., 1981]. As noted in section 2.1, lymphocytes are especially susceptible to the acute effects of irradiation. Changes in the ratio of B- and T-lymphocytes and changes in the proportions of the various subsets of T-cells can be used to quantify a dose of approximately 1.0 Gy [Rezunkova et al., 1988; Schreiber et al., 1989].

The relative expression of cell surface markers on lymphocytes from irradiated animals has been studied in a wide range of radiation exposures (up to 6 Gy), but the information is limited as to radiation quality, type of radiation, and dose rate [Koteles et al., 1987]. The exact mechanism for the effect of radiation on the expression of cell surface markers is yet unknown. It appears that the onset and length of time when an effect of radiation on the subpopulation of lymphocytes can be observed depends on the dose and subsequent recovery of the cell population [Stevenson et al., 1989]. The evaluation of a particular subpopulation of lymphocytes can probably serve as an indicator of radiation dose for a short time period extending from hours to days, but certainly not years, since the effect is either lost or diluted over such a long period. This is a promising area, where considerable improvements in technology and methodology might overcome most of the limitations that interfere with the interpretation of dose estimation.

2.7.2 Lymphocyte stimulation

The mitogen-stimulated lymphocyte assay provides information on the functional capacity of B lymphocytes and their interaction with one T-cell subset [Stewart et al., 1988]. The test is difficult to standardize because of large individual variations in unexposed subjects. Little is known about the time sequence between radiation exposure and immunoglobulin production. As the data base is sparse, the uncertainties in this assay are large. Future studies on the relative production of specific antibodies may provide information on the early radiation damage to immune systems.

2.7.3 Cytotoxic functions

Cytotoxic activities of lymphocytes from irradiated patients exposed to high radiation doses (> 0.5 Gy) demonstrate marked changes in their functions [Workshop on low dose radiation, 1988]. Different effector mechanisms are involved in the destruction of foreign target cells. The cells participating in these reactions are affected by the increasing doses. Some of these changes persist for a long time, but there have been no detailed studies of their potential use as biological indicators of radiation dose.

3. BIOCHEMICAL INDICATORS

Biochemical analysis of the metabolic patterns in the body can provide information on cellular disturbances that may arise due to the radiation exposure [Feinendegen, 1986]. These include disturbances that affect the regulation of renal functions (urea, creatinine, calcaemia, phosphoraemia and blood ingogram), liver functions (lactic dehydrogenase, transaminase, alkaline phosphatase and bilirubin), and nutritional parameters (electrophoresis of peptides and proteins, and serum iron).

3.1 Blood Biochemical Indicators

Changes in blood biochemical parameters following irradiation can be regarded as interesting indicators for biological dosimetry. A dose-dependent appearance of a humoral blood factor in blood serum, which inhibits the incorporation of ^{125}IUdR into cells in culture, has been observed [Feinendegen et al., 1982]. Although this technique has proven to be an effective way to measure radiation exposure, it is far from being developed for man, partly because thymidine concentration in human serum is only one-tenth that in mouse serum. The level of plasma electrolytes and plasma proteins, and the reduction in the electrophoretic mobility of the protein fractions of the blood serum, increase as the radiation dose rises [Spangler and Cassen, 1967].

In recent years, a number of new blood biochemical indicators of severe radiation damage have been proposed [Walden and Farzaneh, 1990]. Methods have been suggested for the quantitative evaluation of damage to red blood cell membranes by measuring the loss of K^+ and sulphyhydry groups. Inhibition of DNA precursors in bone-marrow cells has been used to monitor irradiation at some tenths of a Gy. However, the data are not accurate enough for these indicators to be used quantitatively, mainly because of the variations in the background incidence. Certain infections and other irregularities can influence the observations.

3.2 Urine Metabolite Indicators

Variations in the chemical composition of urine are more significant than those of blood. The disturbance in urinary electrolytes reveals changes in potassium excretion (extra-physiological fluctuations) and in the excretion of sodium and chlorine, which declines during the first few days after exposure [Mole, 1984].

After radiation exposure (0.5-2.5 Gy), there is a considerable enzymatic breakdown of nucleic acids and proteins, especially in lymphatic tissues [Gerber et al., 1961]. As a consequence, the urinary excretion of nucleosides, amino acids and their metabolites increases. An enhanced excretion of deoxycytidine from normal cells in man after radiotherapy, and the increased excretion of β-aminoisobutyric acid (BAIBA) after accidental human irradiation, have been observed [Streffer, 1971]. Any agent which results in cell death in lymphatic tissues would be expected to produce similar effects.

The levels of amino acids and their metabolites in urine also increase during and following the irradiation. The relative amounts excreted depend on the absolute excreted amount and on the metabolism of the specific amino acids. Accordingly, the excretion of amino acids in urine is not usually an appropriate indicator. However, the enhancement in the level of some amino acids and their metabolites shows a dose-dependent change in urinary excretion after irradiation. One of these is taurine, a metabolic end-product of the amino acid cysteine residue in the body, whose excretion increases with radiation dose in the range 0.75-2.5 Gy [Dilley, 1972]. The enhanced urinary level of taurine was ascribed to intracellular taurine elimination due to changes in cellular permeabilities and to the breakdown of lymphatic tissues. As a consequence of such metabolic alterations, the urinary excretion of kynurenic acid and xanthurenic acid, metabolites of the amino acid tryptophan, also increases in the radiation dose range of 4-8 Gy [Deeg, 1983]. The level of urinary metabolites and amino acids may be useful for high dose ranges and is probably best suited for screening the individuals into a lower and higher dose range.

