

Report on an Investigation of the unexplained death of a cow in N.E. Utah, October 16, 1998.

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ABSTRACT - On October 16, 1998 a three-year-old registered polled Hereford cow was found dead by the owner who had seen the animal in good health the previous day. The animal's left eye and periorbital tissue was removed as well as part of its left ear. A full necropsy followed by toxicological, histological and chemical analysis was done on the animal. The following noteworthy features were found: (1) Both the left periorbital tissue and left ear were removed with a sharp instrument(s) as confirmed by two and three veterinary pathologists respectively; (2) no evidence of high heat or cautery was obtained from these cuts; (3) no signs of struggle and no tracks were found near the animal; (4) an unusual, formaldehyde-containing blue gel-like substance was found on the eye, the ear and the anus of the animal; using headspace gas chromatography mass spectrometry analysis, the blue gel-like substance was shown to contain 300-3000 μ g/g of 2,6-bis-dimethylethyl-4-methyl phenol, a synthetic molecule with anti-oxidant and anti-viral properties, as well as contaminants of normal putrefaction breakdown products from cow tissue; (5) the animal's heart was shredded, yet its pericardium was intact; (6) the animal tested positive on two different pregnancy tests, yet there was no fetus present at the time of necropsy and no sign of a recent spontaneous abortion; (7) it is suspected that the animal's blood contained high levels of potassium chloride; (8) the animal had extremely low liver copper levels and low Vitamin A levels; (9) severe hemorrhaging under the hide around the neck of the animal was observed; (10) the animal was normal for routine bacteriology, virology and toxicology scans. In summary, the totality of the data obtained from the necropsy, combined with the histological, toxicological, bacteriological and chemical analyses suggests that the animal did not die a normal death.

BACKGROUND

At 1600 hours on the afternoon of October 16, 1998, the owner called NIDS to report that his best cow was dead on his property, possibly mutilated. The owner had seen the animal, an expensive registered polled Hereford, in perfect health the previous day. The animal was lying in a waterlogged area of his pasture about 20 feet from a paved road that is used by many local residents (see photograph, Figure 1).



Fig. 1 – Shows the position the animal was lying in when found by the owner

To preserve anonymity of the owner, we can say that the property is located in the Uinta Basin, Utah. (The neighbors were subsequently interviewed and it was determined that there was nothing unusual noticed by them in the previous or subsequent days that the dead animal was discovered.) Immediately, two NIDS investigators, both experienced ranchers and animal mutilation investigators, were dispatched to the scene. They arrived as it was beginning to get dark, less than two hours after the initial call. According to the investigators, the animal was lying on its front (sternal recumbency) with front legs tucked in under and rear legs splayed behind. Within feet of the head and sides of the animal the ground was waterlogged. There were no signs of struggle and no visible tracks. Using a compass, the investigator found that the animal was lying in a north-south axis with its head pointing north. This north-south pattern conformed to all 16 cases of mutilations that one of the investigators had investigated in the previous several years in the Uinta Basin. The meaning of this non random placement of mutilated animals in the Uinta Basin is unknown and will not be further commented on except as an item for comparison with other future unexplained livestock deaths.

The animal's left ear had been cut off and its left eye was missing together with a half-inch diameter piece of tissue around the top of the eye. The cut around the eye from visual inspection of photographs appeared to have been made with a sharp instrument (see Figure 2).

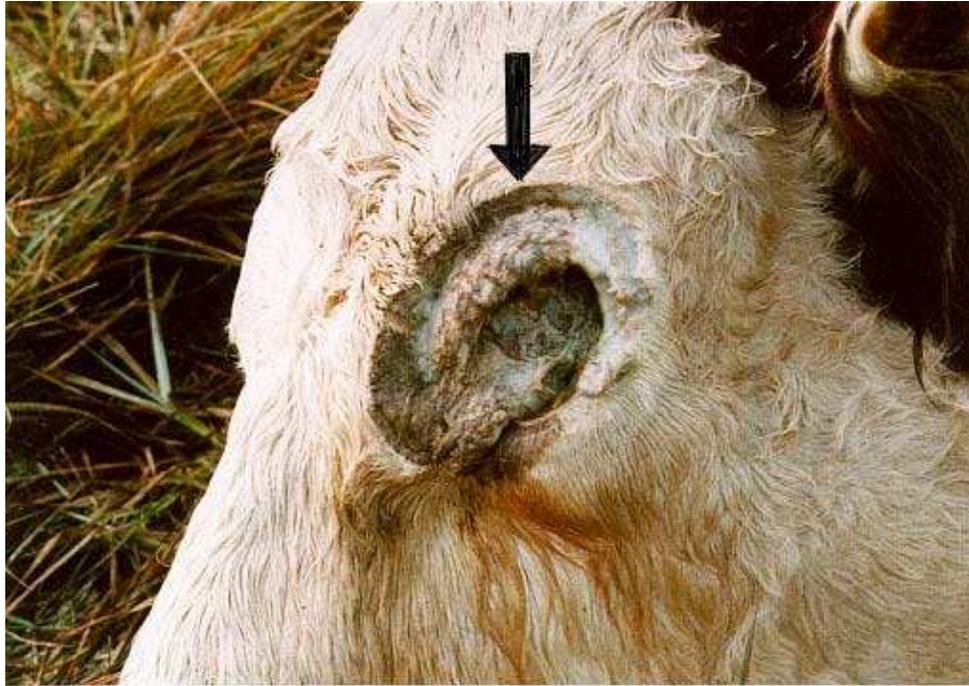


Fig. 2 – Sharp looking cut around the eye

Both investigators and the owner, also noticed an unusual bluish colored gel substance around the eye of the animal (Figure 2) as well as on its anus/vagina and a small amount on its ear. The investigator sampled some of the bluish gel from the anus area into a test tube and within an hour placed the tube in the freezer (-10 °C). He also took a sample of the bluish gel from the eye together with a tissue sample. Finally, he removed a part of the ear which contained the cuts for subsequent histological analysis. A local veterinarian was immediately contacted to conduct a full necropsy. NIDS was informed that because of the lateness of the hour the necropsy would be conducted the next day.

The following morning, October 17, the veterinarian arrived under contract to NIDS to conduct the necropsy. He found:

- The animal appeared to have died instantly on the spot, since there were no signs of struggle.
- Cardiac tissue which was almost unrecognizable. The pericardium was intact. Investigator described the heart as “shredded”. There was no blood in the pericardial sac.
- Enlarged uterus which on palpation yielded no fetus.
- Hemorrhaging around the neck area.

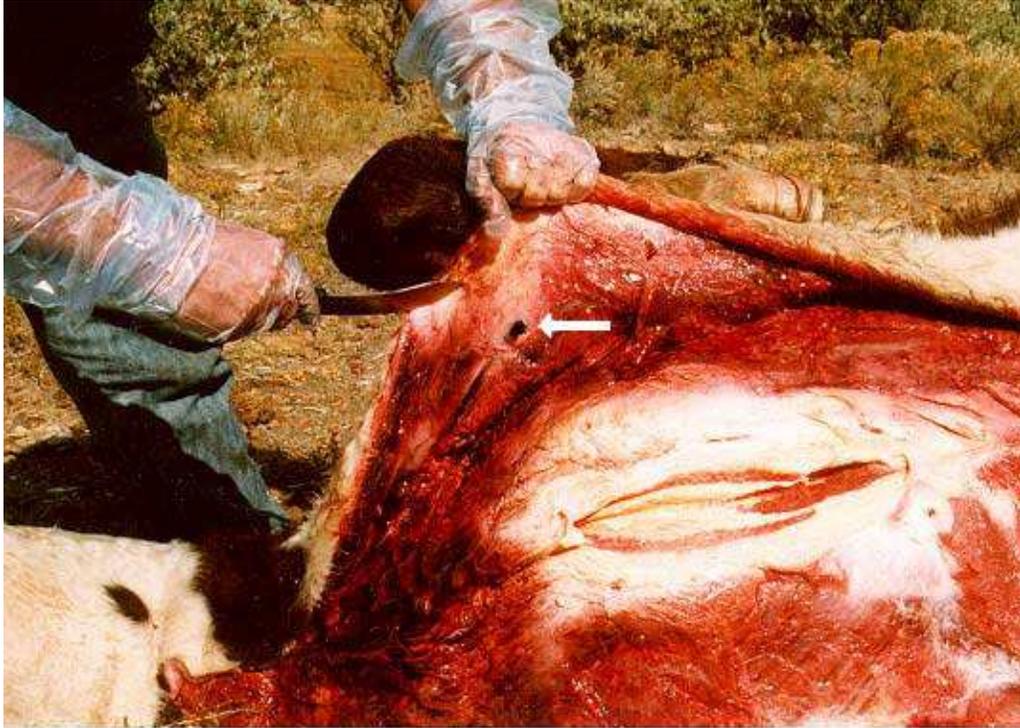


Fig. 3 – The white arrow shows the location of a small hole in the hide near the brisket area

- Small hole in the hide near brisket area (see Figure 3).
- No wound in flesh beneath the hole.
- Large blood clot in intestines.

For full text of veterinarian report see Appendix I at the end of the document.

SUMMARY OF THE ANALYSIS

There are several simultaneous facets of the mutilation that have been investigated. The following is a summary of each facet:

- **CUT AROUND THE EYE:** There was a half-inch diameter cut from around the upper eye (Figure 2). Photographic evidence, showing hair that was obviously cut, suggested strongly that the cut was made with a sharp instrument (see Figure 4).



Fig. 4 – Microscopic image of the hair around the eye (x 6.5)

This was confirmed by a veterinary pathologist from Purdue State University and by the NIDS staff veterinarian using a Wesco dissecting microscope equipped with an Olympus digital camera. The following photographs indicate that under low microscopic power, the hair around the eye appears to have been cut, rather than torn by a scavenger's teeth. It was further established histologically that there was no high heat or cautery used in making the cuts according to veterinary pathologists from Purdue State University and Colorado State University. These two opinions were confirmed by the NIDS staff veterinarian. In summary it was established by three independent experts that the cuts were made with a sharp instrument and not by a predator/scavenger and that no high heat was used to make the cuts.

- **THE ANIMAL WAS PREGNANT:** An enlarged uterus, which can mean pregnancy, was found by the veterinarian (see full necropsy report Appendix I). He palpated the uterus and did not open it because the uterus did not contain a fetus. However, NIDS did two different blood tests, for progesterone and for Pregnancy Specific Protein B, which confirmed that the animal was pregnant very close to the time of death. The blood progesterone levels were high, but by themselves were not conclusive of pregnancy, since progesterone levels are known to cycle during estrus. Therefore, a second confirmatory blood test was ordered-Pregnancy Specific Protein B (PSPB). The blood PSPB test was also positive. Since PSPB is only made by the placenta, which itself is only present during pregnancy, the combination of the two tests shows that the animal was pregnant at or close to the time of death. Yet, there was no fetus present. The half life (clearance from blood) of progesterone following spontaneous abortion is relatively rapid (approx. 24 hours). This indicates that if the animal did spontaneously abort, it did so very close to the time of death. The NIDS investigators examined the rear of the animal for traces of bodily fluids that might indicate a recent abortion (according to the owner, the animal should have been approx. 90 days pregnant) but found no traces of blood/bodily fluids that might indicate this. An ectopic pregnancy was ruled out since (a) the veterinarian did not find any sign of enlarged

fallopian tubes and (b) the enlarged uterus indicated a quasi normal pregnancy. As the necropsy report indicated, the veterinarian was sure there was no fetus present. The blood tests indicated that the animal had been pregnant and secreting both progesterone and PSPB close to the time of death. Another possibility is that the fetus was removed by unknown means at or close to the time of death.

- **THE EAR WAS CUT:** The veterinarian from Purdue University said it was removed with a non-surgically sharp instrument. In other words not a scalpel and more significantly, not a scavenger or a predator. The NIDS veterinarian, using a microscope agreed (see photograph taken of the pattern of cut hair from the ear). However, in comparing sharpness of instruments, a caveat needs to be inserted. It is not appropriate to compare a “sharp instrument” used on a freshly dead animal with a sharp instrument used on tissue that has been soaked in Formalin. Formalin makes the tissue much easier to cut and therefore, these cuts will look sharper with clearer edges than a cut with the same instrument made in the field before the sample is put into formalin.



Fig. 5 – Microscopic image of the hair of the ear (x 6.5)

- **THE HEART:** The veterinarian doing the necropsy found that the heart “was almost unidentifiable as cardiac tissue”. The NIDS investigator said that the heart looked like it had been blown apart. The investigator also said that the pericardium, which is the membrane surrounding the heart, was intact. No blood was found in the pericardial sac. According to two different veterinarian diagnostic labs, the heart was so badly decomposed that it was beyond obtaining useful information. According to the veterinarian who performed the necropsy, the heart was more decomposed than any of the other internal organs from the animal. One diagnostic laboratory reported large numbers of bacterial organisms throughout the tissue. Numerous large round spaces indicative of gas accumulation were present. Sarcosporidia parasites and cysts were also present in expected numbers. Examination by the NIDS veterinarian pathologist under the

microscope at various magnification (10x-60x) revealed multiple white-yellowish amorphous structures in the heart muscles. These structures appeared unevenly sized and irregularly shaped. Cysts usually appear regularly shaped and sized and do not have amorphous structure. Rather, they usually appear translucent. In the opinion of the NIDS veterinarian, there is little probability that the structures found in the dead animal's heart were calcified parasitic cysts. If they were, they still should have been uniformly shaped and sized. One of the diagnostic labs also reported that the structures were possibly bacterial conglomerations.



Fig. 6 – Multiple white-yellowish amorphous structures in the heart (x 6.5)

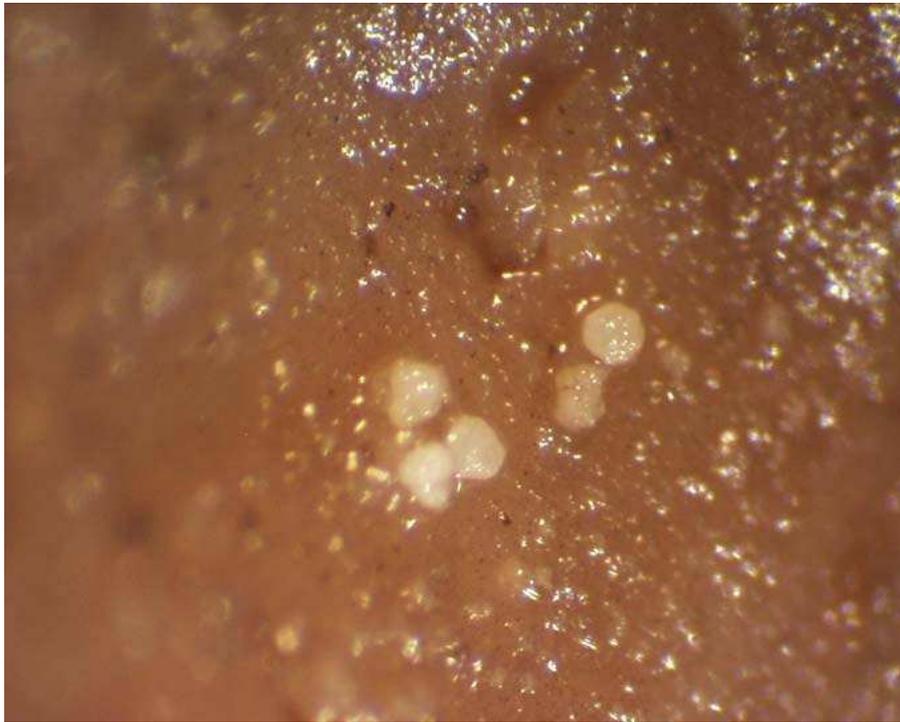


Fig. 7 – Amorphous irregular structures in the heart (x10)

As stated before, the heart contained multiple bacteria and gas pockets. In the opinion of the NIDS veterinarian, neither of these findings explain the extreme friability of the heart. Based on the available laboratory findings it is difficult to explain the profound myocardial changes which resulted in the severe damage described by the investigators when they first opened the animal.

