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CARNEGIE-MELLON INSTITUTE OF RESEARCH, COMPARATIVE TOXICITY OF RATS OF METHOXYACETONE AND FIVE OTHER ALPHATIC KETONES IN THEIR DRINKING WATER, SUBMITTED TO UNION CARBIDE CORP, DANBURY, CT - (USED AS A REFERENCE IN OU 5 RI REPORT - APPENDIX A)

03/23/77

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UNION CARBIDE
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REPORT

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5 December 1995

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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 DOE and U.S. EPA in Region V (Chicago).

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

T J Cawley

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

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Project Report 40-37
17 Pages
March 23, 1977
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CHEMICAL HYGIENE FELLOWSHIP
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Comparative Toxicity to Rats of Methoxyacetone and Five Other Aliphatic Ketones in Their Drinking Water

Sponsor: *Union Carbide Corporation*

* * * * *

Summary

The toxicity of methoxyacetone was compared to that of methyl n-butyl ketone and a series of four other aliphatic ketones (diethyl ketone, ethyl n-butyl ketone, methyl isobutyl ketone, methyl tert-butyl ketone) with particular reference to neurotoxicity. Preliminary studies were performed to establish toxic dosage levels of the ketones, which were administered in drinking water for seven days at concentrations of 0.5% and 1% (estimated to be equivalent to ∿0.3 to 0.9 gm/kg/day). Reduced food and water intake and low body weight gains probably reflected rejection of the water because of taste. The concentrations used were at or near maximum water solubility of most of the ketones, methoxyacetone being the most notable exception. Methoxyacetone produced no toxic effects at these concentrations or at 2%, but 6% and 20% solutions of this material for 7 days produced dosage-related effects similar to those of the other ketones.

In the definitive study, the ketones were administered for 120 days in drinking water. Most of the ketones were administered at saturation concentrations. The concentration of methoxyacetone was escalated during the study to approximate a maximally tolerated dosage level. In addition to the parameters measured in the preliminary studies, toxicity was evaluated by examination of organ weights and gross pathology as well as by a battery of neurologic and neuromuscular function tests.

The administration of approximately 1 gm/kg/day of methyl n-butyl ketone produced the most severe toxicity as indicated by reduced food and water consumption, reduced body weight gain and increased kidney weight/body weight ratio. Also noted was neurologic impairment reflected in hind limb weakness and atrophy. Methoxyacetone, at final dosage levels of approximately 2 and 4 gm/kg/day, resulted in reduced food and water consumption and increased liver weight *per se* and as percentage of body weight. Mild to severe skeletal muscle atrophy of the

hind limbs was present in 2 to 4 of the 5 rats given methyl n-butyl ketone while only slight muscle atrophy was seen in 1 of the 5 given the higher dosage level of methoxyacetone. Effects produced by the other ketones studied included reduced body weight gain and decreased relative kidney weight. None of these other ketones produced significant neurologic alterations.

It is concluded that 1 gm/kg/day of methyl n-butyl ketone administered to rats in their drinking water for 120 days produced severe neurotoxicity and muscle atrophy. Methoxyacetone administration at a dosage approximately four times that of methyl n-butyl ketone produced only slight muscle atrophy. None of the other ketones studied produced discernible gross neurotoxic effects. Histopathologic findings will be reported separately.

Samples

Experimental materials were obtained from Union Carbide Corporation (UCC) as indicated below:

Diethyl ketone - CHF Sample No. 39-113, from UCC - South Charleston, 3-18-76, Register No. 511-01-1270, 1 quart.

Ethyl n-butyl ketone - CHF Sample No. 39-112, from UCC - South Charleston, 3-18-76, Register No. 511-01-1270, 1 quart.

Methyl n-butyl ketone (2-hexanone, H-449) - CHF Sample No. 39-111, from UCC - South Charleston, 3-18-76, Register No. 511-01-1270, 1 kg.

Methyl isobutyl ketone - CHF Sample No. 39-114, from UCC - South Charleston, 3-18-76, Register No. 511-01-1270, 1 quart.

