CBW

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
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Dear Chester and Joe,

Following up on our discussion at dinner in New York last Friday, I telephoned Bill Sarver yesterday to get a clearer understanding of how the analyses done in his laboratory relate and compare to those you have done. This is a summary of what I understand to be the facts:

1) Sarver's lab has analyzed by GC/MS about 60 environmental samples from alleged attack sites in Southeast Asia for T2 and certain other trichothecenes.

2) Based on an earlier telephone conversation with Bill and his summary in Science (15 July 1983, page 242), these samples appear to be 10 of yellow powder, 10 of water and about 40 of foliage. Some of the foliage samples may have included yellow spots or powder.

3) In Bill's lab, all of these samples tested negative for trichothecenes. The detection limit was in the mid parts per billion range, about 60-80 ppb.

4) Only one of these samples was tested by either of you. This was the sample Chester refers to as FS704B (or FS704D). Fifty mg of it are reported to have been received by Chester on 20 (or 23) Oct, 1981 and to have contained 143 ppm of T2, 27 ppm of DAS and a trace of zearalenone. This is the highest T2 level reported for any yellow rain sample. Full mass spectra for T2 and DAS GC fractions of the TFA derivatized material are given in figures 5A and 5B of the manuscript by Watson, Mirocha and Hayes submitted to the JOAC. Sarver's lab analyzed a portion of the same sample about a year after Chester did and found no trichothecenes. During that time, the material had been kept at room temperature.

5) Only one environmental sample has tested positive for trichothecenes in Sarver's laboratory. It was a $CHCl_3$ swab from part of the surface of a Soviet gas mask, said by Gary Crocker to have been purchased in Kabul. Bill's lab found T2 corresponding to approximately 1/2 - 1 microgram per 1/4 of the mask surface. Two other labs, including Mirocha's, tested swabs from the same mask and also found T2.

The Sarver lab finding of no positives out of about 60 environmental samples from sites of alleged attack in Southeast Asia contrasts with Chester's finding of five out of five positives (excluding the 'pink' sample) and Joe's finding of one out of one positive. Some details regarding these samples are summarized in the attached table, which I believe includes all environmental samples from alleged attack sites in Southeast Asia analyzed in your laboratories.

Why does Sarver find no positives out of <u>ca</u> 60 samples from Southeast Asia while Mirocha plus Rosen find six out of

six positive (excluding the pink)? In particular, why does Sarver find no T2 and Mirocha 143 ppm of T2 in aliquots of the same sample? The apparent difference corresponds to a factor of more than 1,000 between what Mirocha reports and what Sarver presumably could have detected. Is this a false negative, a false positive or are both labs right?

(a) False negative. Could Sarver's procedure, while able to detect T2 in spiked controls, be unable to recover even a very small fraction of it from a natural matrix?

(b) False positive. Could laboratory contamination have occurred at so high a level? Could some compound have mimicked derivatized T2 in GC/MS under Mirocha's conditions but not under Sarver's?

(c) Decomposition of T2. Decomposition by a factor of about 1,000 would presumably be required.

(d) Sample heterogeniety. This seems the least likely explanation for so large a discrepancy.

If we require an explanation that also accounts for the overall lack of positives in Sarver's analyses and the high proportion of positives found by Mirocha plus Rosen, explanation (d) above becomes even less likely.

This all seems quite baffling. What do you make of it? And what do you think should be done?

Sincerely,

Matthew

Matthew Meselson

MM/db
Enclosure
cc: Dr. Emery W. Sarver
Dr. Joshua Lederberg

Environmental Samples From Alleged Attack Sites in Southeast Asia Reported to Contain Trichothecenes

Sample	Nature	Origin	Date collected	Received by	Date	Amount received	Findings Reported (ppm) T2	DAS	NIV	DON		Pollen
FS698A FS698B	leaf and stem fragments	Kampuchea	Mar 81	Mirocha	July 81	200mg	3.17 35.7		109 21.7	59.1 0	(unspiked) (aliquot of above spiked with T2)	not tested
FS704A	water with debris	Kampuchea, same site as above	Mar 81	Mirocha	10 Oct 81	10ml; 33mg residue on evaporation	0	trace	0	66 0.22	(residue basis) (water basis)	not tested
FS704B (or D)	rock scraping (yellow powder)	Laos	13 Mar 81	Mirocha	10 Oct 81 20(or23) Oct 81	140mg spilled 50mg	143	27	0	0		yes
				Sarver	Fall(?) 82	?	0	0	0	0		
FS704C	rock scraping (yellow powder, very small sample previously extracted with methanol)	Laos	2 Apr 81	Mirocha	10 Oct 81	0.1mg		10-100(?)				not tested(?)
Thai	yellow spots on vegetation	Thailand	19 Feb 82	Mirocha	?	?	0.086	0.30	0	0		yes
ABC News	yellow powder scraped from vegetation	Laos	Mar 81	Rosen	Fall(?) 81	43mg	48	43		58		yes