- **BLOOD ANALYSIS:** It was originally intended to obtain as much blood as possible from the heart of the animal. As noted above the heart was shredded and fell apart when a syringe with needle was inserted into the tissue. A blood sample was taken from the nearby artery and also blood was collected from the abdominal cavity. Both samples were hemolyzed. The blood from the abdominal cavity showed 10 times the expected Potassium (*K*) levels (50 versus 4-5 milliequiv/liter). Although a large increase in *K* is not unexpected in a post-mortem sample since the intracellular stores of *K* are released back across the concentration gradient into the extracellular space after death, it was decided to test the ratios of *Na*, *K* and *Cl* in the animal's arterial blood. NIDS is cognizant of the difficulty in examining and interpreting post-mortem ion levels. Arterial blood from the dead cow was added to an inert matrix and a SEM EDX was run on both the matrix and the matrix plus blood (Figs. 8 and 9).

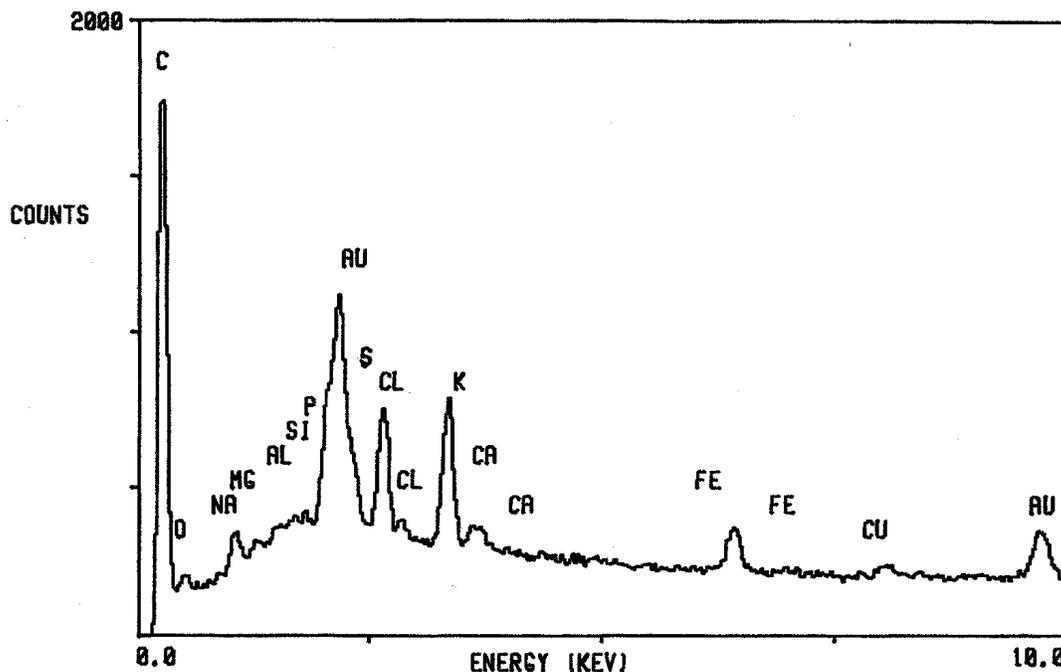


Fig. 8 – SEM/EDX spectrum of matrix to which arterial blood from the cow had been added.

It can be seen that there is a large increase in the *K* (potassium) and *Cl* (chloride) levels in the blood when compared to *Na* (sodium), the other dominant electrolyte in the plasma. It may be inferred from this analysis that there was a higher than usual concentration of Potassium and Chloride in the arterial blood of the animal, the ratios were much more marked than those in the abdominal blood. This discrepancy leads to the possibility that potassium chloride might have been used to kill the animal. Further tests are underway to test this hypothesis. It is noteworthy that potassium chloride is a favored method for killing since (a) it is relatively easy to obtain, (b) its presence is extremely difficult to detect in post mortem samples, and (c) it kills the animal rapidly - if sufficient *KCl* is injected into a vein, the animal dies in seconds.

- **TOXICOLOGY:** Enclosed in Appendix II at the end of this document is a summary of the toxicology report. The salient points are as follows: The animal was negative for heavy metals and all standard toxicology assays were negative. Liver copper was extremely low (4.8 ppm) and liver Vitamin A was low (3.18 µg/g: versus normal Vitamin A liver levels of 30-80 µg/g). Without delineating liver Vitamin A levels from other dead livestock we cannot determine if this finding is significant. For example in another previous animal mutilation that we investigated, the animal had a *large excess* of liver Vitamin A. Such an extremely low liver copper value can be considered unusual since according to the veterinarian, the owner and both NIDS investigators the animal looked in excellent nutritional condition. An extremely copper deficient animal would be expected to suffer pronounced weight loss, have a rough patchy coat. The dead animal did not display any of these symptoms. The Uinta Basin area is not noted for high molybdenum (*Mo*) levels which is a common cause of dietary copper deficiency. Indeed the animal's tissue *Mo* levels were normal. It is well known that an animal's liver copper reserves need to be extremely

depleted before the blood copper levels begin to be affected. The extremely low liver level of 4.8 ppm in the dead animal should have been reflected in abnormally low blood levels in the animal and in other animals in the pasture if the deficiency was caused by dietary imbalances. The SEM/EDX analysis of the arterial blood (compare figures 8 and 9) did not show any copper level in the animal's blood. Two random blood samples were taken from other animals in the same herd to determine if the entire herd was suffering from copper or other mineral deficiency. An ICP scan of all minerals showed that neither animal were mineral deficient. One of the two animals had a serum copper marginally below normal values while the other had a value in the mid-range of normal values. Therefore, the extremely low liver copper in the dead animal was not reflected by the other animals in the herd who were grazing the same pasture. Finally, we have previously found low liver copper levels (5-6 ppm) in another mutilated animal from a different area. The significance, if any, of low liver copper levels in these animals, remains to be determined.

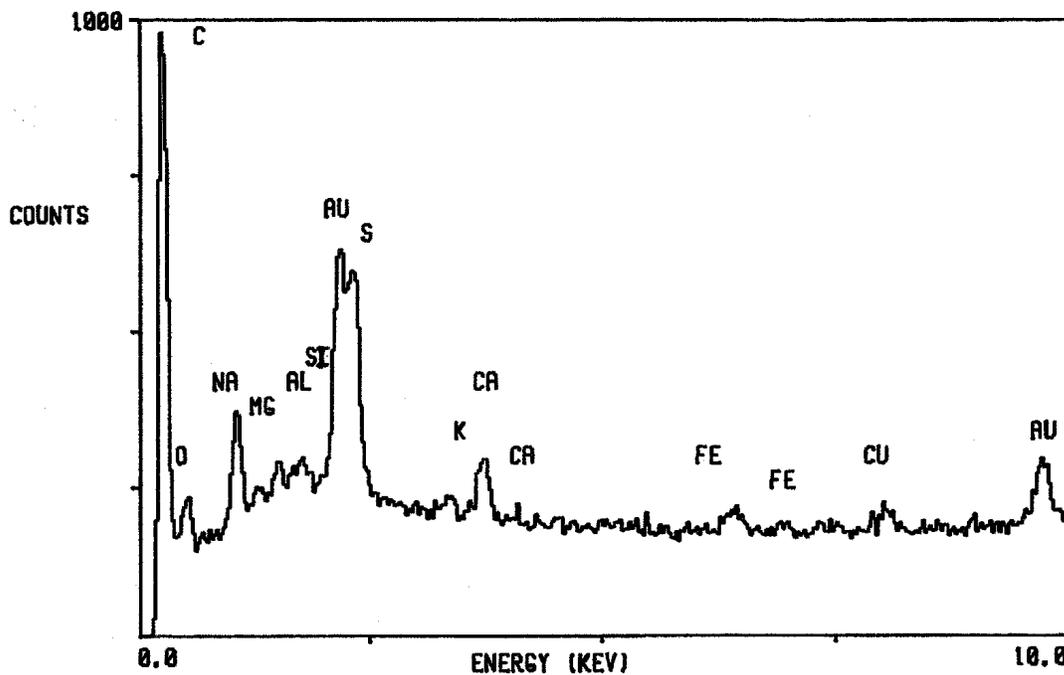


Fig. 9 – SEM/EDX of an inert matrix before addition of arterial blood from dead cow

THE BLUE GEL-LIKE SUBSTANCE - A MULTILEVEL ANALYSIS

Because the blue gel-like substance appeared to be unusual both to the veterinarian who performed the necropsy and to the two NIDS investigators, NIDS decided to conduct a multi-level analysis on the blue substance. This section on the blue gel is divided into descriptions of three levels of testing for the constituents of the blue gel and finally a fourth level which tests the hypothesis that the blue gel was an embalming gel. The four levels comprise:

Level 1:

- SEM/EDX testing of the blue gel
- GCMS analysis
- Infra red spectroscopy
- MBTH analysis for aldehydes

Level 2:

- Inductive coupled plasma mass spectrometry (ICPMS) A complete elemental (Lithium to Uranium) scan of the gel.
- Liquid Chromatography Mass Spectrometry (LCMS) Electrospray analysis of the gel.

Level 3:

- Head-space gas chromatography mass spectrometry (head space GCMS) of the gel.

Level 4:

Testing the hypothesis that the blue gel contains constituents of common embalming gels.

LEVEL 1: The following narrative appeared in the preliminary report.

- **THE BLUE GEL-LIKE SUBSTANCE:** In the experience of the veterinarian and of two NIDS investigators, the blue gel-like substance was unusual and had no precedence being found on a dead animal in the Uinta Basin. Therefore, it was determined that a chemical analysis was warranted. Note the photographs of the blue gel-like substance taken under magnification from around the eye and the ear (Fig. 10). Four different tests were done on the blue gel from the cow by a nationally accredited chemical analysis company. They were (1) Scanning Electron Microscopy/Electron Dispersive X-Ray analysis (SEM/EDX), (2) Gas chromatography mass spectrometry (GCMS) (attempted), (3) Infrared spectroscopy, and (4) Formaldehyde test. Figure

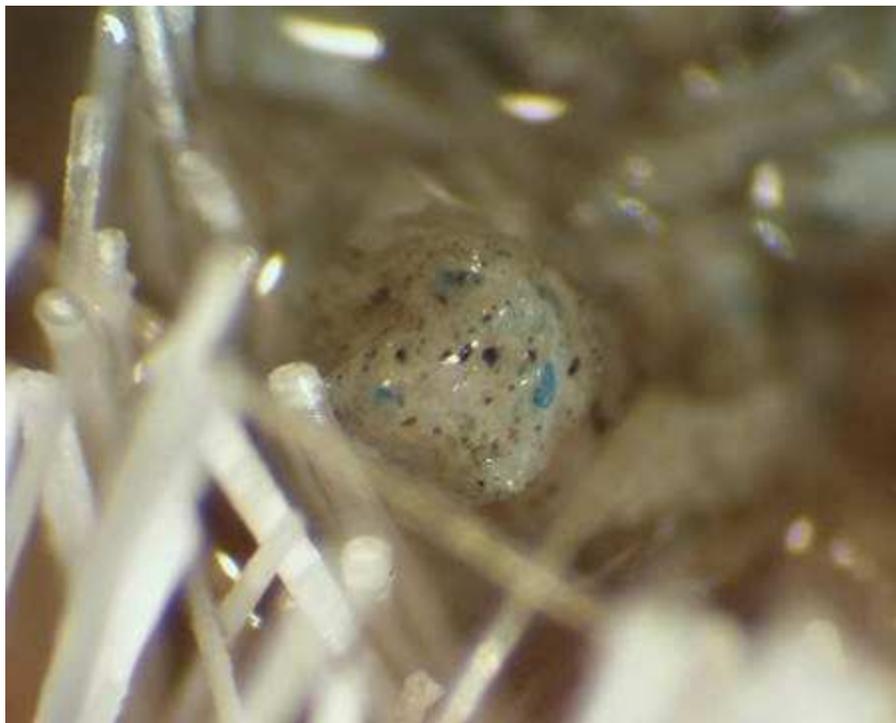


Fig. 10 – Blue gel-like substance on the periorbital skin (x 20)

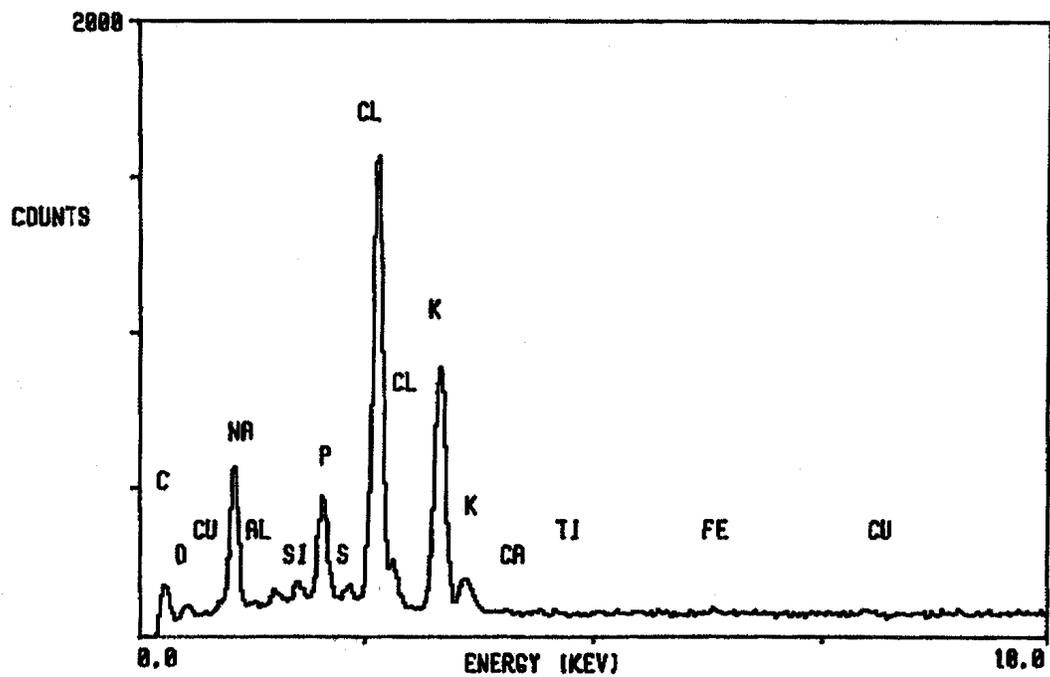


Fig. 11 – SEM/EDX spectrum of the blue gel-like substance

12 (below) shows the infrared spectroscopy profile of the substance.

