Commonwealth Edison
1400 Opus Place
Downers Grove, Illinois 60515

May 14, 1991

Dr. Thomas E. Murley, Director
Office of Nuclear Reactor Regulation
U.S. Nuclear Regulatory Commission
Washington, DC 20555

Attn: NRC Document Control Desk

Subject: 10 CFR 26 Fitness For Duty Programs
Docket No:50-237/249, 50-254/265,50-295/304,
50-373/374, 50-454/455, 50-456/457

Reference: 10 CFR 26 Appendix A

Dear Dr. Murley:

Section 2.8 (e) (4) of reference (a) requires the licensee to investigate any unsatisfactory performance test result and submit a report of the findings within 30 days. The purpose of this letter is to respond to this requirement. Attached is a copy of the investigation conducted by Commonwealth Edison.

This information is being provided for NRC review. Please address any questions regarding this submittal to this office.

Very truly yours,

Allen R. Checca

Allen R. Checca
Nuclear Licensing Administrator

Attachment

cc: A.B. Davis Region III
Resident Inspector - Braidwood
Resident Inspector - Byron
Resident Inspector - Dresden
Resident Inspector - LaSalle
Resident Inspector - Quad Cities
Resident Inspector - Zion

ARC/cag
ZNLD/593

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A022

A REPORT OF UNCONFIRMED LABORATORY TEST RESULTS OF TWO POSITIVE BLIND PERFORMANCE TEST SPECIMENS

Since the implementation of Fitness For Duty testing under 10CFR26, Commonwealth Edison has included testing for Benzodiazepines. The positive threshold for initial screening was established at 300 ng/ml, with a confirmatory threshold of 150 ng/ml.

On October 9, Commonwealth Edison (CECo) received a laboratory test report from Bio-Analytical Technologies (BAT) for Specimen No. 362665 (BAT accession no. 196871). Bio-Analytical Technologies is the NIDA certified laboratory utilized by CECo to perform Fitness For Duty drug testing. This report was negative for all of the six drugs tested on the panel. Chain of Custody No. 362665 documented the October 1, submittal of a blind performance test specimen (Lot # 10133) of Benzodiazepine (Oxazepam) certified at 820 ng/ml.

The BAT Laboratory Director was requested to review laboratory tests for this specimen. The quantitative value was 386.9 ng/ml, above the cutoff, however since all criteria for a GC/MS positive were not met, the results were reported as negative by the laboratory standard operating procedure.

The Commonwealth Edison Director of Corporate Security and back-up Medical Review Officer were notified of this event late morning of October 9.

Commonwealth Edison contracted BR Biomedical Testing of Overland Park, Kansas to supply Blind Performance Test Specimens for the five substances mandated by 10CFR26, as well as Benzodiazepines. Biomedical is not a NIDA certified laboratory, but contracted Medical Arts Laboratory (NIDA certified) of Oklahoma City, Oklahoma to certify Blind Performance Test Specimens.

The specimens are shipped from Biomedical Testing directly to CECo's contract collection agency, CSM. CSM disburses these specimens to the six collection sites where the specimens are maintained in refrigerated storage. The Fitness For Duty Program Administrator directs CSM on the submittal of Blind Performance Test Specimens on a weekly basis. Biomedical Testing prints the expiration date of each lot on the identification label which is attached to the 60 ml shipping bottle of each specimen.

Lot #10133 was manufactured on May 29, 1990 and certified on June 1, 1990, and carried an expiration date of September 30, 1990. When the Fitness For Duty Program Administrator Director advised the collection contractor to submit the Benzodiazipine Blind Performance Test Specimen, it was not anticipated that the submittal date (October 1) would be beyond the expiration date of the specimen.

On October 4, the Fitness For Duty Program Administrator informed of Biomedical Management of the unconfirmed test on the Benzodiazepine spiked sample. Biomedical was also informed the specimen was submitted one day after the expiration date. The Laboratory Director for Biomedical recommended to immediately discontinue use of any outdated spiked Blind Performance Test Specimens. According to the Laboratory Director, other customers of Biomedical had not reported any blind performance test false negatives associated with Lot #10133.

