An Assessment of Radioactivity Level in $^{51}$Cr-contaminated Dry Solid Waste Generated from a Research Facility for Verification of Clearance Levels

Tomohiro Nagamatsu$^{1,2}$, Tadashi Hanafusa$^2$, Toshiro Ono$^{2,4}$ and Kiyonori Yamaoka$^1$

$^1$Department of Radiological Technology, Graduate School of Health Sciences, Okayama University, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan
$^2$Department of Radiation Research, Advanced Science Research Center, Okayama University, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan

Received Jun. 7, 2010; accepted Jul. 21, 2010

Radioactive waste generated from research laboratories and other facilities is regulated by the Law Concerning Prevention from Radiation Hazards due to Radioisotopes etc. (Prevention Law). However, the Prevention Law does not provide the level of clearance or the procedures to follow for compliance monitoring. To assess radioactivity amounts for making decisions about clearance levels, the radioactivity levels in dry solid semi-combustible wastes generated from biomedical research, such as $^{51}$Cr-release assays, were measured and evaluated. Radioactivity of semi-combustible waste was 1.42–6.32% of the initial level. In comparison, records for the past 8 years in the Shikata Laboratory, Department of Radiation Research, Okayama University Advanced Science Research Center, indicated 7% to 90% of the initial radioactivity remained in the waste and was differed widely among researchers. This study determined an accurate radioactivity level in dry solid waste, which could lead to savings in disposal costs.

Key words: low level radioactive waste, $^{51}$Cr, cell-mediated cytotoxicity assay, disposal, clearance, prevention law

1. Introduction

Under the Law Concerning Prevention from Radiation Hazards due to Radioisotopes etc. (Prevention Law), dry solid waste must be compacted into drums and stored in an on-site facility until collection and disposal at the storage facility of the Japan Radioisotope Association (JRIA). Most dry solid waste from hospitals and scientific research laboratories is low-level radioactive waste (LLRW). Some medical wastes are incinerated at the Takizawa Laboratory, JRIA. However, permanent disposal of non-medical wastes is not allowed. These wastes must be stored at the JRIA or the Japan Atomic Energy Agency. However, space at these facilities is limited and decreasing.

Approximately 34,000 50-liter drums of dry solid waste are collected annually by the JRIA. The drums contain approximately 175 different nuclides, more than half of which involve short-lived nuclides (half life < 90 days), including $^{32}$P, $^{35}$S, $^{51}$Cr, and $^{125}$I. Amendment in 2005 of the Prevention Law provided for the construction and operation of a landfill site for disposal of LLRW. The law, however, does not provide a clearance level or outline procedures for compliance monitoring. Therefore, there is a need for clearance level regulation for wastes from research facilities. This study involved the investigation and evaluation of radioactivity levels in $^{51}$Cr-contaminated dry solid waste generated from cell-mediated cytotoxicity assays. This was done to determine radioactivity levels for introduction of clearance levels specified in the RS-G-1.7 of the International Atomic Energy Agency (IAEA).

2. Materials and methods

2.1 Data analysis

$^{51}$Cr used in the Shikata Laboratory, Department of Radiation Research, Okayama University Advanced Science Research Center (Shikata Laboratory), was collected and analyzed for 1999–2006. All of the $^{51}$Cr delivered to the Shikata
An assessment of radioactivity level in $^{51}$Cr-contaminated dry solid waste generated from a research facility for verification of clearance levels

Laboratory has been used for a cell-mediated cytotoxicity assay, performed mainly by researchers in the Department of Immunology. Individual records of radioactivity in semi-combustible waste as estimated by the researchers were analyzed.

2.2 Cell-mediated cytotoxicity assay

The cell-mediated cytotoxicity assay involves target tumor cells labeled with $^{51}$Cr, which were then released into the medium when the cells were lysed by tumor-specific cytotoxic T lymphocytes (CTLs). In brief, as shown in Fig. 1, $1 \times 10^9$ cells (mouse radiation-induced leukemia) were labeled with $2 \text{ MBq} \text{ of Na}_2^{51}\text{CrO}_4$ (PerkinElmer, Waltham, MA, USA) in $15 \text{ ml}$ polypropylene tubes (BD Biosciences, Bedford, MA, USA) for 1 hr at $37^\circ \text{C}$ under 5% CO$_2$ in air. After washing 3 times, cell numbers were counted, adjusted to $5 \times 10^5$, and used as target cells. Then, $5 \times 10^3$ $^{51}\text{Cr}$-labeled target cells (100 µl) were cultured in a 96-well round-bottom plate (BD Biosciences) with $100 \mu$l of effector cells (RL $\phi^1$ specific CTL) for 4 hrs at $37^\circ \text{C}$ at various effector and target cell ratios. The supernatants (100 µl) from each well were removed and collected in small polypropylene tubes. Cotton swabs were then placed in the respective tubes to absorb the supernatants, and their radioactivities were measured with a gamma counter.

