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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE
LARGE-SCALE REVIEW WORKING GROUP[1]

MINUTES OF MEETING

FEBRUARY 7, 1984

The Large-Scale Review Working Group of the Recombinant DNA Advisory Committee (RAC) was convened for its sixth meeting at 9:00 a.m. on February 7, 1984, in Building 31, Conference Room 6, at the National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20205. The meeting was open to the public. Dr. Robert McKinney (Chair) presided. The following were present for all or part of the meeting.

Working group members:

Manuel Barbeito	Robert McKinney
Morris Levin	Henry Miller
Kathryn Mahaffey	Sue Tolin
Gerald McGarrity	Elizabeth Milewski (Executive Secretary)

A committee roster is attached (Attachment I).

Ad hoc consultant:

Debra Hunt, Duke University

National Institutes of Health staff:

Emmett Barkley, OD
John Irwin, OD

Others:

Irene Brandt, Eli Lilly & Company
Theodore DeLoggio, Pennwalt Corporation
Gerry Elman, Biotechnology Law Report
Ernest Falke, Environmental Protection Agency
Jack Fowle, Environmental Protection Agency
Carol Gronbeck, Genentech, Inc.
Anne Hollander, Environmental Protection Agency

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[1]The Working Group is advisory to the RAC, and its recommendations should not be considered as final or accepted.

J. Hospodka, Wyeth Laboratories
James Jones, National Institute for Occupational Safety & Health
Roger Johnson, Genetic Engineering News
John Keene, Abbott Laboratories
Yoshibumi Komeda, University of Tokyo
Agger Langlykke, Genex Corporation
D. S. Mabry, Pfizer, Inc.
Max Marsh, Eli Lilly & Company
Kenneth Martinez, National Institute for Occupational Safety & Health
Charles Morin, Burditt & Calkins
William Muth, Eli Lilly & Company
Elio Passaglia, National Bureau of Standards
LaVaughn Queen, Biotechnology News
William Reed, Corn Products Company
Marvin Rogul
Mark Segal, Environmental Protection Agency
Joseph Van Houten, Schering-Plough Corporation
Patricia Williams, F-D-C Reports, Inc.
James Wu, Hoffman LaRoche, Inc.

Dr. Robert McKinney, Chair, called the meeting of the Large-Scale Review Working Group to order at 9:15 a.m., February 7, 1984. Dr. McKinney introduced the ad hoc consultant, Ms. Debra Hunt of the University of North Carolina to the working group. Dr. McKinney then recounted the history of the proposal which the RAC had remanded to the working group for consideration.

Dr. McKinney said Dr. Irving S. Johnson of Lilly Research Laboratories, a division of Eli Lilly and Company, proposed that procedures be modified for experiments involving more than 10 liters of culture of "exempt" organisms, i.e., those listed in Appendix C of the NIH Guidelines for Research Involving Recombinant DNA Molecules. Specifically, Dr. Johnson proposed the following two changes in the Guidelines:

1. Delete statements in all sections of Appendix C that refer to large-scale experiments, viz: "Large-scale experiments (e.g., more than 10 liters of culture) require prior IBC review and approval. (See Section III-B-5)."

2. Modify Section III-B-5 to read as follows:

   "III-B-5. Experiments With Non-Exempt Organisms Involving More Than 10 Liters of Culture. The appropriate containment will be decided by the IBC. Where appropriate, the large-scale containment recommendations of the NIH should be used (See Appendix K)."

Dr. McKinney said this proposal was reviewed by the RAC at its September 19, 1983, meeting. During the 30 day comment period (prior to the meeting) one comment was received from a RAC member; this comment was discussed by the RAC at the September 19 meeting. The comment encouraged the RAC to reject the proposal and to retain the current provisions of the Guidelines for the following reasons:

> "The current Guidelines provide adequate guidance for establishing containment levels and reflect practices appropriate to these levels. They provide institutions and the NIH the necessary degree of oversight for activities involving large-scale research or production with organisms containing recombinant DNA. This oversight is viewed as essential for activities in which the NIH is a participant. In addition, the experiments exempted under Appendix C of the Guidelines are recommended to be conducted under P1 physical containment conditions. This equates with P1-LS for research or production quantities in excess of 10 liters."

The RAC generally supported this position.