On October 10, The BAT Laboratory Director identified Medtox Laboratories of St. Paul, Minnesota, a NIDA Certified Laboratory with the capability to quantitate Benzodiazepines. An aliquot from the Specimen #362665 was submitted for independent reanalysis. These results were to be used to assist in the determination of the cause of the disparity in the certified results provided by Biomedical and the BAT confirmatory test results.

On October 23, Lot #10133 was recertified by Medical Arts Laboratory to 640 ng/ml of Benzodiazipine (Oxazepam) and an expiration date of 03/31/91 was established. This allowed continued use of this lot as Blind Performance Test Specimens.

The laboratory report from Medtox Laboratories was included in the October 26, document package received from BAT. Medtox quantitated Specimen #362665 at 200 ng/ml and determined the specimen to be positive for the presence of Benzodiazepines.

On October 31, as a result of ten months testing experience and MRO recommendations, Commonwealth Edison entered into an agreement with the Bargaining Unit representing Company employes, that future drug testing under the Fitness For Duty rule shall be limited to the panel of substances listed in 10CFR26. This administratively eliminated testing for Benzodiazepines.

On November 1, prior to receiving notice that Benzodiazepines had been eliminated from FFD testing, the collection contractor for Commonwealth Edison was directed to submit Blind Performance Test Specimen #365447, containing Lot #10133, certified to 640 ng/ml of Benzodiazepine (Oxazepam). The BAT Laboratory Test Report dated November 13, documented that Specimen #365447 (BAT Accession No. 208950) was negative for the six drugs tested on the panel.

The BAT Laboratory Director was immediately notified by the Fitness For Duty Program Administrator of this second unconfirmed laboratory test. The second specimen Immunoassay screened positive for Benzodiazepines on November 5, at 17 absorbance units above the cutoff. It was then extracted and run by GC/MS for confirmation. The analysis indicated no peaks present in appropriate ion channels and was reported as negative.

It was agreed that Medtox would also be requested to reanalyze an aliquot of this specimen and report the results to the Bio-Analytical Laboratory Director.

The Director of Corporate Security and the Medical Review Officer were immediately notified of this second unconfirmed laboratory report. The decision was made to fully investigate the unconfirmed laboratory reports for both Specimen #362665 and #365447 and issue a single report to the NRC within 30 days of completion of the investigation.

Because of the complexity of this issue, the Fitness For Duty Program Administrator desired additional scientific assistance in reaching a determination regarding the unconfirmed laboratory test results of these two Blind Performance Test Specimens. Three NIDA certified laboratories were now involved in the testing of these two Benzodiazepine spiked specimens: The laboratory certifying the spiked specimens - Medical Arts, the laboratory performing the analysis on the Blind Specimens- Bio-Analytical Technologies and the NIDA laboratory selected by BAT to independently analyze the aliquots of the Blind Specimens - Medtox.

The Fitness For Duty Program Administrator contracted the Director of General Chemistry and Toxicology at the University of Illinois Hospital, in Chicago, for assistance in resolving the two unconfirmed test results. The doctor is the certified toxicologist who participated in the effectiveness audit of the Commonwealth Edison Fitness For Duty Program in May of 1990. All available documents for both specimens and a 60ml specimen of Lot #10133 were sent to the toxicologist.

Biomedical manufactured Lot #10133, which included spiking the sample with Benzodiazepine and testing to verify the level. Medical Arts then conducted GC/MS testing of the lot to certify the quantitation. Biomedical does utilize a technique to stabilize the sample against rapid deterioration, however, no chemicals are added to the sample. Biomedical's Laboratory Director would not divulge the actual process. Biomedical recommends that the spiked specimens to be stored refrigerated at 2 - 8°C.

Shipping records for the recertified Lot #10133 indicate that the specimens were shipped from Biomedical testing on 10/25/90 and received by CSM on 10/29/90. Beyond this, CECo could not establish that the specimens may have been subjected to an unusual length of unrefrigerated storage.

On February 8, the Fitness For Duty Program Administrator visited BAT to discuss the unconfirmed blind performance test specimens with the Laboratory Director. Medtox reports for both specimens were reviewed, however, no GC/MS tracings were submitted with the reports.

The conclusion of the Bio-Analytical Laboratory Director regarding the unconfirmed laboratory reports of Specimens #362655 and #365447 is that:

> Extraction efficiencies and quantitative results for Benzodiazepines vary widely between laboratories. Quantitative results and extraction of higher concentrations are more consistent and yield better results. These false negatives are attributed to poor sample integrity and resultant poor extraction behavior in the BAT system.
>
> The BAT Laboratory Director's report is submitted as Attachment 'A' to this document.