Methyl tert-butyl ketone (pinacolone) - CHF Sample No. 39-115, from UCC - South Charleston, 3-18-76, Register No. 511-01-1270, 1 pint.

Methoxyacetone, 98.24% - CHF Sample No. 39-122, from UCC - Tarrytown, 3-19-76, Identification 1-HJD-34, 1245-38, 1 quart.

Except for methoxyacetone materials were assumed to be pure. All these ketones are clear or very pale-yellowish clear liquids and, except for methoxyacetone, of low water solubility. Gas chromatographic examination of saturated solutions and comparison with solutions of known concentration were carried out to determine aqueous solubility. Solubilities of ketones in water were determined as follows:

		Solubility w/v
Diethyl ketone	-	2.4%
Ethyl n-butyl ketone	-	0.03%
Methyl n-butyl ketone	-	1.3%
Methyl isobutyl ketone	-	1.3%
Methyl tert-butyl ketone	-	1.3%
Methoxyacetone	-	miscible at all concentrations

I. Preliminary Studies

A. Comparative Studies of Six Ketones

Procedure for Study 1 and Study 2

The six ketones were administered in drinking water to rats for seven days in two preliminary studies. The purpose of these preliminary studies was to determine acceptability and to estimate toxic levels for the subchronic study. Criteria of effect included food and water consumption, general appearance and behavior, body weight change and gross pathological examination.

In the first two preliminary studies the ketones were administered at concentrations of 0.5% and 1.0% (w/v) in water except for ethyl n-butyl ketone. Solubility of ethyl n-butyl ketone limited its concentration to 0.03% in both studies.

Results

Results of these studies are summarized in Table 40-1. One cage of five rats was treated with each ketone, thus statistical analysis of food and water consumption is precluded. Apparently reduced water consumption over the seven-day period probably reflected a reduction in palatability as a result of the addition of five ketones, the exception being methoxyacetone. A slight increase in water consumption by rats drinking the lower concentration of methoxyacetone is probably not biologically significant. Addition of 1.0% ketones to drinking water for seven days (0.03% ethyl butyl ketone) produced possible reduction in water consumption and food consumption and statistically significant reduction in weight gain by four ketones. Methoxyacetone was conspicuous in its apparent lack of effect on any of the parameters examined. Gross pathological examination revealed pale or mottled kidneys in both treated and control groups. These observations are summarized in Table 40-2. No evidence of gross pathologic effects by any of the ketones used was found.

B. Maximal Tolerated Dosage of Methoxyacetone

Procedure for Study 3

In the third preliminary study, methoxyacetone was administered in drinking water for seven days at concentrations of 2%, 6% and 20% to estimate a maximal tolerated dosage level.

Results

Results of the third preliminary study are shown in Table 40-3. Water and diet consumption and body weight change were all significantly affected in a dosage-related fashion. Rats given 2% methoxyacetone ate, drank and gained weight in an essentially normal manner. As indicated in the table footnote, the control data presented are from the earlier preliminary study and are not a valid basis for statistical comparison. Rats given a 6% solution had reduced food and water intake and failed to gain weight. Water and food consumption by rats at the 20% concentration were essentially nil and

there was a drastic weight loss over the one week period. Significant findings in necropsy were marked reduction in stomach and intestinal contents and a yellow staining of the intestine, probably the result of bile. These findings are most likely attributable to starvation and not a primary toxic effect of methoxyacetone. Hypoactivity and weakness were probably also attributable to starvation.

II. Subchronic Study (120 Days)

Procedure

Rats, approximately 4 weeks of age, used in this study were Wistar females obtained from Hilltop Laboratories, Scottdale, Pa. on April 1, 1976. The rats were identified by toe-clipping. They were weighed and distributed among the treatment groups of five rats each by formal randomization. Only rats whose body weights were within two standard deviational units of the overall mean weight were accepted for the study. Any rat that lost weight or that had poor tone during the preliminary observation period was rejected. The range of weights on the first day of study (April 23, 1977) was 187 to 236 grams.