In response to this letter, Dr. Max Marsh of Eli Lilly and Company offered an alternative revision of the Guidelines (Attachment II) and suggested to the RAC that the issue be remanded to the Large-Scale Review Working Group for review. He specified two areas of large-scale operations that Eli Lilly and Company wished to see reviewed: (1) the types of filters used in the air exhaust system, and (2) handling of large-scale operations outside of the fermentors.

Dr. Max Marsh of Eli Lilly and Company told the working group Eli Lilly was proposing this modification as the organisms listed in Appendix C do not present significant risks to the operators or to the environment. Dr. Marsh said the cost of the equipment required to achieve complete containment, as currently required by Appendix K, is significantly higher than the cost of equipment normally used in fermentation. Thus, there is a processing and an economic advantage in not requiring absolute aerosol containment in large-scale operations using these organisms. He emphasized that each manufacturer would be liable for any effects associated with its product.

Dr. Marsh said the Eli Lilly and Company would use discretion in determining containment conditions. If a gene product were highly toxic, tight containment would be employed to protect the operators.

Dr. Marsh suggested that a fourth containment category, P1-Exempt Large-Scale (P1-ELS) might be introduced to apply to organisms listed in Appendix C. Alternatively language permitting some flexibility in the interpretation of P1-LS might be added to the Guidelines.

Dr. Marsh said the data Eli Lilly and Company had supplied in support of their proposal show that aerosolization of E. coli K-12 in a production atmosphere is minimal under deliberate spill conditions; e.g., there is no major contamination of the environment or the atmosphere in the vicinity of the production process.

Dr. Gerard McGarrity pointed out that these data were generated using an E. coli K-12 host-vector system. The proposal, however, requests modification of P1-LS conditions as applied to S. cerevisiae and asporogenic B. subtilis host-vector systems as well as to E. coli.

In addition, Dr. McGarrity thought the language of the proposal was vague. He preferred that the proposal specify the procedures to be modified.

Dr. Emmett Barkley of the NIH Division of Safety, who had helped to draft Appendix K, said P1-LS conditions were not intended to require absolute containment. Indeed, it was envisioned that under P1-LS viable materials would in some cases have to be processed outside of the primary fermentation vessel. For this reason, Appendix K-II-A states that the material shall be handled in a closed system or other primary containment equipment and the word "minimized" indicates that absolute containment is not required at that step. He felt the language describing P1-LS is flexible with the exception of the word "prevents" in Section K-II-D. Appendix K-II-D specifies that:

> "Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing recombinant DNA molecules to the environment."

Dr. Barkley thought use of the word "prevents" in Appendix K-II-D was inappropriate. Appendix K-II-D specifies that exhaust gases must be passed through a HEPA filter, and a HEPA filter is 99.97 per cent efficient for particles three-tenths of a micron or larger.

Dr. Barkley thought the efficiency limits for processing viable materials under P1-LS should be of the order of those obtained with laminar flow biologic safety cabinets and HEPA filters. Dr. Marsh said particular emphasis was placed in early RAC discussions on the requirement to inactivate the fermentation before transfer from primary containment. The term "primary containment" was interpreted to mean hard pipe connections between the fermentor and subsequent processing units, and centrifuges had to be contained.

Dr. McGarrity asked an Eli Lilly representative to describe the facilities meeting the P1-LS specification as compared to facilities used in conventional fermentations. Dr. Marsh replied that under P1-LS containment the entire fermentor system must be closed. The agitator seals must all be double sealed and operated under steam pressure. The lines must be welded or a validated pressure fitting sealing process must be used. Exhaust gases must be trapped, condensed and burned or passed through redundant filters.

Dr. Mahaffey asked if E. coli had been used in conventional fermentation processes. Dr. Marsh replied that several years ago Eli Lilly produced L-asparaginase through E. coli fermentation. At that time Eli Lilly used the employee protection procedures utilized in antibiotic production.

Dr. Mahaffey asked Dr. Marsh to describe the operator protection procedures designed by Eli Lilly for use under conventional operation conditions. Dr. Marsh replied that Eli Lilly and Company have operator training programs for every process. Training is dependent on (1) the particular process with which the operator is involved, and (2) the requirements of the process.

Dr. William Muth of Eli Lilly and Company said aerosols will be generated at two points inside the plant in conventional production facilities: (1) at the agitator seal, and (2) in the sampling process. A well-ventilated workplace appears to protect the worker as these aerosols are quickly diluted.