2.3 Radioactivity measurement

Liquid waste consisting of medium collected from $^{51}\text{Cr}$-labeled target cell washing and unused $^{51}\text{Cr}$-labeled target cell suspension was mixed and a small fraction (100 µl) was measured using a gamma counter equipped with a $3 \times 3$ inch NaI(Tl) well-type detector (Aloka ARC-2000, Mitaka, Tokyo). Cotton swabs (96 per assay) and papers for wiping slide used for counting $^{51}\text{Cr}$-labeled target cells were combustible waste (Fig. 1). Each cotton swabs and the papers were put in the assay tubes and measured with ARC-2000. Semi-combustible waste generated from each assay consisted of 3 polypropylene tubes (15 ml), one large polypropylene tube (50 ml), 2 polystyrene pipettes (5 and 10 ml), one polystyrene dish (60 x 15 mm), one 96-well polystyrene plate, 96 small polypropylene tubes (500 µl), a hundred pipette tips, and rubber gloves. Since some of them could not be measured directly, radioactivity of semi-combustible waste was calculated as (initially used radioactivity) – (radioactivity of the liquid waste + radioactivity of the combustible waste). The radioactivity of the initially used Na$_2^{51}\text{CrO}_4$ for target cell labeling and the wastes was determined from the relative counting ratio of known $^{51}\text{Cr}$ standard with a similar geometry.

3. Results and Discussion

3.1 Experience of $^{51}\text{Cr}$ used in the Shikata Laboratory

Shikata Laboratory is a core facility for radioisotope research at Okayama University enrolling approximately 200 permitted radiation-handling workers. The majority of the waste generated is in the form of semi-combustible dry solids, containing short-lived radionuclides such as $^{32}\text{P}$, $^{51}\text{Cr}$, and $^{125}\text{I}$. The

![Fig. 1. Schematic representation of the procedure used for cell-mediated cytotoxicity assays.](image-url)
$^{51}$Cr is used solely for cell-mediated cytotoxicity assays by researchers in the Department of Immunology. As shown in Fig. 2, the number of assays performed peaked in 2001. $^{51}$Cr used in 2001 was investigated. Nine researchers used a total of 823 MBq of $^{51}$Cr and disposed of 465 MBq (56% of the initial activity) as semi-combustible wastes. However, radioactivity levels of the semi-combustible wastes recorded differed by researcher (Table 1). For example, researcher A recorded the percentage radioactivity remaining in semi-combustible wastes as 9% of the initial value, while researcher T reported it as 50% for every experiment (Table 2). One of these values is probably inaccurate, because a researcher’s estimate of radioactivity levels remaining in waste material may be just a guess.

### 3.2 Determination of radioactivity levels in semi-combustible wastes generated from cell-mediated cytotoxicity assays

CTLs destroy target cells such as virus-infected cells or tumor cells. One of the most reliable cell-mediated cytotoxicity assays for CTL activity is the $^{51}$Cr-release assay. This assay is widely used in studies of antigen-specific CTLs.\textsuperscript{6,7} In a previous study, the radioactivity levels of semi-combustible wastes generated from $^{51}$Cr-release assay were estimated by comparing the wastes with mock samples containing a $^{51}$Cr standard.\textsuperscript{8} The estimated radioactivity levels were 13% to 38% of the initial radioactivity.

To precisely determine radioactivity levels of semi-combustible waste, 5 independent $^{51}$Cr-release assays were performed. Three types of wastes were identified: liquid waste, combustible waste (including cotton swab and papers), and semi-combustible waste (including plastic labwares such as three types of polypropylene tubes, 96-well plate, pipettes, dishes).

