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U-007-307.73

**CARNEGIE-MELLON INSTITUTE OF RESEARCH, COMPARATIVE
PATHOLOGY ON RATS GIVEN METHOXYACETONE AND FIVE OTHER
ALIPHATIC KETONS IN DRINKING WATER, SUBMITTED TO UNION
CARBIDE CORP, DANBURY, CT, - (USED AS A REFERENCE IN OU
5 RI - APPENDIX A)**

11/08/77

40-146
UNION CARBIDE
16
REPORT

UNION CARBIDE CORPORATION

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5 December 1995

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Pittsburgh Pennsylvania 15220-2745

Subject: Methoxyacetone and Other Aliphatic Ketones

This is in response to your letter of December 4 concerning the reports listed below:

Carnegie-Mellon Institute of Research, 1977. Comparative Toxicity to Rats of Methoxyacetone and Five Other Aliphatic Ketones in Their Drinking Water. Project Report 40-37. Submitted to Union Carbide Corporation, Danbury, Connecticut, OTS 0206068.

Carnegie-Mellon Institute of Research, 1977. Comparative Pathology on Rats of Given Methoxyacetone and Five Other Aliphatic Ketones in Drinking Water. Project Report 40-146. Submitted to Union Carbide Corporation, Danbury, Connecticut, OTS 0206068.

Please be advised that you can reference the Union Carbide Corporation data in your "Remedial Investigation Report" which will be presented to the DOB and U.S. EPA in Region V (Chicago).

Please feel free to contact me if you have any questions.


Mr. T.J. Cawley - Manager
Risk Assessment Information Group

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consent of the C&P Medical Director,
Occupational Health Team Operations
Manager, or Product Safety Director.

Project Report 40-146
Sequel to Report 40-37
16 Pages
November 8, 1977
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Comparative Pathology on Rats Given Methoxyacetone and
Five Other Aliphatic Ketones in Drinking Water
(Ketone Neurotoxicity)

Sponsor: *Union Carbide Corporation*

* * * * *

Summary

Selected aliphatic ketones (methoxyacetone, diethyl ketone, ethyl n-butyl ketone, methyl isobutyl ketone and methyl tert-butyl ketone) were administered in drinking water to groups of 5 female HLA Wistar rats for 120 days. Methyl n-butyl ketone was given as a positive control. Negative controls received tap water.

Peripheral neuropathy was present in all 5 rats given 1.01 gm/kg/day of methyl n-butyl ketone; 3 of these rats had attendant hind limb skeletal muscle atrophy. Four of 5 rats given 0.48 gm/kg/day of methyl n-butyl ketone had peripheral neuropathy; 2 of these rats with peripheral neuropathy and 1 rat with normal sciatic nerves had skeletal muscle atrophy affecting the hind limb. One of 5 rats given 1.99 gm/kg/day of methoxyacetone had skeletal muscle atrophy without associated peripheral neuropathy. Occasional swollen axons were present in the central nervous system of one rat in each of the methyl n-butyl ketone groups.

Based upon analysis of tissue injury, it is concluded that methoxyacetone and other selected ketones studied are less toxic than methyl n-butyl ketone. With the exception of 1 rat given 1.99 gm/kg/day of methoxyacetone and rats given methyl n-butyl ketone, significant treatment-induced pathologic effects were not detected.

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Introduction

This study was designed to compare the relative toxicity of methoxyacetone to methyl n-butyl ketone and 4 of its structural analogs with particular emphasis on relative neurotoxicity. The compounds were administered continuously in drinking water to female HLA Wistar rats for 120 days. Pathology record sheets for each rat are attached to CHF file copy.

Methods

Groups

Fifty rats were randomly divided into groups of 5 and assigned to 1 of 10 groups. The original dosages for different groups of rats given to the pathology department were subsequently changed due to (a) realization of solubility of some of the ketones and (b) the decision to alter a dosage after the study commenced. Consequently, some of the pathology records reflect dosages different from those in the final CHF Report 40-37 (CHF report issued before pathology was completed). We have used the final, corrected dosage in this pathology report but some of the pathology records and notes have original dosages. The listing below should clarify any confusion.

