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~~(2) Jim D.~~
~~(1) Blair: FYI~~

ABC News 7 West 66th Street, New York, New York 10023 Telephone 212 887-3704

Richard Richter
Senior Producer, Documentaries

March 17, 1982

Mr. Richard Burt
Director of Politico-Military Affairs
United States Department of State
Washington, D.C. 20520

Dear Mr. Burt:

Thanks for your note and your comments about "Rain of Terror."

I have enclosed a copy of Arthur D. Little Inc.'s report to us on the chemical analyses done on the "ABC News - Whitney" sample. The results of Dr. Rosen's analysis are part of the Arthur D. Little report.

Dr. Rosen has submitted his report for publication in scientific journals, but as yet no publication has occurred. He has asked us to hold his full report until after it has been printed. We are doing that, but feel that circulation of the Arthur D. Little report is not inconsistent with his wishes.

I see by the New York Times that you may be transferred to another post in State. Maybe our professional paths will cross again if you move. Or maybe even if you don't.

Sincerely,

Richard Richter

Biomedical Mass Spectr
Journal of Biomedical Spectrom

Arthur D. Little Inc. ACORN PARK · CAMBRIDGE MA 02140 · 617 864-5770 · TELEX 921436

December 15, 1981

American Broadcasting Company
7 W. 66th Street, ASCAP 2
New York, NY 10023

Attention: "Rain of Terror" ABC News Closeup, Steve Singer, Producer

Dear Mr. Singer: ADL Ref: 87134

This is submitted as the final report on our activities on your behalf in connection with chemical analyses of a sample of material provided through you. There were several tasks outlined in our proposal dated October 21, 1981, and authorized by you on October 26. Each will be discussed separately.

1. *Arrange for the analyses desired to be performed at an appropriate laboratory or laboratories.*

There were three categories of compounds for which analyses were originally desired: several trichothecenes of the T-2 toxin type; macrocyclic trichothecenes; and other non-mycotoxin chemical agents. Analysis of the carrying compounds, or matrix, of the sample was also desired, if possible after the initial analyses. It soon became clear that no single laboratory was experienced and qualified to do all the analyses, and finally, when the actual size of the sample was known, it was decided that the third category of analyses would be dropped. In the search for appropriate laboratories, several criteria were applied: 1) experience with the several specific trichothecenes of interest and availability of reference samples, 2) willingness to work as rapidly as possible; and 3) reputation in the field. Dr. Chester Mirocha of the University of Minnesota would have fit all of these pre-eminently, but since we wanted to obtain objective confirmation (or not) of his findings with other samples, he was not approached.

Before the final selection of Dr. Bruce Jarvis of the University of Maryland and Dr. Joseph Rosen of Cook College, Rutgers University, fifteen laboratories were considered or approached.

Dr. Edward Smalley, University of Wisconsin was capable of analyzing only for deoxynivalenol (Vomitoxin) and was just developing a technique for T-2 toxin by radio-immunoassay (RIA). He also would require a larger sample than we had, because of the lower sensitivity of his method.

Dr. William Roush, Massachusetts Institute of Technology, had no chemical standards and was not routinely analyzing crude materials, being a synthetic organic chemist. He did suggest other workers, including Dr. Jarvis. Two others were a government employee and a chemist at a pharmaceutical company, neither of which we considered appropriate sites for the task.

Dr. William Busby of MIT has published one of the definitive reviews in the field, and thus has an excellent overview of the scientific community of interest. He did not have analytical capabilities but suggested a number of leading workers we might approach. They are listed as the next eight entries.

Dr. F.S. Chu, University of Wisconsin, was contacted but could only analyze T-2 toxin, by radio-immunoassay.

Drs. Ronald Vesonder and Harland Burmeister, of the Northern Regional Research Laboratories of the U.S. Department of Agriculture (Peoria, IL) were not approached because of potential complications due to their affiliation with the U.S. Government.

Dr. Robert Epply of the U.S. Food and Drug Administration was not approached for the same reason.

Dr. M.A. Hayes and Dr. H.B. Schliefer, University of Saskatchewan, were called, but found to work only with T-2 bioassay by animal skin application, which lacks both the sensitivity and specificity we needed.

Dr. James Bamburg, of Colorado State University, the original isolator of T-2 toxin lacked experience in the most recent analytical methodology.

Drs. P.M. Scott and J. Harwig of the Canadian Food Directorate (equivalent to the U.S. F.D.A.) were not approached for reasons similar to those applying to U.S. Government personnel.

Dr. Bruce Jarvis was contacted in regard to the T-2 type analyses for which he was not equipped. We later went back to him, primarily upon Dr. Mirocha's recommendation, for the macrocyclic analyses and engaged him for that task. It is generally agreed by others we talked to that he is the pre-eminent worker in this specialized area.

Dr. Joseph Rosen was originally suggested by Busby. Initial contacts indicated a capability of analyzing for T-2, vomitoxin (deoxynivalenol) and DAS (di-acetoxyscirpenol). His experience with these analyses had been gained with analyzing a large number of samples, primary of milk and corn. He was not experienced in the analysis of nivalenol.