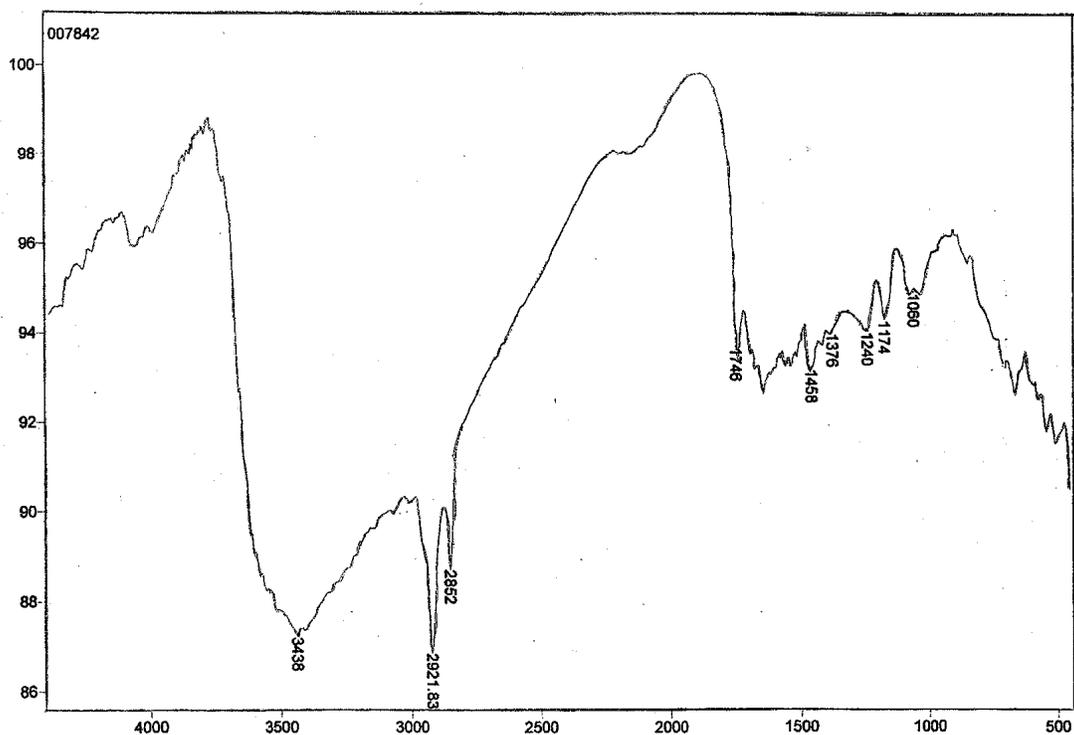


Fig. 12 – Infrared spectrum of the blue gel-like substance.

Figure 11 (previous page) shows the SEM/EDX profile for the blue gel-like substance that was obtained from the cow. Since the sample was ashed, a lower carbon content is shown than would be expected. The figure shows prominent peaks for Sodium (*Na*), Phosphorous (*P*), Chloride (*Cl*) and Potassium (*K*). The ratios and quantities appeared consistent with an organic, possibly a biological origin.

A GCMS analysis was attempted on the sample. It was extracted into methylene chloride/acetone and analyzed on GCMS. To compare against subsequent levels of testing, the following is a description of the initial methodology used:

- Sample extracted with 1:1 methylene chloride/acetone.
- Solvent dried with sodium sulfate
- 1 μ l splitless injection on HP-5 μ s column, 30 x 0.25 mm x 0.25 μ m film thickness
- Oven program: 50 °C for 2 minutes
 6 °C/min to 320 degrees
 Hold 5 minutes
- Inlet temp: 250 °C
- Carrier *He* at 1ml/min
- MS parameters: 35-450 a.m.u.
 2.6 scans/sec
- MS Model: HP 5971 MSD

RESULTS

No peaks whatsoever were detected from two different experiments. Operator or instrument error were ruled out. The flatline behavior on GCMS was unusual. Figure 12 shows the infrared spectroscopy profile of the substance. Characteristic absorbencies were identified by comparison with reference spectra. Strong carbonyl and hydroxyl absorbencies can be seen at 1746 cm^{-1} and 3838 cm^{-1} . The *C-H* stretching bands around 2900 cm^{-1} are also very strong. The spectrum appears to resemble an oxygenated lipid, but this is speculative.

The analytical company then performed an analysis for formaldehyde. The following is quoted from their report: "A spectrophotometric chemical analysis for formaldehyde was performed on the liquid portion of the sample. (Because of its low molecular weight, formaldehyde cannot normally be detected by GCMS analysis). A reagent containing MBTH (3-methyl-2-benzothiazoline) was added to the diluted sample. The test is highly specific and very sensitive. The sample absorbency was measured and quantified against a certified formaldehyde standard. The liquid portion of the sample was found to contain 20 parts per million of formaldehyde. Since formaldehyde is a volatile, highly reactive compound, the concentration measured in the laboratory may be significantly lower than the concentration at the time of sampling".

The analytical conclusions from all four analyses are summarized by the analytical company: "The sample can be characterized as a complex mixture of organic substances of biological origin and an aqueous solution containing formaldehyde. Since there is no biological process that produces formaldehyde and it is not a common environmental contaminant, the source of formaldehyde is unknown".

Suffice it to say, the presence of formaldehyde in the sample was indicative that the blue gel-like substance may have been applied to the animal, it did not come from the animal. This conclusion was borne out by both of the NIDS investigators, both experienced ranchers who had never seen any comparable substances on any of the dead animals.

LEVEL 2

ICP-MS analysis:

The finding of formaldehyde in the gel prompted NIDS to pursue another level of analysis. A 0.25 ml of the sample was added to a Teflon Bomb and 1 ml of Seastar grade nitric acid was added. The Bomb was capped tightly and heated (refluxed) until the sample was entirely digested. The digestate was then diluted with 10 ml of deionised water and analyzed by ICPMS (PE SCIEX Elan 6000) using NIST traceable multielemental solutions as calibration standards. Table I shows the entire duplicate ICPMS results (expressed as ppb-parts per billion). Duplicate assays were done and results of both are shown. The only noteworthy features were mean levels of 752.5 parts per billion Strontium and 400 ppb Barium. The significance, if any of these levels are unclear. Future work will establish local indigenous levels of these elements.

Table I - Levels of elements in blue gel-like substance (parts per billion)

Element	Assay 1	Assay 2
Lithium	149	151
Beryllium	<1	<1
Boron	200	220
Sodium	1220000	1250000
Magnesium	72000	66000
Aluminum	9000	7000
Silicon	40000	50000
Phosphorous	420000	434000
Potassium	904000	921000

Calcium	126000	126000
Scandium	20	10
Titanium	220	150
Vanadium	<50	<50
Chromium	90	90
Manganese	520	450
Iron	12700	12100
Cobalt	5	8
Nickel	30	50
Copper	1200	1110
Zinc	7800	7400
Gallium	5	4
Germanium	<1	<1
Arsenic	<50	<50
Selenium	110	120
Rubidium	336	343
Strontium	800	705
Yttrium	2.8	1.9
Zirconium	14	8
Niobium	1	<1
Molybdenum	24	23
Ruthenium	<0.1	<0.1
Rhodium	<0.1	<0.1
Palladium	<1	<1
Silver	<0.5	<0.5
Cadmium	3	2
Tin	11	9
Antimony	3	2
Tellurium	<1	<1
Caesium	1	<1
Barium	440	360
Lanthanum	10	<10
Cerium	30	10
Praseodymium	3	<1
Neodymium	10	<10
Samarium	<1	<1
Europium	0.5	<0.5
Gadolinium	2	<1
Terbium	<0.5	<0.5
Dysprosium	<1	<1
Holmium	<0.1	<0.1
Erbium	<0.5	<0.5
Thulium	<0.1	<0.1
Ytterbium	<0.5	<0.5
Lutetium	<0.1	<0.1
Hafnium	<1	<1
Tantalum	<0.5	<0.5
Tungsten	<1	<1
Rhenium	<0.1	<0.1
Iridium	<0.5	<0.5
Platinum	<0.5	<0.5

Gold	<0.5	<0.5
Mercury	<4	<4
Thallium	0.2	0.1
Lead	39	26
Bismuth	1.1	1.0
Thorium	6	<5
Uranium	1.7	1.6

LCMS Electrospray Analysis

Next, as part of level 2 analysis, LCMS Electrospray was conducted as follows. The sample was thawed at room temperature and 0.8465 g was weighed into a 20 ml disposable scintillation vial. Five grams of sodium sulfate was added to the vial. The sample was then mixed thoroughly and was doubly extracted with 5 ml dichloromethane (DCM) and the organic layer separated from the particulate sample and dried under Nitrogen gas. The residue of this procedure was then reconstituted with 1 ml of Electrospray buffer (50/5v/v MeOH/H₂O acidified with 0.1% acetic acid) and analyzed by flow injection analysis electrospray mass spectrometry.

A procedural blank sample was prepared by extracting de-ionized water using an identical procedure to that used for the sample. This procedural blank was sequenced immediately preceding and immediately after the sample to be analyzed to serve as a reference for the real sample. The sample was loaded into a HP 1090 auto-sampler and analyzed by flow injection analysis (FIA) with electrospray MS under positive mode. Mass spectral data was collected using full scan mode over the ranges 200 to 1200 AMU (Atomic Mass Units). The sample was injected three times, preceded and followed by two injections of a procedural blank. The mobile phase used was 50% *MeOH* acidified with 0.1% acetic acid. The flow rate of the mobile phase was 0.05 ml/min.

Figure 13 shows a typical spectrum obtained from a procedural blank. The spectrum was clean except for some ions of 242, 412, 506, etc. which are ions from solvent impurities commonly found when methanol and dichloromethane are used as solvents. Peaks of low intensity are expected and can usually be attributed to system background noise. Figure 14 is a spectrum obtained from the sample of the blue gel which shows the presence of some strong low mass ions eg. 323, 370 and 521. These ions were not found in the spectrum of the procedural blank. Note that the two spectra in the figures are scaled to the same intensity to allow direct comparison of signal intensity. In particular, three positive ions are fairly strong and unique to the blue gel sample. Figure 15 shows an extracted ion chromatogram of these three ions. Also notice ionized compounds of 370 and 521 have twin peaks and tailing while eluting from the system, indicating that the compounds are lipophilic in nature.

The identity of the three compounds was unresolved and because the sample size was so low after multiple testings it was decided to try a level 3 analysis which would yield some molecular identities from small sample volume. If there had been more sample available a tandem Liquid Chromatography Mass spectrometry (LC/MS/MS) technique could have been tried.