On May 5, 1991, the investigating toxicologist presented a report to the Fitness For Duty Program Administrator summarizing a detailed and lengthly investigation of BAT Laboratory's failure to confirm Benzodiazepine Blind Specimens. This investigation concurs with the conclusions drawn by the BAT Laboratory Director, that is, not reporting this specimen positive based on chromatographic problems and lack of appropriate ions encountered during analysis by gas chromatography-mass spectrometry. The toxicologist's chronology of the laboratory investigation and the summary report is submitted as Attachment 'B' to this document.

Commonwealth Edison considers this issue closed. Back-up documentation relative to this investigation is on file in the office of the Fitness For Duty Program Administrator. A copy of this report will be provided to the Laboratory Director of Bio-Analytical Technologies, as well as to BR-Biomedical Testing, the supplier of the Blind Performance Specimens.

+4J/278-80

Attachment 'A'

Bio-Analytical Technologies Report of the investigation of unconfirmed positive Benzodiazepine Blind Performance Test Specimens.

Two specimens, later determined to be positive benzodiazepine QC specimens, were reported as negative by our laboratory on 10/8/90 and 11/13/90.

Specimen #362665, BAT# 196871, screened positive for benzodiazepines on 10/2/90, 6 units above the threshold. The threshold control for the batch which contains oxazepam at 375 ng/ml was 12 units above the cut-off. The specimen was extracted for confirmation by GC/MS. The GC/MS analysis demonstrated poor chromatographic behavior and a peak in only one of four required ion channels for oxazepam. The quantitative value was 386.9 ng/ml, above the cut-off, however since all criteria for a GC/MS positive were not met the results were reported as negative as required by the standard operating procedure. The target value was reported to be approximately 820 ng/ml.

At the request of the client, an aliquot was sent to another laboratory for analysis. That laboratory quantitated oxazepam at 200 ng/ml and provided data that indicated that ion ratio criteria were met in their analysis.

The second specimen, #365447, BAT# 208950, screened positive for benzodiazepines on 11/5/90 at 17 absorbance units above the cut-off. It was then extracted and run by GC/MS for confirmation. The analysis indicated no peaks present in appropriate ion channels and was thus reported as negative. An aliquot of that specimen was also sent to Med Tox Labs for analysis. The results of that analysis were + oxazepam = 363 ng/ml. Data provided by the laboratory indicated that on the initial injection, ion ratio criteria were not met. The specimen appears to have been re-injected and the second analysis met ion ratio criteria.

At the request of the client, aliquots were sent to Dr. Robert Williams at the University of Illinois Hospital on 1/23/91 using Airborne Express. The package was apparently lost in transit and never arrived. Airborne was unable to locate the package nor could they verify delivery. No specimen remains for retesting.

The benzodiazepine analysis by GC/MS is a problematic one both due to chromatographic behavior and due to the large number of potential compounds available by prescription. the analytes are better analyzed by HPLC or GC/ECD, however, neither of those confirmatory methods are deemed acceptable under Federal Guidelines. In our experience, extraction of these compounds from individual patient urines at low levels is inconsistent, and repeated analysis often does not improve the result. That is why, in the first case (#196871) the specimen was not reanalyzed prior to reporting.

The target values of 820 and 640 ng/ml were not verified by analysis from either laboratory, indicating that significant degradation may have occurred. This is also indicated by the poor extraction and chromatographic behavior observed in our laboratory. The quantitative results reported by our laboratory and by Med Tox are consistent with the results of the screening assay.

We observed screening results of +6 and +17, respectively, while positive controls at 375 ng/ml and 500 ng/ml yielded immunoassay results of +12 and +23. A specimen containing 820 or 620 ng/ml oxazepam would be expected to screen at a higher level.

As is indicated by results of external proficiency surveys, extraction efficiencies and quantitative results for benzodiazepines vary widely between laboratories. In our experience, quantitative results and extraction of higher concentrations are more consistent and yield better results. I would attribute these false negatives to poor sample integrity and resultant poor extraction behavior in our system.

