Idaho National Engineering Laboratory

LOCKHEED MARTIN

Lockheed Idaho Technologies Company
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TRANSMITTAL OF REPORT, *NATIONAL INSTITUTES OF HEALTH: MIXED WASTE MINIMIZATION AND TREATMENT*, DOE/LLW-218 - PDW-75-95

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Enclosed is a document produced by the National Low-Level Waste Management Program entitled *National Institutes of Health: Mixed Waste Minimization and Treatment*, DOE/LLW-218, August 1995. This report was prepared in response to a request for technical assistance from the Appalachian States Low-Level Radioactive Waste Commission on the issues of possible minimization and treatment approaches for wastes generated by the biomedical community.

This document (DOE/LLW-218) is the second of two reports looking at mixed waste management processes at the National Institutes of Health (NIH). The first report, *National Institutes of Health: Mixed Waste Stream Analysis*, DOE/LLW-208, is a characterization of the liquid low-level radioactive mixed waste (LLMW) generated at the NIH. This second document takes the waste stream analysis data and develops process groups that appear to be derived from similar activities, while specifically looking at ways to prevent or minimize the generation of these more-difficult-to-manage mixed wastes.

The report concludes that the largest quantities of LLMW evaluated for the study are associated with a relatively small number of research techniques. The NIH has made significant progress in reducing LLMW generation rates from previous years and this study indicates that there are mixed waste management options that have the potential for even further reduction.

Should you have any questions concerning this report or wish additional copies, please contact Kathleen Asbell at (208) 526-8330 or Donna Lake at (208) 526-6927.

K. A. Asbell for PDW

Philip D. Wheatley
National Low-Level Waste Management Program

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DOE/LLW-208

National Institutes of Health: Mixed Waste Stream Analysis

N. P. Kirner
G. P. Faison
D. R. Johnson

Published August 1994

Idaho National Engineering Laboratory
EG&G Idaho, Inc.
Idaho Falls, Idaho 83415

Prepared for EG&G Idaho, Inc.
Under Subcontract No. C90-132744
and for the
U.S. Department of Energy
Assistant Secretary for Environmental Management
Contract DE-AC07-76ID01570

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ABSTRACT

This report presents the methodology and findings of a technical analysis conducted on the many biomedical mixed waste streams generated by the National Institutes of Health (NIH) that are believed to be representative of the biomedical mixed waste community. The analysis included the characterization of the mixed waste streams based on information contained in an NIH mixed waste management database; the report combines the characterization information into similar process categories. The resulting information will help define and characterize the mixed waste streams generated by the biomedical community so that an identification can be made of the waste streams that can and cannot be minimized and treated by current options.

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DOE/LLW-208

National Institutes of Health: Mixed Waste Stream Analysis

N. P. Kirner
G. P. Faison
D. R. Johnson

Published August 1994

Idaho National Engineering Laboratory
EG&G Idaho, Inc.
Idaho Falls, Idaho 83415

Prepared for EG&G Idaho, Inc.
Under Subcontract No. C90-132744
and for the
U.S. Department of Energy
Assistant Secretary for Environmental Management
Contract DE-AC07-76ID01570

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ACRONYMS

ATP	Adenosine triphosphate
DNA	Deoxyribonucleic acid
DOE	U.S. Department of Energy
EPA	U.S. Environmental Protection Agency
HPLC	High performance liquid chromatography
LLW	Low-level radioactive waste
LLRWPA	Low-Level Radioactive Waste Policy Act of 1980
NIH	National Institutes of Health
NLLWMP	National Low-Level Waste Management Program
RCRA	Resource Conservation and Recovery Act
RNA	Ribonucleic acid

National Institutes of Health: Mixed Waste Stream Analysis

1. INTRODUCTION

1.1 Background and Purpose

The Low-Level Radioactive Waste Policy Amendments Act of 1985 requires that the U.S. Department of Energy (DOE) provide technical assistance to host States, compact regions, and unaffiliated States to fulfill their responsibilities under the Act. The National Low-Level Waste Management Program (NLLWMP) operated for DOE by EG&G Idaho, Inc. provides technical assistance in the development of new commercial low-level radioactive waste disposal capacity.

The NLLWMP has been requested by the Appalachian Compact to help the biomedical community become better acquainted with its mixed waste streams, to help minimize the mixed waste streams generated by the biomedical community, and to provide applicable treatment technologies to those particular mixed waste streams. Mixed waste is waste that satisfies the definition of low-level radioactive waste (LLW) in the Low-Level Radioactive Waste Policy Act of 1980 (LLRWPA) and contains hazardous waste that either (a) is listed as a hazardous waste in Subpart D of 40 CFR 261, or (b) causes the LLW to exhibit any of the hazardous waste characteristics identified in 40 CFR 261.

The purpose of this report is to clearly define and characterize the mixed waste streams generated by the biomedical community so that an identification can be made of the waste streams that can and cannot be minimized and treated by current options. An understanding of the processes and complexities of generation of mixed waste in the biomedical community may encourage more treatment and storage options to become available.

Specifically, the report includes a technical analysis of the many biomedical mixed waste streams generated by the National Institutes of Health (NIH) that are believed to be representative of the biomedical mixed waste community.

The NIH has numerous processes that generate mixed wastes. The NIH has created a detailed database for use in tracking the low-level radioactive containerized mixed waste generated by these processes. This database contains detailed information on waste origination, hazardous waste numbers, chemical properties, radionuclide assays, waste volume, waste generation processes, treatment processes used on the wastes, and waste storage, shipping, and handling requirements. The database is maintained using dBASE IV and contains approximately 3,800 records.

In addition to the containerized mixed waste that is tracked by the database, there are two supplemental mixed waste streams that were not included in the database as of July 1993. These two supplemental waste streams are tracked outside of the database based on their properties,

waste stream size, and methods of handling and disposal. The supplemental mixed waste is discussed further in Section 2.2 and Section 4.

1.2 Methodology

1.2.1 Preliminary Analyses

Ebasco Environmental performed a preliminary analysis of mixed waste streams using the database maintained by the NIH. The database received from NIH is in a dBASE IV format and contains 17 database groups within a "mixed waste" catalog. The 17 databases contain approximately 4,000 records dating from 1989 through June 1993. In most cases, one database record was created for each waste container received by the waste management group at NIH. Three of the 17 databases contain the majority of the database records. The waste management group at NIH uses the 17 individual database groups to track the waste through the waste handling steps in the NIH waste management process and to separate the database information according to chronological period and container number.

The 17 databases used for the analysis contain records with a total of 187 fields each. The field identifiers used in the records required some clarification for the databases as received. A detailed list of questions was prepared and forwarded to NIH for the fields that were subject to variable interpretation. NIH responded with information that was used to clarify the interpretation of the fields. Copies of these communications were included in an earlier letter report to EG&G Idaho, Inc. on August 27, 1993. Ebasco Environmental personnel also participated in a tour of the NIH Building 21 waste processing area that was conducted by NIH staff. Procedures for mixed waste acceptance, management, and disposal were outlined, as well as use and maintenance of the database.

The NIH database information was used to group the waste streams into process groups that appeared to be derived from similar activities. The processes were grouped based on a comparison of the descriptions of the waste generating processes provided for each container of mixed waste in the database. The groups were used to identify the major process sources and develop characterizations of the waste produced. Preliminary information was developed and presented to the Biomedical Mixed Waste Workshop held in Bethesda, Maryland, on August 4 and 5, 1993. Some of the key preliminary findings were (a) relatively few waste generating processes produced the large majority of the waste, and (b) wastes produced by these processes have similar properties and compositions.

1.2.2 Subtask 1—Identify Processes Responsible for Waste Generation

A waste generating process was identified for each of the database records in two sequential fields containing a total of 128 characters. Generally, the process descriptions consisted of less than 10 words and were therefore somewhat cryptic. A problem with this data was that the descriptive words for the waste generating processes varied between records from what appeared to be similar processes. This created some difficulty in extracting and comparing the process data.

This difficulty was overcome by using key words for each process group to identify records, which described waste that appeared to originate from each of the particular process groups.

When the key word was found in the process description, the record was selected for inclusion in the process group. Section 2 presents the waste generating process groups that were identified in this way.

1.2.3 Subtask 2—Combine Similar Processes

After the waste generating processes had been identified, similar processes were combined based on similar process activities. Wastes were grouped into 31 similar process categories, based on database information, and one unknown category. These 32 categories are used throughout the report for analytical purposes.

1.2.4 Subtask 3—Segregate Waste Streams Generated by One-time Operations

"One-time" waste streams were those waste streams that were produced relatively infrequently with properties and compositions that were markedly dissimilar from other waste streams. The process description information and the waste composition and waste properties data were used to identify these streams. The results from Subtasks 1 and 2 were used to identify and characterize the one-time waste streams. The one-time wastes are discussed in Section 3.

1.2.5 Subtask 4—Assemble Detailed Waste Stream Characterizations

The process information, waste composition data, and waste properties data developed in Subtasks 1 and 2 were used to develop the waste stream characterization for each of the waste generating processes. The available data limited the degree of detail that could be provided for the waste characterization. The characterizations are limited to radioassay parameters, pH, flashpoint, chemical composition data, and U.S. Environmental Protection Agency (EPA) hazardous waste codes. Section 4 presents this information.

1.2.6 Subtask 5—Identify and Categorize Mixed Waste Streams for Which No Current Treatment/Disposal Options are Available

Information was available in the NIH database for treatment and disposal options that have been used for the mixed wastes generated at NIH. This information was combined with information developed in Subtask 4 to identify the waste streams that do not have current treatment and disposal options. This information was used to categorize the waste according to treatment and disposal options. Section 5 includes a discussion of this information.

1.2.7 Development of Final Report

The preparation of the final report required that the five subtasks be completed for all of the information from the databases. Essentially, the required data from each of the databases were combined into a focused database that was then used to complete the analyses of the data required to complete the subtasks. The fields used in this final analysis were primarily the process description, waste volume, generator location, radioassay information, physical properties of pH and flashpoint, waste composition data, and the Resource Conservation and Recovery Act (RCRA) hazardous waste codes. This report presents the results of the analysis.

1.3 Report Organization

Information on the waste generating processes is presented in Section 2 along with the results of an analysis of the waste volume produced by each of the main process groups. Section 3 presents information on the one-time processes that have produced mixed waste. Detailed characterization information by process group is presented on the waste streams in Section 4. Additional detailed information is provided in Section 5 on waste streams that apparently have no current treatment and disposal options. Section 6 presents the conclusions of the analysis and briefly describes the subsequent steps in a waste minimization process.

2. IDENTIFICATION AND COMBINATION OF THE PROCESSES RESPONSIBLE FOR WASTE GENERATION

Radioactively labeled chemicals are widely used in biomedical research to trace the movement of substances through analytical equipment and processes and through biological processes. Technical information about the use of radioactively labeled substances in biomedical research was obtained from references listed at the end of this document. The NIH performs biomedical research activities in many different areas using radioactively labeled chemicals and generates mixed waste from a variety of biochemical research techniques. This section presents a brief overview of these research activities and discusses the interrelationships of the waste generation activities.

The NIH provides a centralized waste management group that is responsible for properly treating, storing, recording, and disposing of all wastes generated at NIH. As part of this responsibility, the waste management group maintains a detailed database on each radioactive and hazardous waste container generated at the NIH. The NIH mixed waste database contains information for the containerized mixed waste received by the waste management group between early 1989 and June 1993 and contains approximately 3,800 records for this period. However, as noted previously, this database does not include data on two supplemental mixed waste streams that are discussed in Section 2.2. The database was set up with a very large number of fields to provide the flexibility necessary to systematically document all of the waste management activities for the mixed wastes. A total of 187 fields were created for each record in the database and, in most cases, one record had been created for each container of waste received at the NIH. A supporting waste tracking system is used at the NIH to gather and enter the data into the database as they become available during the waste management activities.

The NIH waste tracking system begins with the generation of waste at one of the many laboratory and research facilities at the NIH. The facility that is generating the waste contacts the waste management group and completes a waste tracking form for each container of waste that is being generated. The waste tracking form requires the generator to provide complete process and waste composition information. The waste management group then collects the waste and transports it to the waste management building. Liquid wastes are then sampled and tested for pH and flashpoint. A radioassay is performed on the waste that indicates the activity of the radionuclides found to be present. The results of this analytical testing and the information provided on the waste tracking form are entered into the NIH database. A determination is made of the regulatory status and classification of the waste and of the treatment and disposal options available for the waste. The waste then moves through the NIH waste management system based on this determination and is tracked by the waste management group using the computer database.

Because of the complexity of the research activities at the NIH, the information on the process that was used to generate the waste must, by necessity, be provided by the generator. This is because the research activities can be varied, unpredictable, and at the forefront in using heretofore unknown procedures. In the past, the generators have tended to provide very limited and general information about the waste generating activities. This information is entered into

the database in two character fields, each with a length of 76 characters. For most of the records, however, the information entered is sufficiently limited so that only one field is required.

The processes used for waste generation at the NIH were primarily characterized for this study by using key words contained in the process description field. The key words were extracted from the field, grouped according to apparent process similarities, and then sorted and combined to develop groupings of similar activities that seemed to be from similar waste generating processes. Thirty-two process groupings were ultimately developed in this way. These process groups were then used throughout the remainder of the report to analyze and track the waste management activities. Most of these 32 groupings seem to represent unique and distinct process activities. Nonetheless, several of the groups may be subsets of other groups. These subsets could not be combined, however, based on the information available. For example, a significant portion of the waste contains general information such as "radioassay" and is therefore not specific enough to include into one of the more specific process groups. Some of the 32 groupings represent groupings of processes that are dissimilar. An example would be deoxyribonucleic acid/ribonucleic acid (DNA/RNA) extractions/sequencing. While the extraction and sequencing activities are distinct, there appear to be sufficient similarities to justify combining them as a single process group. This method of combining was also used for the gel fixing/washing and staining/destaining processes. For another significant portion of the data, the process information provided is incomplete or lacking and cannot be used to assign the waste to one of the process groups. Generally, this waste is some of the older waste and obtaining detailed information from the generator as the program was being initiated was probably difficult. Waste with incomplete process information is grouped in an "unknown" category.

Figure 2-1 graphically depicts the relative volumes of the containerized mixed wastes received at the NIH in 21 of the largest process groups. This figure does not include the two supplemental mixed waste streams that are described in Section 2.2. The remaining 11 process groups contain a relatively small amount of waste and are grouped as "all others." Table 2-1 lists the waste volume for each of the 32 waste generating processes and lists the relative percentages of each process. As Table 2-1 shows, 67% of the waste is produced by the three largest processes and over 90% of the waste volume is produced by the 10 largest processes. A brief discussion of the largest waste generating processes is presented below. Table 2-2 contains the waste volumes generated for the two supplemental mixed waste streams.

2.1 Description of Waste Generating Processes

The mixed waste generating processes at NIH are highly specialized and complex research techniques that are used by researchers to investigate specific areas of interest. The development of such research techniques is an ongoing process that results in large changes in the procedures in a relatively short period of time. Therefore, any discussion of the processes and techniques used is likely to become quickly dated. A large variety of such processes is used at the NIH as a part of its research activities. However, the analysis of the mixed waste database indicates that most of the processes are variations of a few research techniques and that these techniques produce the majority of the total mixed waste volume. Following are brief and simplified discussions of these research processes that describe the techniques in a general way, but do not represent the full scope and scale of research activities at the NIH. Researchers at the NIH are at the forefront of developing improved biochemical techniques and procedures and are

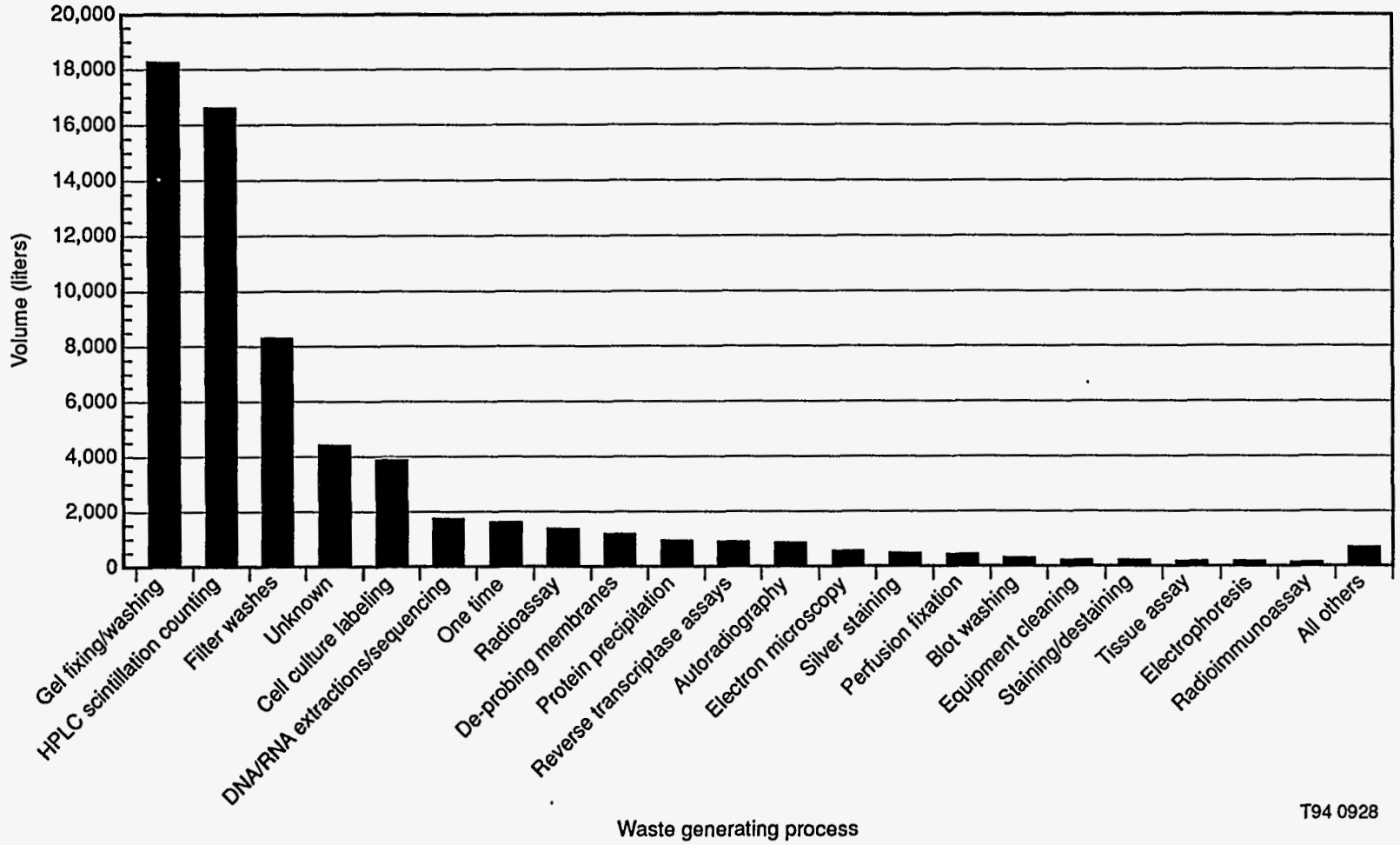

Figure 2-1. Distribution of waste volume by process for containerized mixed waste generated at NIH.

Table 2-1. Volume of NIH mixed waste by process category.

Waste generating process	Contents volume (L)	Container volume (L)	Contents volume percent of total	Cumulative percent
Gel fixing/washing	18,201	20,054	28.4	28.4
HPLC scintillation counting	16,614	18,396	25.9	54.3
Filter washes	8,261	9,230	12.9	67.2
Unknown	4,342	5,392	6.8	73.9
Cell culture labeling	3,913	4,798	6.1	80.0
DNA/RNA extractions/sequencing	1,793	2,070	2.8	82.8
One-time processes	1,652	1,491	2.6	85.4
Radioassay	1,344	1,582	2.1	87.5
Deprobing membranes	1,209	1,336	1.9	89.4
Protein precipitation	1,010	1,200	1.6	90.9
Reverse transcriptase assays	990	1,040	1.5	92.5
Autoradiography	852	1,020	1.3	93.8
Electron microscopy	601	714	0.9	94.8
Silver staining	508	468	0.8	95.5
Perfusion fixation	453	486	0.7	96.3
Blot washing	349	772	0.5	96.8
Equipment cleaning	344	396	0.5	97.3
Staining/destaining	292	305	0.5	97.8
Tissue assay	223	313	0.3	98.1
Electrophoresis	223	246	0.3	98.5
Radioimmunoassay	161	180	0.3	98.7
Operating room wastes	156	156	0.2	99.0
Tissue staining	149	170	0.2	99.2
Cancer treatment	101	101	0.2	99.4
Cell assays	95	131	0.1	99.5
Lipid extraction	88	106	0.1	99.7
ATP extractions	56	60	0.1	99.7
Phosphorylation	50	52	0.1	99.8
Membrane washing	42	68	0.1	99.9
Protein harvesting	34	64	0.1	99.9
Hormonal labeling	31	41	0.0	100.0
Automated amino assay	12	20	0.0	100.0
Total	64,146	72,458	100.0	

HPLC = high-performance liquid chromatography
DNA/RNA = deoxyribonucleic acid/ribonucleic acid
ATP = adenosine triphosphate.

Table 2-2. Volume of supplemental mixed wastes disposed of by NIH.

	Number of containers shipped for disposal	Shipping container volume (L)	Volume shipped (L)
Liquid scintillation vials			
1986	1,212	212	257,392
1987	987	212	209,609
1988	931	212	197,716
1989	850	212	180,515
1990	829	212	176,055
1991	733	212	155,667
1992	612	212	129,970
1993 (Jan.–June)	255	212	54,154
Total			1,361,078
Solid medical pathological waste[a]	220	74	46,721
Vials with mercury/^{3}H	25	0.005–0.5	0.81

a. Solid medical pathological waste is contaminated with cytotoxic agents (melphalan), thus making it a mixed waste.

continuously striving to develop completely new techniques while improving the capabilities of existing ones.

2.1.1 Gel Fixing/Washing Wastes

Gel electrophoresis is a valuable tool for effecting separations of protein or DNA/RNA fragments and is often used to determine the sequence of peptides in a protein or bases in nucleic acids. In this technique, DNA, RNA, or a protein is labeled with nuclides such as ^{32}P or ^{35}S and is broken up into fragments. The mixture of fragments is then placed on a gel where separation occurs due to the differential rates of migration of the fragments across and through the gel medium. After the separation has progressed sufficiently, the gel is "fixed" or washed with solvents such as methanol to remove undesirable impurities that would otherwise decrease the sensitivity of the subsequent photographic analysis. It is the fixing of the gels that can produce a mixed waste depending on the chemicals that are used. Different types of gels and fixing chemicals are used for DNA/RNA sequencing and for protein sequencing. However, the available data do not allow differentiation between these areas.

In the analysis, the fixed gel is exposed to photographic film that is sensitive to the radiation emitted by the radionuclides incorporated in the radioactively labeled peptides or bases. The sequence of peptides or bases in the original DNA, RNA, or protein is determined by an analysis of the exposed film and the repetition of the procedure. In some cases, researchers at NIH have been successful in analyzing gels without first fixing them by using specialized substitute gels that can be analyzed with certain impurities present. This type of procedural modification is dependent upon the particular research application of gel electrophoresis to be successfully implemented.

