Investigation of a Report of Animal Mutilation in Dupuyer, Montana on June 27, 2001

NATIONAL INSTITUTE FOR DISCOVERY SCIENCE

Abstract

NIDS received a call from local law enforcement regarding a six-year old Red Angus cow found dead at 8:00–9:00 AM on June 27, 2001 near Dupuyer, Montana. According to the rancher, the animal had last been seen alive on June 25, 2001. The animal was lying on its right side. The left eye and eyelid were missing, the hide from the left jaw was missing and parts of the tongue were gone. The vagina and rectum were also missing. A thorough examination of the area by law enforcement failed to reveal any tracks, markings or signs of struggle from the animal. When the hide under the left jaw was cut away, investigators noticed a greenish-colored tissue mass just under the jaw. The green color markedly contrasted with the pink color of the surrounding tissue. Because of the ambient temperature and humidity in the area and to prevent further decomposition, the head of the animal was severed and immediately frozen. After the head was thoroughly frozen in Montana, it was then rapidly shipped to NIDS in Las Vegas, Nevada, where it was immediately stored at –85ºC to prevent further decomposition.

NIDS then consulted with a forensic expert, who arranged to fly to Las Vegas to conduct a thorough sampling of the head. An analysis of the eyes and jaw showed no blood in the tissue, indicating that the heart had stopped beating upon removal of the tissues. If the animal was mutilated, the mutilation occurred after death. In addition to the gross pathology, samples of eye fluid from the animal's right eye and tissue from the neck area were collected. A comprehensive set of organic extraction procedures followed by Infrared spectrometry and gas chromatography mass spectrometry (GCMS) analysis were conducted to determine the molecular components in the eye fluid and tissues. Preliminary chemical analysis was also conducted on maggot mass from the animal.

A second animal was obtained from a slaughterhouse and left to decompose for four days as a sham or control animal. Tissue and eye fluid from the control animal was subjected to identical extraction and analytical procedures.

A compound called oxindole was found in both tissue and eye fluid from the mutilated animal but not in the control animal, suggesting oxindole was not a decomposition product. The clinical and pharmacological properties of oxindole have been examined primarily in Europe (Mannaioni et al.(1998) *British J. Pharmac*. 125, 1751-1760). However, prior to these more recent studies, it has been well established that systemic administration of oxindole to rats, dogs or humans has been shown to cause profound sedation, decrease in blood pressure, decrease in muscular tone and loss of consciousness (Orcutt et al. (1964) *Arch. Int. Pharmacodynam*. 152, 121-131). Our failure to find oxindole in the control animal leads us to the working hypothesis that oxindole may have been used to sedate the animal prior to its death and mutilation. Similar

analyses of different mutilated animals in the future will either substantiate or negate this working hypothesis. For example, the pharmacokinetic data on administration of oxindole to large animals is scanty as are the data on the extent of rumen-saliva recycling of tryptophan metabolites. A second hypothesis is that an unknown traumatic event triggered the rapid accumulation of oxindole in the tissues of the mutilated animal but not in the control animal These subjects are currently under study.

Since the summer of 2001, NIDS has received over eight reports of animal mutilations from Montana, the majority of which were too old to seriously investigate. Nevertheless, this number of reports in a few months constitutes by far the largest report frequency received in the history of NIDS investigations of the animal mutilation phenomenon. Therefore, the present case should be seen not as an isolated incident, but in the context of a wave of mutilation reports in 2001 from Montana. It should also be noted that during the period 1974-1977, the Great Falls area of Montana was the locus of one of the most intense and sustained waves of reported animal mutilations in recorded history. For more details on this historical animal mutilation wave see the NIDS report at http://www.nidsci.org/articles/pdf/wolverton_report.pdf.

Finally, NIDS is gratified by the increasing spirit of cooperation and collegiality between our organization and ranchers, law enforcement officials and veterinarians. We believe that the successful investigation of animal mutilations is utterly dependent upon close cooperation between NIDS and three separate groups: (a) ranchers who are willing to make timely reports to NIDS (702-798-1700) or to local law enforcement, (b) open-minded veterinarians who are willing to conduct timely necropsies on mutilated animals, and (c) hard-working law enforcement officials who serve as both investigators and liaisons between NIDS and the ranchers themselves. We emphasize that NIDS absorbs 100% of the costs of these investigations. Secondly, because of the controversial nature of the animal mutilation phenomenon, NIDS does NOT publicize the names of ranchers, law enforcement officials or veterinarians who work with us.

Introduction

NIDS received a call from local law enforcement regarding a six-year old Red Angus cow found dead at 8-9 AM on 6/27/01 near Dupuyer Montana. According to the rancher, the animal had last been seen alive on 6/25/01. An attempt to conduct a necropsy on the animal failed because of lack of availability of local veterinarians. The animal was lying on its right side.

Figure 1.

The left eye and eyelid were missing, the hide from the left jaw was missing and parts of the tongue were gone (Figure 1). The vagina and rectum were also missing. A thorough examination of the area by law enforcement failed to reveal any tracks, markings or signs of struggle from the animal.

When the hide under the left jaw was cut away, investigators noticed a greenish-colored tissue mass just under the jaw (see Figure 2). The green color markedly contrasted with the surrounding pink color of the surrounding tissue.

Figure 2. Greenish colored tissue mass lying below the jaw. The entire greenish mass was excised at the mutilation site and frozen for later analysis (see later).

Because of the ambient temperature and humidity in the area and to prevent further decomposition, the head of the animal was severed and immediately frozen. When the head was thoroughly frozen in Montana, it was rapidly shipped to NIDS in Las Vegas, Nevada, where it was immediately stored at –85ºC to prevent further decomposition.

Section I

Gross Examination and Sampling

NIDS then consulted with one of our forensic experts who arranged to fly into Las Vegas to conduct a thorough sampling of the head. Five days prior to the arrival of the forensic expert, the head was removed from –85ºC and placed in another freezer at 0ºC. Two days prior to the arrival the storage temperature of the head was adjusted to +5ºC. The intent of the slow incremental increases in temperature was to limit fracturing and other tissue damage that is associated with sudden freeze-thaw cycles.

Upon examination, the left hand eye-socket was examined and a prominent maggot mass was noted and sampled (Figures 3 & 4). Subsequent microscopic analysis indicated a first instar maggot growth giving some clues regarding the time of death of the animal.

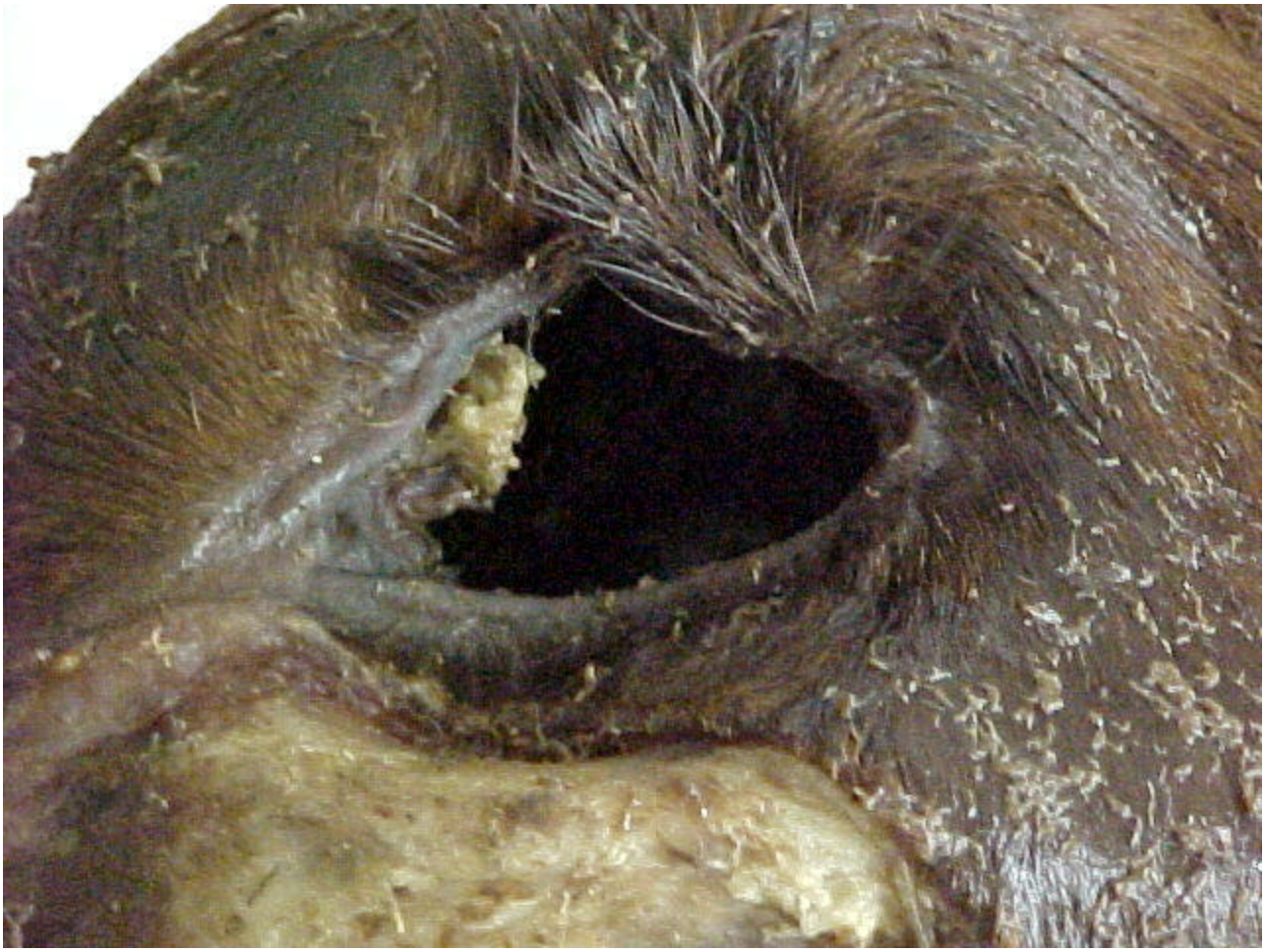


Figure 3. Prominent maggot mass in left eye socket.

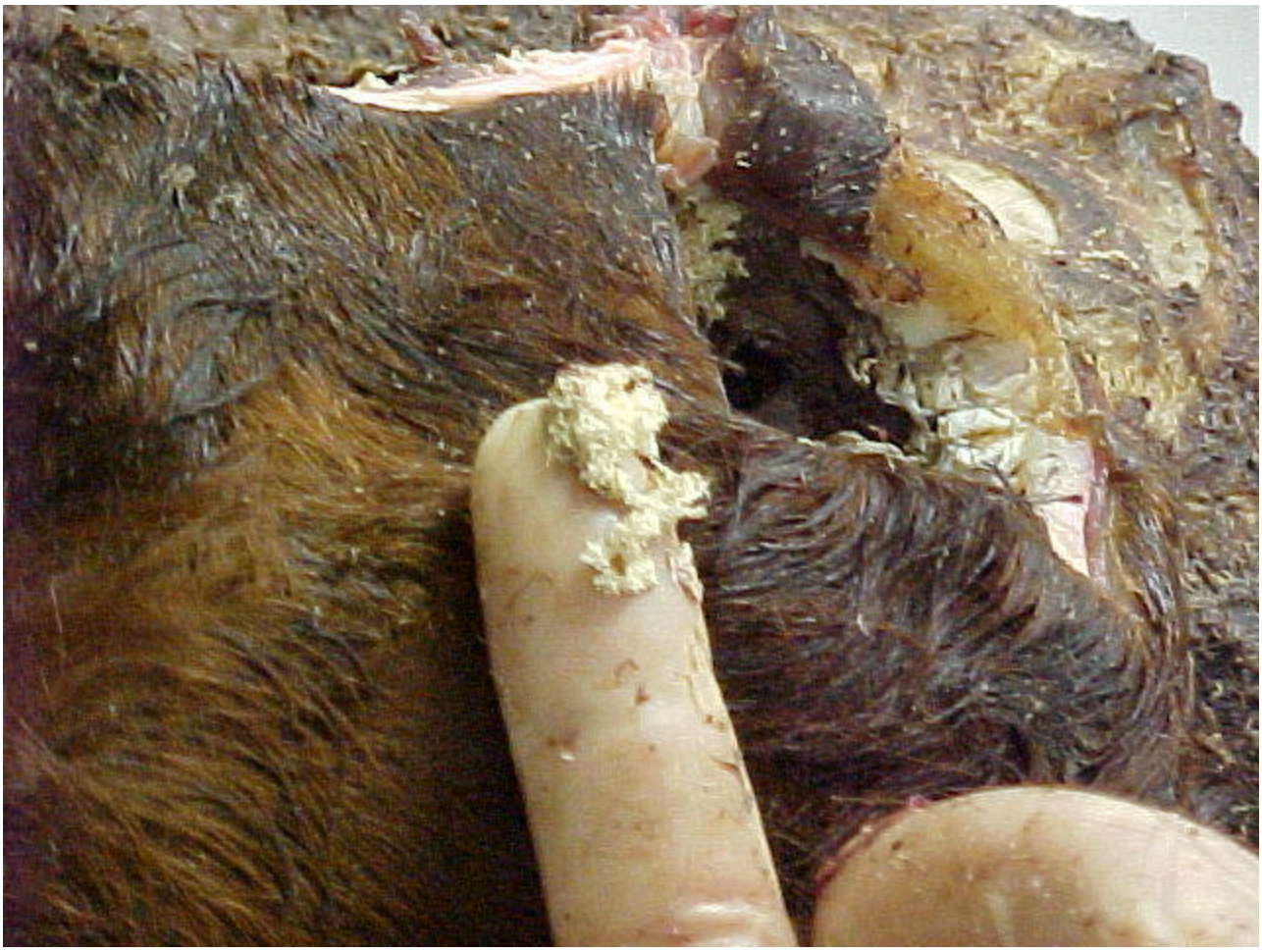


Figure 4: A prominent maggot mass was removed from the left eye and sampled.

It was determined that the right eye of the animal was intact and still contained its full complement of vitreous and aqueous humor. At the beginning of the examination, the eye fluid was still frozen (see Figure 5). During the six-hour gross examination and necropsy, the fluid gradually thawed (see description later).

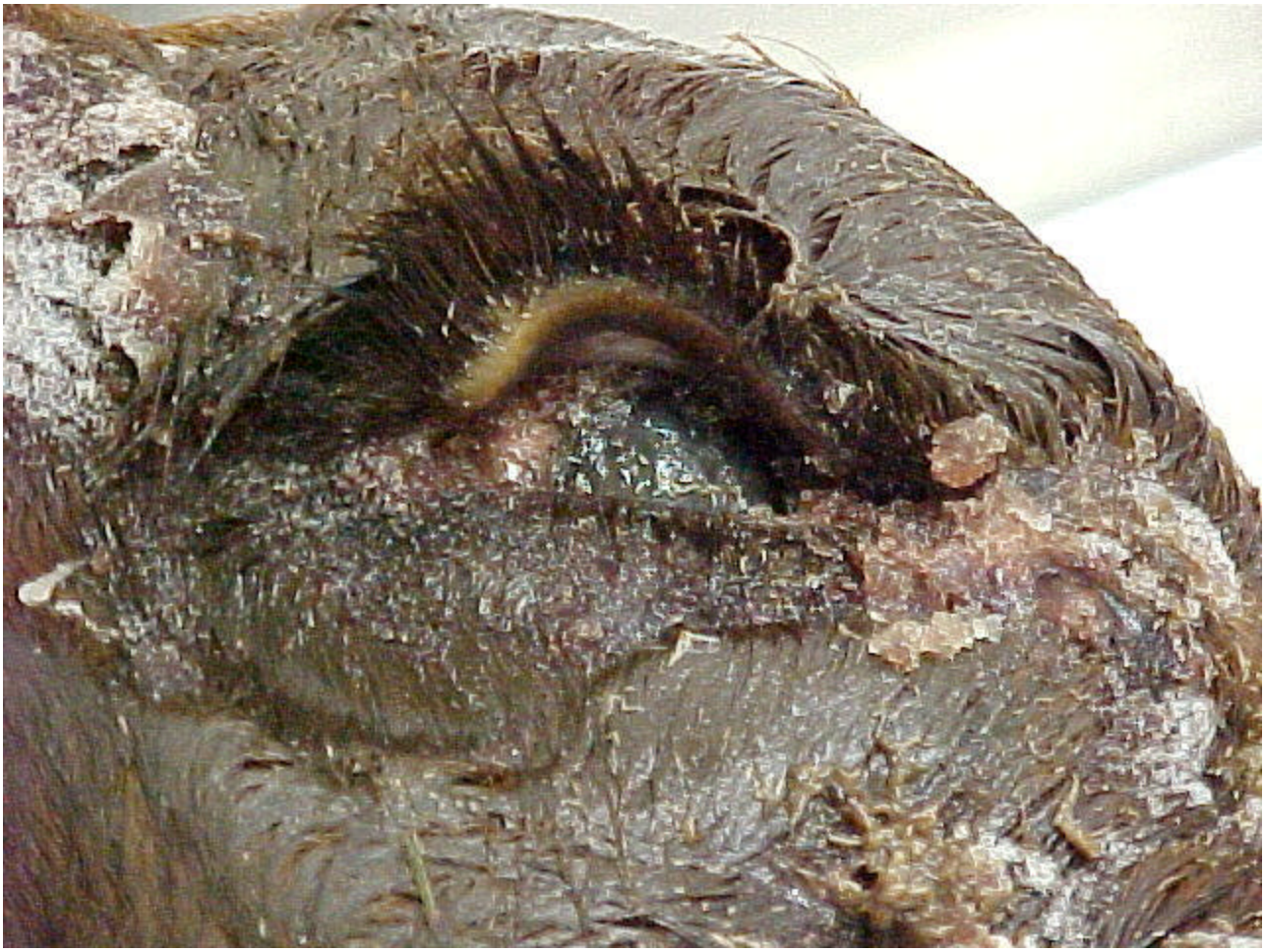


Figure 5. Fully intact right eye, frozen at the beginning of the necropsy.

The teeth of the animal on the left side were deeply blackened (see Figure 6). Close examination showed that the animal had substantial dental calculus. This calculus was not related to the cause of death and was probably nutrition related.