<u>Group</u>	<u>Original Dosage Given to Pathology Department</u>	<u>Dosage in Final CHF Report and Pathology Report</u>
Methoxyacetone I	1 gm/kg	*4.04 gm/kg/day
Methoxyacetone II	2 gm/kg	1.99 gm/kg/day
Methyl n-butyl ketone I	1 gm/kg	0.48 gm/kg/day
Methyl n-butyl ketone II	2 gm/kg	1.01 gm/kg/day
Ethyl n-butyl ketone	0.5 gm/kg	0.03 gm/kg/day
Diethyl ketone	1 gm/kg	1.96 gm/kg/day
Methyl isobutyl ketone	1 gm/kg	1.04 gm/kg/day
Methyl tert-butyl ketone	1 gm/kg	0.88 gm/kg/day
Control-1	Tap water	Tap water
Control-2	Tap water	Tap water

* The mean weighted average daily dose was calculated to be 3.0 gm/kg/day over the entire 120 days of dosing.

Necropsy Procedure

All rats were euthanized by CO₂ narcosis followed by severing the cervical spinal cord and exsanguination *via* jugular and carotid blood vessels. Right and left sciatic and brachial nerves were dissected free and fixed in 3% phosphate buffered glutaraldehyde. The entire vertebral column was removed, divided into sections and fixed in 10% neutral buffered formalin (NBF). Anterior and posterior thigh muscles and distal portions of one rear leg were removed and fixed in 10% NBF. Slices were made through the muscles to allow for proper fixation. All abdominal and thoracic viscera were removed and representative samples were fixed in 10% NBF. The brain and skull were also fixed along with the abdominal and thoracic viscera.

Organ weights were recorded for the liver and kidneys.

Tissue Processing

Tissues were processed by standard procedures with the following exceptions:

A. Sciatic nerves. Sciatic nerves from all 50 rats were post-fixed in osmium tetroxide and whole mount teased preparations as well as sections were made on each nerve according to the following procedure:

1. Nerve briefly rinsed in normal saline.
2. Transferred to 1% osmium tetroxide in normal saline. Protected from light. Left in 1% osmium tetroxide for 24 hours.
3. Rinsed in 3 changes of normal saline.
4. Transferred to 80% glycerine in normal saline and stored overnight or longer.
5. Nerve divided in half by a transverse cut with a sharp razor. Proximal half carefully teased and processed for embedding as follows:

- A) 50% ethanol - 20 minutes
- B) 50% ethanol - 20 minutes
- C) 80% ethanol - 20 minutes
- D) 95% ethanol - 20 minutes
- E) 100% ethanol - 20 minutes
- F) 100% ethanol - 20 minutes
- G) Toluene - 15 minutes
- H) Toluene - 15 minutes
- I) Paraffin - 15 minutes
- J) Paraffin - 15 minutes

Embedded in such a manner that the teased nerve was spread out. Teasing needles were used to facilitate this during embedding.

Sectioned at 5 microns and counterstained with nuclear fast red.

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6. The distal half of the sciatic nerve was teased similarly to the proximal half and mounted on a slide using Permunt [®] to adhere the coverslip.

In several instances where nerve preparations were not suitable for evaluation due to technical artifacts, additional nerve tissue was prepared. All teased and sectioned nerves were evaluated without knowledge of the treatment level.

One-micron sections of toluidine blue stained, epon araldite embedded sections were prepared and evaluated without knowledge of treatment by Dr. Damon R. Averill, Jr., a veterinary neuropathologist at the Children's Hospital Medical Center, 300 Longwood Avenue, Boston, MA. The sciatic nerves examined by Dr. Averill were from all rats in the following groups:

1. Methoxyacetone (4.04 gm/kg/day)
2. Methoxyacetone (1.99 gm/kg/day)
3. Methyl n-butyl ketone (1.01 gm/kg/day)
4. Control-1
5. Control-2

This examination was limited to the above groups since we were primarily concerned with evaluation of methoxyacetone neurotoxicity. The methyl n-butyl ketone was included in this study as a positive control against which we hoped to assess methoxyacetone. Inclusion of the structural analogs of methyl n-butyl ketone in this study was primarily for academic reasons and, hence, sciatic nerve tissues from these animals were not subjected to this special examination.