2.1.2 High-Performance Liquid Chromatography Scintillation Counting Fluids

High-performance liquid chromatography (HPLC) scintillation counting fluid wastes are generated when two powerful analytical techniques, HPLC and liquid scintillation counting, are used. HPLC is a type of liquid chromatography in which liquid samples to be analyzed are injected directly into a column through which a solvent is pumped at high speeds and pressures. The various types of molecules contained within the sample are separated as they pass through the column, depending on the specificity of the column, and exit the column at different times, which are characteristic of the different molecular species.

Liquid scintillation counting is a technique to detect beta radiation (also alpha radiation, positrons, and weak X-rays) emitted by radioactively labeled chemicals. In this technique, ionizing radiation passing through a dilute solution of an organic substance causes ionization and excitation of some of the solvent molecules through promotion of pi-orbital electrons to higher energy states. If a suitable solute is present in the solution, these electrons will transfer their excitation energy to the solute molecules, which then emit multiple light flashes (scintillations) through solute fluorescence as they return to lower energy states. The scintillations are then recorded with photomultiplier tubes. In biomedical research, the technique is widely used to measure ^{14}C and ^{3}H, as well as other nuclides. When the scintillation counting technique is applied to solvents from an HPLC column, it provides a powerful technique for quantitatively detecting the presence of biochemical species of interest. The key points with respect to waste

generation are that the use of a suitable solvent with pi-orbital electrons is required together with a solute appropriate for the solvent being used. Many of these solvents are regulated under RCRA as F-listed spent solvents or as characteristic wastes.

2.1.3 Filter/Blot Washing

Protein residues that are separated using gel electrophoresis techniques as described in Section 2.1.1 can be transferred from the sequencing gel to a glass fiber filter media substrate coated with a thin layer of noncovalently adsorbed polybase or other media such as sheets of polyvinylidene difluoride. This procedure is referred to as "protein blotting" or "Western blotting" and provides a method of identifying gel-separated proteins with specific binding properties. It provides a replica of the protein separation pattern present in the separation gel. After the protein is transferred to the filter paper, it is washed using a variety of chemicals to remove impurities before further analysis. The filter washings can produce a mixed waste depending on the chemicals used. Again, many of these chemicals are regulated as F-listed spent solvents or as characteristic wastes.

2.1.4 Cell Culture Labeling

Many of the research techniques using radioactive labeling of biological molecules require that the radioactive nuclide be biologically incorporated into the structures of cells. This can be done by allowing photosynthesis to occur in an atmosphere containing $^{14}CO_2$, using ^{3}H-labeled water in the culturing of cells, or by using $^{32}PO_4$ as a nutrient in a cell culture. The radiologically labeled biological molecules can be recovered from the cell cultures with techniques that require the use of a variety of chemicals. This process can produce a mixed waste if the chemicals are regulated as F-listed spent solvents or as characteristic wastes.

2.1.5 Protein Precipitation

Protein solutions are developed and used for a variety of analytical purposes. It is often necessary to ultimately precipitate the proteins from solution as a prelude to separating them from the solution or performing a further step. A common protein-precipitating agent is trichloroacetic acid. The anion of trichloroacetic acid and other acids forms an insoluble salt with proteins when the proteins are in solution in the form of cations. Proteins can also be precipitated from solution by solvents that are miscible in water such as methanol, ethanol, and acetone. Methanol and acetone are regulated as F003 spent solvents. A decomposition product of trichloroacetic acid is chloroform, which is regulated as a toxicity characteristic RCRA waste.

2.1.6 Autoradiography

Autoradiography is a technique that allows the resolution and study of structures on the intracellular level. In this procedure, a tissue is cultured and radioactively labeled. Then photographic film is applied to a cut section of the tissue. The portions of the film that are close to radioactive areas in the tissue will darken. When nuclides with suitable characteristics are used, intracellular structures can be resolved to allow the study of cellular structures and functions. Mixed waste can be generated from autoradiography if chemicals, such as methanol, are used to prepare the samples before filming.

2.1.7 Radioimmunoassay

Radioimmunoassays are a technique for detecting the presence and concentration of a variety of biological proteins using a radioactively labeled antigen molecule that binds with specificity to a receptor molecule in a biological system of interest. The technique is used for several hundred different applications such as determining the presence of various hormones, viruses, or other substances. Nuclides commonly used for this procedure are ^{3}H and ^{125}I. Mixed wastes are generated with the procedure by the need to separate the antigen from the receptor to complete the test. This is usually done by some type of differential precipitation of proteins or by using other separation techniques such as gel electrophoresis or chromatography. If F-listed solvents, such as methanol, are used in this process, then mixed wastes can be generated.

2.2 Supplemental Mixed Waste Streams

The NIH generates two large, and one smaller, supplemental mixed waste streams that are not tracked in the mixed waste database. The first waste stream consists of liquid scintillation fluids contained in vials instead of bulk liquid containers. These vials are used in scintillation counting and then placed in shipping containers for shipment to an offsite incinerator. The total volume of the liquid scintillation fluid vials shipped offsite from 1986 to 1993 (January to June) is shown in Table 2-2.

The second supplemental mixed waste stream was created recently as a result of a research study that began in 1993. This waste stream consists of solid medical pathological waste containing melphalan (a carcinogen) and ^{131}I used as a tracer. This waste is placed in shipping containers for radioactive decay and storage before shipment offsite. The NIH will integrate this waste stream into the mixed waste database tracking system in 1994. Table 2-2 lists the waste volume generated for this waste stream from the study inception through June 1993.

The third mixed waste stream consists of small vials of mercury and other compounds contaminated with ^{3}H that were generated in 1989–1990. These wastes are also listed in Table 2-2.

3. DESCRIPTION OF WASTE STREAMS GENERATED BY ONE-TIME OPERATIONS

A significant number of the mixed waste streams generated at the NIH are from activities that could be described as one-time activities. These waste streams are produced relatively infrequently and are produced by activities that appear to be dissimilar. Table 3-1 contains a summary list of one-time waste streams, the dates they were received by the NIH waste management group, and the waste volume produced. The data suggest that a relatively large proportion of the waste streams are produced as a result of spills and activities of an unplanned nature. Other sources of one-time waste are special projects and activities that produce relatively small amounts of waste and maintenance activities for equipment.

The one-time waste streams are characterized and described further in later portions of the report as the one-time process group. Characterization data for the one-time process group are presented in Sections 4 and 5.

Table 3-1. Summary of one-time waste generating processes at the NIH.

Date received	Waste volume (L)	Waste generation process activity
22 October 1991	1	Abandoned vials
9 June 1992	3	Accidental contamination
31 March 1992	18	Accidental contamination of aqueous waste
17 September 1991	20	Affinity chromatograph binding studies
6 August 1991	1	Aldehyde reduction
6 August 1991	<1	Aldehyde reduction
2 April 1991	20	Alkaline glucose
2 April 1991	20	Animal destruction
2 April 1991	20	Animal destruction
20 June 1990	20	Animal destruction (prebiopsy)
20 June 1990	20	Animal injection
4 June 1991	20	Animal injection experiment
4 June 1991	20	Animal injection experiment
8 April 1991	8	Animal studies
8 January 1991	3	Aqueous waste aspirated and unintentionally contaminated with vacuum pump oil
23 October 1990	16	Aspiration dispersion
5 March 1991	4	Aspiration mishap
20 November 1990	2	Broken speed vacuum trap
5 November 1991	1	Broken thermometer contaminated with ^{32}P
17 January 1989	4	Buffer RNA separation
30 July 1991	<1	Cardiac cell experiments
12 April 1991	12	Cell culture waste
22 January 1993	7	Cell staining
5 December 1990	20	Centrifuge waste
27 November 1990	20	Centrifuge waste
27 November 1990	20	Centrifuge waste
15 January 1991	4	Cesium chloride gradients
19 March 1991	20	Chemical assays
16 June 1992	<1	Chemical mishap
4 May 1993	500	Chemical reactive
28 January 1992	1	Cholesterol extractions

Table 3-1. (continued).

Date received	Waste volume (L)	Waste generation process activity
16 July 1991	20	Chromatography
12 February 1991	1	Chromatography waste
9 February 1993	20	Chromium release assays
5 June 1991	10	Clean up of broken bottle
5 June 1991	10	Clean up of broken bottle
5 June 1991	10	Clean up of broken bottle
5 June 1991	10	Clean up of broken tritiated ethyl ether bottle
12 February 1991	20	Cleaning cages
12 February 1991	20	Cleaning cages
21 July 1992	20	Column chromatography solvents
23 August 1990	20	Conjugation of viruses
15 October 1991	4	Contaminated photography chemicals
24 March 1992	4	Contaminated pump oil
9 June 1992	1	Contaminated vacuum pump oil
8 December 1992	2	Copper metabolism in marker
28 January 1992	4	Culture media/unused scintillation fluid
13 August 1991	20	Culture washes and extractions
30 July 1991	18	Cytotoxicity assay
7 May 1991	8	Cytotoxicity assay
21 October 1991	<1	Discarding reagent chemicals
31 March 1992	10	Disinfecting labware
27 March 1990	20	Dismantled film developer
27 March 1990	20	Dismantled film developer
4 May 1993	<1	Animal death
30 March 1993	<1	Animal death
30 March 1993	<1	Animal death
30 March 1993	<1	Animal death
4 April 1993	18	Enhancer radioactive enhancement
8 May 1990	8	Fluorography
16 June 1992	19	Fluorography
16 June 1992	19	Fluorography
27 September 1990	20	Gamma flow through detector
20 August 1991	20	Glassware cleaning mishap

Table 3-1. (continued).

Date received	Waste volume (L)	Waste generation process activity
15 September 1992	18	Immunoprecipitation
10 March 1992	1	Leftover source vials
12 March 1991	1	Lymphilizer waste
12 March 1991	1	Lymphilizer waste
12 March 1991	1	Lymphilizer waste
17 September 1991	1	Lypholizer waste
22 January 1991	20	Microsphere injection
27 April 1993	16	Miltepoposal
2 April 1991	2	Mishap with lipholizer (cleaned up and placed in a flimsy plastic jug)
17 February 1993	18	Mistake
20 April 1993	1	Mistake
26 December 1990	16	Molecular extractions/washings
5 September 1990	1	Oil change
25 February 1989	2	Oil change mishap
29 January 1991	1	Oil contaminated in aspiration mishap
23 August 1990	4	Oil replacement
27 April 1993	20	Overshot neutralization washes
12 February 1993	20	Overshot neutralization washes
2 March 1993	4	Overshot pH
2 March 1993	4	Overshot pH water wash
19 November 1991	2	Peptide iodination
26 December 1990	20	Peptide synthesis
26 December 1990	20	Peptide synthesis
31 October 1990	8	Peptide synthesis, paper fixing
13 November 1991	20	Plate washes
16 May 1990	2	Protein labeling
29 January 1991	8	Radioactive drugs tests
29 August 1990	16	Radiolabeling ^{35}S
13 August 1991	4	Rat plasma extractions
23 October 1990	20	Receptor binding assays
19 November 1991	<1	Resin absorption
1 June 1993	35	Resume breakdown

Table 3-1. (continued).

Date received	Waste volume (L)	Waste generation process activity
1 February 1991	2	Rinsate from pH meter probe
8 August 1990	16	Scintillation counting waste accidently put in aqueous waste container
26 January 1993	16	Solvent extractions
4 June 1991	<1	Source vial
25 February 1992	18	Spectrophotometry
7 March 1990	6	Spill cleanup
7 January 1992	8	Spill cleanup
22 October 1991	1	Spill cleanup
25 February 1992	18	Spill cleanup
19 February 1991	<1	Spill cleanup
5 November 1991	19	Tagged probe waste
7 May 1990	4	Thin layer chromatography
21 July 1987	4	Unknown container
27 February 1991	20	Unused buffer solution
30 October 1991	1	Uptake assay
21 May 1991	1	Urea buffering
21 May 1991	1	Urea buffering
7 August 1992	3	Used filter
7 August 1992	3	Used filter
24 October 1989	4	Vacuum pump oil change
27 June 1989	<1	Vacuum pump oil change

4. WASTE STREAM CHARACTERIZATIONS OF THE WASTE GENERATING PROCESSES

This section presents a chemical and physical characterization of each of the 32 waste generating process groups. These characterizations have been prepared by processing all of the available database information and summarizing the results in compilations that are presented in tabular form. Table 4-1 presents summary information on the chemical properties of pH and flashpoint of the waste stream processes. The flashpoint was entered in the database using comparative operators in many cases. For comparative analytical purposes, these operators were converted to the first possible true value, and this value was used for numerical comparisons. For example, a value of >140°F would be converted in the numerical analysis to 141°F and this value would be reported.

Table 4-2 presents information on chemicals present in the waste streams. For the most part, the database information does not include numerical quantitative chemical composition information. It does contain chemical composition information that generators have provided on tags attached to the waste containers. This information is entered as character data for each record and is inconsistent in format, completeness, and presentation. As a result, comparative numerical data generally cannot be extracted for analytical purposes from this information. However, what can be extracted is the presence or absence of a specific chemical species, which was done. Table 4-2 presents the volumetric percentages of each of the waste streams that contained the chemical species mentioned. For example, HPLC scintillation counting wastes are shown with a value of 55.1% under methanol. This means that 55.1% of all of the waste produced by this process was mentioned as containing methanol. Similarly, a value of 60.6% is shown for this waste under pseudocumene, which means that this percentage of the total waste stream contained pseudocumene.

Information is presented in this way for methanol, acetone, ethanol, phosphoric acid, sodium hydroxide, pseudocumene, acetic acid, trichloroacetic acid, chloroform, acetonitrile, and xylene. These chemicals were selected based on their relatively high frequency of occurrence in the waste stream descriptive data. However, many other chemical species are also present in the waste streams including a wide variety of buffering solutions and a number of proprietary scintillation cocktail fluids such as Flow Scint III, Flow Scint IV, Pico-Aqual, Enhance, and Trucount. Because of the proprietary nature of these fluids, a detailed chemical characterization was not possible.

Table 4-3 presents information on the EPA hazardous waste numbers that were provided for the different waste streams. Multiple waste numbers were used for many waste streams based on the descriptive waste composition data provided by the generators and by testing performed on the waste. Nine waste numbers are presented based on their frequency of occurrence. The percentages shown for each of the waste numbers represent the volume percentage of the waste stream that was designated with the particular EPA waste number. This information therefore shows that a relatively large proportion of the mixed waste received is in the D001, D002, and F003 categories and very little is in the other categories. Some of the wastes do not have an EPA hazardous waste number associated with them. This is particularly true for wastes in the "Cancer Treatment" and "Operating Room" process groups. It is possible that in addition to being

Table 4-1. Chemical properties of NIH mixed waste.

Waste generating process	Waste volume (L)	pH minimum	pH maximum	Flashpoint minimum (°F)
ATP extractions	56	1.6	1.8	96
Automated amino assay	12	4.0	4.0	141
Autoradiography	852	0.2	13.0	84
Blot washing	349	1.0	12.9	135
Cancer treatment	101	N/A	N/A	N/A
Cell assays	95	1.0	12.6	120
Cell culture labeling	3,913	0.1	13.1	83
Deprobing membranes	1,209	1.3	13.0	99
DNA/RNA extractions/sequencing	1,793	0.3	12.5	75
Electron microscopy	601	1.4	8.0	139
Electrophoresis	223	0.9	8.0	70
Equipment cleaning	344	0.5	14.0	90
Filter washes	8,261	0.1	14.0	65
Gel fixing/washing	18,201	0.0	13.9	72
Hormonal labeling	31	1.6	6.8	92
HPLC scintillation counting	16,614	0.2	13.1	60
Lipid extraction	88	1.9	10.1	70
Membrane washing	42	1.0	4.1	141
One-time processes	1,652	0.6	13.1	78
Operating room wastes	156	N/A	N/A	N/A
Perfusion fixation	453	2.7	9.6	99
Phosphorylation	50	1.0	5.7	99
Protein harvesting	34	1.1	4.1	95
Protein precipitation	1,010	0.0	10.6	78
Radioassay	1,344	0.1	12.6	63
Radioimmunoassay	161	1.3	12.5	99
Reverse transcriptase assays	990	0.5	5.8	99
Silver staining	508	1.2	6.7	70
Staining/destaining	292	0.1	9.4	92
Tissue assay	223	0.5	9.1	84
Tissue staining	149	1.3	13.0	138
Unknown	4,342	0.3	14.0	58

N/A = Data not available.

Table 4-2. Chemicals typically present in NIH mixed waste.

Waste generating process	Waste volume (L)	Volume percent of waste with chemicals present										
		Methanol	Acetone	Ethanol	Phosphoric acid	Sodium hydroxide	Pseudo-cumene	Acetic acid	Trichloro-acetic acid	Chloroform	Acetonitrile	Xylene
ATP extractions	56	100.0	100.0	—	—	—	—	100.0	—	—	—	—
Automated amino assay	12	—	—	—	—	—	—	—	—	—	—	—
Autoradiography	852	56.5	2.2	4.5	—	0.2	3.3	87.0	4.5	2.3	—	—
Blot washing	349	12.6	—	—	27.8	22.6	2.0	11.7	5.7	—	—	—
Cancer treatment	101	—	—	—	—	—	—	—	—	—	—	—
Cell assays	95	22.1	—	12.6	—	18.9	13.7	12.6	12.6	—	—	—
Cell culture labeling	3,913	46.1	6.7	22.5	5.5	3.1	5.5	30.2	28.3	24.4	0.7	—
Deprobing membranes	1,209	3.0	—	—	1.6	94.6	—	—	—	—	12.9	—
DNA/RNA extractions/sequencing	1,793	53.5	0.4	18.6	1.1	2.3	12.3	49.9	7.6	12.9	0.0	—
Electron microscopy	601	—	—	3.6	—	—	—	—	—	—	3.5	—
Electrophoresis	223	54.7	—	17.9	—	9.0	—	61.8	9.0	9.0	—	—
Equipment cleaning	344	16.3	10.8	10.5	—	6.3	2.3	2.3	—	7.0	—	—
Filter washes	8,261	48.4	1.6	19.4	18.8	4.8	0.8	18.6	58.4	43.4	3.8	—
Gel fixing/washing	18,201	76.7	2.4	8.9	1.7	2.1	3.7	78.4	9.8	4.2	0.0	—
Hormonal labeling	31	—	—	41.2	—	—	—	1.6	—	58.8	—	—
HPLC scintillation counting	16,614	55.1	0.3	4.0	36.0	0.0	60.6	8.6	0.6	2.9	9.7	6.4
Lipid extraction	88	85.2	2.3	2.3	—	20.5	—	—	—	94.3	3.4	—
Membrane washing	42	41.0	—	—	—	—	—	41.0	1.2	—	—	—
One-time processes	1,652	13.7	2.0	9.4	1.1	9.1	3.6	9.2	8.3	9.1	2.5	0.2
Operating room wastes	156	—	—	—	—	—	—	—	—	—	—	—
Perfusion fixation	453	19.4	—	0.9	—	37.1	—	5.3	—	—	—	—
Phosphorylation	50	40.0	—	16.0	56.0	—	—	—	36.0	—	8.0	—
Protein harvesting	34	—	13.4	23.9	—	—	—	11.9	25.4	—	—	—
Protein precipitation	1,010	14.1	4.4	12.4	2.8	2.5	1.7	6.6	49.3	14.1	—	—
Radioassay	1,344	17.3	3.0	11.2	10.4	2.8	0.4	7.1	18.4	29.2	2.7	—

Table 4-2. (continued).

Waste generating process	Waste volume (L)	Volume percent of waste with chemicals present										
		Methanol	Acetone	Ethanol	Phosphoric acid	Sodium hydroxide	Pseudo-cumene	Acetic acid	Trichloro-acetic acid	Chloroform	Acetonitrile	Xylene
Radioimmunoassay	161	64.6	—	60.2	—	12.4	—	12.4	—	—	23.0	—
Reverse transcriptase assays	990	87.9	—	2.0	—	—	—	20.2	96.0	87.9	—	—
Silver staining	508	35.6	—	28.9	—	—	—	14.0	—	—	3.9	—
Staining/destaining	292	17.8	55.6	2.7	15.1	—	—	19.2	—	—	—	—
Tissue assay	223	11.2	3.1	15.0	—	2.7	—	3.1	4.5	28.0	9.4	—
Tissue staining	149	10.8	—	—	—	—	—	20.2	—	—	—	—
Unknown	4,342	11.6	1.6	1.4	4.1	0.8	2.8	9.2	2.6	4.4	2.4	0.5

Note: A blank indicates the specified waste parameter is not applicable to the indicated waste generating process.

Table 4-3. Typical EPA hazardous waste numbers for NIH mixed waste.

Waste generating process	Waste volume (L)	Volume percent of waste using EPA hazardous waste number									
		D001	D002	D007	D008	D009	D011	D022	F001	F003	F005
ATP extractions	56	100	100	—	—	—	—	—	—	100	68
Automated amino assay	12	—	—	—	—	—	—	—	—	—	—
Autoradiography	852	89	38	—	—	—	—	2	—	63	2
Blot washing	349	7	28	—	—	—	—	11	—	13	—
Cancer treatment	101	—	—	—	—	—	—	—	—	—	—
Cell assays	95	36	54	—	—	—	—	—	—	15	—
Cell culture labeling	3,913	54	31	0.4	—	—	—	39	5.1	47	12
Deprobing membranes	1,209	16	12	—	—	—	—	—	—	2	—
DNA/RNA extractions/sequencing	1,793	37	16	—	—	—	—	13	0.5	55	3
Electron microscopy	601	4	7	—	67.8	—	—	—	—	—	—
Electrophoresis	223	69	38	—	—	—	—	9	—	59	—
Equipment cleaning	344	32	33	—	—	—	—	7	—	26	5
Filter washes	8,261	48	58	—	—	—	—	60	—	48	3
Gel fixing/washing	18,201	60	23	—	—	—	1.1	16	1.3	74	1
Hormonal labeling	31	41	60	—	—	—	—	59	—	—	—
HPLC scintillation counting	16,614	90	42	—	—	—	—	6	0.1	62	2
Lipid extraction	88	47	3	—	—	—	—	60	—	81	36
Membrane washing	42	—	1	—	—	—	—	1	—	—	—
One-time processes	1,652	26	15	—	0.4	1.2	—	10	0.1	17	5
Operating room wastes	156	—	—	—	—	—	—	—	—	—	—
Perfusion fixation	453	17	—	—	—	—	—	—	—	19	—

Table 4-3. (continued).