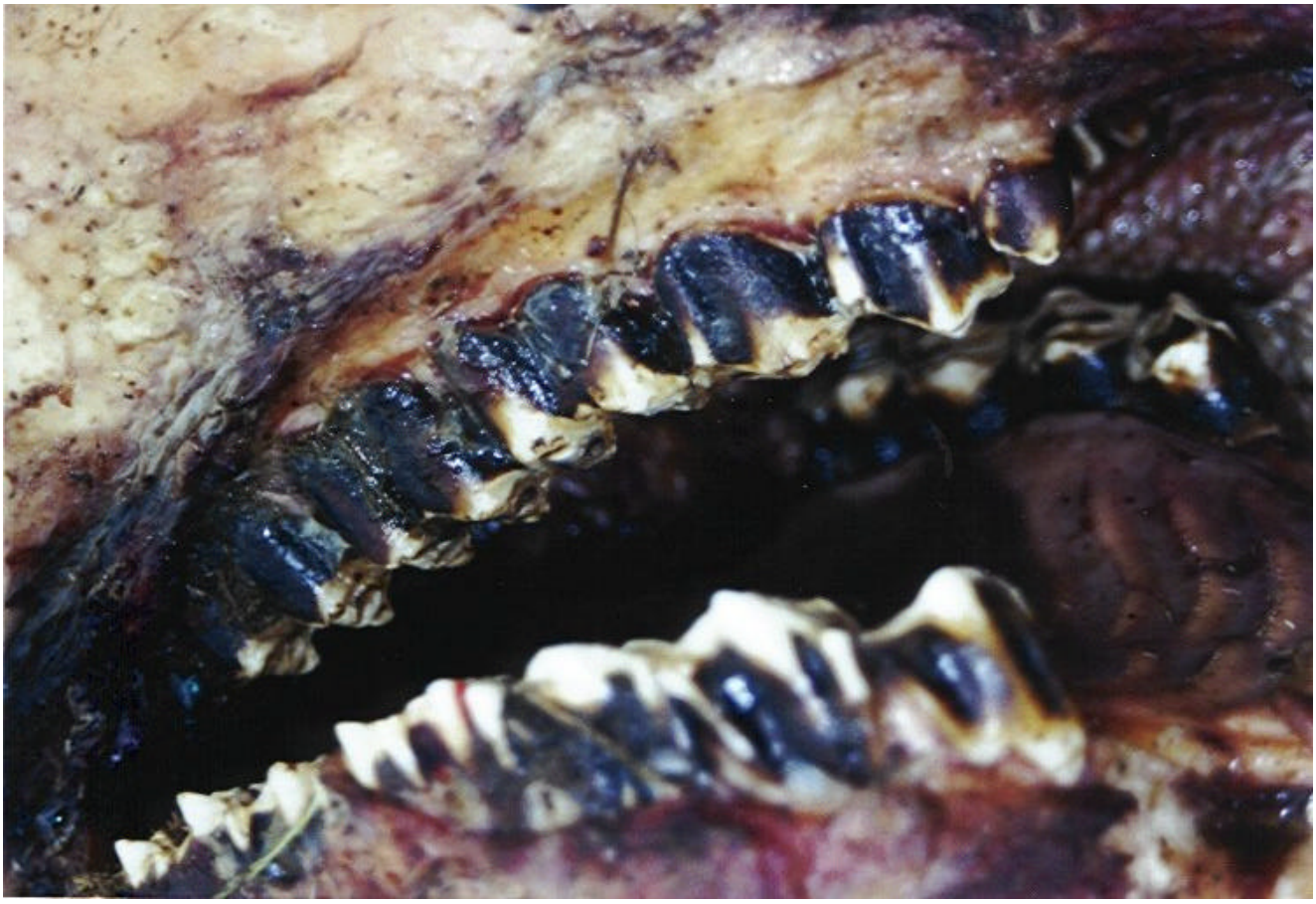


Figure 6. Teeth of the animal showed extensive dental calculus that was probably nutritionally related.

The next phase of the examination involved the removal of the hide from the skull of the animal. The hide was first removed from the top of the skull (Figure 7) and examined.

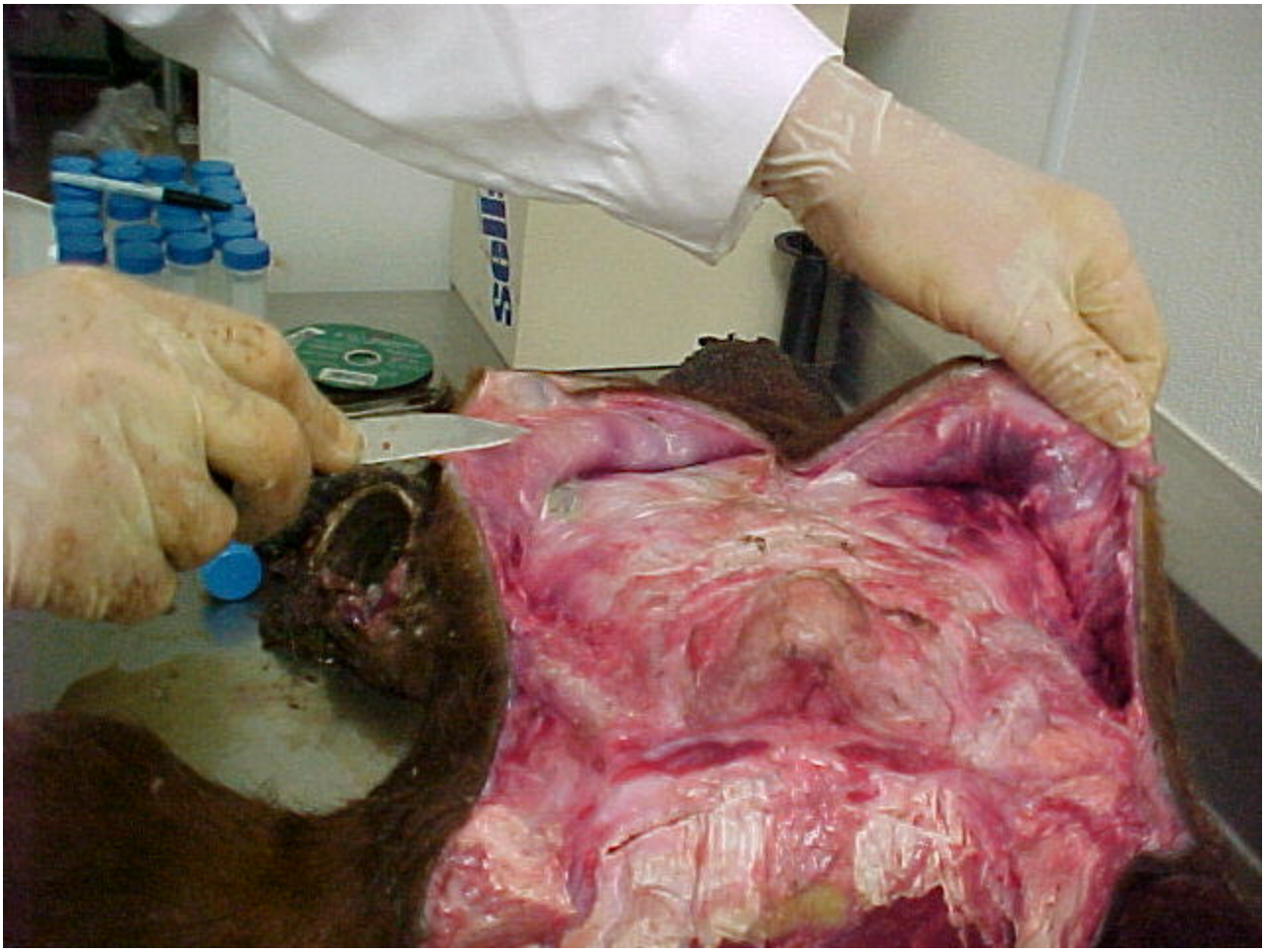


Figure 7. Evidence of hematoma and bruising over the right eye of the animal.

The muscle at the back of the head was normal. There was no sign of hematoma on the left hand side of the skull, no evidence of trauma that might be indicative of a blunt instrument or a stun gun. In contrast, there was evidence of hemorrhaging on the right side of the head (Figure 7). The hemorrhaging continued down the right jaw of the animal. A hematoma over the right eye was noted. The location of the hematoma and hemorrhaging appeared to coincide with the position of the animal's head as it lay on the ground.

Around the eyes and jaw there was no blood in the tissue indicating that the heart had stopped beating when the removal of the tissues occurred. If the animal was mutilated, the mutilation occurred after death.

When the trachea was dissected out, a large mass was noticed clogging the trachea. Figure 8 shows the dissected trachea, the mass compared with a 50 ml Falcon BlueMax tube for size comparison.

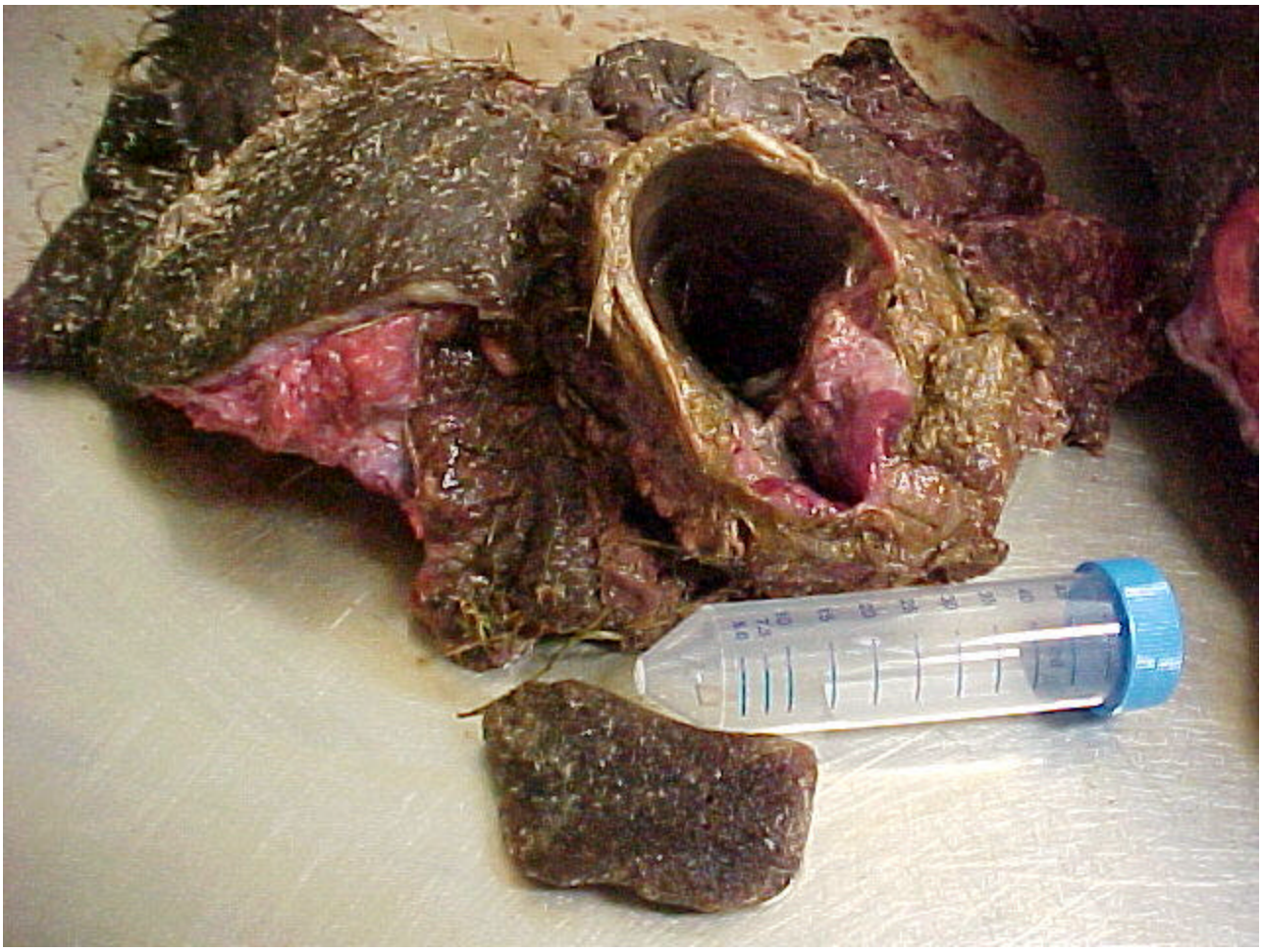


Figure 8. Mass that was removed from the trachea is displayed in the foreground with a 50 ml Falcon BlueMax tube for size comparison.

Initially, it was thought that the mass may have contributed to cause of death of the animal by lodging in the trachea and stopping breathing. Within 60 minutes, the mass had completely melted, indicating that it was merely fluid build up that had frozen in the animal's trachea.

Next, it was determined that the base of the tongue had been cut, but the cut appeared ragged and was not indicative that a sharp instrument had been used. The cut did not extend back to the base of the tongue.

When the hide had been fully stripped off the head, the aqueous fluid in the animal's right eye had finally begun to melt (Figure 9).

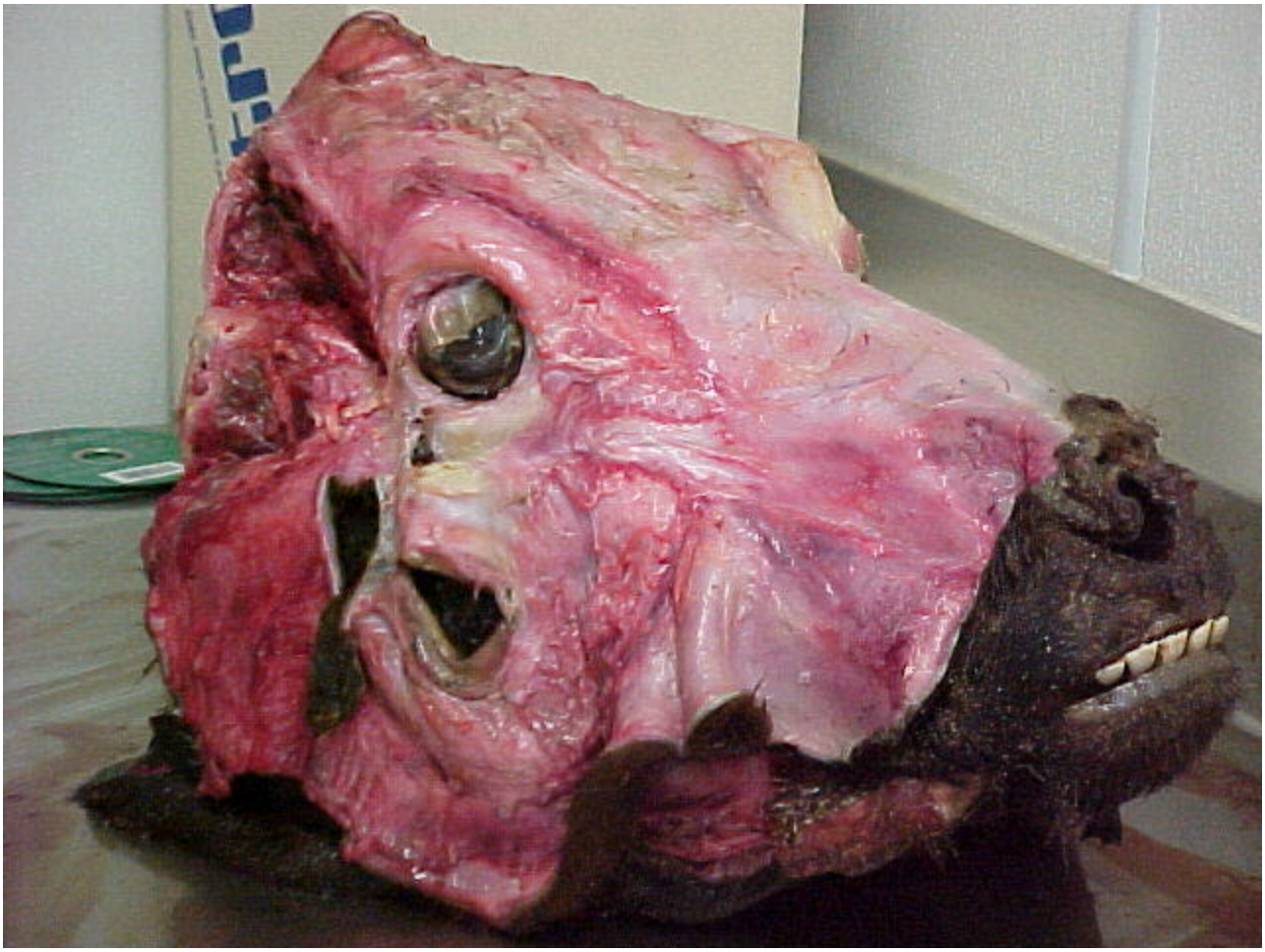


Figure 9. The skull of the animal was stripped of hide, by which time the fluid in the right eye had melted.

The fluid from the right eye was carefully collected using a syringe and needle. Approximately 7.5mls were collected. The eye fluid was immediately frozen for later analysis (see Section II).

As the final and most difficult part of the post-mortem examination, it was decided to see if any of the animal's brain had survived the summer heat in Montana, together with subsequent the freezing and thawing. Brain decomposition is known to be extremely rapid especially at high summer temperatures. The skull of the animal was sawed through (Figures 10) in a laborious process.

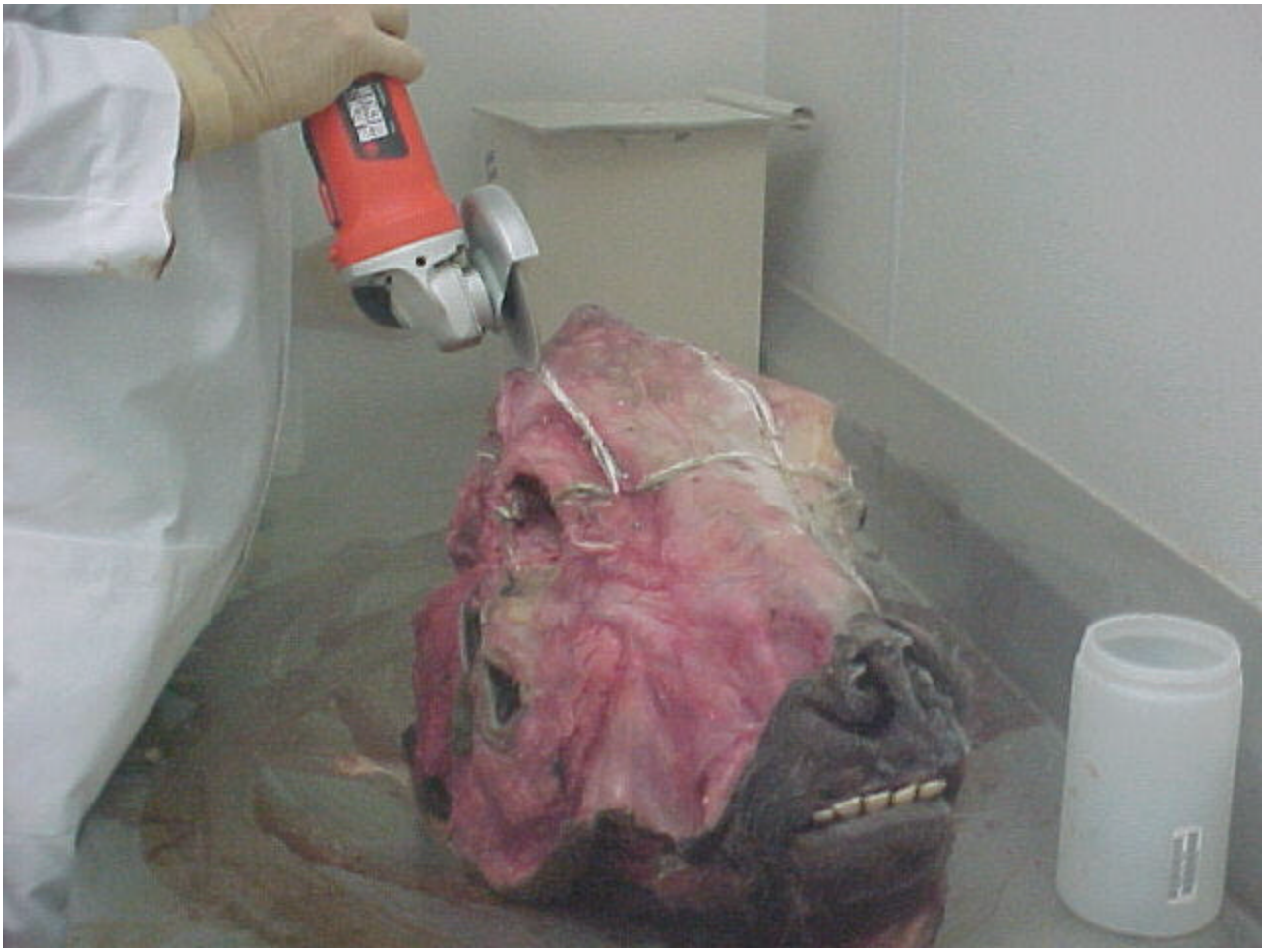


Figure 10. The skull of the animal was sawed through carefully so that the entire top could be lifted off without disturbing the brain.