B. Skeletal muscle. Skeletal muscle tissues from the anterior thigh muscles were processed routinely and evaluated along with other visceral tissues. Due to the desire to better evaluate any possible muscle fiber atrophy in the methoxyacetone treated animals, additional skeletal muscle tissue from anterior thigh as well as gastrocnemius muscles was processed and evaluated without knowledge of treatment. This additional skeletal muscle tissue was from rats in the following groups:

1. Methoxyacetone (4.04 gm/kg/day)
2. Methoxyacetone (1.99 gm/kg/day)
3. Methyl n-butyl ketone (0.48 gm/kg/day)
4. Methyl n-butyl ketone (1.01 gm/kg/day)
5. Control-1
6. Control-2

As an added step in evaluation of these skeletal muscle tissues, representative fields of muscle fibers cut in cross-section were photographed at a standard magnification (200X) and 8 X 10" prints were made, taking care to enlarge all negatives equally. A central 10 cm² portion of each photograph was marked off and cross-sectional areas of each muscle fiber falling within that 10 cm² portion of the photograph were measured by use of a Lasico planimeter (Model N30A). These measurements were then submitted for statistical evaluation.

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C. Spinal ganglia. The lumbar vertebrae were decalcified and cross-sections from 3 levels containing spinal cord were prepared. In several of these sections we were fortunate enough to obtain spinal ganglia. When present, these ganglia were evaluated. However, no special effort was made to obtain spinal ganglia sections from all animals. To do so would require step sectioning several additional pieces of vertebrae and spinal cord and it was judged that this additional work was not warranted since tissue proximal and distal to the ganglia was examined on each rat.

Results and Discussion

Sciatic nerve neuropathy and skeletal muscle atrophy were documented in rats treated with methyl n-butyl ketone; skeletal muscle atrophy was seen in one rat that received methoxyacetone. Axonal spheroids were seen in the spinal cord of one rat that received methyl n-butyl ketone (0.48 gm/kg/day) and similar axonal swelling was found in the brain of one rat that received methyl n-butyl ketone (1.01 gm/kg/day). These histologic changes as well as others commonly seen in rat tissues are listed in Table 1 and discussed below.

Treatment Induced Histologic Findings

Neuropathy. Peripheral neuropathy characterized by myelin clumping and segmental axonal swelling was present in sciatic nerves from all 5 rats which received 1.01 gm/kg/day and in 4 of the 5 rats which received 0.48 gm/kg/day of methyl n-butyl ketone. Several to many nerve fibers in each section were affected. Artifacts such as nerve fiber fractures and banding of myelin were present in most samples. These artifacts resulted primarily from not having fixed nerve tissue *in situ* by perfusion fixation. There was no clear indication of greater severity of sciatic nerve neuropathy in distal *versus* proximal portions of affected nerves. With the exception of 2 animals, morphologic evidence of central nervous system involvement proximal to sciatic nerve was lacking. The two exceptions involved 1 rat (1.01 gm/kg/day of methyl n-butyl ketone) with occasional swollen axons (axonal spheroids) in the cerebellar white matter and 1 rat (0.48 gm/kg/day of methyl n-butyl ketone) with swollen axons at the cauda equina of the spinal cord. These central nervous system lesions are probably treatment related. The prevalence of sciatic nerve neuropathy in methyl n-butyl ketone treated rats was statistically significant relative to controls; significance levels by the Fisher's exact test were $P = 0.0079$ for the 1.01 gm/kg/day level and $P = 0.0476$ for the 0.48 gm/kg/day level.

Further support for lack of sciatic nerve neuropathy in methoxyacetone treated rats *versus* the positive control methyl n-butyl ketone (1.01 gm/kg/day) rats and the negative control rats is obtained from study of the epon araldite one-micron sections. All 5 rats which received 1.01 gm/kg/day had moderate to severe lesions characterized by increased amounts of interstitial stroma, giant axonal fibers, basophilic axons, coagulative and granular axonal degeneration and myelin degeneration. Negative control and methoxyacetone animals had sciatic nerves within normal morphologic parameters. Two rats which received 1.99 gm/kg/day of methoxyacetone had limited sciatic nerve changes which were judged to be artifacts; these 2 animals were in the lower methoxyacetone treatment group.