### Table 1  Total used $^{51}$Cr activity and semi-combustible wastes recorded in the year of 2001

| Researchers | Used (MBq) | Disposed (MBq) | Disposed (Used%) |
|-------------|------------|----------------|------------------|
| A           | 77.787     | 5.629          | (7.2%)           |
| B           | 38.475     | 3.559          | (9.2%)           |
| H           | 49.824     | 4.214          | (8.5%)           |
| K           | 374.039    | 333.637        | (89.2%)          |
| N           | 51.631     | 37.059         | (71.8%)          |
| O-1         | 20.517     | 13.568         | (66.1%)          |
| O-2         | 5.311      | 4.780          | (90.0%)          |
| T           | 66.199     | 32.599         | (49.2%)          |
| U           | 139.655    | 29.984         | (21.5%)          |
| **Total**   | **823.438**| **465.029**    | **(56.5%)**      |
An assessment of radioactivity level in $^{51}$Cr-contaminated dry solid waste generated from a research facility for verification of clearance levels

The generated waste was collected and radioactivity of liquid waste and combustible waste was measured. As shown in Table 3, liquid waste contained the greatest amount of remaining radioactivity: 93.3–98.3% of the initial radioactivity. The radioactivity level of semi-combustible waste was thus calculated as 1.42–6.32% of the initial radioactivity, which was lower than the radioactivity estimated by the researcher (Table 1).

### Table 2 Individual record of $^{51}$Cr activity in semi-combustible wastes in the year of 2001

| Assay | Used (MBq) | Disposed (MBq) | (Disposed/Used) (%) |
|-------|------------|----------------|---------------------|
| 1     | 4.745      | 0.427          | (9.0%)              |
| 2     | 1.780      | 0.160          | (9.0%)              |
| 3     | 6.256      | 0.563          | (9.0%)              |
| 4     | 9.863      | 0.888          | (9.0%)              |
| 5     | 11.100     | 0.999          | (9.0%)              |
| 6     | 17.160     | 0.172          | (1.0%)              |
| 7     | 7.001      | 0.630          | (9.0%)              |
| 8     | 5.920      | 0.533          | (9.0%)              |
| 9     | 3.111      | 0.280          | (9.0%)              |
| 10    | 3.525      | 0.317          | (9.0%)              |
| 11    | 2.886      | 0.260          | (9.0%)              |
| 12    | 4.440      | 0.400          | (9.0%)              |
| **Total** | **77.787** | **5.629**    | **(7.2%)**         |

### Table 3 Measurement of $^{51}$Cr-contaminated waste generated from cell-mediated cytotoxicity assay

| Wastes | Liquid | Combustible | Semi-combustible |
|--------|--------|-------------|------------------|
| Assay | Used (MBq) | Volume (ml) | Activity (MBq) | Weight (g) | Activity (kBq) | Weight (g) | Activity (kBq) |
| 1     | 2.120   | 23.5        | 1.978           | 27.8       | 8             | 165.2       | 134             |
| 2     | 2.116   | 30.0        | 2.021           | 27.4       | 5             | 174.2       | 90              |
| 3     | 2.112   | 26.5        | 2.077           | 27.4       | 5             | 180.6       | 30              |
| 4     | 1.786   | 25.5        | 1.685           | 27.1       | 3             | 169.3       | 98              |
| 5     | 1.838   | 25.5        | 1.772           | 29.5       | 4             | 177.7       | 62              |

1) Calculated as in Materials and Methods.

### 3.3 Application of the clearance level for LLRW

The law for the Regulation of Nuclear Source Material, Nuclear Fuel Material, and Reactors (Reactor Regulation Law) regulates the management of LLRW generated from nuclear facilities. The Nuclear Science Committee and Radioactive Waste Safety Subcommittee in the Cabinet Office evaluated the basic concept of the clearance level and methods for monitoring compliance using clearance level for materials such as concrete...
and metal scraps generated from nuclear facilities. The Reactor Regulation Law was amended in 2005 to provide a clearance level based on IAEA safety standards.

In contrast, the Prevention Law regulates the LLRW from research facilities and other institutions but does not provide the clearance level. Therefore, the Clearance Technological Examination Study Group in the Science and Technology Policy Bureau, the Ministry of Education, Culture, Sports, Science and Technology has been considering a clearance level. More than one-half of the radioactive wastes collected by JRIA from 1999 through 2003 were the LLRW containing nuclides with a half-life shorter than 90 days. In the 2006 interim report, clearance levels for nuclides such as $^{32}$P, $^{33}$P, $^{51}$Cr, $^{125}$I, and $^{35}$S, each with a half-life shorter than 90 days, were estimated based on the records attached to each LLRW-containing drum at JRIA. After one year of storage, nuclides with a half-life shorter than 30 days, such as $^{32}$P, $^{33}$P, and $^{51}$Cr, were expected to be below clearance levels. Nuclides with a half-life shorter than 60 days, such as $^{125}$I, and nuclides with a half-life shorter than 90 days, such as $^{35}$S, were expected to be below clearance levels after 2 and 3 years, respectively.