3.3 Other Biochemical Indicators

A number of biochemical indicators of severe radiation damage are also used in criticality and in radiation accidents. Irradiated muscles that are no longer able to metabolize creatine in the normal way provide an indication of radiological damage [Gerber et al., 1961]. Accidents involving whole-body irradiation, such as occurred at Oak Ridge (Y-12) in 1958, resulted in a significant increase in the levels of creatinuria (creatine/creatinine ratio) and these levels were used to screen the individuals [Gerber, 1986]. However, the data did not reveal a dose effect relationship and the individual values varied greatly. The increase in the α-amylase activity in the body accounts for the radiation dose (>0.6 Gy) received by the salivary glands [Chen et al., 1973; Barrett et al., 1982]. There are also some reports on determining the total content of DNA in the blood and urine of patients irradiated for therapeutic purposes. However, due to the paucity of such studies, it is difficult to assess the value of these indicators.

4. BIOPHYSICAL INDICATORS

Certain technologies that are developed chiefly for their analytical capability are now evolving into *in vivo* methods. Increasing application of these techniques has eventually resulted in their use in radiation dosimetry.

4.1 Electron Spin Resonance (ESR)

The deposition of energy from ionizing radiation produces free radicals in most materials. This is particularly meaningful for biological materials, where such events can be identified with the aid of a proper biophysical technique. Unfortunately, similar types of free radicals are also produced in the normal metabolism of living cells. Electron spin resonance (ESR) is capable of identifying these radicals, but that depends on their stability and concentration in a biological material [Scharman, 1989]. Radiation-induced radicals can only be used for dosimetry if they are stable for some hours or days, and preferably even longer. Most radiation-induced radicals do not survive for long periods of time in the presence of water, e.g., in soft tissues of the body. Thus, this method is limited to the study of damage in solid and dehydrated components such as bone and hair. Typical radiation-induced radicals measured in solid substances by ESR are:
(a) radicals resulting from subtraction or addition of hydrogen radicals,
(b) sulphur radicals, if SH-groups are present in the substances, and
(c) electrons or holes, trapped in the lattice of crystals or by metal complexes.

The measurable free radicals are the result of a chain reaction following the process of radiation absorption. In many instances, the concentrations of these radicals show a linear correlation with the dose over a wide dose range [Regulla and Deffner, 1982]. These reactions occur after irradiation of the bone, teeth, nails and hair in man and, therefore, offer the possibility of a quantitative assessment of the dose received [Ikeya et al., 1984]. It is also possible to assess the dose on the basis of radiation-induced radicals in solid substances, e.g., plastic or other materials worn on the body or lying in the radiation field [Nakajima, 1989].

There have been investigations on radiation-induced radicals in hair and nails concerning the applicability for dose assessment. For hair, a linear relationship between the dose and the number of radiation-induced free radicals has been observed, but different samples show significant variations of the radical yield [Sagstuen et al., 1983]. Moreover, the signal intensity depends on the temperature and humidity during storage of the samples. The nails, too, show radiation-induced radicals and radiation doses in the range of 4.0 Gy even without signal averaging [Daburon, 1986].

Both teeth and bones contain hydroxyapatite and other inorganic ionic components, as well as organic substances. High radical concentration, especially in the organic components of the bone, is found after irradiation [Brady et al., 1968]. The pattern of radical dissipation in these tissues is little known. Thus, it is difficult to decide whether this signal component can be used for free radical dosimetry. However, in teeth a stable radical concentration occurs much earlier than bone [Shimano et al., 1989]. The stable radicals in teeth appear to be suited for dosimetry purposes. The lowest detectable dose for teeth and bone is recorded at the level of 0.5 and 1.0 Gy, respectively [Nakajima, 1982]. A linear dose relationship has been established in the low dose range. The sensitivity and reproducibility depend on the proportion of crystalline fractions in the measured sample. In this regard, the evaluation of the dose could contribute to the estimation of cumulative dose even when the exposure is at a low dose-rate.

The ESR dosimetry method has been applied to emergency populace dosimetry, high-dose measurement and dose estimation of people exposed to radiation in Hiroshima and Nagasaki [Regulla et al., 1989]. The ESR method makes it possible to determine directly the cumulative dose of individuals. For example, the human exposure of A-bomb survivors is being estimated using tooth enamel. Recently, sugar has been demonstrated to be one of the best organic dosimetric materials for emergency populace dosimetry [Nakajima and Otsuki, 1990]. It is possible, therefore, that the development of the ESR dosimetry and its application to radiation emergencies can combine *in vivo* dose measurements from biological materials (tooth, nail) and from bio-organic samples (sugar, amino acids). The supplementary dose estimation from bio-organic samples can provide a way to double-check the absorbed dose in a radiation emergency. An advantage of this approach would be to distinguish the cumulative and integral dose in radiation accidents.