Surprisingly, it was found that the brain was in much condition than expected. Much of the tissue had not liquefied (Figure 11).

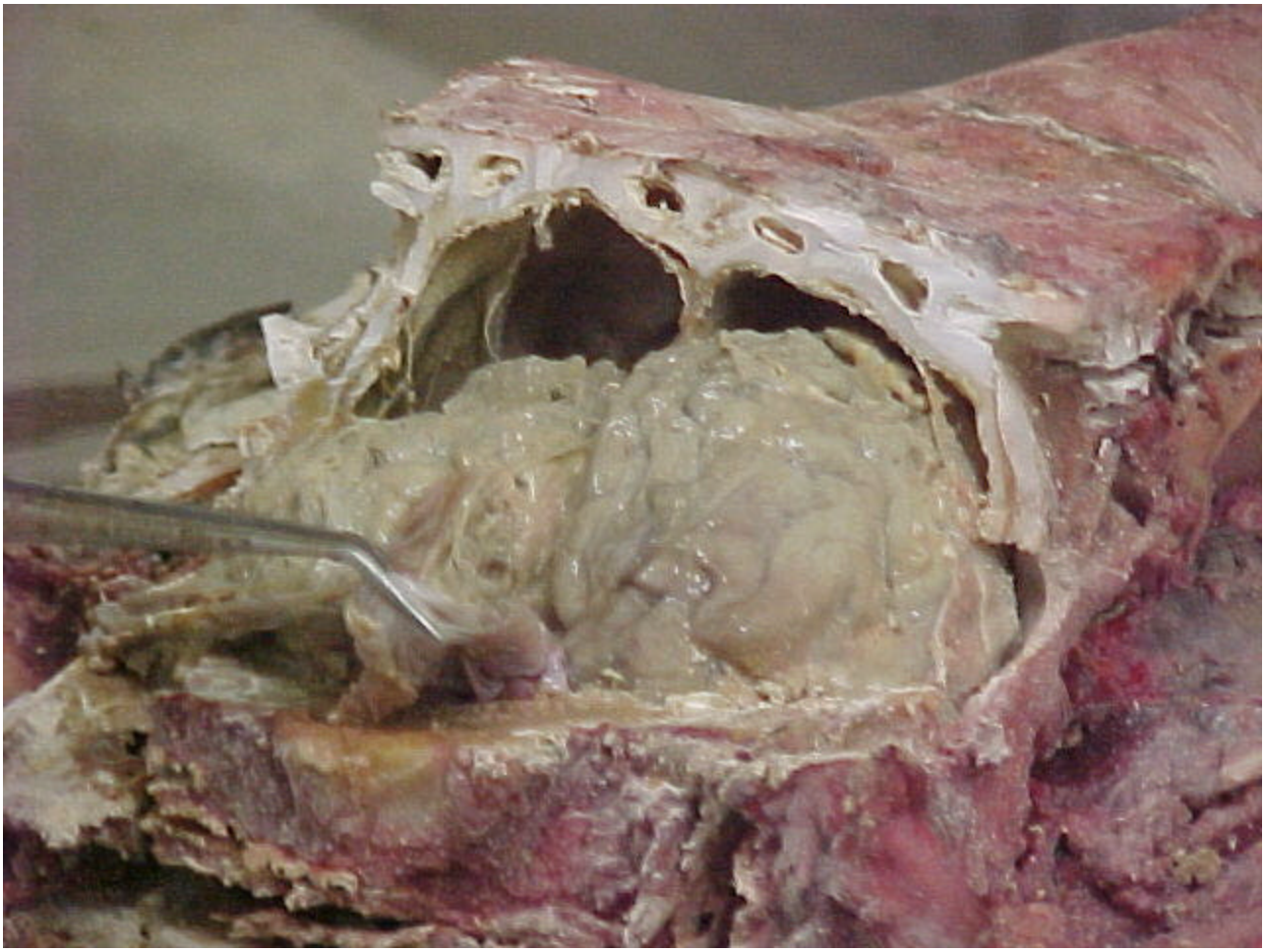


Figure 11. The brain, although beginning to liquefy was in surprisingly good condition.

The brain was carefully removed from the skull and sections of the cortex, midbrain, cerebral nuclei, cerebellum and stem were collected and frozen. Again, no sign of brain trauma was obvious, consistent with the lack of trauma evidence on the outside of the skull.

Section II

Laboratory Investigation: Materials and Methods

Analysis of "green tissue" from under the animal's jaw: analysis of similar tissue from a "mock" mutilation

The greenish colored tissue seen in Figure 2 was deemed of sufficient interest to justify two subsequent levels of analysis. Samples of the green tissue and surrounding pink tissue were extracted in different organic solvents and subjected initially to infrared spectroscopy. A full control experiment was conducted in order to obtain tissue from an animal that had died but had not been mutilated. The animal was obtained from a local slaughter-house at the time of death and was left to decompose under natural conditions for four days. During this period it was protected from normal scavenging activity. After four days of decomposition, samples of vitreous fluid and tissue were obtained from the animal. Throughout this report, these samples from the "mock" mutilation are referred to as control samples.

Secondly, the tissue samples from the control animal and the mutilated animal were extracted and subjected to analysis with gas chromatography mass spectrometry (GCMS). The following section contains a summary of extraction procedures, materials and methods used.

All extractions and Infrared Analyses were performed at Frontier Analysis, Chagrin Falls Ohio. GCMS analysis and spectra interpretation was performed at BP Analytical Laboratory.

Extraction Procedures for IR Analysis

Portions of green tissue and normal pink tissue were excised from two larger 'as received" lumps of tissue. They were allowed to "dry" at ambient temperature in the laboratory to diminish interfering moisture. This took about 3–5 hours. Several infrared spectra were obtained from both tissues. Difference spectra were generated between selected pink and dark spectra. An extraction procedure was then performed. This will be designated "Extraction Procedure #1" in this report, because two other extraction procedures were done on the samples. The two sections of dried pink and dark tissues were extracted with progressively more polar solvents: hexane, chloroform, acetone and water. The procedure involved using approximately 3 mls of solvent per wash and three washes per extractant. For each wash the mixture was agitated manually for two minutes. Infrared spectra were also obtained from each extract. Subtraction spectra were also generated between the extract spectra in order to enhance any differences. The extracts were then combined as follows: hexane + chloroform and acetone + water. The solvents were completely removed and sent for GC/MS analysis. Microscope photographs were taken of tissues using a Leica GZ6 stereomicroscope interfaced to a Kodak digital Science MDS 120 camera.

Two pieces of green tissue from the mutilated cow were additionally submitted. Two different extraction procedures were done on these samples. Using "Extraction Procedure #2" the

sample was extracted first with chloroform and then by acetone. This was done on samples that had not been dried. Roughly 3 mls of solvent was added and the sample agitated for 2 minutes. This was done three times per solvent type. Solvents were reduced to 0.5 to 2 mls, but not completely removed before GC/MS analysis. "Extraction Procedure #3" involved only a chloroform extraction. Solvent was added to the "as received" sample, and it was allowed to soak for 8 days in the refrigerator. The sample was subjected to ultrasonic agitation for approximately one hour a day. The solvent was reduced to 0.5 to 1 ml and not completely removed, as in the #2 procedure. GC/MS analysis was then performed on all the extracts. Infrared spectra were obtained from selected extracts.

Muscle tissue from a control animal which was not mutilated was submitted for reference. It was subjected to "Extraction Procedures #1 and #3". Infrared spectra were obtained from the #1 and #3 extracts. GC/MS analysis was done on the #3 extract.

Extraction Procedures for GCMS Analysis

Three different extraction procedures performed on the tissues were done to establish the best conditions for concentrating and detecting any foreign materials by GC/MS. In other words, some method development was required.

"Extraction procedure #1" involved using a progressively polar solvent sequence on the dried tissues from the mutilated cow. The hexane, chloroform, acetone, water extracts were combined, i.e. hexane + chloroform and acetone + water, from the pink and dark tissues before they were subjected for GC/MS analysis. GC/MS was done in order to detect components that may have been masked by the strongly absorbing esters and acids detected in the infrared analysis. (See the following infrared section.) Numerous individual molecules were identified. However, they seemed to be natural and degradation products from the animal. It was suspected that this analytical approach induced more deterioration of the sample due to the long time of exposure to ambient temperature (many hours) between the extraction and the GC/MS analysis. The four GC chromatograms of the extracts are shown in figures 1, 2, 3 and 4. The MS identifications are displayed in Table I.

"Extraction Procedure #2," done by using chloroform followed by acetone solvents, was performed on the "as received" sample. This reduced the time exposed to ambient temperature. Furthermore, not all the solvent was removed before GC/MS analysis. The GC/MS data show that not much material was extracted by this procedure due to short solvent contact time. For this reason the data were not very informative. The components that were in amounts to be detectable by GC/MS are natural and decomposition products (previously detected). Figures 5 and 6 display the GC chromatograms from the chloroform and the acetone extracts. Table II presents the MS identifications of each peak.

"Extraction Procedure #3" involved a refrigerated chloroform extraction for 8 days. It was successful in removing a large amount of solubles from the tissues of the mutilated cow and the control heifer and minimized as much as possible any further degradation. Both

chromatograms display strong peaks. This analysis shows the expected predominance of natural and degradation products.

Vitreous Fluid:

The vitreous fluid sample was obtained from the mutilated cow's eye and submitted. The "as received" sample was examined by GC/MS analysis. Quantitative values for some components were also obtained by rerunning the fluid using dioxane as an external standard.

Samples of vitreous fluid from the left and right eye were additionally submitted from a control animal for reference. Both were examined by GC/MS using the same conditions as the above vitreous fluid.

Microscope Examination

The "as received" dark and muscle and pink fatty tissues from the mutilated animal, as well as muscle tissues from the control heifer, were observed under the microscope. The cow tissues are darker in color compared to those from the control heifer. Following are the photomicrographs.

Mutilated Cow Tissue

Control Heifer Tissues

Instrumental Data Acquisitions: Conditions

Infrared: Both transmittance and reflectance infrared spectra were obtained from the samples using a Nicolet Avatar 360 spectrometer. Transmittance spectra were obtained from smears on KBr crystals. Reflectance spectra were acquired using the Harrick SplitPea[?] sampling accessory.

GC/MS: A Hewlett-Packard GC/MS (DOS-MSD/ChemStation) employing a 6890 gas chromatography, 5973 Mass selective detector and capillary injection system was used for analysis. Chromatographic separation was accomplished by using a 60m x 0.32mm i.d., 1.0 mm film thickness DB-1 capillary column from J&W Scientific (sn 0433924; Cat # 123-1063). The following GC/MS conditions were used:

Instrument:	GC/MS-4
Injector Temp:	Inj. 300?C
GC Oven Program:	50?C (0.0 min.) to 290?C @ 10.0?C/min. (36.0 min.)
Injection Volume:	1.0 ?l, splitless
Run Time:	60.6 min.
MS Run Type:	Scan
Mass Range:	25-600 Da; Scan threshold: 100
Scan Start Time:	0 min.
Sampling:	No.=5
Multiplier Volt.:	Emv offset=200; resulting volt.=1490
Method File:	RWSVM.M
Tune File:	ATUNE.U

Results

GCMS Analysis of Green Tissue

When comparing the data from the mutilated animal and the control, an unusual compound is uniquely observed in the extract from the mutilated cow. This is oxindole. This molecular structure, as well as some derivatives of this structure such as tryptophan, is known to possess a sedative property. Oxindole has a GC retention time of 17.89 minutes and is positively identified in the mass spectrum. The characteristic masses of oxindole are all present (51, 63, 78, 89, 104 and 133) Masses 104 and 133 are the strongest. The chromatograms of the extracts from the tissues of the mutilated cow and the control heifer are shown in Appendix figures 7 and 8. The mass spectrum along with a reference of oxindole is shown in Appendix figure 9.

GCMS Analysis of Eye Fluid

GC chromatograms of "as received" vitreous fluids from the mutilated cow and control heifer are well endowed with peaks showing the presence of numerous components. As with the tissue analysis, most of these are identified as natural and putrefaction products. Both GC chromatograms are very similar. However, under close inspection there are subtle but very significant and informative differences. There are additional weak GC peaks in the chromatogram of the mutilated animal. Those additional peaks identified by MS as acetic acid, propionic acid, butanoic acid, urea etc., suggests the mutilated cow was in greater state of putrefaction than the control heifer. But there is one additional component at a GC retention time of 18.22 min. which may not be attributable to a decay product. MS identifies it as oxindole, which was also detected in the tissue. The amount of this material could be roughly estimated from the GC chromatogram by comparing it to a chromatogram from another run containing a known amount of dioxane standard. It was determined that the oxindole content is about 50 to 100 ppm (0.005 to 0.01 wt.%). The chromatograms of the fluids from both animals are shown in Appendix figures 28 and 29. The MS spectrum of this material is shown along with an oxindole

reference is shown in Appendix figure 30. Table IV shows the MS identification of many of the components from the mutilated cow. The GC chromatogram from the control heifer does have peaks close to retention times of oxindole. However, an ion scan for the region shows it is definitely not present in the control heifer. The ion chromatogram scans for masses of 104 and 133 from GC retention times 17:00 to 20:00 min. of the vitreous fluid from the right eye of the control animal is shown in Appendix Figure 31. Both of these major peaks would be expected if oxindole is present. They are absent. The ion scan of the left eye fluid is identical.

Tables 1-4 summarize the data from the GC and IR analysis. In addition to the oxindole present, multiple hydrocarbon derivatives were found in the mutilated animal some of which may not have been present in the control animal.

TABLE I
GC/MS Data from Extraction Procedure #1 of Pink and Dark Tissues
(Hexane + Chloroform, Acetone + Water) from the Mutilated Cow

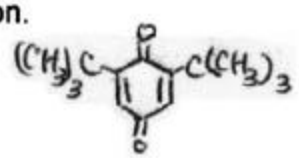
PINK			DARK		
Compound	**Match**	**GC Retention Time (min.)**	**Compound**	**Match**	**GC Retention Time (min.)**
?***Hexane/Chloroform Extracts***					
-	-		C_7H_{16} Heptane Hydrocarbon	80	5.562
C_8H_{18} Octane Hydrocarbon	81	6.727	C_8H_{18} Octane Hydrocarbon	38	6.729
Xylene (Dimethyl Benzene)	64	7.602	Xylene (Dimethyl Benzene)	42	7.604
Indene MW 116	64	9.760	-	-	
Nonanal $(CH_3(CH_2)_{7(}C=O)H$	72	10.168	Nonanal $(CH_3(CH_2)_{7(}C=O)H$	90	10.170
Phthalic Anhydride $C_8H_4O_3$	91	12.326	Phthalic Anhydride	43	12.327
$C_{19}H_{40}$ Nonadecane Hydrocarbon	64	13.259	C_{14} to C_{19} Hydrocarbons		
			Tetradecane	97	13.260
-	-		$C_{14}H_{20}O_2$(Dione) MW 220 See Attached Structure	95	14.019
$C_{16}H_{32}O_2$ Hexadecanoic Acid	93	17.632	$C_{16}H_{32}O_2$ Hexadecanoic Acid	95	17.634
$C_{18}H_{34}O_2$ Heptadecane-(8)Carbonic acid-(1) C_{18} Acid	91	19.324	-	-	
$C_{18}H_{34}O_2$ Octadecanoic Acid	64	19.499	$C_{18}H_{34}O_2$		
			Octadecenoic Acid	91	19.326
			Heptane-(8)-carbonic acid-(1)	90	
$C_{23}H_{48}$ Tricosane MW 321	93	20.140	-	-	
C_{20} to C_{23} Eicosane Hydrocarbon	98	22.881	-		
C_{20} to C_{21} Fatty Acid/Ester	66	23.873	Fatty		
			Acid/Ester/Aldehyde Assorted	10	23.874
			9-Octadecenoic acid-, 9- hexadecenyl ester	38	23.874
				14	24.283
			9-Octadecenal $C_{18}H_{34}O$		
			Hexadecanedioic acid		
$C_{28}H_{58}$ 9-Octyl Eicosane Hydrocarbon	58	24.281	-		
-	-		A Phthalate Ester		
			1,2-Benzene dicarboxylic acid, dicyclohexyl ester	37	25.216

TABLE I (Continued)
GC/MS Data from Extraction Procedure #1 of Pink and Dark Tissues
(Hexane + Chloroform, Acetone + Water) from the Mutilated Cow

PINK			DARK		
Compound	**Match**	**GC Retention Time (min.)**	**Compound**	**Match**	**GC Retention Time (min.)**
MW 330 See Attached Structures	86, 80, 78	25.214	-	-	-
MW 386 See Attached Structure	64	25.564	-	-	-
C_{23} to C_{30} Hydrocarbon			-	-	-
Tricosane $C_{23}H_{48}$	90	26.788			
Heptacosane $C_{27}H_{56}$	86, 90	29.471			
C_{28} Hydrocarbon 9-Octyl-	46	32.679	-	-	-
Eicosane $C_{28}H_{58}$					
C_{30} Hydrocarbon Dotriacontane $C_{32}H_{66}$	45	36.644	-	-	-
MW 386 $C_{27}H_{46}O$ (Another 386 Compound) Cholest-5-en-3-ol (3.beta)- See Attached Structure	96	49.999	MW 386 Cholest-5-en-3-ol (3.beta)-	-	50.059
?***Acetone/Water Extracts***					
Dimethyl Benzene (Xylene) + Butyrolactone	53, 64	7.604	Dimethyl Benzene (Xylene) + Butyrolactone		7.602
3-Methylhydantoin MW=114 $C_4H_6N_2O_2$ See Attached Structure	80	11.220	-	-	-
Phenylacetic Acid MW 136	30	11.511	-	-	-
C_{12} to C_{20} Hydrocarbon Undecane	47	13.261	-	-	-

Structures of Selected Compounds

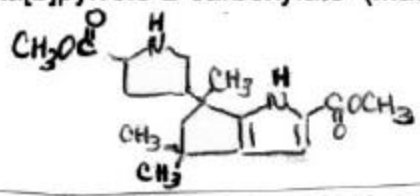
• MW 220 $C_{14}H_{20}O_2$ 2,5-Cyclohexadiene-1,4-dione, 2,6- bis(1,1-dimethylethyl)- This is a positive identification.