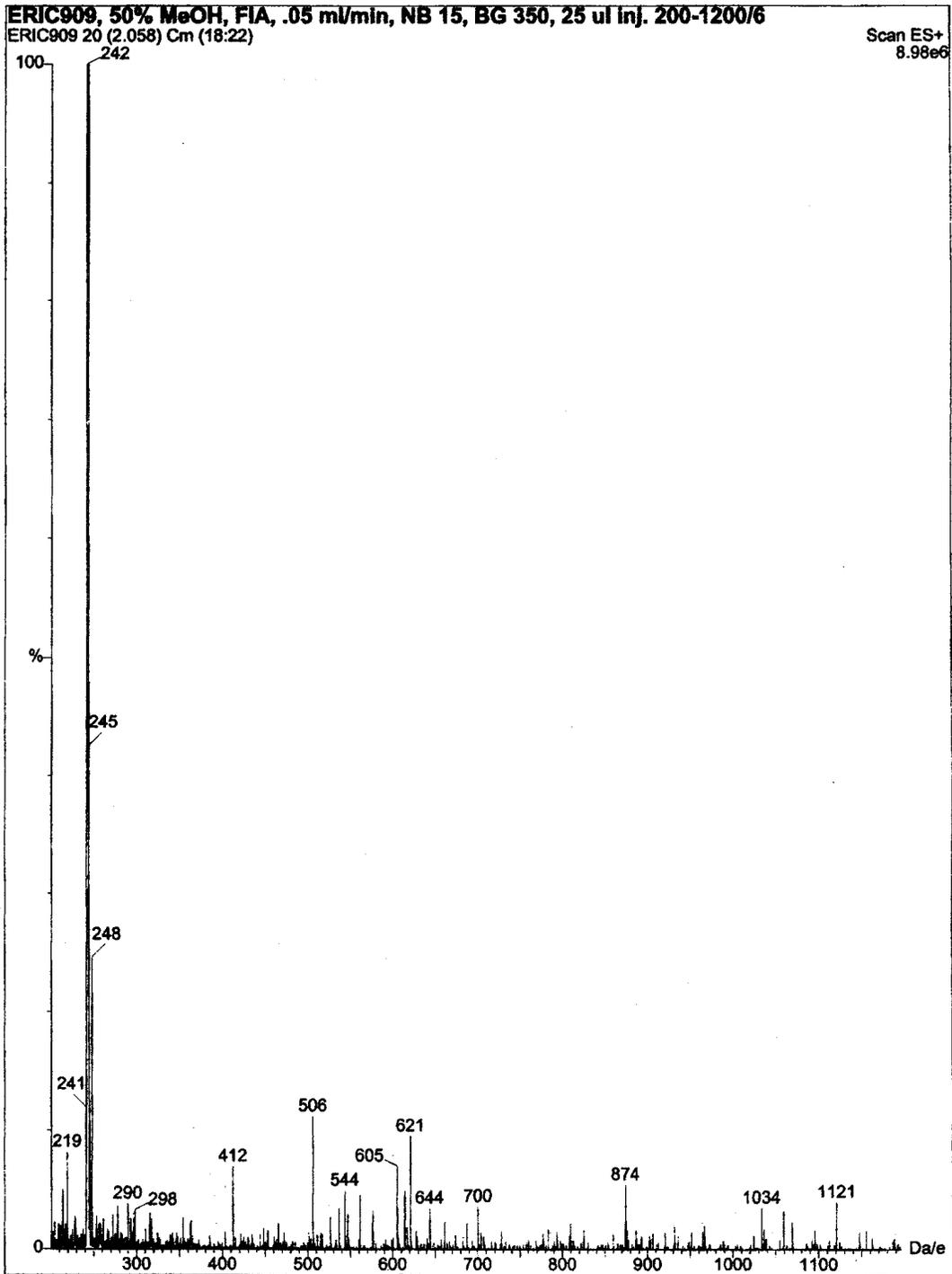


Fig. 13 – Spectrum of procedural blank

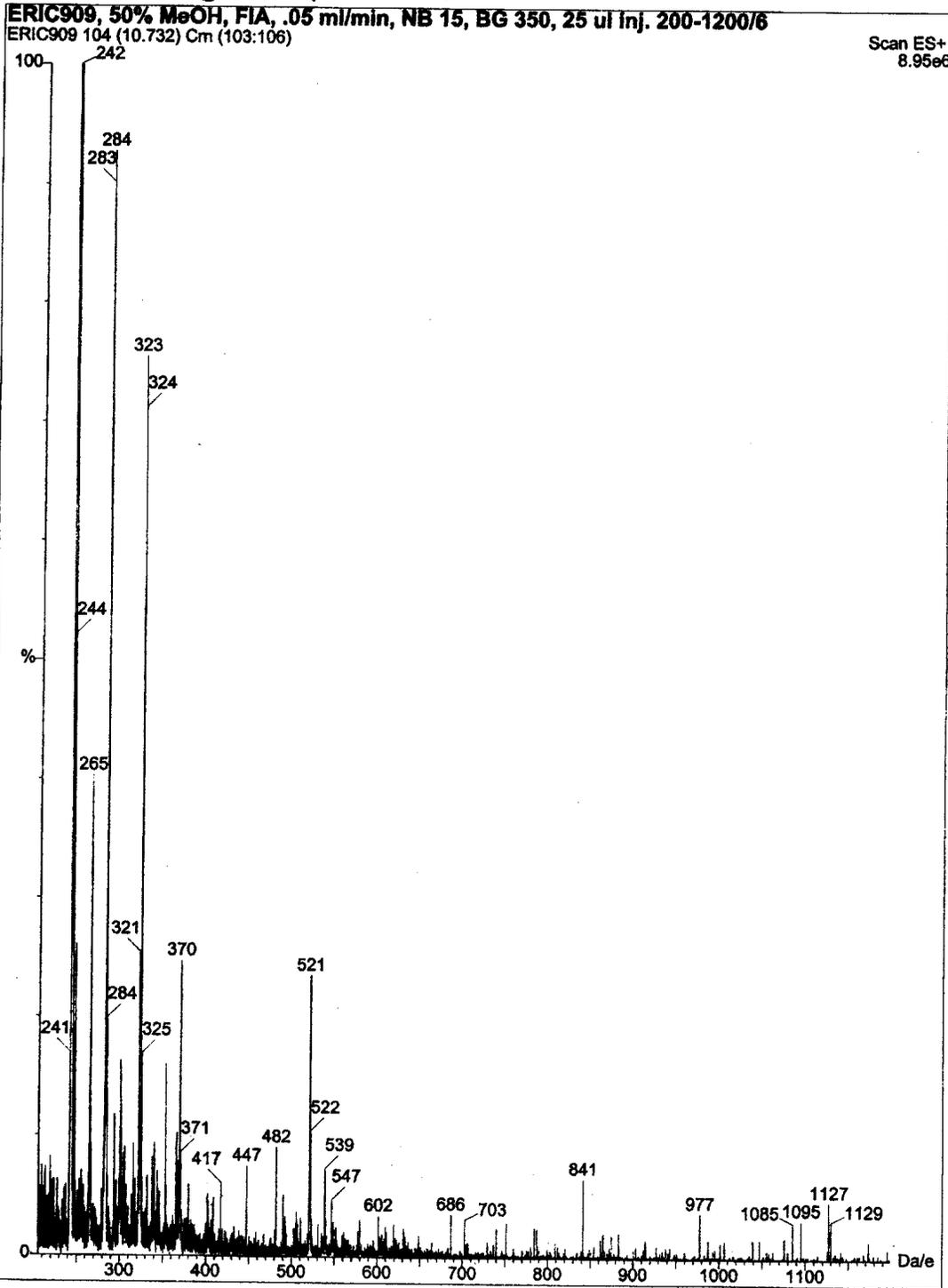


Fig. 14 – Spectrum of blue gel-like substance

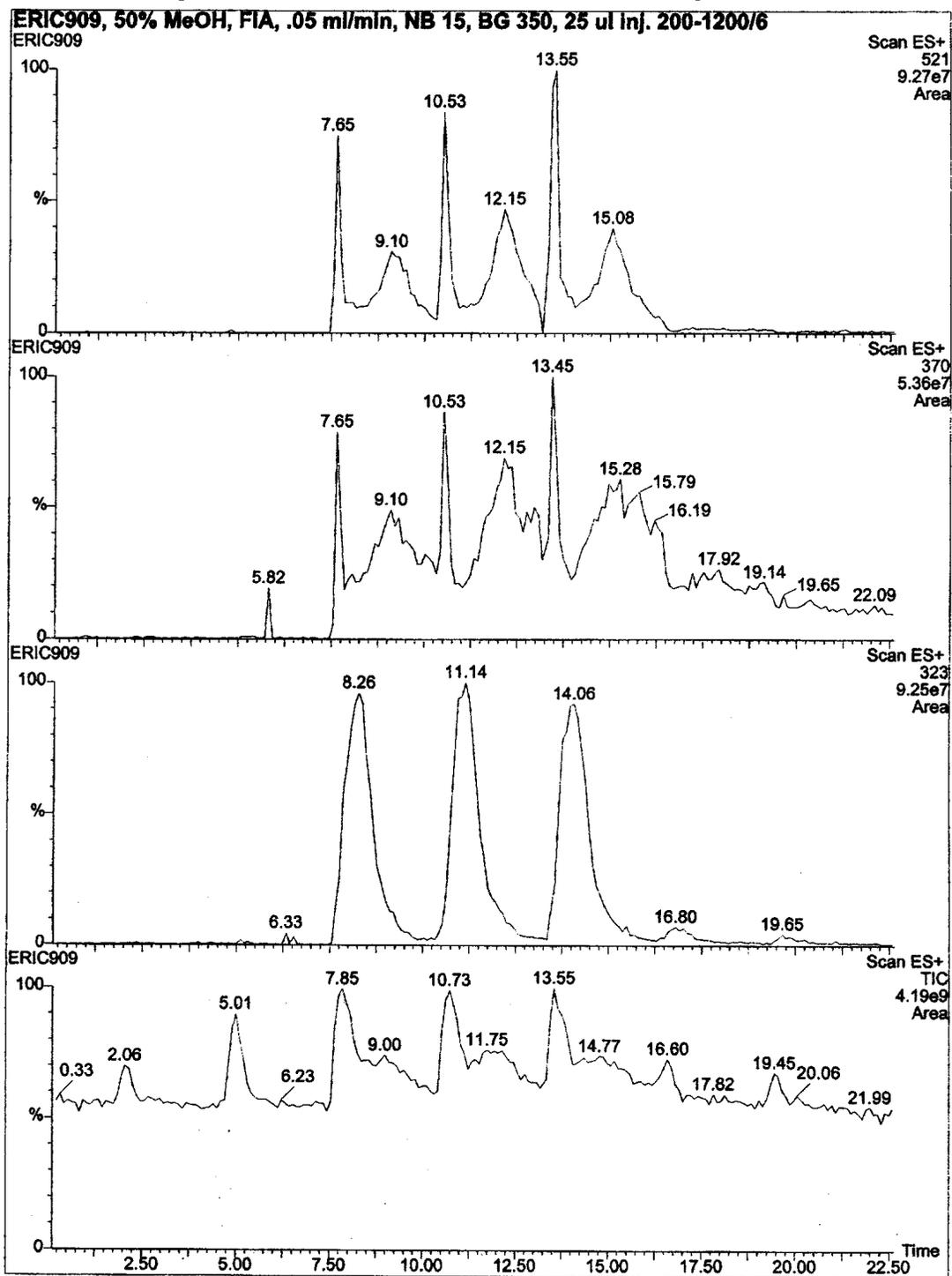


Fig. 15 – Extracted ion chromatogram of procedural blank and blue gel-like substance

LEVEL 3

Headspace GCMS analysis to try to identify volatile unknowns in the sample. The sample was analyzed by headspace GCMS using a Techomar 7000 headspace sampler with 7050 autosampler coupled via a cryotrap module to a Varian 3400 gas chromatograph and a Finnegan Incos 50B quadrupole mass spectrometer. The GC column used was a 30 m x 0.25 mm i.d. Rtx-5 capillary column coupled directly into the MS source. The spectrometer was tuned and mass calibrated immediately prior to use and data acquired using in fullscan mode, scanning from 35 to 400 AMU (Atomic Mass Units) at one scan per second. Instrumental conditions are listed below. Runs were made with and without matrix modifying solution (MMS, nitrogen purged 0.05 M phosphoric acid and saturated sodium chloride, pH<2) as recommended in US EPA method 5021/8260A. Prior to running the blue gel samples, system sensitivity was evaluated by analysis of a headspace vial containing an aliquot of a volatile organics calibration standard (10 ng/component) added to 10 ml of the MMS. Under these conditions a 10 ng spike of a VOC resulted in a detectable peak. Subsamples of the blue gel-like substance (approx 10-15 mg) were placed in 22 ml headspace vials and analyzed in open scan mode with and without the MMS. Empty headspace vials or vials containing only MMS were run to provide method blanks.

Headspace Conditions

Sample Loop	1.00 ml
Vial Size	22 ml
Vial Pressurization	10 psi
Pressurization Gas	Helium
Headspace Vial Temperature	95 °C
Mixing Time	2 minutes
Mixing power	5 (50% of maximum)
Loop Temperature	120 °C
Loop Fill Time	0.3 minutes
Transfer Line Temperature	220 °C
Inject Time (transfer to cryo)	2 minutes
Crytrap Temperature	-78 °C
Transfer Line Pressure	10 psi

Gas Chromatography

The volatiles collected from the headspace sampler were trapped at -78 °C in a cryofocus loop then partially separated using a temperature programmed Varian 3400 GC with a 30 m x 0.25 mm i.d., 0.25 µm film Restek Rtx-5 column coupled directly to the MS ion source. The GC program was initiated with a heating cryotrap using the following 22 minute temperature program.

Initial temperature	30 °C
Initial Hold	2 minutes
Ramp Rate	20 °C/min
Final Temperature	230 °C
Final Hold	10 minutes

Mass Spectrometry and Spectral Analysis

Components eluting from the GC were detected using a Finnigan 3400 Incos 50B quadrupole mass spectrometer operated in electron impact ionisation mode (40 eV) scanned from 35 to 400 AMU at one scan per second. Data were acquired from the start of the GC temperature program using a Data General DG 10 data system running Finnigan Incos software. Major compounds detected in the

blue gel sample were identified or classified based on mass spectra. Compounds for identification were selected based as the major peaks detected in the sample reconstructed ion chromatogram (RIC) which were absent from the method blank. Mass spectra were background subtracted, using local scans as background and forward searched against the NIST library of mass spectra (44,000 entry library). Spectra were also analyzed by manual interpretation.

Table II - Results

Peak #	Scan#	Retention Time	Molecular Weight	Compound Inferred (or class)
1	63	1:03	88	N,N-dimethylethanediamine
2	310	5:10	114	2-heptanone
3	367	6:08	75	2-methoxyethanolamine
4	422	7:03	142	2-nonanone
5	441	7:22	121	2-ethylbenzeneamine
6	599	9:59	220	2,6-bis-dimethylethyl-4-methyl phenol
7	667	11:07	194?	C14 branched alkene?

Compounds 6 and 7 in Table II are of the most interest. Compound 6, which is 2,6-bis-dimethyl-4-methyl phenol, is also known as BHT and was present at low amounts in the analytical blank (see Table III), but was in about 5-30X excess in the blue gel sample. The concentration in the blue gel was estimated to be approximately 300-3000 µg/g. The analytical lab stressed the approximate nature of this figure and the fact that BHT was also found (albeit at greatly reduced amounts) in their reagent blanks. While we stress that it cannot definitely be ruled out that the BHT leached from the plastic test tube container and was therefore artifactual, NIDS considers its presence in the sample of blue gel at relatively high concentrations unusual. See Table II below for comparison of concentrations found in three blanks versus two analyticals. As noted by the analytical company, there was also inhomogeneity in the distribution of the BHT within the blue gel sample. BHT has anti-oxidant as well as anti-virus properties. Its non-polar nature make it ideal for inactivating lipid containing viruses (Snipes, et al. (1975), *Science*, **188**, p. 64). It did not escape our notice that BHT has also been used in animal feeds, but at very low concentrations. In addition, BHT has been used in Bloat Guard (manufactured by Pfizer Inc.) which is a liquid feed supplement for control of legume and wheat pasture bloat in cattle. However the recommended concentration of BHT in the liquid mix even before the animal consumes and further dilutes it, is 2 to 20 times *less* than the concentration found in the blue gel from the cow. In addition, it would be highly unusual for BHT from liquid feed to concentrate around the periorbital or anal area of the animal. Note that the blue gel was found above the anal area, so it was not excreted. And finally, it was confirmed by the rancher that he was *not* using liquid feed mix on his herd.

Thus, in NIDS opinion, the BHT found in the blue gel is unlikely to have come from the cow and its presence at relatively high concentrations in the blue gel sample (compared to the blanks) points away from a laboratory artifact. Rather, the evidence indicates that the substance was added to the cow sometime before the rancher discovered the dead animal. In contrast, compounds 1-5 in Table II are expected putrefaction breakdown products from cow tissue and can be interpreted as contaminants of cow tissue in the sample. Compound 7 was not unambiguously identified but may be a branched chain alkene. If so it is an unusual alkene. Without further unambiguously identifying the compound 7, we cannot further comment on it.

Table III - Levels of 2,6-bis-dimethylethyl-4-methyl phenol in sample and blank runs.

RUN ID	Sample Description	Peak Area (205 ion)
VO97_493	Blank 3	3388
VO97_494	Blank 4	3429
VO97_495	Blue Gel Sample	15248
VO97_496	Blue Gel Sample	61726
VO97_497	Blank 5	2253

LEVEL 4

Comparison of blue gel-like substance with all known blue gels from mortuary samples in the United States. Because of the low levels of formaldehyde present in the blue gel-like substance and under advice from the NIDS science advisory board, it was decided to investigate whether the blue gel was comparable to any known embalming gels currently in use in the United States. The following companies were contacted regarding the production of blue embalming gels: Pierce, Inc. in Texas; Dodge, Inc. in Massachusetts; Hydrols in Philadelphia; Kelco in Minnesota; Frigid in Illinois; Esco in Connecticut; and Bondol Fluid in Arkansas. It was determined that only three blue embalming gels made by different companies existed on the market in the United States. The names of the companies manufacturing these three gels for proprietary reasons are not listed. Finally, it was found that no mortuaries in either Vernal or Roosevelt, Utah admitted to using blue embalming gels.

In order to compare the blue gel found on the dead animal with the three available blue embalming gels, a level 1 analysis (SEM/EDX, GCMS, FTIR and MBTH) was conducted on all three embalming gels. The full comparative report, together with the identification of the constituents of the embalming gels, is given in Appendix 3 at the end of this document. The conclusion from this part of the work was that the blue gel-like substance found on the cow in Utah did not conform to any of the blue embalming gels currently in use in mortuaries in the United States.

CONCLUSIONS

The totality of the evidence that is described in this report (from the results of the investigators, the necropsy findings and the several different lab analyses) suggest that this animal did not die a natural death. In addition, the blue gel-like substance was probably added to the animal shortly prior to, during or shortly after the time of death, and before the arrival of the rancher. It was determined that the gel-like substance was a complex mixture containing decomposed biological tissue (it had been scraped from the cow's anal region) as well as formaldehyde and significant levels of a synthetic phenol containing compound (2,6-bis-dimethylethyl-4-methyl phenol).

The animal mutilation field has been plagued by a severe lack of reproducible results. It is the opinion of the NIDS scientific staff that investigation of animal mutilations is tantamount to being useless unless a full necropsy is performed. As a public service, NIDS has published complete guidelines, on the NIDS web page, for investigators and veterinarians when investigating and necropsying animal mutilations (see, <http://www.accessnv.com/nids/page4.shtml>). Please note that a complete necropsy is essential. After about 48 hours post mortem in the summer (or 96-120 hours in the winter or at high altitudes), the value of in-depth investigation becomes progressively and rapidly diminished. This is because post mortem decay obliterates any useful evidence and renders interpretation almost impossible. Hence, claims of "surgery" involving "high heat" or "laser surgery" being performed on animals found several days after death are dubious at best. Even the highest quality forensic analysis cannot interpret severely decomposed tissue.