4.2 Optically Stimulated Luminescence

This technique is a newcomer in biophysical dosimetry [Pradhan, 1989]. This technique works on the phenomenon known as M-center luminescence. A blue-light laser, emitting at 442 nm, produces a red-light emission in LiF single crystals that have been exposed to ionizing radiation. The M-center luminescence technique can be read out in a fraction of a second, which will allow for fast, cost-effective processing. Unlike conventional thermoluminescent techniques, the M-center luminescence does not require heating of the material. This allows a repeat measurement, and provides for a viable lifetime internal dosimeter. The technique is currently under a feasibility study, to determine how an internal dosimeter for direct measurement of beta radiation inside the body from internal emitters can be developed [Miller and Durham, 1990].

5. OTHER INDICATORS

Other techniques have also been suggested for use in biological dosimetry, but they have not yet been developed. One example is cell death in hair follicles (dose-dependent from about 0.1 to 1.0 Gy) and consequent changes in hair width (1 to 10 Gy).

Neutron activation analysis is another indicator of radiation exposure. It is applicable in cases where neutron radiation or mixed neutron-gamma radiation is involved. In cases of exposure to mixed gamma-neutron fields, the neutron component and its spatial distribution can be estimated by determining the presence of ^{24}Na and ^{32}P in the body [Majborn, 1980]. For instance, this technique focuses on determining the concentration of ^{24}Na in the body by whole body counting. This radiation-induced activity can also be measured in blood. A neutron activation analysis technique is often used in the dosimetry of criticality accidents.

Lyoluminescence, the radiation-induced chemiluminescence of organic molecules, salts and hair, can detect radiation doses in the range of 3 mGy to 1 Gy. It can be used as an alternative method to measure accidental doses. The hindrance in introducing lyoluminescence to routine dosimetry

is the lack of knowledge of environmental influences that affect dose estimation.

Measurement of thermoluminescence from the jewels (synthetic rubies) contained in workers' watches and from other sensitive materials (teeth) has been used in evaluating the data obtained from other bio-indicators [Majborn, 1984]. Such measurement, along with an extended calibration curve and phantom measurements, can provide supplementary information on possible irradiation geometries.

6. CONCLUSION

This report has reviewed the information derived from various investigations on the use of bio-indicators in biological dosimetry. It has considered the appropriateness of bio-indicators in the event of radiation exposures, whether for an operational need or an emergency requirement to monitor individuals. It has considered any guidance for the dose estimation that is likely to be obtainable from clinical evidence or from experimental studies on the standards of screening individuals for medical treatments.

There is a wealth of information on many effects, but so far information is insufficient regarding the mechanisms and the degree of certain radiation effects in man. More studies in animals and determinations in man are required. The test should be first studied in simple experimental models, such as in tissue cultures or in animals, after various times and doses of whole body exposure. Additional studies in whole body and partial body irradiated animals should be performed prior to establishing models that allow the extrapolation of data from whole body exposure of animals to that of man.

It is important to stress that the identification of the individuals requiring urgent medical treatment may be adequately guided by haematological indicators. During the early period, most information can be obtained from karyological analyses and lymphocyte counts. The average bone marrow dose and the prognosis regarding the further course of the illness can be determined on the basis of other biological criteria. The remaining indicators seem to be of an auxiliary nature. For example, the dose value estimated by using haematological indicators for some radiation-exposed individuals correlated with the electron spin resonance study of dental enamel after the individuals' deaths [Brady et al., 1968].

To improve dosimetry and treatment planning of individuals in possible future emergencies, experience gained on the significance of biological dosimetry should be studied. Chromosome analysis is the only procedure developed to applicability, and a practical service is available at Oak Ridge and at Harwell. Research in radiation immunology could also offer a promising future for the development of biological dosimetry. Therefore, immunological concepts and techniques must be followed closely to determine possible applications for bio-indicators. To achieve comparable quantitative information on reproducibility and uncertainty of the data, various parameters, including biological variabilities and interferences, should be established for any bio-indicators. To minimize the expenditure of work and time, all practicable methods of automation should be

considered. Flow cytometry and cell sorting techniques should be considered if immunological techniques are involved in developing the biological dosimetry. Two techniques are recommended as having the highest priority for further study.

6.1 Using Cell Surface Markers on Blood Lymphocytes

The objective of using biochemical changes in the expression of cell surface markers on human blood lymphocytes is to develop an immunological technique to detect and quantify low radiation doses of biological significance. This approach has the potential to be used successfully to quantify previous exposures to radiation, and may serve as a biological indicator for dose estimation in an individual. Immunologically detectable changes in cellular membranes at low doses are one of the most likely choices for new bio-indicators, since the changes will be independent of other cellular mechanisms. For example, the quantification of cytological and other genomic indicators is dependent upon the interplay of dose-dependent DNA damage and genetically mediated DNA repair. Therefore, the net effect may vary among individuals because of variations in the genetically determined capacity for DNA repair; this, of course, might represent a major advantage of the cytogenetic methods.