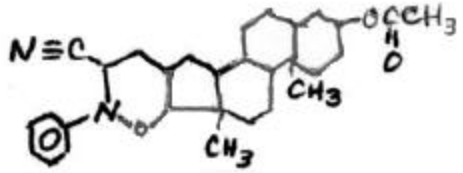
•MW 330 There are three good matches. There's a high probability that it's one of the following structures or a very similar structure.

$C_{23}H_{22}O_2$ 9,10-dihydro-9,10-dimethoxy-9,10-(1',7']-tricyclo(4.1.0.0.(2,7)heptanol anthracene (Not sure of the structure. Match 86)

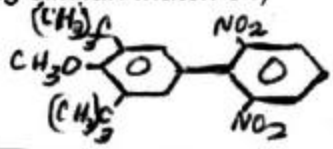
$C_{18}H_{22}N_2O_4$ Methyl-1,4,5,6-tetrahydro-6-[5-(methoxycarbonyl)-1H-pyrrol-3-yl]-4,4,6-trimethylcyclopenta[b]pyrrole-2-carboxylate (Match 80)



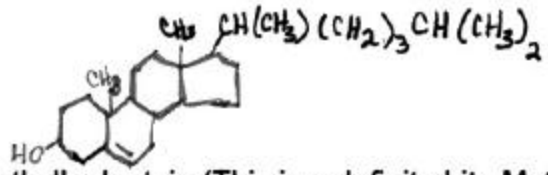
$C_{22}H_{34}O_2$ 3.beta.-Acetoxy-17-methyl-5.alpha.-18(13-17)abeoandrost-13-ene (The literature structure does not correlate with the molecular formula, but here it is. Match 78)



•MW 386 4-Methoxy-2',6'-dinitro-3,5-di-t-butylbiphenyl (The probability is good for this structure or something similar. Match 64)



•MW 386 $C_{27}H_{46}O$ (Another 386 compound) Cholest-5-en-3-ol (3.beta)- (This is a definite hit. Match 96.)



•MW=114 $C_4H_6N_2O_2$ 3-Methylhydantoin (This is a definite hit. Match 80)

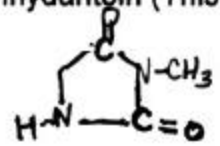


Table II
GC/MS Data from Extraction Procedure #2 Dark (Chloroform, Acetone) from the Mutilated Cow

Green Tissue		
Compound	**Match**	**GC Retention Time (min.)**
?***$CHCl_3$ Extracts***		
2-Ethylhexyl ester of Butanoic Acid	56	9.701
5-Methyl-2,4-Imidazolidinedione	56	14.461
Indole	87	15.778
Hexadecanoic Acid	94	23.779
1,1'-Dodecylidenebis [4-Methyl] Cyclohexane	43	25.703
-	-	-
*4-Methoxy-2''6'-Dinitro-3,5-Di-t-Butylbiphenyl	59	32.083
-	-	-
-	-	-
-	-	-
?*Acetone Extracts*		
Propanoic Acid	95	5.549
2,5-Dimethyl-Furan	81	6.157
C6 Ketone (4-Methyl-3-Penten-2-One)	62	7.524
C6 Ketone (4-Hydroxy-4-Methyl-2-Pentanone)	47	8.183
C5H12N2 (1-Methyl-Piperazine)	56	8.588
Hexadecanoic Acid	97	23.779
Fatty Acid [Heptadecene- (8)- Carbonic Acid- (1)]	81	25.703
Fatty Acid/Ester [Hexadecanoic Acid 2-Hydroxy-1-(Hydroxymethyl)ethyl Ester,	30	27.628
Fatty Acid/Ester [Di-(9-Octadecenoyl)-Glycerol]	41	30.463
M/Z 281 [2-(14-Carboxytetradecyl)-2-Ethyl-4,4-Dimethyl-1,3-Oxazolidine-N-Oxide]	10	30.919
[9,10-Dihydro-9,10-Dimethoxy-9,10-([1',7']-Tricyclo[4.1.0.0(2,7)]Heptano) Anthracene	59	31.729
*M/Z 386, 371 (4-Methoxy-2',6'-Dinitro -3,5-di-t-Butylbiphenol)	45	32.084

*The hit is really not that good. It could be something else, possible siloxane.

Table III
Infrared Analysis of Dried Pink and Dark Tissues and Extraction #1 Fractions from the Mutilated Cow

Spectrum	Infrared Identification	Figures
?**Tissues**		
"Dried" Pink	Significant amounts of both glycerol esters and protein material.	10
"Dried" Dark	Primarily protein type material; trace glycerol esters and carboxylic acids.	11
"Dried" Control		
?**Extracts**		
Hexane Pink	Glycerol triesters; possible trace carboxylic acids.	12
Hexane Dark	Significant amount of glycerol triester; moderate amount carboxylic acids; the esters appear to be of a higher molecular weight compared to the extract from the pink. (See difference spectrum Fig. 22.)	13
Chloroform Pink	Glycerol triester; possible trace carboxylic acids.	14
Chloroform Dark	Significant amounts of both glycerol triester and long chain carboxylic acids.	15
Acetone Pink	Predominantly glycerol triester; some carboxylic acid.	16
Acetone Dark	Significant amounts of carboxylic acid and glycerol triester	17
Water Pink	Primarily protein type material and trace glycerol ester.	18
Water Dark	Protein type material; carboxylic acid salts[1] (see difference spectrum Fig. 25)	19
?**Insolubles**		
Pink	Glycerol triester.	20
Dark	Protein type material; trace ester.	21
?**Difference Spectra**		
C_6 Ext: Dark vs Pink	Long chain carboxylic acid; higher molecular weight ester than in the pink sample.	22
$CHCl_3$ Ext: Dark vs Pink	Long chain carboxylic acid.	23
$(CH_3)_2C=O$ Ext: Dark vs Pink	Long chain carboxylic acid.	24
H_2O Ext: Dark vs Pink	Carboxlic acid salt with a possible ammonium cation; possible sulfate; carboxlic acid.	25

[1] It was noted that the water solubles from the dark tissue foamed when agitated. This implies detergency, which is typical for carboxylic acid salts, i.e. soaps. The water solubles from the pink tissue did not foam.

TABLE IV
GC/MS Data from the Vitreous Fluid of the Mutilated Cow

Vitreous Fluid (As Received)		
Compound	**Match**	**GC Retention Time (min.)**
Acetaldehyde	91	3.380
Trimethylamine	86	3.579
Butane	4	4.077
1-Propanol	72	4.326
Acetic Acid	91	4.824
Methyl Butanal	45	5.421
Propionic Acid	93	5.969
Butanoic Acid	90	7.263
C6 Acid	12	8.159
Dimethyl Sulfone	59	9.055
GBL Butyrolctone	83	9.254
Phenol	91	10.698
Urea	86	10.848 & 10.997 & 11.196
C8H16 Hydrocarbon (1-Ethyl-3-Methyl-Cyclopentane)	83	12.142
4-Methyl-Phenol	95	12.341
Amine? (1-Piperazineethanamine)	12	12.441
2-Piperidinone	35	13.735
2-Piperidinone	50	13.835
5-Methylhydantoin	50	14.581
N-Butyl-1-Hexanamine	42	14.731
Amine (N-Ethyl-Cyclopentanamine)	37	15.030
C3H6N4 Amine (4-Methyl-1,2,4-Triazol-3-Amine)	72	15.278
5-Methylhydantoin	83	15.577
Indole	93	15.926
MW=112 (4,5-Dihydro-6-Methyl-3(2H)-Pyridazinone)	32	16.324
2-Methoxy-5-Methyl-2,5-Cyclohenadiene-1,4-Dione	40	16.573
M/Z 42, 98, 111 (1,1'-Methylenebis-Piperidine)	47	16.772 to 16.822
MW= 152 Aromatic Oxygenate (2-Hydroxy-5-Methoxy-Benzaldehyde)	43	17.120
M/Z 100 Nitrogen Compound (2,4-Imidazolidinedione)	64	17.419
Tyramine	72	17.469
MW=152 ?Oxygenate (3-Hydroxy-2-Isobut-1-Enylcyclopent-2-En-1-One	90	17.817
Oxindole	93	18.216
(4-Hydroxy-3-Methoxy-Benzaldehyde)	23	18.365
M/Z 165 (2-Amino-1,7-Dihydro-7-Methyl-6H-Purine-6-One)	38	18.465
MW=166 [3-(1-Amino Ethylidine)-6-Methyl-1H, 3H-2, 4-Pyridinedione]	35	18.614
M/Z 100 (2-Methyl-2-Butenoic Acid)	49	18.813
(1-Nitroso-Pyrrolidine)	45	
Thymin	87	19.211
MW=180 [4-(Acetyloxy)-Benzoic Acid]	49	19.361
(Glutamic Acid)	72	19.709 to 19.759
MW=194 C12H18O2 Lactone Type (Lactone of 5-Acetyl-1,3,3,4,5-Pentamethylbicyclo[2.1.0]Pentan-2-One)	27	19.958
M/Z 120? Phenylalanine Deriv. (L-Phenylalanine-4-Nitroanilide)	50	20.307
M/Z 168 (Imidazo[2,1-a]Isoquinoline)	11	20.954
M/Z 123, 165 Acetanilide Deriv. (3-Methoxyacetanilide)	25	21.153
M/Z 114, 41, 83 Amine? [3-(Hexylamine)-Propanenitrile]	25	21.302
M/Z 116 Glutaminic Acid Deriv. (Glutaminic Acid Dimethyl Ester)	32	21.551
M/Z 154, 70		22.298 & 22.547
M/Z 154, 70		23.493 & 23.642
C18 Fatty Acid (Octadecanoic Acid)	91	23.891
M/Z 186 Indole Deriv. (Fragments for Indole itself +186)		24.538
M/Z 200 Indole Deriv. (Fragments for Indole + 200)		25.285
Phenoxy Components?		25.883 & 26.082 & 26.530 & 28.222
Cholest-5-en-3-ol	89	56.948

Discussion

Oxindole in the Mutilated Animal, Not in Control Animal

The most noteworthy finding in this investigation is the discovery of oxindole in an animal found mutilated in Dupuyer, Montana in June 2001. NIDS and its contract laboratories spent considerable time and effort to examine the hypothesis that oxindole was merely a degradation product of tryptophan. We obtained an animal from the slaughterhouse and exposed it to weather for 96 hours so that the decomposition process in the mutilated animal was mimicked. We then subjected the eye fluid and tissue from under the jaw from the control animal to exactly the same analysis as we had conducted on the mutilated animal. We found no oxindole in the control animal. The clinical and pharmacological properties of oxindole have been examined primarily in Europe (Mannaioni et al.(1998) *British J. Pharmac*. 125, 1751-1760). However prior to these more recent studies, it has been well established that systemic administration of oxindole to rats, dogs or humans has been shown to cause profound sedation, decrease in blood pressure, decrease in muscular tone and loss of consciousness (Orcutt et al. (1964) *Arch. Int. Pharmacodynam*. 152, 121-131.). It was this interesting property of oxindole that led us to conduct the control experiment. Our failure to find oxindole in the control animal leads us to the working hypothesis that oxindole may have been used to sedate the animal prior to its mutilation. Similar analyses of different mutilated animals in the future will either substantiate or negate this working hypothesis. We are aware that oxindole is not licensed by the FDA for use in the United States as an animal sedative. The compound can be purchased for research purposes by recognized institutions. NIDS is presently investigating the possible sources and origins of oxindole in Montana. The pharmacokinetic data on administration of oxindole to large animals is scanty as are the data on the extent of rumen-saliva recycling of tryptophan metabolites. A second hypothesis is that an unknown traumatic event triggered the rapid accumulation of oxindole in the tissues of the mutilated animal but not in the control animal These subjects are under study and, in conjunction with similar analyses on other mutilated animals in the future, may contribute to our understanding of the mutilation phenomenon.

Other Metabolites or Suspicious Molecules?

A glance at the other compounds found in the GCMS analysis of eye fluid and tissue from the mutilated animal (Tables 1, 2 and 4) reveals that the majority of compounds constitute expected decomposition products. NIDS at present is unsure if the presence of xylene and 4-Methoxy-2',6'-dinitro-3,5-di-t-butylbiphenyl as well as some other hydrocarbon derivatives are suspicious. Unfortunately, the data on decomposition of ruminants are less plentiful than those on human decomposition. Therefore, drawing comparisons between human and ruminant decomposition is fraught with error because of the vast number of poorly characterized metabolites from rumen micro-flora that are found in cattle.

Context of this Investigation

Since summer of 2001, NIDS has received over eight reports of animal mutilations from Montana, the majority of which were too old to seriously investigate. Nevertheless, this number of reports in a few months constitutes by far the largest report frequency received in the history of NIDS investigations of the animal mutilation phenomenon. The present case should thus be seen not as an isolated incident, but in the context of a wave of mutilation reports in 2001 from Montana. It should also be noted that the Great Falls area of Montana was the locus of one of the most sustained waves of animal mutilation in recorded history during the period 1974-1977. For more details on this historical wave see the NIDS report at http://www.nidsci.org/articles/pdf/wolverton_report.pdf.

Investigative Design

For this case, NIDS chose to (i) analyze the animal's eye fluid, (ii) focus on the chemical/toxicology IR+GCMS analysis, and (iii) include a control "mock mutilated" animal in the investigative design. NIDS also began the process of scaling up the methodology for analysis of maggot mass in mutilated animals as a means of determining possible toxic compounds in the animal. In doing this, NIDS is adopting a more forensic approach to animal mutilations and in doing so, we are breaking new ground.

A Note to Ranchers and Law Enforcement Officials

NIDS is gratified by the increasing spirit of cooperation and collegiality between our organization and ranchers, law enforcement officials and veterinarians. We believe that the successful investigation of animal mutilations is utterly dependent upon close cooperation between ranchers who are willing to make timely reports to NIDS (702-798-1700 or toll-free: 888-433-6500) or to local law enforcement, open-minded veterinarians who are willing to conduct timely necropsies on mutilated animals, and hard-working law enforcement officials who serve as both investigators and liaisons between NIDS and the ranchers themselves. We emphasize that NIDS absorbs 100% of the costs of these investigations and secondly that we do NOT publicize the names of ranchers, law enforcement officials or veterinarians who work with us.

Appendix

Raw data, including chromatograms from the investigation of a mutilated animal in Dupuyer Montana.

File : C:\HPCHEM\4\DATA\BSB\PAB90601.D
Operator : [BSB1]RLW 9/6/01
Acquired : 6 Sep 2001 11:32 using AcqMethod DB1SVO
Instrument : GC/MS #4
Sample Name: Dark C6 + CHCl3 Extract 9/601 in methyl Salicylate
Misc Info : Semivol Org Analysis 1 ul Splitless EM+2
Vial Number: 1

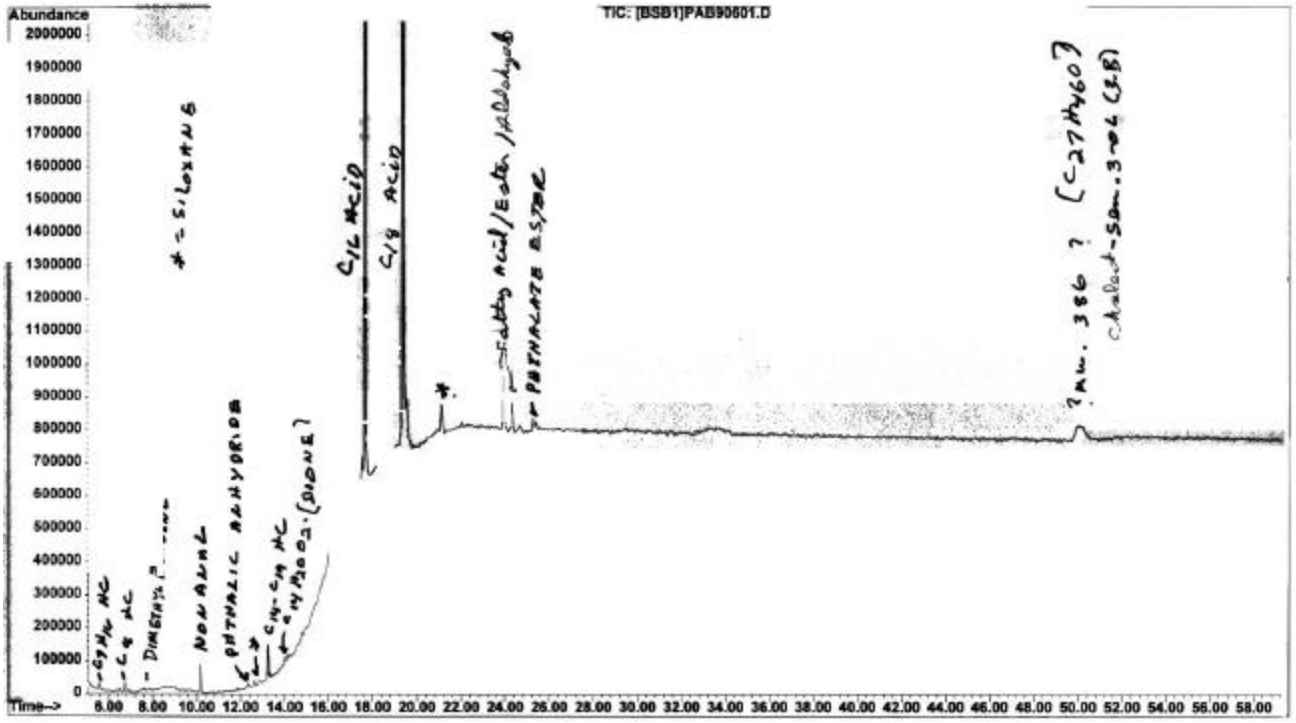


Figure 1. GC chromatogram of the hexane + chloroform extract from the dark tissue of the mutilated cow (extraction #1).

