

Dr. Milewski thanked everyone for participating. She directed the attention of the ad hoc working group to the memorandum dated February 25, 1981 (Attachment I). This language had been drafted following the February 23, 1981 telephone conference call. She said Dr. Gill had suggested some specific language changes and asked him to read those changes to the ad hoc working group. Some discussion concerning these specific changes as well as other modifications arose. These included: (1) In the proposed language for Section I-D-2, unambiguous language for defining lethality or potent was sought. The group agreed that such unambiguous language was difficult to compose but agreed on the following:

"Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins lethal for vertebrates at an LD<sub>50</sub> of less than 100 nanograms per kilogram body weight..."

(2) The abbreviation "etc." in proposed Sections a), b), c), and I-D-2 was redundant and added little in additional information. The group agreed to delete the abbreviation, "etc." (3) Dr. Milewski asked the group if it would be appropriate to explicitly state those toxins known to fall into various categories. She said this would save ORDA telephone calls. The group agreed. (4) Dr. Maas suggested that several heat stable E. coli enterotoxins, in addition to STI and STII, are known. Further, he said differences in nomenclature for these proteins exist. He suggested that reference to STI and STII be deleted from the proposed language. The group agreed. (5) Drs. Gill and Maas suggested that the language related to enterotoxins be modified slightly as monospecific antiserum for cholera toxin will not precipitate the heat stable

enterotoxins of E. coli and of Y. enterocolitica. These five editorial changes were incorporated into an amended document (Attachment II).

The ad hoc working group also determined that polypeptide snake and insect venoms could be covered under this document. Dr. Gottesman suggested that these toxins be added to the toxin list in the future.

Dr. Milewski asked the group to comment on the draft language of the risk assessment request (Attachment III). Dr. Maas asked if 50 milligrams of toxin was the amount which would be produced should the human gut be totally colonized by E. coli devoting one-third of its protein producing capacity to synthesizing the toxin in question. Dr. Gill replied that 50 milligrams was the "worst case" production estimate. He said the proposal was intended to determine risk to the gut and to the whole animal from toxins introduced into the gut. He noted that little data has been accumulated in this area. He asked whether, as an example, diphtheria toxin will necrotize the gut and might subsequently pass into the bloodstream. The proposed protocol attempts to address such concerns.

Dr. Levine said he thought the risk assessment proposal was incomplete; he felt the protocol should address the question of whether E. coli expressing a toxin might present a potential hazard should it colonize the gut. In addition he suggested that piglets should be the experimental animal of choice as E. coli is a normal component of the pig gut flora. E. coli is not, in contrast, a normal component of the gut flora of mice and rats. He said experiments which utilize mice and rats thus may not have any relevance to human gut physiology.

Dr. Gottesman asked if the proposed risk assessment protocol might contribute to knowledge in this area. Dr. Gill said the proposal asked how dangerous pharmacologically a toxin is to the gut. If the maximum toxin dose has no effect on the gut or the animal, then cases where lesser amounts are produced would theoretically not be considered hazardous. Dr. Gottesman said the usefulness of the proposal depends on whether any situation involving expression of a toxin by a host-vector system might be worse than the effect of the toxin alone.

Dr. Milewski said that the proposal as it currently stands might be handled intramurally at the NIH; an RFP or an RFA would not be required. Dr. Gottesman suggested that if the ad hoc working group saw some value in the proposal (Attachment III), experiments might proceed intramurally while proposals reflecting Dr. Levine's concerns were developed. Dr. Maas suggested that the experiments Dr. Levine had suggested might be incorporated into the RFA developed from the Pasadena Risk Assessment Meeting. Dr. Talbot said that the RFA might technically be amended to cover these requests.

Dr. Gill asked Dr. Levine which sites in the gut should be tested for toxin effect. Dr. Levine suggested that the upper bowel, the lower bowel and the jejunum should be tested.

Dr. Milewski asked the group if the draft proposal should be further polished. She asked Dr. Gill and Dr. Levine if they would be willing to develop the proposal further. They replied they would.