Waste generating process	Waste volume (L)	Volume percent of waste using EPA hazardous waste number									
		D001	D002	D007	D008	D009	D011	D022	F001	F003	F005
Phosphorylation	50	48	52	—	—	—	—	36	—	40	—
Protein harvesting	34	27	27	—	—	—	—	—	—	12	—
Protein precipitation	1,010	21	58	—	—	—	—	46	—	17	—
Radioassay	1,344	26	36	—	—	—	—	38	—	20	6
Radioimmunoassay	161	63	22	—	—	—	—	—	—	65	12
Reverse transcriptase assays	990	80	58	—	—	—	—	88	—	86	—
Silver staining	508	70	19	53.7	—	—	51.2	—	—	44	—
Staining/destaining	292	12	8	—	—	—	0.2	—	—	11	—
Tissue assay	223	26	15	—	—	4.5	—	27	—	13	—
Tissue staining	149	11	61	—	57.9	—	—	—	—	11	—
Unknown	4,342	14	21	—	0.5	—	—	4	—	12	1

Note: A blank indicates the specified waste parameter is not applicable to the indicated waste generating process.

low-level radioactive waste, these wastes may be infectious wastes rather than RCRA wastes and may not meet the definition of mixed waste as discussed in Section 1.

Radiological waste properties are presented in Table 4-4. Information is provided for seven radionuclides based on their frequency of use. The percentages shown for each nuclide are the volumetric percentage of the waste stream from a particular process that contained the nuclide. The activity information shown is the volumetric average of the activity information for only that portion of the waste stream which contained the nuclide. These average activities are derived from the radioassay information performed on each waste sample at the NIH. For example, data on the use of tritium with autoradiography indicate that in 44% of the volume of waste generated, tritium was used with an average activity concentration of 0.009 mCi/L. As this information shows, ^{3}H is the most commonly used nuclide and ^{35}S is the next most common. The characterization information presented here provides insight into the types of waste being generated from biomedical research activities such as those occurring at the NIH.

Table 4-5 presents radiological properties for the two supplemental mixed waste streams described in Section 2.2. Information is provided for the seven radionuclides presented for the mixed wastes described in Table 4-4. The liquid scintillation vials also contain other radionuclides at generally lower activity levels including ^{22}Na, ^{36}Cl, ^{51}Cr, ^{46}Sc, ^{45}Ca, and ^{86}Rb.

Table 4-4. Radiological properties of NIH mixed waste.

Waste generating process	Waste volume (L)	^{3}H		^{14}C		^{32}P		^{35}S		^{125}I		^{131}I		^{238}U	
		% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)
ATP extractions	56	—	—	—	—	100.0	0.012	—	—	—	—	—	—	—	—
Automated amino assay	12	—	—	—	—	—	—	—	—	100.0	0.001	—	—	—	—
Autoradiography	852	44.0	0.009	5.4	0.000	30.6	0.000	75.2	0.020	2.1	—	—	—	—	—
Blot washing	349	22.9	0.015	—	—	75.4	0.004	28.4	0.004	2.0	0.004	—	—	—	—
Cancer treatment	101	—	—	—	—	—	—	—	—	—	—	100.0	0.085	—	—
Cell assays	95	33.1	0.053	1.1	0.000	31.5	0.012	45.8	0.183	—	—	—	—	—	—
Cell culture labelling	3,913	77.0	0.081	14.0	0.011	9.6	0.019	29.7	0.030	4.3	0.015	—	—	—	—
Deprobing membranes	1,209	29.9	0.022	—	—	70.1	0.009	3.7	0.000	0.3	0.015	—	—	—	—
DNA/RNA extractions/sequencing	1,793	30.4	0.054	2.7	0.009	23.6	0.008	64.7	0.015	0.2	0.015	—	—	—	—
Electron microscopy	601	28.6	0.027	2.8	0.001	—	—	3.3	0.001	—	—	—	—	89.8	0.008
Electrophoresis	223	27.0	0.018	9.0	0.001	49.3	0.020	40.8	0.048	8.1	0.001	—	—	—	—
Equipment cleaning	344	69.0	0.177	11.9	0.006	17.9	0.052	7.0	0.156	—	—	—	—	—	—
Filter washes	8,261	55.3	0.073	6.6	0.021	34.1	0.012	32.6	0.022	1.5	0.016	—	—	—	—
Gel fixing/washing	18,201	26.5	0.060	4.5	0.002	21.1	0.010	69.1	0.025	3.7	0.004	—	—	—	—
Hormonal labeling	31	39.2	0.000	58.8	0.000	—	—	—	—	—	—	—	—	—	—
HPLC scintillation counting	16,614	86.5	0.023	12.8	0.004	3.0	0.012	9.4	0.047	1.7	0.007	—	—	—	—
Lipid extraction	88	97.7	0.829	20.5	0.151	8.0	0.003	—	—	—	—	—	—	—	—
Membrane washing	42	41.0	0.104	—	—	25.3	0.001	—	—	33.7	0.003	—	—	—	—
One-time processes	1,652	32.7	0.080	6.8	0.282	8.9	0.032	20.0	0.078	4.3	0.015	—	—	—	—
Operating room wastes	156	—	—	—	—	—	—	—	—	—	—	100.0	0.086	—	—
Perfusion fixation	453	41.1	0.005	—	—	0.9	—	0.9	—	7.7	0.002	—	—	—	—
Phosphorylation	50	8.0	0.000	—	—	52.0	0.006	40.0	0.000	—	—	—	—	—	—
Protein harvesting	34	86.6	0.011	—	—	—	—	1.5	2.120	11.9	0.000	—	—	—	—
Protein precipitation	1,010	52.2	0.282	5.7	0.004	21.9	0.179	22.5	0.041	8.6	0.018	—	—	—	—
Radioassay	1,344	71.3	0.174	22.2	0.022	8.7	0.014	44.5	0.019	2.4	0.054	—	—	—	—
Radioimmunoassay	161	47.2	0.246	—	—	12.4	0.003	34.8	0.154	62.7	0.004	—	—	—	—
Reverse transcriptase assays	990	100.0	0.130	—	—	—	—	20.2	—	—	—	—	—	—	—

Table 4-4. (continued).

Waste generating process	Waste volume (L)	^{3}H		^{14}C		^{32}P		^{35}S		^{125}I		^{131}I		^{238}U	
		% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)
Silver staining	508	40.9	0.001	—	—	59.8	0.005	11.4	0.013	—	—	—	—	—	—
Staining/destaining	292	8.2	0.260	—	—	36.4	0.025	29.3	0.157	6.9	0.065	—	—	—	—
Tissue assay	223	52.6	0.073	10.8	0.022	—	—	22.5	0.016	16.1	0.003	—	—	—	—
Tissue staining	149	1.0	0.003	9.4	0.020	12.8	0.008	23.6	0.020	—	—	—	—	57.9	0.001
Unknown	4,342	53.3	0.048	17.7	0.020	20.1	0.039	26.5	0.048	3.7	0.006	—	—	1.1	0.001

Note: A blank indicates the specified waste parameter is not applicable to the indicated waste generating process.

Table 4-5. Radiological properties of supplemental NIH mixed waste.

Waste generating process	Container volume (L)	^{3}H Total activity (mCi)	^{3}H Average activity (mCi/L)	^{14}C Total activity (mCi)	^{14}C Average activity (mCi/L)	^{32}P Total activity (mCi)	^{32}P Average activity (mCi/L)
Liquid scintillation vials—1986	257,392	43.030	0.0002	21.720	0.0001	1.879	0.0000
Liquid scintillation vials—1987	209,609	36.400	0.0002	21.300	0.0001	0.860	0.0000
Liquid scintillation vials—1988	197,716	33.484	0.0002	16.314	0.0001	3.273	0.0000
Liquid scintillation vials—1989	180,515	25.140	0.0001	13.000	0.0001	1.580	0.0000
Liquid scintillation vials—1990	176,055	33.400	0.0002	18.840	0.0001	4.870	0.0000
Liquid scintillation vials—1991	155,667	345.145	0.0022	75.555	0.0005	81.289	0.0005
Liquid scintillation vials—1992	129,970	604.586	0.0047	79.437	0.0006	129.997	0.0010
Liquid scintillation vials—1993 (January–June)	54,154	328.420	0.0061	39.960	0.0007	47.248	0.0009
Solid medical pathological waste	46,721	0.000	0.0000	0.000	0.0000	0.000	0.0000

Waste generating process	Container volume (L)	^{35}S Total activity (mCi)	^{35}S Average activity (mCi/L)	^{125}I Total activity (mCi)	^{125}I Average activity (mCi/L)	^{131}I Total activity (mCi)	^{131}I Average activity (mCi/L)
Liquid scintillation vials—1986	257,392	1.110	0.0000	0.507	0.0000	0.020	0.0000
Liquid scintillation vials—1987	209,609	0.540	0.0000	0.170	0.0000	0.000	0.0000
Liquid scintillation vials—1988	197,716	1.039	0.0000	0.425	0.0000	0.000	0.0000
Liquid scintillation vials—1989	180,515	0.970	0.0000	0.390	0.0000	0.000	0.0000
Liquid scintillation vials—1990	176,055	3.630	0.0000	1.010	0.0000	0.000	0.0000
Liquid scintillation vials—1991	155,667	42.191	0.0003	17.607	0.0001	0.001	0.0000
Liquid scintillation vials—1992	129,970	101.141	0.0008	14.514	0.0001	0.166	0.0000
Liquid scintillation vials—1993 (January–June)	54,154	42.032	0.0008	1.833	0.0000	0.072	0.0000
Solid medical pathological waste	46,721	0.000	0.0000	0.000	0.0000	11.000	0.0002

Table 4-5. (continued).

Waste generating process	Container volume (L)	^{238}U Total activity (mCi)	^{238}U Average activity (mCi/L)
Liquid scintillation vials—1986	257,392	0.000	0.0000
Liquid scintillation vials—1987	209,609	0.000	0.0000
Liquid scintillation vials—1988	197,716	0.000	0.0000
Liquid scintillation vials—1989	180,515	0.000	0.0000
Liquid scintillation vials—1990	176,055	0.000	0.0000
Liquid scintillation vials—1991	155,667	0.000	0.0000
Liquid scintillation vials—1992	129,970	0.000	0.0000
Liquid scintillation vials—1993 (January–June)	54,154	0.000	0.0000
Solid medical pathological waste	46,721	0.000	0.0000

5. IDENTIFICATION AND CATEGORIZATION OF WASTE STREAMS WITH LIMITED TREATMENT/DISPOSAL OPTIONS

The NIH waste management facility includes an extensive waste processing capability. A carbon adsorption system has been successfully tested and placed into operation for removing trace chloroform and phenol contaminants from aqueous waste streams. Tanks and equipment are available for neutralizing acidic and alkaline waste streams. Other tanks can be used for holding waste to allow radioactive decay of nuclides with short half-lives to take place before sewer discharge. Onsite incineration capability exists for a few of the wastes, including facilities to place small volumes of other wastes in bulk containers and transport them to offsite waste treatment and disposal facilities. NIH is currently reevaluating the continuation of onsite medical waste incineration, including radioactive medical waste, and this option may not be available in the future.

The NIH evaluates mixed waste as it is received at the waste processing facility. Based on this evaluation, a determination is made as to whether disposal options exist for the waste, and recommended NIH treatment codes are assigned to the waste. An NIH treatment code of 02 indicates that no treatment or disposal options are available for the waste. The waste is packaged and labeled for indefinite long-term onsite storage until treatment or disposal facilities become available. The NIH determined that approximately one-third of the waste received during the 1989 to 1993 study period cannot be disposed of using currently available disposal options for mixed waste. Because no disposal options are available, this waste has been placed into bulk containers and is currently being stored at the NIH. Tables 5-1 through 5-4 present chemical properties, chemicals present, EPA hazardous waste numbers, and radiological properties for this waste. Generally, the radiological activity levels of this waste are higher than the comparable activity levels of the overall waste stream characterized in Section 4. Since the no disposal option waste is a subset of the overall waste stream, this indicates that the waste streams with the highest activities are being diverted to storage at NIH.

Some of the waste shown as having no disposal options in the database records is produced by waste generating processes that have typically not produced such wastes. An example of this would be HPLC scintillation counting wastes. Even though disposal options exist for HPLC scintillation counting wastes, a small volume of these wastes (approximately 4%) are described in the database and shown in Tables 5-1 through 5-4 as having no disposal options. It appears likely that a small amount of the HPLC scintillation counting wastes were combined with wastes from other processes to produce a combined waste that does not presently have a disposal option.

Another significant proportion of the overall waste stream contains wastes that are believed to have treatment and disposal options but that have not been disposed of and are currently being stored. These wastes are referred to as "waste in process" in this report and are described in detail in Tables 5-5 through 5-8. This waste has not been disposed of due to treatment capacity limitations, transportation limitations, processing and handling limitations, or the recent arrival of the waste at the NIH waste processing area.

Table 5-1. Chemical properties of NIH mixed waste with no disposal options.

Waste generating process	Waste volume (L)	pH minimum	pH maximum	Flashpoint minimum (°F)
ATP extractions	0	N/A	N/A	N/A
Automated amino assay	0	N/A	N/A	N/A
Autoradiography	513	1.0	13.0	94
Blot washing	89	1.0	11.3	135
Cancer treatment	0	N/A	N/A	N/A
Cell assays	35	1.0	4.4	120
Cell culture labeling	1,842	0.3	13.1	88
Deprobing membranes	154	9.4	12.9	99
DNA/RNA extractions/sequencing	729	0.3	12.5	78
Electron microscopy	29	5.4	7.5	139
Electrophoresis	101	1.9	6.7	80
Equipment cleaning	97	3.1	14.0	99
Filter washes	3,798	0.1	12.8	82
Gel fixing/washing	9,721	0.0	13.4	72
Hormonal labeling	30	1.6	6.8	92
HPLC scintillation counting	616	0.9	8.2	70
Lipid extraction	72	1.9	10.1	70
Membrane washing	17	3.1	3.1	141
One-time processes	512	0.6	12.5	78
Operating room wastes	0	N/A	N/A	N/A
Perfusion fixation	110	2.7	9.6	99
Phosphorylation	24	3.8	5.7	99
Protein harvesting	5	1.8	3.7	141
Protein precipitation	455	0.0	6.0	78
Radioassay	562	0.2	12.6	96
Radioimmunoassay	124	1.3	7.7	99
Reverse transcriptase assays	670	0.8	5.8	99
Silver staining	306	1.2	6.7	70
Staining/destaining	45	2.0	9.4	139
Tissue assay	103	0.5	9.1	84
Tissue staining	35	1.3	11.6	139
Unknown	735	0.3	14.0	60

N/A = Data not available.

Table 5-2. Chemicals typically present in NIH mixed waste with no disposal options.

Waste generating process	Waste volume (L)	Volume percent of waste with chemicals present										
		Methanol	Acetone	Ethanol	Phosphoric acid	Sodium hydroxide	Pseudo-cumene	Acetic acid	Trichloro-acetic acid	Chloroform	Acetonitrile	Xylene
ATP extractions	—	—	—	—	—	—	—	—	—	—	—	—
Automated amino assay	—	—	—	—	—	—	—	—	—	—	—	—
Autoradiography	513	47.4	3.7	7.4	—	0.4	1.6	93.6	3.5	—	—	—
Blot washing	89	49.4	—	—	22.5	7.9	7.9	27.0	22.5	—	—	—
Cancer treatment	—	—	—	—	—	—	—	—	—	—	—	—
Cell assays	35	59.8	—	34.2	—	—	37.0	34.2	34.2	—	—	—
Cell culture labeling	1,842	57.0	2.9	28.8	5.2	2.6	1.4	19.4	44.0	39.8	1.6	—
Deprobing membranes	154	10.4	—	—	—	88.3	—	—	—	—	89.6	—
DNA/RNA/extractions/sequencing	729	63.8	1.1	13.0	—	3.3	2.7	52.8	10.4	25.2	0.0	—
Electron microscopy	29	—	—	—	—	—	—	—	—	—	28.4	—
Electrophoresis	101	45.5	—	19.8	—	—	—	63.4	—	19.8	—	—
Equipment cleaning	97	24.7	34.0	37.1	—	—	8.2	—	—	16.5	—	—
Filter washes	3,798	60.0	1.4	19.2	1.7	1.8	1.8	10.1	89.0	75.9	1.5	—
Gel fixing/washing	9,721	93.1	1.2	3.6	1.1	2.2	0.5	86.2	5.4	6.4	0.1	—
Hormonal labeling	30	—	—	40.0	—	—	—	—	—	60.0	—	—
HPLC scintillation counting	616	50.1	—	5.5	27.7	—	41.1	6.7	1.3	7.1	16.2	8.3
Lipid extraction	72	81.9	2.8	2.8	—	25.0	—	—	—	93.1	4.2	—
Membrane washing	17	100.0	—	—	—	—	—	100.0	—	—	—	—
One-time processes	512	44.0	3.3	26.7	—	12.3	8.2	15.8	26.8	29.3	8.2	0.8
Operating room wastes	—	—	—	—	—	—	—	—	—	—	—	—
Perfusion fixation	110	80.0	—	3.6	—	16.4	—	3.6	—	—	—	—
Phosphorylation	24	83.3	—	—	83.3	—	—	—	—	—	16.7	—
Protein harvesting	5	—	100.0	—	—	—	—	88.9	11.1	—	—	—
Protein precipitation	455	31.2	8.8	23.5	—	—	3.7	14.7	74.5	24.2	—	—
Radioassay	562	39.6	5.0	18.7	3.6	4.4	0.9	7.2	36.8	66.7	6.4	—
Radioimmunoassay	124	83.9	—	64.5	—	—	—	—	—	—	16.1	—

Table 5-2. (continued).

Waste generating process	Waste volume (L)	Volume percent of waste with chemicals present										
		Methanol	Acetone	Ethanol	Phosphoric acid	Sodium hydroxide	Pseudo-cumene	Acetic acid	Trichloro-acetic acid	Chloroform	Acetonitrile	Xylene
Reverse transcriptase assays	670	97.0	—	3.0	—	—	—	—	100.0	100.0	—	—
Silver staining	306	42.5	—	37.9	—	—	—	13.1	—	—	6.5	—
Staining/destaining	45	98.9	—	18.0	36.0	—	—	98.9	—	—	—	—
Tissue assay	103	20.3	6.8	24.7	—	—	—	6.8	9.7	60.5	20.3	—
Tissue staining	35	45.7	—	—	—	—	—	45.7	—	—	—	—
Unknown	735	29.0	6.6	6.8	5.4	0.8	5.8	15.5	4.7	22.1	8.7	2.7

Note: A blank indicates the specified waste parameter is not applicable to the indicated waste generating process.

Table 5-3. Typical EPA hazardous waste numbers for NIH mixed waste with no disposal options.

Waste generating process	Waste volume (L)	Volume percent of waste using EPA hazardous waste number									
		D001	D002	D007	D008	D009	D011	D022	F001	F003	F005
ATP extractions	—	—	—	—	—	—	—	—	—	—	—
Automated amino assay	—	—	—	—	—	—	—	—	—	—	—
Autoradiography	513	90	52	—	—	—	—	—	—	55	3
Blot washing	89	27	45	—	—	—	—	45	—	49	—
Cancer treatment	—	—	—	—	—	—	—	—	—	—	—
Cell assays	35	99	4	—	—	—	—	—	—	40	—
Cell culture labeling	1,842	68	54	0.9	—	—	—	48	0.0	59	2
Deprobing membranes	154	100	6	—	—	—	—	—	—	—	—
DNA/RNA extractions/sequencing	729	53	19	—	—	—	—	26	1.2	66	6
Electron microscopy	29	28	—	—	27.9	—	—	—	—	—	—
Electrophoresis	101	71	28	—	—	—	—	20	—	47	—
Equipment cleaning	97	79	21	—	—	—	—	16	—	59	16
Filter washes	3,798	63	80	—	—	—	—	87	—	60	0.4
Gel fixing/washing	9,721	68	29	—	—	—	—	8	0.2	89	0.2
Hormonal labeling	30	40	60	—	—	—	—	60	—	—	—
HPLC scintillation counting	616	78	55	—	—	—	—	6	—	58	—
Lipid extraction	72	54	4	—	—	—	—	71	—	96	44
Membrane washing	17	—	—	—	—	—	—	—	—	—	—
One-time processes	512	76	40	—	—	3.9	—	33	0.2	54	12
Operating room wastes	—	—	—	—	—	—	—	—	—	—	—
Perfusion fixation	110	51	—	—	—	—	—	—	—	80	—
Phosphorylation	24	100	—	—	—	—	—	—	—	83	—

Table 5-3. (continued).

Waste generating process	Waste volume (L)	Volume percent of waste using EPA hazardous waste number									
		D001	D002	D007	D008	D009	D011	D022	F001	F003	F005
Protein harvesting	5	—	—	—	—	—	—	—	—	89	—
Protein precipitation	455	46	82	—	—	—	—	87	—	33	—
Radioassay	562	49	66	—	—	—	—	81	—	45	11
Radioimmunoassay	124	68	13	—	—	—	—	—	—	84	16
Reverse transcriptase assays	670	88	76	—	—	—	—	100	—	94	—
Silver staining	306	69	13	52.3	—	—	54.2	—	—	49	—
Staining/destaining	45	54	36	—	—	—	1.1	—	—	54	—
Tissue assay	103	56	14	—	—	9.7	—	58	—	27	—
Tissue staining	35	46	46	—	—	—	—	—	—	46	—
Unknown	735	36	23	—	2.7	—	—	18	—	25	1

Note: A blank indicates the specified waste parameter is not applicable to the indicated waste generating process.

Table 5-4. Radiological properties of NIH mixed waste with no disposal options.

Waste generating process	Waste volume (L)	^{3}H		^{14}C		^{32}P		^{35}S		^{125}I		^{131}I		^{238}U	
		% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)
ATP extractions	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Automated amino assay	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Autoradiography	513	30.4	0.022	4.8	0.000	9.9	0.000	64.7	0.032	3.5	—	—	—	—	—
Blot washing	89	42.7	0.004	—	—	44.9	0.004	92.1	0.002	7.9	0.004	—	—	—	—
Cancer treatment	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cell assays	35	61.3	0.073	2.8	0.000	—	—	38.7	0.221	—	—	—	—	—	—
Cell culture labeling	1,842	85.6	0.134	9.2	0.026	6.8	0.023	16.6	0.088	8.1	0.013	—	—	—	—
Deprobing membranes	154	93.5	0.000	—	—	—	—	16.9	0.000	—	—	—	—	—	—
DNA/RNA extractions/sequencing	729	39.8	0.058	6.6	0.009	8.3	0.004	60.6	0.020	—	—	—	—	—	—
Electron microscopy	29	—	—	—	—	—	—	—	—	—	—	—	—	100.0	0.000
Electrophoresis	101	19.8	0.000	19.8	0.001	15.8	0.002	62.4	0.064	17.8	0.001	—	—	—	—
Equipment cleaning	97	83.5	0.393	16.5	0.015	—	—	20.6	0.183	—	—	—	—	—	—
Filter washes	3,798	72.1	0.110	10.6	0.025	11.2	0.022	30.9	0.041	1.1	0.001	—	—	—	—
Gel fixing/washing	9,721	20.0	0.127	3.4	0.004	16.2	0.005	80.3	0.031	5.6	0.003	—	—	—	—
Hormonal labeling	30	40.0	0.000	60.0	0.000	—	—	—	—	—	—	—	—	—	—
HPLC scintillation counting	616	85.4	0.176	1.3	0.002	7.1	0.102	23.0	0.394	11.5	0.011	—	—	—	—
Lipid extraction	72	97.2	0.783	25.0	0.151	—	—	—	—	—	—	—	—	—	—
Membrane washing	17	100.0	0.104	—	—	—	—	—	—	—	—	—	—	—	—
One-time processes	512	68.2	0.033	7.8	0.076	5.1	0.018	27.9	0.069	1.6	0.001	—	—	3.1	0.123
Operating room wastes	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Perfusion fixation	110	45.5	0.016	—	—	3.6	—	3.6	—	16.4	0.001	—	—	—	—
Phosphorylation	24	16.7	0.000	—	—	—	—	83.3	0.000	—	—	—	—	—	—
Protein harvesting	5	—	—	—	—	—	—	11.1	2.120	88.9	0.000	—	—	—	—
Protein precipitation	455	81.3	0.292	11.0	0.004	6.6	0.044	35.9	0.021	4.4	0.007	—	—	—	—
Radioassay	562	65.6	0.326	39.4	0.020	0.1	0.000	8.2	0.004	3.5	0.087	—	—	—	—
Radioimmunoassay	124	61.3	0.246	—	—	16.1	0.003	45.2	0.154	51.6	0.001	—	—	—	—
Reverse transcriptase Assays	670	100.0	0.155	—	—	—	—	—	—	—	—	—	—	—	—
Silver staining	306	61.4	0.001	—	—	44.4	0.006	19.0	0.013	—	—	—	—	—	—
Staining/destaining	45	18.0	0.006	—	—	80.9	0.002	82.0	0.004	—	—	—	—	—	—
Tissue assay	103	61.5	0.107	20.3	0.025	—	—	31.2	0.024	—	—	—	—	—	—

Table 5-4. (continued).