In the case of contained P1-LS fermentors, the agitator seal is very carefully constructed. The seal is a double mechanical seal with steam under pressure between the two seams. Samples are taken in a closed fashion at Eli Lilly facilities. Dr. Muth said aerosols also form out of the vent stack. If a thousand cubic feet of air passes into a fermentor, a thousand feet of air comes out; this exhaust air is vented out of the stack. At Eli Lilly air vented out of the fermentor passes through a series of four air treatments; the 10^{12} calculated reduction in organisms is cumulative across the four units. Each exhaust filter has a non-sterile side where condensate accumulates. Condensate also accumulates during the sample-taking procedure. These condensates are taken by hard pipe to a condensate collection tank. The condensate is sterilized before disposal.

Dr. McGarrity asked Dr. Muth to describe exhaust filtration procedures. Dr. Muth said most organizations use two filters, either a wet/dry filter or a dry only filter and one or the other combinations of these filters. Some organizations use a filter and an incinerator. Most facilities, however, employ a pair of sterilizing grade units in the exhaust system to handle the volume of air passed through a 10,000 gallon fermentor. Such filters represent a considerable expense. A Dominic-Hunter filter, for instance, consists of sixty 24-inch cartridges set in a large housing.

Dr. McKinney asked how much air would pass through such a filter. Dr. Muth replied that the amount of air depends on the age of the fermentation. Early in the process, less air is required; characteristically most fermentation plants would operate on a volume of air per volume of medium per minute. For a 10,000 gallon tank, that is approximately 1,500 to 2,500 standard cubic feet per minute (SCFM). Dr. McKinney observed that under such conditions, a large number of organisms could be discharged to the atmosphere. Dr. Muth said other processes are used in the exhaust line in addition to filters. At Eli Lilly the first process consists of a a mechanical coalescer which throws the air into a cyclonic flow. Large particles are dropped out and returned, sterilely, to the fermentor. Behind the coalescer, in series, is a coalescing filter which removes droplets. Together, the coalescer and coalescing filter remove 99.9% of the organisms. Dr. McKinney noted that this procedure is equivalent to the use of a HEPA filter.

Mr. Barbeito asked Dr. Muth whether Eli Lilly facilities are built for a specific purpose or whether they are multipurpose. Dr. Muth replied that each product is anticipated to have a specific plant. Mr. Barbeito said such use contrasts with use of a research laboratory; a laboratory will have multiple uses over a number of years. Mr. Barbeito asked if the data produced by Eli Lilly (Attachment II) were generated in an actual test run. Dr. Marsh replied the data were generated in a "dummy run," i.e., the host vector system without the recombinant gene was tested.

Dr. McGarrity said industry's primary concerns appear to be with the language of Appendix K-II-B and K-II-D. He asked if the problems encountered with this language were unique to Eli Lilly manufacturing procedures. Dr. Muth thought these issues were germaine to most production companies.

Dr. McKinney noted that some data on organism survival in aerosols and on surfaces had been sent to the working group for their information (Attachment III), and that Schering Corporation had supplied some additional information (Attachment IV). Dr. McKinney said Ms. Hunt would present additional data[2] on survival of recombinant DNA containing organisms in aerosols to the working group.

Ms. Hunt said that she had compared the aerosol survival of one recombinant E. coli host-vector system and a wild type E. coli at two relative humidities (45% and 70%) and room temperature. She had also compared one HV1 B. subtilis host-vector system.

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[2]Thesis presented as part of the requirements for Dr.PH at University of North Carolina School of Public Health, Chapel Hill, NC, May 1984, Debra Long Hunt.

The organisms tested included E. coli CS10 which is a wild-type strain, the EK-1 host system E. coli HB101, and strain HB101 with the pBR beta T plasmid (HB101-pBRβT). Plasmid pBRβT is a pBR-322 plasmid into which has been cloned, in the ampicillin resistance gene, DNA complementary to beta tubulin. The following B. subtilis strains were also tested: B. subtilis BR151, and BR151 with the plasmid pHV14 (BR151-pHV14). Plasmid pHV14 was constructed by ligating plasmid pBR322 with plasmid pC194. In B. subtilis hosts, the plasmid expresses chloramphenicol resistance.

Ms. Hunt said preliminary laboratory characterization of the E. coli organisms showed that HB101 and HB101-pBRβT require certain nutrients for replication on agar medium.