Therefore, NIDS requests that all investigators examining a fresh (suspected) animal mutilation to investigate the following aspects:

- The compass direction in which the animal is laying.
- If eye(s) or ear(s) has been removed, note whether left or right.
- Hemorrhaging under the skin around the neck or the back of the animal.
- Liver copper levels need to be ascertained.
- Complete blood chemistry, including electrolytes, be performed being cognizant of the difficulty in interpreting post-mortem (and hemolyzed) blood samples.

APPENDIX 1

NECROPSY REPORT

NAME: COWS
 SPECIES: BOVINE
 BREED: HEREFORD
 DATE: OCTOBER 17, 1998

PATIENT HISTORY FILE (COWS)

11:00 AM NECROPSY ON COW FOUND DEAD YESTERDAY ABOUT 3:00 PM. COW WAS OKAY DAY BEFORE YESTERDAY. COW WAS IN STERNAL RECUMBENCY WITH REAR LEGS STRADDLED OUT. TIP OF LEFT EAR REPORTEDLY WAS CUT OFF AND THE INVESTIGATOR HAD REMOVED END OF EAR LAST NIGHT. THE LEFT EYE WAS MISSING WITH ABOUT 2-3 CM OF ENTIRE UPPER EYELID. NO SIGNS OF STRUGGLE IN WET GROUND COW WAS IN. APPEARED TO HAVE DIED INSTANTLY AT THAT LOCATION. SUBCUTANEOUS HEMORRHAGES WERE FOUND IN NECK AND INGUINAL AREAS, EXTENDING DOWN LEGS WITH ASSOCIATED EMPHYSEMA. ABDOMEN WAS OPENED AND STOMACH WAS NOT VERY DISTENDED WITH GAS, ALTHOUGH FULL OF NORMAL FEED CONTENTS. SMALL INTESTINES WERE SEVERELY DISTENDED WITH GAS. ALSO FOUND A BLOOD CLOT AMONG SMALL INTESTINES ABOUT 4 CM DIAMETER AND ABOUT 40 CM LONG. INTESTINES WERE VERY FRIABLE AND TORE WITH SLIGHTEST TENSION ON THEM. SPLEEN WAS DARK AND SOMEWHAT HEMORRHAGIC. LIVER WAS VERY FRIABLE. VENTRAL DIAPHRAGM WAS ALSO HEMORRHAGIC. THORACIC CAVITY SHOWED GREENISH/BLUE TINGE COLOR ON SEROSAL SURFACE AS DID SURFACE OF THE SKIN AT CUT EDGES AS WELL AS LIPS OF VULVA AND RECTUM. LUNGS SHOWED EVIDENCE OF EMPHYSEMA AND PALER THAN NORMAL. HEARTH WAS EMPTY OF BLOOD AND VERY FRIABLE. WHEN TOUCHED HEART, IT FELL APART AND WAS ALMOST UNIDENTIFIABLE AS CARDIAC TISSUE. SOME BLOOD WAS COLLECTED FROM BRACHIAL ARTERY, AND SOME THAT POOLED ON THE SKIN FROM THE SUBCUTANEOUS HEMORRHAGE IN THE NECK. SAMPLES WERE ALSO TAKEN FROM THE CUT EDGES OF THE SKIN NEAR THE EYE, LIVER, SPLEEN, LUNGS AND HEART. THE HIGH TEMPERATURE YESTERDAY WAS ABOUT 45 DEGREES AND ABOUT 50 DEGREES TODAY. THE AMOUNT OF DECOMPOSITION SEEMED MORE ADVANCED THAN EXPECTED WITH THE CLIMATIC CONDITIONS, ALTHOUGH OTHER TISSUES SUCH AS STOMACH AND SKELETAL MUSCLES SHOWED DECOMPOSITION ABOUT AS EXPECTED. NO DEFINITIVE CAUSE OF DEATH WAS DETERMINED BASED ON RESULTS OF GROSS NECROPSY. UTERUS WAS ENLARGED ABOUT 10-12 CM DIAMETER. ON PALPATION NO EMBRYO OR FETUS WAS PALPATED IN UTERUS. THERE WAS ALSO NO FLUID EVIDENT IN

BD 86	TEST: Lepto 5 L. Canicola	Negative
BD 86	TEST: Lepto 5 L. Grippotyphosa	Negative
BD 86	TEST: Lepto 5 L. Pomona	Negative
	TEST: Chemistry – Sample Preparation	
LIV 86	TEST: Arsenic	Dry Weight Analysis 0.09 ppm
LIV 86	TEST: Cadmium	Wet Weight Analysis 0.07 ppm
LIV 86	TEST: Lead (TISSUES)	Wet Weight Analysis < 0.2 ppm
LIV 86	TEST: Mercury	Wet Weight Analysis < 0.01ppm
LIV 86	TEST: Copper	Dry Weight Analysis 5.48 ppm
LIV 86	TEST: Iron	Dry Weight Analysis 142 ppm
LIV 86	TEST: Molybdenum	Dry Weight Analysis 0.69 ppm
LIV 86	TEST: Selenium	Dry Weight Analysis 1.08 ppm
LIV 86	TEST: Zinc	Dry Weight Analysis 83.1 ppm
LIV 86	TEST: Vitamin A	Wet Weight Analysis 3.18 µg/g
LUNG 86	TEST: Cyanide	None Detected
	TEST: Histopathology 1-3 Slides	
LUNG 86	TEST: Virus Isolation (FOOD ANIMAL)	Negative
LUNG 86	TEST: FA TEST, IBR	Negative

TEST: FA TEST, BVD
LUNG 86 Negative

TEST: FA TEST, BRSV
LUNG 86 Negative

TEST: FA TEST, PI-3
LUNG 86 Negative

TEST: BOV Respiratory Serology Panel

TEST: IBR SN Serology
BD 86 <=1:4

TEST: BVD SN Serology
BD 86 1:16

TEST: BRSV SN Serology
BD 86 <=1:8

TEST: PI-3 SN Serology
BD 86 1:64

TEST: Slide, Extra Slide Per Pathologist

COMMENT CODE COMMENT NARRATIVE

AS5 ARSENIC Diagnostic level: Bovine: Liver & Kidney (DW) – Normal, < 1.5 ppm

CD7 CADMIUM Diagnostic level: Bovine: Liver (WW) – normal, < 1.0 ppm; high, 1.4-9.0 ppm; toxic, > 50 ppm

Cu3 COPPER Diagnostic level: Bovine: Liver (DW) – normal, 100-600 ppm; deficient, < 40 ppm is inadequate to maintain a normal serum copper level; toxic, > 600 ppm. Please note that fetal and newborn liver copper is accumulated at the expense of the dam. Dietary molybdenum and sulfur decrease copper utilization. Excessive zinc and iron may also decrease copper utilization.

IR1 IRON Diagnostic level: Bovine: Liver (DW) – normal, 180-1,200 ppm; deficient, < 120 ppm; toxic, 200-2,800 ppm.

LD6 LEAD Diagnostic level: Bovine: Liver (WW) – normal, < 0.1-1.0 ppm; toxic, > 10.0 ppm.

ME2 MERCURY Diagnostic level: Bovine: Liver (WW) – normal, < 0.06 ppm; toxic, > 2.00 ppm.

MO2 MOLYBDENUM Diagnostic level: Bovine: Liver (DW) – normal,

0.6-6.0 ppm; toxic, < 10.0 ppm. Note that molybdenum toxicity occurs only when copper levels are deficient. Molybdenum levels in the toxic range are not a problem when copper levels are normal.

- SE8 SELENIUM Diagnostic level: Bovine: Liver (DW) – normal 0.3-1.0 ppm; deficient, < 0.2 ppm; toxic, > 5.0 ppm.
- VA1 VITAMIN A Diagnostic level: Bovine: Liver (WW) – normal 30-80 µg/g; < 30 µg/g indicates an inadequate store to maintain a normal serum vitamin A level. Newborn and fetal liver and serum are very low.
- ZI2 ZINC Diagnostic level: Bovine: Liver (DW) –normal, 90-400 ppm; deficient, < 75 ppm.
- s12 Lepto titers of less than 1:100 are reported as negative.
- s2 Titer indicates exposure, infection, or vaccination. Paired Acute and convalescent sera are needed for interpretation.
- s7 Unknown substances in serum interfered with cells used in the test. Titer is equal to or less than that given. Please submit another sample for accurate testing.

APPENDIX 3

Analysis of three blue embalming gels used within the United States.

Background:

The purpose of the Level 4 analysis was to test the hypothesis that the blue gel might be an embalming gel. Upon advice from members of the NIDS science advisory board, and based on the formaldehyde content of the gel, it was decided to test this hypothesis. Additionally, some of the bluish gel was also noticed in the thoracic cavity of the animal. This also was thought to lend some credence to the hypothesis that an attempt at embalming parts of the animal (for an unknown purpose) might have taken place. It was decided to conduct the same Level 1 suite of tests on the three embalming gels that are used nationally. Thus the exact four tests that were done on the blue gel from the dead animal were also conducted on the three embalming gels.

WORK REQUESTED

Chemical Analysis for:

- (1) Elemental Analysis (SEM/EDX)
- (2) Organic Analysis (GC/MS)
- (3) Material Characterization (FTIR)
- (4) Formaldehyde Analysis (MTBH Colormetric)

DESCRIPTION OF SAMPLES

Three (3) samples reported to be a "blue gel" and identified as #1, #2 and #3.

CHEMICAL ANALYSIS

(1) Elemental Analysis (SEM/EDX)

Samples were dried at 105 °C prior to analysis. The residual sample was stud mounted and subjected to EDX spectroscopic analysis.

Sample #1 exhibited a complex X-Ray spectrum (Figure 1) with major peaks corresponding to sodium, chlorine, sulfur and carbon. Lesser concentrations of oxygen, phosphorus, lead, calcium and copper. The spectrum is consistent with an organic material containing high levels of sodium chloride and other inorganic impurities.

The spectrum for sample #2 (Figure 2) was much less pronounced, suggesting a more volatile matrix, which was evaporated during the drying step. The aluminum peak found in both spectra is likely an artifact deriving from the aluminum sample stud. The observed chemistry of sample #2 is similar to sample #1, with carbon, oxygen, sodium and chloride being major constituents. The sample had a greater relative concentration of phosphorus and no indication of sulfur or lead. Traces of calcium and copper were also observed.

Sample #3 had a different X-Ray spectrum again (Appendix 3, Figure 13) showing significant residual carbon and oxygen, indicating the high organic content of the sample. Peaks corresponding to common inorganic salts were also observed. These included calcium and sodium. Additionally, a large sulfur peak can be seen. (The sample was gold sputtered). The compound was obviously different from the first two and different again from the original blue gel (see spectrum Figure 11 in the main body of the report).

CHEMICAL ANALYSIS

(2) Organic Analysis (GC/MS)

The three gel samples were prepared for analysis by extraction with a mixture of methylene chloride and acetone. The extract was collected and dried over sodium sulfate and injected into the GC/MS system, where individual organic compounds were separated and identified by characteristic mass spectra. The results of this analysis are summarized in Table I (below).

Table I - GAS CHROMATOGRAPHY/MASS SPECTROSCOPY

<u>Sample</u>	<u>Identification</u>	<u>Label</u>	<u>Figure No.</u>
1	Total Ion Chromatogram	-	3
	2,2' Oxybis ethanol	A	4
	Cineole	B	5
	2,2',2''-Nitrilotris ethanol	C	6
2	Total Ion Chromatogram	-	7
	Unidentified Compound	D	8
	Terpineol	E	9

The compounds identified as Eucalyptol (1,8-Cineole) and Terpineol are normally used as fragrance additives. Diethylene glycol (2,2'-Oxybis ethanol) is an antifreeze additive and Triethanolamine (2,2',2''-Nitrilotris ethanol) is a viscous liquid which might be used as a thickening agent.

Blue gel #3 did not appear to be soluble in methylene chloride. After extraction, the solvent was collected, filtered and injected into the GCMS system. The developed TIC is shown in Figure 14, Appendix 3. A single major peak was observed which could not be identified by library searches.

The lack of any GCMS spectrum comparisons from either Level 1, 2 or 3 with the three blue embalming gels shown above suggest that they are not similar. The original bluish gel from the cow did not have the same spectral characteristics as the three embalming gels tested in identical manner.

(3) Material Characterization (FTIR)

Each sample was prepared by drying a film of blue gel on a salt slide. The FTIR spectra developed for each sample are listed in Table II (below).

Table II - FTIR SPECTROSCOPY RESULTS

<u>Figure No.</u>	<u>Description</u>
11	Sample 1 (Dark Blue Gel)
12	Sample 2 (Light Blue Gel)
13	Comparative Scan of 1 and 2
15	Sample 3

Sample #1 presented a much richer infrared spectrum than sample #2, probably due to the fact that a larger fraction of the sample remained after drying. A strong hydroxyl absorbance is present at 3350 cm^{-1} . The absorbances at 1590, 1400 and 900 cm^{-1} are all associated with alkene structures (carbon-carbon double bonds). The peak at 3150 cm^{-1} is most likely associated with the nitrogen-hydrogen bond of an amine group. Finally, the two absorbances at 510 and 570 cm^{-1} are indicative of carbon-halogen bonds. No single compound could be identified which would account for all major features of the spectrum. The sample is likely a complex mixture of residual additives and impurities concentrated by the removal of water and other volatile components.

Sample #2 exhibited a much simpler spectrum. Only two major functional groups were identified: Hydroxyl absorbances at 3390 and 1070 cm^{-1} and carbon-hydrogen absorbances at 2900 and 1400 cm^{-1} . (The strong absorbance at 2400 cm^{-1} is an artifact from CO_2). The relative strength of the hydroxyl absorbances is suggestive of a glycol or glycerin, but no conclusive matches could be established.

The FTIR spectrum from sample blue gel #3 is shown in Figure 15 (Appendix 3). The hydroxyl, carbonyl and hydrocarbon absorbencies in the spectrum are clearly defined indicating a simple matrix.

It is to be emphasized that all three FTIR analyses did not match the FTIR spectrum from the original blue gel suggesting they were different mixtures.