Rats were housed in suspended steel wire-mesh cages. The diet fed, provided in 12-oz. opal glass jars, was Purina Laboratory Chow. Diet for each group was stored in a separate glass stock jar with a screw cap. These jars were weighed, full, at the beginning and, again, at the end of each week to determine the amount of diet consumed.

Water was available at all times in nonfouling siphon water bottles equipped with stainless steel tips. Ketones were administered in drinking water at predetermined concentrations, based on body weight and water consumption. Fresh solutions were prepared each week. The concentrations were adjusted as necessary to maintain a relatively constant dosage level for those ketones administered at subsaturation concentrations. Ethyl n-butyl ketone, methyl isobutyl ketone, methyl tert-butyl ketone, and the high dosage level of methyl n-butyl ketone were given as saturated aqueous solutions. Diethyl ketone, the low dosage of methyl n-butyl ketone, and both dosages of methoxyacetone were at subsaturation concentrations. The dosage of methoxyacetone was increased to the maximum level of 4.04 gm/kg/day after 38 days at 0.97 gm/kg/day.

The results of each quantitative continuous variable were intercompared for each of the eight ketone-treated groups and two control groups by use of Bartlett's homogeneity of variance, by analysis of variance and by Duncan's multiple range test. The latter was used, if F for analysis of variance was significantly high ($P < 0.05$), to determine which dosage groups differed from the controls. Any significant difference was denoted on the appropriate table of results as:

a $0.05 > P > 0.01$
b $0.01 > P > 0.001$
c $P < 0.001$

If a mean does not have an a, b or c superscript then $P > 0.05$.

Neurologic evaluation of treatment effects was performed by E. R. Homan on each rat in formal random sequence. Each examination consisted of observation of balance, strength, coordination and behavior according to predetermined sequence of criteria (App. 1). All rats were read blind, i.e., the treatments were not known to the observer at the time of examination. Performance was scored 0, 1, or 2 (0 = normal, 1 = equivocal or slight deficit, 2 = clearly de-

fined deficit) for each of ten criteria and recorded on evaluation forms (App. II). The net score for each rat on each test day was calculated as the sum of individual test scores. These scores were tabulated, ranked, and analyzed using the Kruskal-Wallis rank sum test.

Rats were euthanized on August 18, 1976 by CO_2 narcosis followed by exsanguination by severing of the cervical spinal cord and carotid blood vessels. Both sciatic nerves and brachial plexi were dissected free and fixed in 3% glutaraldehyde. The entire lumbo-sacral spinal cord, anterior and posterior thigh muscles and the distal portions of the hind limbs after removal of the skin, liver and kidneys and any tissues with gross lesions were fixed in 10% neutral buffered formalin. The liver and kidney weights of all rats were measured and tabulated prior to fixation. Results of histopathologic evaluation will be reported separately.

Results

The results of 120 days of administration of ketones on physical parameters are shown in Table 40-4. Four of the six ketones, including methoxyacetone at both dosage levels, had adverse effects on body weight. Methyl n-butyl ketone produced the most drastic reduction, with weight gains of rats at the high level only one-third of those of control rats. Liver weights of methyl n-butyl ketone-treated rats were depressed although the liver weights were roughly proportional to body weight. Methoxyacetone differed from all other ketones in this study in producing significant increases in absolute and relative liver weights. All of the ketones produced significant increases in relative kidney weight and significant increases in absolute kidney weight were observed in rats given diethyl ketone, methyl isobutyl ketone or methyl tert-butyl ketone.

The general observation to be made from these data is that all of the ketones, with the possible exception of the poorly soluble ethyl n-butyl ketone, were administered at definable adverse effect levels, thus providing sufficiently high dosage level in each case to estimate the relative as well as the absolute neurotoxic potential of each chemical. It should be noted that methoxyacetone was administered at dosage levels considerably higher than all the other materials.