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Skeletal muscle atrophy. Evaluation of skeletal muscle fiber atrophy was difficult due to shrinkage artifacts resulting from not fixing muscles *in situ* by perfusion fixation. Several evaluations of original slides plus subsequently processed additional muscle tissue were undertaken; in each instance this was done without knowledge of treatment of the animals. Once the normal range of variations in the morphologic appearance of muscle fibers in different skeletal muscles of rats was assessed, a final judgement was made as to whether there was, in fact, definite muscle fiber atrophy. Criteria for atrophy included diminution of cross-sectional area of muscle fibers, alteration of the shape, texture and tinctorial staining properties of muscle fibers and degenerative changes in muscle fibers.

The skeletal muscle fiber atrophy was, at times, generalized affecting individual fibers in many muscle bundles and, at times, localized where several contiguous muscle fibers within a particular muscle bundle were affected. On occasion, both localized and generalized atrophy was evident in the muscle from the same rat.

Histologic muscle fiber atrophy was documented in 3 of 5 rats which received 1.01 gm/kg/day of methyl n-butyl ketone, in 3 of 5 rats which received 0.48 gm/kg/day of methyl n-butyl ketone and in 1 of 5 rats receiving the lower dosage (1.99 gm/kg/day) of methoxyacetone. These findings as evaluated by Fisher's exact test were not statistically significant *versus* negative controls.

One rat which received 4.04 gm/kg/day of methoxyacetone had gross evidence of slight atrophy of thigh muscles. However, there was no histologic evidence in several pieces of muscle tissue examined to substantiate this gross observation.

Statistical testing of the planimeter measurements of skeletal muscle fiber cross-sectional areas by the rank sum test procedure indicated no significant differences. However, the value for the 0.48 gm/kg/day methyl n-butyl ketone group approached significance with respect to one negative control group ($\Delta = 15.8$ *versus* critical difference of 16.4 at $P = 0.05$).

Histologic Findings Not Related to Treatment

A variety of histologic changes were present in tissues of rats from all groups. These changes are briefly discussed below.

Larynx. In several rats there was minimal infiltration of the laryngeal submucosa by lymphocytes and other mononuclear cells. Although this is technically laryngitis, it was not recorded as a lesion; in no instance was this change significant. Similar submucosal infiltrates are observed in the larynxes of rats on other studies in this laboratory.

Dilation of laryngotracheal submucosal glands is a histologic finding in virtually all rats which come to necropsy. It is present in very young animals as well as adults and does not increase in severity over time. I do not understand the exact significance of this finding but its presence is not associated with any respiratory or general health impairment to the best of my knowledge.

Nasal cavity. Mild submucosal aggregates of lymphoid cells were present in the nasal cavities (turbinates), the nasopharynxes and the maxillary sinuses of most rats. On occasion a small amount of sero-purulent exudate could be found in nasal cavity spaces. In all instances these changes were considered to be within normal limits and were not reported. The rationale behind this judgment is that it is the normal function of the tissues in these anatomic areas to mount a response to inhaled particulates and antigens. The consistent absence of a host response would, in fact, be a remarkable finding somewhat analogous to a human going through life without ever blowing his nose.

Brain. A very peculiar vacuolation artifact was present in the cerebellar white matter of all 50 rats on this study. This change, which was also frequently seen in the medullary and spinal cord white matter, was not characterized by any pattern of progression or development. In some instances birefringent material was present in these vacuoles. We believe this artifact to be associated with the method of euthanasia (cervical severing) and have seen this change in the central nervous system of rats from other studies where the method of euthanasia was similar.

Spinal cord. An artifactually produced vacuolation of the cytoplasm of gray matter neurons was observed in the spinal cords of 42 of the 50 rats. This is believed due to delayed fixation of the spinal cord (the cord was not removed from the vertebral column) and/or exposure to acid during decalcification.

Heart. The mild arteriosclerotic changes characteristic of rat intramural coronary arteries were present in rats on this study. This spontaneous arterial change is common in rats and, while it progresses slowly during life, it is not associated with any significant cardiovascular or general health problems.

Lymph nodes. Cervical lymph nodes were characterized by recent hemorrhage in the lymph node sinuses. Erythrocytes were usually free but occasionally there was phagocytosis of erythrocytes. This histologic finding is interpreted to represent a drainage reaction occurring after euthanasia and is a consequence of the method of euthanasia. Blood from the cervical area drains to these regional lymph nodes while the animal is bleeding out. The cervical nodes are grossly red when the necropsy is performed. This hemorrhagic drainage reaction in cervical lymph nodes is consistently present in animals killed in this manner and is consistently absent if exsanguination is by some other means.

