MOLECULES for HEALTH, Inc.

Cancer Drug Research & Development
16 North 22nd Street
Richmond, VA 23223
804 / 644-8591

September 24, 1985

U.S. Nuclear Regulatory Commission
Region II
101 Marietta Street, N.W.
Atlanta, GA 30323

REFERENCE: 50602
030-28706

Mail Control Number 50602

Gentlemen:

SUBJECT: ADDITIONAL INFORMATION CONCERNING A BYPRODUCT MATERIAL LICENSE APPLICATION (REFERENCE: 50602; 030-28706)

Molecules for Health, Inc. is a small biomedical firm dedicated to bringing some of its newly synthesized anti-tumor compounds into the clinician's arsenal for its fight against cancer. In this endeavor, low energy beta-emitting radioactive precursors are used in in vitro assays of enzyme systems associated with cell proliferation and tumor growth.

With respect to the Application for Byproducts Material License [3.P. All other specific byproduct material licenses], dated March 14, 1985, to authorize a small laboratory research program, the following additional information is provided, as requested on August 29, 1985:

1. EFFLUENT CONTROL.

"If you do not plan to conduct an air sampling program, you should provide information showing your evaluations made to determine that air sampling is not necessary for your program."

Information to show that effluent releases do not exceed established limits shown in Appendix B of 10 CFR 20:

Air sampling is not necessary for the applicant's program because the radionuclide effluents do not exceed the established limits in Appendix B of 10 CFR 20.

In the routine assay procedure for ribonucleotide reductase, the enzymatic reaction is:

Received 9/18/85
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Appendix B: Concentrations in Air and Water above Natural Background

Element (Atomic No.)	Isotope		Table 1 Col 1 Air (uCi/ml)	Table 1 Col 2 Water (uCi/ml)	Table 2 Col 1 Air uCi/ml)	Table 2 Col 2 Water uCi/ml)
Hydogen (1)	H^3	S	$5x10^{-6}$	$1x10^{-1}$	$2x10^{-7}$	$3x10^{-3}$
		I	$5x10^{-6}$	$1x10^{-1}$	$2x10^{-7}$	$3x10^{-3}$
		Sub	$5x10^{-3}$	...	$4x10^{-7}$	...
Carbon (6)	C^{14}	S	$4x10^{-6}$	$2x10^{-2}$	$1x10^{-7}$	$8x10^{-4}$
		Sub	$5x10^{-5}$	...	$1x10^{-7}$	...
Sulfur (16)	S^{35}	S	$3x10^{-7}$	$2x10^{-3}$	$9x10^{-9}$	$6x10^{-5}$
		I	$3x10^{-7}$	$8x10^{-3}$	$9x10^{-9}$	$3x10^{-4}$

1. Incubated in a stoppered vial using approximately 0.1 uCi of ^{3}-H-labeled nucleoside diphosphate (^{3}-Hcytidine-diphosphate), a non-volatile precursor, which is converted to ^{3}-H-labeled deoxynucleoside-diphosphate (^{3}-H-deoxycytidine-diphosphate), a non-volatile product. The ^{3}-H-labeled product is usually less than 1% of added precursor.
2. After incubation, the reaction mixture is acidified with either hydrochloric acid (HCl) or acetic acid (HAc) (both volatile acids) to terminate the enzyme reaction and is then heated at 70° for 20 minutes to aggregate the proteins in the enzyme mixture and hydrolyze the ^{3}-H-labeled nucleoside diphosphates to ^{3}-H-labeled nucleoside monophosphates.
3. From the acidified reaction mixture, the protein aggregate is centrifuged and the supernatant containing the ^{3}-H-labeled nucleoside monophosphates (precursors and products) is removed.
4. The acidified reaction is then applied to a Dowex-50 ion-exchange column for separation of ^{3}-H-deoxy-nucleoside monophosphate (product) from ^{3}-H-nucleoside phosphate (precursor). Eluent fractions are collected which are heated in the hood for removal of excess effluent (water containing volatile acid - HCl or Hac).
5. Since the ^{3}-H-labeled precursor (~99%) and the ^{3}-H-labeled product (~1%) are both non-volatile, these components remain in the fraction vials, whereas the excess acidified aqueous solution is evaporated and vented in the hood. The evaporation of excess eluent is carried out as a batch process, in which the volatile aqueous solvents from 10 vials at a time from

each column (assay) occurs over the course of 1-2 hours.

6. To the vials containing the column eluent fractions at a reduced volume (approximately 10% of the initial eluent volume), scintillation fluids and fluors for counting in the liquid scintillation counter are added.
7. Since the reaction precursors and products are counted, the amounts of ^{3}-H-labeled components are measured directly. With less than 1% of the ^{3}-H-labeled materials (precursors and products) lost during the protein aggregate step, all materials are contained and accounted for.
8. After counting, the liquid scintllation and fluors are collected in a carboy for disposal as organic waste and the ion-exchange resin is recycled for future assays.
9. Throughout the entire assay procedure, the ^{3}-H-labeled molecules are non-volatile and contained in aqueous mixtures within reaction vials, apparatus (ion-exchange columns) and counting vials. Thus, the use of the fume hood is for venting of excess acidified aqueous solvents and not for the venting of ^{3}-H-labeled gases or other ^{3}-H-labeled volatile materials. Emmissions of ^{3}-H-labeled materials to the atmosphere are less than $2x10^{-7}$ uCi/ml in the atmosphere and within the limits defined in Appendix B of 10 CFR 20.