A second characteristic of HB101 and HB101-pBRβT was their decreased growth rate compared to the wild-type CS10 when the organisms were grown in a shaking water bath at 37 degrees Centigrade. Strains HB101 and HB101-pBRβT grew at a reduced rate and reached logarithmic phase later than wild type strain CS10. HB101-pBRβT grew at a reduced growth rate compared to HB101.

Ms. Hunt said E. coli HB101 contains a recombination-deficient mutation which results in increased sensitivity to ultraviolet light (UV). Strains HB101 and HB101-pBRβT demonstrated increased UV sensitivity compared to the wild-type E. coli CS10 strain with HB101-pBRβT demonstrating an even greater sensitivity to UV light than HB101.

Thus, results of preliminary tests indicated that HB101 and HB101-pBRβT are more debilitated in a laboratory environment than wild-type CS10. HB101-pBRβT demonstrated an even greater disadvantage than HB101 under these conditions.

Ms. Hunt described the aerosol test system. She said aerosols of the test organisms were dispersed within a static chamber consisting of a modified Hot Pack incubator. The chamber volume was 842 liters. Air of the desired temperature and humidity flowed through a fiberglass filter (FG-50) into the aerosol chamber. The air was dispersed through the aerosol chamber by a dispersion fan. When the desired temperature and humidity were attained within the aerosol chamber, the fan was turned off and all of the dampers closed.

A three-jet Collison nebulizer produced the test aerosol. The suspension fluid is drawn from the bottom of the reservoir and impacted onto the side of the glass jar. Large particles reflux back down into the reservoir while smaller particles are released through the spray outlet. Pre-trial particle sizing experiments revealed a mean particle size of 2.49 microns when organisms were aerosolized in their growth broths. This particle size is well within the respirable range, and it has been estimated that 97 percent of particles of 2.6 microns or less will contain zero to one organism per droplet. The aerosal was sampled by all glass impingers (AGI) at 5 time intervals within one hour.

Ms. Hunt said organisms contained within the air stream are impinged into a liquid collection medium. The sampling liquid is diluted and plated on agar medium.

Ms. Hunt said organism survival was calculated in percent biological recovery, a ratio of percent viable recovery and percent physical recovery. The fluorescent tracer uranine was added to the bacterial suspension before aerosolization as a control to calculate physical loss. The uranine was also collected in all glass impinger (AGI) samplers and analyzed by a fluorometer.

Ms. Hunt then presented data generated at 45 percent relative humidity and room temperature. She said samples of aerosolized organisms were taken at two, five, ten, and thirty minutes after aerosolization. As might be expected, the wild-type strain, E. coli CS10, survived better than both B. subtilis BR151 and E. coli HB101. B. subtilis BR151 and BR151-pHV14 survived aerosolization somewhat better than E. coli HB101 although this was not statistically significant. BR151 survived aerosolization better than BR151-pHV14.

At 45 percent humidity and room temperature, E. coli HB101 rapidly decreased within the first few minutes after aerosolization. In fact, no viable E. coli HB101 organisms were detected after five minutes following aerosolization. However, HB101-pBRβT survived better than HB101, with small but detectable levels of HB101-pBRβT observable after 30 minutes following aerosolization.

Ms. Hunt then presented data generated at 70 percent relative humidity and room temperature. At 70 percent relative humidity, there was no significant difference between the aerosol survival of the wild-type E. coli CS10 and HB101 for up to 30 minutes after aerosolization. However, E. coli HB101-pBRβT, survived significantly better than E. coli HB101 and wild-type E. coli CS10 for up to the 30 minutes after aerosolization.

Ms. Hunt hypothesized that perhaps pBRβT may code for excretion of beta tubulin to the periplasmic space, as the pBR-322 plasmid expresses ampicillin resistance by excretion of beta lactamase to the periplasmic space, and the cDNA encoding for beta tubulin is inserted within the ampicillin resistance gene. If beta tubulin is excreted to this space, its presence may protect the organism.

Alternatively she suggested that as pBR T expresses tetracycline resistance, the appearance of additional membrane proteins of 14,000, 17,000, and 34,000 daltons associated with tetracycline resistance may affect membrane structure and stability. A recent Russian study demonstrated increased aerosol survival of E. coli K-12 after transfer of the pSA-50 plasmid into the organism. The study[3] correlated increased survival to appearance in the outer membrane of two proteins of molecular weights of 70,000 and 80,000 daltons encoded by the plasmid.