(4) Formaldehyde Analysis (MTBH Colormetric)

<u>Sample</u>	<u>Formaldehyde (%)</u>
1	9.89
2	17.0

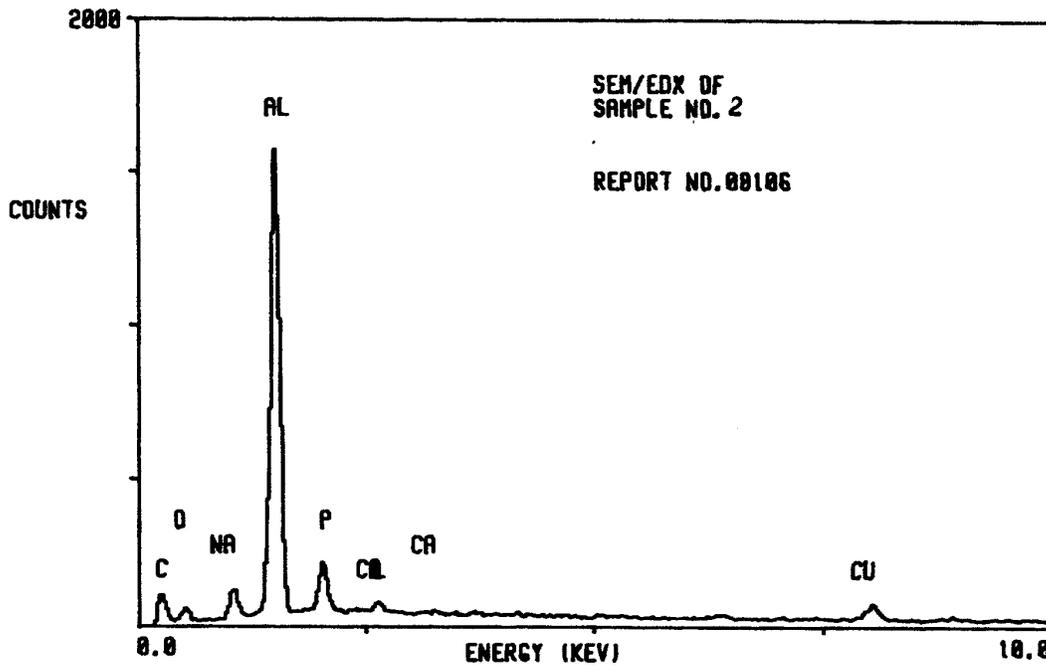
No formaldehyde test was performed for sample #3.

CONCLUSION

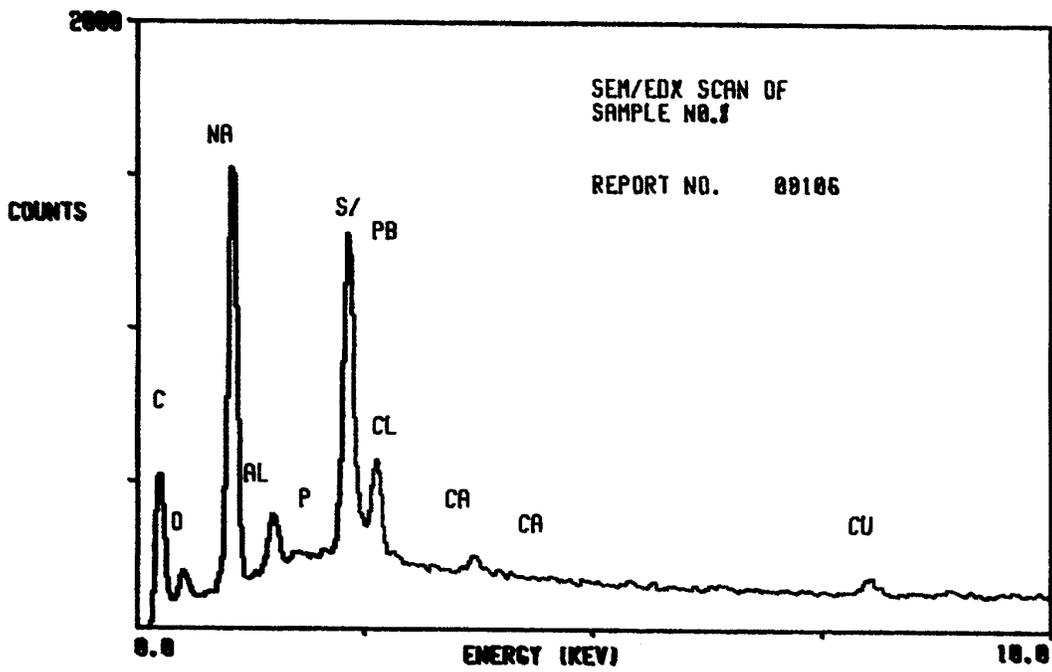
The three blue gel samples, although displaying similar chemical properties, showed considerable differences in product formulations. Although water content was not determined, it is likely that all three samples could be characterized as aqueous formaldehyde preparations containing emulsifying or thickening agents, artificial fragrances and other additives. GC/MS analysis identified

some of these additives, but there may be other non-volatile constituents which cannot be determined by the protocols employed.

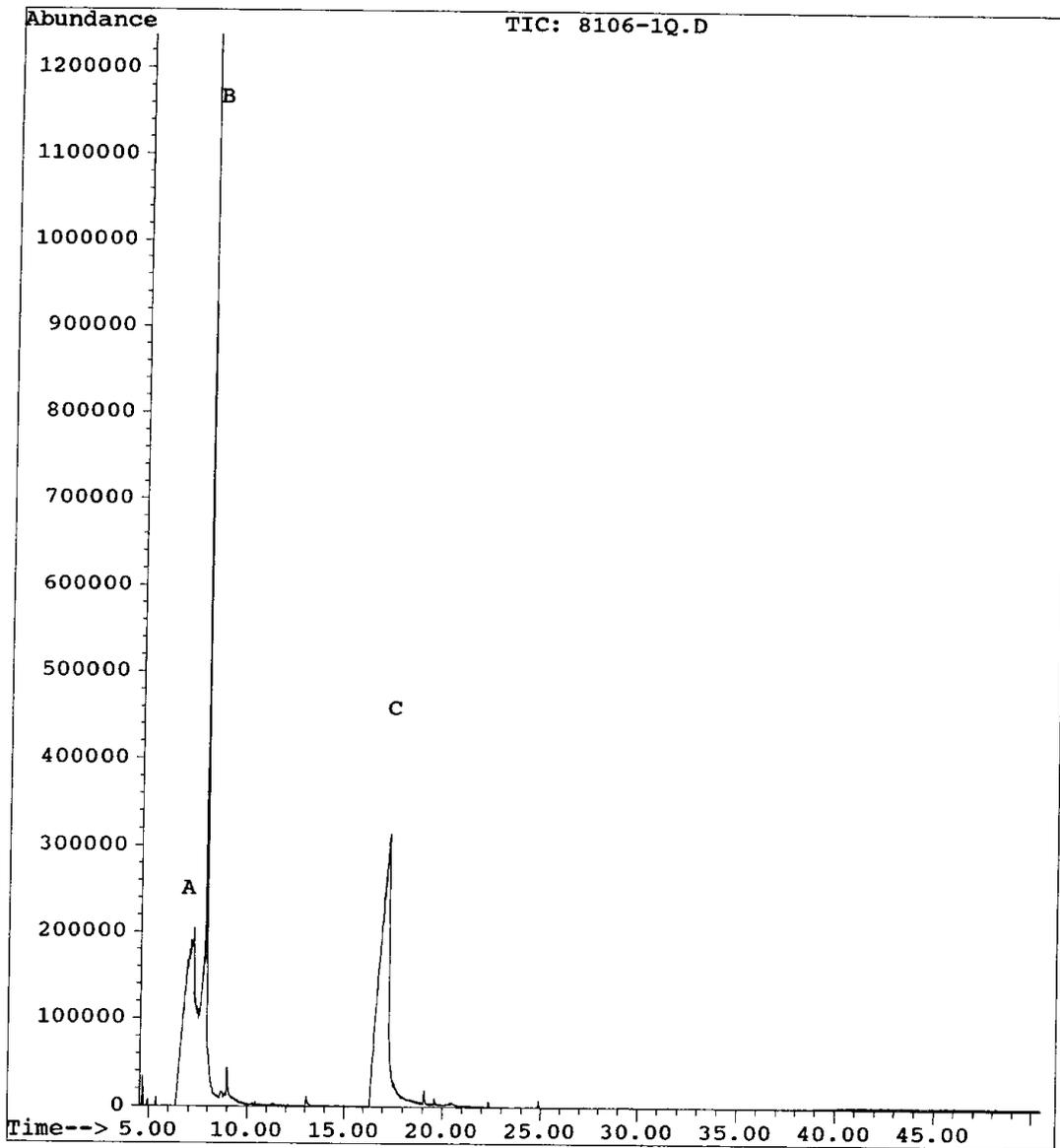
Finally, the SEM/EDX elemental compositions, the different GCMS behavior and the different FTIR profiles all suggest that the blue gel that originated from the cow was NOT any of the three embalming gels tested.



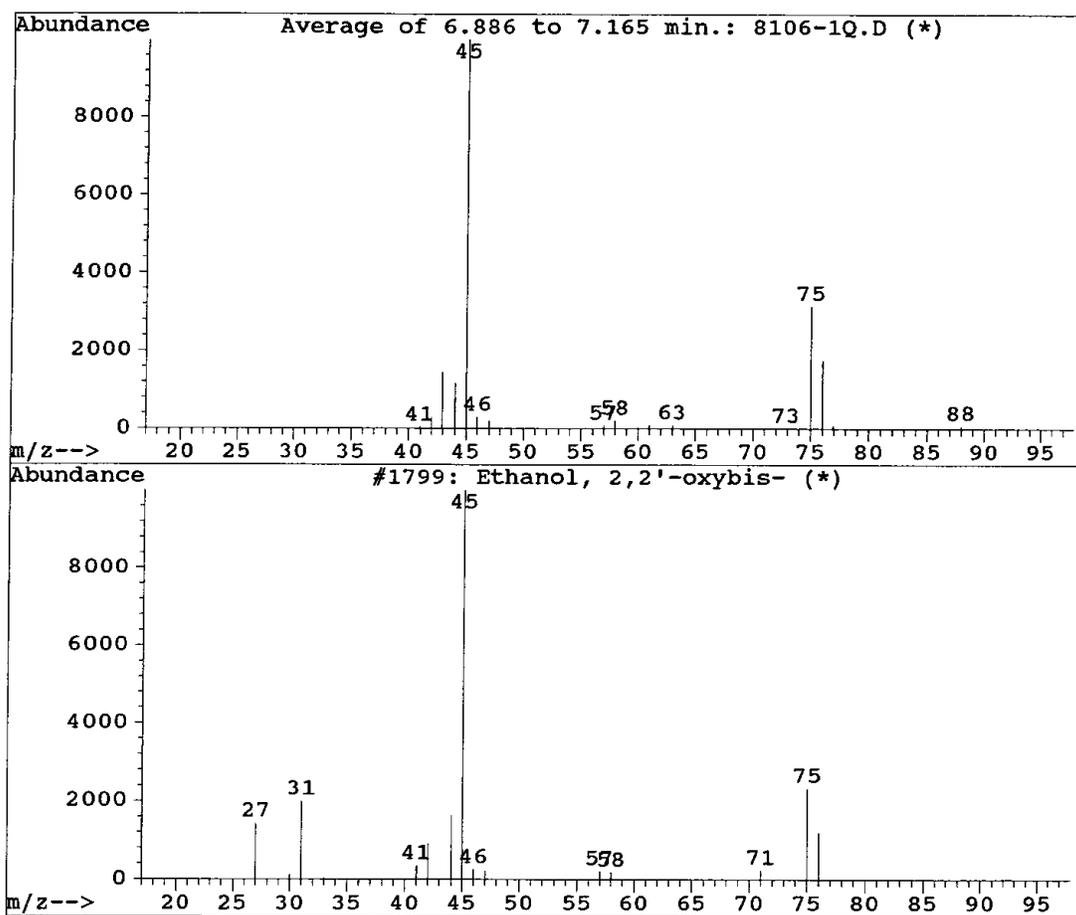
Appendix 3 – Fig 1 – SEM/EDX scan of embalming gel # 1



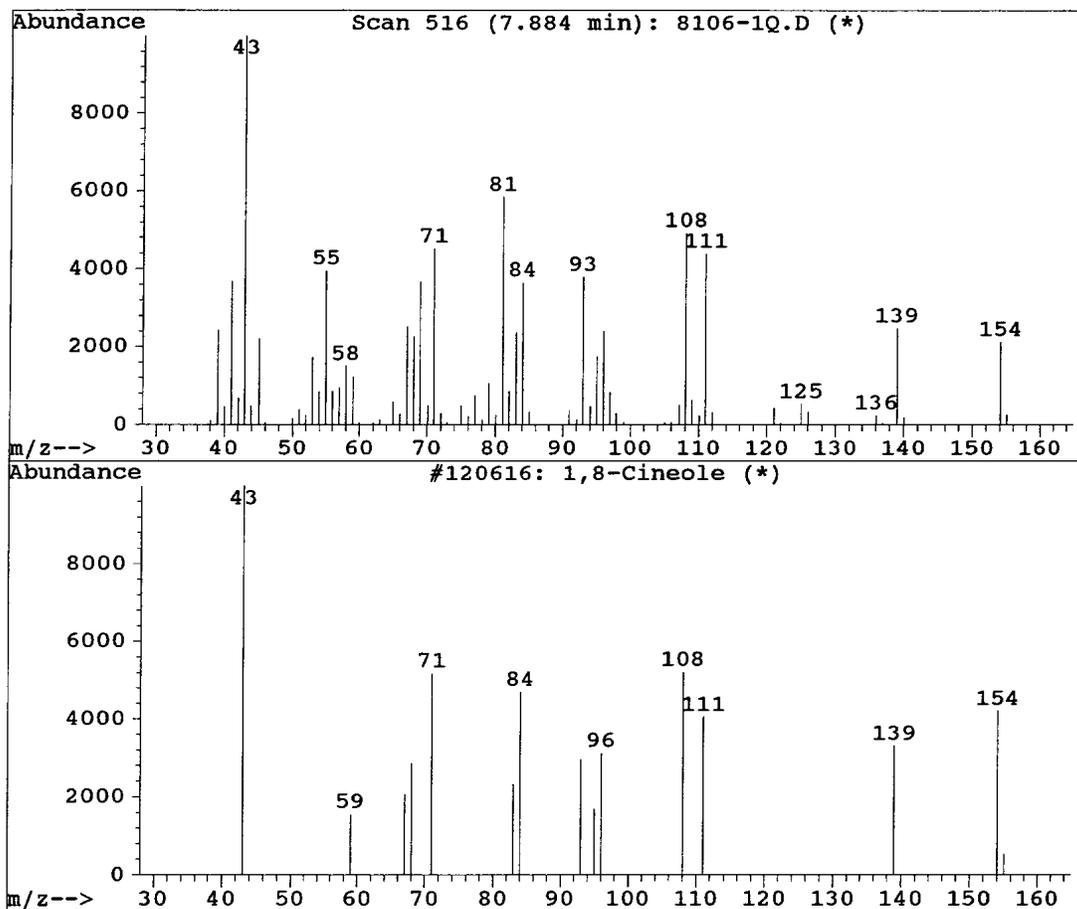
Appendix 3 – Fig. 2 – SEM/EDX scan of embalming gel # 2