Fume Hood Without Filters

As described above, the fume hood is primarily used for venting volatile solvents (either HCl or HAc acidified aqueous solvents) from the ion-exchange effluents which contain non-volatile ^{3}-H-labeled nucleoside monophosphates. Thus, the use of a hood equiped with filters for trapping volatile molecules which are labeled with ^{3}-Hydrogen are not required.

In reactions in which volatile organic compounds (e.g., ^{14}-C-CO_2) are anticipated, a trap containg alkaline absorbers is used as part of the reaction train apparatus.

In the synthesis of radionuclide-labeled organic materials used in the Grignard reactions, the reaction occurs in a closed system in vacuo with the necessary traps in place to protect the vacuum system from contamination. Again, the need for a fume hood with filters for trapping volatile molecules is not necessary because the potential volatile reactants or side-products are trapped by absorbers within the reaction train sequence.

As described in the laboratory survillance procedures in the application, the fume hood is a site to be swipe monitored. An

additional monitoring position will be added, if necessary, at the top of the hood adjacent to the venting oriface.

2. ALARA. Occupational radiation exposures as low as reasonably achievable.

The applicant for the By-Products Materials License is seeking to use low levels of the weak beta-emitting isotopes, Hydrogen-3 (0.0186 mev, half-life = 12.33 years), Carbon-14 (0.156 mev, half-life = 5730 years) and Sulfur-35 (0.167 mev, half-life = 87.2 days). Because the low energies associated with radiactive emissions, these isotopes may be efficiently detected by liquid scintillation counting. Thus, much of the research effort is expended in the detecting and quantitatively measuring the presence of low levels of radioactivity (< 0.001 uCi) in reactions contained within glass reaction vessels.

Radiation hazards from these three radionuclides are minimal as long as such materials remain external to the human body. Thus, precautions and training are prescribed for all persons undertaking work with these radionuclides to avoid any means of entry of reaction materials into the body by such routes as ingestion, inhalation or direct penetration of the skin barrier. Persons with breaks in the skin, particularly, are not permitted to undertake use of such materials until skin breaks are satisfactorily healed. Precautions, including the use of gloves, outer protective garments and remote handling devices (tongs, forceps, pipette bulbs) are recommended at all times.

Reactions in which release of radioactive materials is anticipated are undertaken with appropriate chemical traps as part of the reaction train. Thus, the lines of prevention of exposure to and release of radioactive materials to personnel and the atmosphere include:

1. Appropriate experimental design and review of apparatus
2. Appropriate experimental vessels, apparatus, materials and space and bench set-up with traps and containment trays and absorbent materials in place
3. Protective handling devices
4. Readily accessible emergency outlines, instructions and necessary hazard containment materials in place in case of accident or illness of the principal investigator

With good experimental design, knowledgeable and informed workers, and appropriate operating facilities and equipment, release of radiation is kept ALARA.

The use of the fume hood is primarily for venting of non-radioactive volatile materials and for operating in a safe manner with the added protection of experimental apparatus

contained on all sides possible. Under most conditions (if not all), the radionuclides which are employed are non-volatile.

3. BIOASSAYS

The applicant has requested a license limited to 50 milli curies of ^{3}-H-Hydrogen. However, it is not anticipated that more than 5 milli curies of ^{3}-H-Hydrogen (and most likely, less) will be in active use at any given time. The materials will be obtained from licensed commercial producers or distributors, usually as bio-organic materials in solution. Under most conditions, after an initial transfer is made from the primary container to the working container, the primary conatiner and its contents remain in storage. Because these materials are non-volatile bio-organic substrates for in vitro assays, biological hazard from radiation is minimal as long as the radionuclide-labeled materials do not enter the body.

In recognizing the need for consistent review by the U.S. Nuclear Regulatory Commission, a procedure will be instituted whereby in vitro assays for Hydrogen-3 of the workers' urine will be undertaken, if necessary, on a semi-annual or quarterly basis. The procedure will include the obtaining of the urine sample from each worker for Tritium Bioassay before initiating work under License for By-Product Materials and at quarterly intervals, thereafter, as practicable. The urine samples will be coded, processed in a suitable manner and prepared for assay by liquid scintillation counting using appropriate reference materials. Results will be maintained as part of the necessary log procedures.

4. RECEIPT AND OPENING OF PACKAGES

After a package containing radionuclide-labeled material has been received at the loading dock at 16 North 22nd Street, Richmond, VA 23223, receipt is logged and the package is relegated to a quanantine area. After notification to Molecules for Health, Inc., the material is released from the quarantine area to the Radiation Safety Officer, taken directly to the laboratory and wiped for surface (container) radioactivity. A count of the wipe will be made and logged as soon as practicable. Upon opening the package in the laboaratory, packing materials will be inspected visually, the surface of the inner container wiped and the material stored appropriately (usually in a locked refrigeratorfreezer). A count is be made and logged as soon as practicable.

Under most conditions (if not all), the received materials will fall under Section 20.205:

Procedures for picking up, receiving and opening packages (b)(i) Each licensee upon receipt of a package of radioactive material shall monitor the external surfaces of the package for radioactive contamination caused by leakage of the radioactive contents, except:

(ii) Packages containing no more than 10 millicuries of radioactive material containing solely Tritium, Carbon-14, Sulfur-35 or Iodine-125;

The monitoring shall be performed as soon as possible after receipt, but no later than three hours after the package is received at the licensee's normal working hours, or eighteen hours, if received after normal working hours.

We believe that all questions concerning the communication of August 29, 1985 are answered satisfactorily. If there are any further questions, please contact me directly at 804 / 229-6230 or Dr. Howard L. Elford at 804 / 644-8591.

We appreciate your expediting the review of this application and look forward to hearing from you soon.

Sincerely,

Paul L. Zubkoff

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