MEMORANDUM

DATE: 17 December 1992

TO: Bruce Burlington, M.D.

HFD-02-02

FROM: Murray M. Lumpkin, M.D.

Director

Division of Anti-Infective Drug Products

HFD-520

cc:

RE: The tomatoes that will eat Akron

Bruce:

You really need to read this consult. The Division comes down fairly squarely against the kan gene marker in the genetically engineered tomatoes. I know this could have serious ramifications. If you are uncomfortable or if you feel we need more input, please let me know. As this is an area no-one has great expertise in, I am more than happy to get as many opinions as we need on this.

Take care

Mac

MEDICAL OFFICER'S SUMMARY OF CONSULTATION, MEETING SYNOPSIS, and FINAL COMMENTS

CONSULT FROM:

James H. Maryanski, PhD

CFSAN Biotechnology Coordinator, HFF-300

Office of Compliance

&

Eric Flamm, PhD

Office of Biotechnology, HF-6

SUBMISSIONS:

Original Consultation dated 9/30/92

Follow-up Data Submission dated 11/13/92

(Both submissions contained material from Calgene, Inc. with reference to the FLAVR

SAVR® Tomato)

MEETING DATE:

November 4, 1992

ATTENDEES:

James Maryanski, Office of Compliance Eric Flamm, Office of Biotechnology Linda Ann Sherman, ODEII, DAIDP Albert Sheldon, ODEII, DAIDP

Renata Albrecht, ODEII, DAIDP

Maureen Dillon-Parker, ODEII, DAIDP

CONSULT COMPLETED:

December 3, 1992

BACKGROUND:

The initial request to the Division was to comment on the safety of use of the kanamycin resistance marker (kan) gene in agricultural products intended for human and animal consumption. Although the consultation was in reference to the Calgene product, FLAVR SAVR* tomato; the concept has broader implications. No food or feed products currently contain the marker gene; however, other sponsors plan the use of the kan marker in their foodstuffs.

The FLAVR SAVR® tomato is a variety of tomato that has been modified by anti-sense technology to reduce endogenous tomato polygalacturonase activity and thus slow fruit ripening and softening. To mark the modified DNA, a gene marker (kan¹) has also been inserted. The kan¹ gene product is a protein (enzyme), APH(3')II, that when phosphorylated can inactivate kanamycin or neomycin.

The questions posed were as follows:

 What aminoglycoside antibiotics are administered orally whose efficacy might be impaired by APH(3')II? Are only kanamycin and neomycin of concern?

 Should the sponsor provide evidence that ATP levels in the stomach would not be sufficient to allow significant inactivation of orally administered susceptible antibiotics?

The concern for exposure to the enzyme was raised by Dr. Olempska-Beer in her memorandum dated May 3, 1991. The FDA reviewer calculated, based on an average per capita intake of tomato solids, exposure to APH(3')II protein would be 48 µg/person/day (or 0.8 µg/kg BW/d for a 60 kg person). Background exposure to the protein from kanamycin-resistant bacteria already present in the environment was estimated to be considerably less i.e. 2.8 x 10 µg/person/day (or 5 x 10 µg/kg BW/d for a 60 kg person). Because the kan marker gene will be used as a selectable marker in many future genetically engineered plants, a gradual increase in exposure to APH(3')II protein is anticipated. The reviewer also stated that because the increase in APH(3')II protein could be substantial; the enzyme should be considered as a new constituent of the human diet and safety considerations should be addressed.

In response to an FDA request for more information, Calgene presented written arguments (May 31, 1992) to support the following:

 APH(3')II is degraded under simulated human gastric and intestinal conditions.

APH(3')II does not compromise efficacy of kanamycin.

- APH(3')II was shown not to have significant homology with known toxins.
- APH(3')II was shown not to have significant homology with known allergens.

The kan gene has been used safely in human gene therapy.