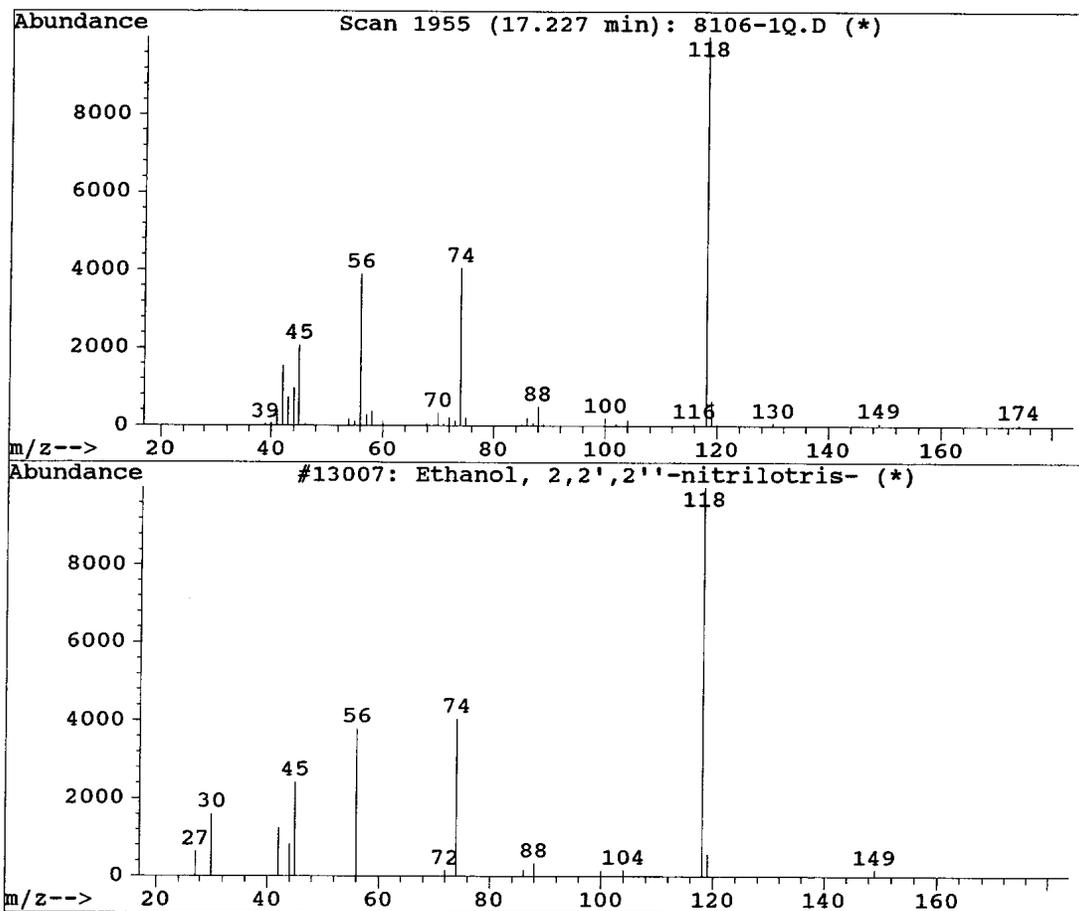
Appendix 3 – Fig. 3 – Total ion chromatogram for embalming gel # 1



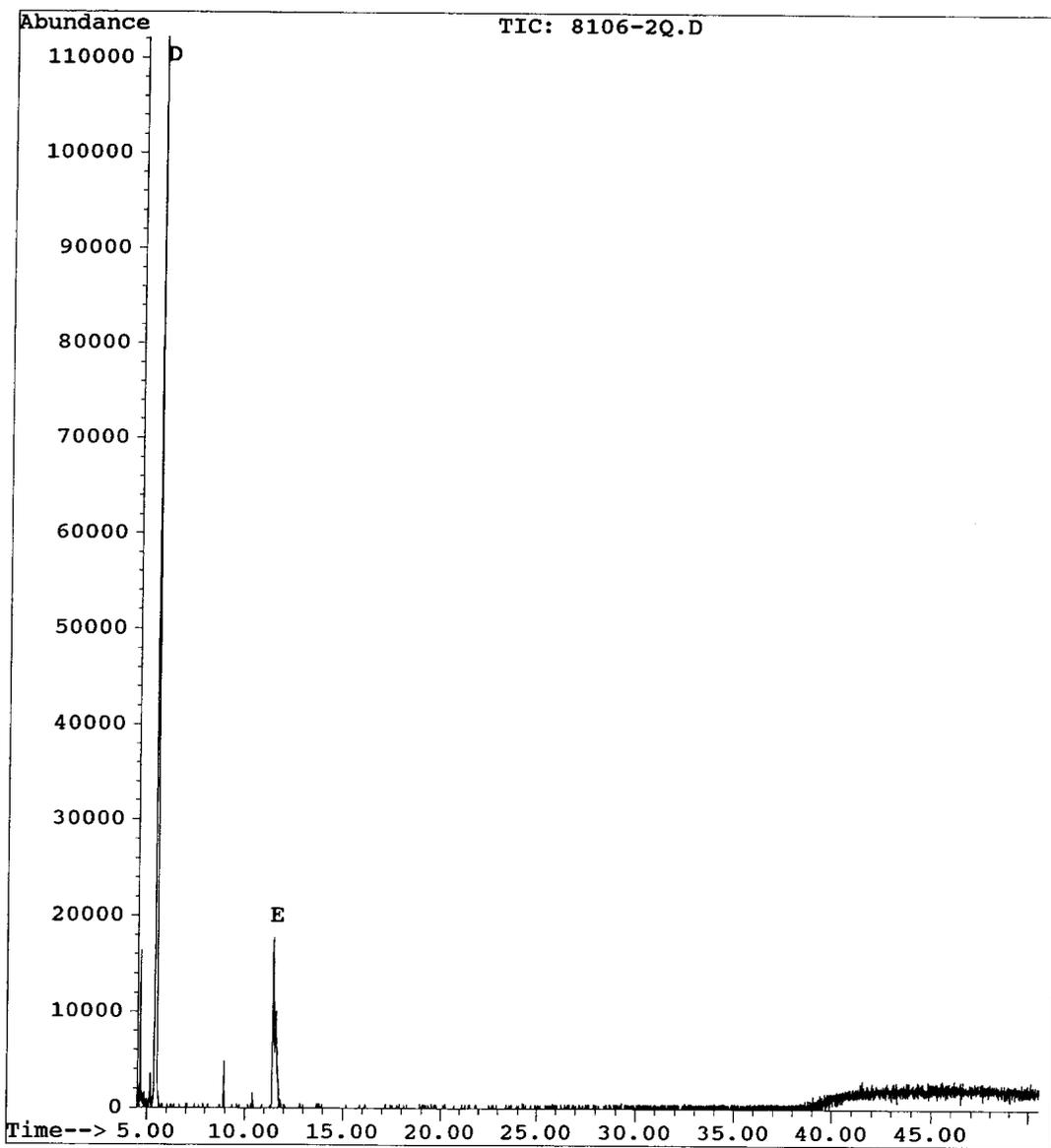
Appendix 3 – Fig. 4 – Compound from TIC identified as 2,2' – oxybis ethanol (embalming gel 1)



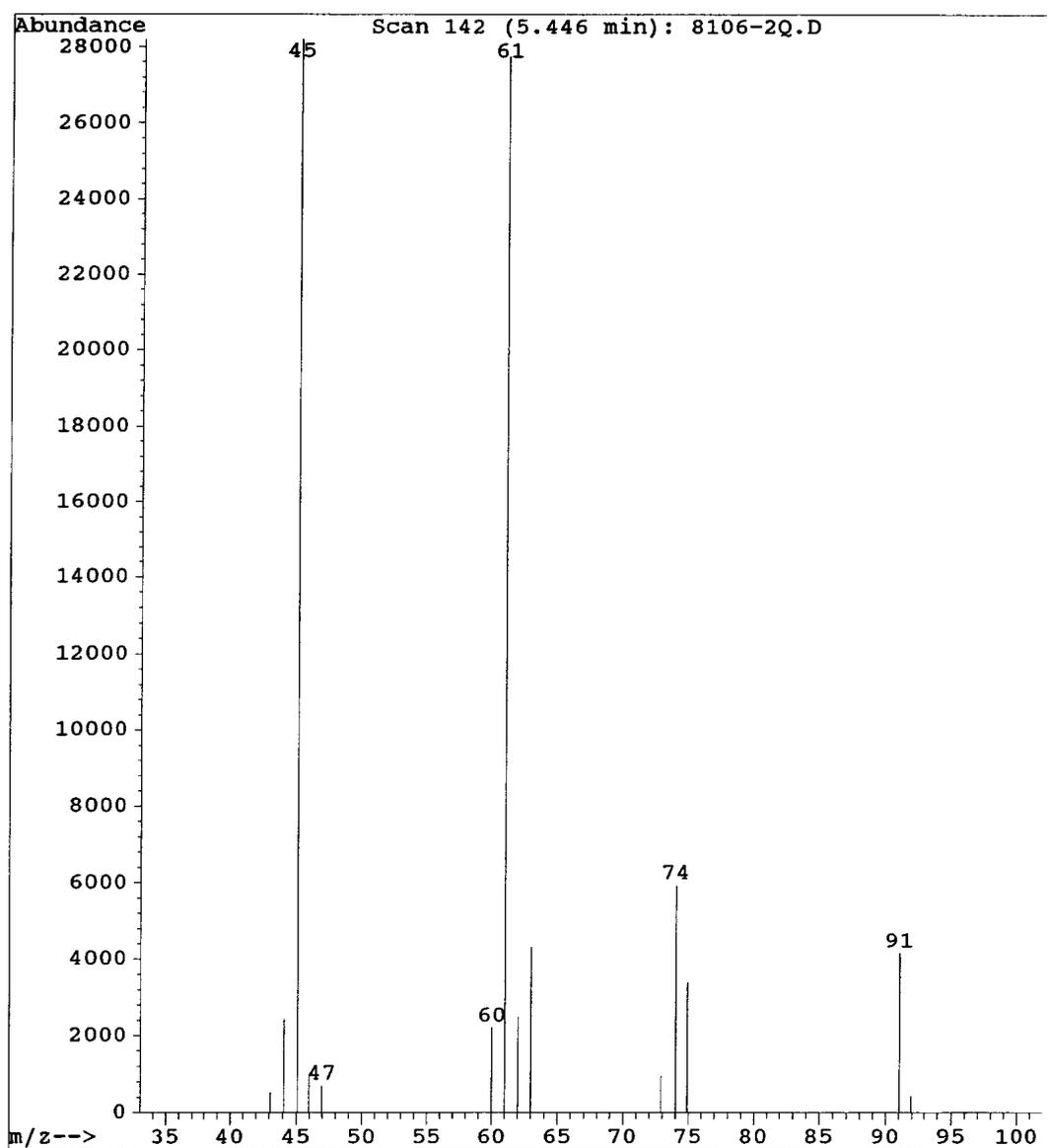
Appendix 3 – Fig. 5 – Compound B from TIC of embalming gel 1 identified as 1,8 -cineole



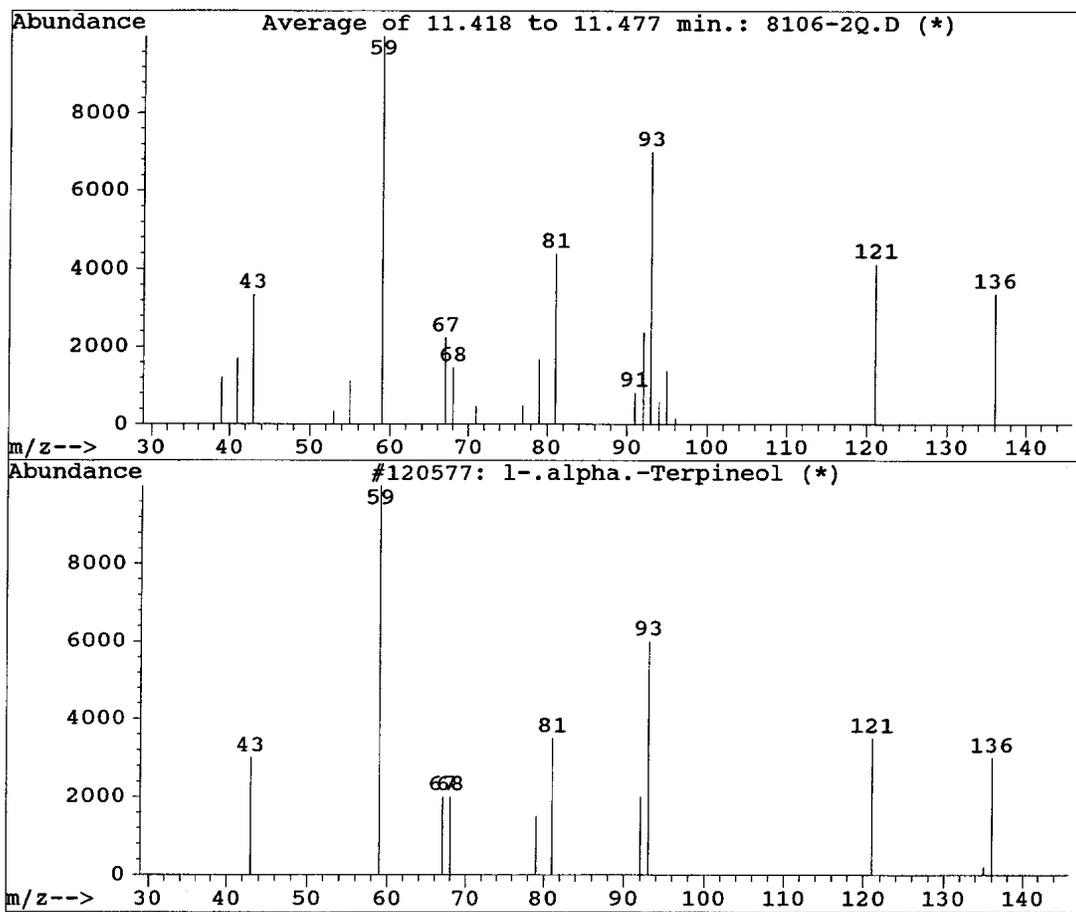
Appendix 3 – Fig. 6 – Compound C from TIC of embalming gel # 1, identified as 2,2',2'' – nitritotris ethanol



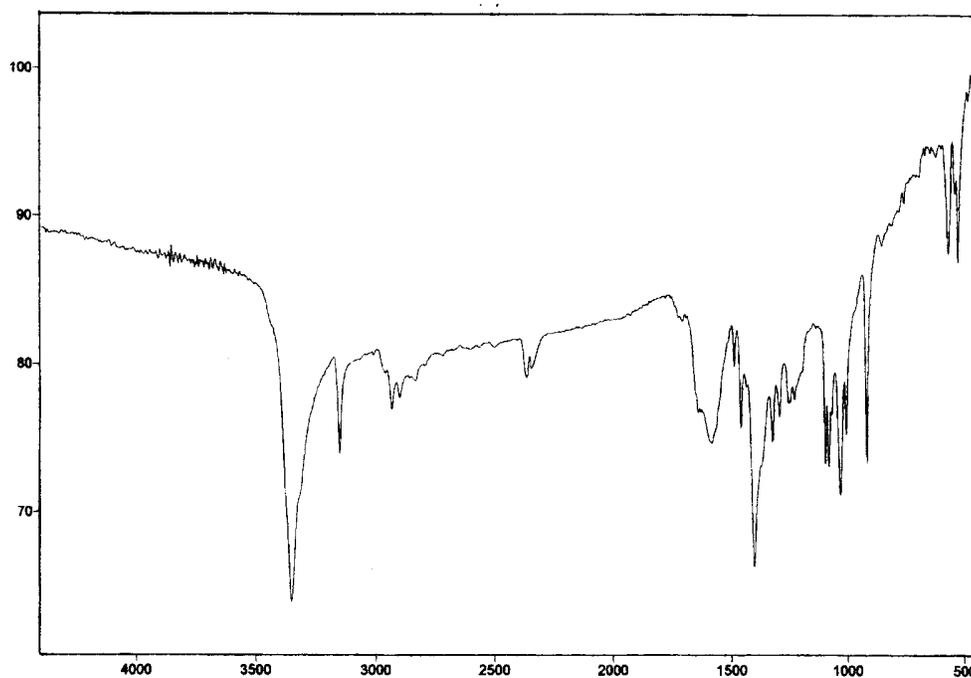
Appendix 3 – Fig. 7 – Total ion chromatogram for embalming gel # 2