Mediastinal lymph nodes are in proximity and adjacent to the thymus and are separate from the bronchial (parabronchial) lymph nodes which are adjacent to the mainstem bronchi. While the mediastinal lymph nodes may indirectly drain pulmonary tissues due to the interconnection of the entire intrathoracic lymph system, they primarily drain the mediastinal tissues. In the present study many mediastinal lymph nodes (34 of 36 present on slides) were characterized by a mild hemorrhagic drainage reaction and/or mild hemosiderosis. This latter finding would indicate that at some previous time the lymph node had erythrocytes in lymphatic sinuses. The most likely source of the blood or hemosiderin in these

nodes is from the thymus. Thymus glands of rats in our laboratory frequently have mild hemorrhage in the medulla of some lobules. In all but two instances, the rats with evidence of a hemorrhagic drainage reaction in the mediastinal lymph nodes had some evidence of thymic hemorrhage. While I do not know the cause of the thymic hemorrhage, I consider it not to be a significant lesion unless severe. It is frequent in rat thymuses that I have examined and is likewise frequent, and equally non-specific, in dog thymus glands.

Lungs. Parabronchial and paravascular aggregates of small numbers of lymphoid cells, medial hypertrophy of occasional pulmonary arteries and occasional intra-alveolar aggregates of foamy macrophages are frequent spontaneous changes occurring in rodent pulmonary tissues. Unless these reactions were exaggerated, they were not documented during slide evaluation.

Spleen. In evaluating slides of spleen it was noted that some rats had splenic hemosiderosis which was more marked than that usually present in rat spleens. Consequently, a separate evaluation of splenic hemosiderosis was undertaken utilizing Prussian blue-stained sections. The results are tabulated in Table 2. There were no significant differences in degree of splenic hemosiderosis between treated and control animals. Hemosiderin was present in all 50 rats and has been tabulated in Table 1 even though it is probably not a significant finding.

Skeletal muscles. Skeletal muscle from 3 of 5 rats in the Control-1 group and 4 of 5 rats in the Control-2 group had occasional muscle fibers which were undergoing mineralization. The significance of this finding is not known.

Other. The sporadic occurrence of a variety of other histologic changes (e.g., Harderian gland dacryoadenitis, focal myocarditis, focal interstitial pneumonia and renal tubular cell hyperplasia) is consistent with similar findings in other studies. These changes are documented in Table 1 and represent part of the background of spontaneous histologic alterations commonly seen in this stock as well as other stocks and strains of rats.

Correlation of Clinical Observations and Histologic Lesions

Indications of neuromuscular deficit were documented throughout the study. Although they were variable and difficult to quantitate, it was concluded that there was significant ($0.05 > P > 0.01$) neurologic deficit in the rats receiving 1.01 gm/kg/day of methyl n-butyl ketone versus Control-1 rats but not versus Control-2 rats. Table 3 presents the occurrence of clinical neuromuscular deficit, sciatic nerve neuropathy and skeletal muscle (gastrocnemius) atrophy in the rats treated with methyl n-butyl ketone and methoxyacetone. In all instances where definite neuromuscular deficit was observed clinically, there were histologic lesions, viz, sciatic nerve neuropathy and/or skeletal muscle atrophy, to substantiate the clinical judgement. Six additional rats had nerve and/or muscle lesions (5 of the 6 had neuropathy) in the absence of definitive clinical indications of neuromuscular impairment suggesting that assessment of neuropathy is the most sensitive of the three criteria compared.

Histopathologic changes which might account for liver and kidney weight changes in treated *versus* control animals were not found. Partial explanation for decreased body weight gain in some treated rats may be a result of the observed skeletal muscle atrophy.