The assay for cell surface markers on human blood lymphocytes employs a specific spectrum of monoclonal antibodies for these markers. It is a relatively rapid and simple procedure, and promises to be useful at low doses. It has the advantage of responding to the average external whole body dose. The practicality of this technique has been speculated upon on the basis of earlier data [Schreiber et al., 1989]. The ability to detect and quantify the radiation exposure to the body is expected to be below 0.1 Gy. A similar approach has received scientific recognition in screening the radiation-exposed individuals during the Chernobyl disaster [Cassel and Leanning, 1989]. Also, it has the potential to become fully automated, since technically advanced instrumentation such as flow cytometers and cell sorters can be operated to monitor the expression of cell surface markers in the peripheral blood population. The value of the technique depends upon the appropriate selection of suitable radiosensitive markers, and the ability to develop stable, highly specific fluorescent-tagged monoclonal antibodies against them.

6.2 Using Bio-Organic Molecules for Free Radical Dosimetry

The objective of using bio-organic molecules for free radical dosimetry is to develop a simple, rapid and reliable method to accurately detect and quantify radiation doses for personnel dosimetry. Electron spin resonance spectrometry can measure radiation-induced free radical production in various organic molecules. This technique is used by the International Atomic Energy Agency in the high dose range. In light of current information, the development of a monitor based on free radical dosimetry (FRD) for measuring low doses of radiation appears possible. Dry bio-organic molecules (e.g., amino acids, saccharides) are sensitive to low levels of radiation dose (<0.1 Gy), and a satisfactory relationship between dose and the intensity of their ESR signals has already been established [Nakajima, 1982].

TABLE 5

BIOLOGICAL INDICATORS

Indicator System	Target Organs	Bioassay Site	Dose Range (Gy)	Time Period of Applicability After Exposure
Haematological				
1. Serum Thymidine	whole-body lymphocytes	blood	≥ 1.0	days
2. Electrophoretic Mobility	lymphocytes	blood	> 2.0 - 4.0	days
3. Mitotic Index	lymphocytes	blood	≥ 1.0	days
Cytological				
1. Micronuclei	lymphocytes	blood	0.25 - 5	months
2. Chromosomal Aberrations	lymphocytes	blood	0.05 Gy (High LET) 0.15 Gy (Low LET)	years
3. Spermatogenesis	testes	sperm	≥ 0.15	months
Genomic				
1. Mutational Expression	lymphocytes	blood	≥ 0.1	weeks/years
2. DNA Base Alteration	somatic cells lymphocytes	blood	≥ 0.1	days/years
Neurophysiological	neurons brains	body	≤ 6.0	hours-week
Immunological				
1. Subpopulation of Lymphocytes	blood system	blood	< 10	days-week
2. Cell Surface Markers	lymphatic tissue, blood	blood	< 6.0	weeks
3. Lymphocyte Stimulation	blood, lymphatic tissue	blood	< 10	weeks
4. Cytotoxic Function	blood, lymphatic tissue	blood	< 5.0	weeks

TABLE 6

BIOCHEMICAL INDICATORS

Indicator System	Target Organs	Bioassay Site	Dose Range (Gy)	Time Period of Applicability After Exposure
1. α-amylase	salivary	urine	≥ 0.5	days
2. Nucleotides and amino acids (deoxycytidine, taurine)	lymphatic tissue	urine	≥ 0.75 - 2.5	days
3. K^+, Na^+, Cl^- excretion	renal system	urine	≥ 0.5	weeks
4. Creatine/ creatinine	whole-body	urine	≥ 0.6	days
5. Serum amylase	salivary gland	blood	≥ 0.5	days
6. Electrolytes	blood	blood	≥ 0.75	days

TABLE 7

BIOPHYSICAL INDICATORS

Indicator System	Target Organs	Bioassay Site	Dose Range (Gy)	Time Period of Applicability After Exposure
Hair	whole-body partial body	hair	1 - 4	months
Nail	partial body	nail	1 - 4	years
Teeth	whole-body oral cavity	teeth enamel	0.5 - 5	years
Bone	whole-body	bone	0.5 - 5	years
Sugar Crystal	-	-	≤ 0.05	weeks/months
Organic Molecule (alanine)	-	-	≤ 0.1	days/months

The ability to measure a real individual dose in a radiation emergency is obvious from this review. Clearly, a need for bio-indicators to measure the radiation response within the dose limit to the lethal dose limit is considerable. Although the biological methods that aim at providing such important information are summarized in Tables 5 to 7, they need to be verified with extreme care before they can be recommended for routine use.

7. SUMMARY

This report recognizes the importance of the time course of early radiation effects for an accurate understanding of the nature of biochemical and biological injuries. More detailed data on the relevance of biological dose indicators, and the reasons for disparities between them, will be of use in preparedness to respond to radiation emergencies. Many of the bio-indicators described are capable of providing reasonable measurements of doses in the region of 0.1 Gy. However, efforts should be expended in further development of the most promising ones. The methods currently available or under development are evaluated on the basis of:

(a) technical considerations such as the specificity, detection limits, precision, reproducibility, possibility of sample storage, and ease of execution;

(b) assessment of whether exposure data obtained by the method are accurate; give information on duration and other time variables of exposure, on the dose that reached the target site and on total dose;

(c) whether the results obtained by the method can be interpreted on an individual or group basis;

(d) whether the method can currently be used for biological monitoring in nuclear operating facilities.