Waste generating process	Waste volume (L)	^{3}H		^{14}C		^{32}P		^{35}S		^{125}I		^{131}I		^{238}U	
		% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)
Tissue staining	35	—	—	—	—	54.3	0.008	100.0	0.020	—	—	—	—	—	—
Unknown	735	67.1	0.097	7.5	0.223	12.9	0.001	25.3	0.177	5.8	0.000	—	—	2.7	0.000

Note: A blank indicates the specified waste parameter is not applicable to the indicated waste generating process.

Table 5-5. Chemical properties of NIH mixed waste in process.

Waste generating process	Waste volume (L)	pH minimum	pH maximum	Flashpoint minimum (°F)
ATP extractions	56	1.6	1.8	96
Automated amino assay	0	N/A	N/A	N/A
Autoradiography	103	0.2	3.8	84
Blot washing	0	N/A	N/A	N/A
Cancer treatment	101	N/A	N/A	N/A
Cell assays	10	N/A	N/A	N/A
Cell culture labeling	560	0.1	4.7	83
Deprobing membranes	0	N/A	N/A	N/A
DNA/RNA extractions/sequencing	422	1.2	8.9	75
Electron microscopy	317	2.1	8.0	139
Electrophoresis	40	0.9	8.0	80
Equipment cleaning	32	2.8	7.4	90
Filter washes	394	0.4	8.4	65
Gel fixing/washing	2,193	0.1	7.8	76
Hormonal labeling	0	N/A	N/A	N/A
HPLC scintillation counting	2,188	0.5	9.2	65
Lipid extraction	16	2.4	2.4	86
Membrane washing	1	1.0	1.0	141
One-time processes	766	3.9	7.6	99
Operating room wastes	81	N/A	N/A	N/A
Perfusion fixation	55	4.5	7.1	105
Phosphorylation	26	1.0	1.9	141
Protein harvesting	8	1.7	1.7	95
Protein precipitation	347	0.1	6.8	139
Radioassay	647	1.0	8.7	63
Radioimmunoassay	17	6.7	6.7	99
Reverse transcriptase assays	40	0.7	1.6	141
Silver staining	52	3.8	9.7	99
Staining/destaining	247	0.1	3.2	92
Tissue assay	10	6.7	6.7	141
Tissue staining	38	5.4	5.9	138
Unknown	1,122	0.5	8.6	58

N/A = Data not available.

Table 5-6. Chemicals typically present in NIH mixed waste in process.

Waste generating process	Waste volume (L)	Volume percent of waste with chemicals present										
		Methanol	Acetone	Ethanol	Phosphoric acid	Sodium hydroxide	Pseudo-cumene	Acetic acid	Trichloro-acetic acid	Chloroform	Acetonitrile	Xylene
ATP extractions	56	100.0	100.0	—	—	—	—	100.0	—	—	—	—
Automated amino assay	—	—	—	—	—	—	—	—	—	—	—	—
Autoradiography	103	27.2	—	0.5	—	—	19.5	25.1	19.5	19.5	—	—
Blot washing	—	—	—	—	—	—	—	—	—	—	—	—
Cancer treatment	101	—	—	—	—	—	—	—	—	—	—	—
Cell assays	10	—	—	—	—	—	—	—	—	—	—	—
Cell culture labeling	560	14.7	—	5.9	3.4	—	—	6.4	3.6	2.9	—	—
Deprobing membranes	—	—	—	—	—	—	—	—	—	—	—	—
DNA/RNA extractions/sequencing	422	68.7	—	0.2	—	—	—	59.2	4.7	0.4	—	—
Electron microscopy	317	—	—	6.3	—	—	—	—	—	—	4.1	—
Electrophoresis	40	99.5	—	—	—	49.8	—	49.8	—	—	—	—
Equipment cleaning	32	100.0	12.5	—	—	—	—	—	—	25.0	—	—
Filter washes	394	34.8	5.1	5.1	11.2	—	—	15.5	38.8	18.3	—	—
Gel fixing/washing	2,193	60.2	2.1	6.7	6.0	—	0.5	54.2	7.4	2.9	—	—
Hormonal labeling	—	—	—	—	—	—	—	—	—	—	—	—
HPLC scintillation counting	2,188	37.7	0.4	6.3	2.7	—	45.4	7.6	1.8	1.6	11.5	5.4
Lipid extraction	16	100.0	—	—	—	—	—	—	—	100.0	—	—
Membrane washing	1	—	—	—	—	—	—	—	100.0	—	—	—
One-time processes	766	0.2	2.1	—	2.5	2.5	2.3	3.0	—	—	—	—
Operating room wastes	81	—	—	—	—	—	—	—	—	—	—	—
Perfusion fixation	55	—	—	—	—	61.8	—	—	—	—	—	—
Phosphorylation	26	—	—	30.8	30.8	—	—	—	69.2	—	—	—
Protein harvesting	8	—	—	100.0	—	—	—	—	100.0	—	—	—
Protein precipitation	347	—	1.2	2.9	—	—	—	—	23.1	9.2	—	—
Radioassay	647	1.5	—	4.3	6.2	—	—	3.1	6.2	2.8	—	—
Radioimmunoassay	17	—	—	100.0	—	—	—	—	—	—	100.0	—
Reverse transcriptase assays	40	50.0	—	—	—	—	—	—	50.0	—	—	—
Silver staining	52	26.9	—	26.9	—	—	—	26.9	—	—	—	—

Table 5-6. (continued).

Waste generating process	Waste volume (L)	Volume percent of waste with chemicals present										
		Methanol	Acetone	Ethanol	Phosphoric acid	Sodium hydroxide	Pseudo-cumene	Acetic acid	Trichloro-acetic acid	Chloroform	Acetonitrile	Xylene
Staining/destaining	247	3.2	65.6	—	11.3	—	—	4.9	—	—	—	—
Tissue assay	10	—	—	—	—	—	—	—	—	—	—	—
Tissue staining	38	—	—	—	—	—	—	—	—	—	—	—
Unknown	1,122	18.8	1.8	1.1	0.4	0.7	1.8	9.9	2.5	2.7	3.2	—

Note: A blank indicates the specified waste parameter is not applicable to the indicated waste generating process.

Table 5-7. Typical EPA hazardous waste numbers for NIH mixed waste in process.

Waste generating process	Waste volume (L)	Volume percent of waste using EPA hazardous waste number								
		D001	D002	D007	D008	D011	D022	F001	F003	F005
ATP extractions	56	100	100	—	—	—	—	—	100	68
Automated amino assay	—	—	—	—	—	—	—	—	—	—
Autoradiography	103	80	47	—	—	—	19	—	47	—
Blot washing	—	—	—	—	—	—	—	—	—	—
Cancer treatment	101	—	—	—	—	—	—	—	—	—
Cell assays	10	—	—	—	—	—	—	—	—	—
Cell culture labeling	560	24	21	—	—	—	3	—	18	—
Deprobing membranes	—	—	—	—	—	—	—	—	—	—
DNA/RNA extractions/sequencing	422	55	18	—	—	—	0	—	64	—
Electron microscopy	317	4	0	—	45.7	—	—	—	—	—
Electrophoresis	40	50	50	—	—	—	—	—	100	—
Equipment cleaning	32	100	—	—	—	—	25	—	100	—
Filter washes	394	6	51	—	—	—	39	—	21	—
Gel fixing/washing	2,193	51	27	—	—	—	9	—	55	—
Hormonal labeling	—	—	—	—	—	—	—	—	—	—
HPLC scintillation counting	2,188	82	15	—	—	—	3	—	49	1
Lipid extraction	16	13	—	—	—	—	13	—	13	—
Membrane washing	1	—	100	—	—	—	100	—	—	—
One-time processes	766	6	—	—	0.8	—	—	—	1	3
Operating room wastes	81	—	—	—	—	—	—	—	—	—
Perfusion fixation	55	36	—	—	—	—	—	—	—	—
Phosphorylation	26	—	100	—	—	—	69	—	—	—
Protein harvesting	8	100	100	—	—	—	—	—	—	—
Protein precipitation	347	1	17	—	—	—	15	—	1	—
Radioassay	647	9	6	—	—	—	9	—	2	3

Table 5-7. (continued).

Waste generating process	Waste volume (L)	Volume percent of waste using EPA hazardous waste number								
		D001	D002	D007	D008	D011	D022	F001	F003	F005
Radioimmunoassay	17	100	—	—	—	—	—	—	—	—
Reverse transcriptase assays	40	—	100	—	—	—	—	—	50	—
Silver staining	52	65	—	—	—	34.6	—	—	27	—
Staining/destaining	247	5	3	—	—	—	—	—	3	—
Tissue assay	10	—	—	—	—	—	—	—	—	—
Tissue staining	38	—	—	—	68.4	—	—	—	—	—
Unknown	1,122	18	5	—	—	—	3	—	19	1

Note: A blank indicates the specified waste parameter is not applicable to the indicated waste generating process.

Table 5-8. Radiological properties of NIH mixed waste in process.

Waste generating process	Waste volume (L)	^{3}H		^{14}C		^{32}P		^{35}S		^{125}I		^{131}I		^{238}U	
		% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)
ATP extractions	56	—	—	—	—	100.0	0.012	—	—	—	—	—	—	—	—
Automated amino assay	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Autoradiography	103	1.5	0.020	19.5	0.000	—	—	79.1	0.012	—	—	—	—	—	—
Blot washing	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cancer treatment	101	—	—	—	—	—	—	—	—	—	—	100.0	0.085	—	—
Cell assays	10	100.0	0.012	—	—	—	—	100.0	0.006	—	—	—	—	—	—
Cell culture labeling	560	69.0	0.049	51.1	0.003	13.0	0.041	14.8	0.084	—	—	—	—	—	—
Deprobing membranes	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
DNA/RNA extractions/sequencing	422	7.6	0.397	—	—	35.5	0.009	69.2	0.013	—	—	—	—	—	—
Electron microscopy	317	41.1	0.034	5.4	0.001	—	—	6.3	0.001	—	—	—	—	80.6	0.002
Electrophoresis	40	50.2	0.017	—	—	99.5	0.041	49.8	0.015	—	—	—	—	—	—
Equipment cleaning	32	87.5	0.242	—	—	—	—	12.5	0.025	—	—	—	—	—	—
Filter washes	394	25.4	0.115	4.6	0.019	60.4	0.028	2.0	0.016	4.1	0.094	—	—	—	—
Gel fixing/washing	2,193	15.0	0.072	1.0	0.005	43.9	0.024	25.4	0.074	2.4	0.015	—	—	—	—
Hormonal labeling	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
HPLC scintillation counting	2,188	67.0	0.030	15.8	0.003	2.7	0.002	14.8	0.014	4.8	0.003	—	—	—	—
Lipid extraction	16	100.0	1.027	—	—	43.8	0.003	—	—	—	—	—	—	—	—
Membrane washing	1	—	—	—	—	100.0	0.010	—	—	—	—	—	—	—	—
One-time processes	766	8.6	0.323	0.1	28.800	9.5	0.011	11.0	0.030	5.3	0.000	—	—	—	—
Operating room wastes	81	—	—	—	—	—	—	—	—	—	—	100.0	0.087	—	—
Perfusion fixation	55	69.1	0.000	—	—	—	—	—	—	30.9	0.003	—	—	—	—
Phosphorylation	26	—	—	—	—	100.0	0.006	—	—	—	—	—	—	—	—
Protein harvesting	8	100.0	0.005	—	—	—	—	—	—	—	—	—	—	—	—
Protein precipitation	347	34.3	0.339	—	—	19.6	0.493	7.5	0.168	5.8	0.032	—	—	—	—
Radioassay	647	86.9	0.082	9.3	0.023	6.8	0.018	78.2	0.000	—	—	—	—	—	—
Radioimmunoassay	17	—	—	—	—	—	—	—	—	100.0	0.004	—	—	—	—
Reverse transcriptase assays	40	100.0	0.227	—	—	—	—	—	—	—	—	—	—	—	—
Silver staining	52	38.5	0.003	—	—	100.0	0.001	—	—	—	—	—	—	—	—

Table 5-8. (continued).

Waste generating process	Waste volume (L)	^{3}H		^{14}C		^{32}P		^{35}S		^{125}I		^{131}I		^{238}U	
		% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)	% of volume	Average activity (mCi/L)
Staining/destaining	247	66.5	0.388	—	—	28.3	0.036	19.8	0.271	8.1	0.065	—	—	—	—
Tissue assay	10	—	—	—	—	—	—	—	—	100.0	0.009	—	—	—	—
Tissue staining	38	—	—	—	—	—	—	—	—	—	—	—	—	68.4	0.000
Unknown	1,122	69.1	0.064	48.9	0.001	19.8	0.012	14.7	0.012	2.6	0.005	—	—	2.3	0.000

Note: A blank indicates the specified waste parameter is not applicable to the indicated waste generating process.

Table 5-9 presents a summary by process of the overall waste stream, waste disposed of, waste in process, and waste with no disposal options. As can be seen, approximately one-third of the overall waste stream has no disposal options. About 15% of the overall waste stream exists as waste in process and slightly over one-half of the waste has been disposed of using one of the disposal options available. Figures 5-1, 5-2, and 5-3 graphically depict the distribution of the waste streams by process category. Generally, gel fixing/washing and filter washes produce the largest proportionate volume of wastes with no disposal options. In contrast, HPLC scintillation counting wastes generally have disposal options available.

Table 5-9. Volume of NIH mixed waste by handling steps.

Waste generating process	Total waste received by NIH (L)	Waste disposed (L)	Waste disposed: Percent of received volume	Waste in process (L)	Waste in process: Percent of received volume	Waste with no disposal options (L)	Waste with no disposal options: Percent of received volume
ATP extractions	56	0	N/A	56	100.0	0	N/A
Automated amino assay	12	12	100.0	0	N/A	0	N/A
Autoradiography	852	236	27.7	103	12.1	513	60.2
Blot washing	349	260	74.5	0	N/A	89	25.5
Cancer treatment	101	0	N/A	101	100.0	0	N/A
Cell assays	95	50	52.6	10	10.5	35	36.9
Cell culture labeling	3,913	1,511	38.6	560	14.3	1,842	47.1
Deprobing membranes	1,209	1,055	87.3	0	N/A	154	12.7
DNA/RNA extractions/sequencing	1,793	642	35.8	422	23.5	729	40.7
Electron microscopy	601	256	42.5	317	52.7	29	4.8
Electrophoresis	223	82	36.7	40	18.0	101	45.3
Equipment cleaning	344	215	62.4	32	9.3	97	28.2
Filter washes	8,261	4,069	49.3	394	4.8	3,798	46.0
Gel fixing/washing	18,201	6,287	34.5	2,193	12.0	9,721	53.4
Hormonal labeling	31	1	2.0	0	N/A	30	98.0
HPLC scintillation counting	16,614	13,810	83.1	2,188	13.2	616	3.7
Lipid extraction	88	0	N/A	16	18.2	72	81.8
Membrane washing	42	24	57.8	1	1.2	17	41.0
One-time processes	1,652	374	22.7	766	46.4	512	31.0
Operating room wastes	156	75	48.3	81	51.7	0	N/A
Perfusion fixation	453	288	63.6	55	12.1	110	24.3
Phosphorylation	50	0	N/A	26	52.0	24	48.0
Protein harvesting	34	21	62.7	8	23.9	5	13.4
Protein precipitation	1,010	208	20.6	347	34.4	455	45.0

Table 5-9. (continued).

Waste generating process	Total waste received by NIH (L)	Waste disposed (L)	Waste disposed: Percent of received volume	Waste in process (L)	Waste in process: Percent of received volume	Waste with no disposal options (L)	Waste with no disposal options: Percent of received volume
Radioassay	1,344	135	10.0	647	48.1	562	41.8
Radioimmunoassay	161	20	12.4	17	10.6	124	77.0
Reverse transcriptase assays	990	280	28.3	40	4.0	670	67.7
Silver staining	508	150	29.5	52	10.2	306	60.2
Staining/destaining	292	0	N/A	247	84.7	45	15.3
Tissue assay	223	110	49.3	10	4.5	103	46.2
Tissue staining	149	76	50.8	38	25.6	35	23.6
Unknown	4,342	2,485	57.2	1,122	25.8	735	16.9
Total	64,146	32,730	51.0	9,887	15.4	21,529	33.6

N/A = Data not available.

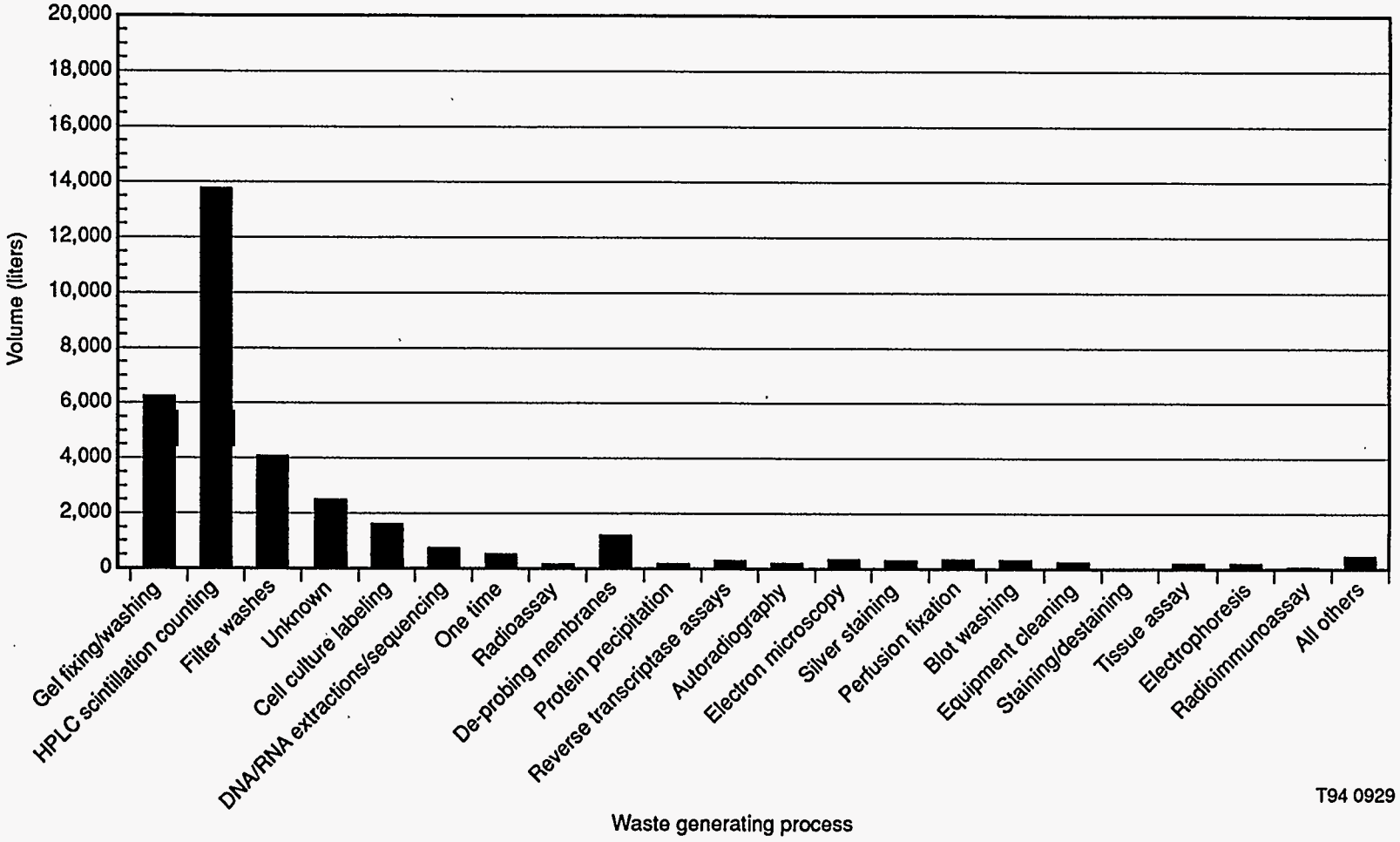

Figure 5-1. Distribution of waste volume by process for waste with disposal options.