Appendix 3 – Fig. 8 – Unidentified compound from TIC embalming gel # 2



Appendix 3 – Fig. 9 – Compound E from TIC of embalming gel # 2 identified as 1- α -terpineol



Transmittance / Wavenumber (cm-1)

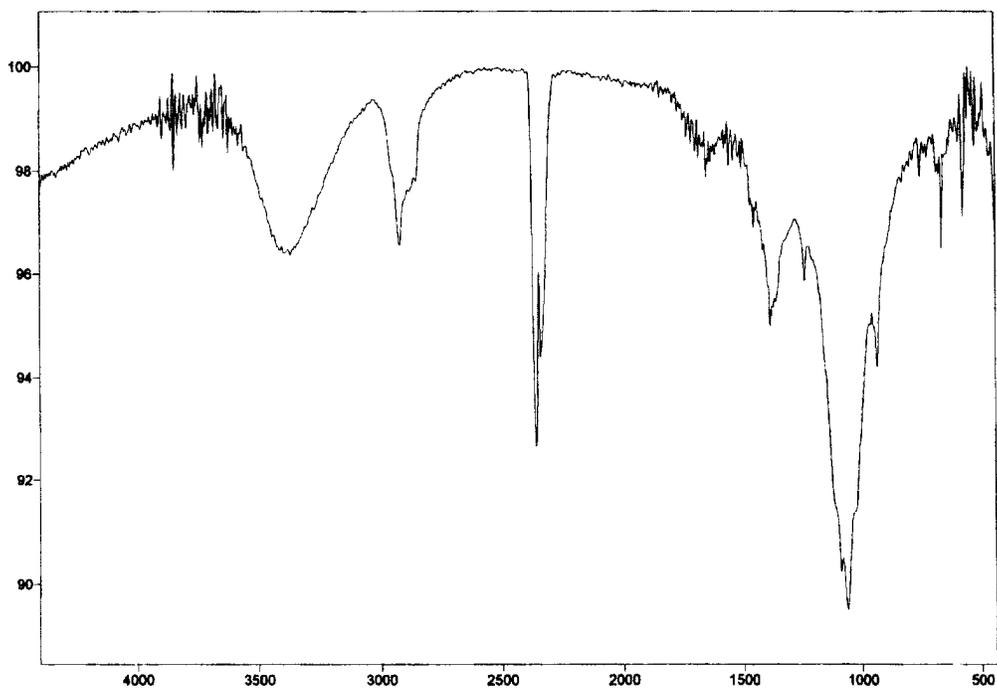
Number of Scans= 10 Apodization= Weak

File # 1 : 81061

4/24/99 1:37 PM Res=8 cm-1

dark blue gel

Appendix 3 – Fig. 10 – FTIR spectroscopy of embalming gel # 1



Transmittance / Wavenumber (cm-1)

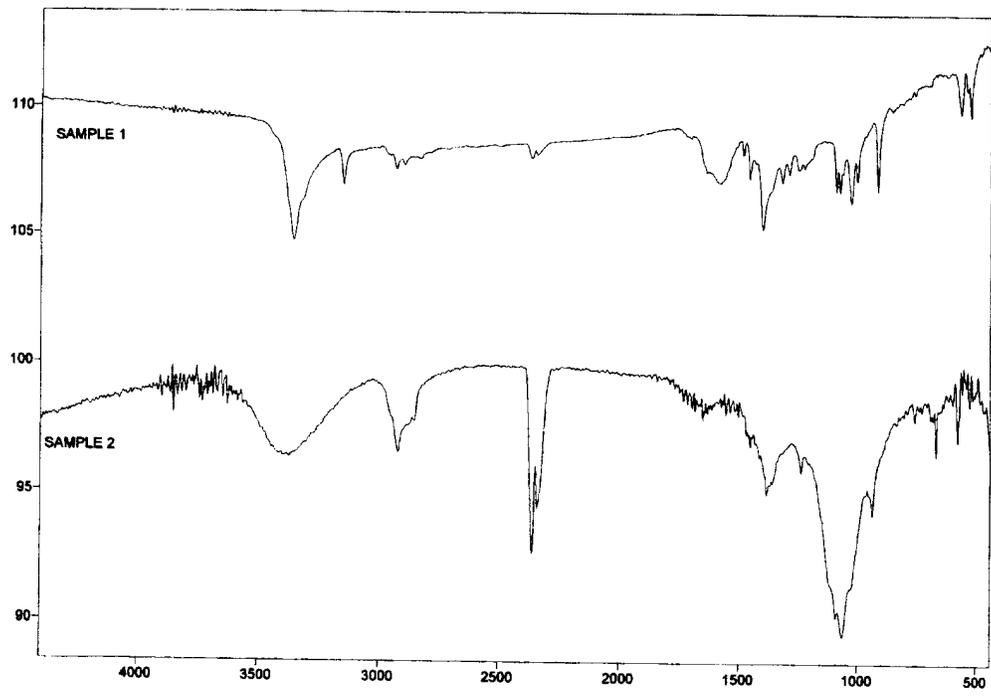
Number of Scans= 10 Apodization= Weak

File # 2 : 81062

4/24/99 1:39 PM Res=8 cm-1

light blue gel

Appendix 3 – Fig. 11 – FTIR of embalming gel # 2



Transmittance / Wavenumber (cm-1)

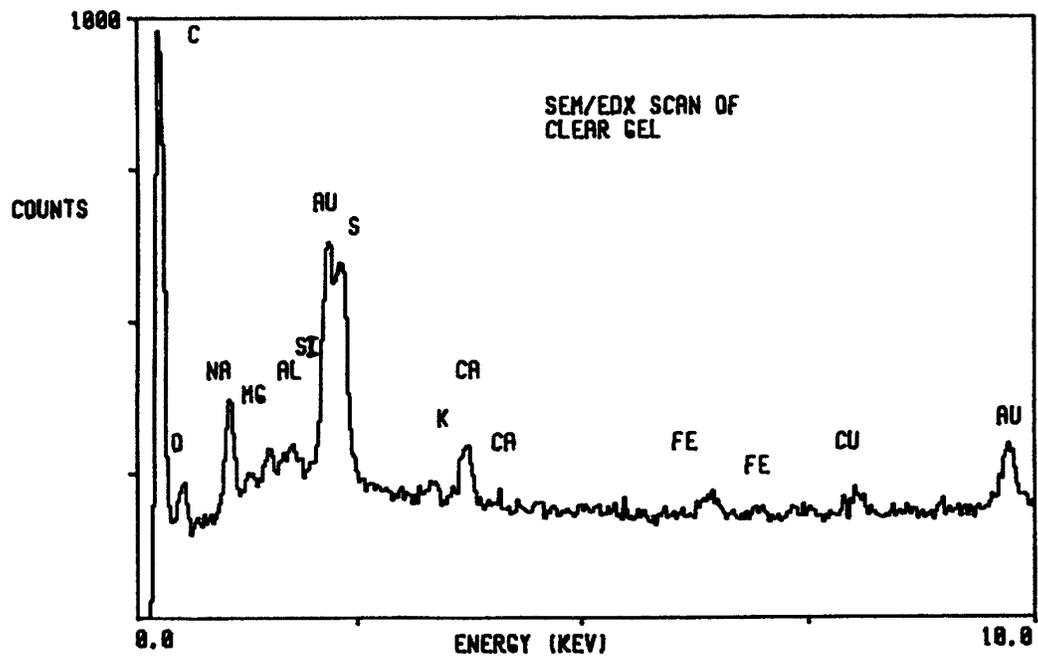
File # 2 : 81062

light blue gel

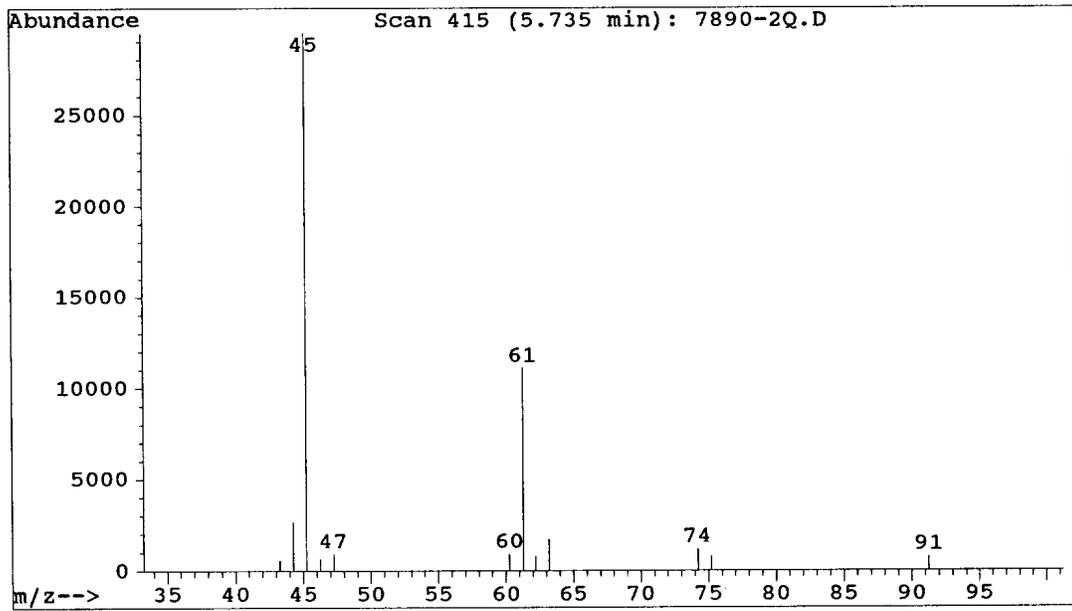
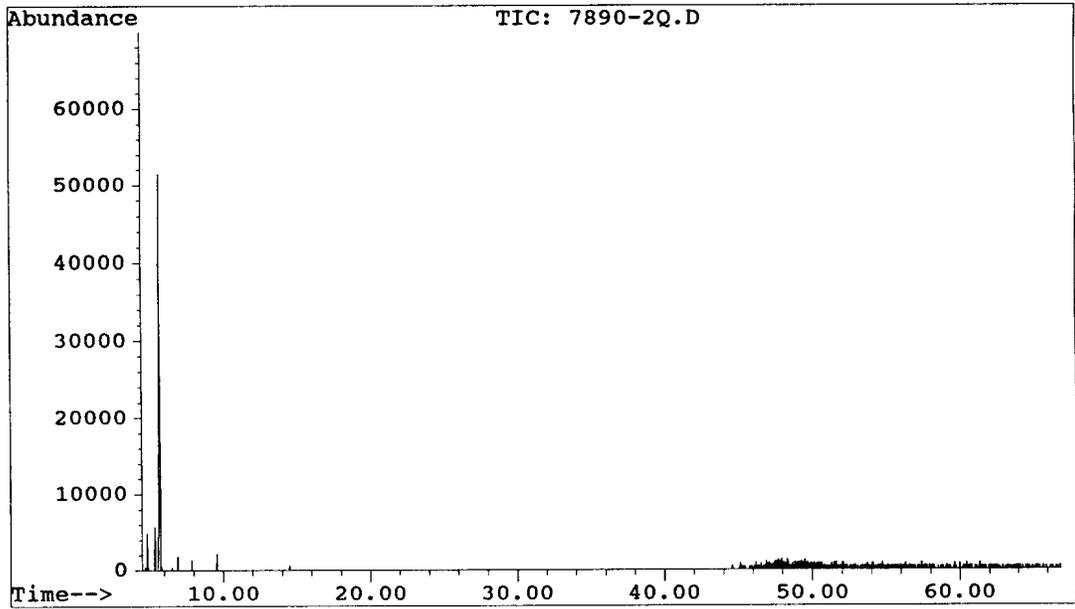
Number of Scans= 10 Apodization= Weak

4/24/99 1:39 PM Res=8 cm-1

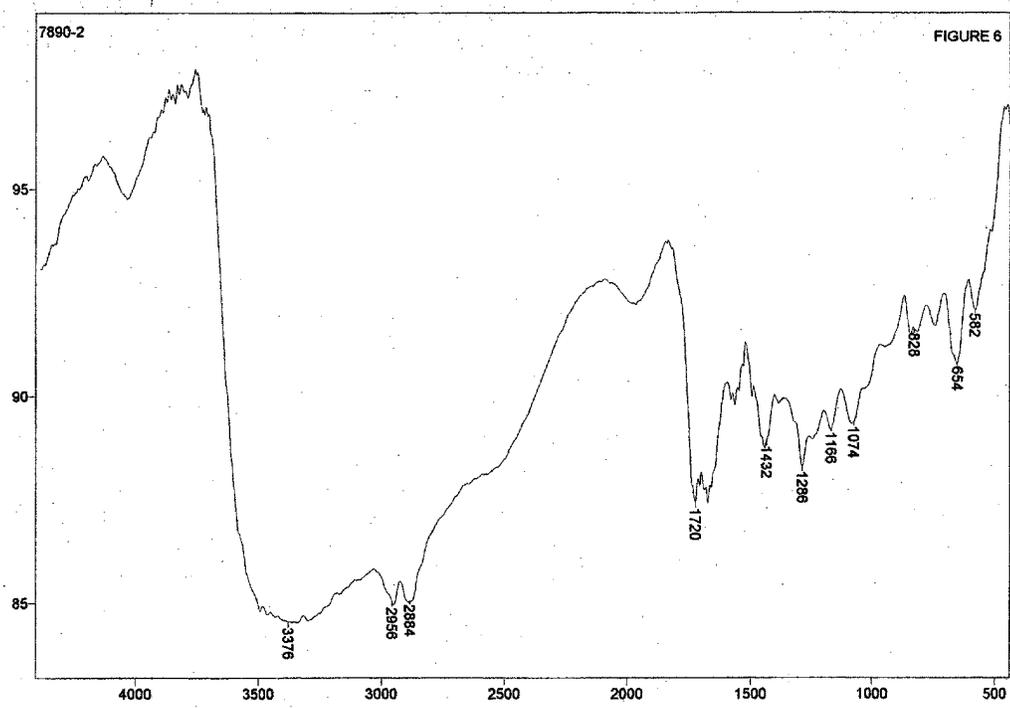
Appendix 3 – Fig. 12 – Comparative FTIR for embalming gel # 1 and # 2



Appendix 3 – Fig. 13 – SEM/EDX scan of embalming gel, sample # 3



Appendix 3 – Fig. 14 – GCMS profile of embalming gel # 3



Transmittance / Wavenumber (cm-1)

Number of Scans= 150 Apodization= Weak

File # 2 : 78902

12/11/98 2:05 PM Res=8 cm-1

UNKNOWN 2

Appendix 3 – Fig. 15 – FTIR analysis of embalming gel # 3