6. An acute toxicity study in rats exposed to modified tomatoes demonstrated no pathological abnormalities post mortem.
A review of this data was completed by Dr. Carl Johnson, of the additives evaluation branch. The data submitted was limited;

however, no major inaccuracies were noted. MEETING SUMMARY:

Drs. Maryanski and Flamm initiated the discussion by summarizing the potential risks of the kan gene product, APH(3')II protein, to patients receiving oral antibiotic therapy. Calgene has submitted data that support the findings that APH(3')II is inactive in acidic environments and is substantially degraded in simulated gastric and intestinal fluids. The risk to the achlorhydric patient may be theoretically valid, but was considered by the discussants as a whole to be practically inconsequential. In practice, oral aminoglycosides are administered to either pre-operative (NPO)

patients or patients with hepatic encephalopathy. Neither patient population would be ingesting foodstuffs; therefore the risk of inactivating the oral antibiotic drug is essentially a moot point. In sum, the collective opinion of the DAIDP's representatives was that the health risk of the APH(3')II protein product of the kangene was negligible.

The Division representatives expressed that their main concern focussed on the gene itself. Concern was expressed that the endogenous bacterial population could be transformed by the insertion of the kan gene. Kanamycin resistance in the intestinal flora would be a health hazard. The kan protein has homology with other aminoglycoside transferases such as amikacin. The presence of these genes in commensal intestinal bacteria could have farreaching implications with respect to antimicrobial treatment of patients and in particular, the immunosuppressed patient. Flamm and Maryanski stated that the gene transfer from the eucaryotic tomato plant genome to the procaryotic bacterial genome Even if the gene transfer were successful, in was improbable. order to have expression of the gene product i.e. the enzyme; the bacterial genome would require a procaryotic promotor region. The kanamycin-resistance gene originated in a prokaryotic system, i.e. a plasmid; can it be assumed that the prokaryotic promotor region is not available?

The documents from the sponsor state that food already is colonized with bacteria containing the kanamycin resistance gene. This accounts for the background exposure of individuals to the APH(3')II protein. By ingesting the genetically engineered foodstuffs and thus increasing the background exposure of the kanamycin resistant gene manyfold, are we creating a selective pressure to induce natural transformation of bacteria?

The sponsor, Calgene, submitted a preliminary report of an acute toxicity study completed in rats fed the tomatoes by gavage. The preliminary report stated that there were no apparent pathological changes in the animals. It is unfortunate that the intestinal flora of the rats was apparently not monitored for the appearance of the kanamycin resistance gene.

The meeting concluded. The following recommendations were made:

- In addition to the consideration of the safety of the gene product, APH(3')II; careful evaluation should also be made of the possibility of gene incorporation in the genomes of the intestinal micro-organisms.
- The potential health hazard risk of the gene product, APH(3')II, is probably negligible in this scenario.
- 3. The sponsor should provide information with regard to whether

the bacterial promotor for the kan gene is present on the T-DNA region.

A brief study examining the effect of ingestion of genetically modified tomatoes on the intestinal flora of animals or man might be encouraged.

Another avenue to explore is the possibility of using another gene marker that has no clinical implications.

RESPONSE TO THE DISCUSSION:

On November 13, 1992, Dr. Flamm assembled information from the Calgene submission which addressed the theoretical transformation frequency from ingested tomatoes with the kan gene. (November 26, 1990: Request for Advisory Opinion on the Kan Gene Safety and Use in the Production of Genetically Engineered Plants, Docket no. 90A-0416). The author of the sponsor's document presents clinical assumptions which are not entirely valid. However, these statements do not impact on the final conclusions determined by the Division of Anti-Infective Drug Products and will not be discussed in this document. The bulk of the submission deals with a model that addresses the potential uptake and expression of the kan gene in humans consuming genetically engineered fresh tomatoes.

The survival and possible expression of naked DNA (from disrupted cells) has been the subject of several papers. These papers were summarized in the Calgene paper. The conclusions are presented as follows:

Curtiss mixed extracted chromosomal and plasmid DNA from E. coli with diluted extracts of rat intestinal contents and found the subsequent degradation rapid enough to state: "in vivo transmission of naked recombinant DNA in the rat intestinal tract [is] highly improbable..." (page 000174, Calgene submission)

While nucleic acids are for the most part unaffected by proteolytic gastric enzymes, acid pHs of 3.0 or less result in extensive depurination of the DNA rendering it unusable as a template and destroying its biological potential...(page 000174-5, Calgene submission)

It is generally assumed, therefore, that the DNA humans consume (in the form of animal and plant food eaten) is degraded in the gastrointestinal tract to the point that the probability of a functional gene sequence surviving intestinal digestion may be considered to be zero...