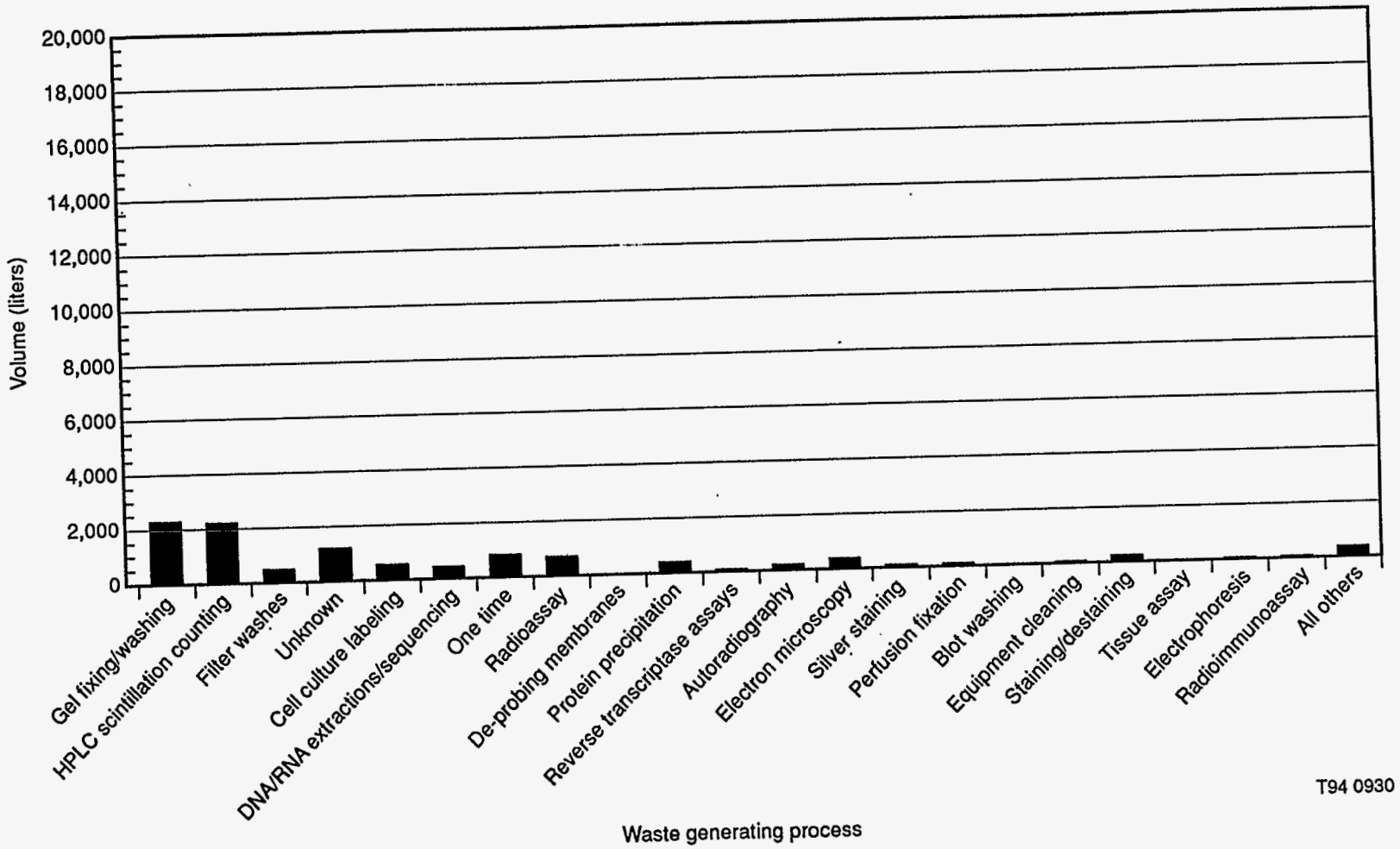

Figure 5-2. Distribution of waste volume by process for waste in process at NIH.

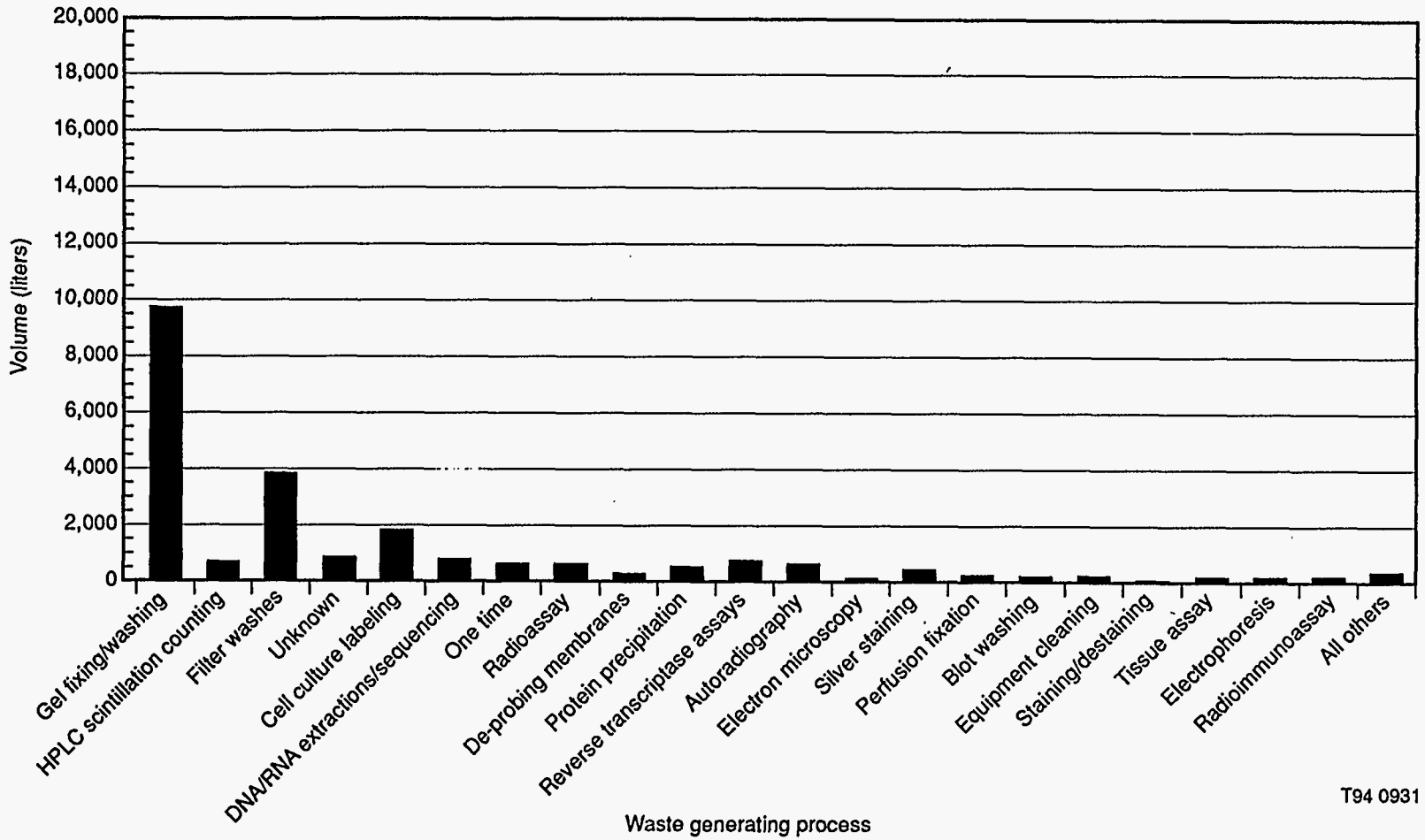

Figure 5-3. Distribution of waste volume by process for waste with no disposal options.

6. CONCLUSIONS

Section 2 of this report presents the results of the study in which the NIH wastes were combined into similar process groups. The results of this procedure reveal two significant findings: (a) relatively few waste generating processes produced the large majority of the mixed wastes, and (b) waste produced by these processes has relatively similar properties and compositions. In Section 3, it can be seen that a relatively small percentage (2.8%) of the total containerized mixed waste volume shown in Table 2-1 can be attributed to one-time processes. The detailed characterization information presented in Sections 4 and 5 provides a valuable insight into the chemical and radiological properties of the waste based on the waste generating processes from which it was generated. This information provides a unique picture of biomedical mixed wastes. Figures 5-1, 5-2, and 5-3 are particularly helpful in pointing out areas upon which waste minimization efforts might profitably focus. These figures show that the majority of the waste with no disposal options is generated by the gel fixing/washing process group. To a lesser extent, the filter washing group also generates a large portion of the waste with no disposal options. The remaining process groups are almost insignificant in their waste generation volume by comparison with these processes.

The two supplemental mixed waste streams constitute approximately 96% by volume of the total waste volumes listed in Tables 2-1 and 2-2. The relatively large volume of these waste streams makes them candidates for waste minimization efforts. However, disposal options currently exist for these waste streams and they are not presently being accumulated onsite.

This report presents a review of the available data regarding mixed waste generation at the NIH. A follow-on study will be conducted to fully characterize waste generating processes. Specifically, individual NIH mixed waste generators will be interviewed to gain an in-depth understanding of the processes and analytical techniques that generate the waste stream and of the chemicals used. The goal of the analysis will be to identify potential changes in procedures and materials that would reduce the volume of mixed waste with no disposal options without sacrificing the quality of the research activities.

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8. CONTACTS

For additional information please contact:

Mr. Edward H. Rau
Chief, Hazardous and Solid Waste Management Section
Environmental Protection Branch
Division of Safety
National Institutes of Health
Building 13, Room 2E63
Bethesda, MD 20892

Phone: (301) 496-7990
Fax: (301) 480-8056

or

Mr. Sean M. Austin
Chief, Radioactive Materials Control Section
Radiation Safety Branch
Division of Safety
National Institutes of Health
Building 21, Room 104
Bethesda, MD 20892

Phone: (301) 496-5774
Fax: (301) 496-3544

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DOE/LLW-218

National Institutes of Health: Mixed Waste Minimization and Treatment

Published August 1995

National Low-Level Waste Management Program

MASTER

Prepared for Lockheed Idaho Technologies Company
Under Subcontract No. C90-132744
and for the
U.S. Department of Energy
Assistant Secretary for Environmental Management
Contract DE-AC07-94ID13223

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EXECUTIVE SUMMARY

The Appalachian States Low-Level Radioactive Waste Commission requested the U.S. Department of Energy's National Low-Level Waste Management Program (NLLWMP) to assist the biomedical community in becoming more knowledgeable about its mixed waste streams, to help minimize the mixed waste streams generated by the biomedical community, and to identify applicable treatment technologies for these mixed waste streams. As the first step in the waste minimization process, liquid low-level radioactive mixed waste (LLMW) streams generated at the National Institutes of Health (NIH) were characterized and combined into similar process categories. The results of these evaluations were outlined in the *National Institutes of Health: Mixed Waste Stream Analysis* report (DOE/LLW-208).

This report identifies possible waste minimization and treatment approaches for the LLMW generated by the biomedical community identified in DOE/LLW-208. In development of the report, onsite meetings were conducted with NIH personnel responsible for generating each category of waste identified as lacking disposal options. Based on the meetings and general waste minimization guidelines, potential waste minimization options were identified. Conclusions from the study are as follows:

- Most of the reagents that are used in gel electrophoresis for gel fixing and washing (the largest portion of the difficult-to-manage LLMW at NIH) do not have substitutes acceptable to the research community at this time. However, there do appear to be substitution opportunities for using alternative gels that would require either a smaller volume of fixatives or would not require fixing or washing.

- Procedural changes appear to be a potential waste minimization approach for some research applications. In particular, chemiluminescent detection techniques could be used more widely as a substitute for techniques involving autoradiography. Also, additional expenditures for new equipment such as automated DNA sequencers could reduce waste volumes.

- There does not appear to be significant opportunities for LLMW volume reduction by segregation of radioactive and hazardous waste streams.

- The NIH radiation safety group appears to have done a good job of educating and informing the generators about the additional expense and effort required to manage mixed waste.

- Recycling opportunities are limited by both the precautions required for radioactive materials and by the lack of a simple, quick, and effective means of treating the waste reagents prior to their reuse.

- The waste produced by gel electrophoresis and filter washing techniques appears to be surprisingly uniform in character and composition, regardless of the specific research application. Such uniformity makes treatment options a potentially effective means of reducing LLMW volumes requiring disposal.

- An opportunity may exist for LLMW minimization through improved waste classification. Experience to date has shown that generators may be overestimating solvent concentrations in the waste resulting in misclassification of the waste as listed hazardous waste. Treatment residues of such listed waste must continue to be managed as hazardous waste, thereby significantly limiting disposal options.

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ACRONYMS AND ABBREVIATIONS

AEA	Atomic Energy Act
ALARA	as low as reasonably achievable
CFR	Code of Federal Regulations
DNA	deoxyribonucleic acid
DSSI	Diversified Scientific Services, Incorporated
ELISA	enzyme linked immuno sorbent assay
EPA	U.S. Environmental Protection Agency
GAC	granulated activated carbon
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
HRP	horseradish peroxidase
HSSDS	Hazardous Solvent Substitution Data System
LLMW	low-level radioactive mixed waste
LLW	low-level radioactive waste
LSC	liquid scintillation counting
ml	milliliter
NaOH	sodium hydroxide
NIH	National Institutes of Health
NLLWMP	National Low-Level Waste Management Program
nm	nanometer
ppm	parts per million
PPO	Solvent manufactured by Eastman Chemical with formula 2,5 diphenyloxazole
PVDR	polyvinylidene difluoride
RCRA	Resource Conservation and Recovery Act
RNA	ribonucleic acid
RT	reverse transcriptase
SDS	sodium dodecyl sulphate
SEG	Scientific Ecology Group, Incorporated
SFC	supercritical fluid chromatography
TCA	trichloroacetic acid
TIGR	The Institute for Genomic Research
UV	ultraviolet

NATIONAL INSTITUTES OF HEALTH: MIXED WASTE MINIMIZATION AND TREATMENT

1.0 INTRODUCTION

1.1 Background

Currently, management of mixed waste[a] is a challenge for states, regional compacts, and biomedical mixed waste generators because offsite treatment and disposal facilities are limited. The U.S. Department of Energy's National Low-Level Waste Management Program (NLLWMP) has been requested by the Appalachian States Low-Level Radioactive Waste Commission to assist the biomedical community to become better acquainted with its mixed waste steams, to help minimize the mixed waste streams generated by the biomedical community, and to identify applicable treatment technologies for those particular mixed waste streams. As the first step in the waste minimization process, liquid low-level radioactive mixed waste streams (LLMW) generated at the National Institutes of Health (NIH) were characterized and combined into similar process categories. The results of these evaluations were outlined in the *National Institutes of Health: Mixed Waste Stream Analysis* report (DOE/LLW-208).

That analysis compiled information on each container of LLMW at the NIH and consolidated mixed waste stream data into process groups that appeared to be derived from similar activities. Because liquid scintillation wastes were being actively and adequately managed, they were not included in the scope of that study. The container inventory information is maintained by the NIH in the form of a computerized waste stream database. The processes were grouped into 32 categories (31 similar processes and 1 unknown category). The groupings were based on a comparison of the descriptions of the waste generating processes provided for each container of mixed waste in the database. The groupings were used to identify the major processes and to develop characterizations of the waste produced.

The objective of this report is to identify possible minimization and treatment approaches for those LLMW generated by the biomedical community that were identified in DOE/LLW-208. This scope of work includes further characterization of the generating

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a. Mixed waste is defined in the Federal Facilities Compliance Act amendments to the Resource Conservation and Recovery Act (42 USC 6901 et seq.) as a waste that contains both hazardous waste and source, special nuclear, or byproduct material subject to the Atomic Energy Act of 1954, as amended.

processes for LLMW previously identified as being difficult to dispose of. Specifically, ways to prevent or minimize the generation of these more difficult-to-manage mixed wastes are identified, keeping in mind that biomedical research will need to continue. Options for treatment, minimization, alternative processes, and chemical substitutions are also evaluated.

1.2 Approach

As a first step, onsite meetings were conducted with NIH personnel responsible for generating each of the categories of waste identified as lacking disposal options. The onsite discussions also confirmed the accuracy of characterization information contained in the NIH waste stream database. The meetings focused on in-depth analyses of the specific processes generating the waste and identifying potential opportunities for waste minimization.

Based on the meetings and general waste minimization guidelines, potential waste minimization options were identified. The range of potential options included:

- Material substitution of nonhazardous or nonradioactive process inputs
- Procedural changes concerning material handling
- Process modifications
- Segregation of radioactive and hazardous waste streams
- Recycle/reuse of a mixed waste stream
- Waste treatment to reduce toxicity or remove hazardous constituents
- Volume reduction
- Stabilization.

Potential waste minimization options identified were then evaluated relative to their applicability to the NIH waste streams.

The following sections outline the results of the NIH mixed waste stream analyses. Section 2.0 discusses general biomedical mixed waste generating processes and waste minimization approaches. Section 3.0 provides an overview and summary of the 20 interviews with NIH LLMW generators. Section 4.0 identifies various waste minimization options for the waste streams generated at the NIH. Section 5.0 discusses general waste treatment options for previously generated NIH LLMW. Section 6.0 summarizes the overall results of the evaluations and provides recommendations.

2.0 PROCESS INFORMATION

A total of 20 interviews were conducted with NIH researchers in organizations that represented a broad cross-section of NIH activities. Based on those interviews, Section 2.1 provides background information on the main NIH processes that generate LLMW and Section 2.2 summarizes general waste minimization approaches for such LLMW.

2.1 Background Information on Waste Generating Processes

Most of the waste produced by the LLMW generators who were interviewed were produced by six analytical procedures and techniques. Each of these is described in general in the following sections.

2.1.1 Electrophoresis Gel Fixing/Washing for Protein, DNA Separation

An analysis of the NIH mixed waste streams found that the largest portion of the LLMW, which did not have disposal options at the time of the analysis, was derived from electrophoresis gel fixing and/or washing (DOE/LLW-208). Gel electrophoresis is a valuable tool for making separations of protein or deoxyribonucleic acid/ribonucleic acid (DNA/RNA) fragments. This technique is used to separate, identify, and purify fragments and is often used to determine the sequence of bases in nucleic acids. As discussed below, radiolabeling of the protein or DNA fragments, coupled with fixing and washing of the gels, results in generation of the LLMW.

The amount of waste generated depends on the type of gel used and the type and amount of reagent used in processing the gel. All of the gels are constructed of polyacrylamide or agarose. These chemicals act as porous media that behave like sieves by retarding or obstructing the movement of the protein or DNA/RNA molecules while allowing smaller molecules to migrate freely. The size of the gel pore determines the extent of the molecular sieving. The mobility of molecules through gels is sensitive to pH, which must be controlled. To control pH, the molecules being evaluated are dissolved in an aqueous buffer system that maintains the pH required for the separation. In some systems, two buffers are used, a "running" buffer for the sample and a gel buffer. The molecules migrate through the gel media when subjected to an electric field. The mobility of the molecules through the electric field is determined by the strength of the field and the charge, size, and shape of the molecule.

Generally, different types of gels are used for protein separation and for DNA/RNA separation. Large pore gels such as those made with agarose are used to separate macromolecules such as DNA/RNA and large proteins. The pore size of polyacrylamide gels is determined by the concentration of acrylamide in the gel. Acrylamide gels are characterized by the concentrations of acrylamide monomer and bisacrylamide crosslinking

agent that are used to produce the gel. It is important to use an appropriate gel for the separation being studied. The size and nature of the molecule and the nature of the sample are all factors in selecting the correct gel.

When polyacrylamide gels are used to separate proteins, a dissociating or nondissociating buffer system can be used. In a dissociating system, the protein is first dissociated or broken up into polypeptide subunits by using sodium dodecyl sulphate (SDS) and by heating the protein sample. These subunits are then solubilized and can be separated in gels. This type of system is referred to as SDS PAGE. Nondissociating buffer systems can also be used. These systems are designed to fractionate a protein mixture such that subunit interaction, protein conformation, and biological activity are preserved.

For protein separation, the protein can be removed for further use or autoradiographic images can be made of the radiolabeled proteins on the gel. In both of these cases, the gel is often fixed with reagents such as trichloroacetic acid (TCA), or methanol/acetic acid to precipitate the protein and remove such contaminants as urea.

For gel electrophoresis of DNA fragments, agarose is typically used for analyzing larger molecules. However, due to its large pore size, agarose does not provide optimal resolution for smaller molecules. Polyacrylamide gels can be used for smaller DNA fragments up to about 2,000 base pairs in length. Another advantage of polyacrylamide gels is that the DNA recovered is relatively pure and can be used for additional applications without extensive purification. Double-stranded DNA can be separated using nondenaturing polyacrylamide gels. Most of these DNA strands will migrate through nondenaturing polyacrylamide with a mobility inversely proportional to the number of base pairs. However, this mobility is affected by the actual base composition and sequence of the DNA strand such that DNA fragments of identical size can migrate at significantly different rates. Therefore, it is not always possible to determine the DNA molecular weight in these non-denaturing systems.

A double strand of DNA can be denatured, or split into single strands, by using urea, formamide, or sodium hydroxide (NaOH) that suppresses the base pairing in the double strands. Typically, the denaturing agent is incorporated in the gel by polymerizing the gel in its presence. Denatured, single-stranded DNA will migrate through denaturing gels at a rate that is nearly independent of the base composition and sequence of the DNA that allows a more accurate determination of the molecular weight of the DNA strand. This is less true for smaller single-stranded DNA fragments, however.

After the gel electrophoresis step is complete, the separated DNA can be removed from the gel for further work, or photographs of the separated bands of DNA on the gel surface can be made. There are two methods of photographing the gel. The first method does not use radioactive materials. In this method, the DNA is stained with a powerful

fluorescent dye such as ethidium bromide. The dye bound to DNA displays an increased fluorescent yield compared to dye in free solution when exposed to ultraviolet (UV) radiation. The energy is re-emitted at 590 nanometers (nm) in the red-orange region of the visible spectrum. A photograph is made of the stained DNA by directing a UV source at the gel and photographing the resultant phosphorescence. This dye can be used to detect both single- and double-stranded DNA. However, the affinity of this dye for single-stranded nucleic acid is relatively low and the fluorescent yield is poor. The second method of DNA detection requires that the DNA be labeled with a radionuclide such as ^{32}P or ^{35}S. After separation, the gel is placed in contact with photographic film and the radiation emitted from the radionuclides will expose a characteristic pattern on the film. This second method is termed autoradiography. Some researchers use both autoradiography and fluorescent techniques.

The volume of buffer used for gel electrophoresis is typically quite small relative to the volume of fixing and washing solutions that are used. The buffer, therefore, is not a significant contributor to the volume of mixed waste produced with radiolabeled DNA. If DNA is labeled with a radionuclide such as ^{32}P and an agarose gel is being used, it is typically soaked in a 7% solution of TCA following the electrophoresis. This solution becomes a mixed waste after coming into contact with the radiolabeled DNA. Following this fixing step, the gel is dried for several hours. When polyacrylamide gels are used with DNA containing radionuclides, they are typically immersed in a fixative solution of acetic acid followed by drying. The drying step is required for DNA labeled with ^{35}S (a weak Beta emitter) or very small quantities of ^{32}P to reduce the exposure time required for a satisfactory autoradiographic image.

2.1.2 Electrophoresis Gel Fixing/Washing for DNA Sequencing

Perhaps the most widely used application for gel electrophoresis is to determine the sequence of the bases comprising a segment of unknown DNA. Most of the LLMW produced during DNA sequencing activities is a result of fixing the gels prior to autoradiography. The gels are often fixed by soaking them in a bath of 10% methanol and 10% acetic acid in water. The fixing removes the urea that might otherwise prevent the gel from drying out and might cause it to stick to the autoradiographic film.

Two common, but differing, methods are used for DNA sequencing. In the Sanger method, a single-stranded template DNA fragment is used to synthesize new DNA with DNA polymerase until a DNA chain terminates. By using chain termination agents that are specific for each of the four bases in four sets of reactions, a population of oligonucleotides are produced for each reaction that terminate at four positions in the template DNA strand. These four reaction mixtures are then loaded into adjacent lanes of a DNA sequencing gel and the order of the bases in the DNA can be read directly from an autoradiographic image of the gel. To produce this image, the DNA is usually labeled with ^{32}P or ^{35}S. The ^{32}P is

less commonly used because the high-energy beta particles that it emits form larger and more diffuse bands on the autoradiograph due to scattering and also cause radiolysis of the DNA in the sample if it is stored for more than 1 to 2 days. The beta particles emitted by the ^{35}S produce a higher resolution autoradiograph image. From the standpoint of waste minimization, ^{33}P can be used as a substitute for ^{35}S. It is reported to provide better resolution and sensitivity than ^{32}P and shorter autoradiographic exposure times than ^{35}S but is more costly than either of these radionuclides (Party and Gershey, 1995).