3/4/90

ATTACHMENT 'B'

SUMMARY REPORT OF INVESTIGATION OF BR-BIOMEDICAL LABORATORIES - LOT # 10133

BY THE INVESTIGATIVE TEAM AT THE UNIVERSITY OF ILLINOIS

PREPARED

BY

DR. ROBERT H. WILLIAMS
DR. IAN TEBBETT

LIST OF AGENCIES INVOLVED WITH COMMONWEALTH EDISON'S FITNESS FOR DUTY PROGRAM AND BLIND PERFORMANCE TEST SAMPLE PROGRAM

BR-Biomedical Testing (BR):

Manufacturer of blind performance testing specimens for NIDA laboratories located in Overland Park, Kansas. Sample is shipped as 60 mL aliquots. **Lot # 10133** was manufactured on May 29, 1990 - **positive for benzodiazepines**; sample spiked as **"oxazepam"**.

Medical Arts Laboratory (MAL):

NIDA certified laboratory located in Oklahoma,City, Oklahoma that certifies drug levels for blind performance testing specimens for BR-Biomedical Testing. **Lot # 10133** certified at **820 ng/mL** on June 1, 1990; expiration date of September 30, 1990. **Lot # 10133** was re-certified at **640 ng/mL** on October 23, 1990; expiration date of March 31, 1991.

MedTox Laboratories (MTL):

NIDA certified laboratory that analyzed Bio-Analytical Technologies Specimen # **196871** ****** Commonwealth Edison Control Sample # **00362665** and Bio-Analytical Technologies Specimen # **208950** ****** Commonwealth Edison Control Sample # **365447**.

Bio-Analytical Technologies (BAT):

NIDA certified laboratory that analyzes Commonwealth Edison's employee specimens and blind performance specimens. Failed to confirm positive benzodiazepine (oxazepam) on Com-Ed samples # **362665 and 365447** equivalent to BAT samples # **196871 and 208950**, respectively.

University of Illinois Hospital/University of Illinois - College of Pharmacy (U. of I.)

Investigative laboratory directed by Dr. Robert H. Williams (U.of I. Hosp.) and Dr. Ian Tebbett (U. of I. - Coll. of Pharm.). Dr. Williams was also a member of the investigative team for audit of Bio-Analytical Technologies laboratory conducted - May 1990.

INCIDENT SUMMARY: REPORT OF FALSE NEGATIVE SAMPLES FROM BIOANALYTICAL TECHNOLOGIES FOR BENZODIAZEPINES FROM BLIND PERFORMANCE TEST SAMPLES SUBMITTED BY COMMONWEALTH EDISON

September 11, 1990 - Com-Ed receives report from BAT that fails to confirm a positive blind performance test specimen: **BR Lot # 10133 - Benzodiazepine as "Oxazepam"** - **Test date:** 9-25-90 **Expiration date:** 9-30-90 **Certified Level:** 820 ng/mL

October 9, 1990 - Com-Ed receives another report from BAT regarding a blind performance test specimen reported as a false negative result for Benzodiazepines: **Com-Ed Control Sample # 00362665 ****** BAT Sample # 196871 ****** BR - Lot # 10133** (Oxazepam - 820 ng/mL). This specimen tested 6 absorption units above cutoff level for benzodiazepines using EMIT; quantitated at 386 ng/mL by GC-MS, however peaks in chromatogram were not aligned for a positive result (inappropriate retention times).

October 10, 1990 - Dr. Collins identifies another NIDA certified lab, **MedTox** in Minneapolis, MN, to re-analyze this specimen: **Com-Ed Control Sample # 00362665**

October 12, 1990 - MedTox receives specimen **Com-Ed Control Sample # 003622665** from BAT.

October 23, 1990 - MedTox quantifies/reports that **Com-Ed Control Sample # 00362665 ***** BAT Specimen # 199871** as positive for Benzodiazepines as "**Oxazepam**" at a level of 200 ng/mL. However, they do not send a copy of the GC-MS chromatograms with report.