Tables 40-5 through 40-8 summarize the neurologic examination test scores at various periods throughout the study. The Kruskal-Wallis rank sum test, appropriate to such nonparametric data, demonstrated a statistically significant difference between the neurologic effects of the higher dosage level of methyl n-butyl ketone (1.01 gm/kg/day) after 110 doses (Table 40-8) as compared to one of the untreated control groups. Methyl n-butyl ketone also produced unequivocal neurologic deficits reflected in posture, weakness, incoordination, and muscle wasting. This muscle wasting, most conspicuous in the hind limbs, was also noted in other groups as indicated in Table 40-9, which summarizes the gross pathological evaluation of the rats given the ketones in their drinking water for 120 days. In the 1.01 gm/kg/day methyl n-butyl ketone group, four of the five rats were affected and the severity ranged from slight to severe. At the 0.48 gm/kg/day dosage level, mild hind limb muscle atrophy was noted in two of five rats. One of five rats that received 4.04 gm/kg/day of methoxyacetone, a dosage approximately four times the highest level of methyl n-butyl ketone, exhibited slight atrophy. It should be noted that these animals were dosed at this higher rate for only 81 days since dosage was increased from 0.97 gm/kg/day at this time.

Several potentially rewarding areas for further investigations of methoxyacetone toxicity are suggested by these data. These are the metabolism of methoxyacetone, the possible use of liver and kidney biochemical and functional tests as sensitive indicators of toxicity, and the rate of recovery from neurotoxic and other toxic effects on termination of exposure. It would also be valuable to define the relationship between dosage and duration in producing toxic effects.

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Approved:

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Summary of Results of Seven Days of Inclusion of Ketones in the Drinking Water of Rats
(Study 1 and Study 2)

	Methyl n-Butyl Ketone	Ethyl Butyl Ketone	Diethyl Ketone	Methyl Isobutyl Ketone	Methyl tert-Butyl Ketone	Methoxy-acetone	Control I	Control II
	0.5% Ketones							
Concentration in water, %	0.5	0.03	0.5	0.5	0.5	0.5	0.0	0.0
Dosage attained, gm/kg/day	0.415	0.032	0.450	0.396	0.329	0.562	-	-
Water consumption, ml/rat/day	28.4	28.0	30.3	28.7	25.7	35.6	31.9	34.9
, % of control mean	85.0	83.9	90.9	85.9	77.0	106.8	-	-
Diet consumption, gm/kg/day	23.0	22.5	22.7	22.6	21.4	24.7	23.1	23.5
, % of control mean	98.7	96.6	97.4	96.9	91.8	106.0	-	-
Body weight change, gm	59.3	47.3	50.0	49.0	37.0	62.7	54.0	53.0
, % of control mean	110.9	88.5	93.5	91.6	69.2	117.1	-	-
	1% Ketones							
Concentration in water, %	1.0	0.03	1.0	1.0	1.0	1.0	0.0	0.0
Dosage attained, gm/kg/day	0.585	0.038	0.634	0.652	0.502	0.939	-	-
Water consumption, ml/rat/day	27.2	28.7	23.7	28.6	24.4	33.7	33.9	32.0
, % of control mean	80.1	84.5	69.8	84.2	71.8	99.3	-	-
Diet consumption, gm/kg/day	20.8	21.8	18.9	19.4	20.3	24.0	23.0	21.7
, % of control mean	93.1	97.5	84.6	86.8	90.8	107.4	-	-
Body weight change, gm	30.0	20.7[a,-*]	8.3[a,-]	14.7[a,-]	15.3[a,-]	37.3	31.3	44.7
, % of control mean	78.9	54.5	21.8	38.7	40.3	98.2	-	-

[a] $0.05 > P > 0.01$

* First letter of superscript denotes degree of significance *versus* control group A; second letter denotes degree of significance *versus* control group B.