In the Maxam-Gilbert sequencing method, a DNA fragment, radiolabeled at one end, is chemically separated in five separate chemical reactions, each of which is specific for a particular base or type of base. This produces five populations of radiolabeled molecules that extend from the common radiolabeled end-point to the site of the chemical separation. Each population consists of a mixture of molecules whose lengths are determined by the locations of a particular base along the length of the original DNA fragment. The populations are then separated by gel electrophoresis and the end-labeled molecules are detected by autoradiography. By comparing the separations from each of the five populations, the sequence can be determined.

2.1.3 HPLC Scintillation Counting

Another large volume waste identified in the NIH database is the high-performance liquid chromatography (HPLC) scintillation counting. LLMW HPLC scintillation counting fluid wastes are generated when two powerful analytical techniques, HPLC and liquid scintillation counting are used. HPLC is a type of liquid chromatography in which liquid samples to be analyzed are injected directly into a column through which a solvent is pumped at high pressures. The various types of molecules contained within the sample are separated as they pass through the column, depending on the specificity of the column, and exit the column at different times that are characteristic of the different molecular species.

Liquid scintillation counting (LSC) is a technique to detect beta radiation (also alpha radiation, positrons, and weak x-rays) emitted by radioactively labeled chemicals. In this technique, ionizing radiation passing through a dilute solution of an organic substance causes ionization and excitation of some of the solvent molecules through promotion of pi-orbital electrons to higher energy states. If a suitable solute is present in the solution, these electrons will transfer their excitation energy to the solute molecules, which then emit multiple light flashes (scintillations) through solute fluorescence as they return to lower energy states. The scintillations are then recorded with photomultiplier tubes. In biomedical research, the technique is widely used to measure ^{14}C and ^{3}H, as well as other nuclides. When the scintillation counting technique is applied to solvents from a HPLC column, it provides a powerful technique for quantitatively detecting the presence of biochemical species of interest.

The key points with respect to waste generation are that the use of a suitable solvent with pi-orbital electrons is required together with a solute appropriate for the solvent being used. The solvent used with LSC is typically referred to as a "cocktail" and is usually a proprietary vendor-supplied mixture. During the laboratory process, materials such as acetonitrile and methanol may be added potentially resulting in the generation of an F-listed hazardous waste. The automatic HPLC equipment can operate 24 hours per day generating approximately 10 milliliters (ml) of LLMW per minute of operation.

LSC is also used without a HPLC column. In this case, the LSC cocktail is usually enclosed within a small vial that is used and then discarded. The discarded vials can usually be disposed of by shipping them to an off-site commercial facility in Florida for processing and fuel recovery. This waste stream is, therefore, not a part of this study.

2.1.4 Blotting

Protein residues, which are separated using gel electrophoresis techniques, can be transferred from the sequencing gel to a membrane in a process called "blotting." Membranes are constructed of media such as nitrocellulose, Nylon™, or polyvinylidene difluoride (PVDF). This electrophoretic transfer of proteins or "western blotting" consists of the transfer of proteins from the gel onto the membrane surface. Typically, after electrophoresis, the protein molecule is trapped within the gel matrix and is relatively inaccessible. However, if it is transferred to a membrane surface, it becomes easily accessible to many detection and analysis techniques. In the blotting process, a gel is layered next to the membrane. After the protein is transferred to the filter paper, it is washed using reagents such as methanol or ethanol to improve retention on the membrane and to remove impurities prior to further analysis. The membrane or blot washings can produce a LLMW depending on the reagents used.

2.1.5 Enzyme Assays/Cell Harvesting

Frequently, the presence of a particular protein in a cell culture must be determined. The protein typically has been previously radiolabeled with a radionuclide such as ^{3}H in a cell culture. Then, the cultured cells will be lysed with TCA and the resultant protein fragments will be aspirated and filtered with a cell harvester. The fragments can be washed with methanol to precipitate them and a radioassay via scintillation counting can be made of the protein fragments to determine if the radiolabeled protein is present. LLMW is generated from both the washes and liquid scintillation counting.

Assays for reverse transcriptase (RT) activity in specific viruses, such as human immunodeficiency virus (HIV), are also frequently performed on cell cultures. In this procedure, thymidine labeled with ^{3}H is incorporated into acid precipitable material. Then, the non-incorporated thymidine is removed and the cell culture is rinsed with 10% TCA

followed by rinsing with 100% methanol. The remaining material is filtered in a cell harvester and the precipitated, labeled material is radioassayed. The rinses and washes in this procedure produce a LLMW, as well as the waste generated from LSC.

In addition to being a LLMW, many such wastes are also potentially infectious. The potential presence of infectious agents or components that are also regulated as medical waste often precludes the few available treatment options. Onsite inactivation of biohazards by autoclaving or chemical disinfection may alter the waste, increase volumes, or introduce new contaminants. The treatment and management of infectious mixed waste were not within the scope of this report.

2.1.6 Staining/Destaining

Silver staining is a method of creating light and dark bands from proteins or DNA in electrophoresis gels that can be directly photographed. Silver in an ionic form is added to the gel and reduced to metallic silver. Protein bands appear in the gel due to differences in the oxidation/reduction potentials between sites in gels occupied by protein and adjacent sites not occupied by protein. If protein-occupied sites have the higher reducing potential, then positive images are formed. If sites unoccupied by protein have the higher reducing potential, then negative images are formed.

Prior to the staining procedure, the gel is first fixed in a solution of ethanol, glacial acetic acid, and water, followed by an additional washing with 30% ethanol. LLMW can be produced if the silver staining is being used in conjunction with radiolabeled proteins or DNA. The silver reduction reaction is typically quenched by washing the gel in 1% acetic acid for several minutes.

A second method of staining is the use of Coomassie Blue stain for proteins separated on SDS polyacrylamide gels. With this stain, the gels are first fixed with methanol and glacial acetic acid followed by Coomassie Brilliant Blue R250. LLMW can also be produced using this stain if radiolabeled proteins are being stained. Generally, silver staining offers much more sensitivity than Coomassie Blue.

Following the staining with Coomassie Blue, the gel is typically destained by soaking it in a 30% methanol/10% acetic acid solution. This process will again generate a LLMW if radioactivity is present.

2.2 Background Information on Waste Minimization Approaches

Waste minimization concepts that may be appropriate for the waste streams outlined above are described in the U.S. Environmental Protection Agency's (EPA's) *Waste Minimization Opportunity Assessment Manual* (EPA/625/7-88/003). In the manual, waste

minimization is defined as "source reduction to reduce or eliminate waste generation, followed by recycling or reuse where wastes are unavoidable, and finally, minimization of the volume, toxicity, and mobility of waste generated through treatment and stabilization." Thus, a range of methods and technologies is available that can be used to implement an effective waste minimization program. The following section discusses general waste minimization options determined to be appropriate for most biomedical operations. Specific application of these techniques to the NIH mixed waste streams is discussed in Section 4.0.

2.2.1 Substitution of Reagents

To reduce the volume and/or the chemical or radiological hazard of the waste produced, nonhazardous short half-life radioactive or nonradioactive reagents can be substituted into the process. Three main factors determine the applicability of substitution: an evaluation to identify the reaction inputs that produce the hazardous or radioactive component of each mixed waste stream; the purpose of introducing the input that produced the mixed waste into the component; and identification of a substitute that will acceptably perform the intended function of the original input while at the same time reduce the volume or hazard of the waste stream.

The primary radionuclides that biomedical facilities use in their research are: ^{125}I as a tracer for labeled proteins in research; ^{3}H (tritium) and ^{14}C in certain analytical techniques; ^{35}S and ^{32}P for labeling proteins and DNA with autoradiography; and ^{3}H, ^{14}C, ^{32}P, ^{35}S, and ^{125}I for sample counting with liquid scintillation techniques. ^{33}P, ^{51}Cr, and others are also used.

Material substitution options for such activities include substituting short-lived radionuclides such as ^{131}I for ^{125}I, and ^{32}P in place of longer-lived radionuclides such as ^{35}S. Also, ^{33}P can be substituted for ^{35}S or ^{32}P. ^{33}P has a shorter half-life (25 days) than ^{35}S (88 days) but a longer half-life than ^{32}P (14 days). Consequently, ^{32}P is preferred over ^{33}P from a waste minimization perspective. However, as noted in Section 2.1.2, ^{33}P provides better resolution and sensitivity than ^{32}P and comparable results to ^{35}S. If substitution of short-lived radionuclides is to be used in conjunction with storage for decay, the radioactive materials license of the facility must specifically allow such a waste management strategy. Release limits are specifically included in the facility license and are usually set so that no residual activity can be detected by normal means of measurement. Also, the potential for increased radiation exposure of laboratory personnel arising from use of substitute radionuclides must be considered before substitutions are made. Waste minimization strategies should not conflict with the principles of maintaining radiation exposures of personnel to levels that are as low as reasonably achievable (ALARA). Following decay and release, the waste can be managed without regard to its radioactivity and can be managed as hazardous waste only.

Use and generation of lower concentrations of radioactive liquid waste can also increase the management options that are available. One regulatory standard in 10 CFR §20.2003 allows disposal by release into a sanitary sewerage system provided the material is readily soluble in water or is readily dispersible biological material, and the concentrations are at or below specified levels. Another regulatory provision, 10 CFR §20.2005, allows disposal of LSC medium or animal carcasses without regards to their radioactivity provided the concentration of ^{3}H or ^{14}C is 0.05 microcurie or less per gram under certain conditions. Other techniques include the use of higher flash point solvents to avoid generation of Resource Conservation and Recovery Act (RCRA) hazardous wastes, and the elimination of organic solvents during preparation and analytical processes by using supercritical fluid chromatography (SFC). Highly automating the process and providing reproducible characteristics of a SFC can reduce the need for organic solvents.

2.2.2 Changes in Techniques and Procedures

Changing laboratory techniques and procedures, and providing more detailed guidance concerning material handling, can result in significantly reduced wastes for a minimal cost. For instance, a mixed waste is often generated as a result of the way a chemical or material is used, handled, or stored. Operational practices and procedures can be examined to determine if changes can be made.

An example of a change in technique is researchers who use smaller volumes of reagents to accomplish a particular objective. Illustrative of this approach are those researchers who were found to fix DNA sequencing gels with a much smaller volume of reagents, thereby reducing LLMW volume, than other researchers who were performing the same procedure.

An additional example of a change in practice would be inventory control. Laboratories can consider purchasing based on the amount of material needed rather than purchasing based on price per volume. If a facility determines that only one liter of a solvent-based reagent is used before the shelf life expires, it can request one-liter containers of this material in the future. This approach can reduce LLMW volume if the facility previously was purchasing larger containers that provide more material than was required.

Specific process modifications can also reduce the volume or degree of hazard (either chemical or radiological) of the waste stream. Factors determining if a process modification is applicable include the ability to identify the step in the process that actually produces the mixed waste; the purpose of the step that produces the mixed waste; and the availability of modifications that can be made to the process or alternative process that will acceptably perform the objective of the original process step, while at the same time reducing the volume or hazard of the waste stream.

An example of a process modification could be the use of small-bore tubing on HPLC. The tubing would reduce the amounts of solvents and buffers required to perform analytical procedures.

The waste minimization practices that the NIH employs for lead are also an example of procedural change. The procurement of lead containers is discouraged, as is the use. Contamination of lead pigs is prevented by using liners of various types. If the lead becomes contaminated, it is decontaminated, allowing it to be recycled or reclaimed.

2.2.3 Improved Waste Segregation

This mixed waste minimization technique can be accomplished by simply preventing the hazardous and radiological components of the waste from being mixed. To implement this approach, the current laboratory process must be examined to determine if there is any mixing of hazardous and radioactive waste streams. If mixing of streams is indicated, the next step is to determine if the streams can be separated without unacceptable effects on the process.

Often, several wastes from many processes are combined, and segregation options can be applied to the operation. The mixed waste volume can be reduced if the combination of wastes is controlled at the source, and mixed waste fractions are segregated from other waste forms. A major contribution to mixed waste volume minimization includes separating aqueous components from the organic components. Examples of successfully eliminating contact between hazardous and radioactive waste streams include the use of segregated areas for hazardous and radioactive waste to avoid cross-contamination, and sorting hazardous and radioactive wastes into designated bins at the point where they are first generated, such as low-level radioactive waste (LLW), sanitary waste, RCRA hazardous waste, and mixed waste.

One example of waste segregation at NIH is a simple valve apparatus that can be added to washings of cell cultures. The aqueous washings are valved into a separate collection flask from the organic washings.

2.2.4 Recycling

Many mixed waste streams generated in the laboratory contain recoverable components that can be recycled and reused. An example of reuse would be to recover methanol from gel fixing/washing wastes. However, the recovery, decontamination, and purification of the methanol presents many difficulties that make its reuse impractical. These difficulties include providing and operating the equipment, final product purity, processing cost, and licensing and permitting requirements. Because of these difficulties, there are no outside facilities available for recycling radioactively contaminated solvents for reuse as a solvent. One other recycling option is the reuse of waste materials as alternative

fuels. Diversified Scientific Services Incorporated (DSSI), a subsidiary of Chem-Nuclear Systems Inc., operates a facility in Kingston, Tennessee, which will accept certain types of solvent mixed wastes for use as a fuel in power-generating combustion equipment.

Another more feasible approach to recycling is one in which specific solvent mixtures with relatively low levels of contamination are reused by the generator before being discarded. For example, some researchers may use a solvent mixture to fix more than one electrophoresis gel before discarding the solvent as a waste. Also, lead shielding "pigs" used for radionuclide shipping containers can be recycled in several ways. Uncontaminated pigs can be reused in laboratories or shipped to reclamation facilities. Contaminated pigs are decontaminated and shipped to reclamation facilities, although the decontamination procedure can also produce a LLMW. No lead shielding materials are disposed of as a LLMW by NIH.

2.2.5 Improved Waste Classification

Individual researchers can overestimate the concentrations of hazardous constituents in their waste. Regulatory characterizations are performed at the NIH waste processing facility by waste management personnel based on data provided by the researchers. Overestimation by the laboratories of the concentrations of solvents may result in mischaracterization of the waste as a listed waste. To be classified as mixed waste, the laboratory waste material must contain both radionuclides subject to the Atomic Energy Act (AEA) regulation and a RCRA waste (see mixed waste definition in Section 1.1). Mixed wastes that do not contain both of these components may be disposed of as a radioactive, hazardous, or solid waste.[b]

EPA listed approximately 130 industrial waste streams as hazardous waste (the F- and K-listed wastes). These wastes were listed because they exhibit one or more of the characteristics of hazardous wastes, or they contain specific components known to be toxic or otherwise hazardous at levels of regulatory concern.

The definition of F-listed wastes includes (1) spent halogenated and non-halogenated solvents, and (2) mixtures containing, before or after use, a total of 10% or more by volume of these solvents. Experience of NIH personnel responsible for waste disposal indicates that laboratories, by overestimating these solvent concentrations, may mischaracterize their wastes as F-listed solvents rather than ignitable characteristic wastes. Such mischaracterization can result in greater difficulty for disposal of treatment residues of listed wastes since they remain as hazardous until delisted, an administrative process which may require months or years to complete. (Note: the state definition for F-listed waste is more stringent than the

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b. Because the State of Maryland has not received mixed waste authorization from the EPA, classification and management of the hazardous component of mixed waste is conducted in accordance with the State's Controlled Hazardous Substance Regulations.

federal definition which only applies the 10% volume criterion to the spent solvent before use.)

EPA also designated 123 discarded commercial chemical products as acute hazardous wastes (the P-listed wastes) and 249 other chemical products as hazardous wastes (the U-list wastes). To qualify as a listed waste under the P or U lists, the chemical product must be disposed of as an unused, commercially pure grade of the chemical (any technical grade of the chemical and all formulations in which the chemical is the sole active ingredient). However, if the chemical enters into a mixture or a reaction that is part of a laboratory process, the process waste is not a listed waste unless the process itself is listed (F- or K-listed wastes) or the waste exhibits hazardous characteristics (e.g., ignitability, corrosivity, reactivity, or toxicity characteristic). Thus, the mere addition of a reagent appearing on the lists of P- or U-chemicals, does not, by itself require that waste from that usage qualifies as a P- or U-listed waste. Wastes generated from these processes usually remain listed wastes.

In contrast to the F-listed solvents, NIH personnel have not encountered significant mischaracterization of process wastes as U-listed or P-listed wastes to date. No K-listed wastes are generated by biomedical research procedures.

It should also be emphasized that individual NIH laboratories generating wastes are not required to classify the wastes as described above. The laboratories are only required to indicate the generating process, and to list all of the hazardous constituents on the container label regardless of regulatory status of the wastes. Chemists from the NIH Chemical Recycling and Disposal Service determine the regulatory characterization.

Biomedical research procedures also generate waste containing hazardous constituents which are not regulated under RCRA, and some radioactive materials that are not regulated under AEA. Although mixed waste management standards are not applicable to such material, the potential risks posed by these wastes dictate that prudent management procedures with the same or greater precautions be applied. At NIH, unregulated, potentially hazardous mixed wastes are collected, labeled, tracked and handled in the same manner as regulated wastes.

3.0 LLMW GENERATOR INTERVIEWS

The 20 NIH LLMW generator interviews that were conducted are individually summarized in Appendix A. The interviewees were selected by examining the database of LLMW generation for the years 1989 to 1993 and identifying those laboratories that had generated the largest volumes of LLMW without readily available disposal options. The largest volumes of the more difficult-to-manage LLMW at NIH tended to be associated with usage of TCA, methanol, and acetic acid. In particular, TCA is used to denature proteins, useful for protein separations. Acetic acid, in association with methanol, is used in fixing and washing electrophoresis gels for DNA sequencing.

DNA sequencing waste typically consisted of fixes and washes of 10% TCA and 10% methanol, with past generation of up to 2 liters per DNA sequencing gel. This waste qualifies for regulation as a hazardous waste due to toxicity, corrosivity, the potential for ignitibility, and as a listed F003 waste (methanol). NIH wastes containing TCA are typically toxicity-characteristic RCRA wastes due to the presence of chloroform (D022). Research at NIH has confirmed that TCA, and its neutralization salts, continuously decarboxylate in the wastes to form chloroform.

Seven of the 20 generators interviewed were, or are, performing DNA sequencing. Each of these researchers described how waste volumes from gel fixing and washing have been reduced within the last year or two. For example, one generator no longer fixes and washes the gels in a 2 liter "bath" of acetic acid and methanol, but judiciously pours approximately 200 ml of the fixative/wash over the gel. Another researcher reuses the same fix/wash bath for approximately 10 gels. Other researchers have switched to gels or techniques that can be used without fixing or washing with acetic acid and methanol, a practice that virtually eliminates the generation of LLMW from gel fixing and washing.

Another large-volume, potentially difficult-to-manage waste at NIH is generated from the use of automated HPLC equipment used to evaluate cell metabolism. This waste typically results from a series of cell culture extractions that are evaluated using a combination of liquid chromatography and LSC as described in Section 2.1. The resulting waste consists of a mixture of aqueous buffers (the carrier for the liquid chromatograph) and organic liquid scintillation cocktail (the counting medium for the liquid scintillation counter). LSC cocktails have typically included solvents such as toluene which, when discarded, were RCRA-listed wastes and/or were RCRA-characteristic wastes due to their low flashpoint. More recently, several vendors have offered LSC cocktails which include phenylxylylethane and/or long-chained alkylbenzene solvents that are not RCRA-listed wastes and have flashpoints sufficiently high that they are not RCRA-characteristic wastes. Depending on local permitting requirements, these cocktails can often be drain-disposed and are advertised as being "safe," "high flashpoint," and "biodegradable."

The cocktails using newer toluene-free formulations do not appear to be more environmentally biodegradable than the earlier cocktail formulations using toluene. The use of the word "biodegradable" to describe these may be more of a promotional description than a description of their fate when released into the environment. Additionally, samples used with LSC cocktails can be mixed within a variety of solvents including toxic and ignitable compounds (e.g., methanol, acetonitrile). Because the hazardous solvent becomes mixed with the LSC cocktail during the analytical technique, the solvent and not the LSC cocktail would likely dictate if the waste could be drain disposable.

Most researchers are attempting to use these newer cocktails. However, the counting efficiency of some of these cocktails is lower than the older toluene-based LSC cocktails when they are used with ^{3}H, which emits beta particles with a relatively low energy compared with other commonly used beta-emitters such as ^{14}C. This is very dependent on other variables that can also affect the counting efficiency. Some LSC cocktail manufacturers claim that the ^{3}H counting efficiency of some of their newest biodegradable cocktails is better than the older toluene-based cocktails.

One researcher generated significant volumes of waste with chloroform extractions from cell cultures and subsequent washings with water, sodium borate, and acetic acid (approximately 5 gallons of 7% chloroform waste every month). Another cell culture laboratory used 10% TCA to stop a RT reaction followed by 100% methanol rinses. The researcher interviewed in this laboratory appeared frustrated because the TCA corroded a more modern cell harvester, requiring additional procedural steps and generation of approximately 12 times the waste volume of TCA and methanol. Current LLMW generation in this laboratory is approximately 15 gallons per month. ELISA (enzyme linked immuno sorbent assay), an alternative laboratory technique that generated less waste, can also be used to obtain the same result. However, it is a more expensive assay than the reverse transcriptase assay, if waste disposal costs are not considered.

Smaller volumes of difficult-to-manage LLMW at NIH are generated from silver staining in conjunction with radiolabeling of the protein. Mixed wastes from silver staining are typically a solution of ethanol, acetic acid, and water with the potential for a characteristic silver waste. As discussed in Section 2.1, another staining technique that generates mixed waste is staining with Coomassie Blue in conjunction with radiolabeling of the protein. With the Coomassie Blue stain the mixed waste contains a 30% methanol/10% acetic acid solution, in addition to any radioactive contamination (typically ^{32}P or ^{35}S).

Small volumes of difficult-to-manage LLMW are also generated at NIH from other procedures. One example is the use of a phenol/chloroform mixture to extract isotopically-labelled nucleic acids for subsequent analysis. As noted in Appendix A, this process appeared to produce a relatively small proportion of the total waste stream.

Regarding steps to minimize waste, earlier efforts to educate the researchers in the importance of waste minimization appear to have had a significant impact on waste generation. Each researcher who was interviewed was genuinely concerned about the types and volumes of waste generated and eager to minimize generation of LLMW provided alternative methods produced valid research results. However, researchers expressed significant reluctance over the possibility of adopting an invalidated or unproven technique or substitution just to reduce mixed waste generation. Several researchers were concerned that a major change in protocol may require anywhere from 2 months to 2 years to repeat past work to validate and standardize the new technique or substitution. If a new technique was published in a peer-reviewed journal, however, the reluctance to adopt it was considerably less.

Gel fixing and washing, being the largest volume of difficult-to-manage LLMW, was the focal point of many generator interviews. The issue of whether or not to fix and wash electrophoresis gels for DNA sequencing appears to be as much a matter of individual preference and laboratory technique as an issue is driven by research needs. Table 3-1 summarizes some of the comments on the issue of whether or not to fix and wash the gels prior to autoradiography from different researchers performing DNA gel sequencing.