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Approved:

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Acknowledgments:

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1/2 0
3 0

Table 1

Frequency of Histologic Findings for 25-Week Old Female HLA Wistar Rats Given Selected Ketones in Drinking Water for 120 Days

ORGANS/Lesions	Treatment Groups									
	Methyl n-butyl ketone II	Methyl n-butyl ketone I	Methoxyacetone I	Methoxyacetone II	Ethyl n-butyl ketone	Diethyl ketone	iso-butyl ketone	Methyl tert-butyl ketone	Control I	Control 2
	1.01 gm/kg/day	0.48 gm/kg/day	4.04 gm/kg/day	1.99 gm/kg/day	0.03 gm/kg/day	1.86 gm/kg/day	1.04 gm/kg/day	0.88 gm/kg/day	1	2
DORSAL ROOT GANGLIA, Normal	1/1*	2/2	3/3	3/3	3/3	5/5	1/1	--*	2/2	2/2
SPINAL ROOTS, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
SCIATIC NERVE, Normal	0/5	1/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Neuropathy	5/5	4/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
SKELETAL MUSCLE, Normal	2/5	2/5	5/5	4/5	5/5	5/5	5/5	5/5	2/5	1/5
Atrophy	3/5	3/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Calcification	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	3/5	4/5
FEMUR, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	4/4	5/5	5/5
FEMORAL MARROW, Normal	5/5	5/5	5/5	5/5	4/4	5/5	5/5	4/4	5/5	5/5
NASAL CAVITY, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
TONGUE, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
EYES, Normal	5/5	5/5	5/5	5/5	4/4	5/5	5/5	4/4	4/4	5/5
HARDERIAN GLANDS, Normal	5/5	5/5	4/5	2/5	5/5	5/5	4/5	5/5	4/5	5/5
Dacryoadenitis	0/5	0/5	1/5	3/5	0/5	0/5	1/5	0/5	1/5	0/5
EARS, Normal	5/5	5/5	3/3	5/5	5/5	5/5	5/5	5/5	5/5	5/5
ADRENALS, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
PITUITARY, Normal	5/5	5/5	5/5	5/5	4/4	4/4	5/5	5/5	5/5	5/5
BRAIN, Normal	3/4	5/5	5/5	5/5	4/4	5/5	5/5	5/5	5/5	5/5
Axon spheroids	1/4	0/5	0/5	0/5	0/4	0/5	0/5	0/5	0/5	0/5
SPINAL CORD, Normal	5/5	2/4	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Hemorrhage, focal	0/5	1/4	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Axonal spheroids	0/5	1/4	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
VERTEBRAE, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
VERTEBRAL MARROW, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
AORTA, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
HEART, Normal	5/5	5/5	2/5	5/5	5/5	5/5	4/5	5/5	5/5	5/5
Edema, interstitial	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Myocarditis, focal	0/5	0/5	1/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5
Conventio	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

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Table 1
(Continued)

	Treatment Groups								Control 1	Control 2
	Methyl n-butyl ketone II	Methyl n-butyl ketone I	Methoxy-acetone I	Methoxy-acetone II	Ethyl n-butyl ketone	Diethyl ketone	Methyl iso-butyl ketone	Methyl tert-butyl ketone		
ORGANS/Lesions	1.01 gm/kg/day	0.48 gm/kg/day	4.04 gm/kg/day	1.99 gm/kg/day	0.03 gm/kg/day	1.86 gm/kg/day	1.04 gm/kg/day	0.88 gm/kg/day		
SALIVARY GLANDS, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
CERVICAL LYMPH NODE, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Hyperplasia, lymphoid	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
SPLEEN, Normal	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Hemosiderosis	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
PANCREAS, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
THYMUS, Normal	0/5	0/5	0/5	0/5	1/5	0/5	0/5	1/5	1/5	1/4
Hemorrhage	5/5	5/5	5/5	5/5	4/5	5/5	5/5	4/5	4/5	3/4
MEDIASTINAL LYMPH NODE, Normal	0/4	0/4	0/4	0/4	0/5	0/4	0/5	0/2	0/3	0/3
Hemorrhage	4/4	3/4	4/4	3/4	4/5	4/4	5/5	2/2	2/3	3/3
Hemosiderosis	3/4	3/4	0/4	2/4	4/5	4/4	4/5	2/2	3/3	2/3
Hyperplasia, lymphoid	0/4	0/4	0/4	0/4	0/5	0/4	0/5	0/2	0/3	1/3
TRACHEA, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
ESOPHAGUS, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
LARYNX, Normal	5/5	5/5	5/5	5/5	5/5	5/5	4/4	5/5	5/5	5/5
THYROID, Normal	5/5	5/5	5/5	4/5	5/5	5/5	3/3	5/5	5/5	4/4
Atrophy, early, unilateral	0/5	0/5	0/5	1/5	0/5	0/5	0/3	0/5	0/5	0/4
PARATHYROID, Normal	4/4	2/2	3/3	4/4	-	2/2	3/3	2/2	2/2	3/3
LUNGS, Normal	5/5	5/5	4/5	4/5	4/5	5/5	5/5	1/5	3/5	5/5
Congestion	0/5	0/5	0/5	1/5	1/5	0/5	0/5	1/5	1/5	0/5
Pulmonary arteries, hypertrophy	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Pneumonia, interstitial, focal	0/5	0/5	0/5	0/5	1/5	0/5	0/5	2/5	0/5	0/5
Alveolar macrophages, focal	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5
Hyperplasia, lymphoid	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
KIDNEYS, Normal	5/5	5/5	5/5	4/5	5/5	5/5	4/5	4/5	5/5	5/5
Congestion	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Hyperplasia, tubular cell	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5	0/5	0/5
Nephritis, focal	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
URINARY BLADDER, Normal	5/5	4/4	5/5	5/5	5/5	5/5	5/5	5/5	4/4	5/5

