

## Memorandum

Date

October 27, 1993

From

Director, Office of Special Research Skills (HFS-500)

Subject

Response to Calgene Amended Petition

To

See Below

## Comments on the Submission:

The overall case in Calgene's response is made better than in the original submission. The sponsor has tried to answer our questions seriously and presented his arguments in a more comprehensive fashion. But very little new information has been added.

The one new argument presented in Vol 1 of Calgene's response is based on the historical control data. Here I believe the sponsor overstates his case. The historical control data he provides consists of two groups of studies (1) - studies previously conducted at IRDC laboratories in the same strain of rat (Sprague - Dawley CD) and (2) - studies previously conducted at Hazleton (HLA) laboratories for P&G in the same strain of rat.

The (IRDC) studies consist of 17 individual control groups, both males and females. In the males all 17 of these groups were free of erosions while in the females 16 groups were free from erosions and one group had 2/10 animals (20%) with erosions.

The HLA studies consist of 22 control groups, also both males and females. In the males 21 of the groups were free from erosions while one group had 2/10 animals (20%) with erosions. In the females 18 groups were free from erosions while four of the groups had incidences of: 3/10 (30%); 2/10 (20%); 4/10 (40%) and 1/10 (10%).

Of the two groups of historical data the most relevant to the Calgene study is the IRDC data. This is because the laboratory is the same as in the original Calgene submission and variables in animal husbandry and treatment can be introduced in different laboratories. The HLA group of historical controls is not without interest, but all things being equal the IRDC group has to be given greater weight.

In summary: the IRDC data reported 1/17 studies or 2/265 animals (females) with erosions and 16/17 studies or 263/265 animals without erosions. This is hardly strong evidence that gastric erosions are random and highly variable, taken alone this control data suggests that these gastric erosions are rare. The second group of data (the HLA controls) is more supportive of variability with 4 of 22 studies with erosions or 10/191 animals (females) with erosions and 18/22 studies or 181/191 animals without erosions.

## Comments on the Cause of the Erosions

The sponsor says the erosions "occur spontaneously in a random manner and at a highly variable incidence as demonstrated by historical data."

This is at least a single explanation. Originally the sponsor also attributed the erosions to the stress possibly induced by fasting. But he now indirectly includes a reference to a paper by Higaki and Kowa, Pharmacometrics 8: 157-169 (1974) which reportedly states that a three day fast is needed to produce gastric erosions in the rat. (page 000060). This 72 hour fast is far longer than any of the rats in the current studies experienced. So in essence the sponsor admits that no cause for the erosions is established and instead he argues that they are spurious and unrelated to treatment.

I've read the Higaki and Kowa paper and in fact it offers some indirect evidence that the fasting may indeed be responsible for the appearance of the erosions. It is surprising to me that the sponsors did not mention these findings in detail and furthermore it is puzzling why they accepted an unhelpful and a misleading characterization of these findings.

The aim of the Higaki paper was to study the relationship between gastric acid secretion and fasting of the rat. The pathology was limited to the qualitative reporting of ulcers after 2 - 7 day fasting. No effort was made to report on the formation of erosions within a one to two day period. So the fact that no early erosions were found in the Higaki and Kowa study is not surprising. But it is not accurate to say that it takes at least a three day fast to produce an erosion; the paper was talking about ulcers not erosions. Erosions must certainly be produced sooner but the authors did not look for them. However, since the ulcers are the result of the gastric secretion and erosions are very likely to be their preliminary indications, information bearing on acid secretion is relevant. In the paper the authors report:

- That maintenance of normal body temperature is necessary for obtaining stable gastric secretion in the rat. Also stomach contents affect the extent of gastric secretion.
- Gastric secretion in the rat is hormonally dependent and females were more sensitive to Aoc-tetrapeptide-stimulated gastric secretion than males.

These observations suggest a causal mechanism for the erosions (stressinduced gastric secretion) and are at least consistent with the variable nature of the findings and also the greater sensitivity of female rats to the reported erosions.

## Overall Evaluation of the Calgene Data

The overall evidence cited by the sponsor for his conclusion that the erosions are spurious are:

- Lack of reproducibility and consistency in the findings.
- (2) No differences in the morphology and severity of the gastric lesions among treated and control groups.
- (3) No increase in incidence or severity of the lesions when the dose of the tomatoes was doubled.
- (4) Comparability of the findings to historical controls.

I have listed the sponsor's arguments in the order that they seem strongest to me. This historical control data is not very convincing and that is why I've listed it last in the list of supporting evidence for safety.

Overall their position that the studies do not show harm is strong. I don't believe that the data show either that gastric erosions are consistently found or that they are likely to be reproduced or that they are caused by treatment. The data merely indicate the possibility that erosions may be caused by treatment, but no more than a possibility. They raise a question of safety, an unlikely one I think, but a question nonetheless.

The issue is: Is this safety concern significant and can it be resolved on the basis of the present data base. Given the large exposure intended for the product the questions raised by the data could be important. I don't believe they are, but my reasons are based on the weakness of the adverse data not on a positive demonstration of safety.

In my opinion the data do not show the Calgene product to be unsafe but the data fall short of "a demonstration of safety" or of "a demonstration of reasonable certainty of no harm" which is the standard we typically apply to food additives. To do that we would need, in my opinion, a study that resolves the safety question raised by the current data. For example, a stronger study attempting and failing to reproduce the gastric erosions would be sufficient. I believe this is what the safety standard we operate under means by "a demonstration of reasonable certainty of no harm.

One might well ask: If the adverse data is so weak, why is the question it raises worth considering at all? The answer, I think is that we have real data (however questionable) that raises the safety question and real data is necessary

to resolve it. If the data were absolute junk it wouldn't raise a question, but no one has suggested that the data is junk.

However, it has been made clear to us that this present submission is not a food additive petition and the safety standard is not the food additive standard. It is less than that but I am not sure exactly how much less.

All I can do is to state my opinion that the data does not show any real toxicity. The requirements that one should have in this data (and in data generally) for a real finding are:

- (1) A strong association between the alleged cause (treatment) and the effect. Here the association is weak and variable and the effect seems spurious and unlikely to be reproduced.
- (2) Specificity (One should not find the effect produced by other extraneous factors. (Here we find the effect produced in the controls.)
- (3) A biological gradient evidenced by a clear dose response. (This feature was not present in these studies.)
- (4) Consistent data shown by positive results in repeated studies. (Here a second study is negative.)
- (5) A plausible biological mechanism for the treatment and the effect observed. (Here the tomatine levels were shown to be the same as in regular tomatoes and the acid in tomatoes is much weaker than stomach acid itself. No other likely treatment related cause is apparent.) On the other hand the stressinduced fasting is plausible.

Robert J. Scheuplein, Ph.D.

T. Schuple

Addressees:

James H. Maryanski, Ph.D. (HFS-13)

Alan M. Rulis, Ph.D. (HFS-200)

Laura M. Tarantino, Ph.D. (HFS-206)