Table 3-1. Pros and cons of fixing and washing electrophoresis gels.

If Gels are Fixed...	If Gels are Not Fixed...
More certainty that the gel will transfer correctly to the filter paper	With care, the gel transfers to the filter paper without incident
Shorter drying time as gels dry faster without the hydroscopic urea present	Quicker and simpler procedure since less steps are involved
	Resolution may not be as good in the upper regions of the plate

Another alternative to gel washing and fixing is the use of chemofluorescence coupled with automated equipment. It was apparent from the interviews that many researchers did not trust the results from automatic DNA sequencers except when the research became repetitive and standardized. Several researchers commented that for an occasional DNA sequence, it was far more reliable and less costly in terms of time, equipment, and labor to run the electrophoresis gel using traditional autoradiography than to standardize and calibrate the automated equipment. This alternative is discussed in greater depth in Section 4.1 of this report.

It also became apparent from generator interviews that equipment and chemical vendors play an important role in improving laboratory technique and waste minimization. Vendors promote alternative methodology and can invest the time required to validate a new

technique, methodology, or new product or reagent. Several researchers were testing new products given them by vendors, although such testing was not a focal point of any of the research. Initial reports from researchers indicated that some of the products worked well, but several researchers were frustrated that the vendors considered some chemical constituents proprietary information.

In general, those interviewed stated that LLMW generation at NIH has been reduced over the past several years. Several reasons were given for waste reductions including the "maturation" of the research, development of techniques that used less radioactive material, and development of techniques that relied more on chemiluminescence instead of radioactivity. There was a general preference among many researchers to use nonradioactive techniques, if available and consistent with reliable research results.

The interviews indicate that minor procedural or process modifications require less costly validation and can generally be implemented with only a minor investment of time and lost research productivity. A bias towards minor modification is demonstrated by the fact that every researcher interviewed is using less acetic acid/methanol to fix and wash electrophoresis gels while very few researchers actually changed gels to avoid fixing and washing.

Some of the researchers are recycling waste materials within their laboratory when the same procedure is performed repetitively. However, in general, it appears that recycling opportunities are limited by both the precautions required for radioactive materials and by the lack of a simple, quick, and effective means of treating the waste reagents prior to their reuse. Recycling techniques do not appear to be any more effective at minimizing mixed waste generation than judicious and limited usage of gel fixing/washing solutions.

4.0 ASSESSMENT OF WASTE MINIMIZATION OPTIONS

The information obtained from interviews with LLMW generators at the NIH was used to identify various waste minimization options. These options would reduce LLMW generation for research activities such as those conducted at the NIH. These options were identified and developed based on the approaches described in Section 2.2 and have, as their objective, a reduction in the quantity of LLMW generated rather than improvements in treatment and disposal of LLMW already generated. The waste minimization options are described and discussed below. Specific waste treatment and disposal options for previously generated waste are outlined in Section 5.0.

4.1 Electrophoresis Gel Fixing/Washing Options

Electrophoresis gels are one of the most powerful tools being used in biomedical research today. As discussed in Section 2.1, they are being used to separate proteins for identification, concentration, and/or analysis, to determine the peptide sequences in proteins (less commonly done now due to improvements in DNA sequencing), and to determine the sequence of bases in DNA. The development of these procedures required major breakthroughs to make them effective and they are unlikely to be discontinued until further breakthroughs occur that make possible improved alternative methods. A large proportion of the difficult-to-manage LLMW generated by biomedical research is produced through the use of electrophoresis gels with radiolabeled molecules. The following describes methods by which the volume of LLMW generated from electrophoresis gel usage might be reduced.

4.1.1 Changes in Procedures

Several methods for using electrophoresis gels exist that do not require the use of radiolabeled compounds or that require a much smaller volume of acid/methanol solution for fixing and washing the gels.

4.1.1.1 Elimination of Autoradiography. Nonradioactive alternatives exist to identify DNA and proteins in electrophoresis gels. As discussed in Section 2.2.1, ethidium bromide is frequently used as a dye for DNA detection. While it is useful in some applications, one drawback is that it does not work effectively with single-stranded DNA, which is often used in DNA sequencing studies. An additional difficulty is that ethidium bromide is a known mutagen and a suspected carcinogen and requires careful procedures to avoid exposure to laboratory workers. Coomassie Blue R-250 is often used to stain polypeptides separated by SDS-polyacrylamide gels. After staining, the proteins can be detected, photographed, and/or stored for further use.

Silver staining techniques are another alternative to autoradiographic techniques for both proteins and nucleic acids. As an alternative to radiolabeling, silver staining offers

several advantages according to manufacturers of commonly used silver staining kits. It is more rapid, equivalent in sensitivity to autoradiography, less expensive, avoids altering the physical characteristics of proteins, and may detect proteins that are not detected in radiolabeled cell lysate. It also is claimed to offer advantages over Coomassie Blue R-250 staining agents due to its greater sensitivity and its ability to analyze dilute samples.

A final consideration is that the use of nonradioactive methods, such as those described here, can result in the generation of hazardous wastes that must be disposed of at an offsite RCRA treatment, storage, and disposal facility. LLMW minimization efforts must include consideration of the effect on waste volume, the total disposal costs, risks to workers, and other factors before recommending an option that substitutes the generation of RCRA hazardous waste for the generation of LLMW.

4.1.1.2 Reduction in Reagent Volumes. If autoradiography cannot be eliminated, the volume of reagents required to wash and fix electrophoresis gels can be significantly reduced, or fixing/washing steps can be eliminated or scaled down. When using DNA sequencing gels, the fixing/washing procedure that generates the largest volume of waste is soaking the gel after electrophoresis in a solution of 10% acetic acid and 10% methanol. Gel sizes used for DNA sequencing are typically quite large and require a significant volume of fixing solution of approximately 2 liters per gel. Some researchers have been able to reduce the volume of fixing solution by treating the surface of the gel rather than immersing the entire gel, or by reusing the fixing solution repeatedly. With measures such as these, the volume of fixing solution can be reduced by as much as 90% for each gel.

Some gels currently available can be used without performing the fixing step. One such gel is the "Long Ranger" gel provided by AT Biochem. This manufacturer states that the gel does not require fixing to remove urea prior to autoradiography using ^{35}S labeled molecules. The manufacturer also states that the ^{35}S signals are quenched by less than 20% without a fixing step resulting in both time savings and reduction in LLMW volume.

4.1.2 Substitution of Reagents

The reagents most commonly used in gel electrophoresis for gel fixing and washing are methanol, acetic acid, and TCA. In some cases, ethanol can be substituted for methanol. Ethanol has a much lower acute and chronic toxicity and would not produce a LLMW unless it were used in high enough concentrations to produce an ignitable-characteristic hazardous waste. Acetone can sometimes be substituted for TCA, methanol, or ethanol but does not represent an improvement because a waste acetone solution would still be a listed F003 hazardous waste. The Hazardous Solvent Substitution Data System (HSSDS) at the Idaho National Engineering Laboratory was investigated to determine if substitute reagents could be identified using its resources. No substitutions were found for methanol, trichloracetic acid, or acetic acid for biochemical applications such as gel fixing.

Prompted by the waste minimization interviews, one group of researchers investigated alternative methods and materials for fixing and washing DNA sequencing gels.[c] Previously, the laboratory used 10% acetic acid and 10% methanol to fix the DNA in the gel, reduce sample loss and diffusion, and remove urea, permitting proper drying of the gel prior to autoradiography. This group of researchers found that deionized water could be substituted for the 10% acetic acid and 10% methanol solution with indistinguishable results in the autoradiograph. When using deionized water, a 0.2-mm-thick gel is washed for about 20 minutes. On very humid days the wash time needs to be lengthened to prevent the gel from sticking to the x-ray film. Alternatively, the gel could be covered with thin plastic wrap without affecting the quality of the sequencing data, even when ^{35}S is used.

4.1.3 Equipment Changes

Much of the work with electrophoresis gels is being done for the purpose of determining the base sequences in DNA of interest. The volume of work in this area is so large that a number of companies have developed sophisticated automated DNA sequencing equipment. This automated equipment uses electrophoresis gels with laser activation of fluorescent dyes and subsequent computer scanning and interpretation of the signals in real-time. In the more advanced systems, a four-color dye system is used with one color specific to each of the four DNA bases by means of dye-labeled primers or dye-colored terminators. Laser activation of the dye during electrophoresis in conjunction with a photomultiplier tube produces a computer input signal that is interpreted and converted to a direct readout of the DNA base sequence. Such automated sequencing equipment has the potential to significantly reduce the volume of LLMW generated as a result of DNA sequencing activities. The cost of a complete automated scanner system is approximately $120,000 depending on the selected options.

Typically, a facility using automated sequencing equipment sets up a "core" facility that includes the sequencers, computing equipment, and support facilities for preparing and processing the electrophoresis gels. The Institute for Genomic Research (TIGR) is one such facility. TIGR is a nonprofit research institute founded by several former NIH researchers that is currently operating 30 automated sequencers in pursuit of sequence-based characterization of gene expression (Adams et al. 1994). Researchers interviewed at NIH expressed reluctance to use automatic scanning equipment for intermittent and exploratory sequencing operations. However, for more standardized sequencing, the automated systems were highly desirable.

Another possible equipment change would be improving gel dryers. In some cases, fixing is required to remove urea to reduce the drying time of the gel and improve the dried

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c. Memo dated November 3, 1994 from K. Usdin, Ph.D to Wm. Walker, Ph.D., Department of Health and Human Services, Public Health Service.

gel handling characteristics. Improved gel drying equipment can sometimes be used to maintain drying time requirements while decreasing the quantity of reagent required for gel fixing by increasing the tolerance for urea in the finished gel.

4.2 HPLC Scintillation Options

HPLC scintillation counting is a powerful and widely used technique that has extensive value in identifying and characterizing unknown compounds. While most LLMW generated from HPLC has a disposal option, there are very few options for reducing the volume of LLMW generated from HPLC scintillation counting. As noted in Section 3.0, some scintillation fluids available that are claimed to be "biodegradable" and would not seem to be a listed or characteristic RCRA waste. However, one manufacturer of such a fluid recommends that it not be disposed to the sewage system due to its aquatic ecological toxicity. Other manufacturers claim that the EPA has performed aquatic toxicity testing on their fluids and approved them for drain disposal, subject to state and local discharge permits.

As noted previously, if solvents such as methanol or acetonitrile are used with LSC cocktails, the resultant waste would likely be regulated and would not be drain disposable. The composition of LSC cocktails is not usually known due to their proprietary nature; but, if they contain priority pollutants they would also be subject to drain disposal restrictions. Another option for LLMW volume reduction can come from careful attention to buffer flow rates during the running of an HPLC procedure. A flow rate higher than necessary can increase the volume of LLMW waste generated while not providing any increased research benefit.

4.3 Blotting Options

Several options exist for reducing LLMW during blotting activities. These are briefly described below.

4.3.1 Use of Nonradiolabeled Molecules

Several chemiluminescent reagents are available for use with blotting techniques that allow proteins to be detected without the radiolabeled molecules. Most of these use the horseradish peroxidase (HRP)-mediated luminol oxidation reaction in which the oxidation of luminol is catalyzed by peroxidase to produce light. Vendors claim that chemiluminescence offers advantages over radioactive methods due to its speed, sensitivity, and convenience.

4.3.2 Reagent Substitution

Methanol and other reagents are frequently used in blotting techniques. Ethanol can be used in place of methanol in some systems for final membrane washing. This approach avoids generating a LLMW provided that the ethanol concentration is sufficiently low that an ignitable-characteristic waste is avoided. Another possible substitution involves the reagents used for the transfer buffer for the blotting. In western blotting, an older transfer procedure used an electrophoresis buffer consisting of tris-glycine electrophoresis buffer at a pH of approximately 8.3. This procedure typically requires approximately 12 hours to complete, but does not appear to produce a LLMW. In the newer procedure, a concentration of 20% methanol is used in the transfer buffer, which results in the generation of a LLMW. The advantage of the newer procedure, however, is that it is much more rapid and uses a smaller amount of electric current.

4.3.3 Equipment Substitutions

One vendor supplies a multi-purpose fluorescence scanner for gels and membranes that can be used as a substitute for radiolabeling techniques. The vendor claims that the system can be used to detect and quantify a variety of complex protein separations by using fluorescent dye kits specific to the test being performed. The system uses an argon scanning laser with a 50 micron scanning width that is combined with a photomultiplier tube and a computer analysis system. The time required for analysis is reduced from 72 hours for film autoradiography to as little as 2 hours with fluorescence. A drawback to the system is that the cost of the scanning system is approximately $57,000.

4.4 Options for Enzyme Assays/Cell Harvesting

There are at least two options available for reducing waste generation in processing activities of this kind.

4.4.1 Reagent Substitutions

A nonradiometric test is available for the detection of RT.[d] The manufacturer claims that the test has equal or greater sensitivity than the more commonly used ^{3}H-based radiometric test and does not result in the generation of LLMW. The test costs approximately $400 to $650 and can be completed in 4 hours according to the manufacturer.

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d. "RT-Detect" DuPont Medical Products.

4.4.2 Equipment Modifications

In some cases, the provision of additional equipment can reduce the volume of LLMW generated. In cell harvesting, the same quantity of reagent is used for a 16-unit harvester or a 96-unit harvester due to the type of procedure being performed. However, the larger harvester processes six times more samples in a single batch resulting in the potential for a smaller total volume of LLMW generated.

4.5 Options for Staining/Destaining

Staining/destaining does not itself generate a mixed waste; however, a mixed waste is generated when staining/destaining is performed in addition to autoradiography. Therefore, the only option identified for reducing LLMW generation in conjunction with staining/destaining procedures is to use nonradiolabeled molecules. However, the use of radiolabeling is, to a high degree, specific to the particular project in progress. Therefore, the requirement for radiolabeling is determined by the other processing activities being used in combination with staining.

4.6 Option Evaluations

Evaluating waste minimization options generally includes the screening of options based on criteria such as efficacy, safety, short-term, long-term, and life-cycle costs, and estimates of potential waste reduction. Because many of the research applications were unique, no overall waste minimization screening methodology could be applied to the waste generating processes. Each process needs to be evaluated in relationship to its research purpose and the opportunities for waste reduction through material substitution, process change, equipment change, and waste segregation.

In addition to the microscopic review of each individual researcher's protocols, a macroscopic approach to the issues of short-term, long-term, and life-cycle costs should be considered at large institutions such as the NIH. While each of several departments may not have sufficient justification to purchase waste minimization or pollution prevention equipment involving large capital expenditures, taken together there may be sufficient utilization to warrant the expenditure.

5.0 WASTE TREATMENT OPTIONS

Much of the waste produced by the processes described in Section 2.1 was determined to be difficult to manage at the time of the first NIH study (DOE/LLW-208) in 1993. Since that time, NIH has sent most of this waste to an offsite treatment facility, DSSI in Tennessee. NIH also treats and disposes of wastes using various methods that are able to process a larger proportion of the waste since 1993. Described below are in-house treatment technologies that can be applied to these previously generated wastes to create disposal options.

5.1 Waste Stream Properties

The results of a previous 1993 study (DOE/LLW-208) and the information obtained from generator interviews indicate that the waste produced by the processes described in Section 2.1 is relatively consistent in the physical and chemical properties that apply to treatment of the waste. The chemical reagents used in these processes are predominantly trichloroacetic acid, methanol, acetic acid, and ethanol. The concentrations of these reagents vary between 1 and 30 percent, with the exception of a small amount of 100 weight percent methanol used in the reverse transcriptase assay procedure described in Section 2.1.5. Other reagents that might be present in smaller quantities would be phosphoric acid and chloroform. The radioisotopes used are predominantly ^{3}H, ^{14}C, ^{32}P, and ^{35}S.

Other reagents and compounds are also present in LLMW generated at the NIH, but would be found less frequently and at relatively low concentrations depending on the source of the waste. Examples of these would be phenol, silver compounds, acetonitrile, and pseudocumene. Biological materials, gel solids, surfactants, and other waste solids are commonly found in LLMW at NIH. Lipids and oils can also be present. These waste materials could have specific effects on waste treatment processes that would affect a proposed treatment strategy. Such effects might include clogging of filters by solids or increased turbidity.

The generated wastes containing these reagents would have some common physical and chemical properties. The wastes would be aqueous liquid wastes with small amounts of suspended solids present. The pH would likely be low enough that an adjustment would be required. Most of the organic reagents present are completely miscible in water though a few, such as chloroform, could form a separate organic layered phase. The concentration of organic reagents is likely to be high and could potentially be as high as 300,000 parts per million (ppm).

A number of treatment technologies exist that can provide treatment for waste streams with the reagents and properties described above. A preliminary screening of the feasibility of available treatment technologies was provided by using the information from the waste

generating processes and generator interviews to develop a generic waste profile for the NIH LLMW. The majority of the LLMW, which was found to have no disposal options in the 1993 study (DOE/LLW-208), would fall within the parameters of this waste profile. To be effective, the proposed waste treatment technologies should be able to successfully treat wastes that lie within the generic waste profile. The composition and properties of this generic waste stream are identified in Table 5-1 below.

Table 5-1. Generic waste stream profile.

Reagents in Waste	Volume Percent
Methanol	0-30
Acetic acid	0-10
Trichloroacetic acid	0-10
Phosphoric acid	0-1
Chloroform	0-1
Ethanol	0-5
Balance of Waste	
Water	70-100
Ph	1-4
Max. waste volume (liters/year)	10,000
Radionuclides in Waste	
^{35}S	
^{32}P	
^{3}H	
^{14}C	

5.2 Treatment Technologies/Processes

Following pretreatment, disposal of the waste stream to the sanitary sewer system, described in Section 5.1, may be possible. The pretreatment objectives would be to remove compounds from the waste stream that prevent it from being discharged, increase the pH to acceptable discharge limits, and create a smaller secondary waste stream that could be transferred to a disposal outlet.

Except for chloroform, the reagent chemicals listed in Section 5.1 are miscible in water. With the exception of phosphoric acid, they are also organic compounds that are

potentially subject to further oxidation. The ^{32}P and ^{35}S radionuclides have relatively short half-lives and can be stored for decay prior to disposal.

Treatment technologies for this waste stream would be focused on removal or destruction of the organics and adjustment of the pH to acceptable discharge levels. Technologies for accomplishing these objectives are outlined below. NIH performs pretreatment activities and some of the treatment processes similar to those described here.

5.2.1 Organics Removal

The following sections describe several technologies for removing organics from the waste stream. Each technology is briefly evaluated for its applicability, feasibility, and effectiveness for the proposed application.

5.2.1.1 Phase Separation. The solvents used in liquid scintillation fluids, as well as chloroform, and other organics are immiscible in water and would exist as a second liquid phase if they were present along with the aqueous wastes described in Section 5.1. In this case, the nonpolar hydrophobic nonaqueous organic phase would separate from the aqueous phase lying below. This organic liquid phase could be separated and disposed of offsite at DSSI or by steam-reforming technology (Section 5.2.1.5). At NIH, phase separation is employed where feasible. However, emulsions are commonly encountered and difficult to separate. Surfactants are widely used in research procedures and contribute to emulsion formation.

5.2.1.2 Chemical Oxidation. The most commonly used chemical oxidation process for wastewater uses UV light and hydrogen peroxide to oxidize organic compounds to carbon dioxide and water. The UV light activates organic molecules making them more amenable to oxidation, and assists in the creation of hydroxyl radicals from the hydrogen peroxide. The hydroxyl radicals are powerful oxidants that then act on the organic molecules present to produce carbon dioxide and water. Chlorinated compounds will contribute some acidity to the water requiring adjustment of the pH.

The hydrogen peroxide is added to the influent stream and the mixture is passed through a closed vessel equipped with powerful lamps that generate light energy in the UV range of the spectrum. Typically, versions of this technology that use high-intensity lamps operate continuously at ambient temperature and require residence times of 1 to 5 minutes to effect near-complete oxidation of most organic compounds. An advantage of chemical oxidation is that no secondary waste is produced because organics are completely oxidized. The high concentration of the organics in the anticipated waste stream would require a recirculating water stream to which the waste would be slowly added to maintain an influent organics concentration in the desired range.

NIH has completed testing of a UV-enhanced chemical oxidation system and has successfully placed a unit into service. A feature of the NIH system is recirculation of the treated waste. The recirculation flow is controlled based on the oxidant concentration, not the concentration of organics. Process control is by measurement of the peroxide concentrations. A possible advantage of a UV oxidation system is that it may sterilize the waste, eliminating any potentially biohazardous agents. However, there is no data currently available on effects of this type.

5.2.1.3 Carbon Adsorption. Commercially available granulated activated carbon (GAC) is often used to adsorb organics contained in aqueous feed streams. GAC can also be partially effective in adsorbing selected inorganic radionuclides. GAC is most effective when used to adsorb relatively high-molecular-weight hydrocarbons that exhibit low solubility in aqueous solution. GAC is not as effective in adsorbing many types of organics such as some types of chlorinated hydrocarbons and polar solvents such as acetone or methanol. The GAC has a limited capability for adsorbing organic molecules. When this limit is reached, the organics will pass through the GAC bed in a condition referred to as "breakthrough." When this occurs, the GAC must be removed from the adsorption vessel and replaced with fresh GAC. The spent GAC can be reactivated or disposed of as a waste.

A drawback to using GAC for the anticipated waste stream is that the adsorption capacity of the GAC for the anticipated organic compounds is low and the potential organic loading is proportionately high. This might result in a large waste volume of spent GAC relative to the size of the initial waste stream. The spent GAC would become a mixed waste that would have to be disposed if it was used to treat a listed waste, or if it exhibited a RCRA waste characteristic such as chloroform toxicity (DO22).

NIH uses a GAC system to process aqueous waste streams to remove chloroform and has found it to be quite effective. No facility currently exists to accept mixed waste GAC for reactivation. Land disposal of spent GAC may be possible but would depend on its EPA waste designation, treatment to land disposal restrictions, and the degree of radioactivity that it exhibited. The potentially large volume of spent GAC that could be produced and the limited treatment and disposal outlets for this secondary waste limit the feasibility of GAC as a LLMW treatment technology.

5.2.1.4 Solvent Extraction. The high concentrations of organics in the anticipated waste stream could be removed via extraction with a solvent selected for the purpose. The extraction solvent would be a nonpolar organic compound with a low solubility in water. It would be contacted countercurrently with the waste stream in a liquid/liquid contacting vessel where the organic reagent chemicals would be transferred out of the aqueous phase and into the extraction solvent phase. Solvent extraction would be technically difficult for some of the organics anticipated to be present and would require extensive additional pretreatment and post-treatment of the waste stream. In addition, a solvent recovery system would be

required to recover solvent from the extracted organic wastes. The extracted organics could be disposed of via offsite incineration, steam reforming, or other thermal or nonthermal destruction technology.

Recycling of recovered organics is a possible treatment approach if influent waste streams could be segregated by reagent chemical and processed in a sequential fashion. The recovered organics would still contain radioactive materials and would require management as a mixed waste. Depending on the specific conditions, the aqueous waste portion might also contain radioactive materials and require management as a LLW. These difficulties make it doubtful that solvent recovery would significantly reduce the volume of LLMW and it is not considered to be a viable treatment technology.