October 23, 1990 - Medical Arts Laboratory re-certifies **BR - Lot # 10133** for Benzodiazepines: **Certified Level:** 640 ng/mL

November 13, 1990 - BAT reports another false negative for Benzodiazepines on **Com-Ed Control Sample # 365447 ******* BAT Sample # 208950 ****** BR - Lot # 10133** certified positive for Benzodiazepines (Oxazepam - 640 ng/mL). This specimen tested positive at 17 absorbance units above the cutoff level for Benzodiazepines upon screening with EMIT. The extracted specimen did not confirm by GC-MS due to a lack of GC peaks in the appropriate ion channels. BAT sends another aliquot to MedTox for confirmation and quantitation of **BAT Sample # 208950 ****** BR - Lot # 10133.**

November 27, 1990 - MedTox quantifies/reports that **Com-Ed Control Sample # 365447 ****** BAT Sample # 208950** as positive for Benzodiazepines as "**Oxazepam**" at a level of **363 ng/mL.**

December 3, 1990 - Garey Toleski, Program Administrator - Fitness for Duty, contacts Dr. Jerrold B. Leiken, Associate Director of Emergency Services at Rush Presbyterian St.

Lukes Medical Center in Chicago, Illinois, to assist in resolving the discrepancies involved with reporting of the two false negative samples that were certified positive for benzodiazepines. Dr. Leiken was involved with the May 1990 audit of Bioanalytical Laboratories.

December 10, 1990 - Dr. Jerrold Leiken contacted Dr. Robert H. Williams, Director of General Chemistry and Toxicology at the University of Illinois Hospital in Chicago, Illinois, to also assist with the investigation. Dr. Williams was also involved with the May 1990 audit of Bioanalytical Technologies.

January 5, 1991 - After reviewing the initial report and data submitted to Dr. Leiken and Dr. Williams it was decided that analysis of **Lot # 10133** was warranted. It was requested that Dr. Ian Tebbett, Director of Forensic Toxicology at the University of Illinois at Chicago, would assist Dr. Williams in the analysis of this particular lot.

January 7, 1991 - A vial of **BR - Lot # 10133** was shipped by federal express to Dr. Williams' attention for, sample analysis. After receiving sample, it was requested that Dr. Collins ship an aliquot of **BR - Lot # 10133 ***** Com-Ed Sample #'s 362665 and 365447** corresponding respectively to **BAT Sample #'s 196871 and 208950**.

January 23, 1991 - As per Dr. Williams request, aliquots of both samples (BAT 196871 and 208950) were shipped by courier (Airborne Express), however, Dr.Williams never received samples. No additional samples were available for testing. At this time it was decided that analysis of the **BR - Lot # 10133** sample that was shipped to Dr. Williams by Com-Ed would still be performed.

March 14, 1991 - Dr. Williams laboratory screened **Lot # 10133** for Benzodiazepines. Initial results indicated a positive reading that showed a rate change that was 27 absorbance units above the cutoff level of 300 ng/mL using the EMIT System. Repeating the sample gave a positive reading of 30 absorbance units above the cutoff level. The positive control (low calibrator) which had a value of 500 ng/mL gave a positive reading that was 53 units above the cutoff level. Based on the initial screen, it was estimated that the level of benzodiazepines was around **400 ng/mL** (the change in absorbance rate was mid-way between the negative cut-off of 300 ng/mL and the positive control of 500 ng/mL).

March 18, 1991 - Dr. Williams/Dr. Tebbett confirmed the presence of oxazepam using solid phase extraction (10, um Bondapak) Reverse Phase (C 18 column - 4.6 mm x 30 cm) High Performance Liquid Chromatography (HPLC) according to the attached protocol with Diazepam as an internal standard. The unknown sample was quantified four times. Using the attached standard curve and the absorbance ratio of Oxazepam/Diazepam, the average level of benzodiazepine as oxazepam was determined to be **435 ng/mL ± 11.5 % (n = 4)**. These results were in accordance with the screening results using EMIT.