Ms. Hunt said the E. coli strains were examined for retention of plasmid function after aerosolization at 70 percent relative humidity. Aerosolized E. coli HB101-pBRβT were sampled and grown on media either containing (tet^+)

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[3]Koniukhov, V. G., M. E. Liaiman, L. Likhoded, and L. A. Maltseva, 1982. Analysis of the basic protein fractions of the outer membrane in E. coli strains differing in survivability in air. Zh. Mikrobiol. Epidemiol. Immunobiol. 1:54-58. [English Abstract].

or lacking (tet^-) tetracycline. This procedure showed that a substantial portion of the bacteria had lost their functioning plasmids. However, at 45 percent humidity, no difference in numbers of colonies on tet^+ and tet^- media was seen. Ms. Hunt hypothesized that the effects of aerosolization at higher humidities, may affect either cellular processes that specifically inhibit the tetracycline-resistant mechanisms, or damage the plasmid itself either by excision of the gene segments or by replication errors. At low relative humidities the decrease in organism survival due to dehydration may be so rapid that other effects are masked.

At 70 percent relative humidity, no difference in survival between Bacillus subtilis BR151 or BR151-pHV14 is seen. Both strains rapidly decreased 99 percent in survival within five minutes of aerosolization.

In summary, bacterial survival in aerosols under different environmental conditions may vary depending on specific organism characteristics. These characteristics should be considered in developing control measures.

Ms. Hunt said the unexpectedly high survival of E. coli HB 101-pBRβT at 70 percent relative humidity necessitates evaluation of survival advantages conferred upon the host by each specific recombinant plasmid. Such evaluations would not only provide information for designing proper environmental control measures, but might be useful to those interested in increasing airborn stability, such as producers of bacterial pesticides.

Following Ms. Hunt's presentation, Dr. McGarrity asked Dr. Muth how the treatment of exhaust gases in conventional fermentations differs from exhaust gas treatment in fermentations using recombinant organisms. Dr. Muth replied that in conventional fermentations there are no filters in the exhaust lines. Dr. Tolin asked Dr. Muth if he knew the relative humidity in the exhaust lines. Dr. Muth said he suspected the relative humidity is 100%. He said Eli Lilly performs the first two exhaust gas treatments at ambient temperature. The temperature is then raised above the dew point for passage through the dry filters.

Drs. Levin, McKinney, and Tolin agreed the word "prevents" in Appendix K-II-D is too restrictive for work with the organisms listed in Appendix C. Dr. McKinney said that the word is inappropriate in that the efficiency of HEPA filters is 99.97 per cent for particles three-tenths of a micron or larger.

Dr. Miller offered the following proposals:

1. that the word "minimizes" be substituted for the word "prevents" in Appendix K-II-D, and

2. that a statement of the following type be addressed to the RAC:

   "The Guidelines should be amended to explicitly permit latitude by the IBC in interpreting Appendix K-II, A through F. Such a decision by the IBC should be based on data on the given host-vector combination to be fermented. This alteration in the Guidelines may be accomplished by amending Appendix C-II, III, IV or Appendix K."

Dr. Tolin suggested that Appendix C be modified: Appendix C currently applies to laboratory experiments. She suggested the second paragraph of Appendix C-II, C-III and C-IV might read:

> "For these exempt experiments, P1 physical containment conditions are recommended; for large-scale experiments, P1-LS is recommended."

Dr. Levin felt the Eli Lilly proposal was vague and that if any language is modified, the working group should specify the minimum amount of data the IBC would review. Dr. Miller said the organisms listed in Appendix C are benign, and the procedures employing them are subject to the regulatory reviews of agencies such as OSHA, FDA, etc.

Dr. Levin suggested that the data submitted to the IBC should be equivalent to the data presented to the working group. Dr. Miller disagreed; he thought the conditions would vary from case to case. Dr. McKinney felt the duties and obligations of the IBCs are defined in the Guidelines.

Dr. Tolin refined her proposed language to read:

> "For these exempt experiments in the laboratory, P1 physical containment conditions are recommended. For large-scale fermentation experiments with exempt organisms, P1-LS physical containment conditions are recommended. However, following review by the IBC of appropriate survival data for the particular host-vector systems, certain alternatives may be recommended."