5.2.1.5 Steam Reforming. Steam reforming is a technology for chemically converting organic wastes to CO, H_2, CO_2, H_2O, and CH_4 by using steam reforming chemistry. In steam reforming, reforming reactions occur in a near oxygen-free environment under high pressure and temperature and allow an extremely high conversion of organics to the primary conversion products of CO_2, H_2O, and H_2. A significant advantage is that the process can be used to convert organic compounds contained within a solid matrix, thus allowing the processing of mixed waste forms such as GAC that are not presently treatable using conventional incineration or combustion treatment. The steam reforming process requires specialized equipment that is not commercially available for installation at a location such as the NIH. Scientific Ecology Group, Incorporated (SEG) in Oak Ridge, Tennessee, is currently developing a commercial mixed waste treatment facility using this technology. One possible advantage of steam reforming is that it may sterilize the waste, eliminating any potentially biohazardous agents. However, there is no data currently available on effects of this type.

5.2.2 pH Adjustment

Following and/or preceding treatment, the pH of the aqueous waste stream would require adjustment to meet anticipated discharge requirements. The pH would be increased with the addition of sodium hydroxide and would be decreased, if required, with sulfuric acid. The pH adjustment would be performed in mixing tanks that preceded and followed the organics removal process. The pH adjustment would be controlled via metering pumps and pH control equipment.

5.3 Proposed Treatment Process

The recommended organics removal process would be physical phase separation followed by chemical oxidation. Chemical oxidation is recommended based on its demonstrated ability to remove organics from water and on its ability to effectively treat the waste stream without producing a secondary waste. As noted in Section 5.2.1.2, NIH has

installed a UV-enhanced chemical oxidation system. The recommended process arrangement is shown on Figure 5-1. Following phase separation, the influent concentrated waste stream would be mixed with a recirculating water stream to create a combined waste stream with an organics concentration suitable for the UV oxidation reactor system. The two streams would be combined in a mixing tank and the pH would be adjusted, as required, to obtain optimum conditions for oxidation. Following the oxidation of the organics, the combined stream would enter a second pH adjustment tank where the pH would be increased to meet final effluent discharge requirements. A portion of the final treated effluent would be discharged and the remainder would be recycled to be combined with additional concentrated influent waste. A scrubbing system might be required for the offgas from the UV oxidation system depending on site requirements and final operating conditions. The scrubbing system could also be used to reduce ^{3}H, ^{14}C, and aerosols, if required. Monitoring of effluent and offgas concentrations for radioactivity and processing effectiveness would be required. Additionally, a RCRA permit is required for such a treatment facility.

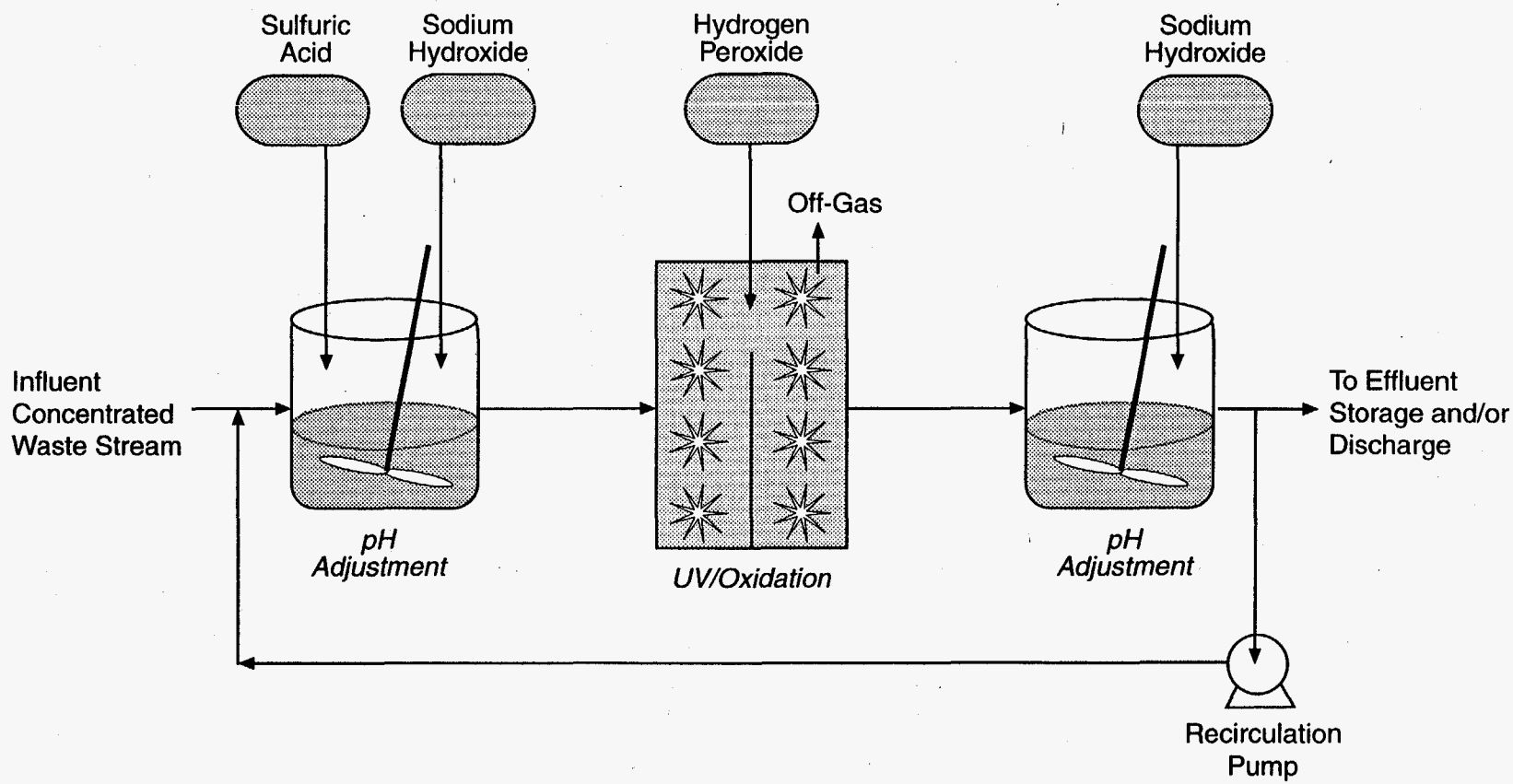

Figure 5-1. Recommended process for organic LLMW treatment.

6.0 CONCLUSIONS AND RECOMMENDATIONS

The largest quantities of LLMW evaluated for this study are associated with a relatively small number of research techniques and processes, namely, gel electrophoresis, HPLC/scintillation counting, cell harvesting, staining and destaining, and protein blotting. Researchers generating LLMW from these processes were interviewed to obtain information that might suggest generally applicable waste minimization techniques. Virtually all generators who were interviewed have reduced LLMW generation rates from previous years; however, most of the waste minimization efforts were the result of either minor process changes in research protocol or a maturation of the research effort whereby more focused studies are now pursued.

Just as each area of research is uniquely pursued, each of the waste minimization options needs to be evaluated in the context of the particular research application. No panacea or generally applied procedure can be used to minimize the LLMW from each generating process. Nonetheless, generalizations regarding greater or less suitability can be made for each generating process and each type of waste minimization option. Based on the analysis outlined in Section 4.0, Table 6-1 summarizes waste minimization option feasibility.

Table 6-1. Summary of feasibility of waste minimization options.

	Substitution of Reagents	Minor Changes in Techniques	Improved Waste Segregation	Recycling	Changes in Equipment
Gel Fixing/ Washing	•	●	▪	•	●
HPLC Scintillation Counting	•	▪	•	▪	•
Blotting	•	•	▪	▪	•
Cell Harvesting	●	•	•	▪	●
Staining/ Destaining	●	▪	▪	▪	▪

▪ Not considered feasible.
• Limited feasibility.
● Likely feasible.

The analysis also indicates that these unique research applications inhibit formal evaluation of waste minimization options on a facility-wide basis. Evaluating waste minimization options generally includes the screening of the options based on criteria such as safety, short- and long-term and life cycle costs, and estimates of potential waste reduction.

Due to the uniqueness of each area of research, waste generation, cost, and implementation procedures differed significantly for each waste stream in a particular research area. Accordingly, no facility-wide waste minimization option screening could be applied to a particular waste stream.

Based on generator interviews, independent evaluation of LLMW generating processes, and the analysis of waste minimization options contained in the previous sections, the following additional conclusions are made:

- Most of the reagents that are used do not appear to have substitutes acceptable to the research community at this time. This situation is particularly true for TCA and methanol. However, there do appear to be substitution opportunities for using alternative gels that would require either a smaller volume of fixatives or would not require fixing or washing.

- Procedural changes appear to be a potential waste minimization approach for some research applications. In particular, chemiluminescent detection techniques could be used more widely as a substitute for techniques involving autoradiography. Also, additional expenditures for new equipment could reduce waste volumes. Such equipment might include 96-unit cell harvesters instead of 12-unit harvesters, automated DNA sequencers, and gel dryers, all of which were identified by researchers as having the potential to reduce waste volumes. Established project protocols, however, tend to limit the application of newer techniques in the near term. Additionally, funding arrangements limit the ability of an individual researcher to unilaterally obtain such equipment.

- There does not appear to be significant opportunities for LLMW volume reduction by segregation of radioactive and hazardous waste streams. The techniques being used require the contacting of reagents with radioactive materials with the resultant transfer of radioactive materials to the reagent waste. The researchers interviewed already strive to segregate radioactive waste from nonradioactive wastes.

- The NIH radiation safety group appears to have done a good job of educating and informing the generators about the additional expense and effort required to manage mixed waste, including the need to segregate radioactive and nonradioactive wastes. However, additional efforts in the area of generator education should be pursued. One possible mechanism would be more interdisciplinary seminars on techniques for laboratory waste minimization.

- Recycling opportunities are limited by both the precautions required for radioactive materials and by the lack of a simple, quick, and effective means of treating the waste reagents prior to their reuse.

- The waste produced by gel electrophoresis and filter washing techniques appears to be surprisingly uniform in character and composition, regardless of the specific research application. Such uniformity makes treatment options a potentially effective means of reducing LLMW volumes requiring disposal. Such techniques as stabilization and biotreatment of portions of the LLMW waste streams at the NIH appears to be a possibility if conducted in conjunction with other waste treatment options.

- An opportunity may exist for LLMW minimization through improved waste characterization. Experience to date has indicated that laboratories may be overestimating solvent concentrations, thus resulting in a mischaracterization of the hazardous component of some LLMW as an F-listed spent solvent, instead of an ignitable characteristic waste. The treatment residues of listed wastes require disposal as a hazardous waste.

Wastes for which treatment is not readily available are generally aqueous with organic concentrations of up to 30%, often having pH less than 4, and contamination with ^{3}H, ^{14}C, ^{32}P, and ^{35}S. Recommended treatment for these wastes consists of a RCRA-permitted pretreatment facility involving a combination of storage for decay, organic recovery from multi-phase waste containers, waste aggregation in mixing tanks, adjustment of pH, treatment by chemical UV oxidation, and final adjustment of pH prior to release. Recovered organics would be treated by offsite incineration or other thermal destruction process. Offgases would be monitored, and if necessary, treated for limited removal of radioactive contaminants. Most of the effluent would be aqueous, releasable to the sanitary sewerage system following successful operation of the treatment facility.

This evaluation of the more difficult-to-treat LLMW from biomedical processes demonstrates that much of the biomedical LLMW can be reduced, if not eliminated, and that which cannot be reduced further can likely be treated to an effluent that is releasable to the sanitary sewerage system.

7.0 REFERENCES

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APPENDIX A

SUMMARY OF GENERATOR INTERVIEWS

APPENDIX A
SUMMARY OF GENERATOR INTERVIEWS

The 20 NIH LLMW generator interviews that were conducted are summarized in this appendix. This information identifies the researcher by number sequence of interview, a brief description of the waste generation process as described in Section 2.1 in the main report, previous generation practices based on information in the LLMW database, and a brief description of current or future generation processes. Because either the nature of the research has changed or the waste generation techniques have changed, this more recent waste generation information was sought. Emphasis was placed on those wastes that do not have a readily available means of disposal, primarily the aqueous wastes with relatively high organic content.

Researcher Number	Process Descriptions	Previous Generation	Current Generation/ Waste Minimization
1.	Gel fixing/washing for DNA sequencing	2 L of 10% acetic acid/ 10% methanol per gel for fixing/washing	90% volume reduction achieved by using less fixing/washing solution.
2.	Protein separation gel fixing/washing with ^{32}P for autoradiography	Trichloroacetic acid (TCA) and 66% ethanol about 1 liter per week	No significant change. TCA waste is being generated with improved segregation. Ethanol usage is 95% pure.
		^{3}H Borohydride reactions with dimethylformamide as solvent	No change.
	Protein labeling with ^{35}S	Silver staining kit	No change. This waste, while not necessarily a RCRA-regulated waste, is nonetheless managed as a hazardous waste at NIH.
3.	DNA sequencing with ^{32}P for autoradiography	Gels fixed with TCA and methanol	Eliminated generation of LLMW by switching to different gel, Long Ranger™, which does not require fixing and washing.
	Protein separations with ^{14}C	100% acetic acid fix, then rinse with a moderately toxic solvent (PPO)	Considering the use of DuPont's Entensify™: Initial fix with 10% acetic acid and 30% methanol; then addition of proprietary Solutions A and B (contains halogens and is managed as radioactive mixed waste). This procedure generates 4 liters per month which is more LLMW than the procedure with the toxic solvent. The laboratory technician preferred Entensify™ due to improved occupational safety.

Researcher Number	Process Descriptions	Previous Generation	Current Generation/ Waste Minimization
4.	Previously gel fixing/ washing		No longer doing gel fixing and washing.
	Binding assays with ^{3}H	55% ethanol washes (25 ml ethanol per sample) one carboy per year of ^{3}H waste	Optimized volume of ethanol.
	Kinase assays with ^{32}P	^{32}P-labeled enzyme is denatured with TCA, sulfacylic acid, or phosphoric acid generating four carboys per year of ^{32}P characteristic waste	Kinase assay waste can be neutralized.
5.	Amino acid and protein synthesis with LSC	With ^{35}S: 10% TCA to precipitate and wash protein, acetone to reduce quench for improved LSC counting (10 ml of 10% TCA + 2 ml acetone per filter). Annual generation is one to five carboys per year.	Lab is now using less TCA, and segregating long/short half-life radionuclides better.
	Protein separation for autoradiography	Gel fixing used acetic acid, and 5% ethanol for drying (~20 ml per gel).	No LLMW generation, as the lab is currently not fixing the gel; using Acrylease (a silicon spray) to improve transfer of gel from plate.

Researcher Number	Process Descriptions	Previous Generation	Current Generation/ Waste Minimization
6.	Protein precipitation with LSC	With ^{35}S uses 10% TCA for precipitating proteins; protein collected on nitrocellulose filters, then washed 3 times with approximately 2 ml TCA. Filter dried and counted in 7 ml LSC vial. Previous generation 180 liters per year.	In general, using more immunofluorescence antibodies instead of radiolabels, but images do not appear as sharp as when the procedure was performed with radioactivity.
	DNA hybridization with autoradiography	DNA is hybridized using ^{32}P-labeled oligoprobe. Add 10 ml 50% formamide solution to denature DNA. This waste is managed as LLMW, even though it is not strictly regulated as such.	Lab now uses less activity in the oligoprobe primer, now ~1.5 μCi per reaction.
	Gel fixing for DNA sequencing with autoradiography.	None	Directly transferring gel to paper, thereby generating no LLMW.
7.	Gel fixing for sequencing nucleic acids and separating proteins (Western Blots)	Uses 5% methanol, 10% acetic acid solution for fixing and washing proteins. Previously generated 5 gallons/2 weeks.	Seeking an automatic sequencer using chemi-fluorescence for some analyses. Also contracting some sequencing to contractor laboratories.
8.	High Performance Liquid Chromatography (HPLC) with ^{3}H, ^{14}C	HPLC process varies amounts of 0.02 M Buffer A (0% methanol and ammonium phosphate) and 0.7 M Buffer B (10% methanol and pH ~5 or 6). Process results in less than 5% methanol in waste jug. Waste contains 35% ammonium phosphate and 60% Monofluor4 [a liquid scintillation counting (LSC) fluor]. This lab also manages ammonium formate buffer as LLMW. Previous generation was 5 to 10 gallons per week of HPLC waste.	Switched to Monofluor4, a non-RCRA regulated LSC fluor.

Researcher Number	Process Descriptions	Previous Generation	Current Generation/ Waste Minimization
9.	DNA sequencing with autoradiography	Approximately 1 liter of 10% methanol and 10% acetic acid solution for gel fixing/washing.	Now use less fixer to just wet the surface of the gel. Filter is laid directly on top of gel and lifted off. Approximately 90% of waste volume reduced by this technique. Automatic sequencer is used when the research is standardized and repetitive. Not cost or time effective for smaller number of gels.
	Protein separations using staining solution	Uses 10% acetic acid and 40% methanol for fixing.	Nonradioactive staining procedure does not generate LLMW, except when used in addition to autoradiography. Because protein gels are small, little LLMW is generated per gel.
10.	DNA sequencing with autoradiography	Uses ^{35}S and ^{32}P with large polyacrylamide gels; acetic acid and methanol fix/wash.	Can reuse one liter of wash/fixate for about 10 gels. (This was the only researcher who claimed to recycle gel washes/fixes)
	Phenol/chloroform extractions	Uses ^{32}P in 100 tubes for each run. Generates approximately 100 μl water and 200 μl 95% ethanol waste per run.	Potential for better waste segregation between aqueous and organic.
	RNA-sol	Generates waste containing chloroform, phenol, and alcohol.	Segregated and managed as mixed waste.
	Cell extractions with LSC	Various organic solvents.	Segregated and managed as mixed waste.
	HPLC	Potential for acetonitrile in waste in addition to LSC fluor and buffers.	No change in procedure.

Researcher Number	Process Descriptions	Previous Generation	Current Generation/ Waste Minimization
11.	Gel fixing/washing	Uses ^{32}P fixing with 40% methanol and 10% acetic acid; previously generated approximately 1 liter per gel.	Previously had large volumes of gel fixate and wash and silver staining of gels. Now using an antibody to isolate protein and generating only a small amount of LLMW.
	Silver staining	Silver staining waste consisted of 200 ml oxidizer, 100 ml silver stain, 500 ml silver reagent, 600 - 1,000 ml developer.	When used in conjunction with antibody technique, no LLMW is generated.
	HPLC	Aqueous and organic waste solutions resulting from multiple extractions and LSC analysis.	HPLC uses a smaller volume of Flo Scint scintillation cocktail than equivalent manual technique.
	Other activities	Waste also contains perchloric acid (hydrolyses/destroys ATP), molybdate (precipitates phosphate), triethylamine (buffer), and acetone (precipitates protein). Lipid extractions involve 50 μCi ^{3}H and ^{14}C with acetone (pure) or chloroform/methanol (50% concentration)	No specific waste minimization techniques were employed or suggested for these relatively infrequent processes.

Researcher Number	Process Descriptions	Previous Generation	Current Generation/ Waste Minimization
12.	DNA sequencing with autoradiography	Uses ^{32}P and ^{35}S. Does not fix or wash gel, therefore no LLMW generation from DNA sequencing.	Does not fix the DNA gel.
	Nonradioactive staining following autoradiography	Use some nonradioactive DNA staining with ethidium bromide (a mutagen) or Coomassie Blue after autoradiography. Staining requires 10% to 50% methanol and 10% acetic acid for fixing and washing, generating LLMW.	No specific waste minimization techniques.
	Protein separations	Uses 20% isopropanol fixer/washer to get rid of the SDS, but it does not denature the protein.	Effective product substitution, as isopropanol is not a LLMW.
13.	TCA protein precipitations	Waste consists of ~/μCi/ml ^{3}H in 5% TCA. Generation rate of 10 gallons per year.	Discontinued adding hydrochloric acid to mixed waste container. This waste can be neutralized in laboratory and does not require management as LLMW.
	TCA protein precipitation on filter paper	Waste consists of ~0.1 μCi/ml ^{14}C in 5% TCA	
14.	HPLC and FPLC with LSC	Uses ^{14}C labeled enzymes. LLMW constituents include isobutanol and hydrochloric acid along with buffers and LSC fluors.	Generates approximately one liter of LLMW per month. Waste separates into two phases; one organic and the other acidic.

Researcher Number	Process Descriptions	Previous Generation	Current Generation/ Waste Minimization
15.	Gel fixing, washing for DNA sequencing with autoradiography	Gel fix/wash had been one liter 10% methanol and of 10% acetic acid.	Currently does not fix/wash gels, but uses plastic sheet to avoid gel sticking problems with unwashed gel. Distrusts automatic gel sequencers. Would like larger plates and dryers. Has attempted to neutralize acid waste in lab, but considers it an unsafe operation.
16.	DNA sequencing	No longer generates RMW, but uses several hazardous compounds: ethydium bromide for staining in a gel -methanol and acetic acid for staining a protein gel with Coomassie Blue stain -phenol for extractions. Generates hazardous waste only and LLW only.	Changed to a labeled primer which is obtained from commercial suppliers (Lovestrand Labs of Gaithersberg attaches ^{32}P to primer DNA). Volumes and concentrations of radioactive waste are much smaller. Process does not generate LLMW. Uses chemiluminescence for blotting for a particular compound. (obtained from Tropix, Amersham, DuPont NEN). Considers chemiluminescence easier to read than autoradiography.

Researcher Number	Process Descriptions	Previous Generation	Current Generation/ Waste Minimization
17.	Cell culture	Multiple cell culture tubes, each generating approximately 25 ml of chloroform/aqueous wastes. LLMW generation is approximately 5 gallons with 7% chloroform every month.	No significant effort for waste minimization is currently employed. Potential exists to separate chloroform from aqueous waste, as waste jug has 2-phases. Potential for development of RIA procedure for melanin. Impediment to development and use is the 6 months to 1 year required for validation of alternate procedure, without guarantee of success.
18.	Gel washing/fixing, largest volume of waste from HPLC	Had generated large volumes of HPLC wastes. Disposal and management of HPLC waste was a nuisance. Occasional spills resulted if waste jug was not changed frequently. Laboratory now gathers as much data using radioimmunoassay technique and does not generate LLMW.	Currently generates little RMW, as research now focuses on 3 metabolites (down from 15, previously). Prefers enzyme immunoassay over radiographic and spectrophotometric methods. (Suppliers include Amersham, DuPont NEN, Advanced Magnetics, Serogen).
19.	Cell culture	Uses ^{3}H-thymidine in reverse transcriptase (RT) assay. Uses 10% TCA to stop reaction and 100% methanol rinse. Waste is estimated at 60% TCA and 40% methanol. TCA attacks cell harvester. Researchers must use 12 cell harvester, instead of 96 cell harvester. 10-15 gallons of waste from RT.	Use more modern 96 cell harvester to reduce volume of waste.
20.	Chloroform/phenol extractions; Western blots	5 gallons/month from chloroform/phenol extractions. Waste also included 10-20% methanol and 10% acetic acid.	Now uses gel that does not require fixing. Uses isotopically labeled DNA-probes that do not generate LLMW.