Conversations were held with Raltech Scientific Services of Madison, Wisconsin, primarily with James Kinsinger. They would have been appropriate for the non-trichothecene analyses, but were not further considered when that aspect was dropped.

A Dr. Romer, of Ralston Purina, was suggested by Rosen as one of three laboratories capable of doing the multiple trichothecene analyses. He was not approached because of the slight chance of the laboratories of a commercial product company being willing to participate. (The other two laboratories indicated by Rosen were his own and Mirocha's).

We established contact through our Tokyo office with Dr. Yoshio Ueno of the Tokyo University of Science. He expressed willingness to perform most of the analyses we requested, but we did not carry this through because of the logistics and communication difficulties likely to be encountered.

The laboratories of ICI in England were suggested, at least for DAS analyses. Attempts were made to contact Drs. Grove and Aldridge, but were abandoned because of finding the U.S. laboratories and because of the commercial implications, as with Ralston Purina.

Thus, the selection of the two laboratories was arrived at after an international search in which all the experts in the field were evaluated. Dr. Jarvis was a clear choice for the macrocyclic analyses. Dr. Rosen was equally clear for the T-2-type analyses, especially after other laboratories had been eliminated for the various reasons and criteria indicated. Both have performed in the most professional manner, and we are satisfied that their data are the result of carefully planned and executed analyses using the most advanced methods and instrumentation available.

2. *Open and sub-divide the sample for submission to the analytical laboratories*

The sample carried by Mr. Charles Whitney was received by Mark Goldman of our laboratories on October 23, 1981. The sample was in a glass bottle with a rubber stopper. The contents were removed in a Bio-hazard hood and portions transferred to five tared (pre-weighed) ampules and sealed with teflon-lined rubber stoppers and capped with aluminum. An Arthur D. Little, Inc., special safety plan for handling toxic chemicals was followed. The receipt and transfer were witnessed and filmed by your crew.

Subsequent re-weighing of the ampules gave the following net contents:

Ampule No.	Weight (mg)
1	21.3
2	20.6
3	15.7
4	17.7
5	12.1

Thus, the total weight of the original sample, as portioned out, was 87.4 mg (0.003 ounce).

On November 4, the vials were packed, boxed and sealed and given to Ms. Diane Rhodes for delivery to Drs. Jarvis and Rosen (Vials #3 and 4 to Jarvis, #1, 2 and 5 to Rosen). Receipts were received by us indicating delivery to Dr. Rosen on November 4 and Dr. Jarvis on November 5.

3. *Receive the analytical results and assemble them for your use.*

Dr. Bruce Jarvis of the University of Maryland looked for macrocyclic trichothecenes in the sample, and concluded that there were none present. His letter report (attached) describes his findings. Dr. Mirocha, using GC/MS, found no evidence of any of the macrocyclic compounds. Jarvis' techniques, described in a recent publication (Science, 214:460-461, 1981), were not designed to separate the "simple" trichothecenes (T-2 toxin, etc.) so that analysis of his preparations would not necessarily be expected to confirm analyses done specifically for only these substances. Dr. Rosen (see below), on examining Jarvis' Science paper, has suggested that the "simple" trichothecenes were most likely to be still on the chromatographic column when Jarvis stopped collecting fractions because no UV-absorbing materials were coming off. A higher concentration (20%) of methanol than Jarvis used (2-6%) would be required to elute T-2 and related materials.

Dr. Rosen has reported the presence of three trichothecenes, a non-trichothecenoid mycotoxin, and a polyethyleneglycol-derived substance in the sample. The toxins are discussed in his letter of November 23 (attached). These observations are presented in some technical detail. We have visited Dr. Rosen and reviewed his results in even more detail, but only present here what we believe is essential to an understanding of the analytical niceties involved.

The ultimate aim of the chemical manipulations is to prepare a solution that would be expected to be as highly enriched as possible with the suspected trichothecenes so that the Selected Ion Monitoring technique on the

mass spectrometer would reveal their presence. The various steps involved -- extraction with methanol, removal of fatty materials with hexane, reverse-phase chromatography on a Setpack column, and thin-layer chromatography (TLC) for some of the suspected components -- narrow the likelihood that extraneous materials would be present.

The gas chromatograph/mass spectrometer (GC/MS) system as employed by Rosen involves the use of a GC column for which the elution time is another diagnostic for the presence of trichothecenes, and the use of an internal standard, deuterated silyl derivatives of authentic samples of the suspected components of the unknown. This is a potent method for the identification of fragments produced by the electron impact (EI) method of analysis in the mass spectrometer, which sorts out molecular fragments according to their weight. The deuterated reference materials come out of the GC column very close to the silylated materials from the sample, and thus provide another cross-check on the identity of the fragments observed.