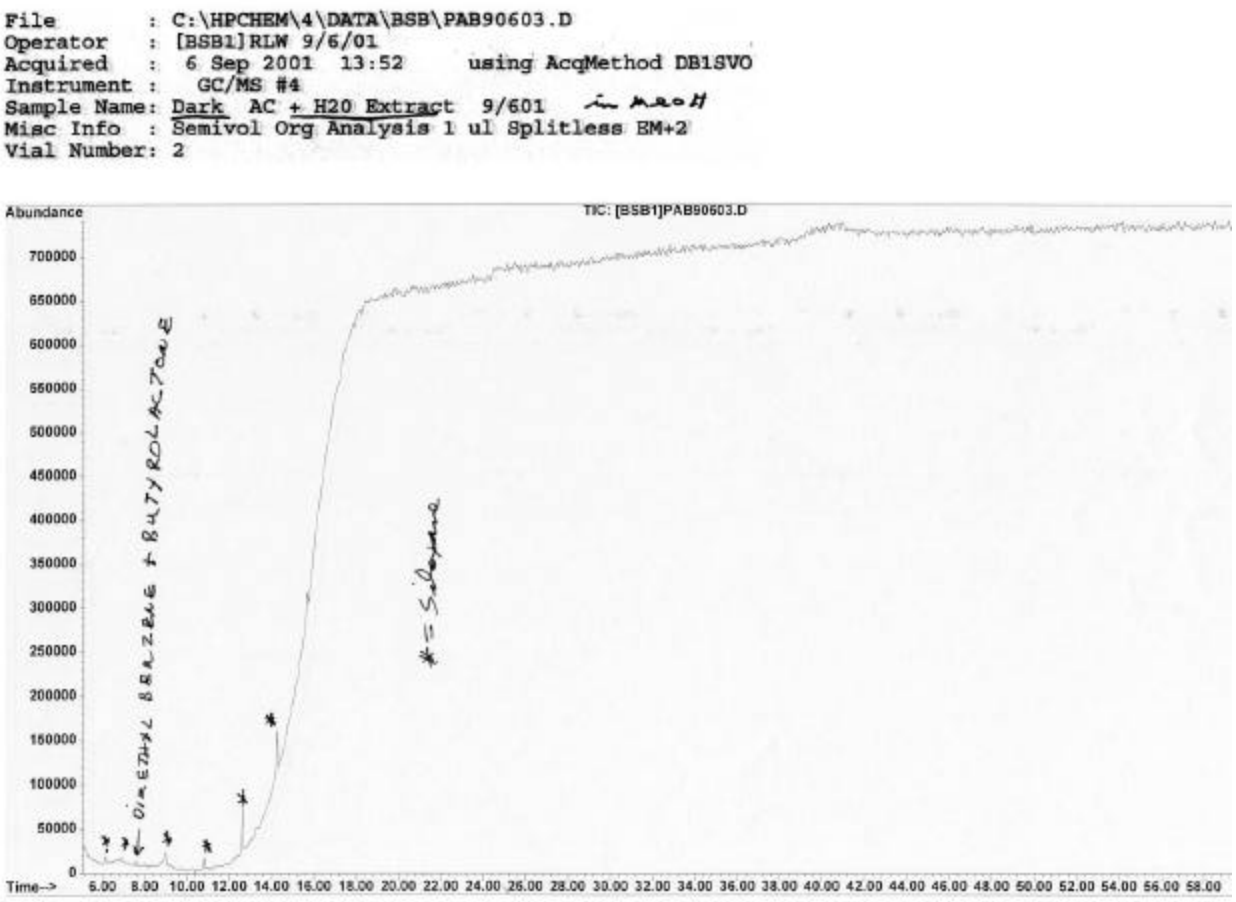


Figure 2. GC chromatogram of the acetone + water extract from the dark tissue of the mutilated cow (extraction #1).

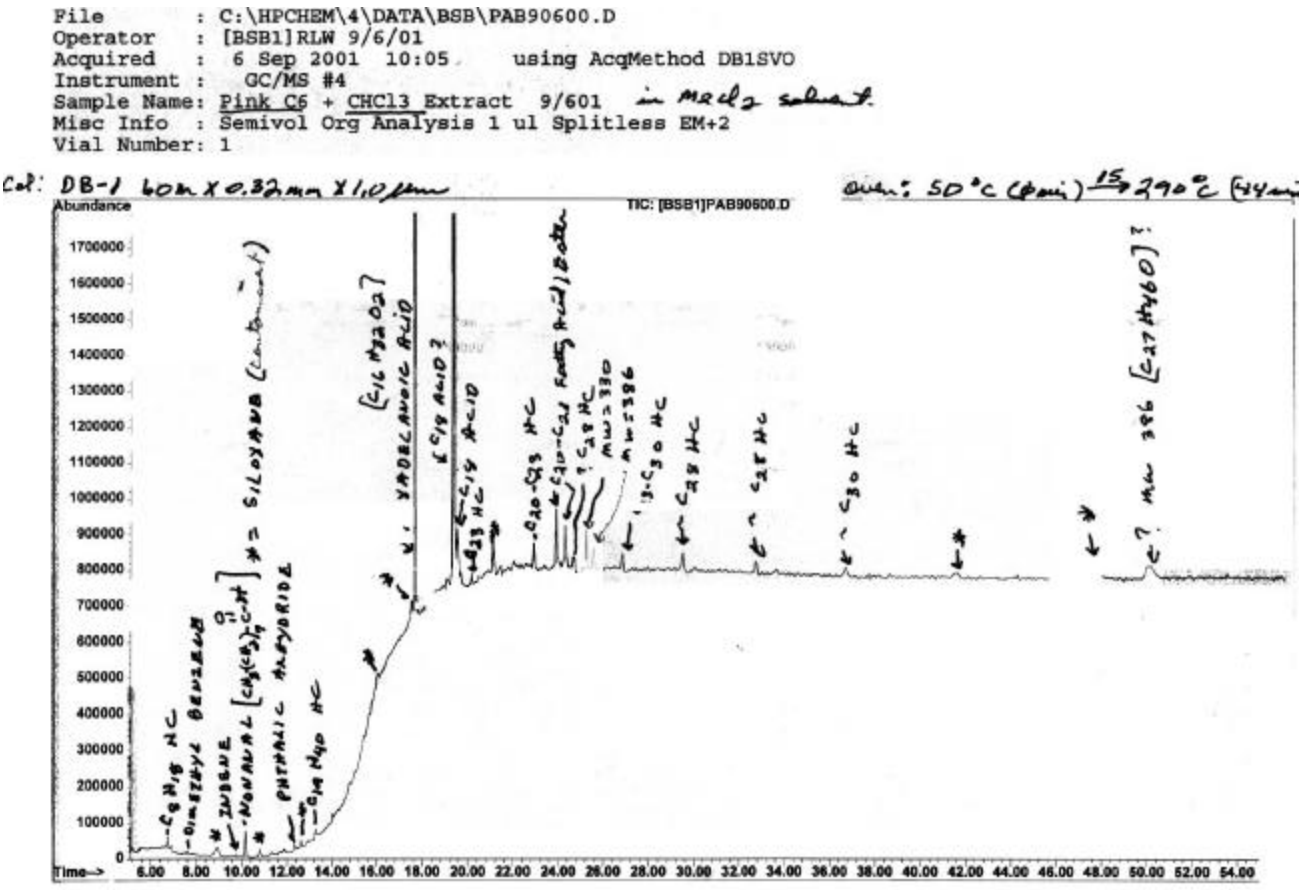


Figure 3. GC chromatogram of the hexane + chloroform extract from the pink tissue of the mutilated cow (extraction #1).

```
File       : C:\HPCHEM\4\DATA\BSB\PAB90602.D
Operator   : [BSB1]RLW 9/6/01
Acquired   :  6 Sep 2001  12:42      using AcqMethod DB1SVO
Instrument :   GC/MS #4
Sample Name: Pink  AC + H20 Extract  9/601   in methanol
Misc Info  : Semivol Org Analysis 1 ul Splitless EM+2
Vial Number: 1
```

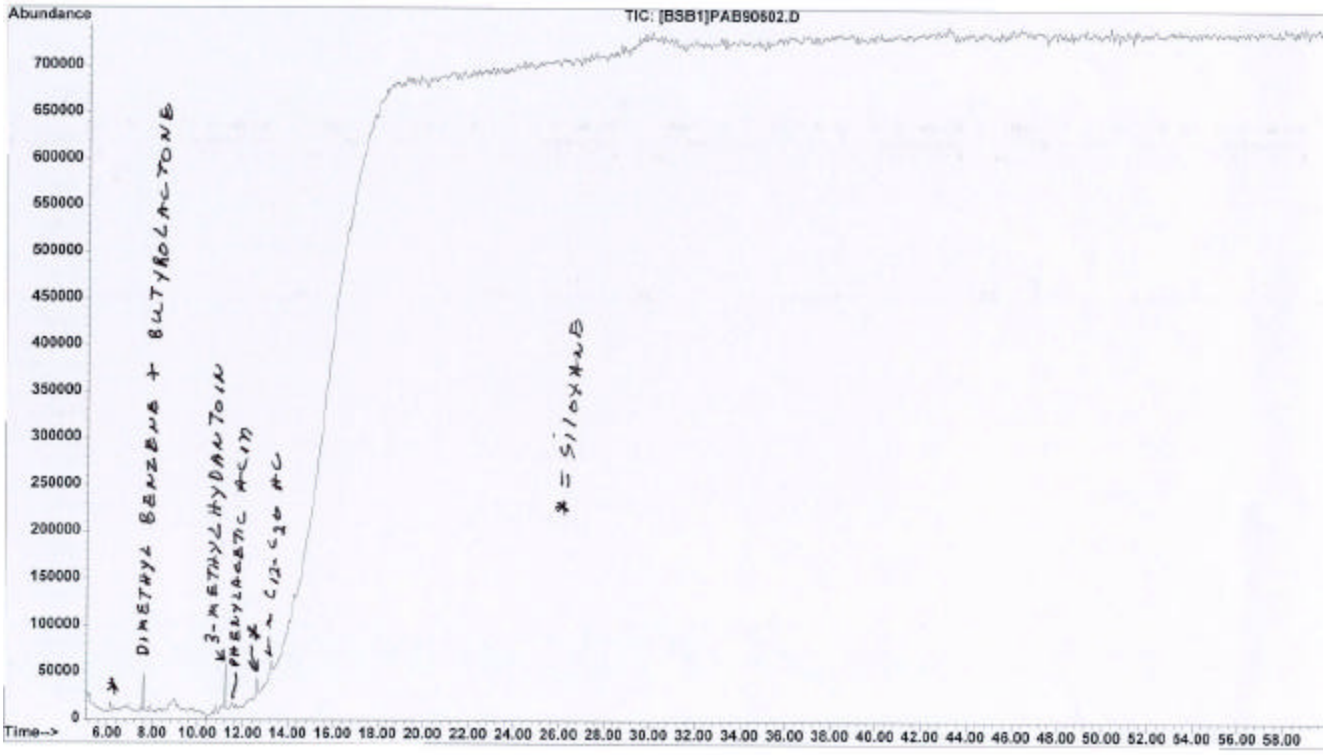


Figure 4. GC chromatogram of the acetone + water extract from the pink tissue of the mutilated cow (extraction #1).

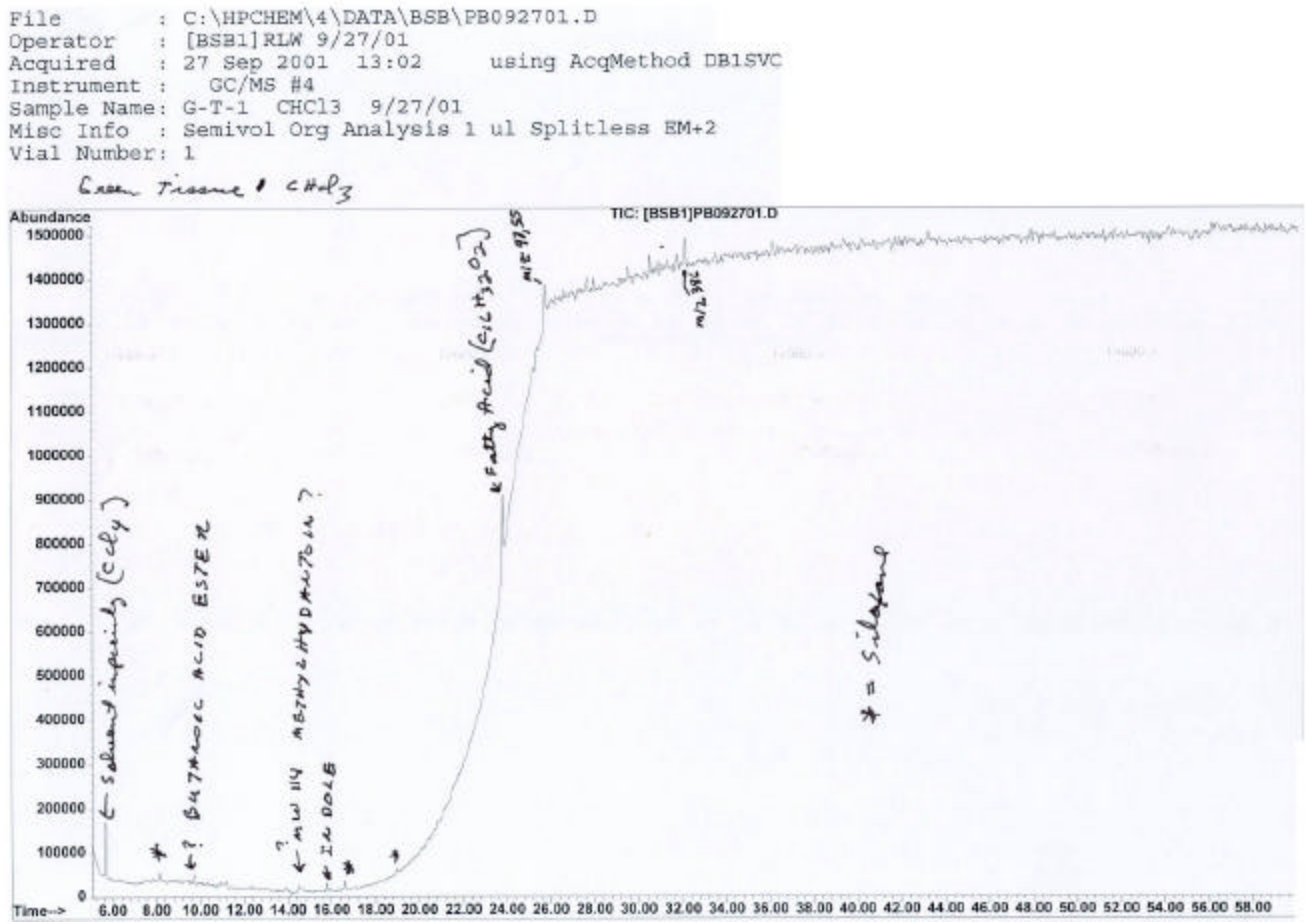


Figure 5. GC chromatogram of the chloroform extract from the mutilated cow tissue (extraction #2).

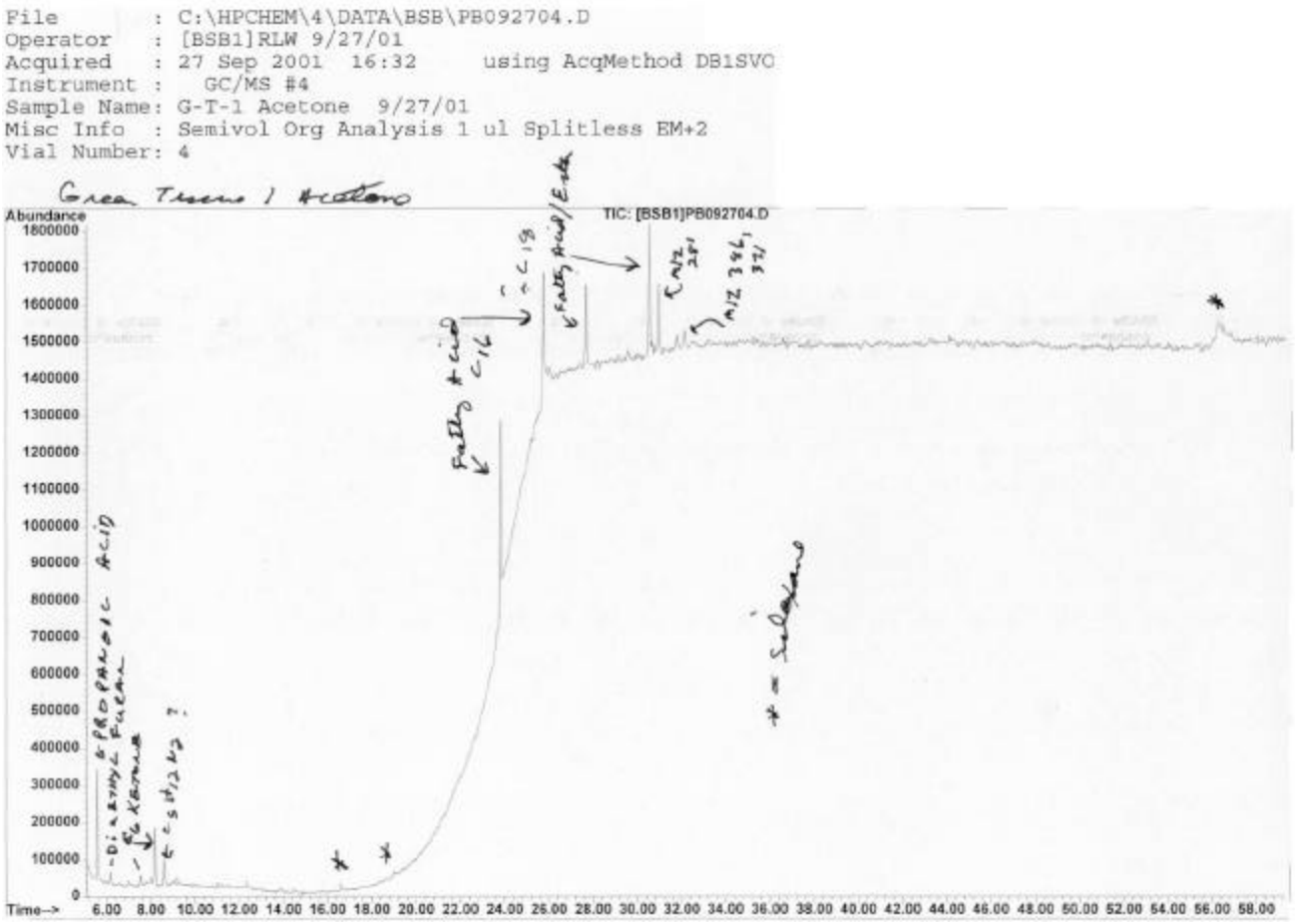


Figure 6. GC chromatogram of the acetone extract from the mutilated cow tissue (extraction #2).

```
File       : C:\HPCHEM\4\DATA\BSB\PAB10162.D
Operator   : [BSB1]RLW 10/16/01
Acquired   : 16 Oct 2001  17:54     using AcqMethod RWSVM
Instrument :   GC/MS #4
Sample Name: Green Tissue  10/16/01
Misc Info  : Semivol Organic Analysis 1ul splitless EM+2
Vial Number: 4
```