Some of the bio-indicators described above appear to be suited for limited use when there is reason to suspect exposure. However, practical implementation of these methods must still be evaluated with appropriate precautions. These methods include:

- detection of chromosomal aberration,
- detection of sister chromatid exchange,
- testing for micronuclei in lymphocytes and/or epithelial calls,
- determination of sperm morphology (in selected situations),
- determination of biochemical metabolites in biological fluids and tissues,
- detection of protein and DNA adducts,
- detection of protein variants in blood, and
- detection of enzymatic activity in urine.

The criteria for selection of bio-indicators has resulted in identifying some of the methods considered most promising at present. But they require extensive development and validation before they can be employed in the workplace for screening. This would make possible a common understanding

of the significance, current stage of development and potential use of these methods:

- detection of point mutations in blood cells,
- investigation of DNA repair mechanism in somatic cells,
- monitoring of cell surface markers on the lymphocytes, and
- measurement of free radical concentrations in bio-organic molecules.

The work to be performed in order to evaluate these principles involves collection and evaluation of data, as well as experimental studies directly related to questions asked. The experimental data obtained, performance criteria defined, and the conditions developed for optimum use of biological dosimeters can provide an assessment of the value of techniques in emergency dosimetry. Also, options to utilize molecular techniques in detecting the mutational frequency and DNA base sequences in genomes after radiation exposure must be considered. The experimental evidence to correlate absorbed radiation dose to either absolute or relative molecular response is needed for establishing the suitability of genomic indicators. The evaluation, validation and research and development of these techniques, utilizing different aspects of biotechnology, should be the focus of subsequent years.

This report has also recognized that a certain confusion has existed in distinguishing "biological monitoring" during accidental and normal operating situations. Therefore, once a method has been properly validated from a technical point of view, monitoring of individuals for exposure baseline reference values should be established, keeping in mind the special problems of selecting control groups.

Research should also be supported in obtaining the data on the quantitative importance of non-occupational confounding factors and to determine the extent to which biological endpoints measured in the above methods are predictors of accurate response to radiation exposure. It would be desirable to establish the scientific relevance of these methods in order to provide guidance for occupational health physicists and physicians who may be required to provide such information in the workplace.

8. ACKNOWLEDGMENTS

The comments and advice received from members of the Health Sciences Division have greatly improved the content and format of this report. The author is particularly indebted to Dr. D.K. Myers for his suggestions after critically reading the manuscript. This work was supported, in part, by CANDU Owner's Group (COG).

9. REFERENCES

Anderson, R.E. and Standefer, J.C. (1983) Radiation injury of the immune system. In: Cytotoxic Insult to Tissues: Effects on Cell Lineage (Potten, C.S. and Hendery, J.H., eds.), Churchill-Livingstone, Edinburgh, U.K., pp. 67-104.

Ash, P. (1980) The influence of radiation on fertility in man. Br. J. Radiol. 53: 271-278.

Ashby, J. (1988) Comparison of techniques for monitoring human exposure to genotoxic chemicals. Mut. Res. 204: 543-553.

Baranov, A.Y. (1981) Dose estimates and the prediction of the dynamics of the peripheral blood neutrophil count according to haematological indicators in human gamma-irradiation. Med. Radiologya 26: 11-16.

Barrett, A., Jacobs, A. and Kohn, J. (1982) Changes in serum amylase and its isoenzymes after whole-body irradiation. Brit. Med. J. 285: 170-171.

Basu, A.K. and Essigmann, J.M. (1988) Site-specifically modified oligonucleotides as probes for the structural and biological effects of DNA-damaging agents. Chem. Res. Toxicol. 1: 1-18.

Bender, M.A., Ava, A.A., Brooks, A.L., Evans, H.J., Groer, P.G., Littlefield, L.G., Pereira, C., Preston, R.J. and Wachholz, B.W. (1988) Current status of cytogenetic procedures to detect and quantify previous exposure to radiation. Mut. Res. 196: 103-159.

Bradley, W.E.C. and Messing, K. (1989) Studies of mutation at the HPRT locus. Mut. Res. 216:75.

Brady, J.M., Aarestad, N.O. and Swartz, H.M. (1968) In vivo dosimetry by electron spin resonance spectroscopy. Health Phys. 14: 201-212.

Cassel, C.K. and Leanning, J. (1989) Chernobyl: Learning from experience. New. Eng. J. Med. 321:254-255.

Chen, I.W., Kereiakes, J.G. and Silberstein, E.B. (1973) Radiation-induced changes in serum and urinary amylase levels in man. Rad. Res. 54: 141-151.

Chiyoko, S. (1988) Biochemical mutations detected in the children of A-bomb survivors. Radiation Effects Research Foundation HIRGA 4: 386- 390.

Cobb, C.E. and Kasmauski, K. (1989) Living with Radiation. Natl. Geographic 175: 403-437.

Commission of the European Communities (1988) Radiation Protection in the European Community--Evaluation and Suggestions, Report EUR 11449 EN, Brussels, Luxembourg.

Conklin, J.J. and Monroy, R.L. (1987). Management of radiation accidents. In : Military Radiobiology (Croklin, J.J. and Walker, R.I., eds.) Academic Press, New York, USA, pp. 347-366.

Court, L., Dufor, R. and Bassant, M.H. (1984) Trav. Scient. 5: 63-68.

Daburon, F. (1986) Biophysical methods for assessing the radiation dose. Brit. J. Radiol. (Supplement 19), 75- 81.