Dr. Miller suggested that the phrase "by the IBC" be deleted from the proposed language. Dr. McKinney asked if the proposal should limit the type of data reviewed to "survival" data. Dr. Miller suggested that if the modifier "survival" is used before "data" the phrase should read "survival and other data." He felt data characterizing the organism were also important. Dr. Tolin agreed.

Dr. McGarrity felt any proposed modifications should be introduced into Sections Appendix K-II-B and K-II-D rather than into Appendix C.

Dr. Tolin moved that the following new paragraph be added to Appendix C-II, C-III, and C-IV:

> "For large-scale fermentation experiments, P1-LS physical containment conditions are recommended. However, following a review by the IBC of appropriate data for the host-vector system, some latitude in the application of P1-LS requirements outlined in Appendix K-II-A through K-II-F is permitted, provided operator exposure and environmental losses are minimized."

Dr. Muth asked if a reference to this language should be included in Appendix K. Dr. McKinney suggested that a reference be added to the end of Appendix K-I. Dr. Tolin suggested the following sentence be added to the end of Appendix K-I:

"The P1-LS level is recommended for experiments involving exempt host-vector systems described in Appendix C."

Dr. Milewski suggested that the language might read:

"For experiments involving exempt host-vector systems, see Appendix C."

She suggested a second possibility would be to add the phrase "see Appendix C" after the third sentence of Appendix K-I. Drs. McKinney and Tolin agreed.

Dr. Tolin then read her three part motion:

1. The second paragraph of Appendix C-II, Experiments Involving E. coli K-12 Host-Vector Systems; Appendix C-III, Experiments Involving Saccharomyces cerevisiae Host-Vector Systems; and Appendix C-IV, Experiments Involving Bacillus subtilis Host-Vector Systems; would read as follows:

   "For these exempt laboratory experiments, P1 physical containment conditions are recommended."

2. A paragraph would be added following the second paragraph of Appendix C-II, Appendix C-II-I, and Appendix C-IV. That paragraph would read as follows:

   "For large-scale fermentation experiments P1-LS physical containment conditions are recommended. However, following review by the IBC of appropriate data for a particular host-vector system, some latitude in the application of P1-LS requirements as outlined in Appendix K-II-A through K-II-F is permitted provided operator exposure and environmental losses are minimized."

3. Additional language would be added to the fourth sentence of Appendix K-I, Selection of Physical Containment Levels. That sentence would read as follows:

   "The P1-LS level of physical containment is required for large-scale research or production of viable organisms containing recombinant DNA molecules which require P1 containment at the laboratory scale (See Appendix C)."

Mr. Barbeito suggested the word "personnel" be substituted for "operator." Personnel would encompass all workers in the facility as well as people walking in the street. Dr. Muth said any individual working in the Eli Lilly production area has operator training; these individuals might include maintenance, housekeeping personnel, people who transport materials, etc. Dr. Tolin accepted Mr. Barbeito's proposed modification.

Dr. McKinney suggested that the phrase "personnel and environmental exposures be minimized" be substituted for the phrase "operator exposure and environmental losses are minimized. Dr. Levin questioned the definition of "minimized."

Dr. McGarrity asked what constituted "environmental loss." Dr. Muth said environmental loss referred primarily to aerosols; it could occur at exhaust vents or when materials are transferred from vessel to vessel. Dr. Tolin said she would delete the phrase "provided personnel and environmental exposures are minimized" from her proposed motion.

Dr. Mahaffey expressed concern about the protection afforded operators should the word "personnel" be deleted.

Dr. Miller seconded Dr. Tolin's motion. Dr. McGarrity called the question. Dr. McKinney reminded the group that any language accepted by the working group would be a recommendation to the RAC. The language would be published in the Federal Register, considered by RAC and acted on by the NIH. By a vote of six in favor, none opposed and no abstentions, the working group accepted Dr. Tolin's motion which reads as follows:

"1. The second paragraph of Appendix C-II, Experiments Involving E. coli K-12 Host Vector Systems; Appendix C-III, Experiments Involving Saccharomyces cerevisiae Host-Vector Systems; and Appendix C-IV, Experiments Involving Bacillus subtilis Host-Vector Systems; would be modified to read as follows:

'For these exempt laboratory experiments, P1 physical containment conditions are recommended.'