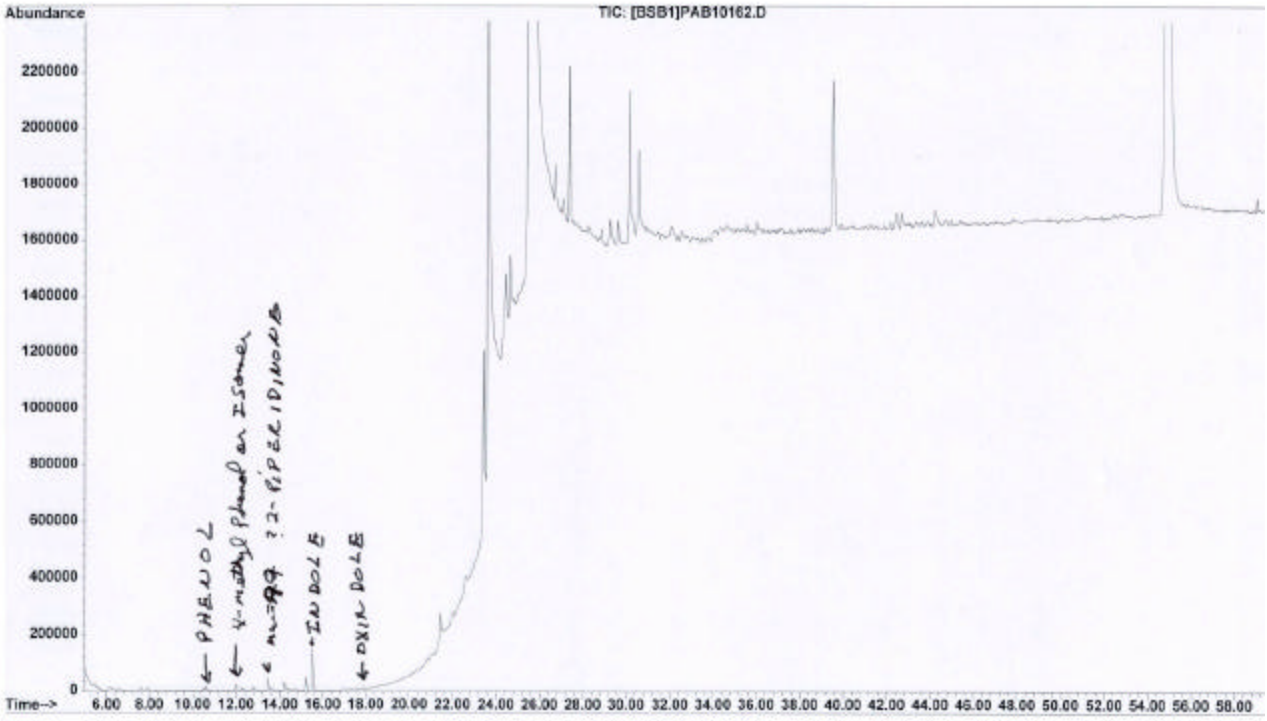


Figure 7. GC chromatogram of the refrigerated chloroform extract from the mutilated cow tissue (extraction #3).

```
File       : C:\HPCHEM\4\DATA\BSB\PAB1204.D
Operator   : [BSB2]RLW 12/4/01
Acquired   :  4 Dec 2001  17:10      using AcqMethod RWSVM
Instrument :   GC/MS #4
Sample Name: Control ( CHCl3 Ext Muscl2) 12/4/01
Misc Info  : Semivol Organic Analysis 1ul Splitless EM+2
Vial Number: 2
```

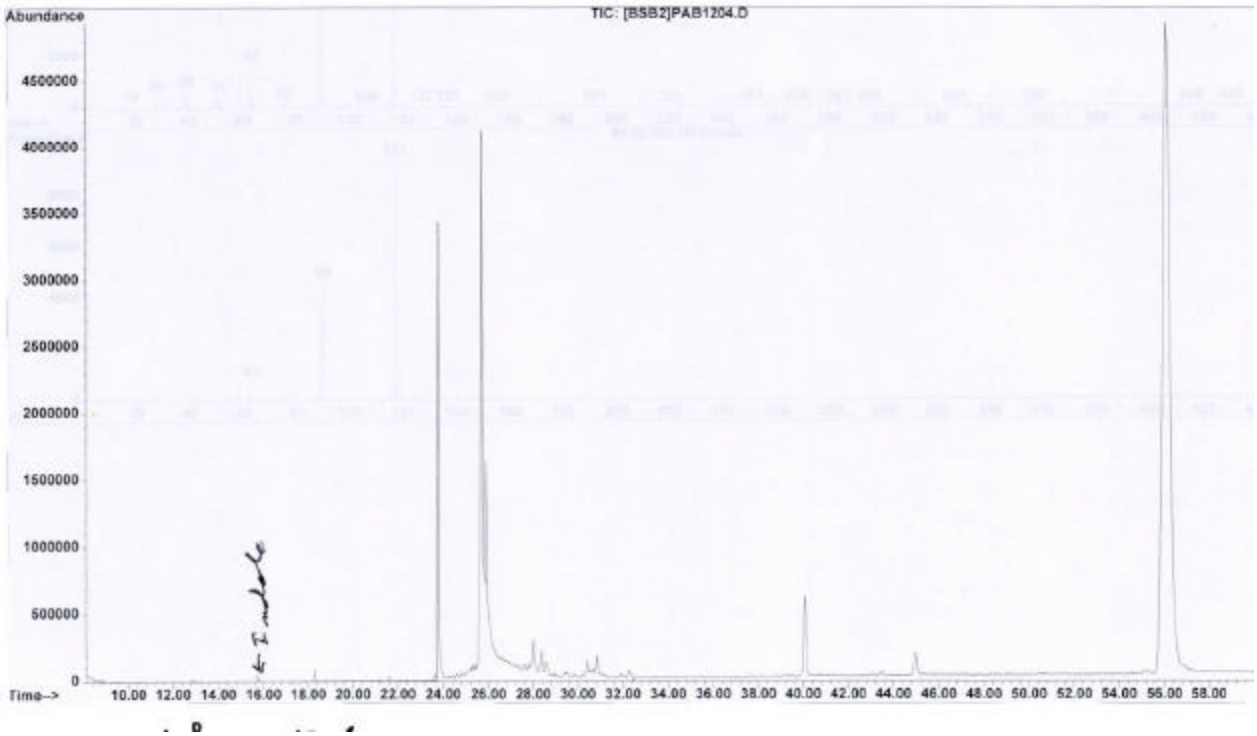


Figure 8. GC chromatogram of the refrigerated chloroform extract from the control heifer tissue (extraction #3).

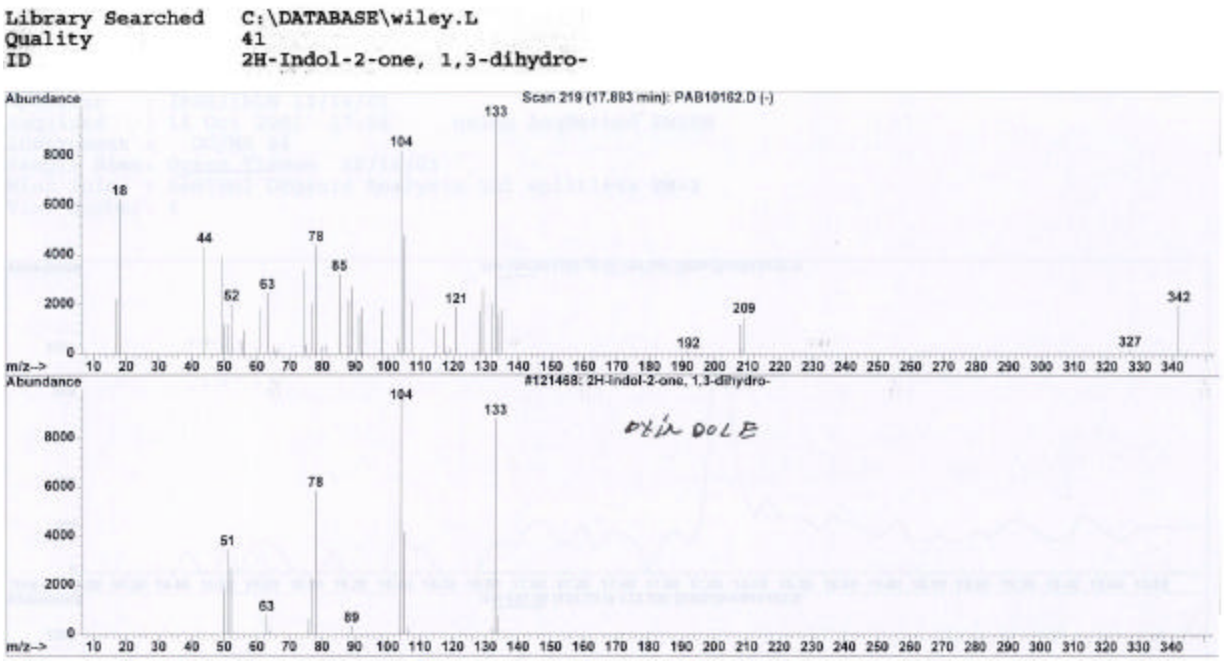
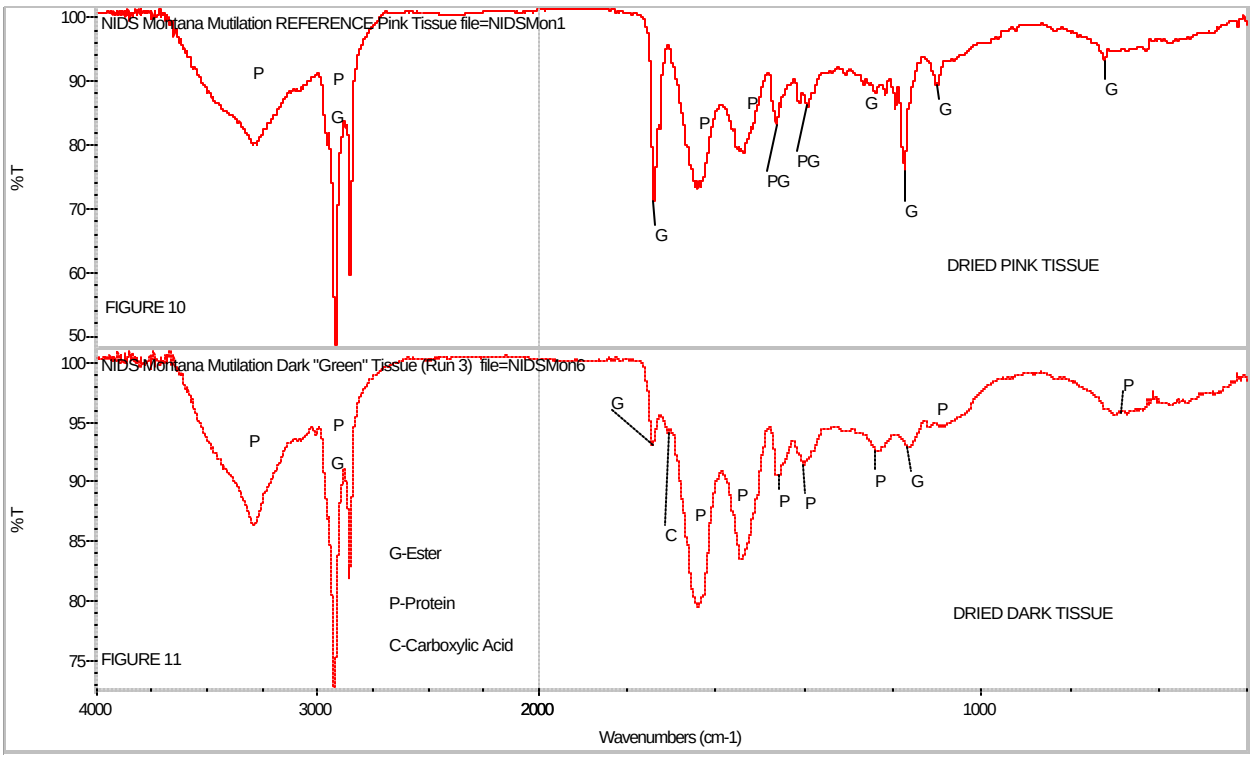
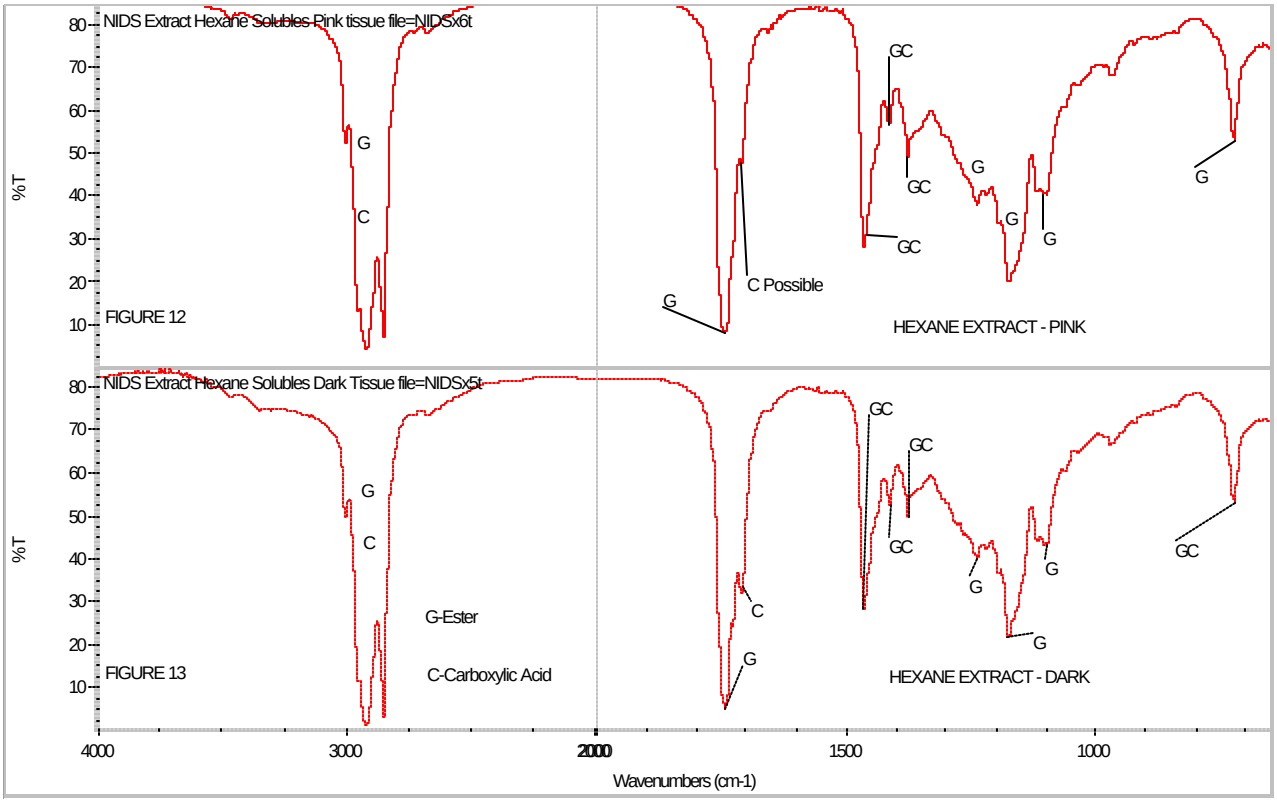


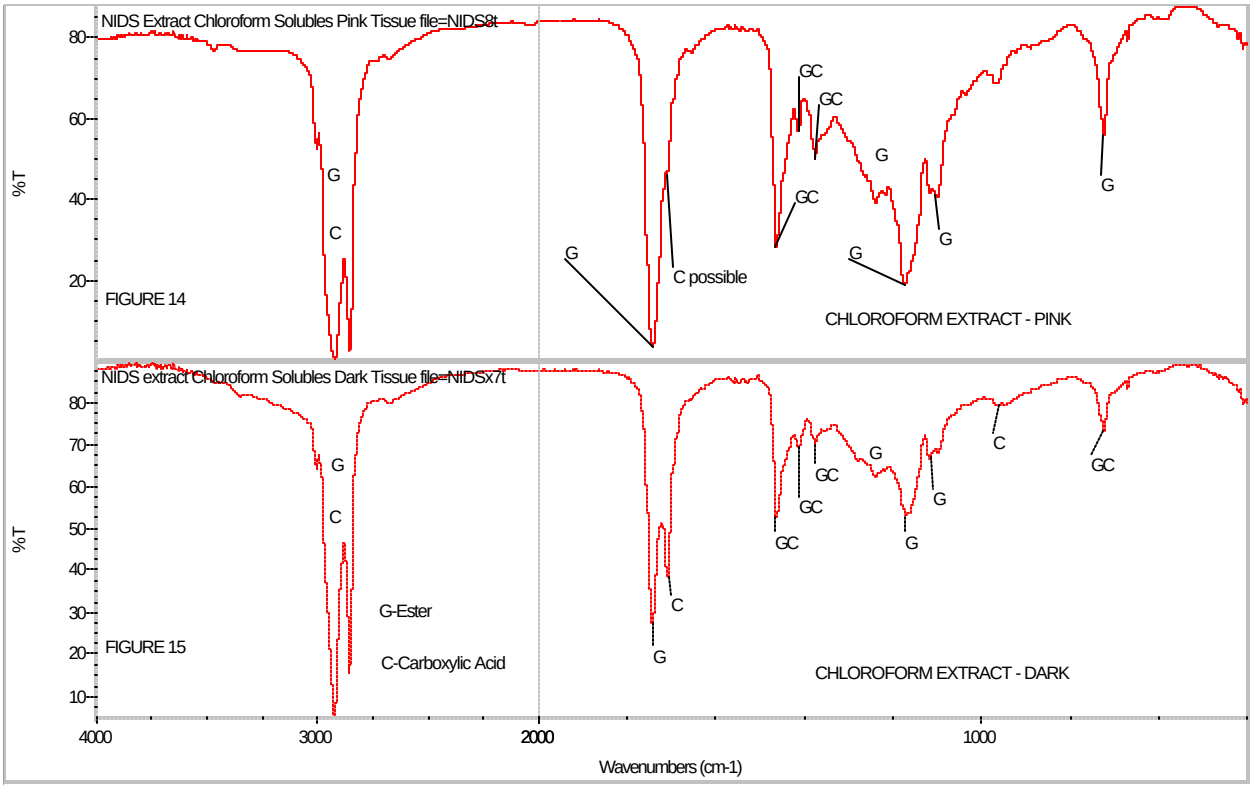
Figure 9. MS spectrum of oxindole from GC peak with retention time 17:54 min. of the chloroform extract from the mutilated cow (extraction #3) and oxindole reference.