(Continued)

Table I
(Continued)

ORGANS/Lesions	Treatment Groups									
	Methyl n-butyl ketone II	Methyl n-butyl ketone I	Methoxy- acetone I	Methoxy- acetone II	Ethyl n-butyl ketone	Diethyl ketone	iso-butyl ketone	Methyl tert-butyl ketone	Control I	Control 2
	1.01 gm/kg/day	0.48 gm/kg/day	4.04 gm/kg/day	1.99 gm/kg/day	0.03 gm/kg/day	1.86 gm/kg/day	1.04 gm/kg/day	0.88 gm/kg/day		
OVARIES, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
OVIDUCTS, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
UTERINE HORNS, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
UTERINE BODY, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
STOMACH, Normal	5/5	4/5	4/5	2/5	4/5	3/5	4/5	2/5	4/5	5/5
Gastric glands, dilation	0/5	1/5	1/5	3/5	1/5	2/5	1/5	3/5	0/5	0/5
Congestion	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
DUODENUM, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
JEJUNUM, Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
ILEUM, Normal	5/5	5/5	4/5	5/5	4/4	5/5	5/5	3/5	5/5	3/5
Hyperplasia, lymphoid	0/5	0/5	1/5	0/5	0/4	0/5	0/5	2/5	0/5	2/5
CECUM, Normal	3/5	5/5	5/5	4/5	5/5	4/5	5/5	5/5	5/5	3/5
Nematodiasis	2/5	0/5	0/5	1/5	0/5	1/5	0/5	0/5	0/5	1/5
Hyperplasia, lymphoid	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5
COLON, Normal	5/5	5/5	4/5	3/5	4/5	5/5	2/5	3/5	5/5	5/5
Nematodiasis	0/5	0/5	0/5	2/5	1/5	0/5	2/5	0/5	0/5	0/5
Hyperplasia, lymphoid	0/5	0/5	1/5	0/5	0/5	0/5	2/5	2/5	0/5	0/5
MESENTERIC LYMPH NODE, Normal	5/5	5/5	5/5	4/4	5/5	5/5	5/5	4/5	5/5	4/5
Hyperplasia, lymphoid	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5
LIVER, Normal	5/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	4/5
Triaditis	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5
Hepatitis, focal	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5

* = Numerator equals number of rats with specified lesions.
 Denominator equals number of rats for which specified organ was examined.
 ** = Tissue not present in slides prepared.

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Re-evaluation of Original Slides of Thymus, Mediastinal and Parabranchial Lymph Nodes
and Anterior Thigh Muscle and Evaluation of Prussian Blue Stain on Spleen