"2. A paragraph would be added following the second paragraph of Appendix C-II, Appendix C-III, and Appendix C-IV. That paragraph would read as follows:

'For large-scale fermentation experiments P1-LS physical containment conditions are recommended. However, following review by the IBC of appropriate data for a particular host-vector system, some latitude in the application of P1-LS requirements as outline in Appendix K-II-A through K-II-F is permitted.'

"3. Additional language would be added to the fourth sentence of Appendix K-I, Selection of Physical Containment Levels. That sentence would read as follows:

'The P1-LS level of physical containment is required for large-scale research or production of viable organisms containing recombinant DNA molecules which require P1 containment at the laboratory scale (See Appendix C).'"

Dr. Miller then moved that the word "minimizes" be substituted for the word "prevents" in Appendix K-II-D of Appendix K-II, P1-LS Level. Dr. Miller interpreted minimize to mean the greatest efficiency obtainable by a recognized procedure. Dr. McGarrity seconded the motion.

Dr. McKinney thought this motion recognized the fact that the HEPA filter does not absolutely prevent release of organisms into the environment.

Dr. Tolin asked if Appendix K-III-D and K-IV-D should also be so modified. Dr. McKinney felt the concept "prevent" should be operative in these sections which apply to P2-LS and P3-LS; through the combined use of HEPA filters and incinerators release can be prevented.

By a vote of four in favor, two opposed, and no abstentions, the working group recommended the proposed modification be published in the Federal Register and forwarded to RAC for consideration.

"Section Appendix K-II-D would read:

> 'Appendix K-II-D. Exhaust gases removed from a closed system or other primary containment shall be treated by filters which have efficiencies equivalent to HEPA filters or by other equivalent procedures (e.g., incineration) to minimize release of viable organisms containing recombinant DNA molecules to the environment.'"

Dr. McKinney adjourned the sixth meeting of the Large-Scale Review Working Group at 12:10 p.m. on February 7, 1984.

Respectively submitted,

May 9, 1984
Date

Elizabeth Milewski
Elizabeth Milewski, Ph.D.
Executive Secretary

May 14, 1984
Date

Robert W. McKinney
Robert W. McKinney, Ph.D.
Chair
Large Scale Review Working Group
Recombinant DNA Advisory Committee

I hereby certify that, to the best of my knowledge, the foregoing Minutes are accurate and complete

RECOMBINANT DNA ADVISORY COMMITTEE

LARGE-SCALE REVIEW WORKING GROUP

CHAIR

McKINNEY, Robert W., Ph.D.
Occupational Safety and
Health Branch, 13/3K04
Division of Safety
National Institutes of Health
Bethesda, Maryland 20205
301 496-2960

BARBEITO, Manuel
Occupational Safety and
Health Branch, 13/3E85
Division of Safety
National Institutes of Health
Bethesda, Maryland 20205
301 496-2346

FOWLE, John R., III, Ph.D.
Office of Research & Development,
RD-689
Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460
202 382-7335

LEVIN, Morris, Ph.D.
Innovative Research Program, RD 675
Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460
202 382-2575

McGARRITY, Gerard J., Ph.D.
Department of Microbiology
Institute for Medical Research
Copewood Street
Camden, New Jersey 08103
609 966-7377

MILLER, Henry I., M.D.
Office of Biologics
Food and Drug Administration, HFN-823
8800 Rockville Pike
Bethesda, Maryland 20205
301 443-4064

MILLS, Mark C., M.D.
Department of Pathology
Good Samaritan Hospital
Vincennes, Indiana 47591
812 882-5220

RICHARDSON, John, Ph.D.
MPH Program
Emery School of Medicine
1518 Clifton Road
Atlanta, Georgia 30322
404 329-7806

TOLIN, Sue, Ph.D.
Department of Plant Pathology
and Physiology
VA Polytech Institute
Blacksburg, Virginia 24061
703 961-5800

WEST, David, Ph.D.
National Institute for
Occupational Safety & Health
5600 Fishers Lane, Mail Stop 8A53
Rockville, Maryland 20857
301 443-2100

MILEWSKI, Elizabeth, Ph.D. (Executive Secretary)
Office of Recombinant
DNA Activities, 31/3B10
National Institute of Allergy
and Infectious Diseases
Bethesda, Maryland 20205
301 496-6051

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