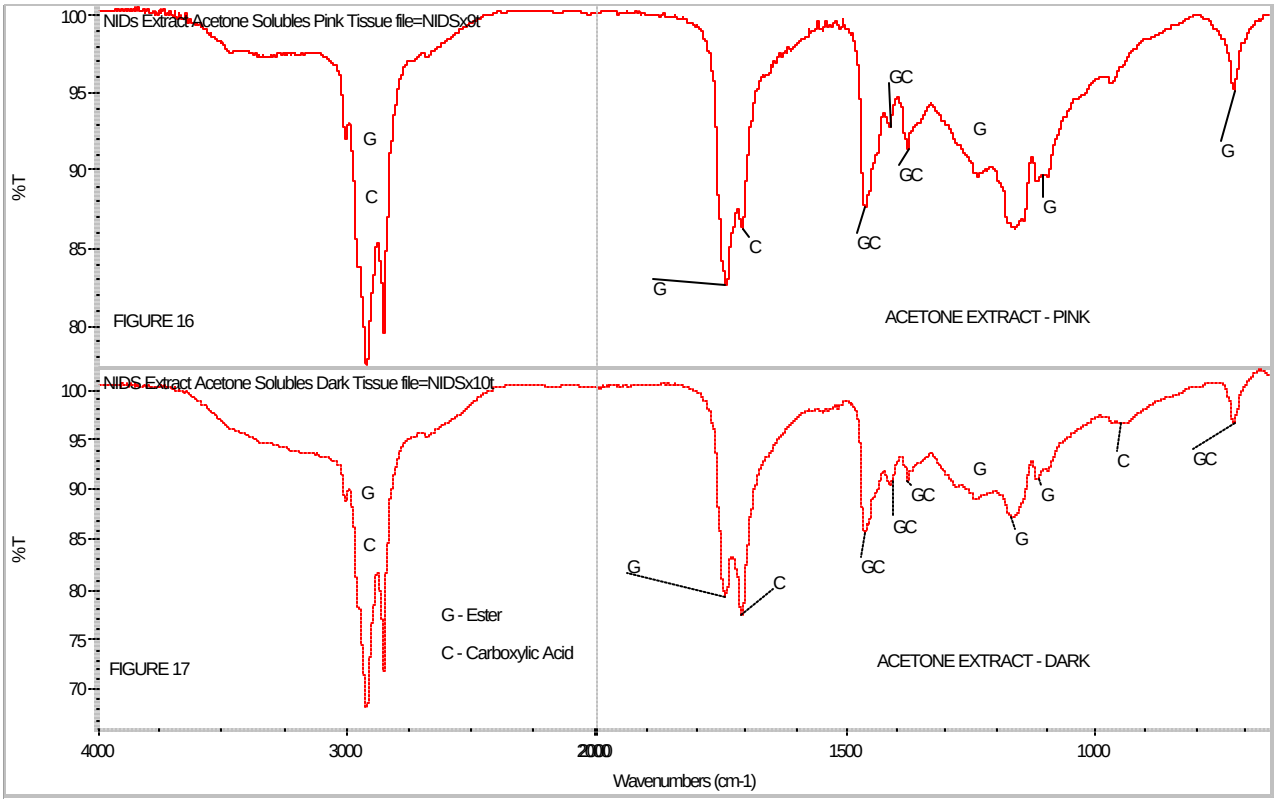
Figures 10, 11: Infrared spectra of dried pink and dark tissues from the mutilated cow.



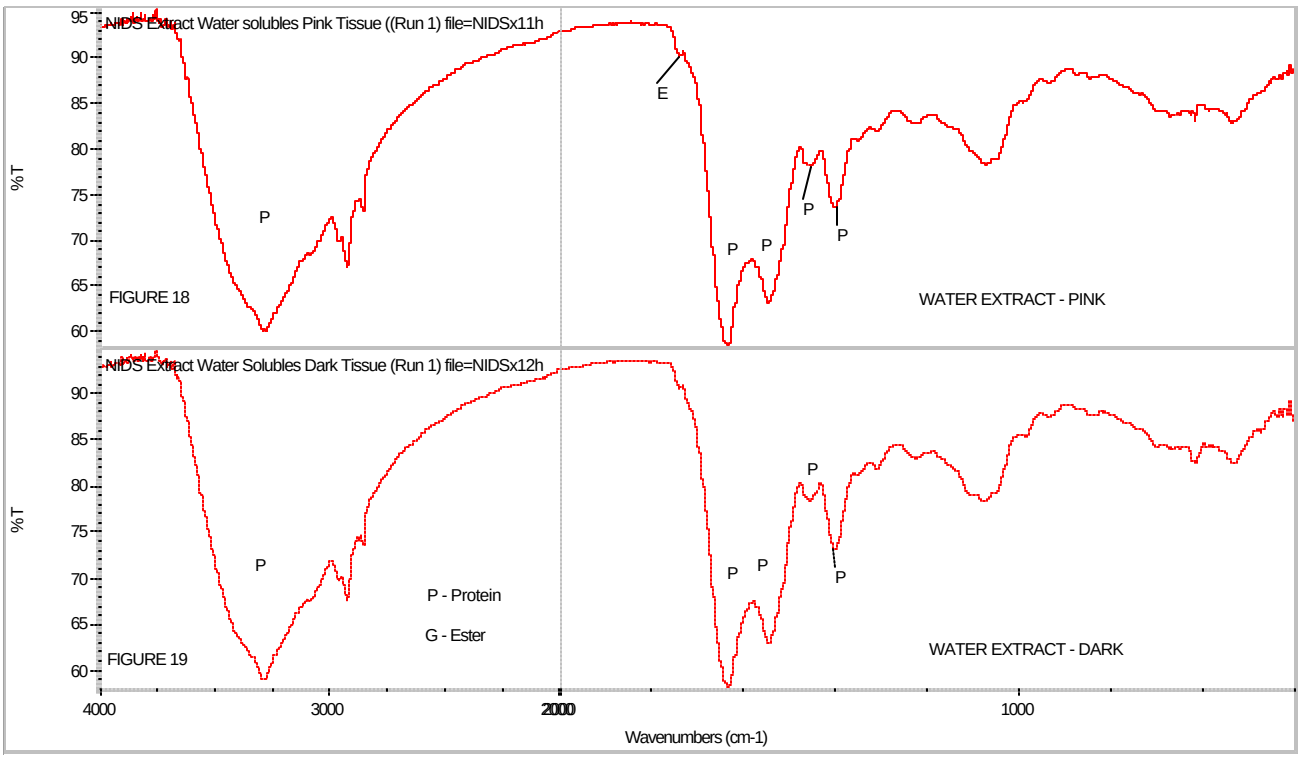
Figures 12,13: Infrared spectra of hexane extracts from the pink and dark tissues from the mutilated cow.



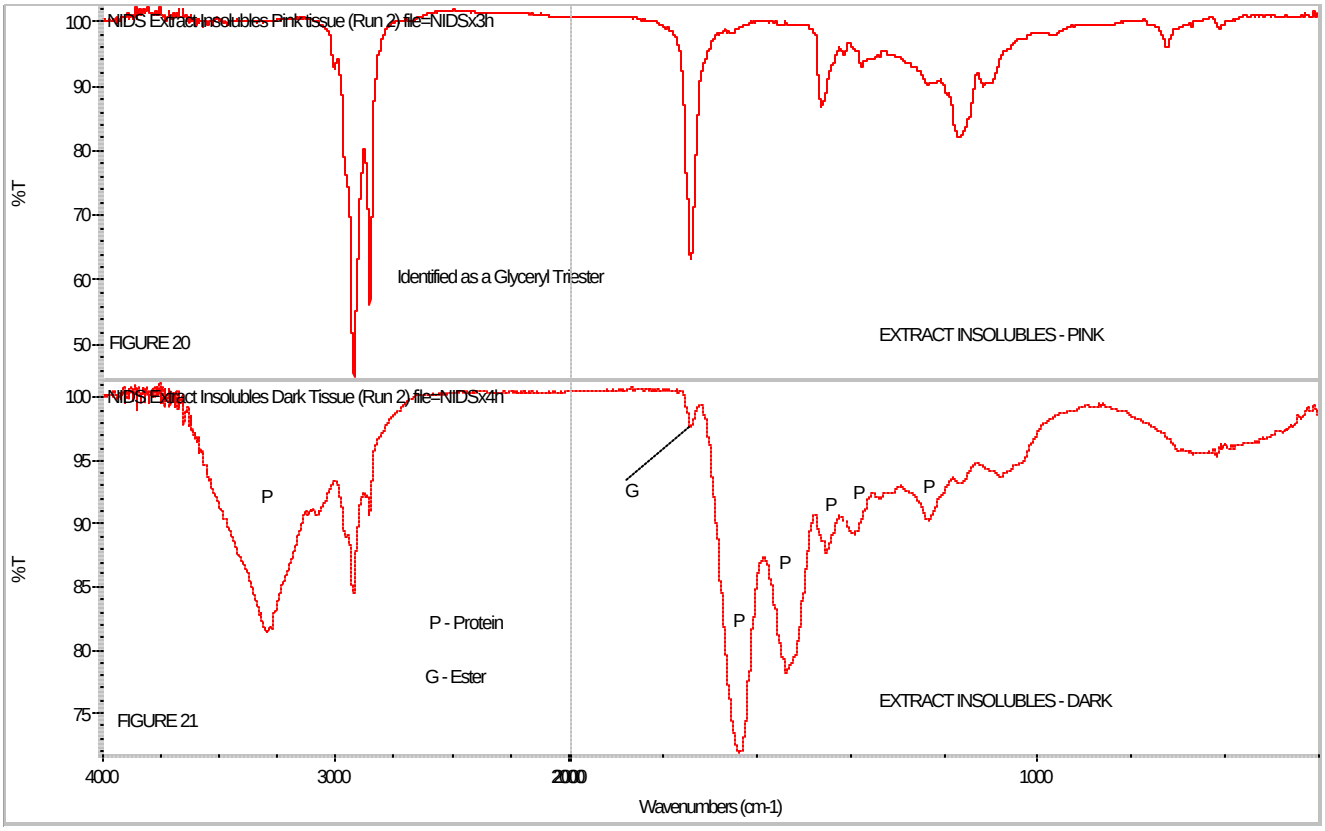
Figures 14,15: Infrared spectra of chloroform extracts from the pink and dark tissues from the mutilated cow.



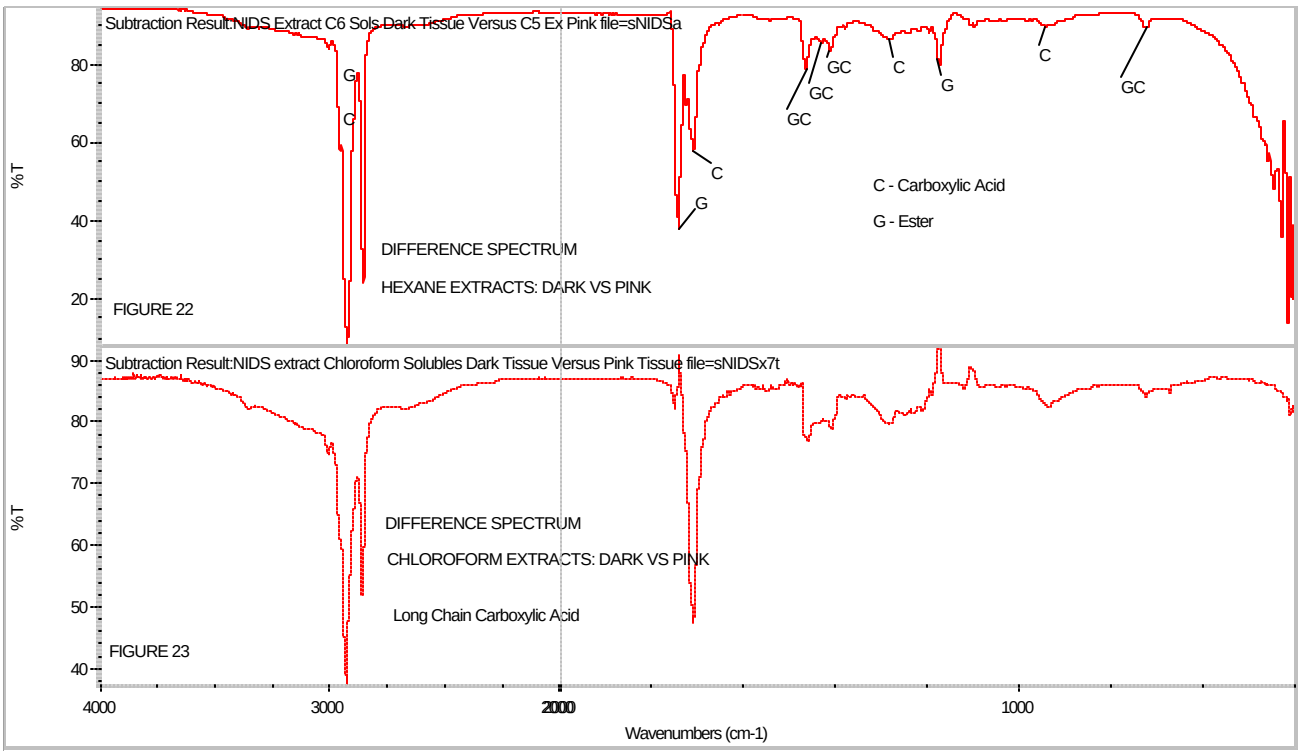
Figures 16, 17: Infrared spectra of acetone extracts from the pink and dark tissues from the mutilated cow.



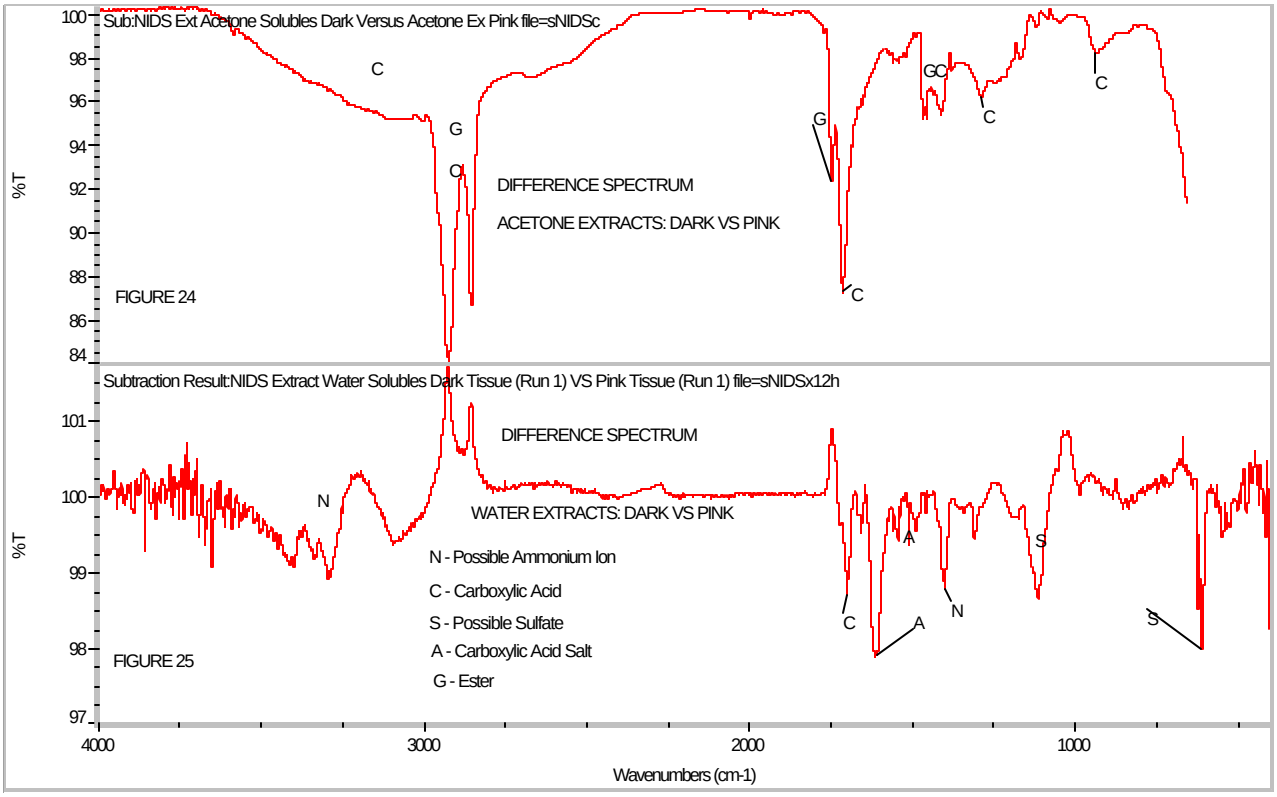
Figures 18, 19: Infrared spectra of water extracts from the pink and dark tissues from the mutilated cow.



Figures 20, 21: Infrared spectra of water extracts from the pink and dark tissues from the mutilated cow.

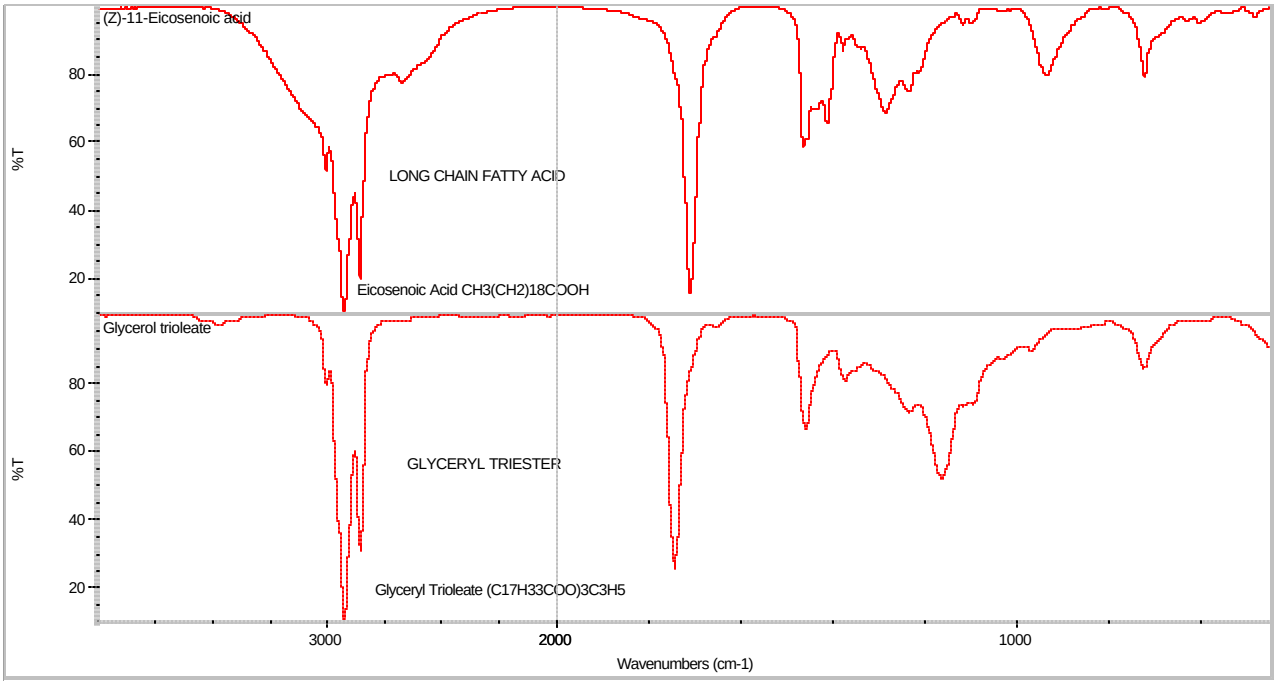


Figures 22, 23: Difference spectra of hexane and chloroform exacts from the pink and dark tissues from the mutilated cow.