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Table 40-2

Necropsy of Rats Given Ketones in Drinking Water for Seven Days
(Study 1 and Study 2)

Ketone	Concentration in Drinking Water	
	1%	0.5%
Methyl n-butyl ketone	Kidneys pale - 3/3	Kidneys pale - 3/3
Ethyl n-butyl ketone (0.03%)	Spleen rough - 2/3	Kidneys pale - 2/3, mottled - 3/3
Diethyl ketone	No significant gross lesions	Kidneys pale - 2/3, mottled - 2/3
Methyl isobutyl ketone	Kidneys pale - 2/3	Kidneys pale, mottled - 3/3
Methyl tert-butyl ketone	Kidneys pale - 2/3	Kidneys pale - 2/3, mottled - 3/3
Methoxyacetone	Kidneys sl. pale - 2/3	Kidneys pale, mottled - 3/3
Control I	No significant gross lesions	Kidneys mottled - 2/3
Control II	No significant gross lesions	Kidneys pale, mottled - 3/3

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Table 40-3

Summary of Results of Seven Days of Inclusion of Methoxyacetone in the Drinking Water of Female Rats
(Study 3)

	Methoxyacetone; Concentration in Water			
	2%	6%	20%	Control*
Dosage attained, gm/kg/day	2.29	3.89	2.31	0.00
Water consumption, ml/rat/day	31.4	13.7	2.1	33.4
, % of control mean	94.0	41.0	6.3	-
Diet consumption, gm/kg/day	21.8	12.3	3.9	23.3
, % of control mean	93.6	52.8	16.7	-
Body weight change, gm	52.0	-1.67	-69.3	53.5
, % of control mean	97.2	-3.1	-129.5	-

* Control = Mean control data of Study 1. No control rats included in this study (rats not available).

Table 40-4

Results of 120 Days of Inclusion of Aliphatic Ketones in the Drinking Water of Female Rats

	Diethyl Ketone	Ethyl Butyl Ketone	Methyl Isobutyl Ketone	Methyl tert-Butyl Ketone	Methoxy-acetone I	Methoxy-acetone II	Methyl n-Butyl Ketone I	Methyl n-Butyl Ketone II	Control I	Control II
Concentration in water, %	2.4	0.03	1.3	1.3	4.8	2.4	0.65	1.3	0.00	0.00
Final Dosage, gm/kg/day	1.86	0.027	1.04	0.88	4.04	1.9	0.48	1.01	0.00	0.00
Diet Consumption, gm/rat/day	15.10	16.48	16.84	14.80	13.69	13.99	16.90	12.90	17.39	15.48
Water Consumption, ml/rat/day	19.28	24.80	24.33	18.53	20.17	22.92	17.98	17.33	32.29	27.57
Body Wt. Gain, gm	$63.6^{c,c*}$	99.6	101.2	$78.0^{b,b}$	$68.6^{c,c}$	$75.6^{b,b}$	$60.0^{c,c}$	$34.3^{c,c}$	110.2	112.6
Liver Wt., gm	9.04	9.71	9.43	9.77	$11.71^{a,a}$	10.77	9.01	$7.80^{c,c}$	10.30	10.13
Liver Wt., % of body wt.	3.33	3.13	3.05	3.41	$4.26^{c,c}$	$3.83^{c,c}$	3.35	3.38	3.10	3.04
Kidney Wt., gm	$2.43^{c,a}$	2.18	$2.39^{b,a}$	$2.47^{c,b}$	2.12	2.07	2.21	2.10	1.97	2.13
Kidney Wt., % of body wt.	$0.90^{c,c}$	$0.71^{a,-}$	$0.77^{c,a}$	$0.87^{c,c}$	$0.77^{c,a}$	$0.73^{b,a}$	$0.82^{c,c}$	$0.92^{c,c}$	0.60	0.64

a $0.05 > P > 0.01$
b $0.01 > P > 0.001$
c $P < 0.001$

* First letter of superscript denotes degree of significance *versus* control group A; second letter denotes degree of significance *versus* control group B.