To apply clearance value to LLRW generated from research facilities, radioactivity levels in the wastes must be verified to be below the concentrations of all of the nuclides values adopted in RS-G-1.7. Evaluation of the nuclides and their concentration by direct measurement from outside of the drums containing the radioactive wastes is sometimes difficult. In this study, 5 independent $^{51}$Cr-release assays were performed to precisely determine radioactivity levels of semi-combustible waste. It revealed that liquid waste contained 93.3–98.3% of the initially used radioactivity (Table 3). Thus, radioactivity levels in $^{51}$Cr-contaminated semi-combustible wastes generated from cell-mediated cytotoxicity assays were determined to be 1.42–6.32% of the initial radioactivity. These actual radioactivity level results cast suspicion on the accuracy of the records in the Shikata Laboratory from the past 8 years. Thus, it is necessary to verify the method of determining radioactivity levels in LLRW generated from research facilities to incorporate a clearance system under the Prevention Law. The present study proposes a method of assessing radioactivity levels in wastes that could lead to savings LLRW disposal costs.

4. Conclusion

In this study, the radioactivity in $^{51}$Cr-contaminated semi-combustible wastes generated from cell-mediated cytotoxicity assays was determined to be 1.42–6.32% of the initial radioactivity level. $^{51}$Cr has been used solely for cell-mediated cytotoxicity assays by researchers in the same Department using the same methodology since 1999. The records of radioactivity remaining in $^{51}$Cr-contaminated semi-combustible wastes as estimated by the researchers over the past 8 years in the Shikata Laboratory varied widely from 7 to 90% of the initial radioactivity. The present study offers a method for the accurate assessment of radioactivity levels in LLRW generated from research facilities for future application of clearance under the Prevention Law.

Acknowledgements

The authors thank Mr. I. Kinno for technical assistance, and Ms. K. Nakamura and Ms. T. Terada for preparation of the manuscript. We also thank Dr. M. Yamada, (Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University) for his continuous encouragement throughout this study.

References

1) The Clearance Technological Examination Study Group in the Science and Technology Policy Bureau, the Ministry of Education, Culture, Sports, Science and Technology: Technological analysis on the management of clearance level under the Prevention Law (Interim report), (2006), [in Japanese].

2) International Atomic Energy Agency: Application of the Concepts of Exclusion, Exemption and Clearance, IAEA Safety Guide No. RS-G-1.7, JAEA, Vienna (2004).

3) Nakayama, E., Shiku, H., Takahashi, T., Ottegen, H. F. and Old, L. J.: Definition of unique cell surface antigen of mouse leukemia RL $\sigma^1$ by cell-mediated cytotoxicity, Proc. Natl. Acad. Sci. USA, 76, 3486–3490 (1979).

4) Uenaka, A., Ono, T., Akisawa, T., Wada, H., Yasuda, T. and Nakayama, E.: Identification of a unique antigen peptide pRL1 on BALB/c RL $\sigma^1$ leukemia recognized by cytotoxic T lymphocytes and its relation to the akt oncogene, J. Exp. Med., 180, 1599–1607 (1994).

5) Hata, H., Uenaka, A., Takada, I., Kenjo, A., Takahashi, M., Ono, T., Fujita, T. and Nakayama, E.: Occurrence of tumor antigen pRL1a specific CD8 T cells in spleen cells from syngeneic BALB/c, semi-allogeneic (BALB/c × C57BL/6) F1, and allogeneic C57BL/6 mice, Int. J. Oncol., 20, 1019–1025 (2002).

6) Ono, T., DiLorenzo, T. P., Wang, F., Kalergis, A. M. and Nathenson, S. G.: Alterations in TCR-MHC contacts subsequent to cross-recognition of class I MHC and singly substituted peptide variants, J. Immunol., 161, 5454–5463 (1998).

7) Van der Bruggen, P., Traversari, C., Chomez, P., Lurquin, C., De Plaen, E., Van den Eynde, B., Knuth, A. and Boon, T.: A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma, Science, 254, 1643–1647 (1991).
8) Nagamatsu, T., Sakoda, A., Hanamoto, K., Kinno, I., Hanafusa, T., Yamaoka, K. and Ono, T.: Methodological approach for assessment of the radioactivity level in dry solid waste, Radiat. Safety Manag., 7, 6–10 (2008).

9) Government of Japan: Joint Convention on the Safety of Spent Fuel Management and on the Safety of Radioactive Waste Management, National Report of Japan for the Second Review Meeting, (2005).

10) Radioactive Waste Evaluation Office Safety Standard Division, Incorporated Administrative Agency, Japan Nuclear Energy Safety Organization: Clearance System, (2006).