Figures 24, 25: Difference spectra of acetone and water exacts from the pink and dark tissues from the mutilated cow.

Infrared References



Figures 26, 27: Infrared reference spectra of a long chain fatty acid and a glyceryl triester.

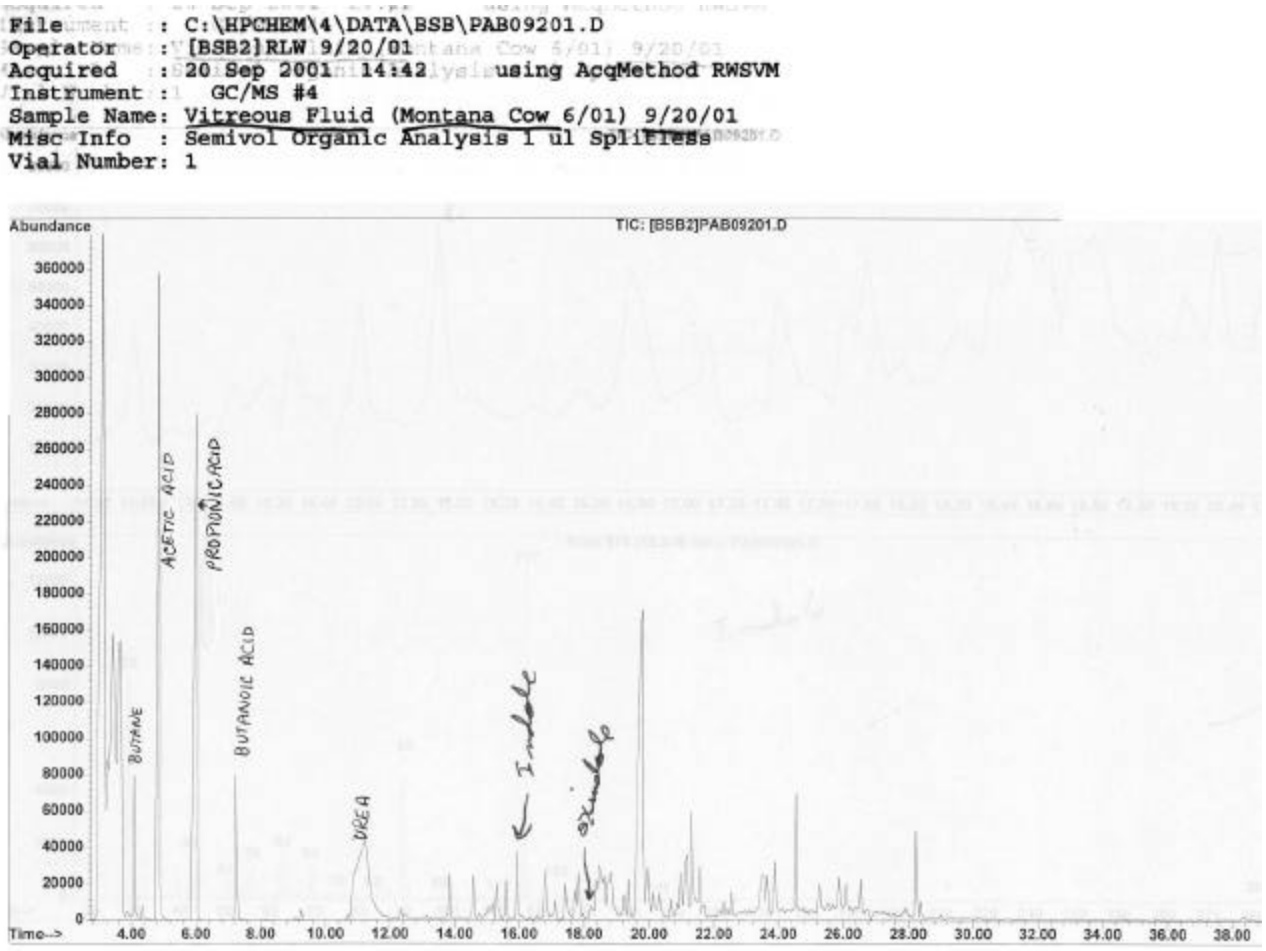


Figure 28. GC Chromatogram of the vitreous fluid from the mutilated cow.

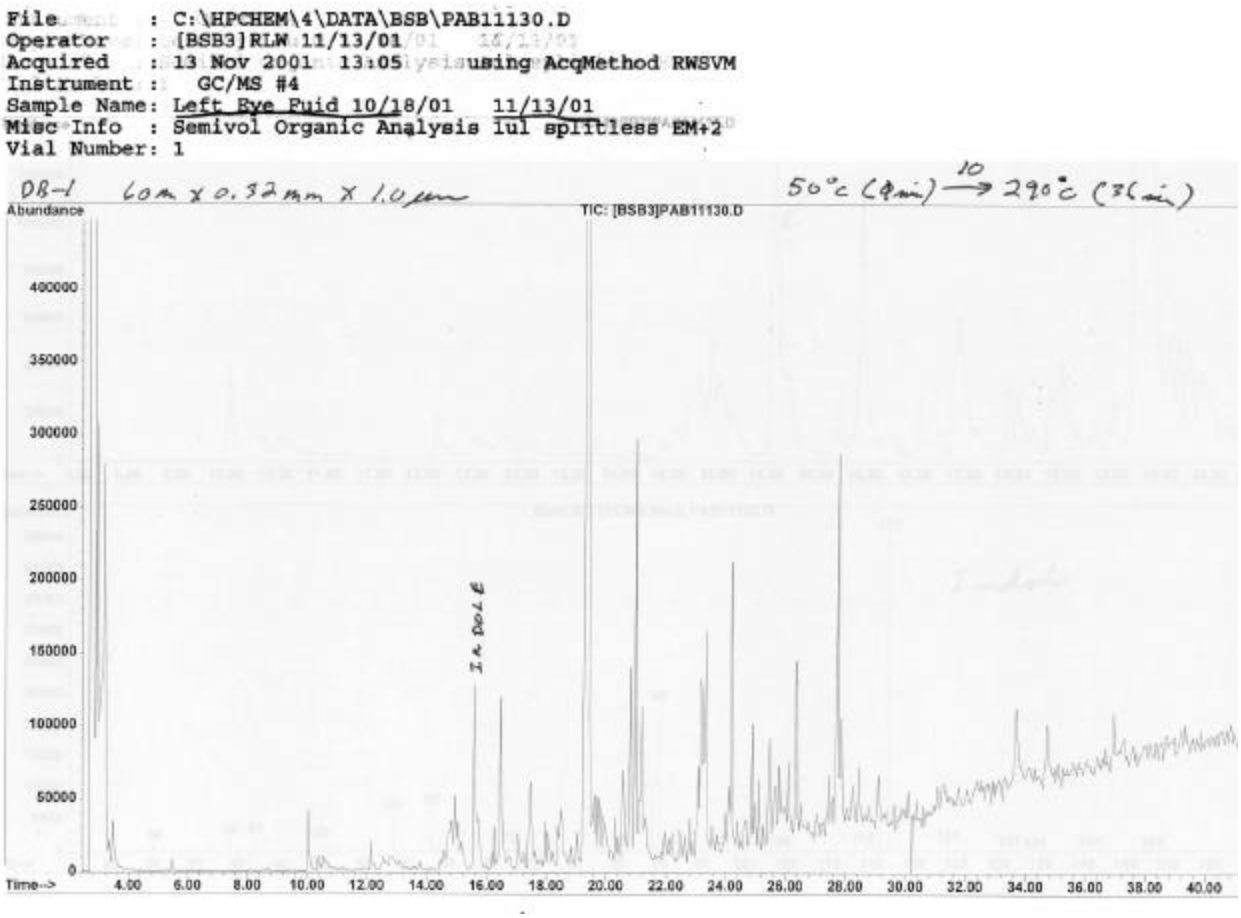


Figure 29. GC Chromatogram of the vitreous fluid from the control heifer.

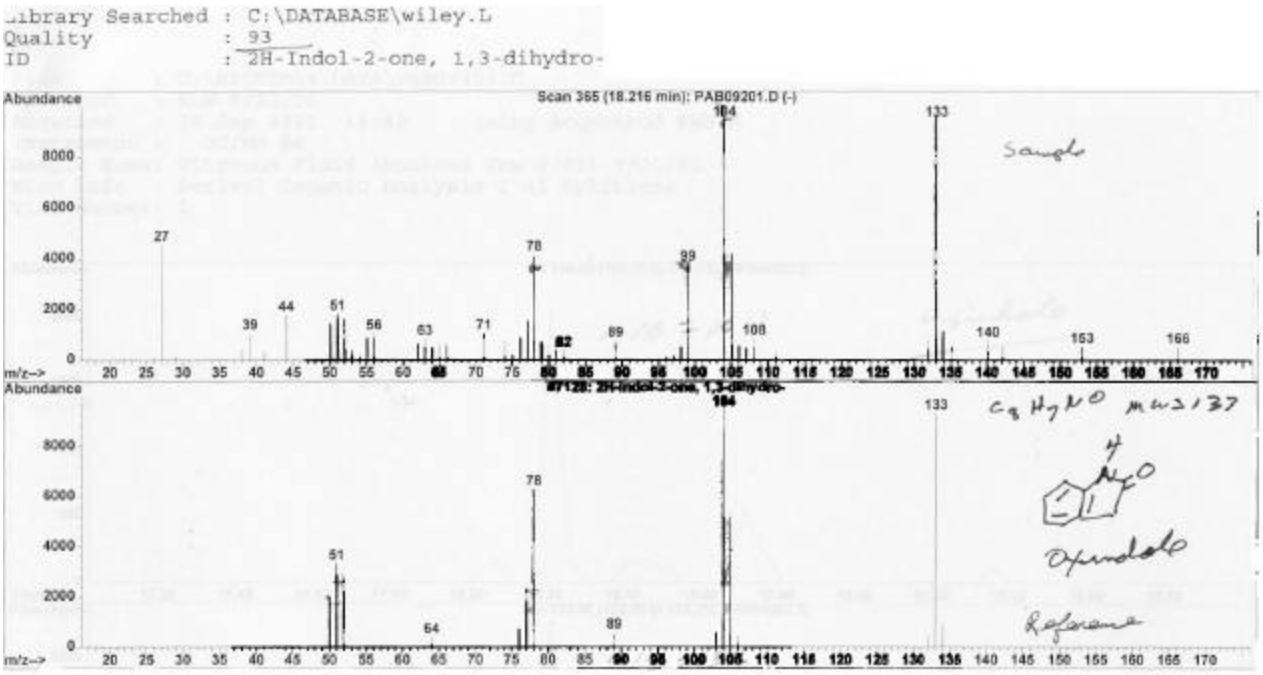


Figure 30. MS spectrum of oxindole from GC peak with retention time 18.22 min. from the vitreous fluid of the mutilated cow.

```
File        : C:\HPCHEM\4\DATA\PAB11131.D
Operator    : RLW 11/13/01
Acquired    : 13 Nov 2001  15:23      using AcqMethod RWSVM
Instrument  :    GC/MS #4
Sample Name: Right Eye Fluid  11/13/01
Misc Info   : Semivol Organic Analysis 1ul splitless EM+2
Vial Number: 3
```

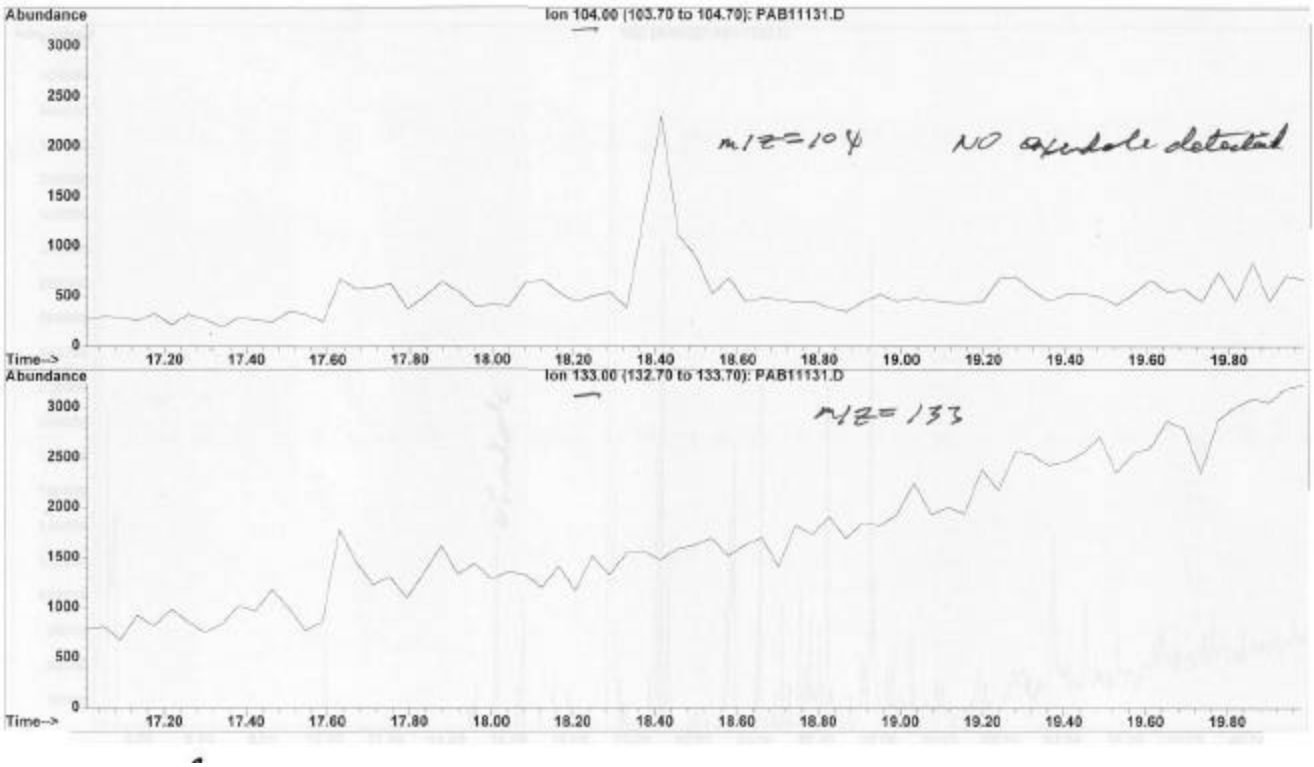


Figure 31. Ion scan for masses of 104 (Top) and 133 (Bottom) of the vitreous fluid from the control heifer.

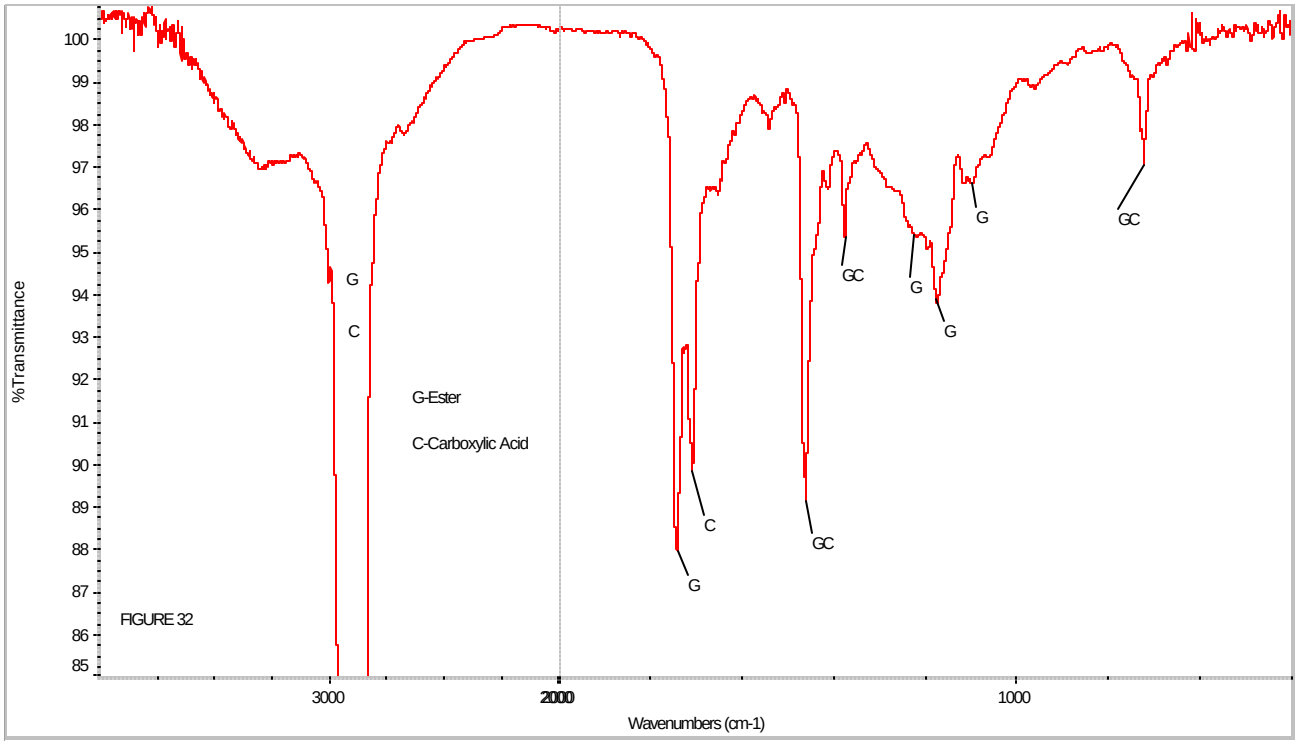


Figure 32. Infrared spectrum of the maggot mass chloroform extract (extraction#3) from the mutilated cow.