March 21, 1991 - Dr. Williams/Dr. Tebbett attempted to confirm the presence of oxazepam for **Lot # 10133** using full scan gas chromatography-mass spectrometry (GC-

MS) with a Hewlett Packard MSD Quadrupole Mass Spectrometer (Column = DB5, 10 meters, Temperature program 200 - 270 degrees at 15 degrees C./min., column pressure = 10 psig, injection 100:1 split). Diazepam was used as the internal standard (1000 ng/mL). Urine spiked with diazepam was extracted and analyzed by GC-MS. Retention time was determined and the internal standard was shown to have the appropriate ions **(M/Z 284, 256, 221)**. A spiked sample of oxazepam (500 ng/mL) in methanol was also analyzed in a similar manner; the retention time was determined and ions generated in the mass spectra were indicative of oxazepam **(M/Z 268, 239, 205, 77)**. An extract of **Lot # 10133** was analyzed in the same manner. Numerous peaks were seen in the gas chromatogram. Only one prominent peaks was noted in the chromatogram having a retention time similar to oxazepam. However, a scan of this peak generated mass spectra not indicative of oxazepam; major ions were **(267, 254, 253)** suggesting the presence of a oxazepam artifact which may occur due to thermolability of the compound. The major ions referenced for this artifact (Mass Spectral and GC Data of Drugs, Poisons and Their Metabolites, K. Pfleger, H. Maurer, A. Weber, VCH Publishers, Deerfield Beach, FL) are **M/Z 254, 253, 219**. Scans of the small peaks around the same retention time of this peak gave mass spectra that were not indicative of oxazepam. Additional attempts to recover oxazepam from **Lot # 10133** were not successful.

March 22, 1991 - Dr. Williams laboratory repeated the screening procedure for Benzodiazepines to determine if the oxazepam in the sample was breaking down (degrading). **Lot # 10133** gave a rate reading of 38 absorbance units above the cut-off of 300 ng/mL; a repeat sample gave a rate reading of 35 units above the cut-off. The positive control gave a rate reading of 60 absorbance units above the cut-off level. Based on these readings **Lot # 10133** was again estimated to have a value of **400 ng/mL**.

March 25, 1991 - Dr. Williams/Dr. Tebbett again confirmed the presence of oxazepam based on retention time using the aforementioned HPLC procedure (protocol attached). Based on the ratio of oxazepam/diazepam the amount of benzodiazepine as oxazepam was calculated to be **406 ng/mL $\pm$ 16.8 %**.

March 29, 1991 - Dr. Williams/Dr. Tebbett again attempted to confirm the presence of oxazepam in **Lot # 10133** using full-scan GC-MS. Several concentrations of oxazepam in methanol were analyzed by GC-MS to note the sensitivity of the instrument near the range of oxazepam determined by HPLC (around 400 ng/mL). The following standards were analyzed: 1000, 500, 250 (ng/mL). The lowest standard still provided an adequate response for quantitative analysis and demonstrated ions indicative of oxazepam. Fresh urine spiked with the internal standard (1000 ng/mL of diazepam) was extracted using the same procedure as for HPLC. The extract was dried under nitrogen and reconstituted in methanol/chloroform. The reconstituted extract generated a retention time and ions indicative of the internal standard. **Lot # 10133** was treated in a similar fashion. The scan time of the internal standard peak was confirmed as diazepam by its mass spectra, however, no peaks were noted for oxazepam. Peaks in the area indicative of oxazepam did not generate ions that would confirm presence of this compound. This approach was repeated several times with the same results. Fresh urine was then spiked with oxazepam to note if there was a recovery problem with extraction technique. Regardless

of the extraction technique employed (Dr. Tebbett's - U. of I. versus Dr. Collins - BAT), oxazepam could not be recovered. However, the same extracts applied to HPLC gave retention times indicative of oxazepam and in amounts in accordance with the EMIT screen performed earlier. Methanolic solutions of oxazepam gave excellent GC-MS scans of the compound indicating that no active groups were apparent in the injection port or present on the column that could cause oxazepam to adsorbed to the GC column. Unlike diazepam, oxazepam has a free hydroxyl group that could cause the compound to be readily adsorbed onto such sites. The results suggested that alkaline extracts regardless of method were affecting the thermolability of oxazepam and thus, the ability to analyze oxazepam in **Lot # 10133**.