Deeg, H.J. (1983) Acute and delayed toxicities of total body irradiation. Int. J. Radiat. Oncol. Biol. Phys. 4: 845-851.

DeMarini, D.M., Brockman, H.E., de Serres, F.J., Evans, H.H., Stankowski, L.F. and Hsie, A.W. (1989) Specific-locus mutations induced in eukaryotes by radiation and chemicals: a perspective. Mut. Res. 220: 11-29.

Dilley, J.V. (1972) The origin of urinary taurine excretion during chronic radiation injury. Radiat. Res. 50: 191-196.

Dutereix, J., Griniski, T. and Cosset, J.M. (1987) Blood cell kinetics and total body irradiation. Radiother. Oncol. 9: 119-129.

Edwards. M. and Raymer, S. (1987) Chernobyl - one year after. National Geographic 171: 633-653.

Eisert, W.G. and Mendelsohn, M.L. (1984) Biological Dosimetry-Cytometric Approaches to Mammalian Systems, Springer-Verlag, Heidelberg, FRG.

Feinendegen, L., Muhlensiepen, H. and Porschen, W. (1982) Acute non-stochastic effect of very low dose whole-body exposure, a thymidine equivalent serum factor. Int. J. Radiat. Biol. 41: 139-150.

Feinendegen, L.E. (1986) Biochemical Indicators. In: Biological Indicators for Radiation Dose Assessment (Kaul, A., Dehos, A., Bogl, W., eds.). MMV Medizin Verlag, Munich, pp. 70-81.

Gentner, N.E., Morrison, D.P. and Myers, D.K. (1988) Impact on radiogenic cancer risk of persons exhibiting abnormal sensitivity to ionizing radiation. Health Phys. 55: 415-425.

Gerber, G.B., Gerber, G. and Kuyohara, S. (1961) Urinary excretion of several metabolites in persons accidentally exposed to ionizing radiation. Radiat. Res. 15: 314-318.

Gerber, G.B. (1986) Biochemical indicators of radiation injury. The current state of knowledge and possible expectations for the future. In: Biological Indicators for Radiation Dose Assessment. MMV Medizin Verlag, Munchen, pp. 237-239.

Gerber, G.B. (1989) Panel discussion. In: Radiation Protection: Past and Future. AECL-9959, Chalk River, Ontario, Canada, pp. 302.

Hacker, U., Schumann, J. and Gohde, W. (1982) Mammalian spermatogenesis as a new system for biological dosimetry of ionizing radiation. Acta. Radiol. Oncol. 21: 349-351.

Hall, S.C. and Wells, J. (1988) Micronuclei in human lymphocytes as a biological dosemeter: preliminary data following beta irradiation *in vitro*. J. Radiol. Prot. 8: 97-102.

Huber, R., Streng, S. and Bauchinger, M. (1983) The suitability of the human lymphocyte micronucleus assay for biological dosimetry. Mut. Res. 111: 185-193.

Ikeya, M., Miyajima, J. and Okajima, S. (1984) ESR dosimetry for atomic bomb survivors using shell buttons and tooth enamel. Jap. J. Appl. Phys. 23: 697-699.

International Atomic Energy Agency. (1986) Technical Report No. 260 on Biological Dosimetry: Chromosomal Aberration Analysis for Dose Assessment. IAEA, Vienna, Austria.

Janatipour, M., Trainor, K.J., Kutlace, R., Bennett, G., Hay, J., Turner, D.R., Morley, A.A. (1988) Mutations in human lymphocytes studied by an HLA selection system. Mutat. Res. 198: 221-226.

Jones, A.R. (1989) The performance of some personal dosemeters for measuring $H_p(10)$ over a range of photon energies. Radiat. Prot. Dosim. 28: 111-114.

Kaul, A., Dehos, A., Bogl, W., Hinz, G., Kossel, F., Schwarz, E, Stamm, A., Stephan, G. (eds.) (1986) Biological Indicators for Radiation Dose Assessment, MMV, Medizin Verlag, Munchen, FRG.

Klasen, E.A., Rigutti, A., Box, A. and Bernini, L.F. (1982) Development of a screening system for detection of somatic mutations. I. Enzyme immunoassay for detection of antibodies against specific haemoglobin determinants. J. Immunol. Methods 54: 241-250.

Kodama, Y., Hakoda, M., Shimba, H., Awa, A.A. and Akiyama, M. (1989) A chromosome study of 6-thioguanine-resistant mutants in T lymphocytes of Hiroshima atomic bomb survivors. Mut. Res. 227: 31-38.

Kondo, S. (1988) Mutation and cancer in relation to the atomic-bomb radiation effects. Jpn. J. Cancer Res. 79: 785-789.

Koteles, G.J., Somosy, Z. and Kubasova, T. (1987) Radiation-induced changes on cell surface charges. Radiat. Phys. Chem. 30: 389-399.

Langlois, R.G., Bigbee, W.L., Kyoizumi, S., Nakamura, N., Bean, M.A., Akiyama, M. and Jensen, R.H. (1987) Evidence for increased somatic cell mutations at the glycophorin A locus in atomic bomb survivors. Science 236: 445-448.

Langlois, R.G., Bigbee, W.L., and Jensen, R.H. (1988) New developments in the glycophorin A human mutation assay. A US patent-4767710.