For each of the trichothecenes, there were two or three fragment peaks looked for on the mass spectrometer, and in each case, the results in comparison with the deuterated standard were convincing. Thus, we accept Dr. Rosen's conclusion that T-2 toxin, vomitoxin (deoxynivalenol), and DAS (diacetoxyscirpenol) are present in the sample. Zearalenone is a separate situation, since Dr. Rosen did not have a deuterated standard available for comparison. However, its level of occurrence was high enough to permit a full-scale mass spectrum, which provided conclusive evidence of its presence, and the quantity present calculated by reference to authentic material. Dr. Rosen attempted to detect nivalenol in the TLC cut containing the other materials using a reference sample supplied by Dr. Mirocha, but did not find positive indications. Because he has not developed methods for nivalenol, this is an uncertain finding.

The quantitation of trichothecenes identified in the sample is dependent on comparison of peak heights for the sample with those of the internal standard which is present in a known quantity. Confirmation is obtained by determining whether the ratios of the heights of the various peaks are the same for sample and standard. These criteria were met in all cases. The calculated amount of each (in ppm) was: zearalenone, 228; T-2 toxin, 48; DAS, 42; and deoxynivalenol, 58.

After the results on the mycotoxins were reported orally, it became of interest to determine what else was present in the sample as received, since the toxins accounted for less than 0.05% of the mass involved. Discussions between Arthur D. Little, Inc., Dr. Rosen and ABC News suggested a search

for polyethyleneglycol-related materials. This was indicated because PEG (and its derivatives) are commonly used as surface-active agents (detergents, emulsifiers, etc.), and thus might be an expected component of a mixture designed for spreading and persistence of active material, as is sometimes done in pesticide applications.

The material analyzed was the same solution (from the thin-layer chromatogram) which contained T-2, DAS and zearalenone. The conclusions from Rosen's data are based on agreement with two properties of PEG after silylation:

- There is successive loss of a repeating monomeric unit of 44 atomic-mass-units (amu), equivalent to the ethoxylate group ($-CH_2-CH_2-O-$)
- Silylation of PEG (or derivatives) yields a fragment of 161 amu [$(CH_3)_3-Si-O-CH_2-CH_2-O-CH_2-CH_2-$]

Furthermore, if there is another substituent on the terminal (nonsilylated) end of the polyethoxylate, fragments will be produced which decrease successively by 44 amu.

Both the reference standard of PEG 400 (average amu) and the unknown show peaks at 161, 205, and 249 amu, representing the silylated fragment, plus one and two ethoxylate units (see Figure).

In the spectrum of the unknown sample, there are peaks showing the successive loss of 44 amu, for example, 279, 235 and 191 in one GC peak (see Figure) and 478, 435, 391, 346, 302 and 258 in another. From these data it cannot be determined what the original substituent on the polyethoxylate chain was, but it is clear that the chain is losing fragments of the size of ethoxylate, and that something other than a silyl group is attached.

Quantitation of PEG or its derivatives by mass spectrometry is difficult because any such product contains a mixture of polymers of various chain-lengths, which may elute from a column at different times and also fragment differently in the mass spectrometer. Dr. Rosen, however, by analogy with the results with PEG 400, estimates that the unknown sample contained a minimum of 1250 ppm of the PEG-like material.

A possible explanation of the presence of PEG-based materials in the sample would be that they were inadvertently introduced during the handling of the sample and the chemical manipulations involved in preparation for the GC/MS analysis. Dr. Rosen has run a "blank" sample and found no indications of

Mr. Steve Singer
American Broadcasting Company

PEG, that is, no peak at 161 amu, nor any peaks showing increments (or decrements) of 44 amu. The preparation of this blank sample involved all steps from extraction of methanol with hexane, through Setpack reverse-phase chromatography, thin-layer chromatography, silylation and gas chromatography. Prior to the chemical processing, all physical handling of the sample, both in our laboratory and his, was with the dry powder. The vials in which the sample was transmitted to him were new and not washed, and had Teflon-lined stoppers. We conclude that the PEG-like material was not introduced by any of the procedures used.

4. *Provide you with our assessment of the validity and significance of the results insofar as possible*

- All four of the compounds found by Rosen (3 trichothecenes and zearalenone) are known to be produced by Fusarium roseum. Vomitoxin, according to the review by Busby and Wogan (1979), has not been reported from F. tricinctum. It is thus possible, if not necessarily likely, that this material could derive from a single culture source.

- The absence of the macrocyclic compounds is not surprising, since they have not been reported to be produced by the same micro-organisms as the T-2 type trichothecenes are. This may be taken to indicate that the sample is not a gross sample of soil, or a contaminated "natural" material.

- The fact that the trichothecenes are found in nearly equivalent amounts is believed to be unexpected from a natural source. They could be artificially produced in these amounts either by manipulation of growth conditions in a single culture, or by pooling cultures grown under different conditions.

- The presence of a polyethoxylate material (PEG-like) is completely unexpected in a natural sample. It is a man-made chemical polymer, and the only one known to us which would give repeated fragment losses of 44 mass units as reported by Rosen. If the PEG-like material is intrinsic to the sample, the implications of how and why it is there must be considered.

Sincerely yours,

Philip S. Thayer, Ph.D.
Consultant

PST:jt