April 20, 1991 - Dr. Williams/Dr. Tebbett attempted to determine if thermolability was a problem with oxazepam if a urine matrix was employed during the extraction process by derivatizing the standards and extracts with TRI-SIL, a Trimethylsilylating (TMS) agent. Oxazepam was added in excess to a spiked urine sample to note the relative scan times for the TMS-derivatized sample and the underivatized sample. The TMS derivative could be separated from underivatized standard. However, all attempts to form the trimethylsilyated derivatives oxazepam using a urine matrix (spiked fresh urine and **Lot # 10133)** and generate a molecular ion were futile. The mass spectra did not appear to be of sufficient molecular mass to account for complete derivatization (major ions were **309, 293, 247, 239, 205**). It was thought that the TMS-derivative would have fragments that were greater than 400, although molecular rearrangement could have prevented some of the available oxygen and nitrogen atoms from reacting with the derivatizing agent. It is also possible that some of the ions were derivatized fragments and that higher molecular weight fragments were metastable. Since the urine extracts were alkaline due to the presence of residual buffer salts, it was thought that the high pH was promoting cleavage of the silyated bonds and thus, preventing complete derivatization. However, use of acidified methanol as a solvent prior to derivatization did not resolve the problem. Although complete derivatization did not appear to be occurring, the same fragments were generated as the fresh urine that was spiked with oxazepam. The intensity of these ions, however, were considerably lower. If the most prominent ion, **309**, was used to quantitate the concentration of derivatized oxazepam in **Lot 3 10133**, it would be <u>less than 250 ng/mL</u>. However, this is only an estimate given the problems encountered with GC-MS analyses of the TMS-derivatives of oxazepam and problems known to exist with its extraction from biological fluids such as urine.

****** CONCLUSIONS ******

<u>The investigative team has concluded that</u>:

1. Screening of **Lot # 10133** generated an approximate value of **400 ng/mL** on two separate occasions (3-14-91 and 3-22-91) using the **Emit (DAU) System**. The estimation was based on the change in absorbance rate of a positive control (500 ng/mL) and the negative control (cut-off level = 300 ng/mL).

2. Quantitation of oxazepam in **Lot # 10133** using high performance liquid chromatography (HPLC) was determined to be **435 and 406 ng/mL** on two separate occasions (3-18-91 and 3-25-91). These findings were in accordance with the estimate determined using EMIT and indicated that the sample appeared quite stable once opened for analysis.

3. Since NIDA guidelines require confirmation by gas chromatography-mass spectrometry (GC-MS), several attempts were made to confirm the presence of oxazepam in a urine matrix (fresh urine spiked with oxazepam and BR - Lot # 10133) without success using two extraction procedures (Dr. Collins - BAT and Dr. Tebbett - U. of I.). Although these extracts that did not generate ion fragments indicative for oxazepam, they could be re-chromatographed/quantitated for this compound using HPLC. Attempts to derivatized the sample and form the Trimethylsilylated derivatives of oxazepam in Lot # 10133 were also without success.

4. It has been our experience in the field that benzodiazepines can be readily quantitated using HPLC or GC using an electron capture detector. However, these methods of analysis are not acceptable under Federal Guidelines using criteria set forth by the National Institute of Drug Abuse (NIDA). Quantitation by GC-MS often leads to spurious results due to problems with chromatography (benzodiazepines react quite readily with active sites in the injection port and on capillary columns) and thermolability during analysis, especially if long capillary columns are used (greater than 10-15 meters). These problems result in anomalous retention times and formation of metastable ion fragments. It is also our experience that a large degree of variability exists in quantitating oxazepam by GC-MS due to recovery problems encountered with the extraction process and formation of metastable products due to the high alkaline pH employed during the extraction. As noted with the results furnished by MedTox, values of 200 ng/mL and 363 ng/mL were generated on Lot # 10133, however, this lot number furnished by BR-Biomedical testing was re-certified by another NIDA lab, Medical Arts Laboratory, at a value of 640 ng/mL. It has been my experience (Dr. Williams) as an inspector for many toxicology laboratories that the variation in quantitation of benzodiazepines (oxazepam) can vary so much as to question its validity. For example, I have seen company records that show certified samples of oxazepam, spiked in an urine matrix at a level of 850 ng/mL, to have quantitative values ranging as low as 220 ng/mL and as high as 810 ng/mL (same lot #). Considering the problems often associated with benzodiazepines during analysis by GC-MS and the problems that we encountered with this class of compounds during our investigations particularly with **Lot # 10133**, we conclude that any laboratory that can only analyze a blind sample once could easily have encountered analytical problems with this sample (Lot # 10133). Therefore, we concur with the conclusions drawn by Dr. Jennifer Collins, that is, not reporting this specimen positive based on chromatographic problems and lack of appropriate ions encountered during analysis by gas chromatography-mass spectrometry.