Leake, J.W. and Rawlings, K.J. (1988) Developments in solid state detectors. Radiat. Prot. Dosim. 25: 157-166.

Lloyd, D.C. (1987) A collaborative exercise on cytogenetic dosimetry for simulated whole- and partial-body accidental irradiation. Mutat. Res. 179: 197-208.

Lucas, J.N., Tenjin, T., Straume, T., Pinkel, D., Moore, D., Litt, M. and Gray, J.W. (1989) Rapid human chromosome aberration analysis using fluorescence _in situ_ hybridization. Int. J. Radiation Biol. 56: 35-44.

Majborn, B. (1980) A critical review of nuclear accident dosimeters. EUR 6838 (Luxembourg: Commission of the European Communities).

Majborn, B. (1984) Estimation of accidental gamma dose by means of thermoluminescence from watch jewels. Health Phys. 46: 917-919.

Mann, W.B., Ayres, R.L., and Garfinkel, S.B. (1988) Radioactivity and its measurement. Appl. Radiat. Isotopes 39:

Martin, R.H., Rademaker, A., Hildebrand, K., Barnes, M., Arthur, K., Ringrose, T., Brown, I.S. and Douglas, G. (1989) A comparison of chromosomal aberrations induced by in vivo radiotherapy in human sperm and lymphocytes. Mut. Res. 226: 21-30.

Maruyama, T., Kumamoto, V., and Noda, V. (1988) Reassessment of q doses from the atomic bombs in Hiroshima and Nagasaki. Radiat. Res. 113: 1-14.

Mazurick, V.K.M., Mikhailov, V.F. and Tarakanova, M.P. (1981) Post-irradiation changes in erythrocyte membranes as a radiological basis for biochemical indicators of radiation damage to the organism. In: SAAS-280, 206-219.

McFarland, W. and Pearson, H.A. (1963) Haematological events as dosimeters in human total-body irradiation. Radiology 80: 850-855.

Mendelsohn, M.L. (1990) New approaches for biological monitoring of radiation workers. Health Physics 59: 23-28.

Miller, S.D. and Durham, J.S. (1990) Internal dosimeter based on optically stimulated luminescence. PNL-7200 Pt. 5, Pacific Northwest Laboratory, Richland, Washington, USA.

Mole, H. (1984) Sodium in man and the assessment of radiation dose after criticality accidents. Phys. Med. Biol. 29: 1307-1327.

Morley, A.A., Trainor, K.J., Seshadri, R., Ryall, R.G. (1983) Measurement of in vivo mutations in human lymphocytes. Nature 302: 155-156.
National Research Council (US National Academy of Sciences) (1987) An assessment of the new dosimetry for A-bomb survivors. National Academy Press, Washington.

Myers, D.K. (1990) Health Sciences Division, AECL Research, Chalk River Laboratories, Chalk River, Ontario, personal communication.

Nakajima, T. (1982) The use of organic substances as emergency dosemeters. Int. J. Appl. Radiat. Isot. 33: 1077-1084

Nakajima, T. (1989) Possibility of retrospective dosimetry for persons accidentally exposed to ionizing radiation using electron spin resonance of sugar and mother-of-pearl. British J. Radiol. 62: 148-153.

Nakajima, T. and Otsuki, T. (1990) Dosimetry for radiation emergencies: radiaton-induced free radicals in sugar of various countries and the effect of pulverizing on the ESR signal. Appl. Radiat. Isot. 41: 359-365.

Ogunranti, J.O. (1989) Haematological indices in Nigerian exposed to radioactive waste. Lancet 11: 667-668.

Pinkel, P., Straume, T., Gray, J.W. (1986) Cytogenetic analysis using quantitative, high sensitivity, fluorescence hybridization. Proc. Natl. Acad. Sci. (USA) 83: 2934-2938.

Potten, C.S. (1986) Biological dosimetry of local radiation accidents of skin: possible cytological and biochemical methods. Brit. J. Radiol. (supplement 19), 82-85.

Pradhan, A.S. (1989) Cooled optically stimulated luminescence of TLDs for radiation dosimetry. Radiat. Prot. Dosim. 29: 213-215.

Ramalho, A.T., Nascimento, A.C.H. and Natarajan, A.T. (1988) Dose assessments by cytogenetic analysis in the Goiania (Brazil) radiation accident. Radiat. Prot. Dosimetry 25: 97-100.

Regulla, D.F. and Deffner, U. (1982) Dosimetry by ESR spectroscopy of alanine. Int. J. Radiat. Isot. 33: 1101-1114.

Regulla, D.F., Scharmann, A. and McLaughlin, W.L. (Eds.) (1989) ESR dosimetry and applications. Appl. Radiat. Isot. 40: 829-1246.

Rezunkova, O.P., Dubur, G., Deme, A. and Sunguorv, A.Y. (1988) Biophysical characteristics of thymocytes following radiation. Analysis of cell surface alterations with the aid of the cationic fluorescence probe DSM. Studia Biophys. 128: 199-205.

Sagstuen, E., Theisen, H. and Henriksen, T. (1983) Dosimetry by ESR spectroscopy following a radiation accident. Health Phys. 45: 961-968.