Using the model that Calgene has developed, it is estimated that

after arrival of the disrupted tomato in the proximal intestinal tract, less than 0.1% of the ingested kan gene will remain intact for potential transformation of the intestinal microorganisms.

Another theoretical argument expressed by the sponsor is that the introduction of eukaryotic DNA sequences (from the tomato) into prokaryotic (bacterial) chromosomes are unlikely to "match" the prokaryotic sequences with which they would combine. Even if successful, gene expression may not occur or may be poor.

Using the mathematical model, the sponsor estimates that at the highest average daily consumption of modified tomatoes; there would be at most 2.6 x 10 transformed bacteria per person.

In addition, the sponsor reports that the human intestinal microflora already has a substantial population of organisms with kanamycin resistance. The sponsor discusses a paper in which 75% of 34,321 clinical isolates of Streptococcus faecalis were resistant to kanamycin. However, it is not clear from this statement whether the sponsor clarified that the mechanism of kanamycin resistance in this study was the same as that which occurs with the kan^f resistance gene.

The sponsor concludes with the following statements:

Selectable marker genes from genetically engineered food products might potentially represent a new, but certainly unique source of antibiotic resistance in Substantial levels of gastrointestinal microflora. background antibiotic resistance have been noted in clinical isolates and fecal samples from humans. addition, significant numbers of antibiotic resistant bacteria are present on the food we eat, particularly on raw vegetables...For all of these reasons it has been concluded that any contribution to the existing populations of antibiotic resistant bacteria that might be made by organisms resulting from transformation of human gut microorganisms to kanamycin resistance by the kan gene present in genetically engineered tomatoes is insignificant. (page 000185-6, Calgene submission)

If the mathematical model and supporting data are valid, then the arguments presented by the sponsor, Calgene are persuasive.

CONCLUSIONS AND RECOMMENDATIONS:

 The major issue of concern from a clinical standpoint is the introduction of the gene kan^f into significant numbers of



micro-organisms in the general population of human microflora. IT WOULD BE A SERIOUS HEALTH HAZARD TO INTRODUCE A GENE THAT CODES FOR ANTIBIOTIC RESISTANCE INTO THE NORMAL FLORA OF THE GENERAL POPULATION. The Division is aware that kanamycin resistance exists; however, in the hospital population or in immunocompromised hosts, micro-organisms do not remain isolated to the gut microflora.

- The arguments proposed by the sponsor with regard to the 2. degradation of the protein product and the naked DNA during transit in the alimentary canal are plausible. It behooves the sponsor to demonstrate these arguments as fact.
- The sponsor should consider a brief controlled animal study designed to determine the rate of transformation in the 3. intestinal microflora after a dietary challenge of gene modified food. Levels of statistical significance would have to be established. The ability to detect change would be facilitated by the presence of a control group.
- The sponsor should also consider implementing a program of post-marketing surveillance, similar to a Phase IV drug safety surveillance, to monitor for increases in frequency in the kanamycin resistance gene.
- The sponsor should address the presence or absence of a bacterial promotor region for kan in the t-DNA region. 5.
- Finally, the sponsor should seek an alternative gene marker, one that does not involve antibiotics used in human therapy. Although there is, at present, no proof that the introduction of the kan gene in the tomato genome will result in widespread bacterial incorporation of the resistance gene, the potential risk of this happening would have enormous implications.

We hope that this memorandum will be helpful to you. If there are further questions, please do not hesitate to contact us.

Jude and Jelymeen Linda Ann Sherman, MS, MD

cc: Original CONSULT

HFD 520

HFD 224

HFD 520/SHERMAN

HFD 520/SHELDON

HFP2/528/MDILLQNE/PARKER

Concurrence Only:

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