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Table 40-5

Analysis of Neurologic Evaluation Scores

Aliphatic Ketones in Drinking Water

after 46 Doses

	Diethyl Ketone	Ethyl Butyl Ketone	Methyl Isobutyl Ketone	Methyl tert-Butyl Ketone	Methoxy-acetone I	Methoxy-acetone II	Methyl n-Butyl Ketone I	Methyl n-Butyl Ketone II	Control I	Control II
Concentration in water, %	2.4	0.03	1.3	1.3	4.8	2.4	0.65	1.3	0.00	0.00
Final Dosage, gm/kg/day	1.86	0.03	1.04	0.88	4.04	1.99	0.48	1.01	0.00	0.00
Test Scores	0	1	0	2	2	3	5	6	1	0
	0	1	0	2	2	2	4	4	1	0
	0	0	0	0	2	0	1	2	1	0
	0	0	0	0	2	0	0	1	0	0
	0	0	0	0	1	0	0	0	0	0
Rank Values	15	33.5	15	41.5	41.5	46	49	50	33.5	15
	15	33.5	15	41.5	41.5	41.5	47.5	47.5	33.5	15
	15	15	15	15	41.5	15	33.5	41.5	33.5	15
	15	15	15	15	41.5	15	15	33.5	15	15
	15	15	15	15	33.5	15	15	15	15	15
Mean Rank	15.0	22.4	15.0	25.6	39.9	26.5	32.0	37.5	26.1	15.0

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Table 40-6

Analysis of Neurologic Evaluation Scores

Aliphatic Ketones in Drinking Water

after 57 Doses

	Diethyl Ketone	Ethyl Butyl Ketone	Methyl Isobutyl Ketone	Methyl tert-Butyl Ketone	Methoxy-acetone I	Methoxy-acetone II	Methyl n-Butyl Ketone I	Methyl n-Butyl Ketone II	Control I	Control II
Concentration in water, %	2.4	0.03	1.3	1.3	4.8	2.4	0.65	1.3	0.00	0.00
Final Dosage, gm/kg/day	1.86	0.03	1.04	0.88	4.04	1.99	0.48	1.01	0.00	0.00
Test Scores	1	2	0	3	0	4	2	6	0	0
	0	1	0	2	0	2	2	2	0	0
	0	0	0	2	0	2	2	2	0	0
	0	0	0	2	0	1	0	2	0	0
	0	0	0	2	0	1	0	1	0	0
Rank Values	32	41	15	48	15	49	41	50	15	15
	15	32	15	41	15	41	41	41	15	15
	15	15	15	41	15	41	41	41	15	15
	15	15	15	41	15	32	15	41	15	15
	15	15	15	41	15	32	15	32	15	15
Mean Rank	18.4	23.6	15.0	42.4	15.0	39.0	30.6	41.0	15.0	15.0

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Table 40-7

Analysis of Neurologic Evaluation Scores

Aliphatic Ketones in Drinking Water

after 80 Doses

	Diethyl Ketone	Ethyl Butyl Ketone	Methyl Isobutyl Ketone	Methyl tert-Butyl Ketone	Methoxy-acetone I	Methoxy-acetone II	Methyl n-Butyl Ketone I	Methyl n-Butyl Ketone II	Control I	Control II
Concentration in water, %	2.4	0.03	1.3	1.3	4.8	2.4	0.65	1.3	0.00	0.00
Final Dosage, gm/kg/day	1.86	0.03	1.04	0.88	4.04	1.99	0.48	1.01	0.00	0.00
Test Scores	0	2	3	2	3	9	2	14	5	4
	0	1	1	1	3	8	2	7	1	1
	0	0	1	0	1	3	2	6	0	0
	0	0	0	0	0	1	2	5	0	0
	0	0	0	0	0	0	0	0	0	0
Rank Values	12.5	35.5	40.5	35.5	40.5	49	35.5	50	44.5	43
	12.5	28.5	28.5	28.5	40.5	48	35.5	47	28.5	28.5
	12.5	12.5	28.5	12.5	28.5	40.5	35.5	46	12.5	12.5
	12.5	12.5	12.5	12.5	12.5	28.5	35.5	44.5	12.5	12.5
	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Mean Rank	12.5	20.3	24.5	20.3	26.9	35.7	30.9	40.0	22.1	21.8