Animal Group Animal Number	Splenic hemosiderosis	Thymic hemorrhage	Mediastinal lymph node		Parabranchial lymph node hemorrhage	Slide #5 Mineralization
			Hemorrhage	Erythrophagocytosis		
Control						
35912	2	1	2	1	-	0
35911	2	-	-	-	-	0
35902	2	0	-	-	-	0
35896	3	3	1	0	0	0
35874	2	2	1	1	-	0
35871	1	2	1	0	-	0
35892	2	1	1	0	-	0
35918	2	1	-	-	-	0
35905	2	2	-	-	0	0
35889	3	0	0	1	-	0
Diethyl ketone (1.86 gm/kg/day)						
35908	1	-	-	-	-	0
35900	2	2	1	1	-	0
35891	1	1	1	1	0	0
35877	1	2	3*	2	0	0
35875	2	1	1	1	0	0
Methyl n- butyl ketone (0.48 gm/kg/day)						
35906	2	1	2	3	0	0
35886	2	3	1	2	-	0
35883	4	2	-	-	-	0
35876	3	2	1	1	0	0
35873	2	3	0	1	1	0

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(Continued)

* also large amount of serum in sinuses
** also hemosiderin in sinuses

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Table 2
Ketone Neurotoxicity
(Continued)

Animal Group	Animal Number	Spleenic hemosiderosis	Thymic hemorrhage	Mediastinal lymph node		Parabronchial lymph node hemorrhage	Slide #5 Mineralization
				Hemorrhage	Erythrophagocytosis		
chyl n-butyl ketone (1.01 gm/kg/day)	35915	4	1	1	2	1	0
	35909	2	2	1	0	1	0
	35907	3	4	4	4	0	0
	35888	3	2	-	-	-	0
	35880	4	2	1	0	3	0
chyl n-butyl ketone (0.03 gm/kg/day)	35913	1	0	1	1	1	0
	35904	3	2	1	0	2	0
	35898	2	2	0	0	0	0
	35893	2	1	2	0	1	0
	35887	2	2	1	0	2	0
ethoxyacetone (1.99 gm/kg/day)	35901	3	1	0	0	0	0
	35882	3	1	1	0	0	0
	35879	3	1	-	-	-	0
	35919	2	1	2	0	1	0
	35916	2	1	2	2	2	0
ethoxyacetone (4.04 gm/kg/day)	35917	2	1	1	0	0	0
	35903	2	1	-	-	-	0
	35899	3	1	1	0	0	0
	35894	2	1	1	0	0	0
	35885	2	1	1	1	1	0

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TABLE 4
Ketone Neurotoxicity
(Continued)

Animal Group Animal Number	Splenic hemosiderosis	Thymic hemorrhage	Mediastinal lymph node		Parabronchial lymph node hemorrhage	Slide #5 Mineralization
			Hemorrhage	Erythrophagocytosis		
Methyl iso- butyl ketone (1.04 gm/kg/day)						
35920	1	1	1	1	-	0
35914	3	1	1	0	0	0
35910	2	2	2	1	-	0
35897	2	1	1	1	-	0
35881	2	1	1	1	-	0
Methyl tert- butyl ketone (0.88 gm/kg/day)						
35895	2	2	-	-	-	0
35890	3	0	2	2	-	0
35884	3	3	-	-	-	0
35878	3	3	2	1	0	0
35872	2	2	-	-	0	0

0 = none
1 = mild
2 = moderate
3 = marked
4 = severe
- = not examined

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Table 3

Concurrence of Clinically Evident Neuromuscular Deficit, Sciatic Nerve Neuropathy
and Gastrocnemius Muscle Atrophy

<u>Treatment Group</u> <u>Animal Number</u>	<u>Neuromuscular</u> <u>Deficit</u>	<u>Sciatic Nerve</u> <u>Neuropathy</u>	<u>Gastrocnemius</u> <u>Muscle Atrophy</u>
Methyl n-butyl ketone (0.48 gm/kg/day)			
35873	-*	+	+
35876	-	+	+
35883	-	+	-
35886	-	-	+
35906	-	+	-
Methyl n-butyl ketone (1.01 gm/kg/day)			
35880	+	+	-
35888	+	+	+
35907	+	+	+
35909	-	+	-
35915	+	+	+
Methoxyacetone (1.99 gm/kg/day)			
35875	-	-	-
35882	-	-	-
35901	-	-	-
35916	-	-	-
35919	+	-	+
Methoxyacetone (4.04 gm/kg/day)			
35885	-	-	-
35894	-	-	-
35899	-	-	-
35903	-	-	-
35917	-	-	-

* - = Not present
+ = Present

Table prepared by RRMDate 9-6-77Table approved by PABDate 9/6/77
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