Savage, J.K. (1989) The production of chromosomal structural changes by radiation. Experientia 45: 52-59.

Scharman, A. (1989) ESR - a scientific tool for applications. Appl. Radiat. Isot. 40: 829-834.

Schreiber, G.A., Spiegelberg, A., Willich, N., Hofmann, R. and Bogl, K.W. (1989) Changes in oligosaccharide of human blood cells measured by tritium- and FITC-labelled lectins after in vitro- and in vivo-irradiation-lectin binding as a "biological dosimeter". Int. J. Radiat. Biol. 55: 843-859.

Seifert, A.M., Missing K., Bradely, W.E.C. (1987) Exposure of nuclear medicine patients to ionizing radiation is associated with rises in HPRT mutant frequency in peripheral T lymphocytes. Mut. Res. Lett. 191: 57-63.

Shimano, T., Iwasaki, M., Miyazawa, C., Miki, T., Kai, A.A. and Ikeya, M. (1989) Human tooth dosimetry for gamma-rays and dental X-rays using ESR. Appl. Radiat. Isot. 40: 10-12.

Shimizu, Y., Kato, H. and Shull, W.J. (1990) Studies of the mortality of A-bomb survivors. Radiat. Res. 121: 120-141.

Skinner, R.W., Kalos, F. and Bond, J.A. (1985) An introduction to radiation protection principles. AECL-8057 (Rev.1), Chalk River, Canada.

Spangler, G. and Cassen, B. (1967) Electrophoretic mobility, size distribution and electron micrograph responses of lymphocytes to radiation. Radiat. Res. 30: 22-37.

Springer, D.L. (1989) Use of adducts to haemoglobin and DNA as molecular dosimeters. Health Physics 57 (supplement 1): 245.

Stephan, G. and Oestericher, U. (1989) An increased frequency of structural chromosome aberrations in persons present in the vicinity of Chernobyl during and after the reactor accident. Is this effect caused by radiation exposure? Mut. Res. 223: 7-12.

Stevenson, A.P., Habbersett, R.C., Voelz, G.L. and Stewart, C.C. (1989) Leucocyte subset analysis on blood from workers exposed externally and internally to radiation. Abstracts of papers for the thirty-seventh meeting of the Rad. Res. Society, Seattle, pp. 175.

Stewart, C.C., Stephenson, A.P. and Habbersett, R.C. (1988) The effect of low-dose irradiation on unstimulated and PHA-stimulated human lymphocyte subsets. Int. J. Radiat. Biol. 53: 77-87.

Streffer, C. (1971) Biochemical post-irradiation changes and radiation indicators. In: Biochemical Indicators of Radiation Injury in Man. IAEA, Vienna, pp. 11-32.

Twan, E.J. and Binks, K. (1989) A cytogenetic study of radiation workers: The influence of dose accumulation patterns and smoking. Radiat. Prot. Dosimetry. 28: 173-180.

Unrau, P. (1989) Radiation Biology Branch, AECL Research, Chalk River Laboratories, Chalk River, Ontario, personal communication.

Van Dilla, M.A., Deaven, L.L., Albright, K.L., Allen, N.A., Aubuchon, M.R., Bartholdi, M.F., Brown, N.C., Campbell, E.W., Carrano, A.V., Clark, L.M., Cram, L.S., Crawford, B.D., Fuscoe, J.C., Gray, J.W., Hildebrand, C.E., Jackson, P.J., Jett, J.H., Longmire, J.L., Lozes, C.R., Luedemann, M.L., Martin, J.C., McNinch, J.S., Meincke, L.J., Mendelsohn, M.L., Meyne, L., Moyzis, R.K., Munk, A.C., Perlman, J., Peters, D.C., Silva, A.J., Trask, B.J. (1986) Human chromosome-specific DNA libraries: construction and availability. Bio/Tech 4: 537-522.

Walden, T.L., Jr. and Farzaneh, N.K. (1990) Biological dosimeters, In Biochemistry of Ionizing Radiation. Raven Press, New York, pp. 160-175.

Watt, D.E. (1989) An approach towards a unified theory of damage to mammalian cells by ionising radiation for absolute dosimetry. Radiat. Prot. Dosim. 27: 73-84.

Wogan, G.N. and Gorelick, N.J. (1985) Chemical and biochemical dosimetry of exposure to genotoxic chemicals. Environ. Health Perspec. 62: 5-18.

Workshop on Low Dose Radiation and the Immune System (1988) Proceedings published as special issue of Int. J. Radiat. Biol. 53. 1-201.

ISSN 0067-0367

To identify individual documents in the series we have assigned an AECL- number to each.

Please refer to the AECL- number when requesting additional copies of this document

from

Scientific Document Distribution Office
Atomic Energy of Canada Limited
Chalk River, Ontario, Canada
K0J 1J0

Price: A

ISSN 0067-0367

Pour identifier les rapports individuels faisant partie de cette série nous avons assigné un numéro AECL- à chacun.

Veuillez faire mention du numéro AECL- si vous demandez d'autres exemplaires de ce *rapport*

au

Service de Distribution des Documents Officiels
Énergie atomique du Canada limitée
Chalk River, Ontario, Canada
K0J 1J0

Prix: A

© ATOMIC ENERGY OF CANADA LIMITED, 1990

0176-91