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Table 40-8

Analysis of Neurologic Evaluation Scores

Aliphatic Ketones in Drinking Water

after 110 Doses

	Diethyl Ketone	Ethyl Butyl Ketone	Methyl Isobutyl Ketone	Methyl tert-Butyl Ketone	Methoxy-acetone I	Methoxy-acetone II	Methyl n-Butyl Ketone I	Methyl n-Butyl Ketone II	Control I	Control II
Concentration in water, %	2.4	0.03	1.3	1.3	4.8	2.4	0.65	1.3	0.00	0.00
Final Dosage, gm/kg/day	1.86	0.03	1.04	0.88	4.04	1.99	0.48	1.01	0.00	0.00
Test Scores	1	1	0	2	6	4	1	15	0	2
	1	0	0	0	4	1	0	14	0	0
	0	0	0	0	0	1	0	14	0	0
	0	0	0	0	0	0	0	10	0	0
	0	0	0	0	0	0	0	2	0	0
Rank Values	37.5	37.5	17.5	42	46	44.5	37.5	50	17.5	42
	37.5	17.5	17.5	17.5	44.5	37.5	17.5	48.5	17.5	17.5
	17.5	17.5	17.5	17.5	17.5	37.5	17.5	48.5	17.5	17.5
	17.5	17.5	17.5	17.5	17.5	17.5	17.5	47	17.5	17.5
	17.5	17.5	17.5	17.5	17.5	17.5	17.5	42	17.5	17.5
Mean Rank	25.5	21.5	17.5	22.4	28.6	30.9	21.5	47.2[a,-]	17.5	22.4

[a] $0.05 > P > 0.01$

* First letter of superscript denotes degree of significance *versus* control group A; second letter denotes degree of significance *versus* control group B.

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Table 40-9

Gross Pathologic Evaluation of Rats Given Ketones in Drinking Water for 120 Days

	Concentration in water, %	Final Dosage Level, gm/kg/day	Pathologic Observations
Diethyl ketone	2.4	0.78	No significant gross lesions
Ethyl n-Butyl Ketone	0.03	0.03	No significant gross lesions
Methyl Isobutyl Ketone	1.3	1.04	No significant gross lesions
Methyl tert-Butyl Ketone	1.3	0.88	No significant gross lesions
Methoxyacetone I	4.8	3.89	Slight atrophy of thigh muscles affecting 1 of 5 animals examined
Methoxyacetone II	2.4	1.92	No significant gross lesions
Methyl n-butyl Ketone I	0.65	0.48	Mild atrophy affecting skeletal muscles of the hind limbs in 2 of 5 animals examined
Methyl n-butyl Ketone II	1.3	1.01	Slight to severe atrophy of skeletal muscles affecting 4 of 5 animals examined, Most pronounced in muscles of the hind limbs
Control I	0.0	0.00	No significant gross lesions
Control II	0.0	0.00	No significant gross lesions

Appendix I

Neurologic Examination of Rats

Performance Standards and Criteria

Posture:

Place animal on horizontal surface; watch for normal arch of back, position and orientation of legs and feet, head carriage.

Gait:

Urge walking if necessary; observe regularity, symmetry, coordination of movement.

Palpebral reflex:

Touch cornea with a wisp of cotton fiber or similar substance; observe blink.

Startle response:

Clap hands or snap fingers out of sight of animal; watch for crouch and/or jump.

Flexor reflex:

Pinch toe; observe for limb flexion, struggle, and vocalization.

Extensor reflex:

With rat in dorsal recumbency, place two fingers on bottoms of hind footpads and press on one; observe clutching of claw in both feet, marked increase in tension of pressed limb.

Placing reaction:

Touch dorsal surface of paw to table edge; watch for attempt to place ventral surface on table.

Hopping reaction:

Hold three legs off table, drag other limb across surface; observe hopping response.

Righting reaction:

Drop rat in dorsal recumbency a few inches from table; rat should land on feet.

Clinging reaction:

Place rat on a vertical mesh surface of a cage; rat should cling securely and move to a horizontal surface in a coordinated and purposeful fashion.

E. R. H.
May 27, 1976

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Appendix II

Neurologic Examination of Rats

Date: _____

Experiment: _____

Examiner: _____

Scoring: 0 = normal 1 = equivocal 2 = abnormal

Rat No.	Posture	Gait	Palpebral reflex	Startle reflex	Flexor reflex	Extensor reflex	Placing reflex	Hopping reaction	Righting reflex	Clinging reaction

Notes: